Estimation of Withanolides (Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastromonolide, Withanolide A, Withanolide B) in *Withania somnifera*

Liquid Chromatography

A. Principle

Withanolides are compounds specific to *Withania somnifera*. The method proposes extraction of withanolides from the sample matrix using methanol and separating the compounds using gradient high performance liquid chromatography on a C-18 column and measure in UV at 227nm.

B. Apparatus

(**a**) *LC system*.— Shimadzu High Performance Liquid Chromatographic system equipped with LC10A pump with SPD-M 10Avp Photo diode Array Detector or UV detector in combination with Class-VP software or LC 2010 A and LC 2010HT integrated system equipped with Quaternary gradient, auto injector in combination with Lab solution software or any other suitable HPLC system with similar configuration can be used.
(**b**) *Column*.— Phenomenex- Luna C18(2), 250 × 4.6 mm with 5 µm particle size; Part No. : 00G-4252- E0 (Phenomenex, Torrance, CA, USA; www.phenomenex.com).
(c) *Analytical balance.—Read ability, 0.1 mg.*
(**e**) *Filtration apparatus*.—0.45 µm nylon filter.
(**f**) *Ultrasonic bath*
(g) *Syringe filter – 0.45µm PES filter*

C. Reagents

(**a**) *Degassed mobile phase*.—
1) Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH2PO4) in 900 ml of HPLC grade water (obtained from Millipore, Milli-Q Water purification system) and add 0.5ml of orthophosphoric acid. Make upto 1000 ml with water, filter through 0.45 µ membrane and degas in a sonicator for 3 minutes **(Solvent A).**
2) Acetonitrile **(Solvent B)**

Time (min)	Solvent A Conc	Solvent B Conc
0.01	95.0	5.0
18.0	55.0	45.0
25.0	20.0	80.0
28.0	20.0	80.0
35.0	55.0	45.0
40.0	95.0	5.0
45.0	95.0	5.0

(**b**) *Diluent*.—Methanol.
(**c**) Individual *Withanolide standards.* — M/s Natural Remedies Pvt. Ltd. (Bangalore, KA, India; www.phytocompounds.com), or other suppliers.

D. Standards

Weigh accurately each 5mg of Withanoside IV, Withanoside V Withaferin A, 12-Deoxywithastramonolide, Withanolide-A and Withanolide-B reference standards to 50 ml volumetric flask. Dissolve in 10ml of methanol with the aid of gently heating and cool then make up to 50ml with methanol.

E. Preparation of Test Solutions

(**a**) *Raw material*. — Weigh accurately a sample quantity of *Withania somnifera* raw material equivalent 5 mg (about 2.5 g will be sufficient) of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastramonolide, Withanolide A and Withanolide B in a 250ml beaker, Extract with 100 ml of methanol boiling on water bath for 10-15minutes and repeat the procedure 3 - 4 times till the raw material is completely extracted or till the extracts turn colorless. Combine all the fractions, concentrate and make up the volume to 50ml with methanol. Filter through 0.45microns membrane filter paper.
(**b**) *Standardized (common) extract*. — Weigh accurately a sample quantity of *Withania somnifera* extract equivalent 5 mg (about 0.5 g will be sufficient) of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastramonolide, Withanolide A and Withanolide B in a 250ml beaker, Extract with 100 ml of methanol boiling on water bath for 10-15minutes and repeat the procedure 3 - 4 times till the raw material is completely extracted or till the extracts turn colorless. Combine all the fractions, concentrate and make up the volume to 50ml with methanol. Filter through 0.45microns membrane filter paper.

F. Analysis

(**a**) *Chromatographic conditions*.—
Column.— Phenomenex- Luna C18(2), 250 × 4.6 mm with 5 µm particle size; Part No. : 00G-4252- E0 (Phenomenex, Torrance, CA, USA; www.phenomenex.com).

Temperature: Maintained at a constant temperature between 20 to 30°C (preferably 27°C)
Detector: SPD-M 10Avp Photo diode array detector or UV Detector
Wave length: 227 nm
Flow rate: 1.5ml/ min
Run time: 45minutes.

(**b**) *Retention time relative*.—

Relative retention time of

Withanoside IV - 0.7
Withanoside V - 0.89
Withaferin A - 0.92
12-Deoxywithastramonolide - 0.96
Withanolide A - 1.0
Withanolide B - 1.15

(**c**) *System suitability*.—(*1*) *Repeatability.*— The RSD of each of the individual withanolides peak area for at least 5 consecutive injections of the level 4 linearity standard solution must be ≤ 2.5%.

(*2*) *Resolution*.—Calculate the resolution between Withanoside V and Withaferin A peaks as follows:

$$R = 2 \times \frac{T2 - T1}{W1 + W2}$$

Where T1 and T2 are the retention times of Withanoside V and Withaferin A respectively and W1 and W2 is their peak widths measured at the base line between tangents drawn to the peak sides. The resolution between ephedrine and pseudoephedrine in should be ≥ 3.0.

(*3*) *Tailing.*—Calculate the tailing factor (F) as follows:

$$F = \frac{L + R}{2L}$$

where L is the width from start of the peak to the perpendicular from the peak apex at 5% of the peak height; R is the width from the perpendicular from the peak apex to the peak end at 5% of the peak height. The tailing factor must be ≤1.5 for all individual withanolides in the linearity standard solution chromatograms.

(*4*) *Determination coefficient*.—the r^2 for the regression line of peak area vs. concentration for each withanolide must be ≥0.998.

Procedure: Inject three times the standard preparation and calculate the mean area and the RSD. The RSD should not be more than 2%. Inject 20µl of sample preparation and record the chromatogram at 227nm. Calculate the percentage of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxywithastramonolide, Withanolide A and Withanolide B content from the peak areas using the formula:

Peak integration: Base to base

Individual Withanolide (%w/w)

$$= \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \frac{\text{Weight of standard (mg)}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight (mg)}} \times \text{Purity of standard \%}$$

Compound name	CAS no.
Withanoside IV	362472-81-9
Withanoside V	256520-90-8
Withaferin A	5119-48-2
12-Deoxywithastramonolide	60124-17-6
Withanolide A	32911-62-9
Withanolide B	56973-41-2

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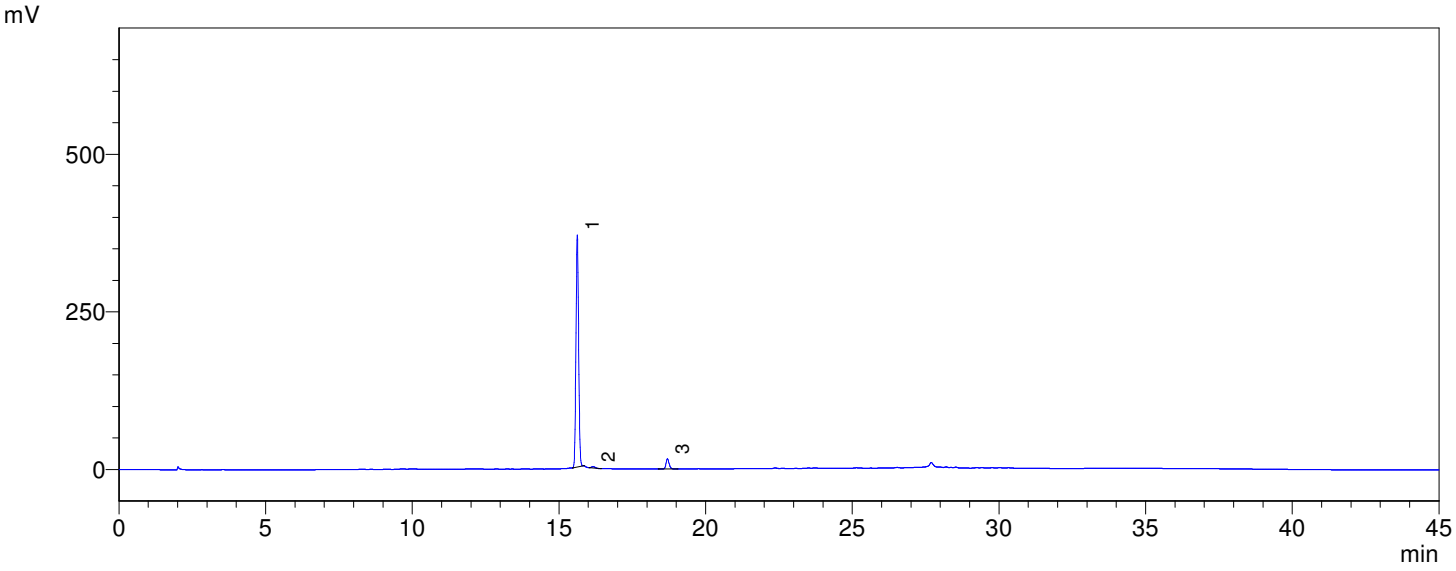
Sample Information

Acquired by	**: Admin**
Sample Name	**: Withanoside IV**
Sample ID	**: 310 mcg / ml**
Vail#	**: 1**
Injection Volume	**: 20 uL**
Data Filename	**: spec001.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 8:00:06 PM**
Data Processed	**: 3/17/2007 4:35:24 PM**

Enclosure: 01



1 Det.A Ch1/227nm

PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	15.621	2297769	367570	94.520	Withanoside IV
2	16.159	16891	1982	0.695	RT16.159
3	18.696	116329	16102	4.785	RT18.696
Total		2430989	385655	100.000	

NATURAL REMEDIES PRIVATE LIMITED
QUALITY CONTROL DEPARTMENT

Sample Information

Acquired by	**: Admin**
Sample Name	**: Withanoside V**
Sample ID	**: 360 mcg / ml**
Vail#	**: 2**
Injection Volume	**: 20 uL**
Data Filename	**: spec002.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 8:45:51 PM**
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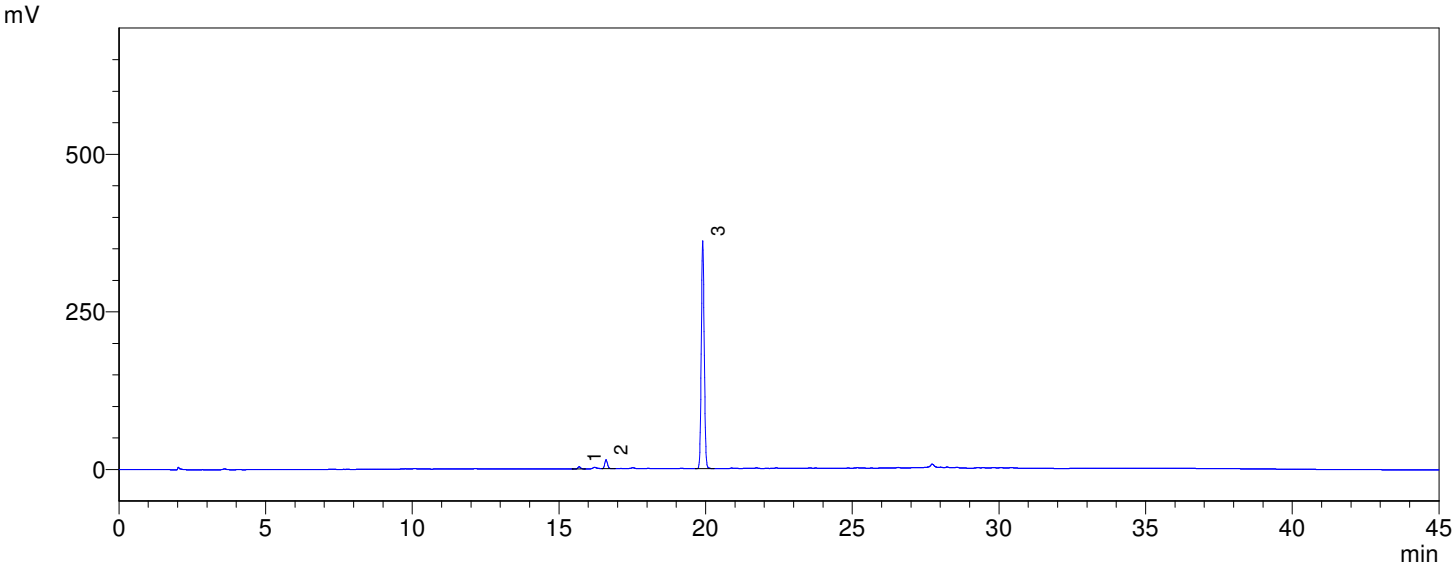


1 Det.A Ch1/227nm

PeakTable

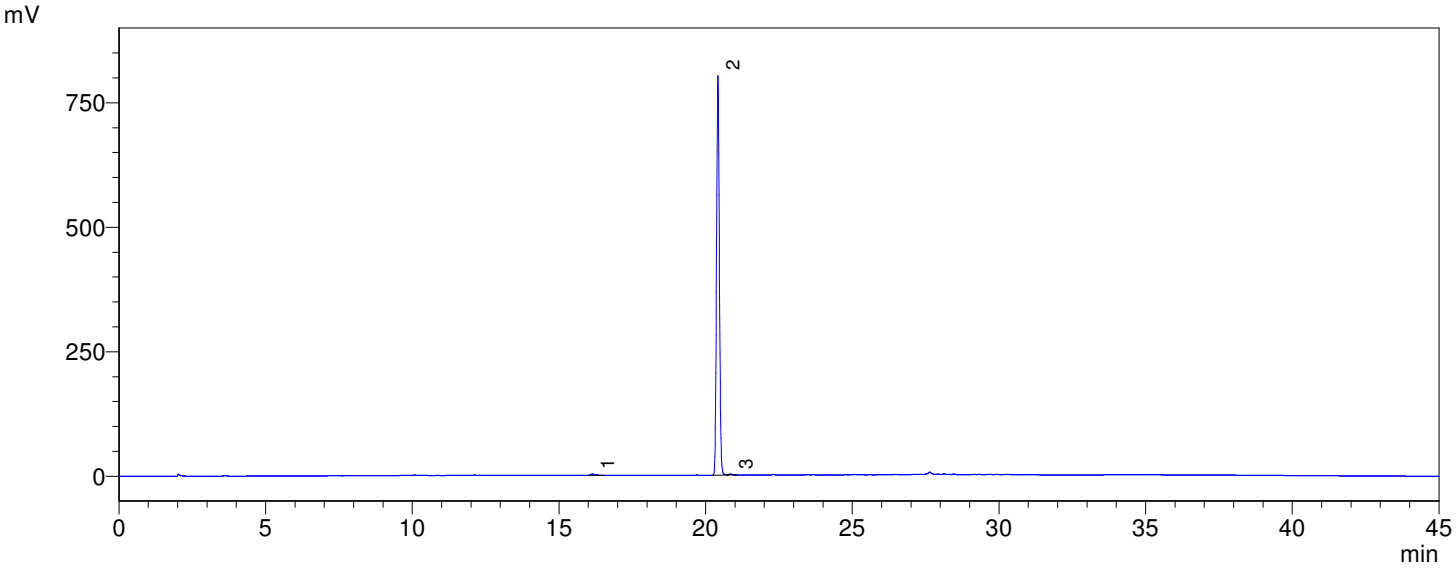
Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	15.687	20820	3456	0.788	RT15.687
2	16.602	90713	13975	3.431	RT16.602
3	19.899	2532314	361517	95.781	Withanoside V
Total		2643847	378948	100.000	

NATURAL REMEDIES PRIVATE LIMITED
QUALITY CONTROL DEPARTMENT

Sample Information

Acquired by	**: Admin**
Sample Name	**: Withaferin A**
Sample ID	**: 210 mcg / ml**
Vail#	**: 3**
Injection Volume	**: 20 uL**
Data Filename	**: spec003.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 9:33:53 PM**
Data Processed	**: 3/17/2007 4:37:03 PM**



1 Det.A Ch1/227nm

PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	16.137	26074	2835	0.478	RT16.137
2	20.416	5416080	802762	99.345	Withaferin A
3	20.851	9622	1700	0.177	RT20.851
Total		5451776	807297	100.000	

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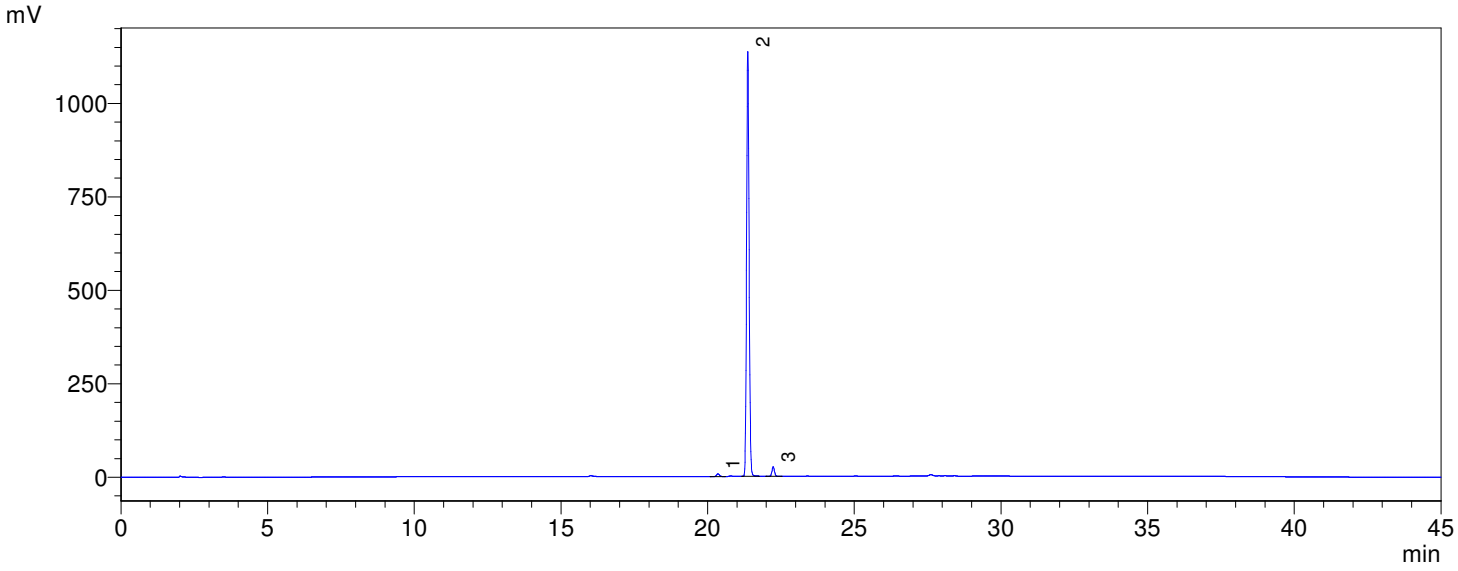
Sample Information

Acquired by	**: Admin**
Sample Name	**: 12-Deoxy withastramonolide**
Sample ID	**: 300 mcg / ml**
Vail#	**: 4**
Injection Volume	**: 20 uL**
Data Filename	**: spec004.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 10:19:39 PM**
Data Processed	**: 3/17/2007 4:37:44 PM**



1 Det.A Ch1/227nm

PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	20.348	48366	7114	0.687	RT20.348
2	21.367	6832966	1135453	97.123	12-Deoxy withastramonolide
3	22.231	154048	25447	2.190	RT22.231
Total		7035380	1168014	100.000	

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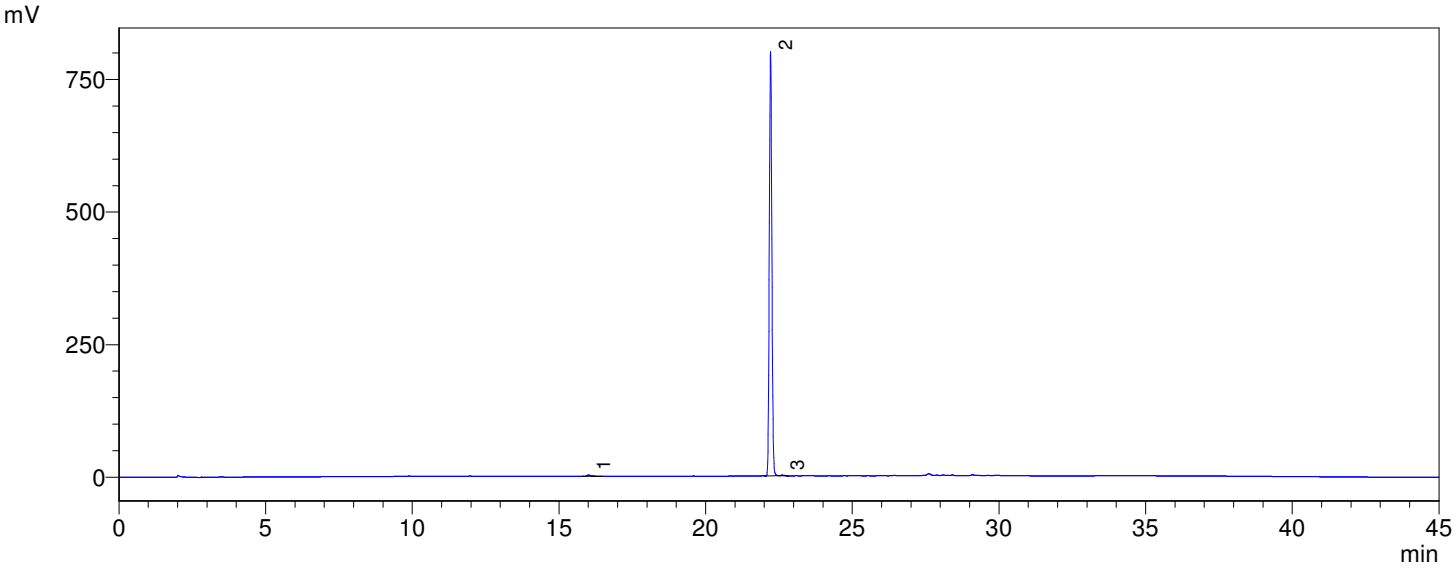
QUALITY CONTROL DEPARTMENT

Sample Information

Acquired by	**: Admin**
Sample Name	**: Withanolide A**
Sample ID	**: 240 mcg / ml**
Vail#	**: 5**
Injection Volume	**: 20 uL**
Data Filename	**: spec005.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 11:05:22 PM**
Data Processed	**: 3/17/2007 4:38:16 PM**



1 Det.A Ch1/227nm

PeakTable

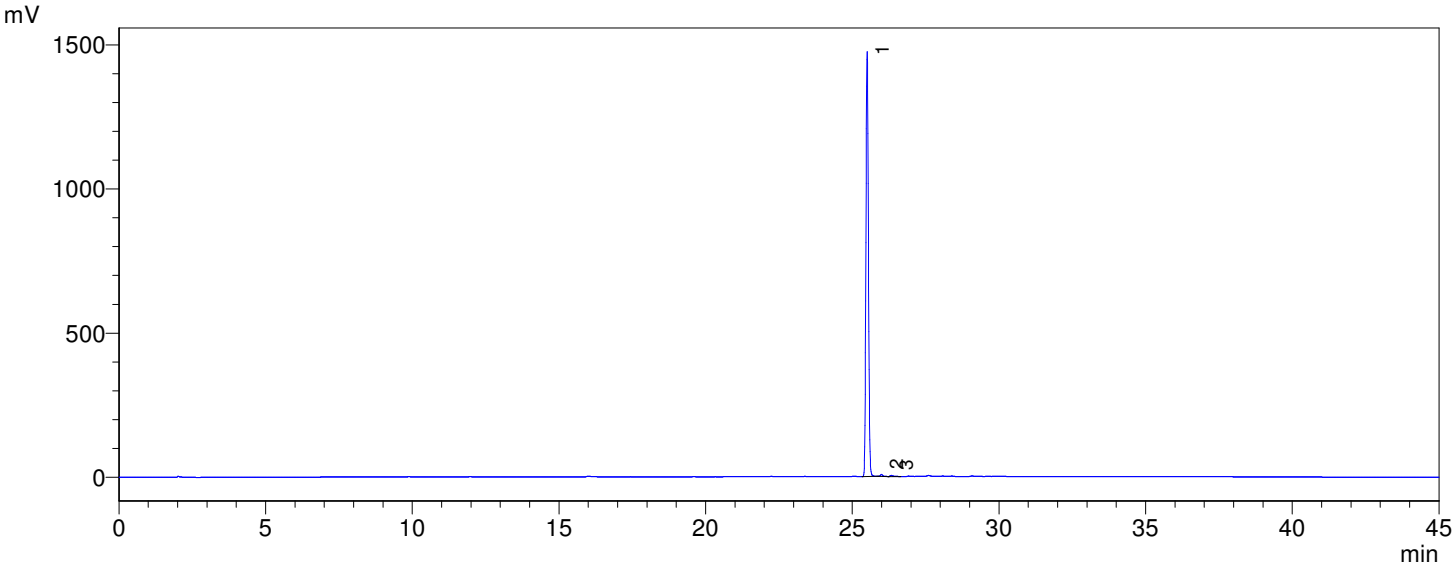
Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	16.006	24336	2445	0.501	RT16.006
2	22.210	4827594	799965	99.306	Withanolide A
3	22.607	9420	1092	0.194	RT22.607
Total		4861350	803501	100.000	

NATURAL REMEDIES PRIVATE LIMITED
QUALITY CONTROL DEPARTMENT

Sample Information

Acquired by	**: Admin**
Sample Name	**: Withanolide B**
Sample ID	**: 350 mcg / ml**
Vail#	**: 6**
Injection Volume	**: 20 uL**
Data Filename	**: spec006.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/13/2007 11:51:07 PM**
Data Processed	**: 3/17/2007 4:38:55 PM**



1 Det.A Ch1/227nm

PeakTable

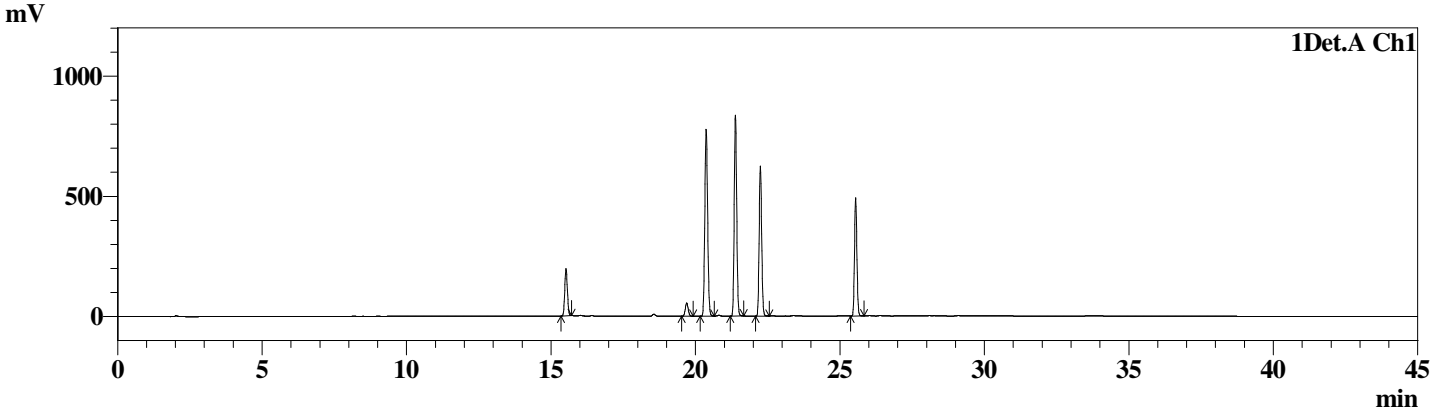
Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Name
1	25.503	8607191	1473066	99.245	Withanolide B
2	25.991	39170	6709	0.452	RT25.991
3	26.328	26273	3790	0.303	RT26.328
Total		8672634	1483566	100.000	

QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: 13.01.07
Vail #	: 40
Injection Volume	: 20 uL
Data File Name	: Std-rep005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 7:15:23 AM
Data Processed	: 3/29/2007 4:24:59 PM

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Std-rep005.lcd

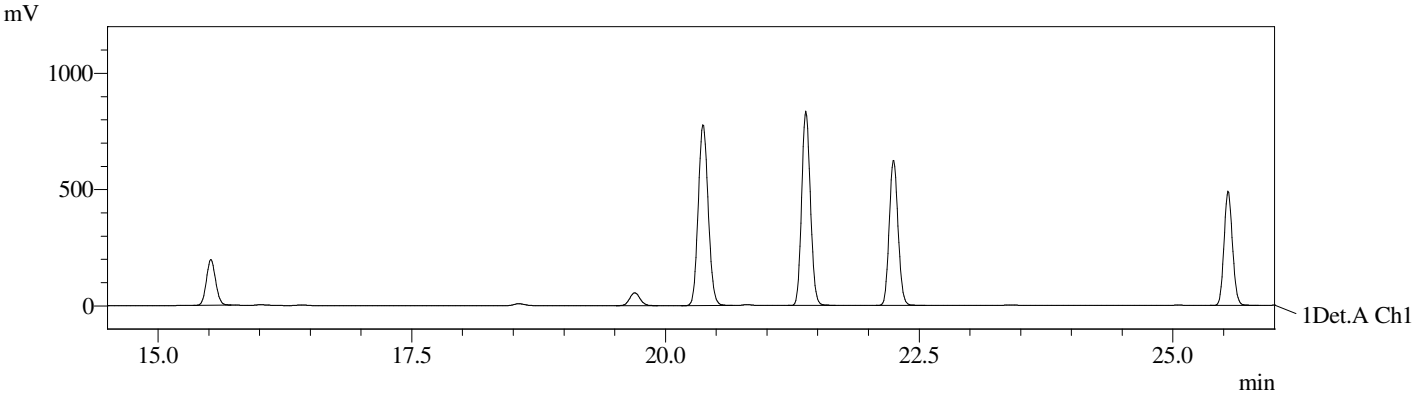


1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.516	Withanoside IV	1234072	6.645	0.000
2	19.692	Withanoside V	391438	2.108	22.163
3	20.366	Withaferin A	5318748	28.640	3.454
4	21.379	12- Deoxy withastramonolide	5001196	26.930	5.576
5	22.242	Withanollide A	3786871	20.391	5.030
6	25.539	Withanolide B	2839005	15.287	19.518
Total			18571330	100.000	

Chromatogram
D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Std-rep005.lcd

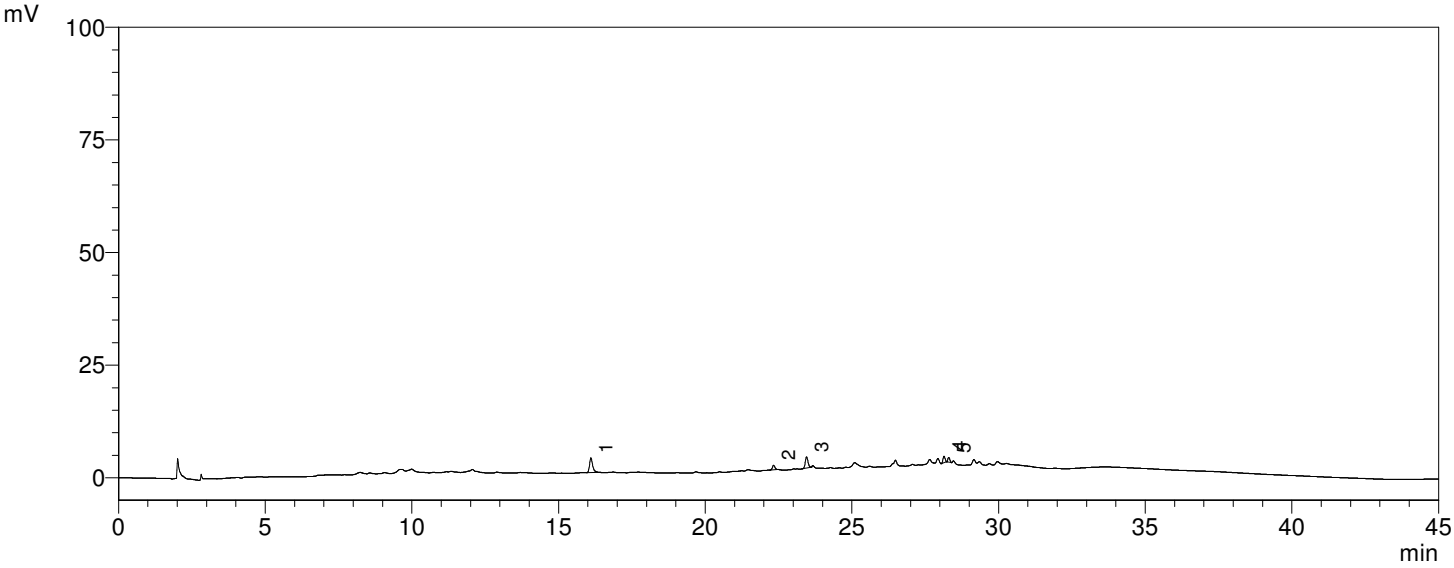


1 Det.A Ch1 / 227nm

NATURAL REMEDIES PRIVATE LIMITED
QUALITY CONTROL DEPARTMENT

Sample Information

Acquired by	**: Admin**
Sample Name	**: Methanol solvent**
Sample ID	**: Blank**
Vail#	**: 41**
Injection Volume	**: 20 uL**
Data Filename	**: Blank.lcd**
Batch Filename	**: Batch 01.lcb**
Report Filename	**: Calibration.lcr**
Date Acquired	**: 1/15/2007 8:13:15 PM**
Data Processed	**: 3/17/2007 4:31:21 PM**



1 Det.A Ch1/227nm

PeakTable

Detector A Ch1 227nm

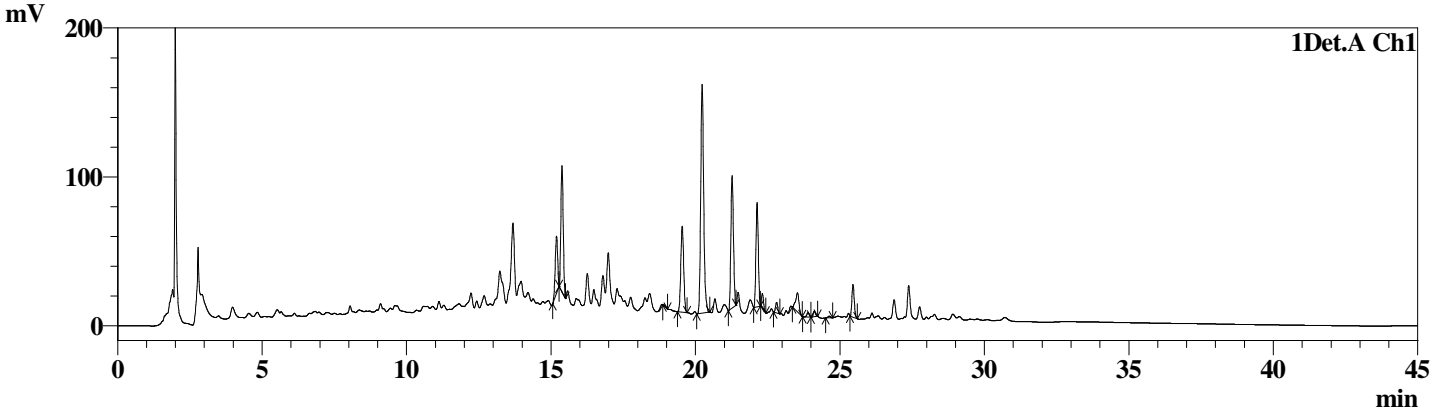
Peak#	Ret. Time	Area	Height	Area %	Name
1	16.101	24794	3252	41.980	RT16.101
2	22.329	5908	1013	10.003	RT22.329
3	23.452	16110	2466	27.277	RT23.452
4	28.140	7305	1439	12.369	RT28.140
5	28.301	4944	1030	8.370	RT28.301
Total		59061	9200	100.000	

QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 1
Vail #	: 43
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 1:45:20 PM
Data Processed	: 3/29/2007 5:01:03 PM

Enclosure: 02

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Rep-RP-II001.lcd

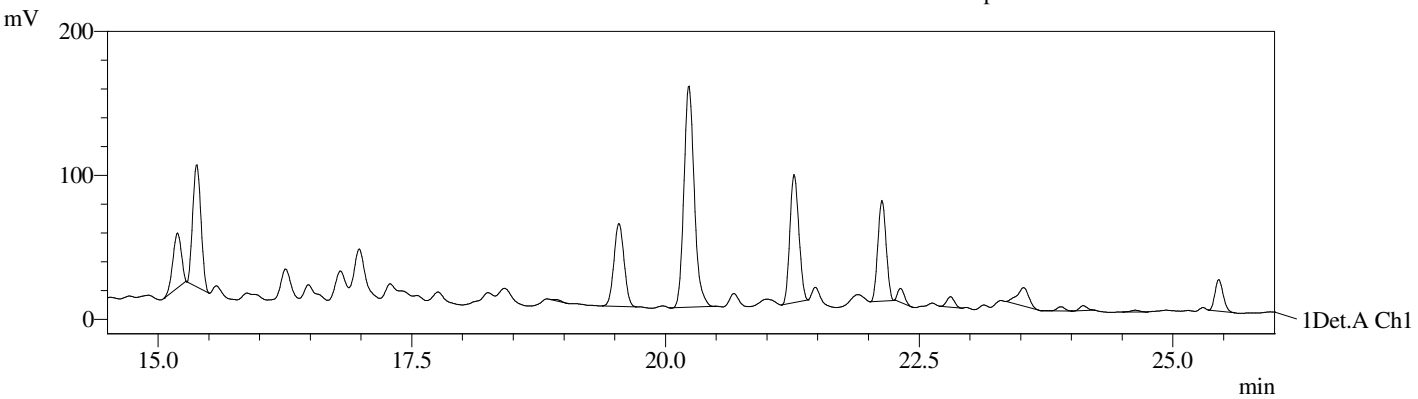


1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.187	RT15.187	225004	6.435	0.000
2	15.376	Withanolside IV	478462	13.685	1.135
3	18.909	RT18.909	3921	0.112	23.432
4	19.537	Withanoside V	400277	11.448	3.776
5	20.226	Withaferin A	1097206	31.381	3.516
6	21.263	Withastramonolide	548663	15.692	5.550
7	22.128	Withanolide A	398098	11.386	5.034
8	22.311	RT22.311	43911	1.256	1.219
9	22.804	RT22.804	36992	1.058	3.406
10	23.524	RT23.524	100800	2.883	3.854
11	23.891	RT23.891	15874	0.454	1.951
12	24.112	RT24.112	17872	0.511	1.423
13	24.625	RT24.625	6589	0.188	3.252
14	25.447	Withanolide B	122702	3.509	5.045
Total			3496369	100.000	

Chromatogram
D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Rep-RP-II001.lcd



1 Det.A Ch1 / 227nm

QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: RM - ERH - 46
Vail #	: 21
Injection Volume	: 20 uL
Data File Name	: Quan008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 1:21:18 PM
Data Processed	: 3/29/2007 4:45:13 PM

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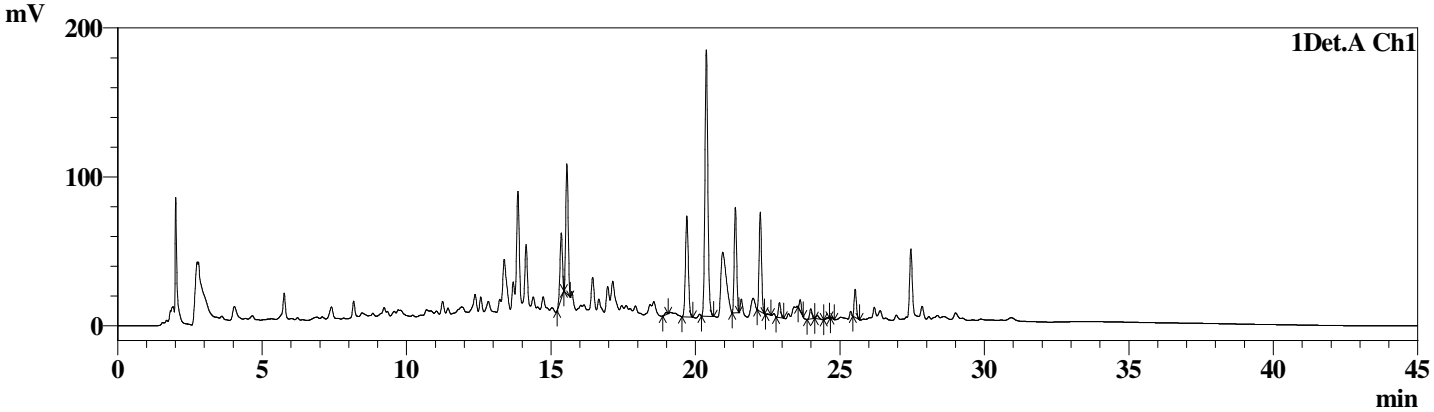
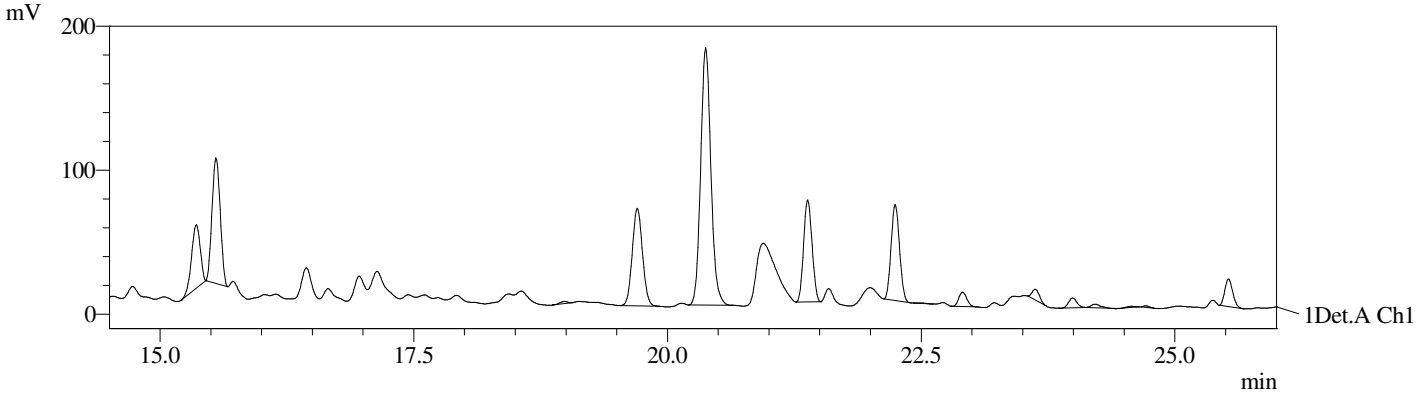


1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.352	RT15.352	265649	7.501	0.000
2	15.547	Withanoside-IV	491157	13.869	1.158
3	18.980	RT18.980	8341	0.236	21.729
4	19.698	Withanoside V	473414	13.368	4.128
5	20.372	Withaferin A	1250895	35.323	3.455
6	21.377	Withastramonolide	410796	11.600	5.561
7	22.239	Withanolide A	384409	10.855	5.143
8	22.491	RT22.491	1440	0.041	1.589
9	22.903	RT22.903	54369	1.535	2.662
10	23.619	RT23.619	36688	1.036	4.654
11	23.987	RT23.987	38306	1.082	2.373
12	24.208	RT24.208	14767	0.417	1.282
13	24.567	RT24.567	4931	0.139	2.088
14	24.709	RT24.709	4074	0.115	0.994
15	25.523	Withanolide B	102080	2.883	5.790
Total			3541316	100.000	

Chromatogram
D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Quan008.lcd



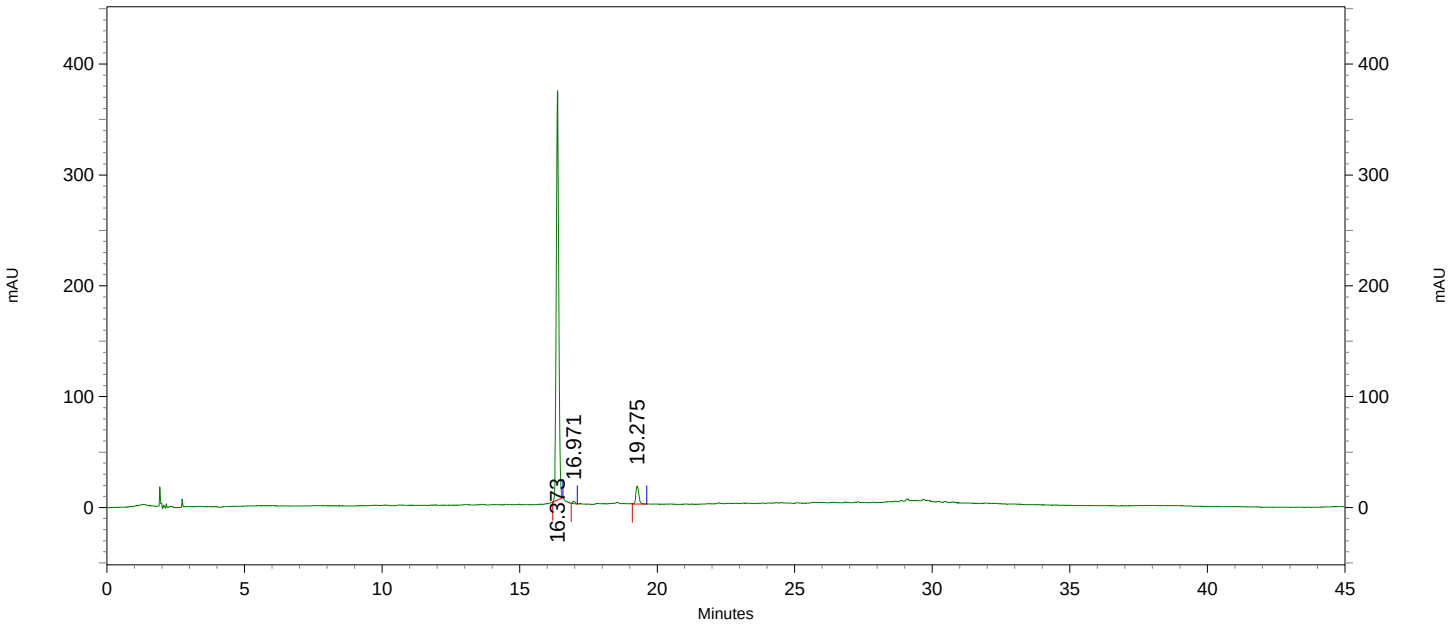
1 Det.A Ch1 / 227nm

Enclosure: 03

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : **Withanoside IV**
Method Name : **C:\CLASS-VP\Methods\METHODS\Withania.met**
Data Name : **D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-01a1-Rep-2**
Injection Volume : **20**
Acquired : **1/16/07 5:13:17 PM**

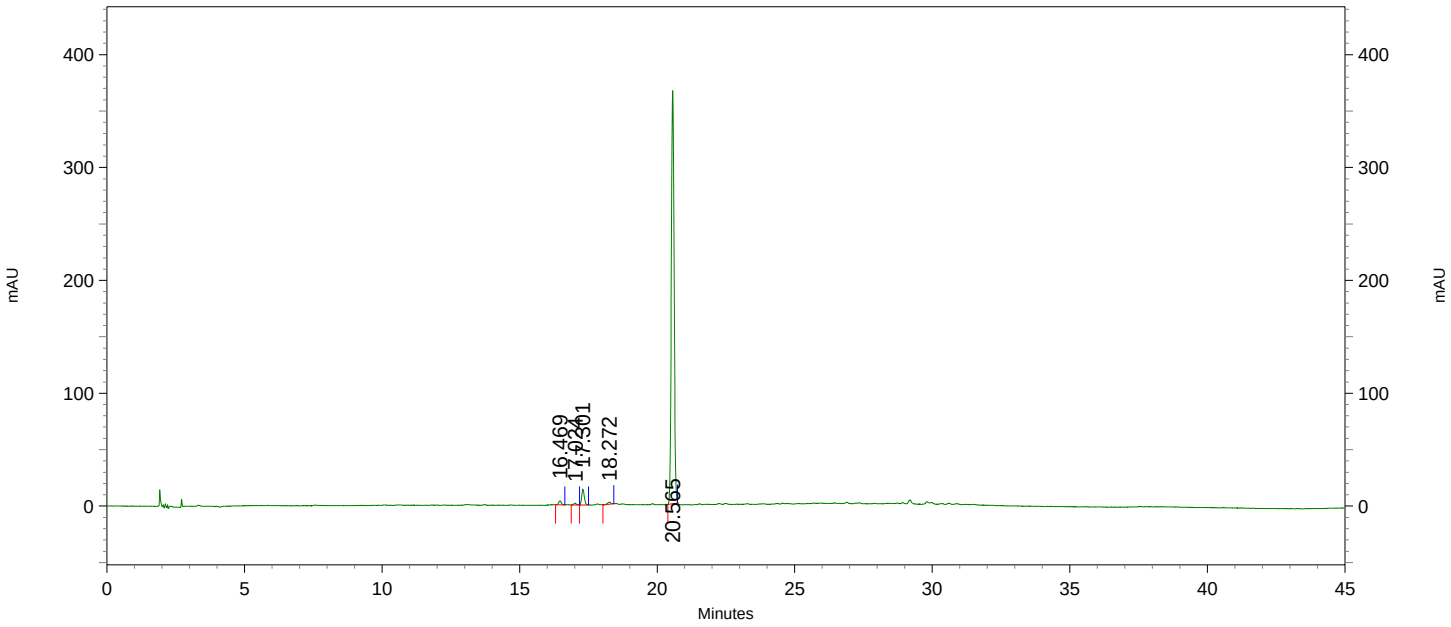


Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.373	2346882	94.68	0.00	1.10	138825.47	-0.05	0.37	0.18	369468
16.971	13273	0.54	3.32	0.00	135239.38	-0.02	0.22	0.18	2153
19.275	118703	4.79	12.20	1.23	159399.92	0.12	0.53	0.19	16352
Totals	**2478858**	**100.00**							**387973**

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : Withanoside V
Method Name : C:\CLASS-VP\Methods\METHODS\Withania.met
Data Name : D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-02a
Injection Volume : 20
Acquired : 1/16/07 6:48:29 PM



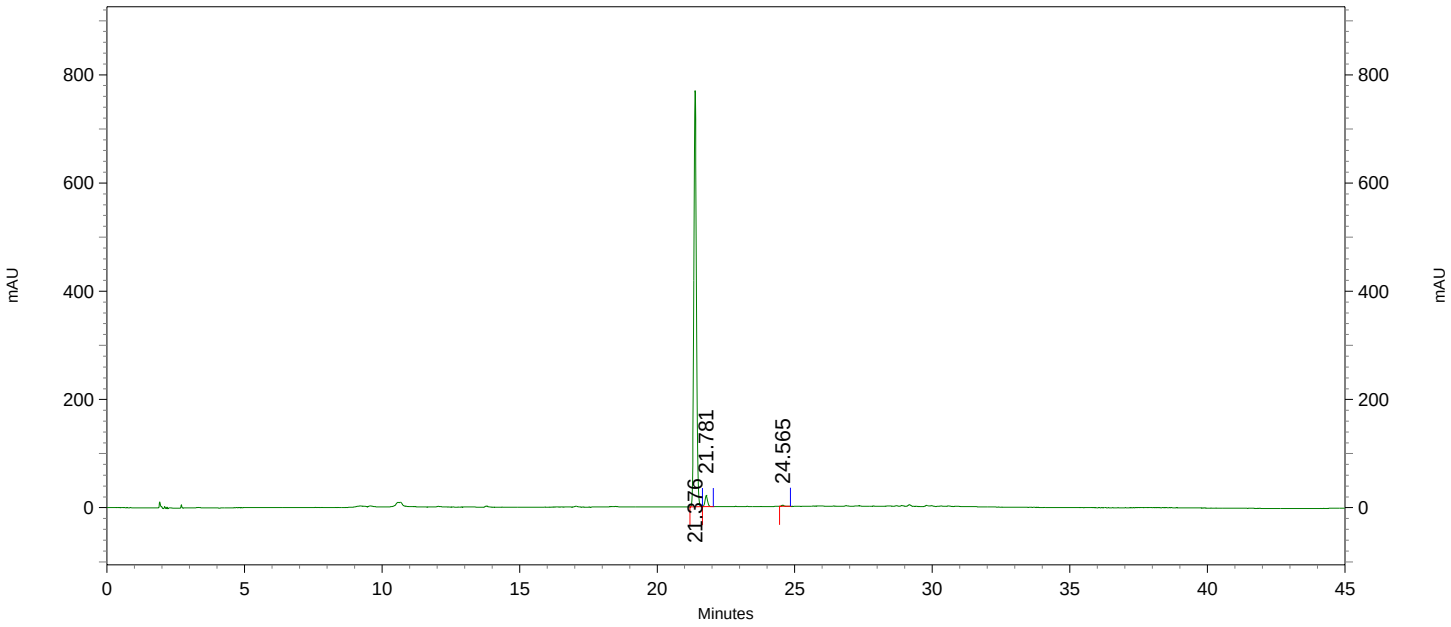
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.469	23248	0.87	0.00	1.07	126689.00	-0.05	0.34	0.19	3544
17.024	11761	0.44	3.01	0.97	137036.11	-0.01	0.30	0.18	1619
17.301	94069	3.53	1.52	1.13	146967.23	0.00	0.33	0.18	14261
18.272	15926	0.60	4.98	0.74	122179.84	0.06	0.39	0.21	1877
20.565	2522780	94.56	11.48	1.05	186867.95	0.19	0.35	0.19	366195

Totals									
	2667784	100.00							387496

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : Withaferin A
Method Name : C:\CLASS-VP\Methods\METHODS\Withania.met
Data Name : D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-03a
Injection Volume : 20
Acquired : 1/16/07 7:36:05 PM



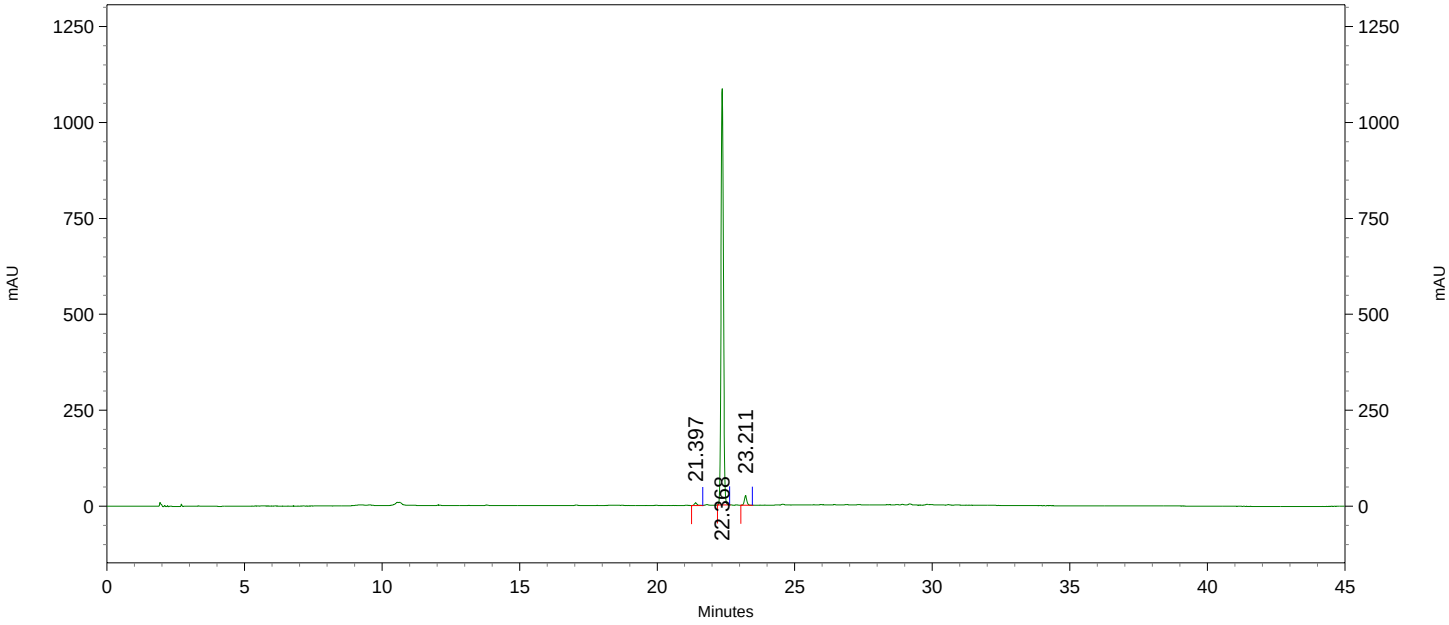
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
21.376	5088622	97.13	0.00	1.11	221434.52	0.24	0.45	0.18	769328
21.781	134321	2.56	2.25	1.16	235949.08	0.26	0.39	0.18	20879
24.565	15855	0.30	15.94	1.56	334448.38	0.42	0.39	0.17	2343

Totals									
	5238798	100.00							792550

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : 12-Deoxy withastramonolide
Method Name : C:\CLASS-VP\Methods\METHODS\Withania.met
Data Name : D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-04a
Injection Volume : 20
Acquired : 1/16/07 8:23:39 PM

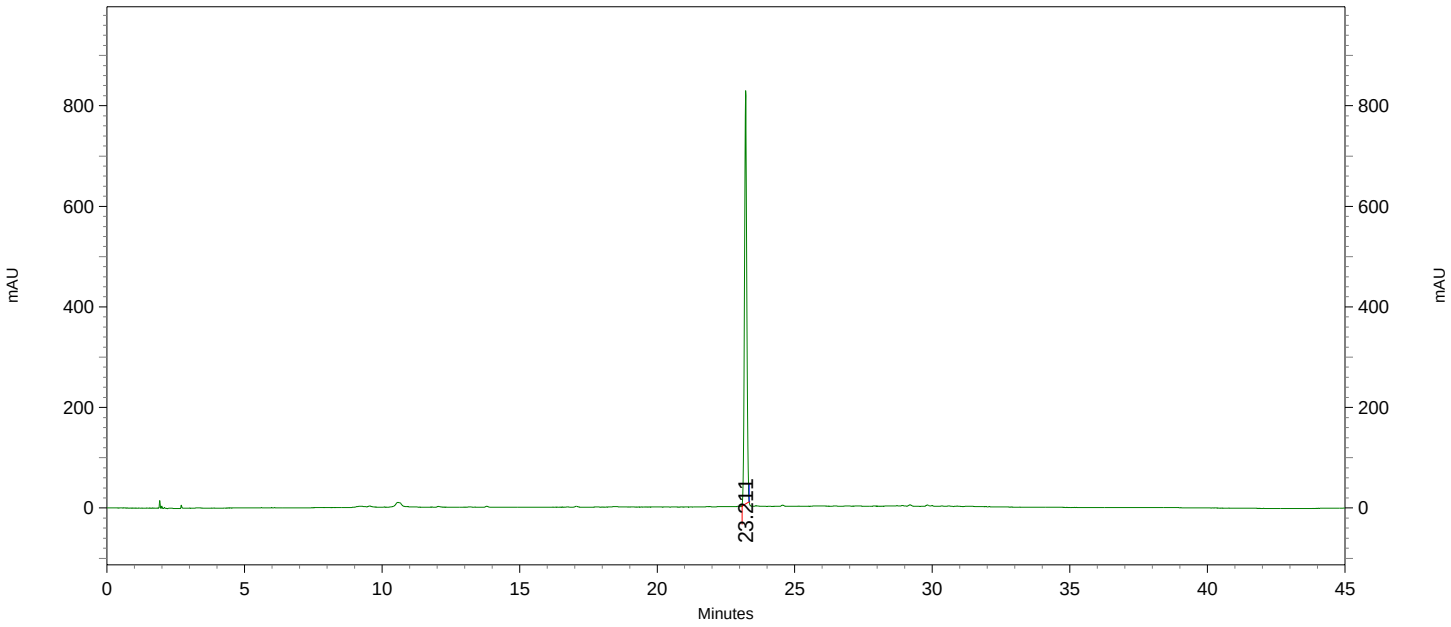


Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
21.397	47071	0.70	0.00	1.14	218371.22	0.24	0.41	0.18	6927
22.368	6561291	97.03	5.56	1.03	290232.75	0.30	0.43	0.17	1085611
23.211	153754	2.27	5.02	1.14	298543.09	0.35	0.42	0.17	25530
Totals	**6762116**	**100.00**							**1118068**

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : Withanolide A
Method Name : C:\CLASS-VP\Methods\METHODS\Withania.met
Data Name : D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-05a
Injection Volume : 20
Acquired : 1/16/07 9:11:15 PM

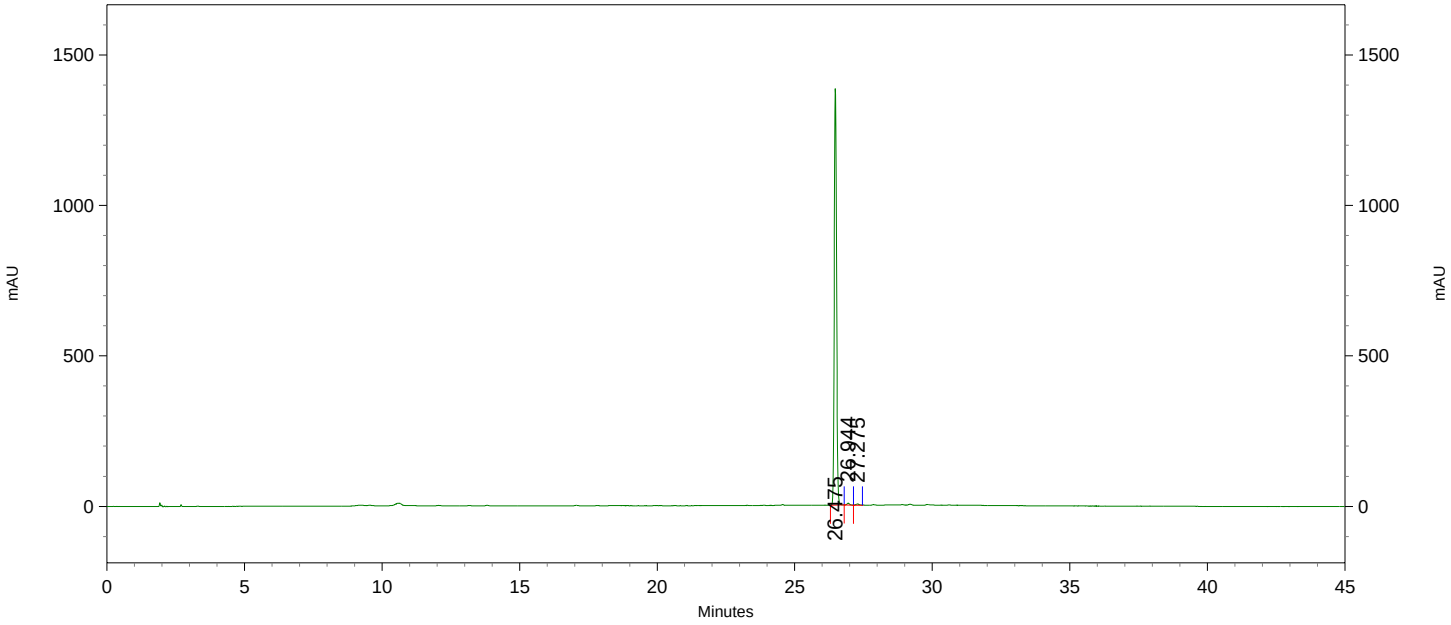


Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
23.211	4806845	100.00	0.00	1.10	315411.41	0.35	0.26	0.17	822204
Totals	4806845	100.00							822204

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : Withanolide B
Method Name : C:\CLASS-VP\Methods\METHODS\Withania.met
Data Name : D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-06a
Injection Volume : 20
Acquired : 1/16/07 9:58:51 PM

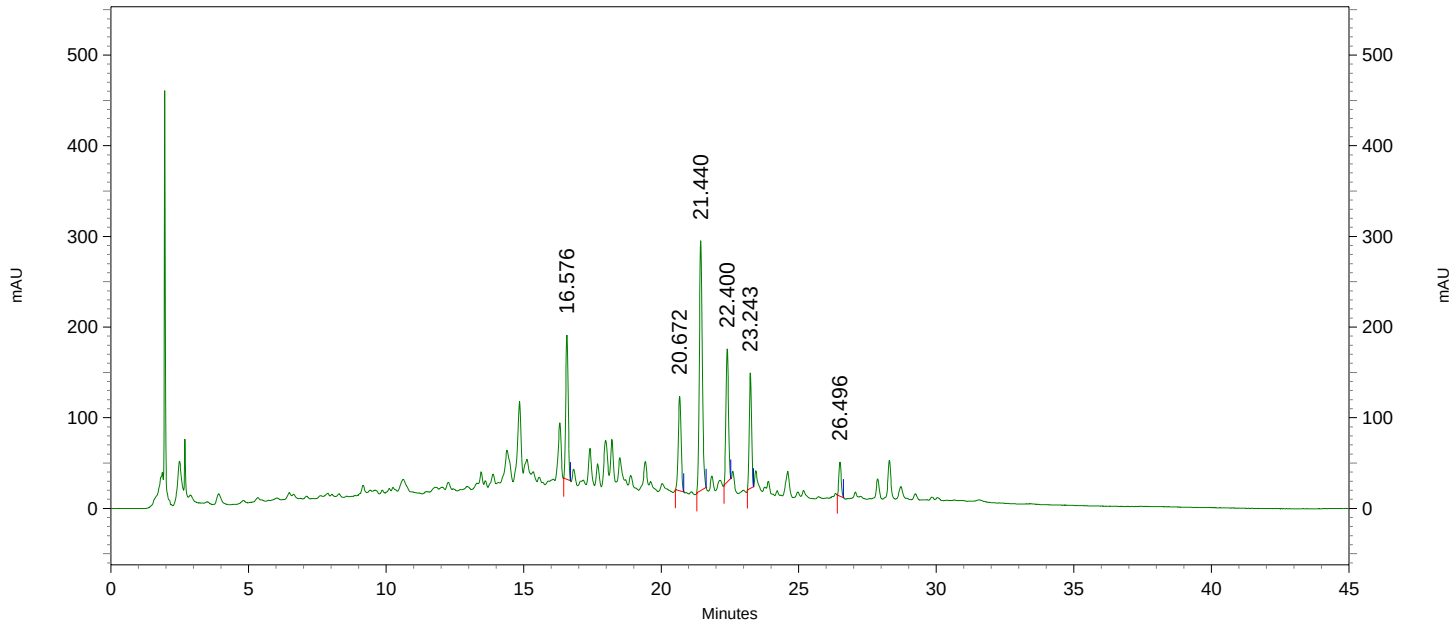


Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
26.475	8139020	99.20	0.00	1.13	413752.75	0.53	0.49	0.16	1384293
26.944	40938	0.50	2.81	1.23	407283.75	0.56	0.34	0.17	6620
27.275	25063	0.31	1.87	1.12	349493.94	0.58	0.33	0.18	3846
Totals	**8205021**	**100.00**							**1394759**

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : **Withania somnifera : WS - 06 lot 10**
Method Name : **C:\CLASS-VP\Methods\METHODS\Withania.met**
Data Name : **D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-16**
Injection Volume : **20**
Acquired : **1/17/07 6:41:54 AM**



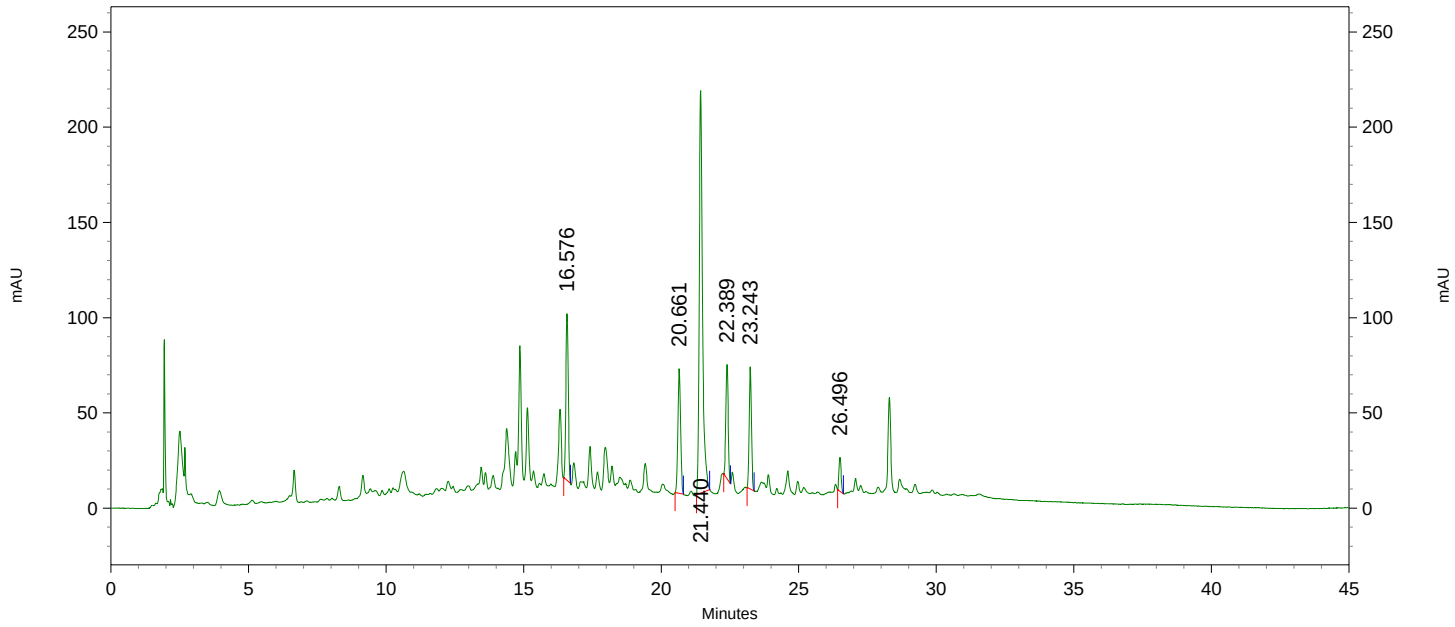
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.576	998797	17.55	0.00	1.04	137409.30	-0.04	0.24	0.18	159064
20.672	745691	13.11	21.84	1.02	177685.13	0.20	0.30	0.20	104250
21.440	2024836	35.59	3.87	1.13	182807.92	0.24	0.34	0.20	275305
22.400	940711	16.53	5.02	1.04	243337.77	0.30	0.25	0.18	146645
23.243	757964	13.32	4.78	1.02	296866.34	0.35	0.22	0.17	127311
26.496	221905	3.90	19.32	1.19	406992.84	0.54	0.22	0.17	37559

Totals									
	5689904	100.00							850134

Natural Remedies Pvt Ltd.

Quality control Dept.

Sample ID : **Withania somnifera : ERH - 46**
Method Name : **C:\CLASS-VP\Methods\METHODS\Withania.met**
Data Name : **D:\2007\Method validation\Withania somnifera\Jan 2007\160107\spl-13**
Injection Volume : **20**
Acquired : **1/17/07 4:19:17 AM**



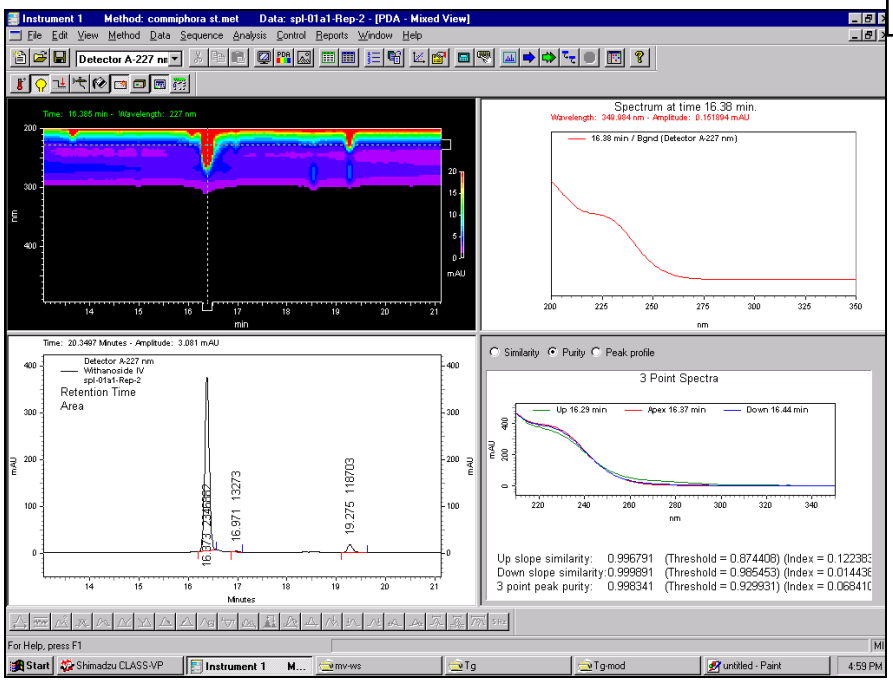
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.576	537308	15.13	0.00	1.08	145898.22	-0.04	0.24	0.17	87496
20.661	453713	12.78	22.24	1.01	181706.16	0.20	0.29	0.19	65400
21.440	1723297	48.54	3.82	1.39	161359.55	0.24	0.48	0.21	211277
22.389	357061	10.06	5.00	1.02	291271.69	0.30	0.24	0.17	59849
23.243	380726	10.72	5.14	1.04	314259.59	0.35	0.26	0.17	64237
26.496	98366	2.77	20.08	1.23	449097.84	0.54	0.21	0.16	17761

Totals									
	3550471	100.00							506020

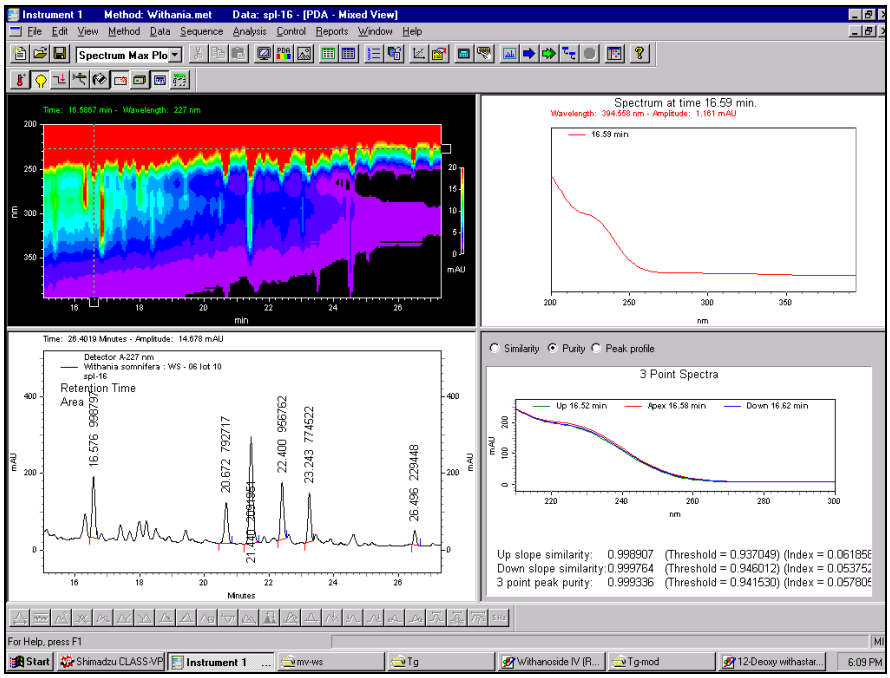
Enclosure: 04

PDA spectrum and Peak purity spectrum

Withanoside-IV – Standard

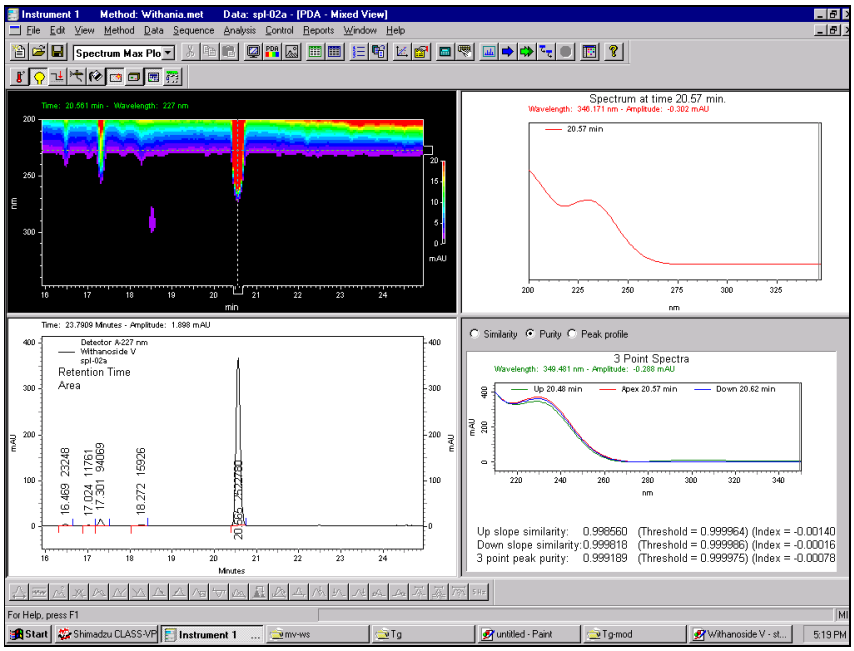


Withanoside-IV - Sample

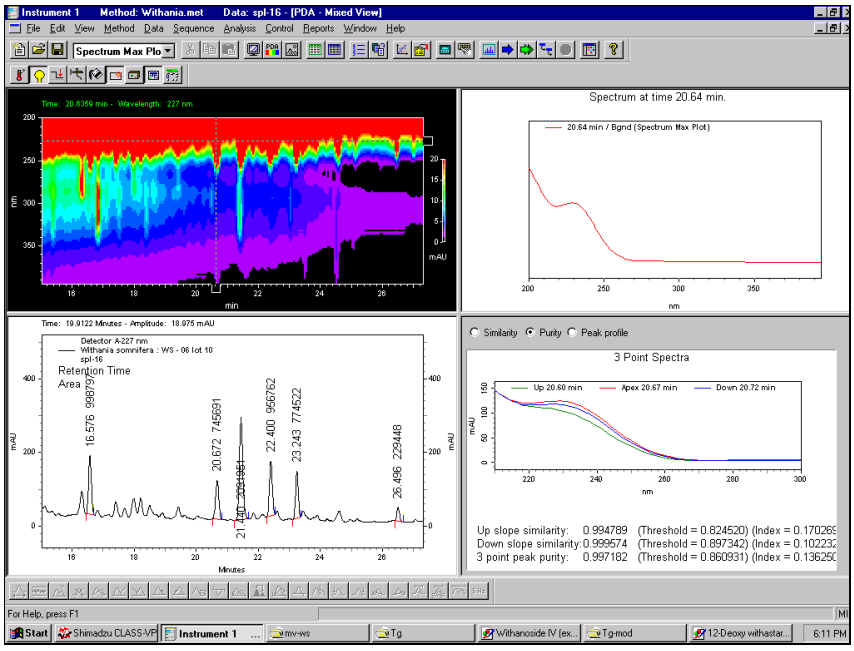


PDA spectrum and Peak purity spectrum

Withanoside-V – Standard

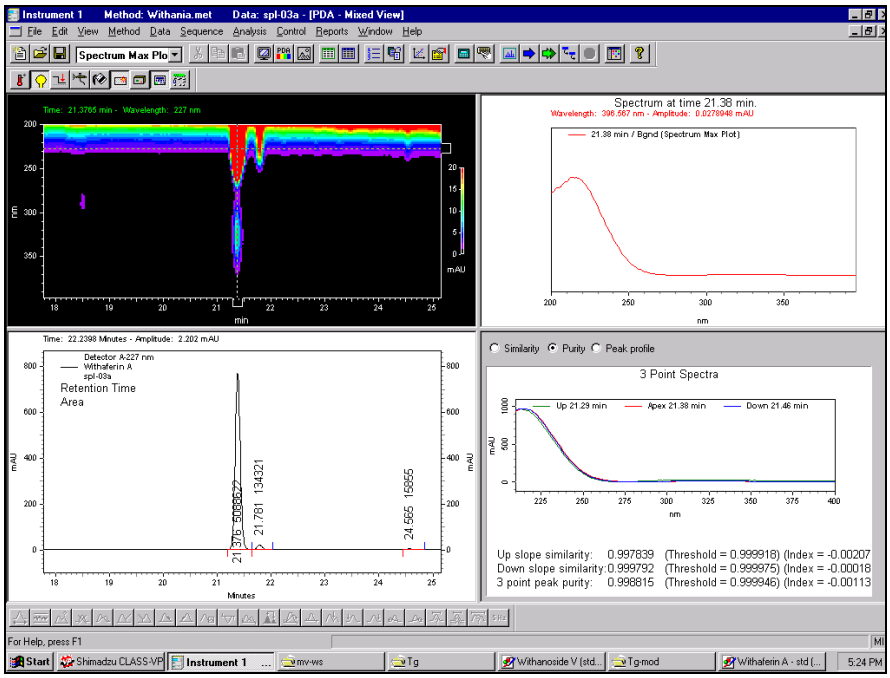


Withanoside-V - Sample

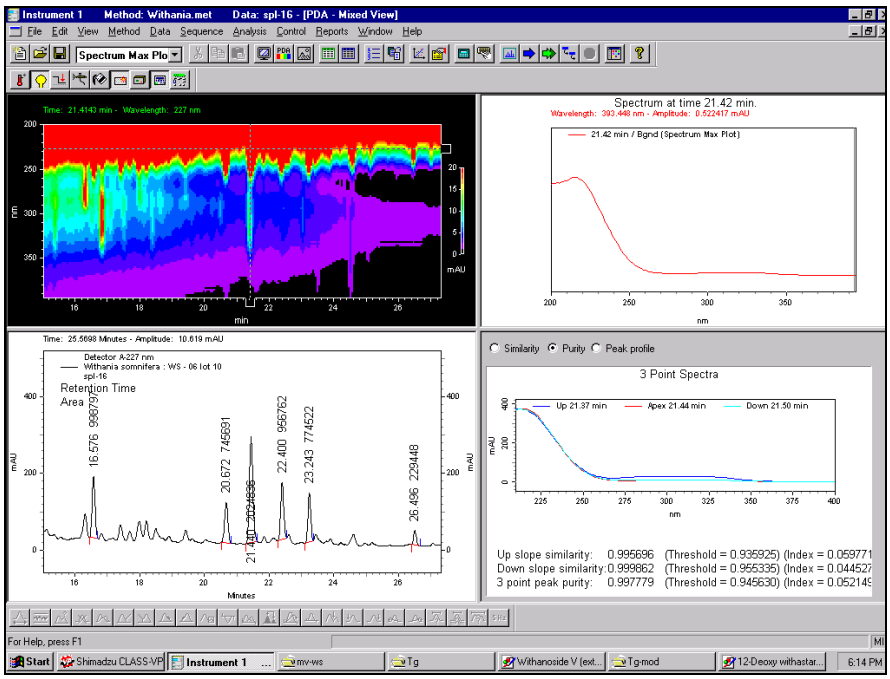


PDA spectrum and Peak purity spectrum

Withaferin-A – Standard

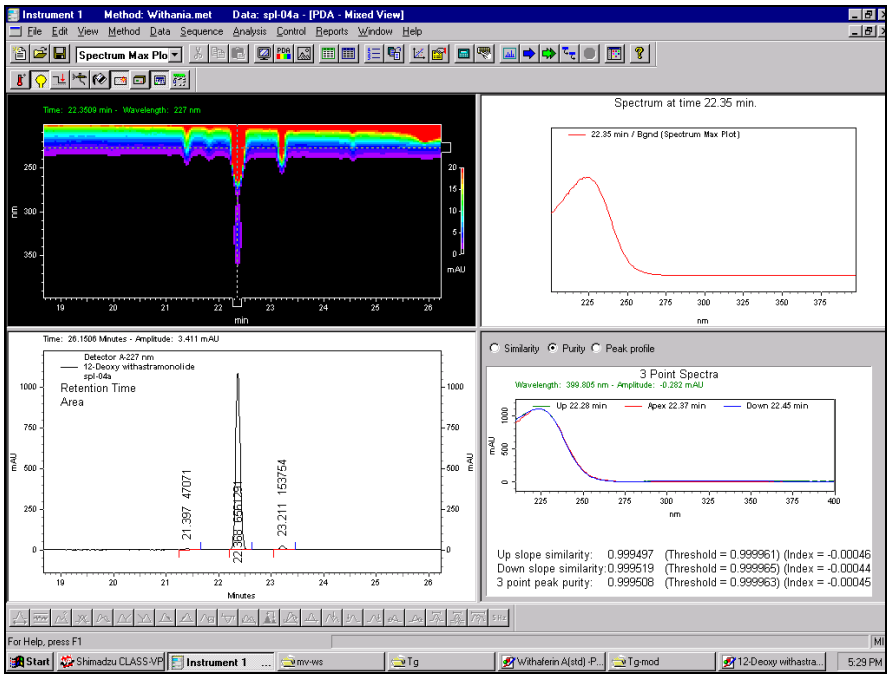


Withaferin-A - Sample

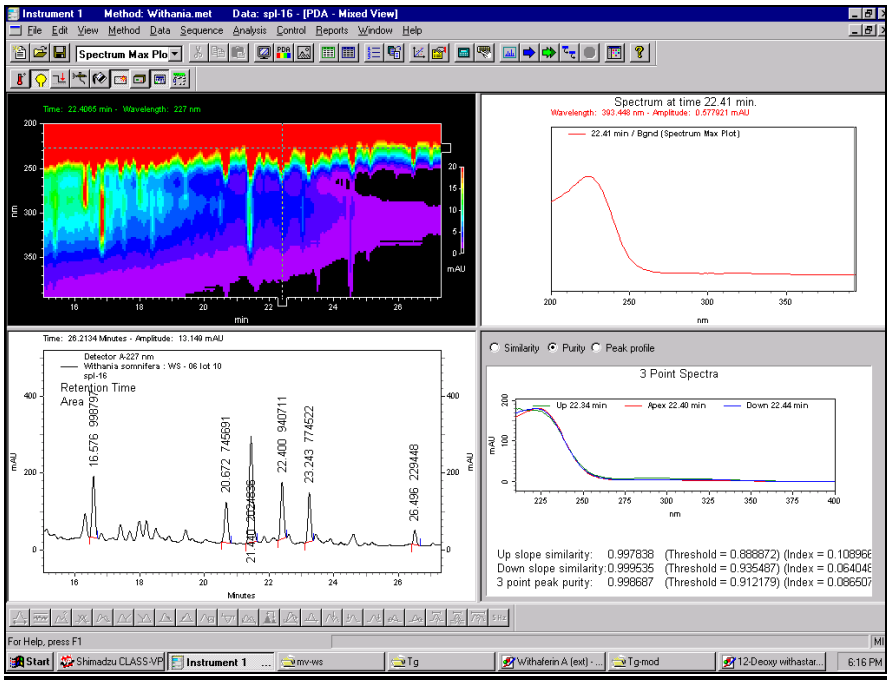


PDA spectrum and Peak purity spectrum

12-Deoxy withastramonolide – Standard

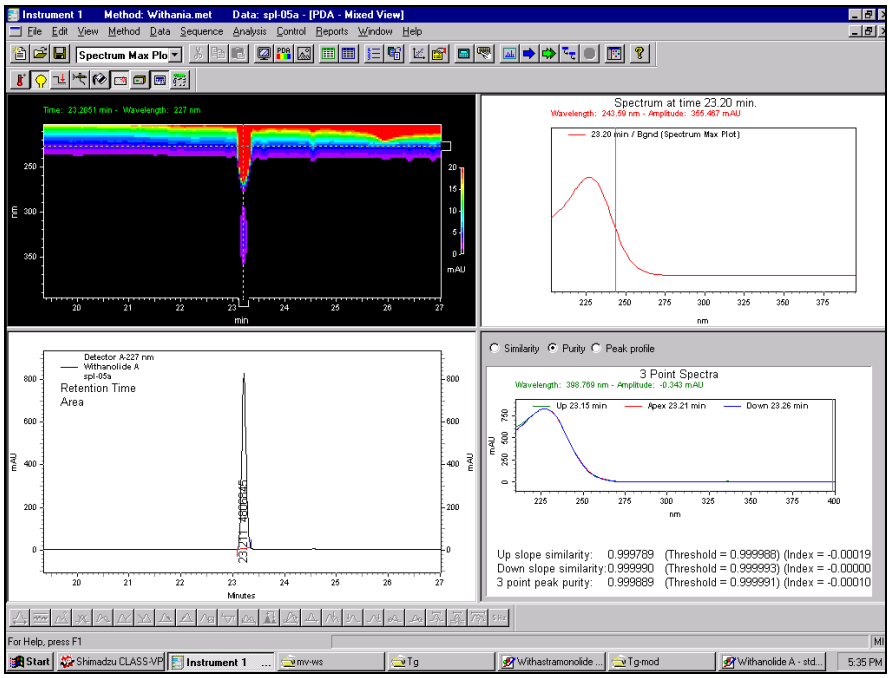


12-Deoxy withastramonolide - Sample

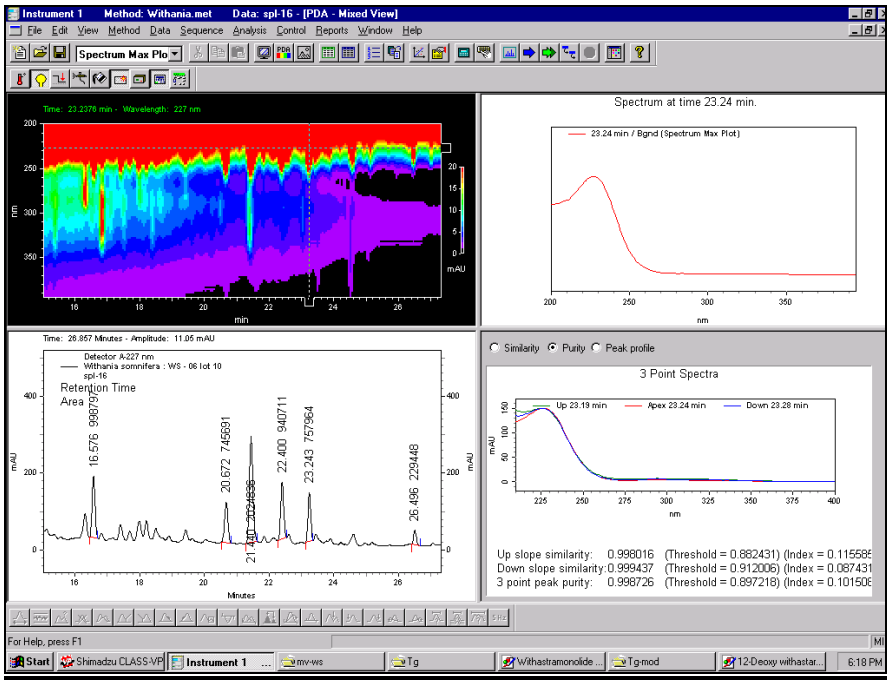


PDA spectrum and Peak purity spectrum

Withanolide A – Standard

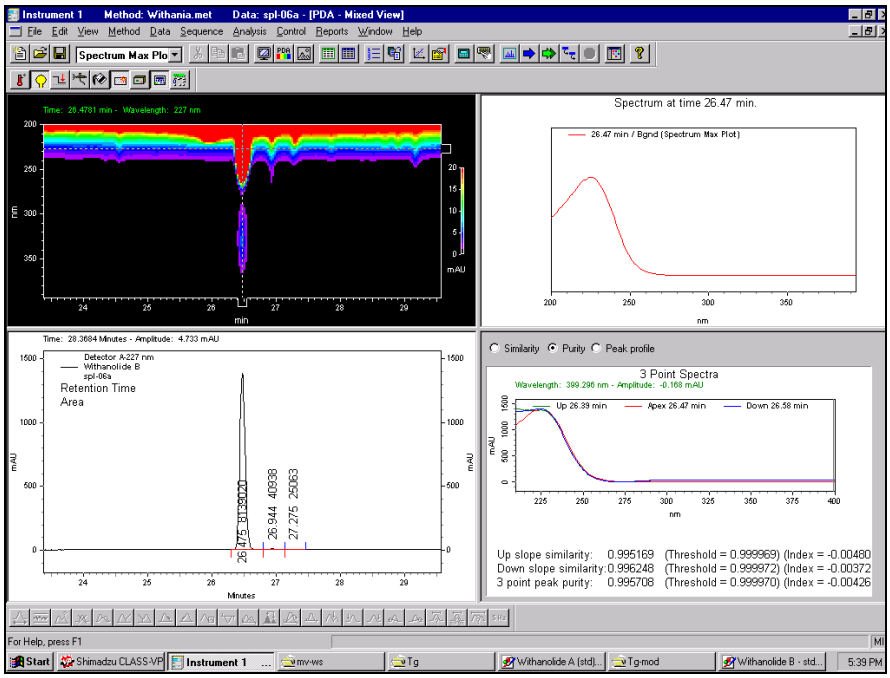


Withanolide A - Sample

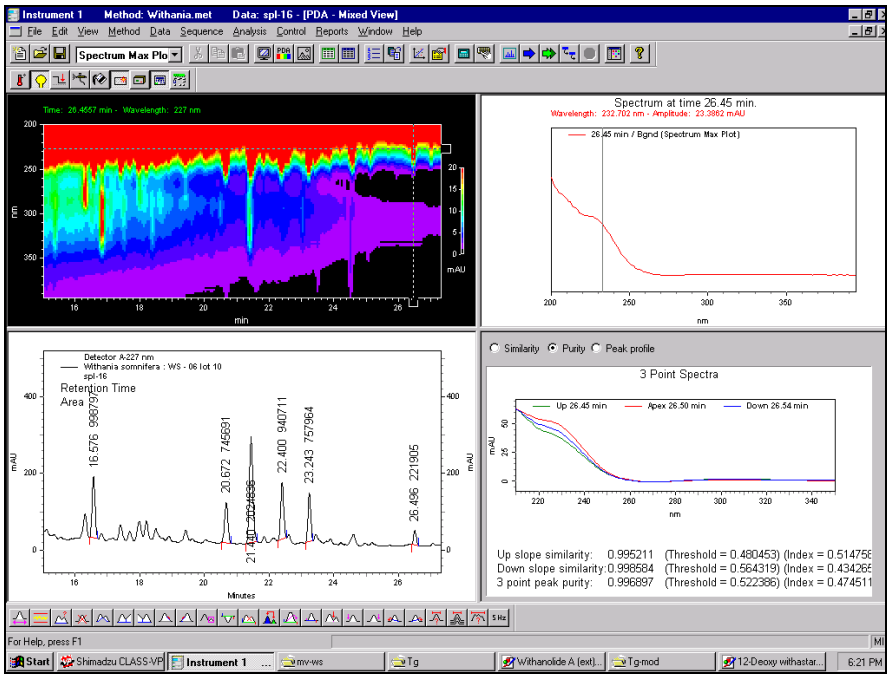


PDA spectrum and Peak purity spectrum

Withanolide B – Standard



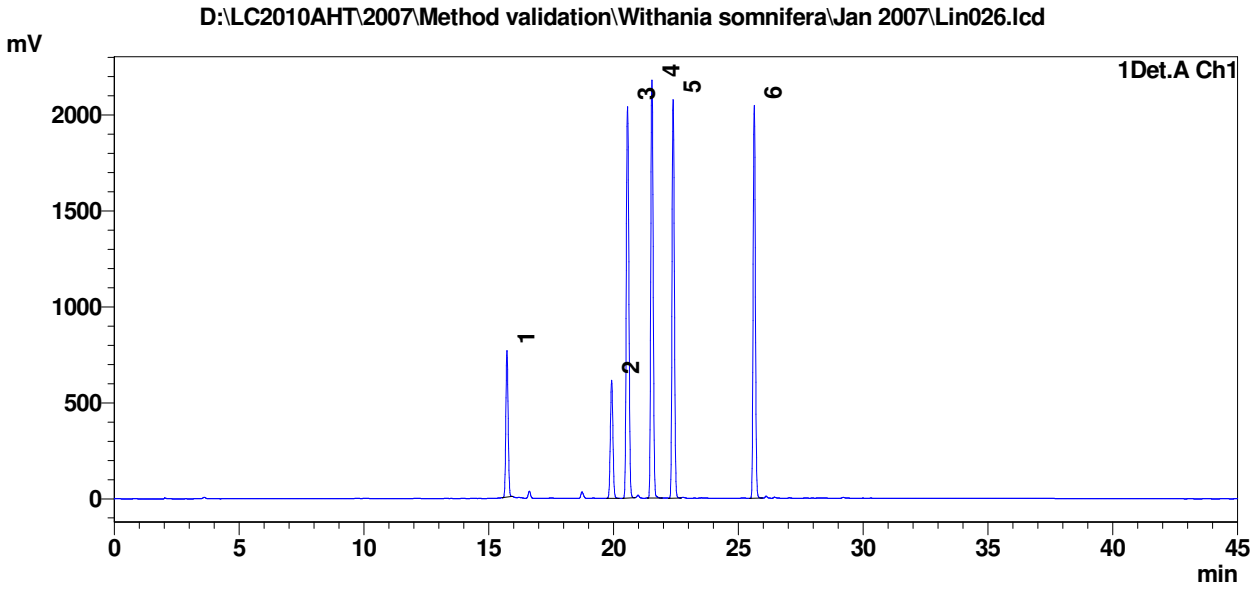
Withanolide B - Sample



NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Enclosure: 05

Acquired by : Admin
Sample Name : Withania : std mix
Sample ID : Dilution : I
Vail # : 12
Injection Volume : 20 uL
Data File Name : Lin026.lcd
Method File Name : Withaina.met.lcm
Batch File Name : Batch 01.lcb
Report File Name : Calibration.lcr
Data Acquired : 1/14/2007 7:40:57 PM
Data Processed : 1/23/2007 2:23:54 AM



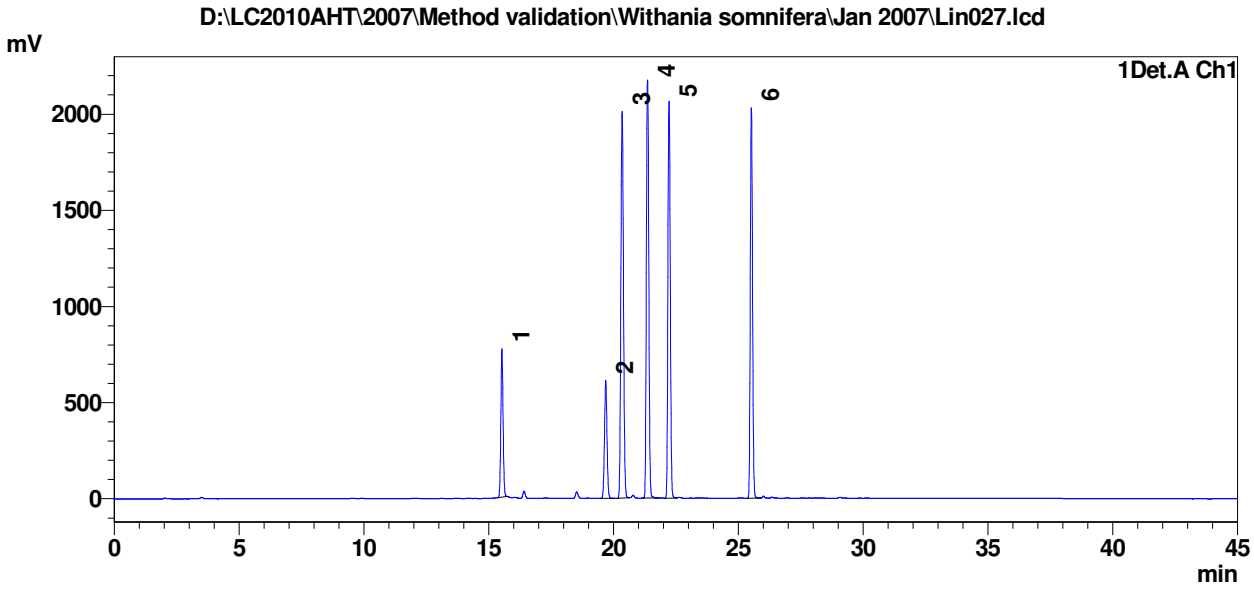
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.732	4815460	764233	7.429	Withanoside IV
2	19.926	4334899	615202	6.688	Withanoside V
3	20.563	14666731	2039992	22.627	Withaferin A
4	21.542	14809446	2177743	22.847	12- Deoxy withastramonolide
5	22.391	13463661	2076735	20.771	Withanollide A
6	25.639	12728719	2047502	19.637	Withanolide B
Total		64818916	9721408	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : I
Vail #	: 12
Injection Volume	: 20 uL
Data File Name	: Lin027.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 8:26:43 PM
Data Processed	: 1/23/2007 2:28:22 AM



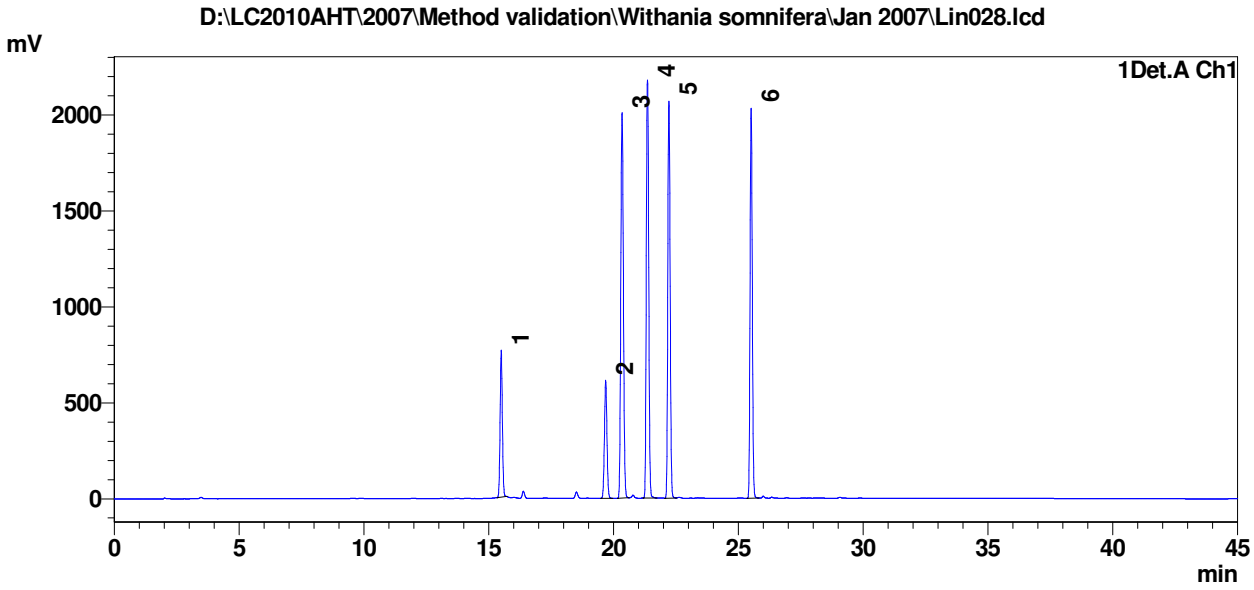
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.530	4811411	771154	7.408	RT15.530
2	19.689	4327804	613706	6.663	RT19.689
3	20.347	14756049	2010233	22.719	RT20.347
4	21.366	14845283	2172899	22.856	RT21.366
5	22.227	13489137	2063880	20.768	RT22.227
6	25.525	12721830	2029980	19.587	RT25.525
Total		64951514	9661853	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : I
Vail #	: 12
Injection Volume	: 20 uL
Data File Name	: Lin028.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 9:12:30 PM
Data Processed	: 1/23/2007 2:27:45 AM



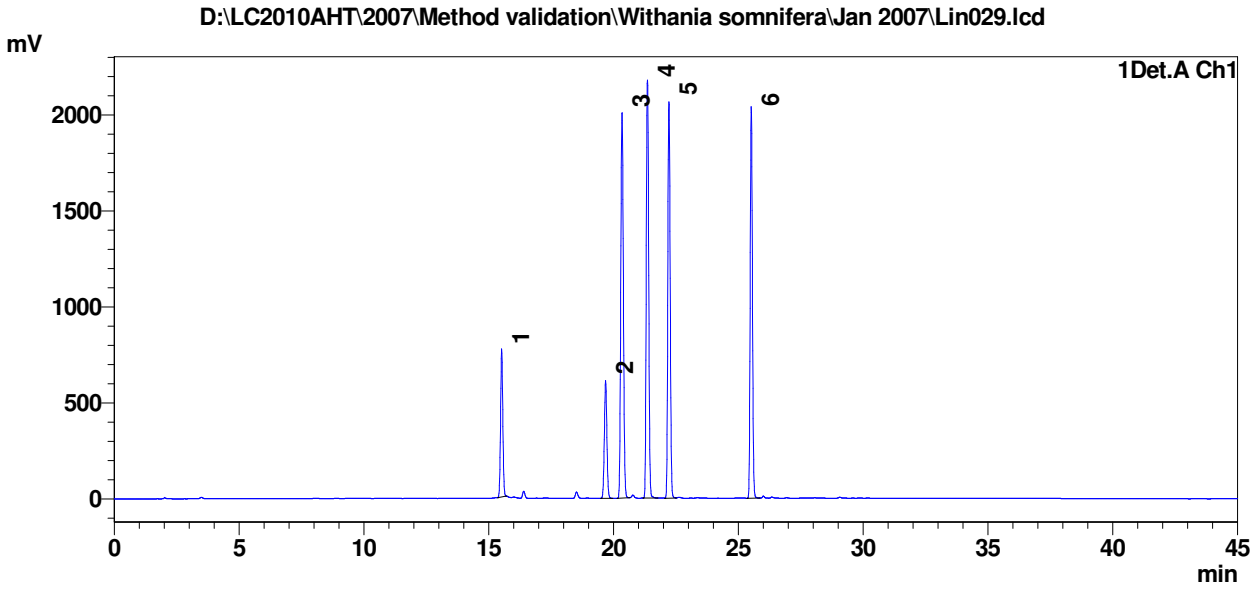
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.502	4819826	765666	7.418	Withanoside IV
2	19.687	4330341	614859	6.665	Withanoside V
3	20.344	14770374	2008055	22.732	Withaferin A
4	21.363	14833377	2176286	22.829	12- Deoxy withastramonolide
5	22.221	13484780	2067542	20.754	Withanollide A
6	25.516	12736068	2030965	19.602	Withanolide B
Total		64974766	9663373	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : I
Vail #	: 12
Injection Volume	: 20 uL
Data File Name	: Lin029.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 10:06:23 PM
Data Processed	: 1/23/2007 2:32:07 AM



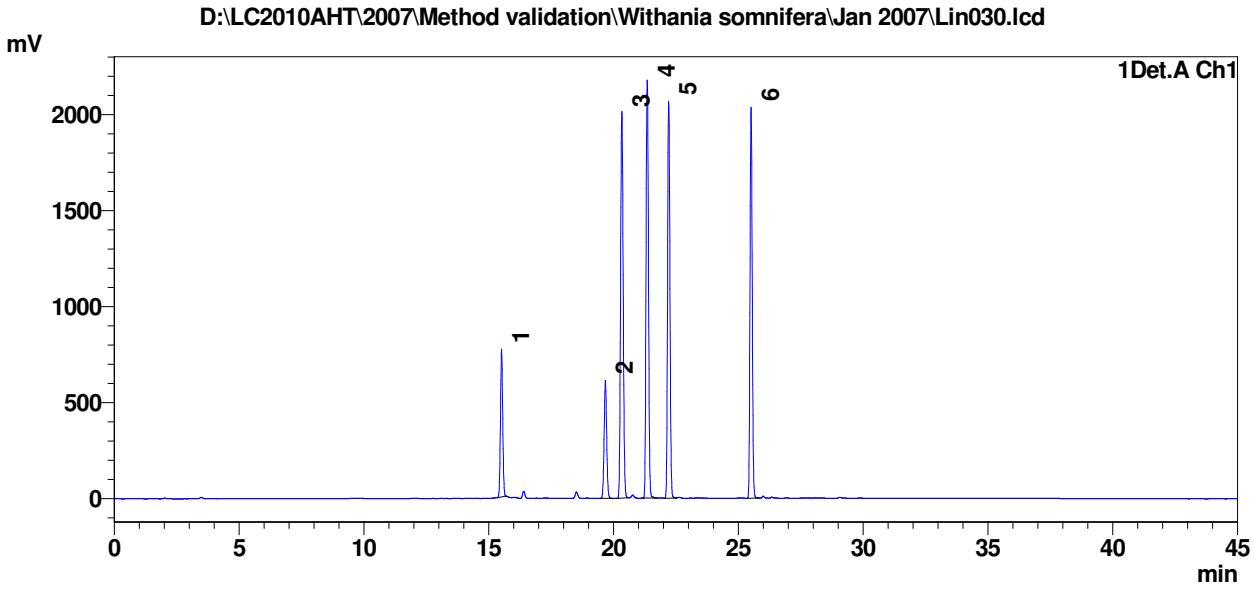
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.519	4815725	770932	7.404	Withanoside IV
2	19.684	4338646	612656	6.670	Withanoside V
3	20.342	14788044	2008178	22.736	Withaferin A
4	21.362	14874117	2176641	22.868	12- Deoxy withastramonolide
5	22.223	13502916	2064485	20.760	Withanollide A
6	25.521	12723957	2038641	19.562	Withanolide B
Total		65043405	9671533	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : I
Vail #	: 12
Injection Volume	: 20 uL
Data File Name	: Lin030.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 10:52:01 PM
Data Processed	: 1/23/2007 2:31:16 AM



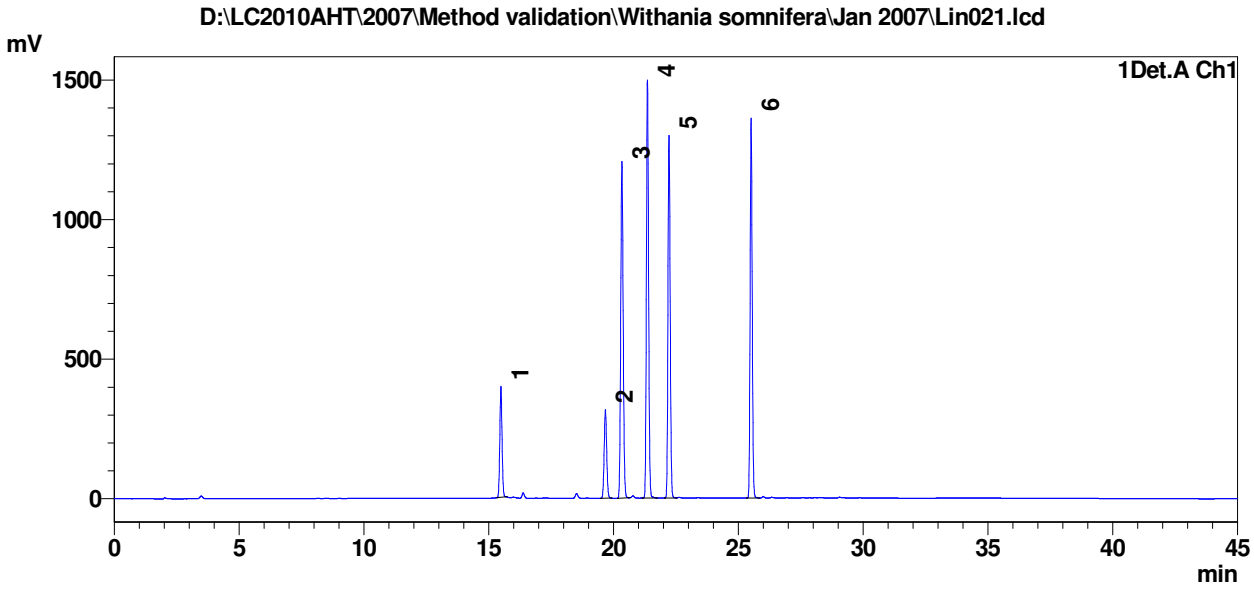
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.515	4818317	768546	7.411	Withanoside IV
2	19.676	4345473	613872	6.684	Withanoside V
3	20.335	14782253	2013446	22.736	Withaferin A
4	21.353	14859008	2175533	22.854	12- Deoxy withastramonolide
5	22.213	13491381	2064396	20.751	Withanollide A
6	25.513	12720153	2034360	19.564	Withanolide B
Total		65016585	9670152	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : II
Vail #	: 11
Injection Volume	: 20 uL
Data File Name	: Lin021.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 3:52:03 PM
Data Processed	: 1/23/2007 2:16:33 AM



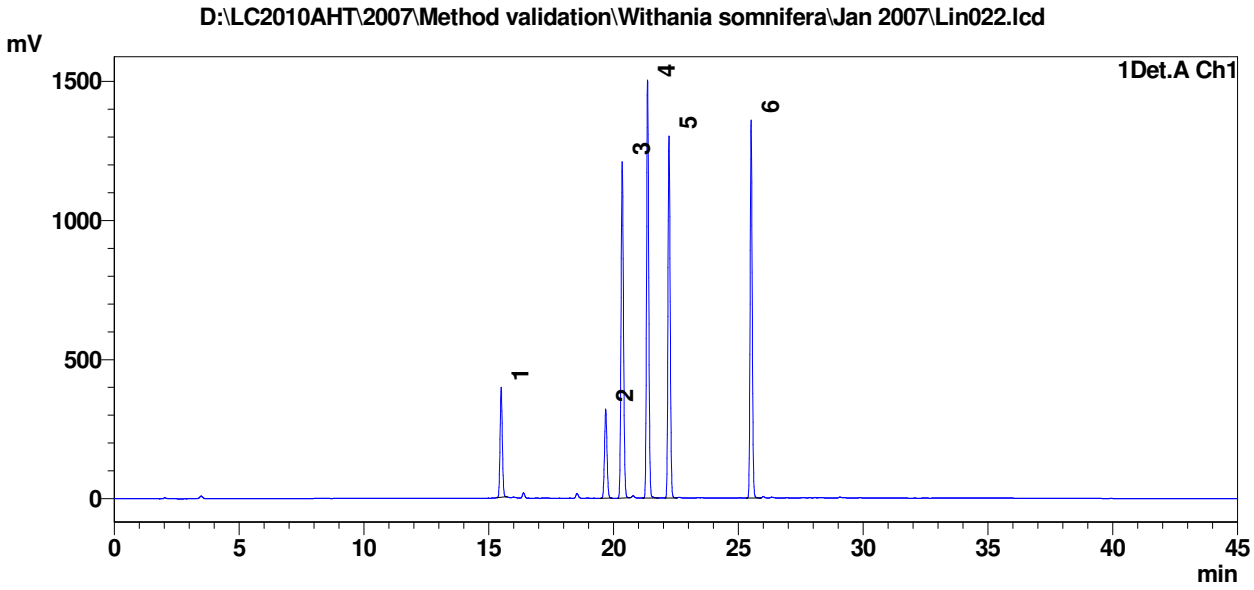
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.489	2493967	397258	6.546	Withanoside IV
2	19.676	2238822	317354	5.876	Withanoside V
3	20.337	8325259	1206681	21.852	Withaferin A
4	21.360	9145644	1496756	24.005	12- Deoxy withastramonolide
5	22.225	7955602	1298608	20.882	Withanollide A
6	25.514	7938851	1360472	20.838	Withanolide B
Total		38098145	6077129	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : II
Vail #	: 11
Injection Volume	: 20 uL
Data File Name	: Lin022.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 4:37:50 PM
Data Processed	: 1/23/2007 2:19:11 AM



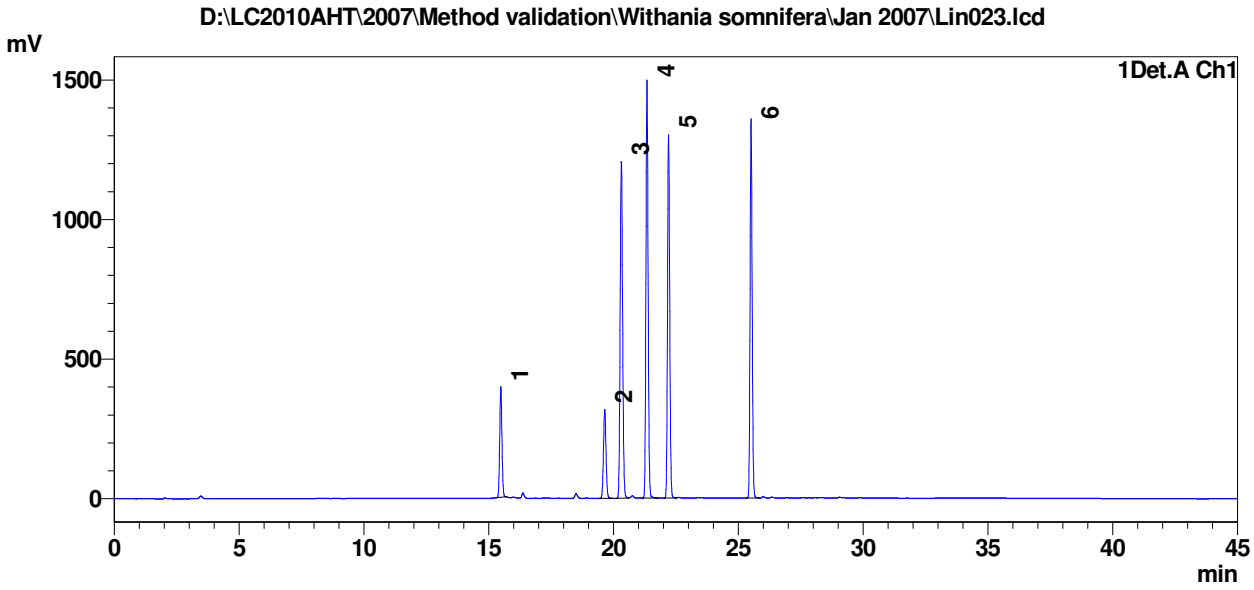
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.500	2493747	395202	6.546	Withanoside IV
2	19.689	2237319	319880	5.873	Withanoside V
3	20.349	8322031	1208671	21.845	Withaferin A
4	21.366	9138372	1501621	23.988	12- Deoxy withastramonolide
5	22.225	7957220	1300693	20.887	Withanollide A
6	25.514	7947620	1358431	20.862	Withanolide B
Total		38096308	6084499	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : II
Vail #	: 11
Injection Volume	: 20 uL
Data File Name	: Lin023.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 5:23:35 PM
Data Processed	: 1/23/2007 2:20:43 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.485	2491031	396845	6.539	Withanoside IV
2	19.648	2243282	318405	5.889	Withanoside V
3	20.314	8317825	1206269	21.835	Withaferin A
4	21.341	9140140	1496559	23.993	12- Deoxy withastramonolide
5	22.206	7958121	1300544	20.890	Withanollide A
6	25.512	7944196	1358501	20.854	Withanolide B
Total		38094594	6077124	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : II
Vail #	: 11
Injection Volume	: 20 uL
Data File Name	: Lin024.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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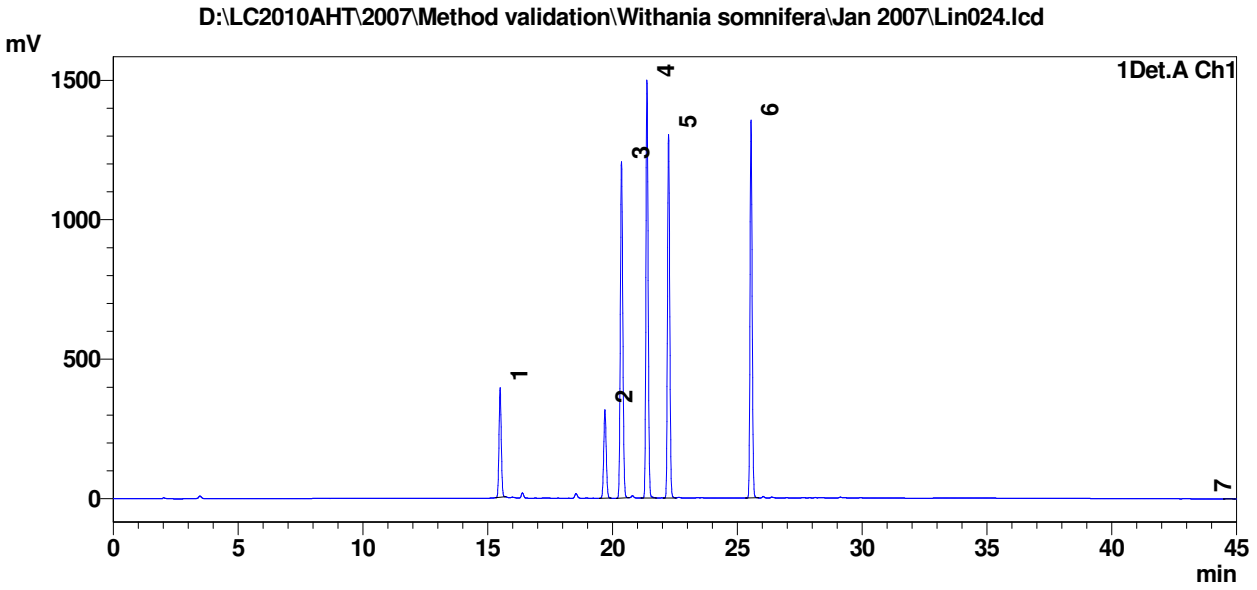
Data Processed	: 1/23/2007 2:20:56 AM



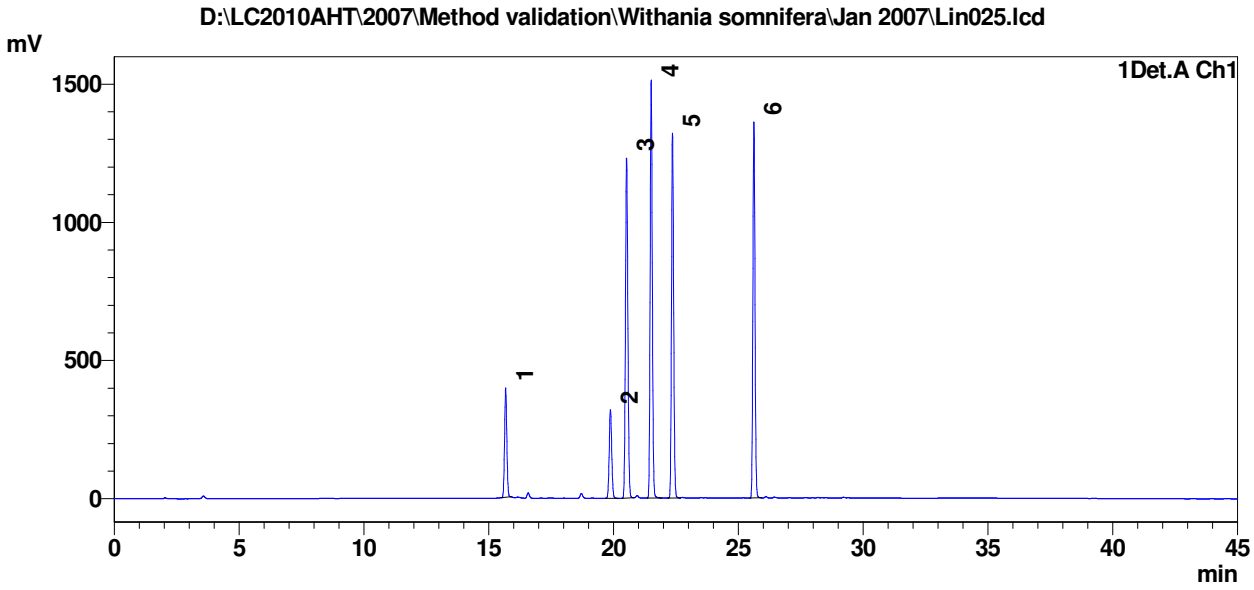
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.497	2492809	393646	6.538	Withanoside IV
2	19.697	2237337	316713	5.868	Withanoside V
3	20.361	8328440	1206071	21.843	Withaferin A
4	21.382	9151223	1497652	24.001	12- Deoxy withastramonolide
5	22.247	7963123	1302715	20.885	Withanollide A
6	25.551	7953428	1354058	20.859	Withanolide B
7	44.827	2416	116	0.006	
Total		38128776	6070972	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : II
Vail #	: 11
Injection Volume	: 20 uL
Data File Name	: Lin025.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 6:55:09 PM
Data Processed	: 1/23/2007 2:22:35 AM



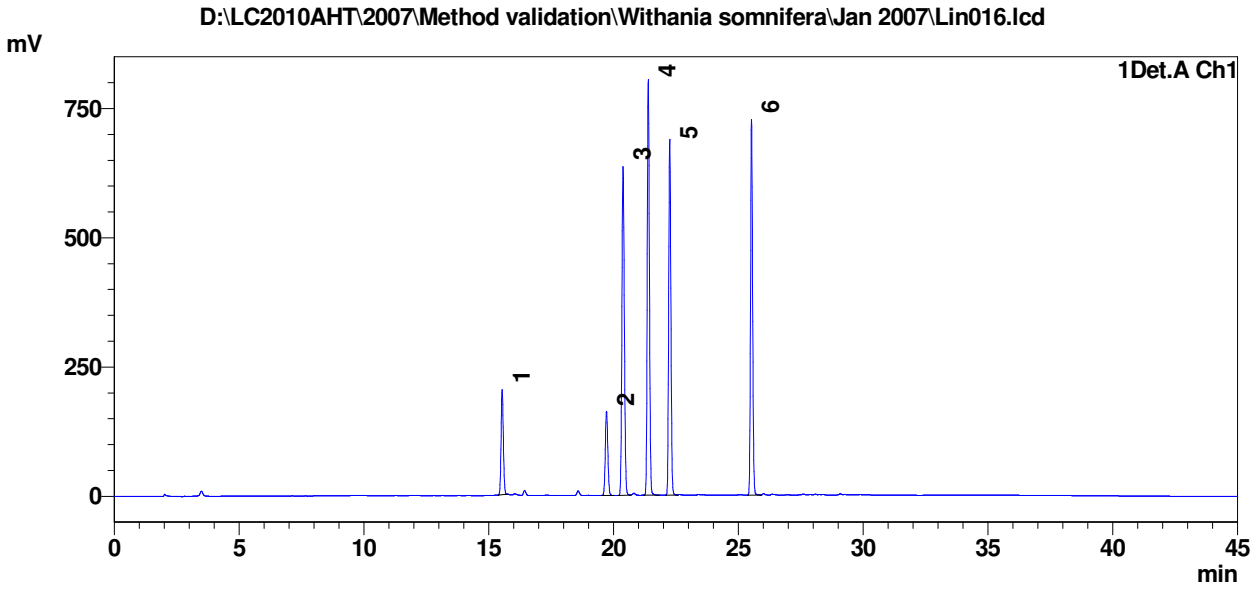
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.680	2497552	396047	6.542	Withanoside IV
2	19.877	2248262	320496	5.889	Withanoside V
3	20.525	8338317	1230031	21.841	Withaferin A
4	21.510	9156566	1511496	23.984	12- Deoxy withastramonolide
5	22.364	7973789	1319908	20.886	Withanollide A
6	25.625	7963457	1360236	20.859	Withanolide B
Total		38177944	6138214	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : III
Vail #	: 10
Injection Volume	: 20 uL
Data File Name	: Lin016.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 12:03:10 PM
Data Processed	: 1/23/2007 2:08:01 AM



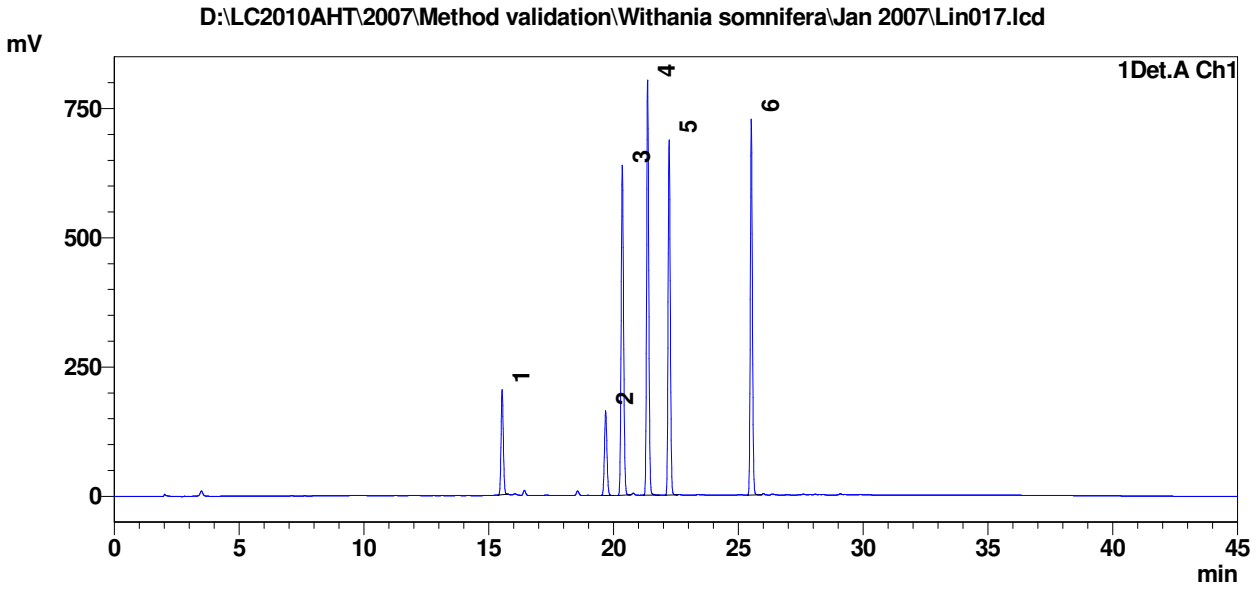
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.539	1275513	203131	6.413	Withanoside IV
2	19.721	1146136	163005	5.763	Withanoside V
3	20.383	4332623	635519	21.784	Withaferin A
4	21.394	4800626	804013	24.137	12- Deoxy withastramonolide
5	22.253	4137256	688329	20.802	Withanollide A
6	25.530	4196971	726582	21.102	Withanolide B
Total		19889126	3220579	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : III
Vail #	: 10
Injection Volume	: 20 uL
Data File Name	: Lin017.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 12:48:57 PM
Data Processed	: 1/23/2007 2:08:43 AM



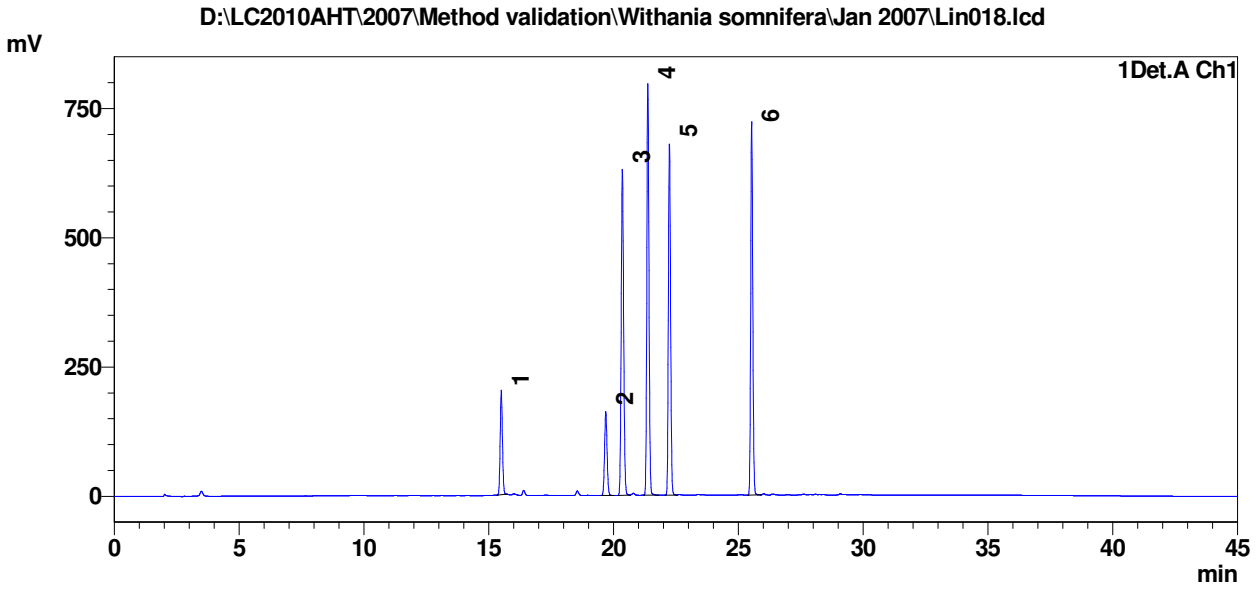
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.538	1278440	203561	6.418	Withanoside IV
2	19.686	1146843	163686	5.758	Withanoside V
3	20.353	4337796	638520	21.777	Withaferin A
4	21.367	4809678	802935	24.146	12- Deoxy withastramonolide
5	22.230	4140860	687017	20.789	Withanollide A
6	25.521	4205282	727155	21.112	Withanolide B
Total		19918898	3222873	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : III
Vail #	: 10
Injection Volume	: 20 uL
Data File Name	: Lin018.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 1:34:44 PM
Data Processed	: 1/23/2007 2:10:33 AM



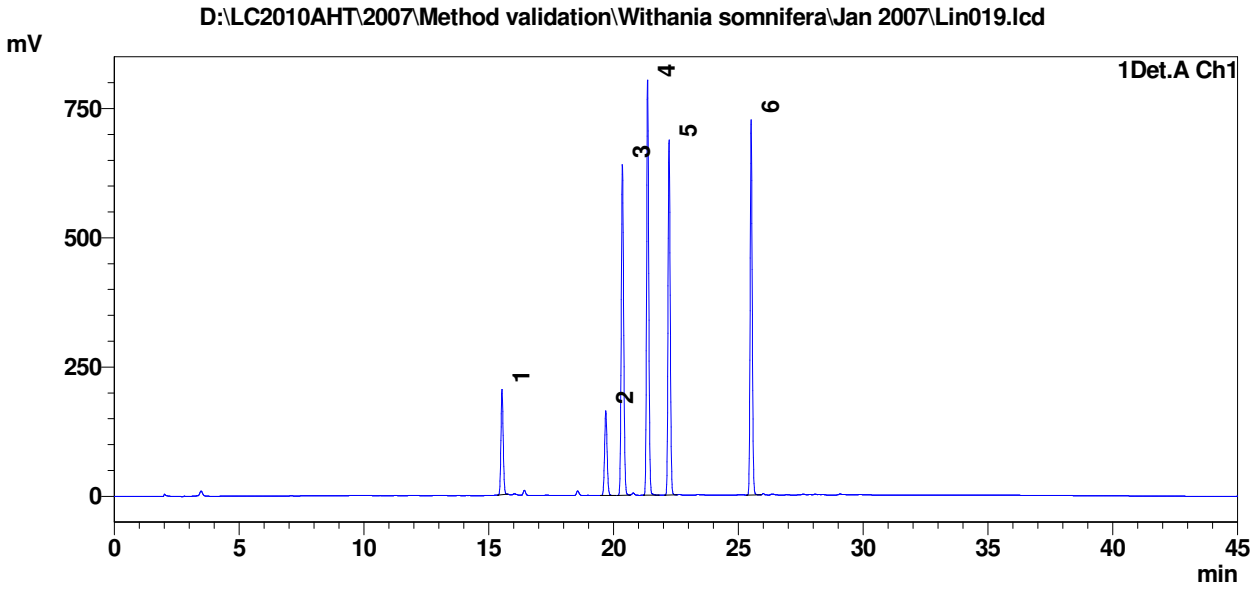
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.504	1269142	201799	6.413	Withanoside IV
2	19.691	1141122	162126	5.766	Withanoside V
3	20.355	4310236	630249	21.778	Withaferin A
4	21.376	4778467	796159	24.144	12- Deoxy withastramonolide
5	22.241	4118314	678874	20.808	Withanollide A
6	25.536	4174239	722038	21.091	Withanolide B
Total		19791519	3191245	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : III
Vail #	: 10
Injection Volume	: 20 uL
Data File Name	: Lin019.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 2:20:29 PM
Data Processed	: 1/23/2007 2:12:10 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.534	1278566	203336	6.417	Withanoside IV
2	19.691	1152990	163525	5.787	Withanoside V
3	20.357	4335168	639586	21.758	Withaferin A
4	21.367	4808531	802957	24.133	12- Deoxy withastramonolide
5	22.227	4144205	687096	20.799	Withanollide A
6	25.514	4205370	725686	21.106	Withanolide B
Total		19924828	3222187	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : III
Vail #	: 10
Injection Volume	: 20 uL
Data File Name	: Lin020.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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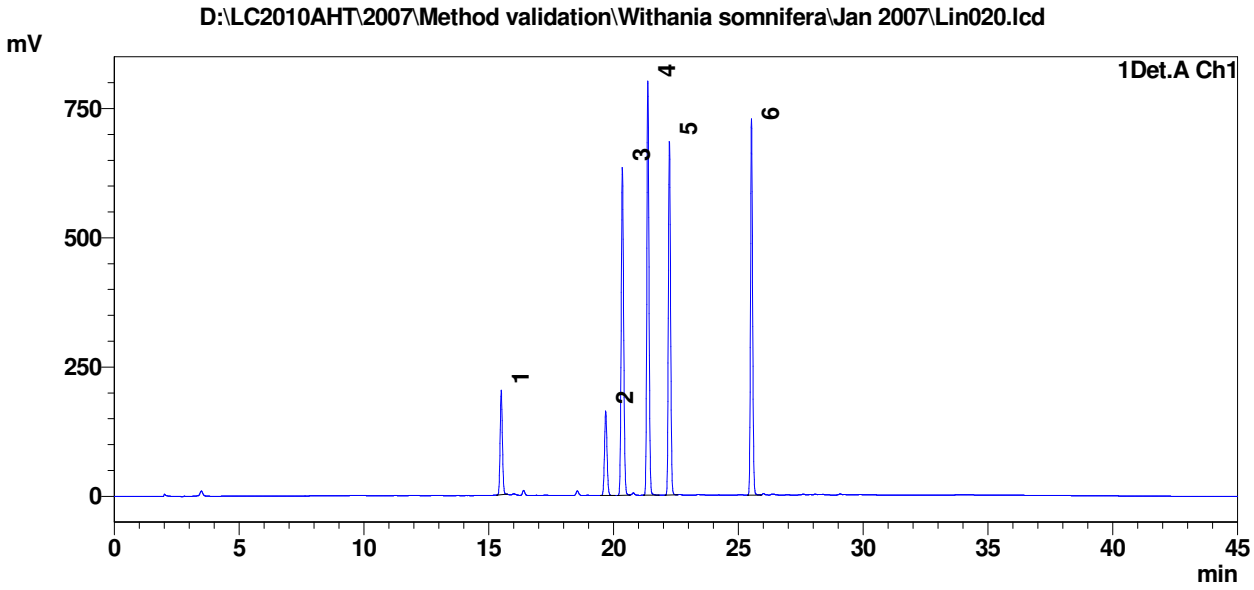
Data Processed	: 1/23/2007 2:13:49 AM



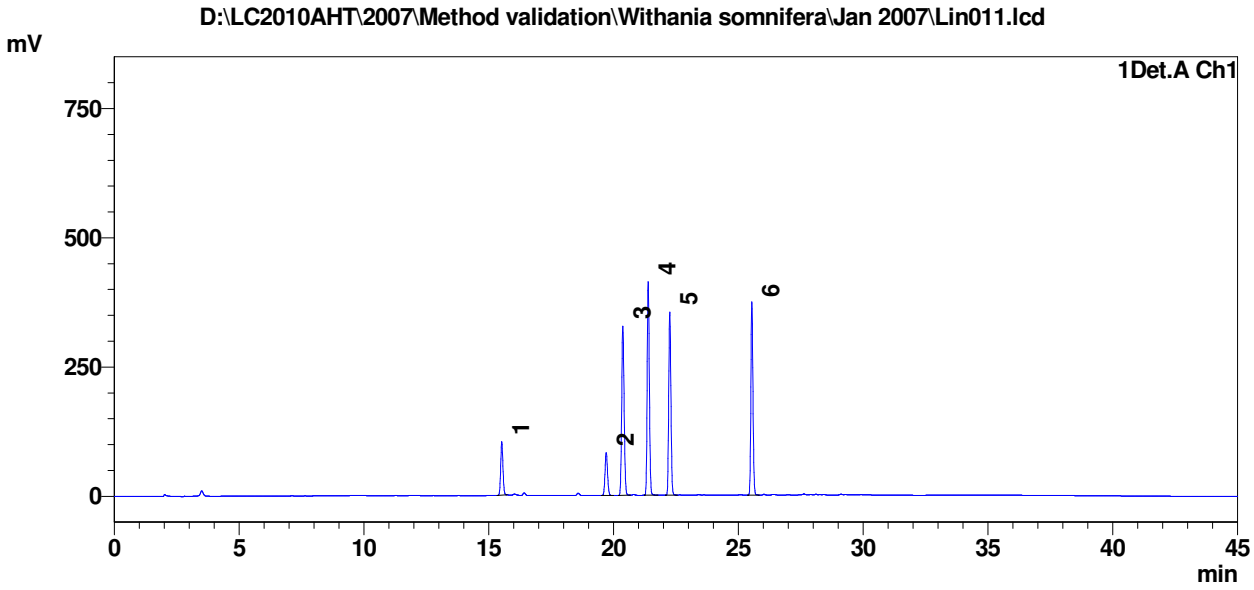
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.502	1279224	202273	6.415	Withanoside IV
2	19.688	1151745	163396	5.776	Withanoside V
3	20.355	4340882	634261	21.769	Withaferin A
4	21.376	4813165	801113	24.138	12- Deoxy withastramonolide
5	22.241	4149692	684028	20.810	Withanollide A
6	25.529	4205743	728042	21.092	Withanolide B
Total		19940451	3213114	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : IV
Vail #	: 9
Injection Volume	: 20 uL
Data File Name	: Lin011.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 8:14:18 AM
Data Processed	: 1/23/2007 2:02:00 AM



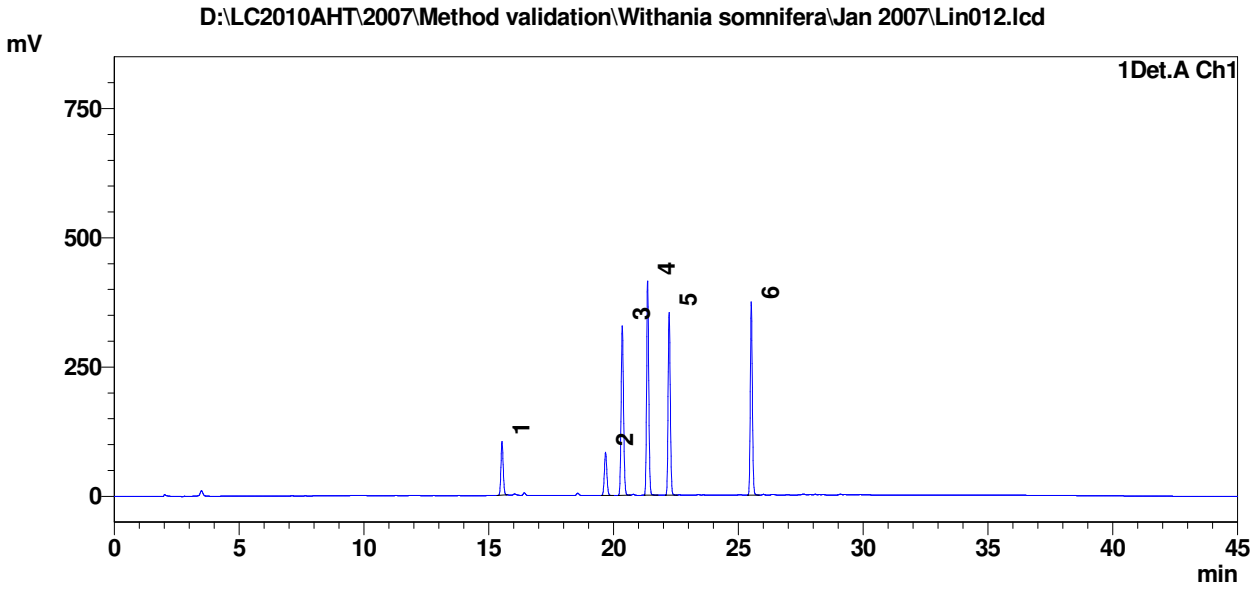
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.523	651382	103380	6.367	Withanoside IV
2	19.708	586819	83106	5.736	Withanoside V
3	20.374	2231492	327201	21.810	Withaferin A
4	21.391	2472835	413032	24.169	12- Deoxy withastramonolide
5	22.255	2130939	354194	20.828	Withanollide A
6	25.544	2157826	374002	21.090	Withanolide B
Total		10231294	1654916	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : IV
Vail #	: 9
Injection Volume	: 20 uL
Data File Name	: Lin012.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 9:00:04 AM
Data Processed	: 1/23/2007 2:02:33 AM



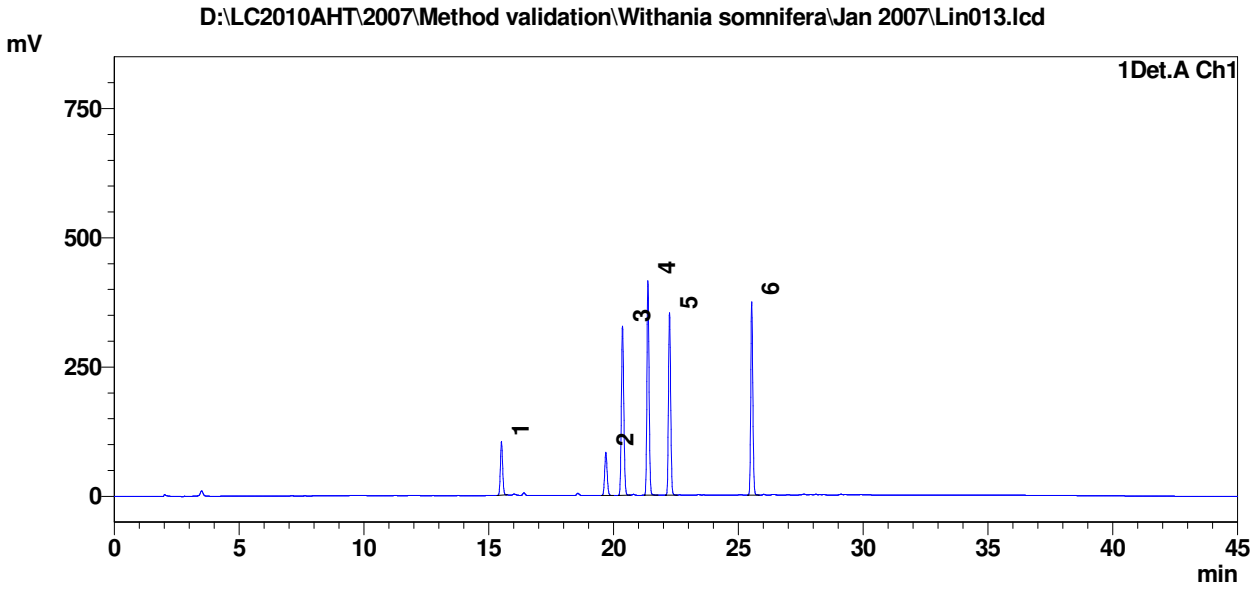
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.533	651009	103534	6.370	Withanoside IV
2	19.682	585559	83536	5.729	Withanoside V
3	20.351	2227105	327740	21.791	Withaferin A
4	21.367	2469751	414098	24.165	12- Deoxy withastramonolide
5	22.230	2130222	353707	20.843	Withanollide A
6	25.520	2156794	374004	21.103	Withanolide B
Total		10220438	1656618	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : IV
Vail #	: 9
Injection Volume	: 20 uL
Data File Name	: Lin013.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 9:45:52 AM
Data Processed	: 1/23/2007 2:02:54 AM



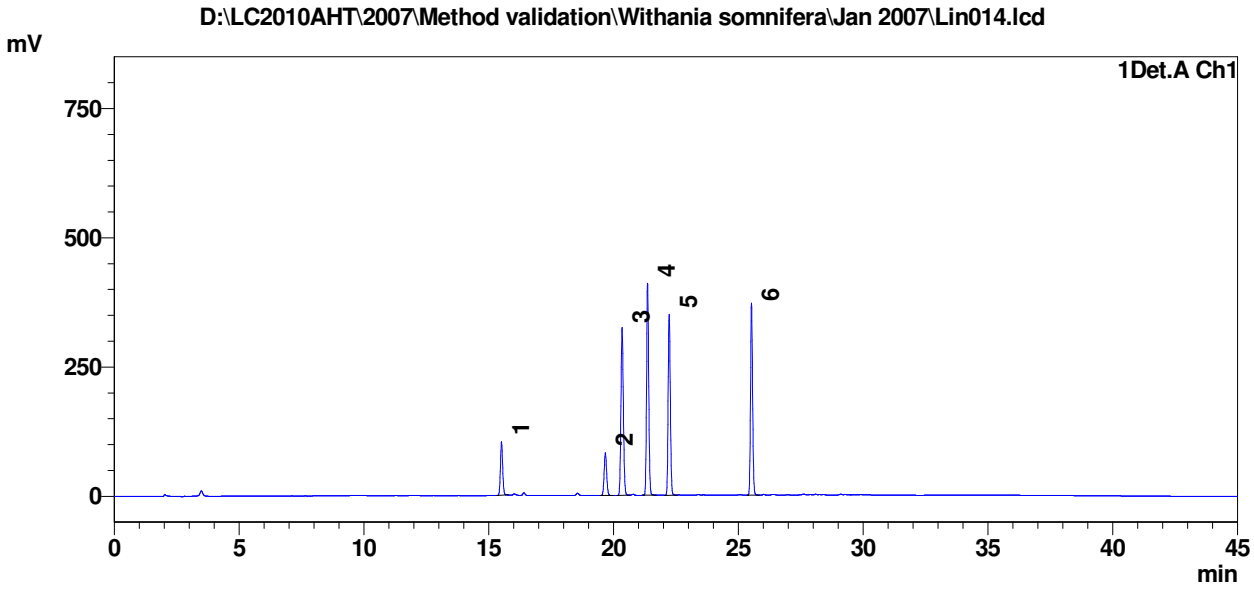
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.512	651630	103712	6.374	Withanoside IV
2	19.694	585906	83388	5.731	Withanoside V
3	20.362	2228288	327394	21.795	Withaferin A
4	21.380	2471386	414714	24.172	12- Deoxy withastramonolide
5	22.245	2130126	352980	20.835	Withanollide A
6	25.538	2156648	374025	21.094	Withanolide B
Total		10223983	1656213	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : IV
Vail #	: 9
Injection Volume	: 20 uL
Data File Name	: Lin014.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 10:31:38 AM
Data Processed	: 1/23/2007 2:04:41 AM



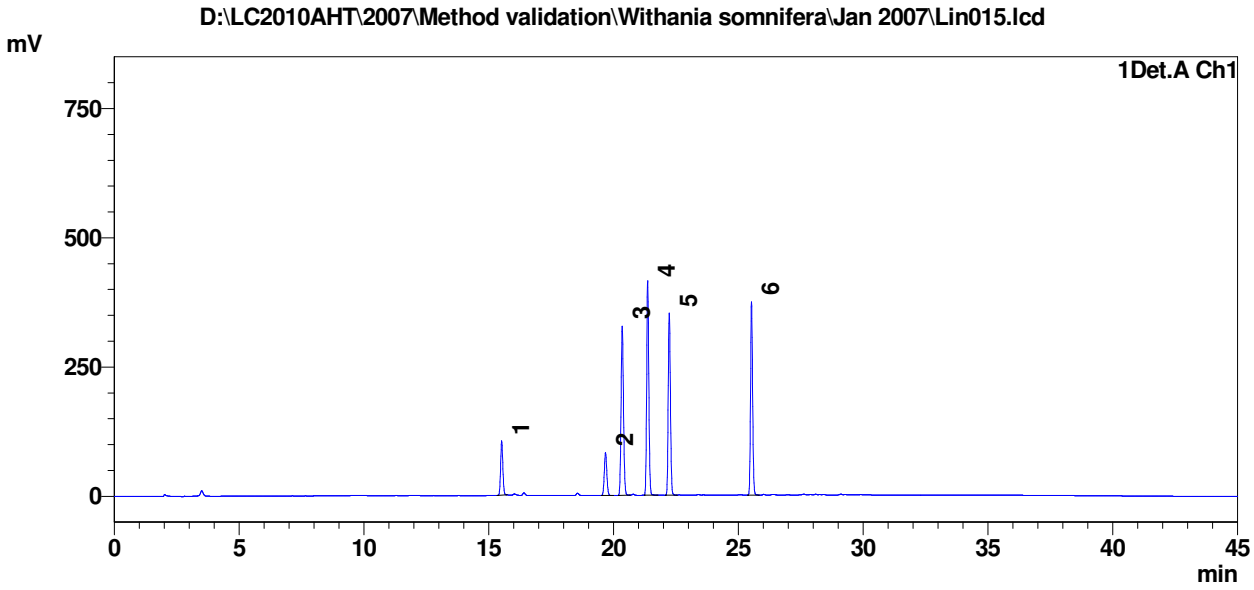
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.513	649769	103300	6.405	RT15.513
2	19.674	581818	82696	5.736	RT19.674
3	20.345	2211324	325031	21.799	RT20.345
4	21.365	2448908	410098	24.141	RT21.365
5	22.231	2112327	349764	20.823	RT22.231
6	25.529	2139922	371296	21.095	RT25.529
Total		10144068	1642185	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : IV
Vail #	: 9
Injection Volume	: 20 uL
Data File Name	: Lin015.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 11:17:23 AM
Data Processed	: 1/23/2007 2:04:54 AM



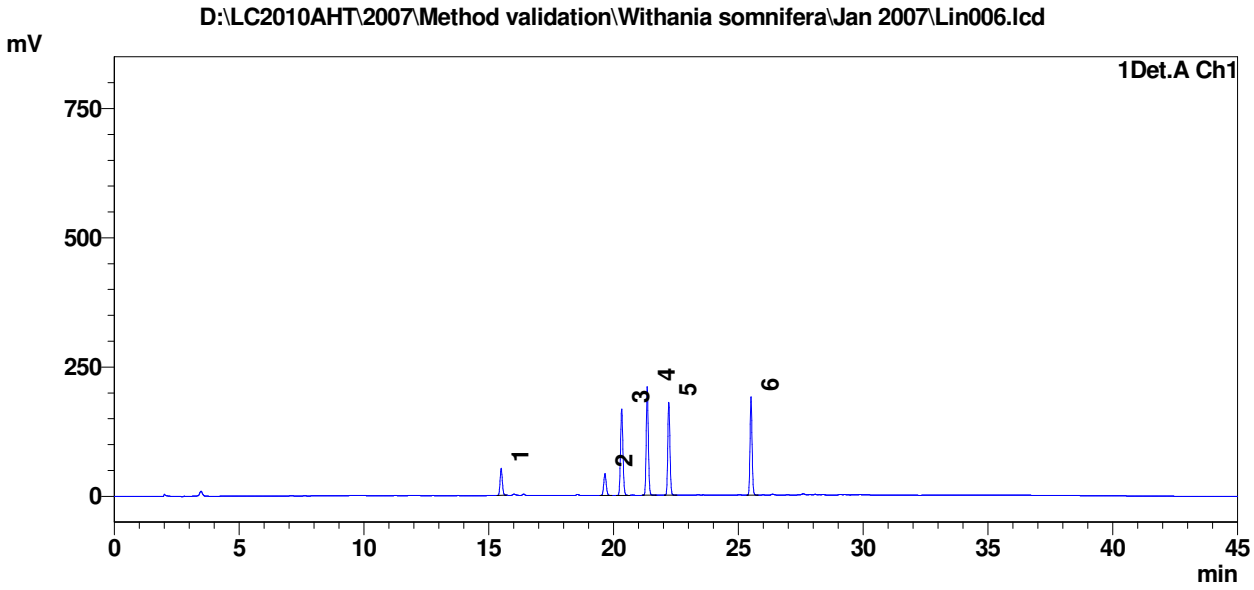
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.520	651869	105067	6.377	Withanoside IV
2	19.680	584964	83301	5.722	Withanoside V
3	20.349	2224755	326954	21.764	Withaferin A
4	21.370	2471248	414982	24.175	12- Deoxy withastramonolide
5	22.235	2131439	352505	20.851	Withanollide A
6	25.530	2158084	374085	21.111	Withanolide B
Total		10222360	1656894	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : V
Vail #	: 8
Injection Volume	: 20 uL
Data File Name	: Lin006.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 4:25:32 AM
Data Processed	: 1/23/2007 1:54:34 AM



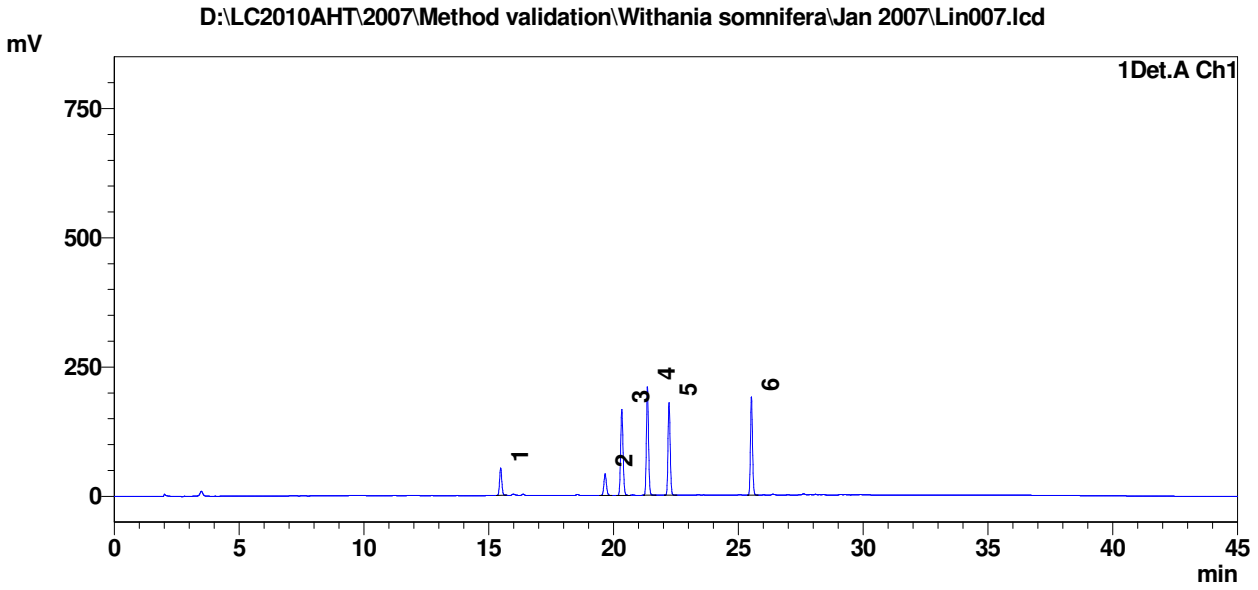
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.499	329878	52391	6.345	Withanoside IV
2	19.657	298008	42420	5.732	Withanoside V
3	20.328	1134850	167103	21.829	Withaferin A
4	21.351	1256078	210138	24.161	12- Deoxy withastramonolide
5	22.216	1084798	179593	20.866	Withanollide A
6	25.509	1095189	189856	21.066	Withanolide B
Total		5198801	841502	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : V
Vail #	: 8
Injection Volume	: 20 uL
Data File Name	: Lin007.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 5:11:18 AM
Data Processed	: 1/23/2007 1:55:57 AM



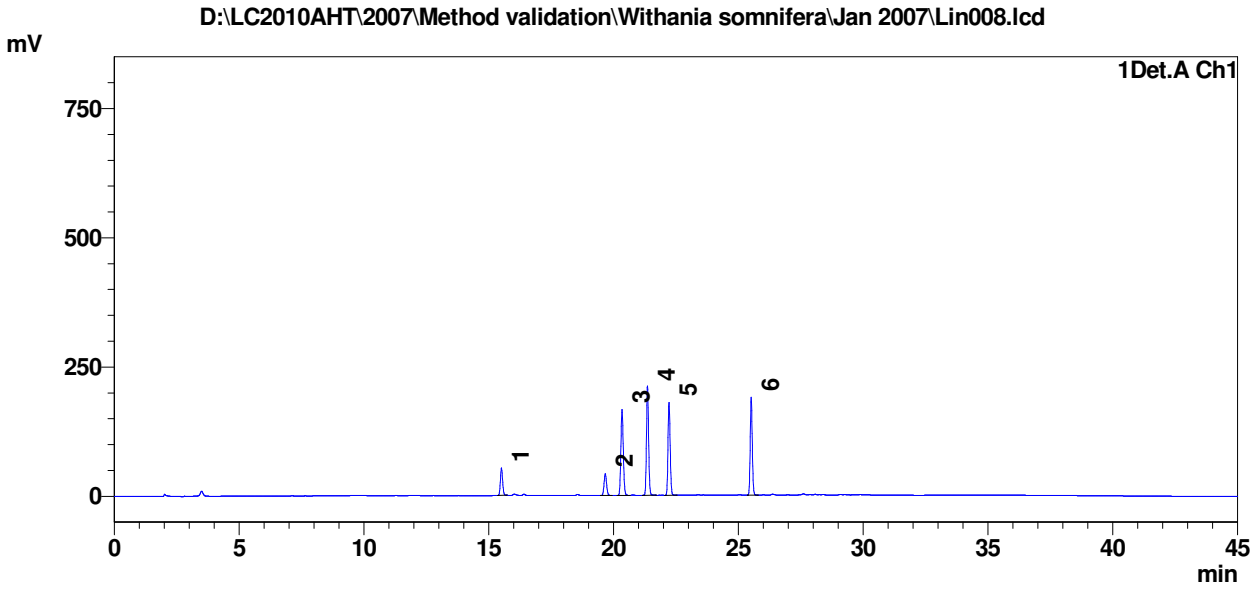
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.479	330019	53099	6.350	Withanoside IV
2	19.664	297953	42274	5.733	Withanoside V
3	20.332	1134742	166376	21.835	Withaferin A
4	21.358	1254992	209684	24.149	12- Deoxy withastramonolide
5	22.226	1084624	179021	20.871	Withanollide A
6	25.526	1094573	189990	21.062	Withanolide B
Total		5196904	840443	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : V
Vail #	: 8
Injection Volume	: 20 uL
Data File Name	: Lin008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 5:57:02 AM
Data Processed	: 1/23/2007 1:58:21 AM



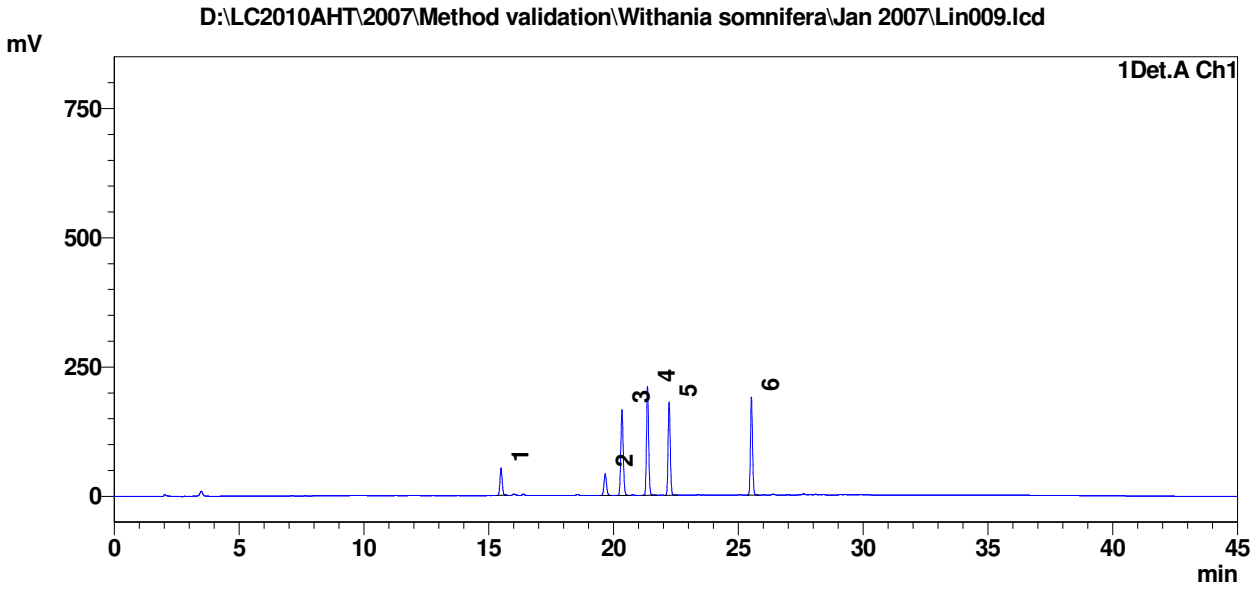
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.511	329943	52980	6.345	Withanoside IV
2	19.670	298036	42397	5.731	Withanoside V
3	20.341	1134589	166172	21.817	Withaferin A
4	21.361	1256714	211311	24.165	12- Deoxy withastramonolide
5	22.225	1084955	179459	20.863	Withanollide A
6	25.516	1096209	189401	21.079	Withanolide B
Total		5200447	841721	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : V
Vail #	: 8
Injection Volume	: 20 uL
Data File Name	: Lin009.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 6:42:47 AM
Data Processed	: 1/23/2007 1:59:17 AM



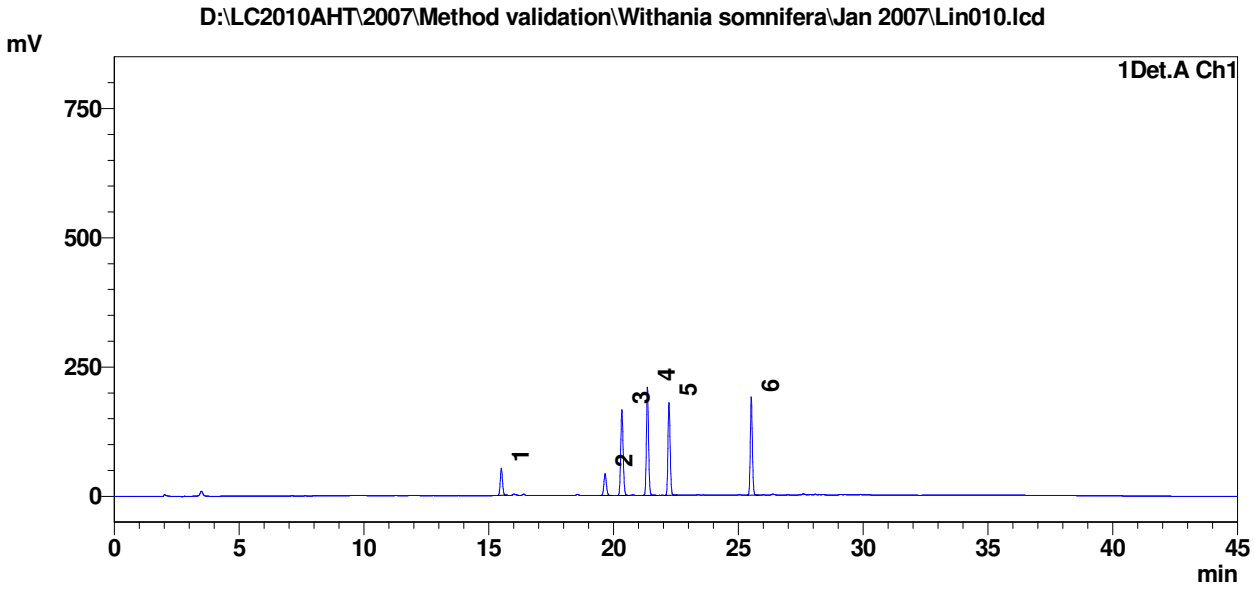
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.494	329827	52958	6.345	RT15.494
2	19.668	297352	42157	5.721	RT19.668
3	20.339	1134172	166159	21.820	RT20.339
4	21.362	1256803	210666	24.179	RT21.362
5	22.229	1084918	180075	20.872	RT22.229
6	25.526	1094840	189492	21.063	RT25.526
Total		5197911	841507	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : V
Vail #	: 8
Injection Volume	: 20 uL
Data File Name	: Lin010.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 7:28:32 AM
Data Processed	: 1/23/2007 2:00:32 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.506	329140	52333	6.343	Withanoside IV
2	19.664	297517	42403	5.733	Withanoside V
3	20.336	1131663	166080	21.807	Withaferin A
4	21.358	1254538	209221	24.175	12- Deoxy withastramonolide
5	22.224	1082926	179282	20.868	Withanollide A
6	25.520	1093649	189781	21.075	Withanolide B
Total		5189432	839100	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Lin001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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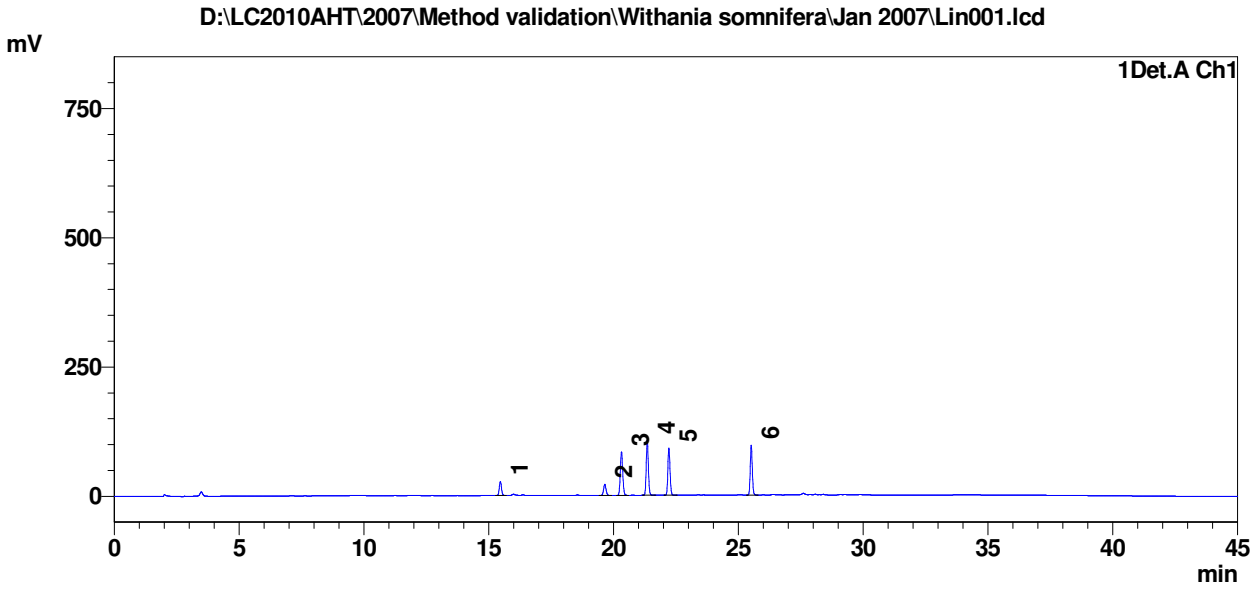
Data Processed	: 1/20/2007 4:58:40 PM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.466	166047	26493	6.310	Withanoside IV
2	19.651	151294	21445	5.749	Withanoside V
3	20.320	573810	84100	21.805	Withaferin A
4	21.351	635487	106310	24.149	12- Deoxy withastramonolide
5	22.219	551317	91243	20.950	Withanollide A
6	25.517	553596	96384	21.037	Withanolide B
Total		2631550	425976	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Lin002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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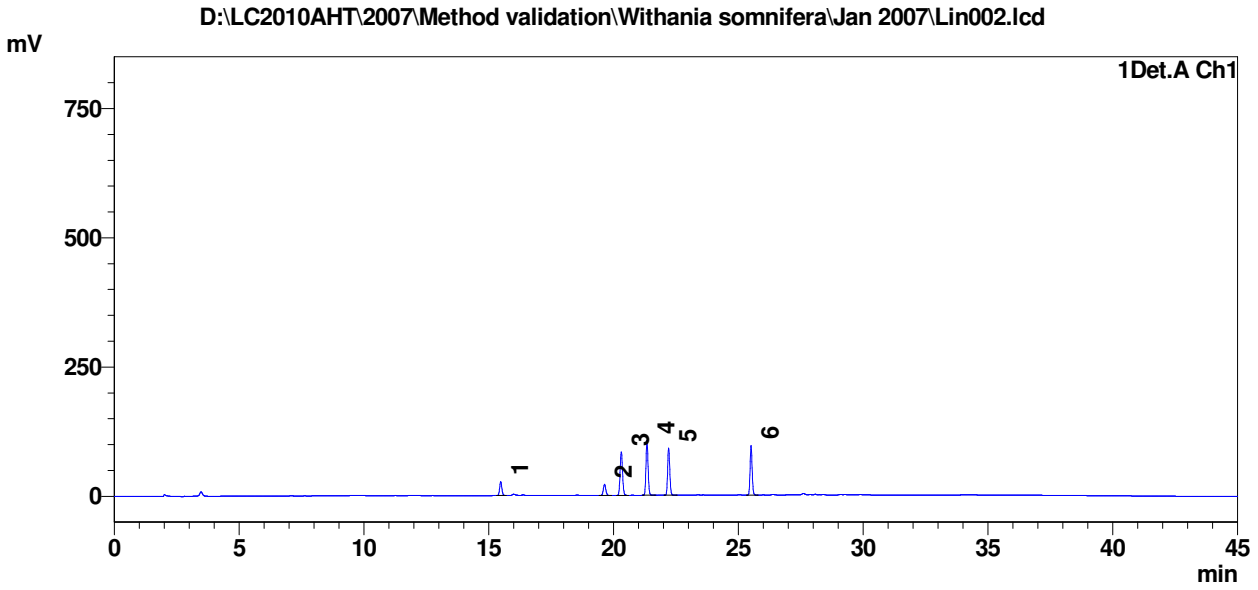


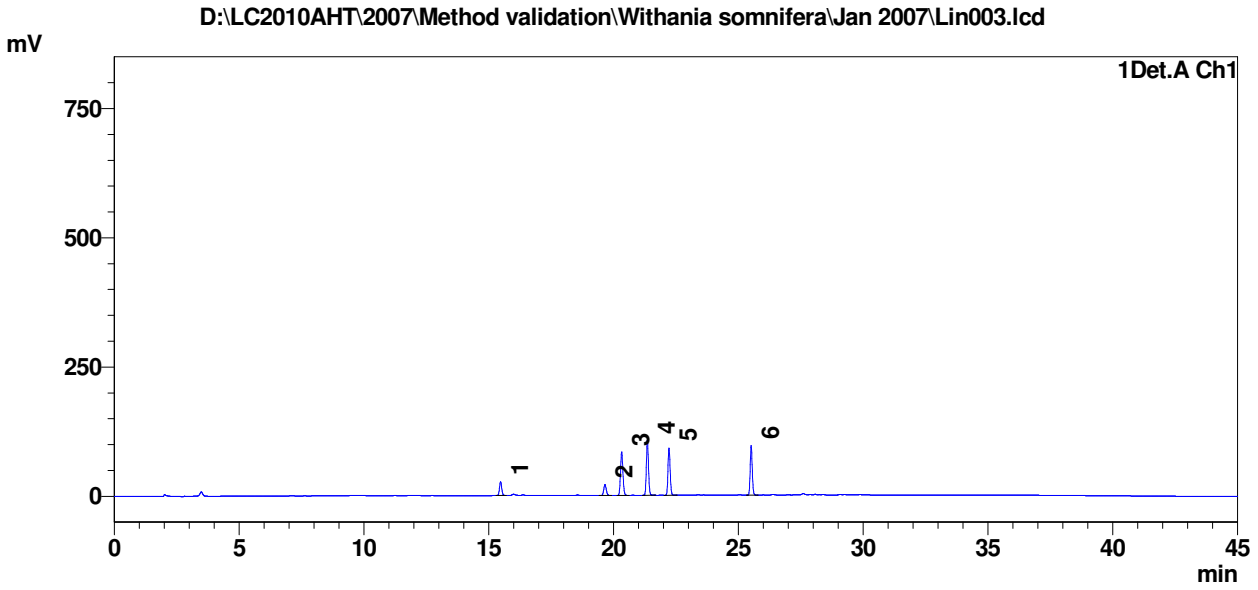
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.481	166038	26677	6.317	Withanoside IV
2	19.641	151121	21442	5.749	Withanoside V
3	20.311	573224	84096	21.807	Withaferin A
4	21.341	634565	105947	24.141	12- Deoxy withastramonolide
5	22.209	550821	90976	20.955	Withanollide A
6	25.511	552800	96186	21.030	Withanolide B
Total		2628569	425324	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Lin003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 2:08:21 AM
Data Processed	: 1/23/2007 1:49:22 AM



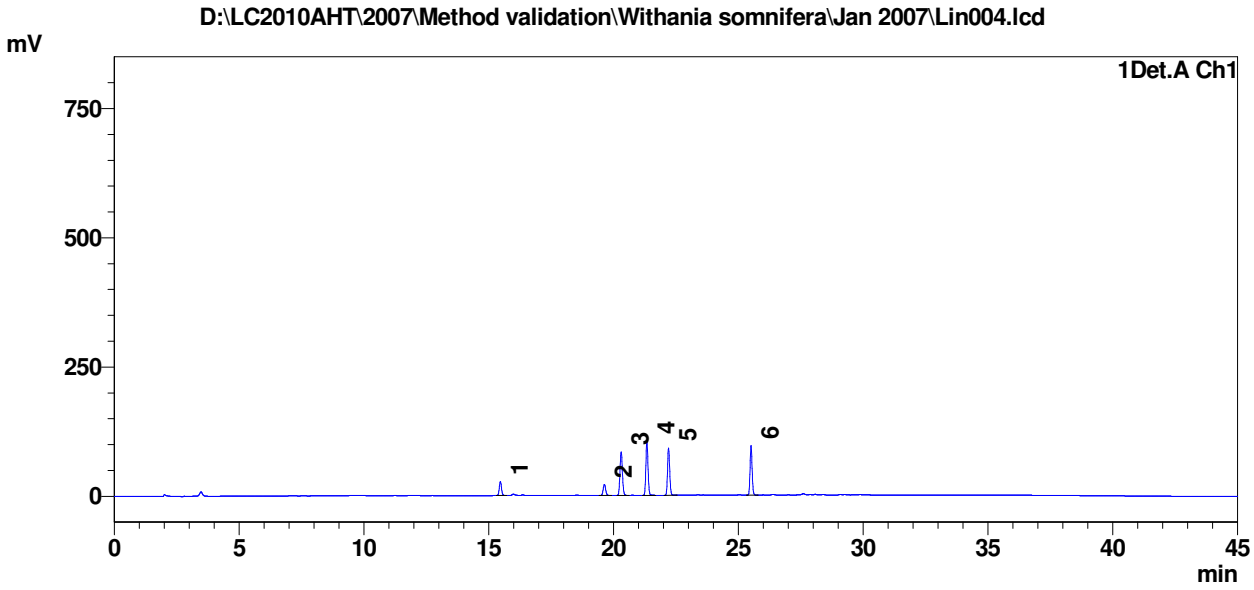
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.475	166015	26501	6.314	Withanoside IV
2	19.658	151156	21357	5.749	Withanoside V
3	20.327	573669	84147	21.819	Withaferin A
4	21.357	634904	106253	24.148	12- Deoxy withastramonolide
5	22.224	550930	91150	20.954	Withanollide A
6	25.515	552601	96154	21.017	Withanolide B
Total		2629275	425561	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Lin004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 2:54:06 AM
Data Processed	: 1/23/2007 1:51:11 AM



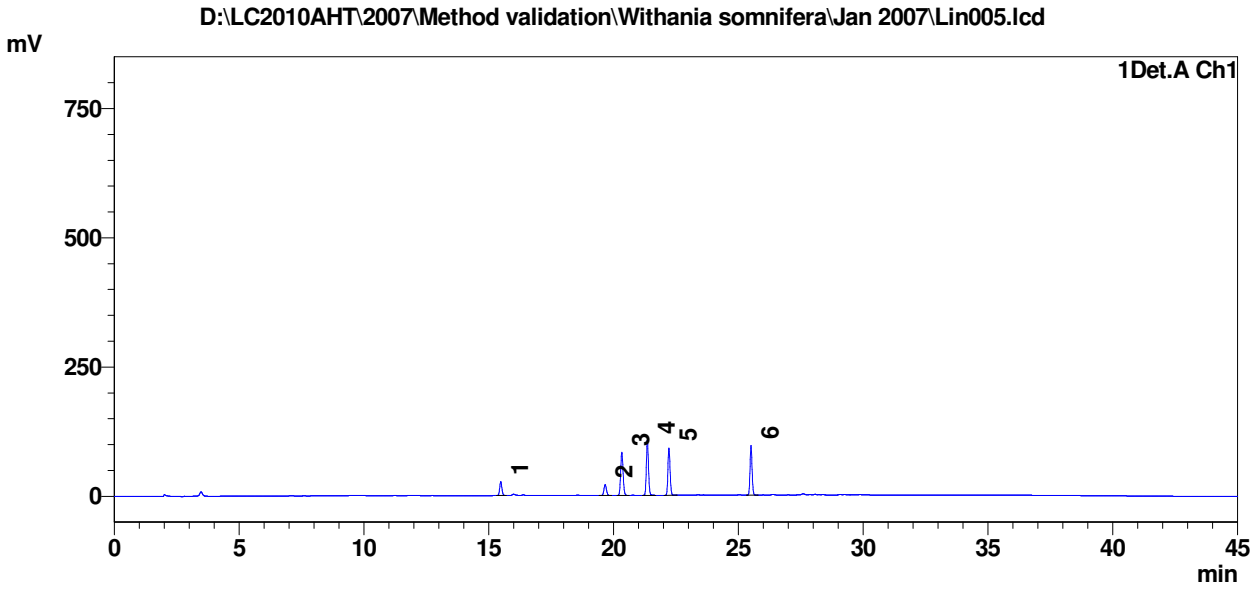
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.467	166025	26498	6.315	RT15.467
2	19.635	151277	21394	5.754	RT19.635
3	20.306	573097	84028	21.799	RT20.306
4	21.338	634821	106301	24.147	RT21.338
5	22.206	550882	91145	20.954	RT22.206
6	25.510	552865	96093	21.030	RT25.510
Total		2628969	425458	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Lin005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 3:39:50 AM
Data Processed	: 1/23/2007 1:52:14 AM



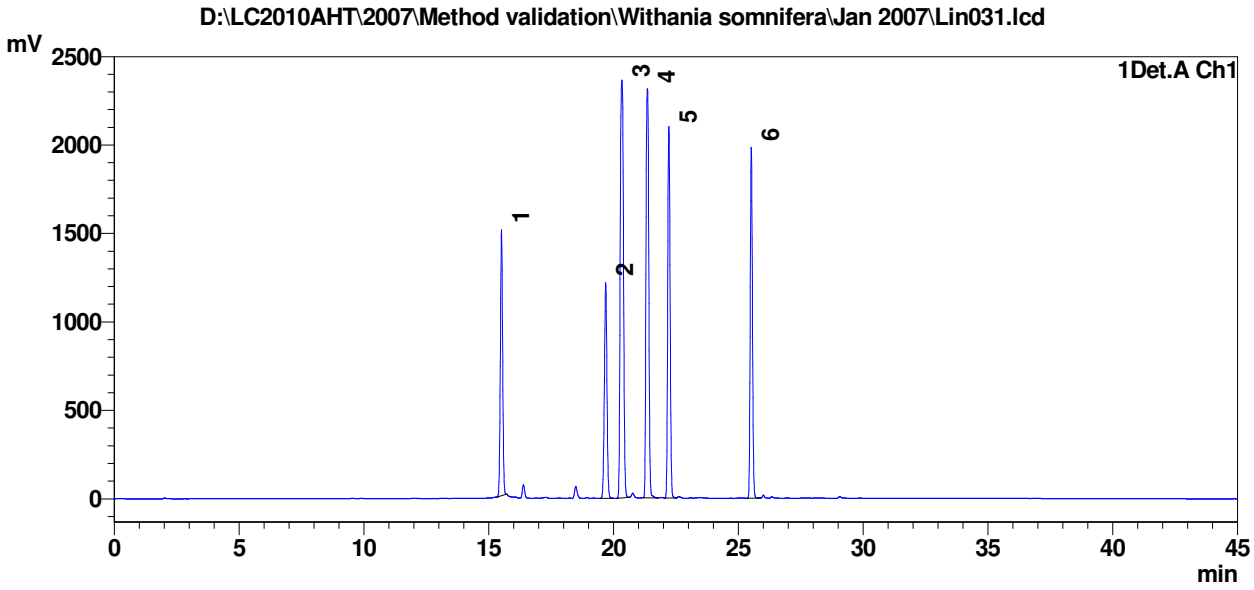
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.483	165993	26475	6.317	Withanoside IV
2	19.665	150985	21409	5.746	Withanoside V
3	20.333	572942	83865	21.805	Withaferin A
4	21.356	634615	106347	24.152	12- Deoxy withastramonolide
5	22.221	550635	91431	20.956	Withanollide A
6	25.509	552430	95695	21.024	Withanolide B
Total		2627600	425221	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Stock solution
Vail #	: 13
Injection Volume	: 20 uL
Data File Name	: Lin031.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/14/2007 11:37:48 PM
Data Processed	: 1/23/2007 2:33:30 AM



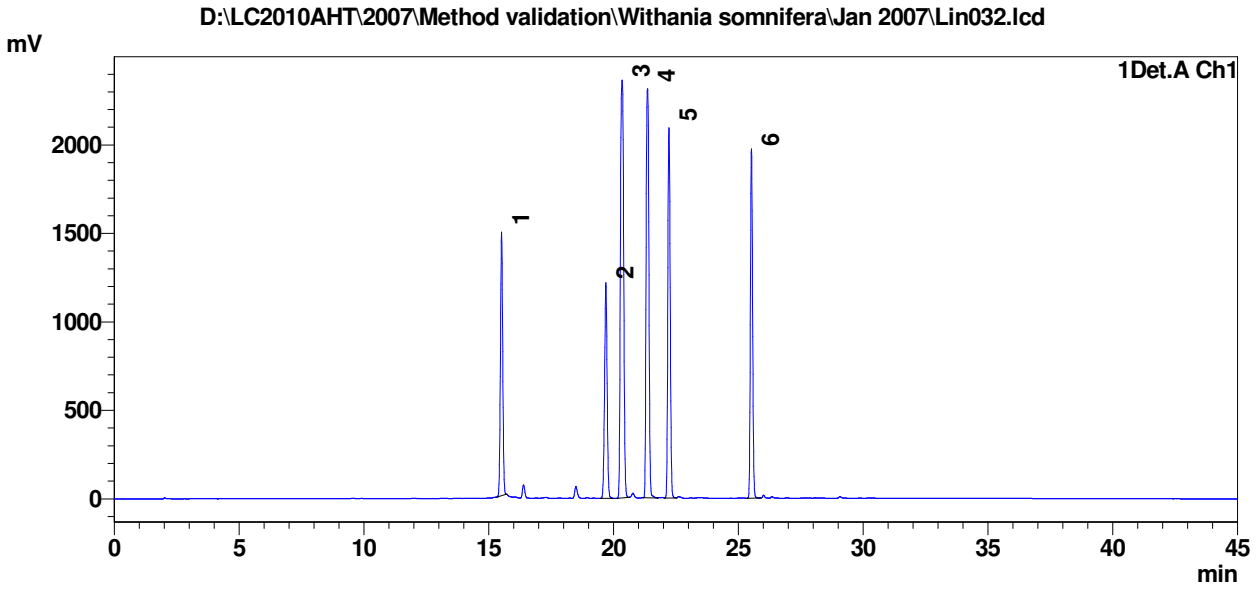
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.513	9597190	1502923	11.422	Withanoside IV
2	19.688	8731747	1218885	10.392	Withanoside V
3	20.337	21469032	2361628	25.552	Withaferin A
4	21.360	18080882	2312241	21.519	12- Deoxy withastramonolide
5	22.219	13878628	2099972	16.518	Withanollide A
6	25.521	12264511	1984228	14.597	Withanolide B
Total		84021990	11479877	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Stock solution
Vail #	: 13
Injection Volume	: 20 uL
Data File Name	: Lin032.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 12:23:36 AM
Data Processed	: 1/23/2007 2:34:01 AM



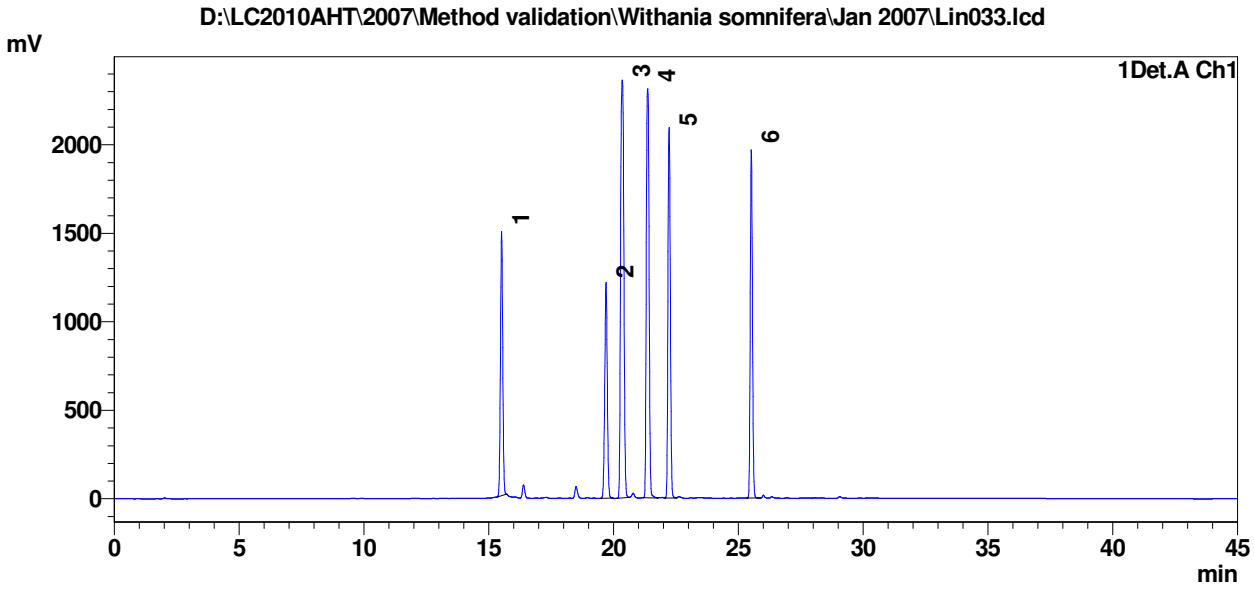
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.516	9587035	1490793	11.434	Withanoside IV
2	19.695	8721728	1218646	10.402	Withanoside V
3	20.344	21438480	2361255	25.568	Withaferin A
4	21.365	18085009	2311064	21.569	12- Deoxy withastramonolide
5	22.224	13809664	2092715	16.470	Withanollide A
6	25.527	12206630	1974740	14.558	Withanolide B
Total		83848546	11449213	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Stock solution
Vail #	: 13
Injection Volume	: 20 uL
Data File Name	: Lin033.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 1:09:22 AM
Data Processed	: 1/23/2007 2:34:21 AM



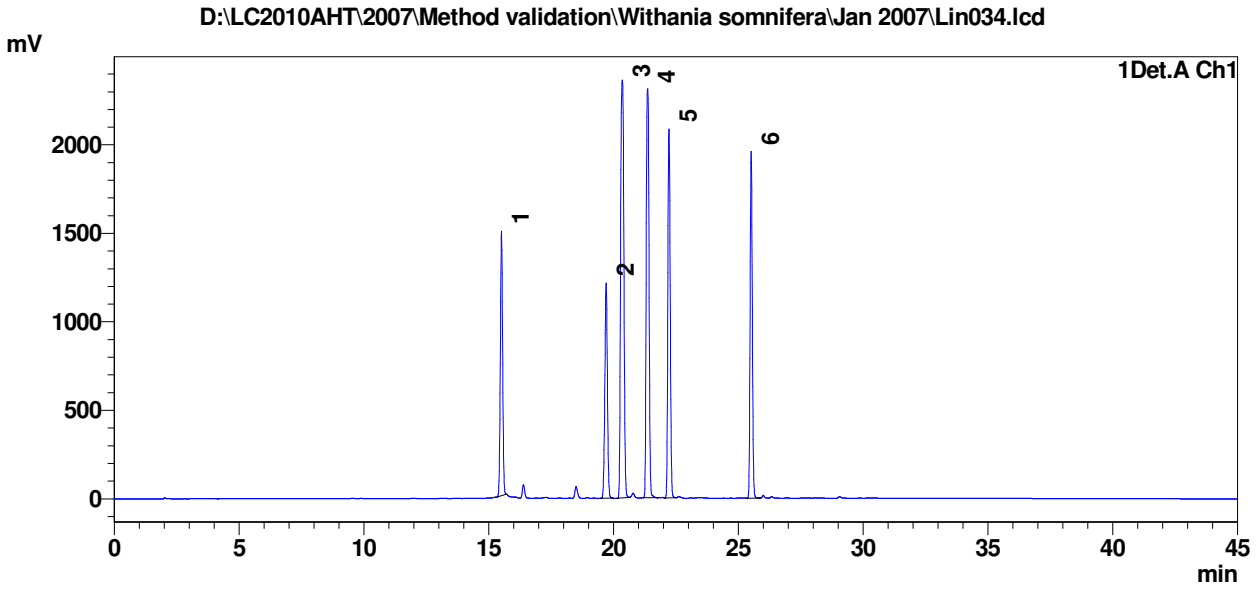
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.517	9584608	1492343	11.439	Withanoside IV
2	19.702	8719358	1219371	10.406	Withanoside V
3	20.352	21482780	2360406	25.639	Withaferin A
4	21.373	18068450	2311083	21.565	12- Deoxy withastramonolide
5	22.229	13766317	2092979	16.430	Withanollide A
6	25.522	12166339	1968443	14.520	Withanolide B
Total		83787852	11444625	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Stock solution
Vail #	: 13
Injection Volume	: 20 uL
Data File Name	: Lin034.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 1:55:08 AM
Data Processed	: 1/23/2007 2:34:42 AM



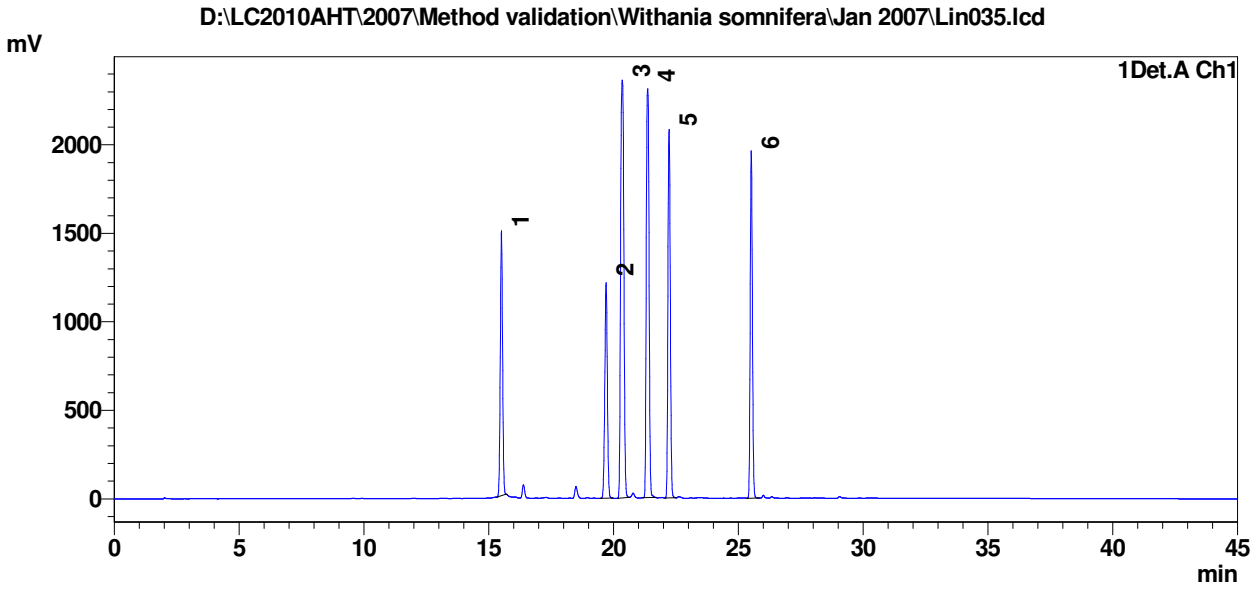
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.513	9588582	1495937	11.464	Withanoside IV
2	19.706	8718285	1216857	10.424	Withanoside V
3	20.352	21438918	2360616	25.633	Withaferin A
4	21.370	18045786	2311081	21.576	12- Deoxy withastramonolide
5	22.224	13721170	2084765	16.405	Withanollide A
6	25.516	12126384	1959630	14.498	Withanolide B
Total		83639125	11428886	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Stock solution
Vail #	: 13
Injection Volume	: 20 uL
Data File Name	: Lin035.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 2:40:53 AM
Data Processed	: 1/23/2007 2:43:56 AM



1 Det.A Ch1/227nm

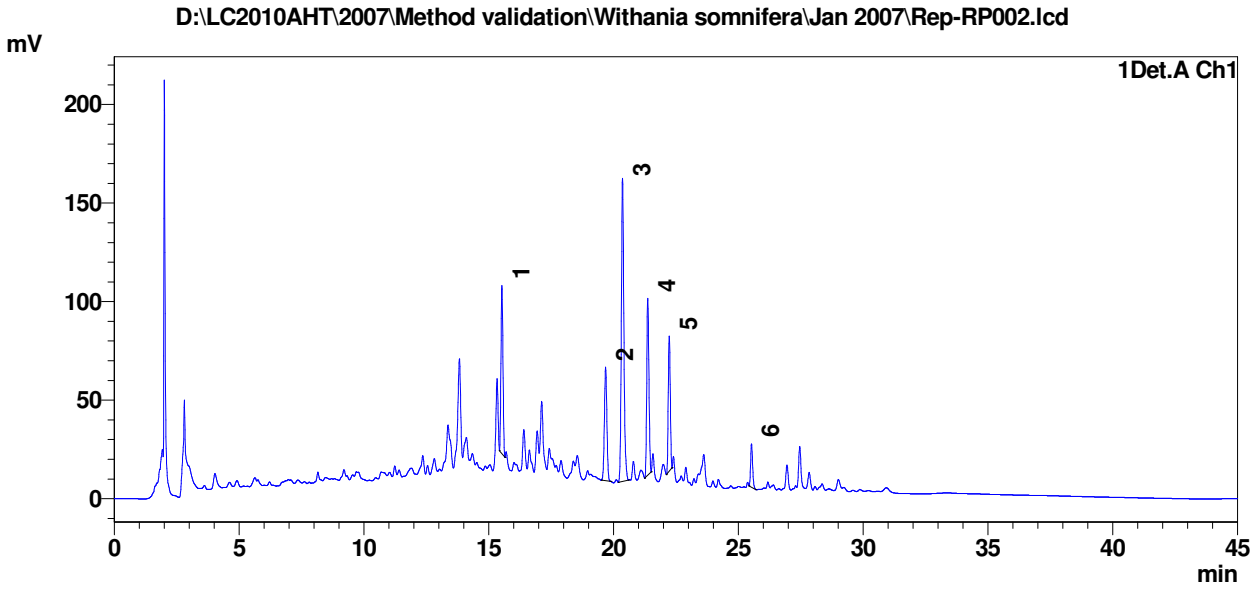
Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.511	9579369	1496492	11.479	Withanoside IV
2	19.704	8714428	1219106	10.443	Withanoside V
3	20.352	21442185	2360662	25.694	Withaferin A
4	21.372	17998894	2309818	21.568	12- Deoxy withastramonolide
5	22.228	13664575	2082704	16.374	Withanollide A
6	25.520	12051739	1961756	14.442	Withanolide B
Total		83451189	11430539	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Enclosure: 06

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 2
Vail #	: 31
Injection Volume	: 20 uL
Data File Name	: Rep-RP002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 2:19:13 AM
Data Processed	: 1/23/2007 3:09:21 AM



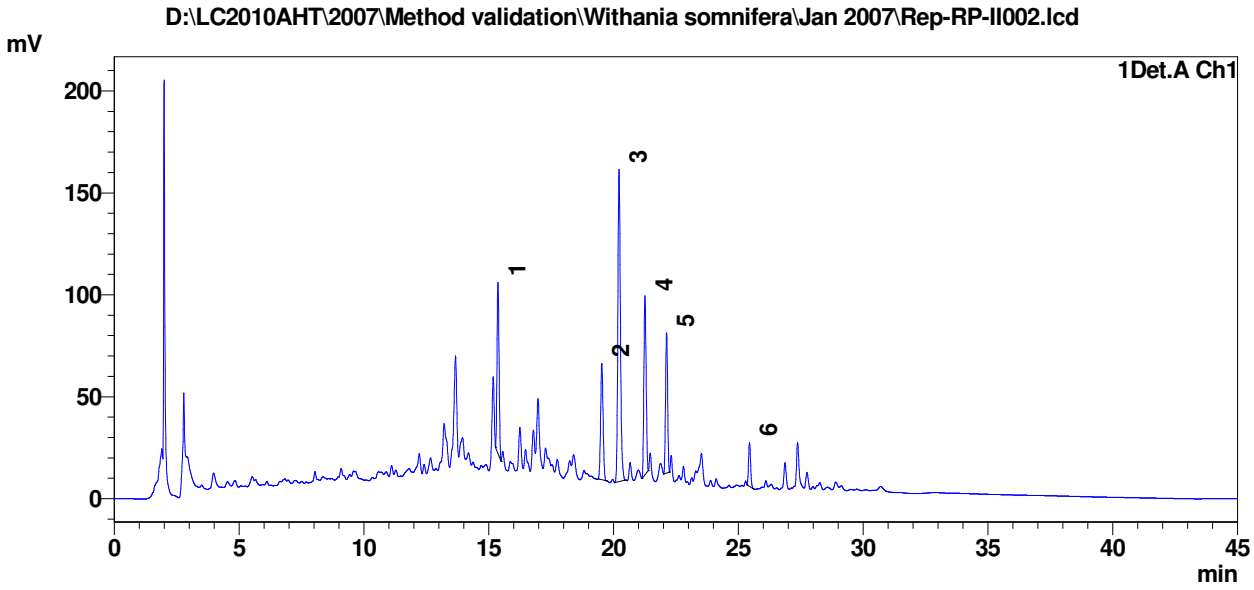
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.529	478028	85286	15.819	Withanoside IV
2	19.684	401702	57571	13.294	Withanoside V
3	20.362	1091994	153766	36.138	Withaferin A
4	21.373	545128	89813	18.040	12- Deoxy withastramonolide
5	22.234	382010	68586	12.642	Withanollide A
6	25.530	122902	21932	4.067	Withanolide B
Total		3021764	476953	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 2
Vail #	: 44
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 2:31:04 PM
Data Processed	: 1/23/2007 2:57:48 AM



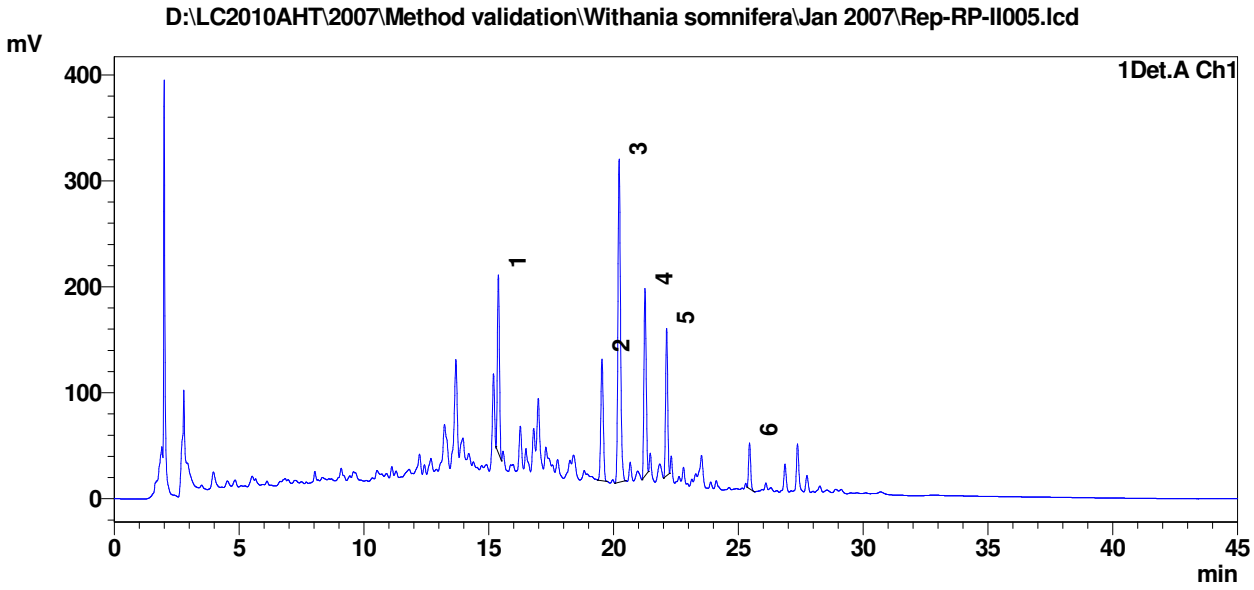
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.372	479376	84003	15.865	Withanoside IV
2	19.532	394834	57054	13.067	Withanoside V
3	20.223	1091327	153105	36.119	Withaferin A
4	21.260	541636	87896	17.926	12- Deoxy withastramonolide
5	22.126	393611	68735	13.027	Withanollide A
6	25.448	120722	21488	3.995	Withanolide B
Total		3021506	472281	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 1
Vail #	: 47
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 4:50:36 PM
Data Processed	: 1/23/2007 3:02:09 AM



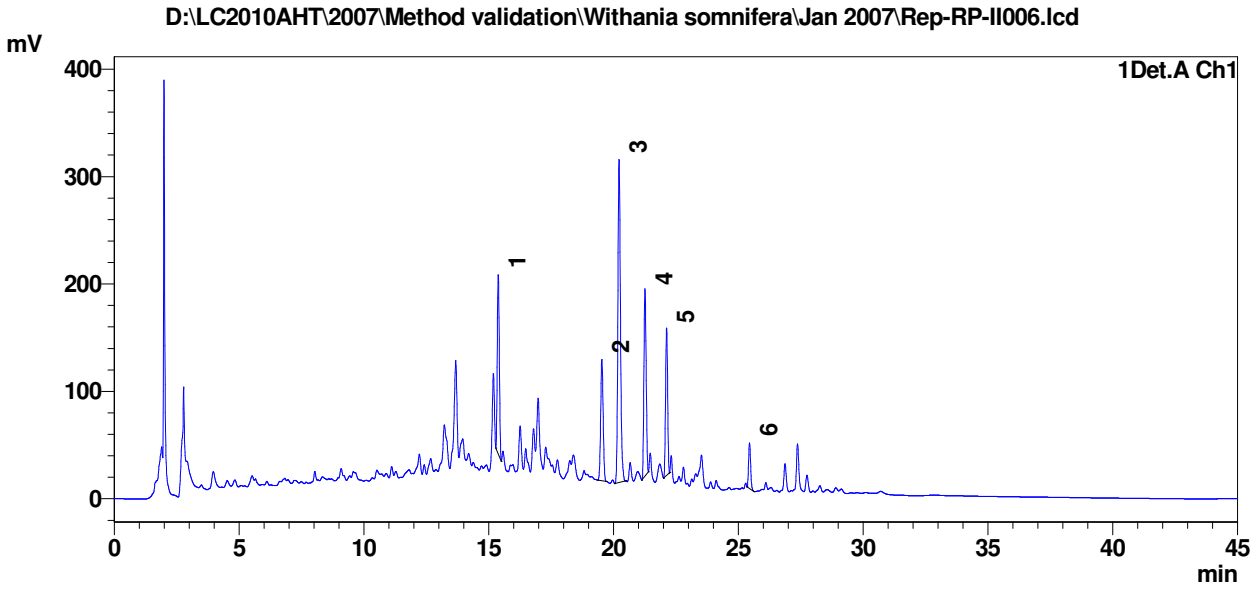
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.388	949957	167823	15.716	Withanoside IV
2	19.540	793298	114653	13.124	Withanoside V
3	20.227	2173830	304832	35.963	Withaferin A
4	21.262	1088292	177034	18.004	12- Deoxy withastramonolide
5	22.128	797140	139023	13.187	Withanollide A
6	25.450	242191	43112	4.007	Withanolide B
Total		6044708	946478	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 2
Vail #	: 48
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II006.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 5:36:20 PM
Data Processed	: 1/23/2007 3:03:19 AM



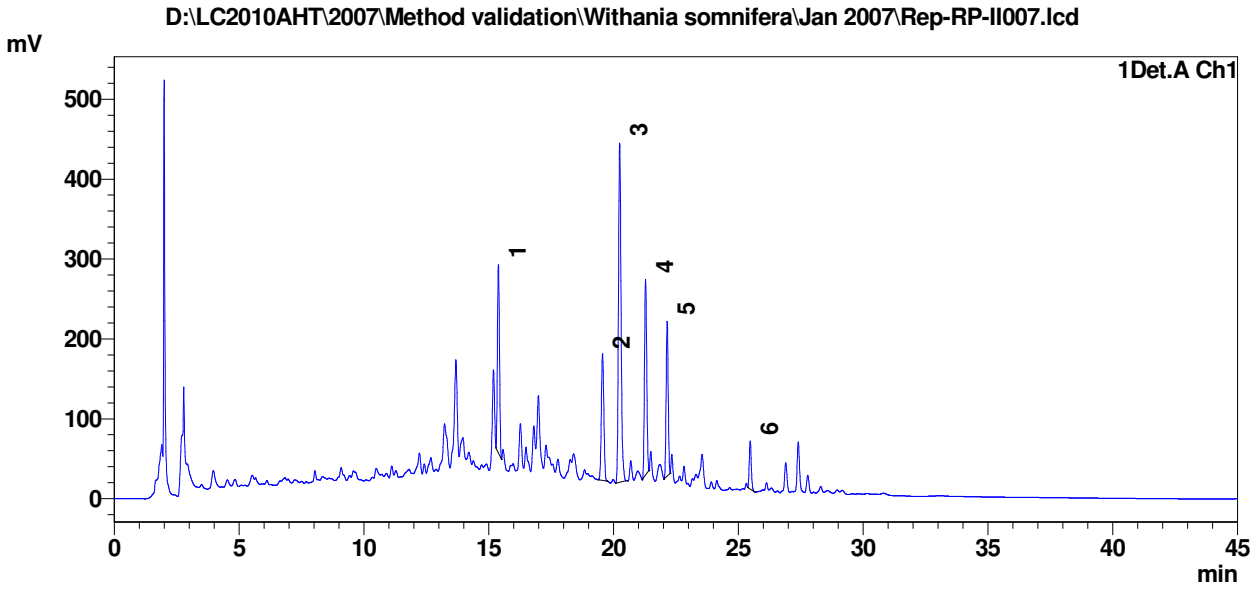
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.383	949797	166616	15.870	Withanoside IV
2	19.534	783540	112934	13.092	Withanoside V
3	20.224	2152710	300796	35.970	Withaferin A
4	21.261	1075418	174559	17.969	12- Deoxy withastramonolide
5	22.126	784022	137155	13.100	Withanollide A
6	25.449	239205	42555	3.997	Withanolide B
Total		5984692	934615	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 1
Vail #	: 49
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II007.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 6:22:05 PM
Data Processed	: 1/23/2007 3:04:28 AM



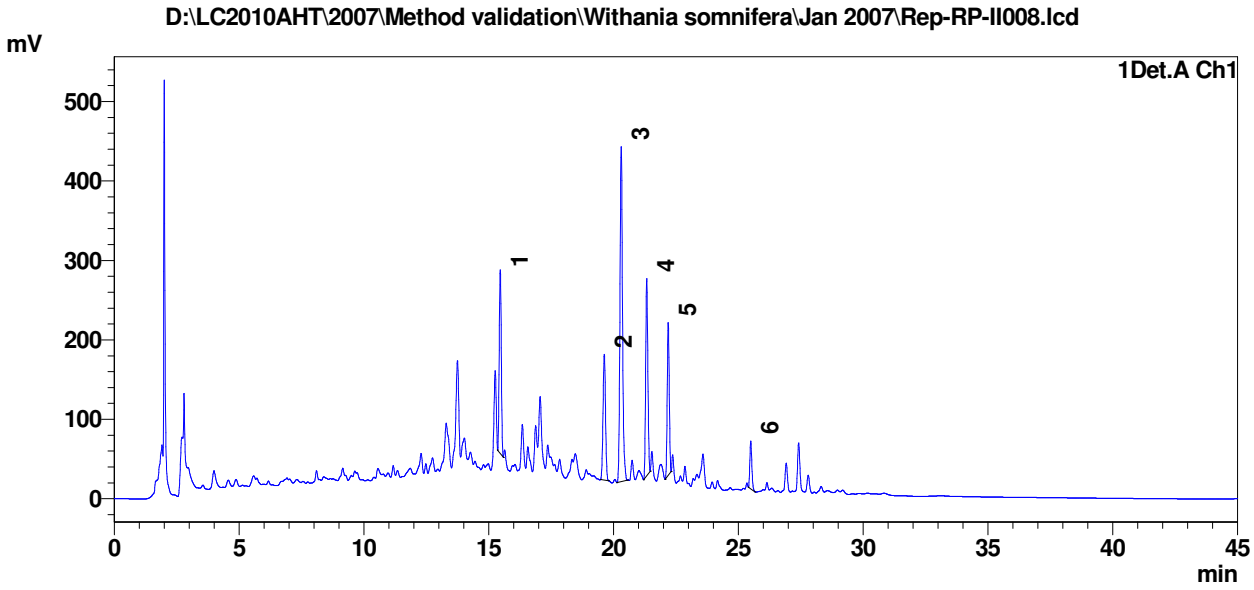
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.390	1333648	234986	15.796	Withanoside IV
2	19.562	1102054	158403	13.053	Withanoside V
3	20.248	3036695	424260	35.968	Withaferin A
4	21.284	1511759	245224	17.906	12- Deoxy withastramonolide
5	22.149	1120023	193935	13.266	Withanollide A
6	25.476	338649	60102	4.011	Withanolide B
Total		8442826	1316911	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 2
Vail #	: 50
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 7:07:50 PM
Data Processed	: 1/23/2007 3:05:39 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

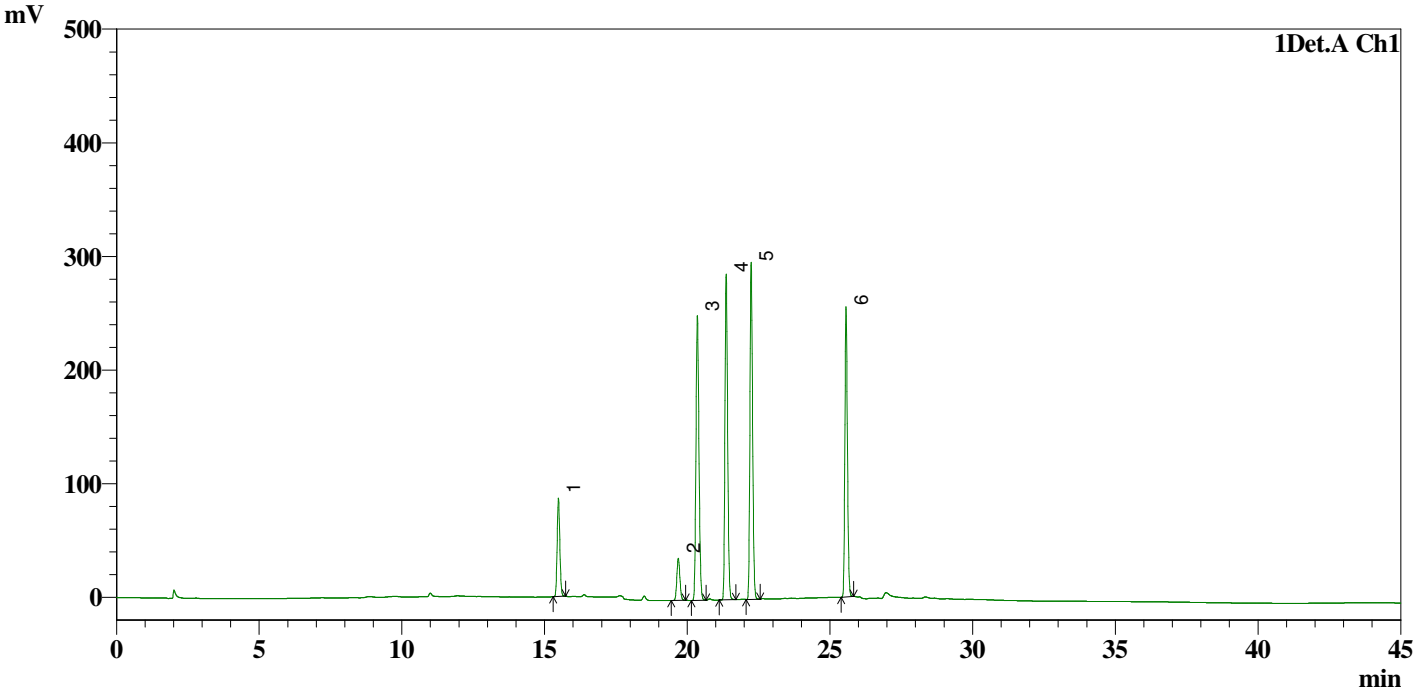
Peak #	Ret. Time	Area	Height	Area %	Name
1	15.462	1320439	230805	15.782	Withanoside IV
2	19.630	1090118	158305	13.029	Withanoside V
3	20.308	3006238	421639	35.930	Withaferin A
4	21.330	1512807	248164	18.081	12- Deoxy withastramonolide
5	22.192	1101225	192750	13.162	Withanollide A
6	25.499	336094	60035	4.017	Withanolide B
Total		8366920	1311697	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Ref Standard**
Sample ID	**: Std001**
Vail #	**: 1**
Injection Volume	**: 20 uL**
Data File Name	**: Std001.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 3:50:17 PM**
Data Processed	**: 4/3/2007 8:52:15 AM**

Enclosure: 07

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Std001.lcd



1 Det.A Ch1/227nm

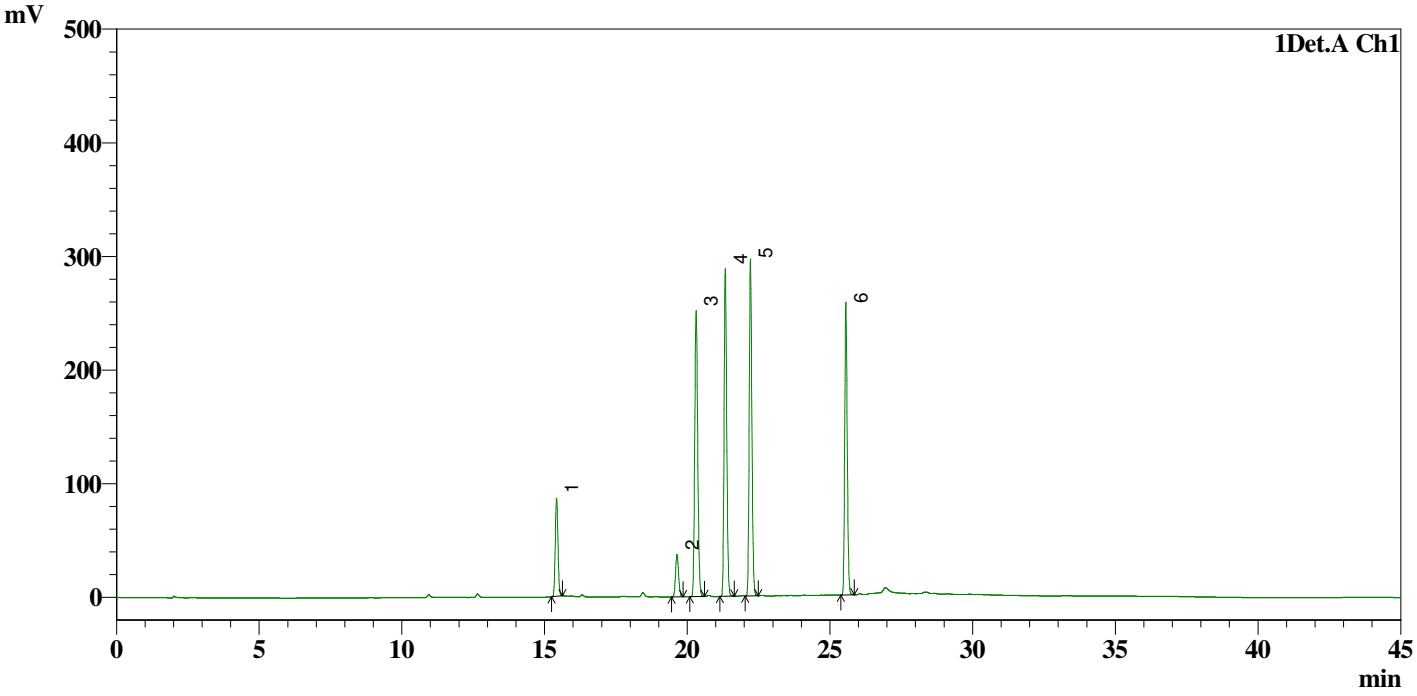
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.482	Withanoside IV	570544	7.053	86373	7.125
2	19.681	Withanoside V	274439	3.393	37134	3.063
3	20.350	Withaferin A	1847990	22.845	250451	20.660
4	21.361	12-Deoxywithastramonolided	1859378	22.986	286472	23.631
5	22.236	Withanolide A	1935284	23.924	296581	24.465
6	25.560	Withanolide B	1601622	19.799	255262	21.056
Total			8089257	100.000	1212272	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Ref Standard**
Sample ID	**: Std002**
Vail #	**: 1**
Injection Volume	**: 20 uL**
Data File Name	**: Std002.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 4:36:00 PM**
Data Processed	**: 4/3/2007 8:53:21 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Std002.lcd



1 Det.A Ch1/227nm

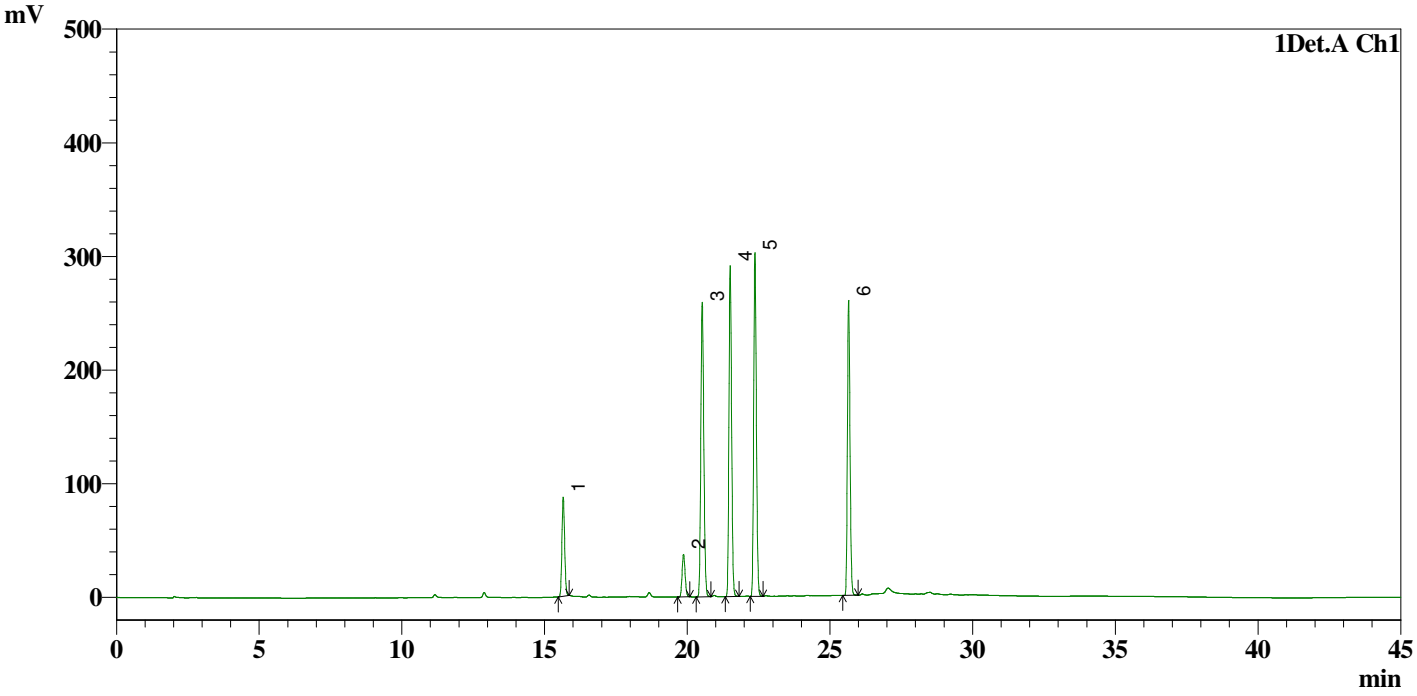
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.415	Withanoside IV	569355	7.009	86362	7.096
2	19.636	Withanoside V	273195	3.363	37249	3.061
3	20.307	Withaferin A	1856433	22.853	251529	20.668
4	21.330	12-Deoxywithastramonolided	1864964	22.958	288126	23.675
5	22.211	Withanolide A	1945947	23.955	296367	24.352
6	25.554	Withanolide B	1613416	19.862	257376	21.148
Total			8123310	100.000	1217010	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Ref Standard**
Sample ID	**: Std003**
Vail #	**: 1**
Injection Volume	**: 20 uL**
Data File Name	**: Std003.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 5:21:47 PM**
Data Processed	**: 4/2/2007 6:24:39 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Std003.lcd



1 Det.A Ch1/227nm

Detector A Ch1 227nm

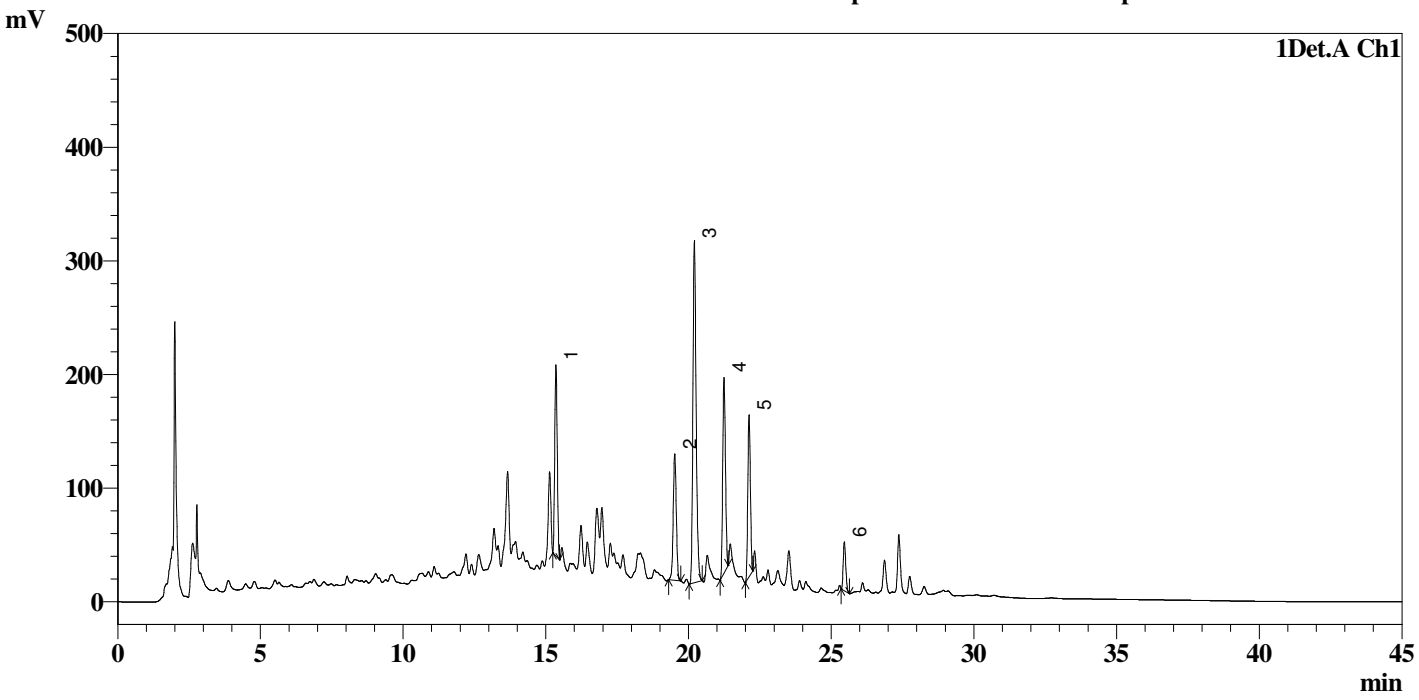
Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.650	Withanoside IV	571295	7.015	87077	7.043
2	19.866	Withanoside V	272912	3.351	37389	3.024
3	20.521	Withaferin A	1862675	22.873	259238	20.967
4	21.500	12-Deoxywithastramonolided	1869244	22.954	290958	23.533
5	22.368	Withanolide A	1951754	23.967	302203	24.442
6	25.651	Withanolide B	1615544	19.839	259521	20.990
Total			8143423	100.000	1236387	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 8-Rep-1**
Vail #	**: 3**
Injection Volume	**: 20 uL**
Data File Name	**: Sample004.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 11:27:48 PM**
Data Processed	**: 4/3/2007 9:41:33 AM**

Enclosure: 08

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample004.lcd



1 Det.A Ch1/227nm

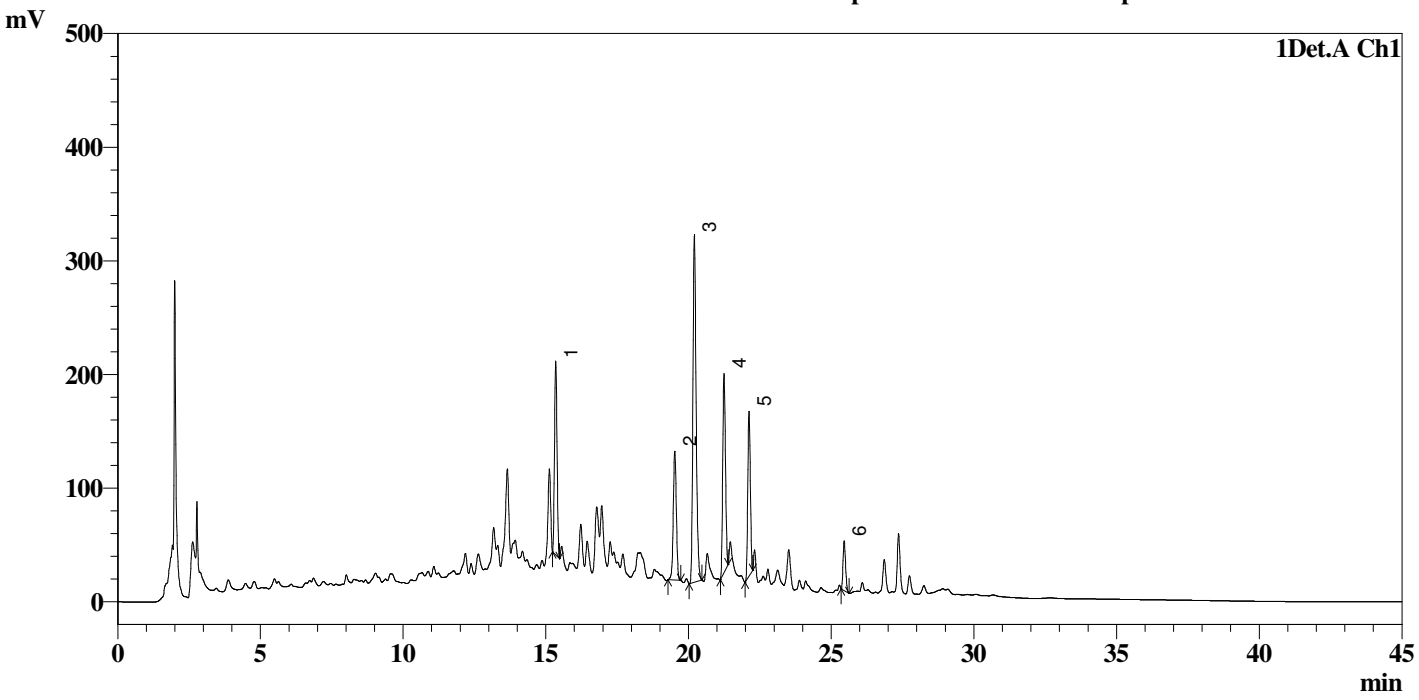
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.351	Withanoside IV	1023792	15.803	168061	17.910
2	19.515	Withanoside V	827144	12.768	111245	11.855
3	20.202	Withaferin A	2328659	35.945	301244	32.103
4	21.239	12-Deoxywithastramonolide	1150183	17.754	172411	18.373
5	22.117	Withanolide A	886037	13.677	142514	15.187
6	25.457	Withanolide B	262613	4.054	42899	4.572
Total			6478428	100.000	938374	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 8-Rep-2**
Vail #	**: 3**
Injection Volume	**: 20 uL**
Data File Name	**: Sample005.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 12:13:31 AM**
Data Processed	**: 4/3/2007 9:42:38 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample005.lcd



1 Det.A Ch1/227nm

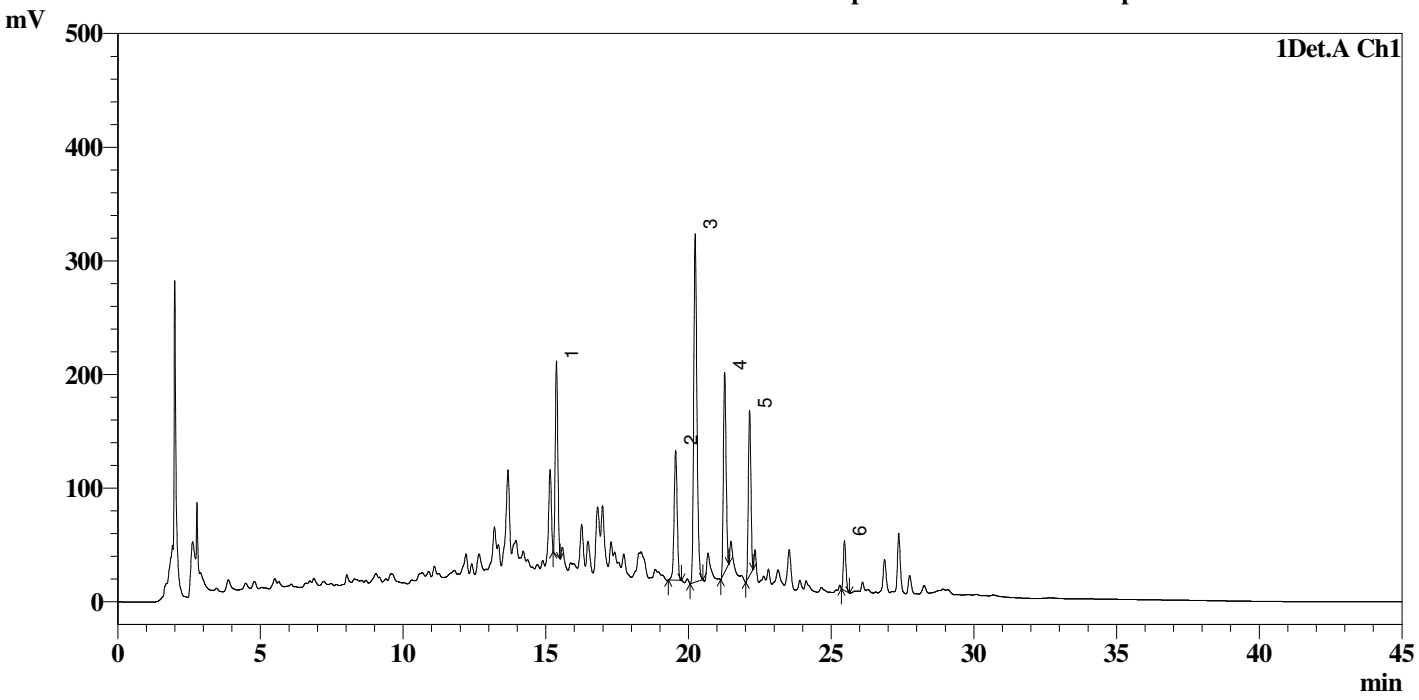
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.343	Withanoside IV	1039148	15.769	170452	17.864
2	19.517	Withanoside V	847222	12.857	113393	11.884
3	20.202	Withaferin A	2366023	35.905	306188	32.090
4	21.240	12-Deoxywithastramonolide	1169829	17.753	175380	18.381
5	22.116	Withanolide A	901239	13.677	145188	15.216
6	25.450	Withanolide B	266145	4.039	43560	4.565
Total			6589606	100.000	954161	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 8-Rep-3**
Vail #	**: 3**
Injection Volume	**: 20 uL**
Data File Name	**: Sample006.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 12:59:15 AM**
Data Processed	**: 4/3/2007 9:47:25 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample006.lcd



1 Det.A Ch1/227nm

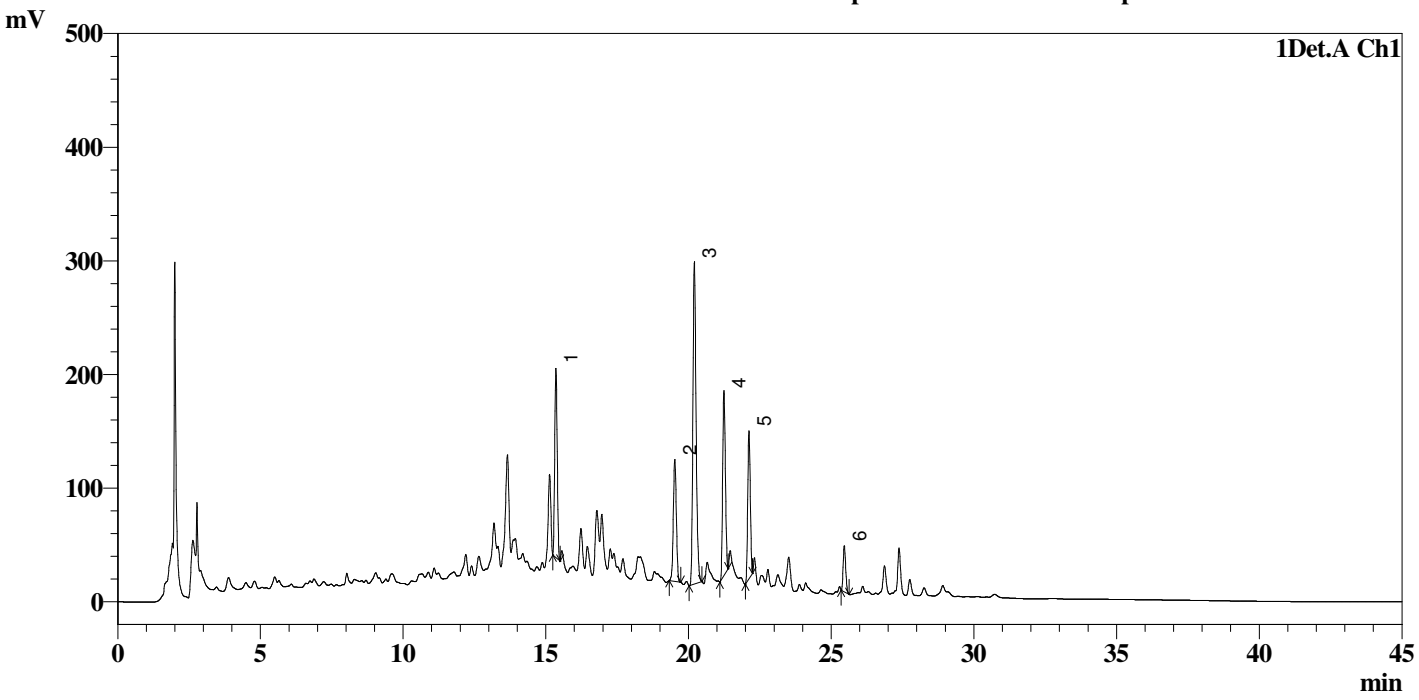
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.370	Withanoside IV	1038717	15.775	170520	17.825
2	19.546	Withanoside V	852993	12.955	114159	11.933
3	20.231	Withaferin A	2364071	35.903	306644	32.054
4	21.262	12-Deoxywithastramonolide	1167766	17.735	176184	18.417
5	22.136	Withanolide A	895427	13.599	145472	15.206
6	25.462	Withanolide B	265549	4.033	43679	4.566
Total			6584523	100.000	956658	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 10-Rep-1**
Vail #	**: 2**
Injection Volume	**: 20 uL**
Data File Name	**: Sample001.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 9:10:31 PM**
Data Processed	**: 4/3/2007 9:37:22 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample001.lcd



1 Det.A Ch1/227nm

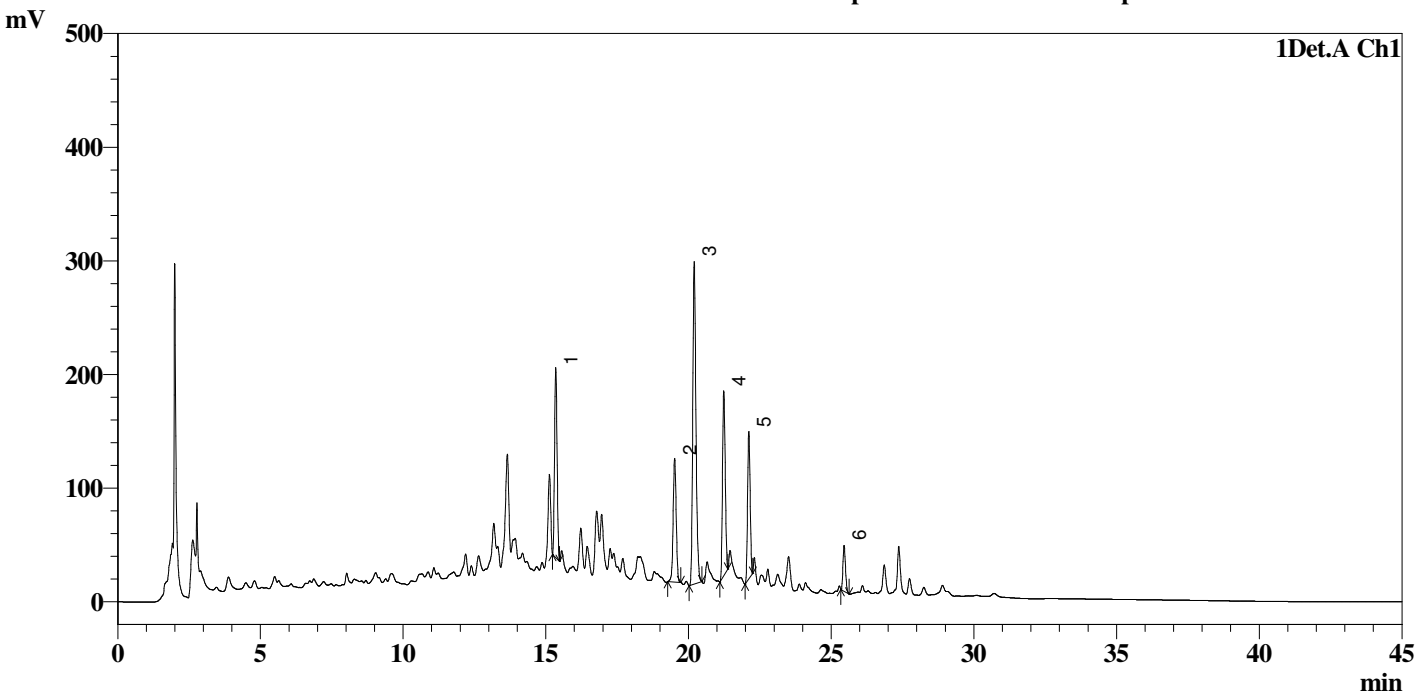
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.350	Withanoside IV	1014379	16.609	166482	18.673
2	19.517	Withanoside V	788092	12.904	107186	12.022
3	20.202	Withaferin A	2172950	35.578	283705	31.822
4	21.237	12-Deoxywithastramonolide	1080192	17.686	163276	18.314
5	22.115	Withanolide A	804364	13.170	130244	14.609
6	25.455	Withanolide B	247511	4.053	40658	4.560
Total			6107488	100.000	891550	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 10-Rep-2**
Vail #	**: 2**
Injection Volume	**: 20 uL**
Data File Name	**: Sample002.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 9:56:14 PM**
Data Processed	**: 4/3/2007 9:39:06 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample002.lcd



1 Det.A Ch1/227nm

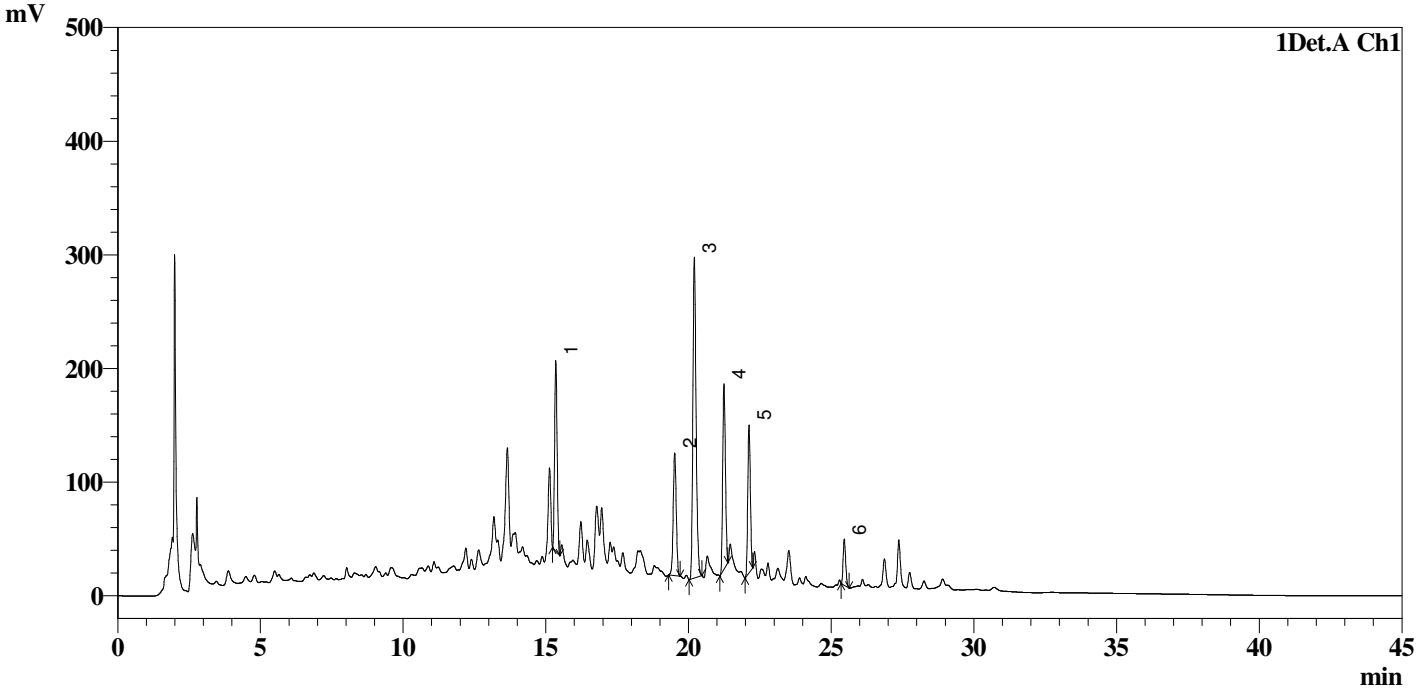
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.344	Withanoside IV	1018518	16.609	167301	18.737
2	19.512	Withanoside V	803499	13.103	108696	12.174
3	20.197	Withaferin A	2176460	35.491	283768	31.781
4	21.231	12-Deoxywithastramonolide	1082433	17.651	162874	18.241
5	22.109	Withanolide A	805495	13.135	129840	14.542
6	25.448	Withanolide B	245982	4.011	40409	4.526
Total			6132387	100.000	892888	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 10-Rep-3**
Vail #	**: 2**
Injection Volume	**: 20 uL**
Data File Name	**: Sample003.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 10:42:02 PM**
Data Processed	**: 4/3/2007 9:40:11 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample003.lcd



1 Det.A Ch1/227nm

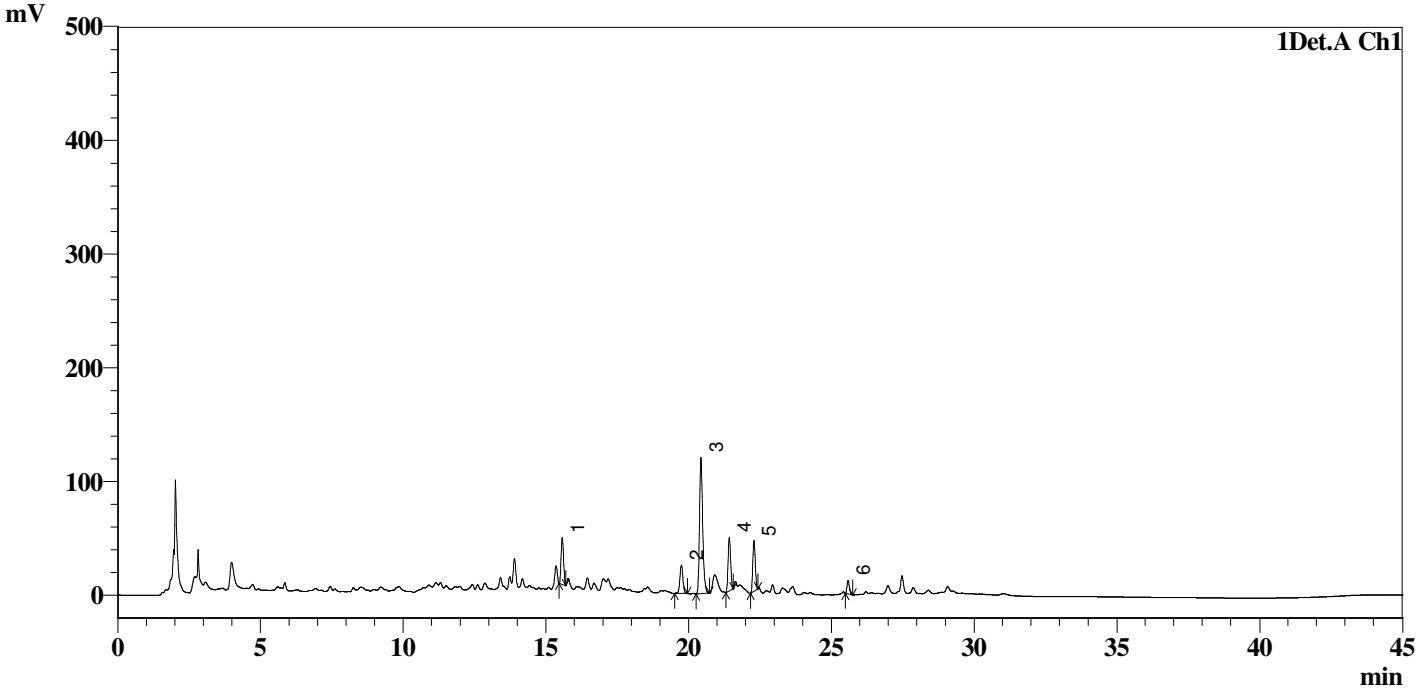
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.346	Withanoside IV	1017234	16.622	167594	18.792
2	19.514	Withanoside V	796155	13.009	107931	12.102
3	20.201	Withaferin A	2172974	35.506	282306	31.654
4	21.238	12-Deoxywithastramonolide	1078140	17.617	163313	18.312
5	22.116	Withanolide A	808865	13.217	130154	14.594
6	25.454	Withanolide B	246600	4.029	40545	4.546
Total			6119968	100.000	891843	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/05-Lot20-Rep-2**
Vail #	**: 7**
Injection Volume	**: 20 uL**
Data File Name	**: Sample017.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 9:22:27 AM**
Data Processed	**: 4/3/2007 11:32:19 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample017.lcd



1 Det.A Ch1/227nm

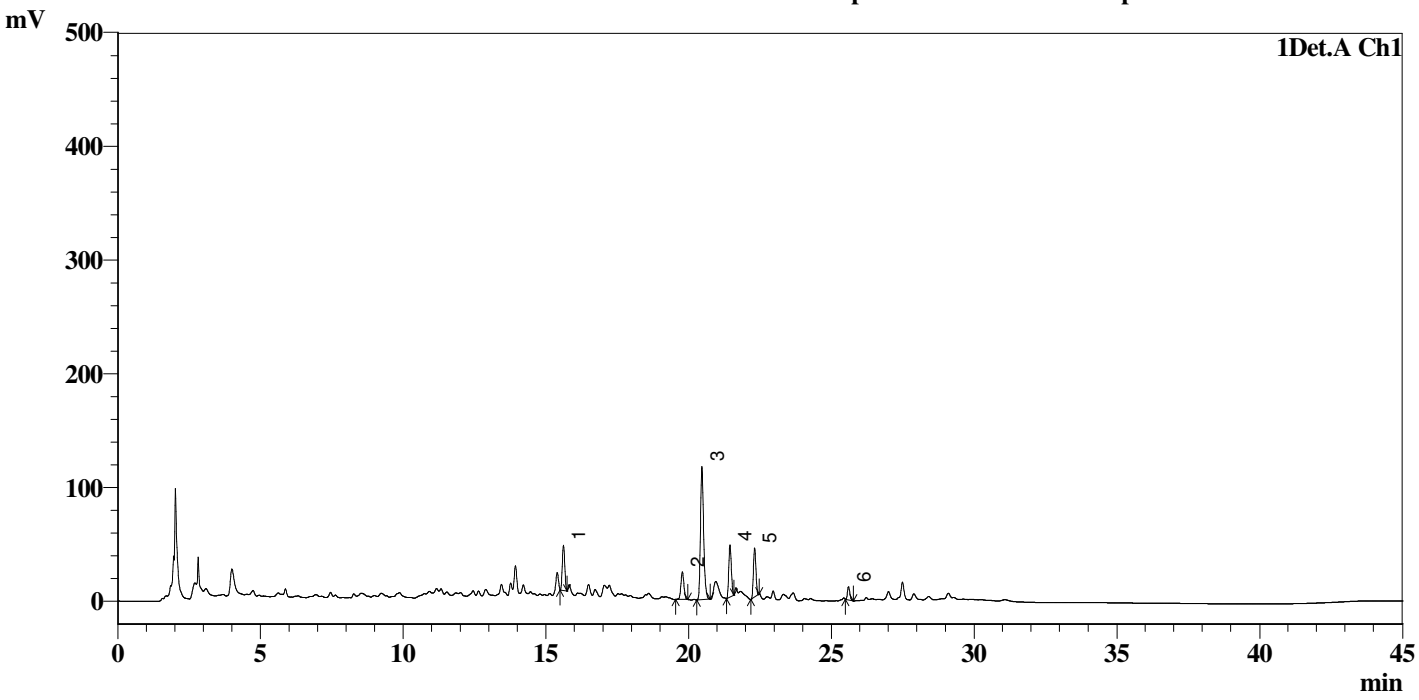
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.557	Withanoside IV	245963	12.365	41238	14.239
2	19.726	Withanoside V	185139	9.308	24936	8.610
3	20.413	Withaferin A	924671	46.486	119868	41.389
4	21.404	12-Deoxywithastramonolided	291928	14.676	47131	16.274
5	22.269	Withanolide A	270490	13.598	44521	15.373
6	25.564	Withanolide B	70945	3.567	11920	4.116
Total			1989135	100.000	289614	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/05-Lot20-Rep-3**
Vail #	**: 7**
Injection Volume	**: 20 uL**
Data File Name	**: Sample018.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 10:08:12 AM**
Data Processed	**: 4/3/2007 11:33:20 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample018.lcd



1 Det.A Ch1/227nm

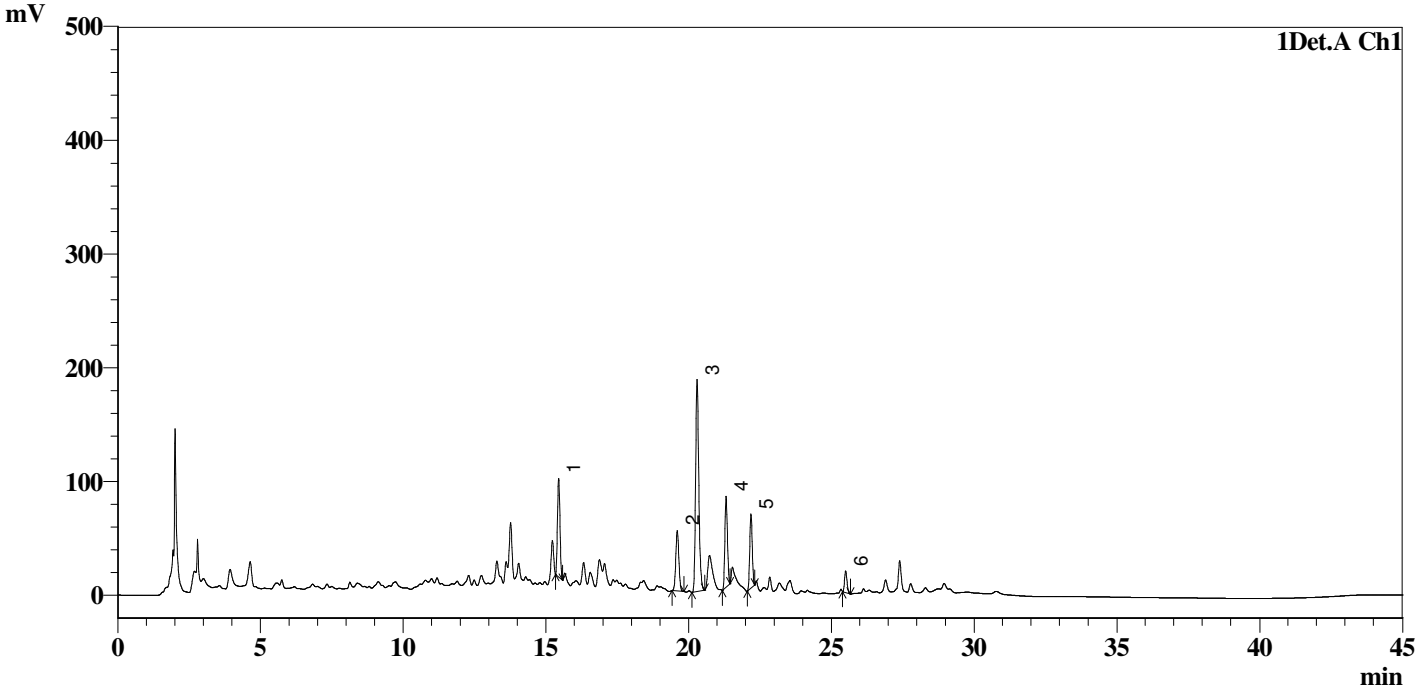
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.599	Withanoside IV	236488	12.264	39751	14.102
2	19.761	Withanoside V	178918	9.278	24299	8.621
3	20.446	Withaferin A	899663	46.654	117283	41.608
4	21.429	12-Deoxywithastramonolided	283582	14.706	45892	16.281
5	22.292	Withanolide A	260793	13.524	43022	15.263
6	25.579	Withanolide B	68927	3.574	11630	4.126
Total			1928372	100.000	281878	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/05-Lot21-Rep-1**
Vail #	**: 6**
Injection Volume	**: 20 uL**
Data File Name	**: Sample013.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 6:19:24 AM**
Data Processed	**: 4/3/2007 9:56:38 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample013.lcd



1 Det.A Ch1/227nm

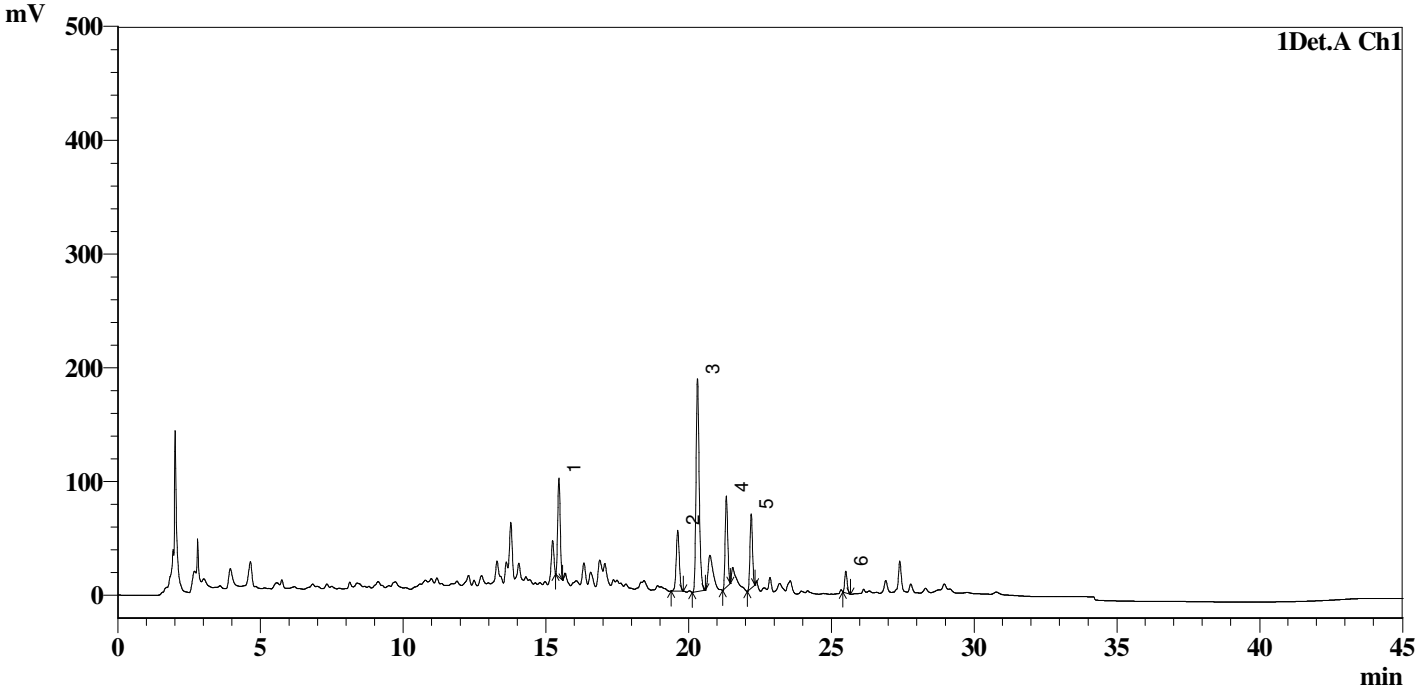
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.429	Withanoside IV	528920	15.668	86430	17.630
2	19.583	Withanoside V	391588	11.600	52945	10.800
3	20.276	Withaferin A	1438974	42.627	186541	38.051
4	21.291	12-Deoxywithastramonolide	504042	14.931	80052	16.329
5	22.163	Withanolide A	399966	11.848	65311	13.322
6	25.478	Withanolide B	112255	3.325	18958	3.867
Total			3375745	100.000	490237	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/05-Lot21-Rep-2**
Vail #	**: 6**
Injection Volume	**: 20 uL**
Data File Name	**: Sample014.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 7:05:09 AM**
Data Processed	**: 4/3/2007 9:58:27 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample014.lcd



1 Det.A Ch1/227nm

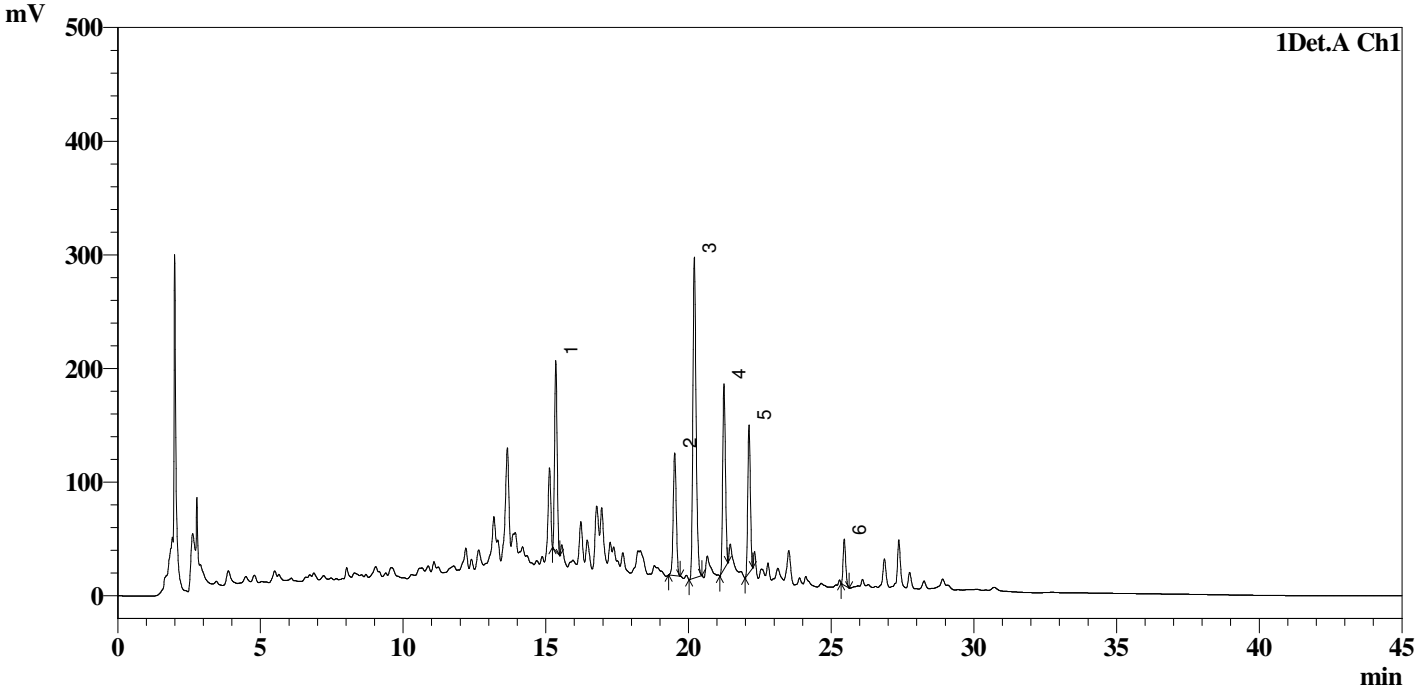
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.438	Withanoside IV	530024	15.666	86767	17.645
2	19.599	Withanoside V	396155	11.709	53298	10.839
3	20.290	Withaferin A	1440076	42.564	187030	38.034
4	21.302	12-Deoxywithastramonolide	503953	14.895	80281	16.326
5	22.171	Withanolide A	399905	11.820	65409	13.301
6	25.483	Withanolide B	113178	3.345	18957	3.855
Total			3383292	100.000	491741	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: WS/06-Lot 10-Rep-3**
Vail #	**: 2**
Injection Volume	**: 20 uL**
Data File Name	**: Sample003.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/2/2007 10:42:02 PM**
Data Processed	**: 4/3/2007 9:40:11 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample003.lcd



1 Det.A Ch1/227nm

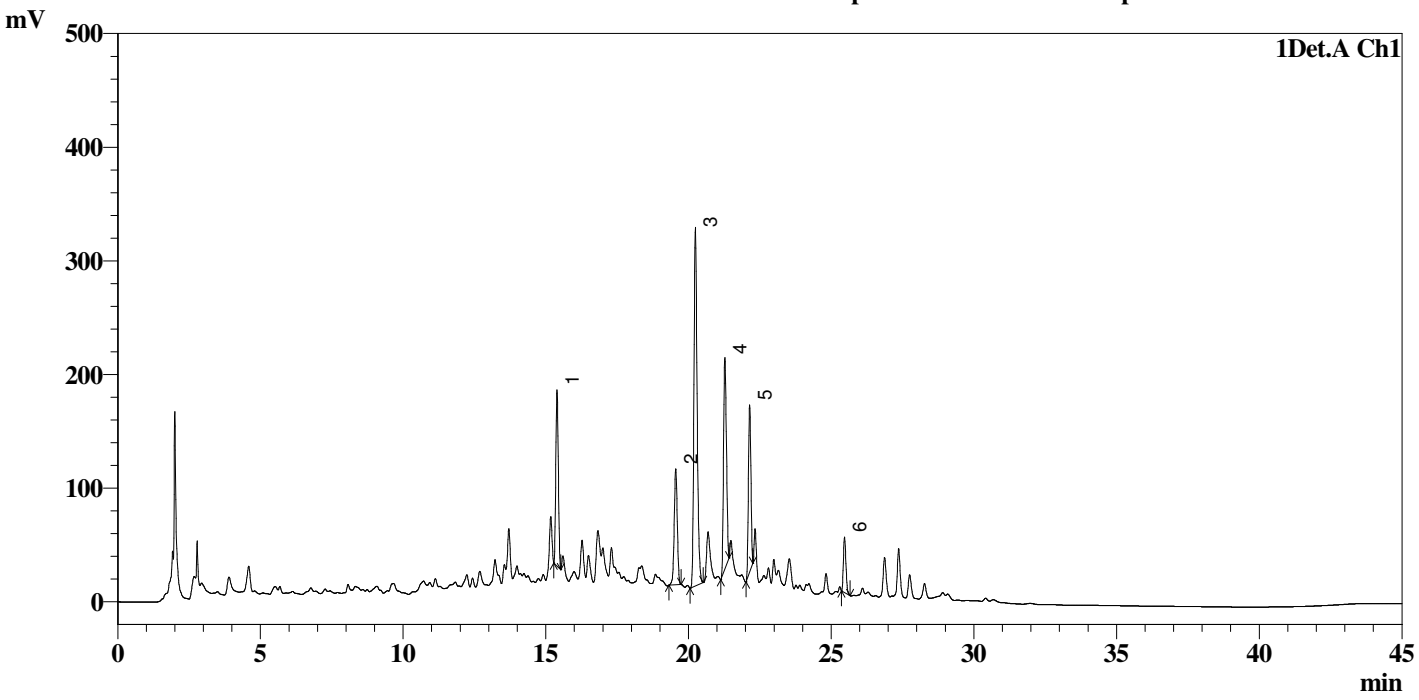
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.346	Withanoside IV	1017234	16.622	167594	18.792
2	19.514	Withanoside V	796155	13.009	107931	12.102
3	20.201	Withaferin A	2172974	35.506	282306	31.654
4	21.238	12-Deoxywithastramonolide	1078140	17.617	163313	18.312
5	22.116	Withanolide A	808865	13.217	130154	14.594
6	25.454	Withanolide B	246600	4.029	40545	4.546
Total			6119968	100.000	891843	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1170-Rep-1**
Vail #	**: 5**
Injection Volume	**: 20 uL**
Data File Name	**: Sample010.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 4:02:09 AM**
Data Processed	**: 4/3/2007 9:52:46 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample010.lcd



1 Det.A Ch1/227nm

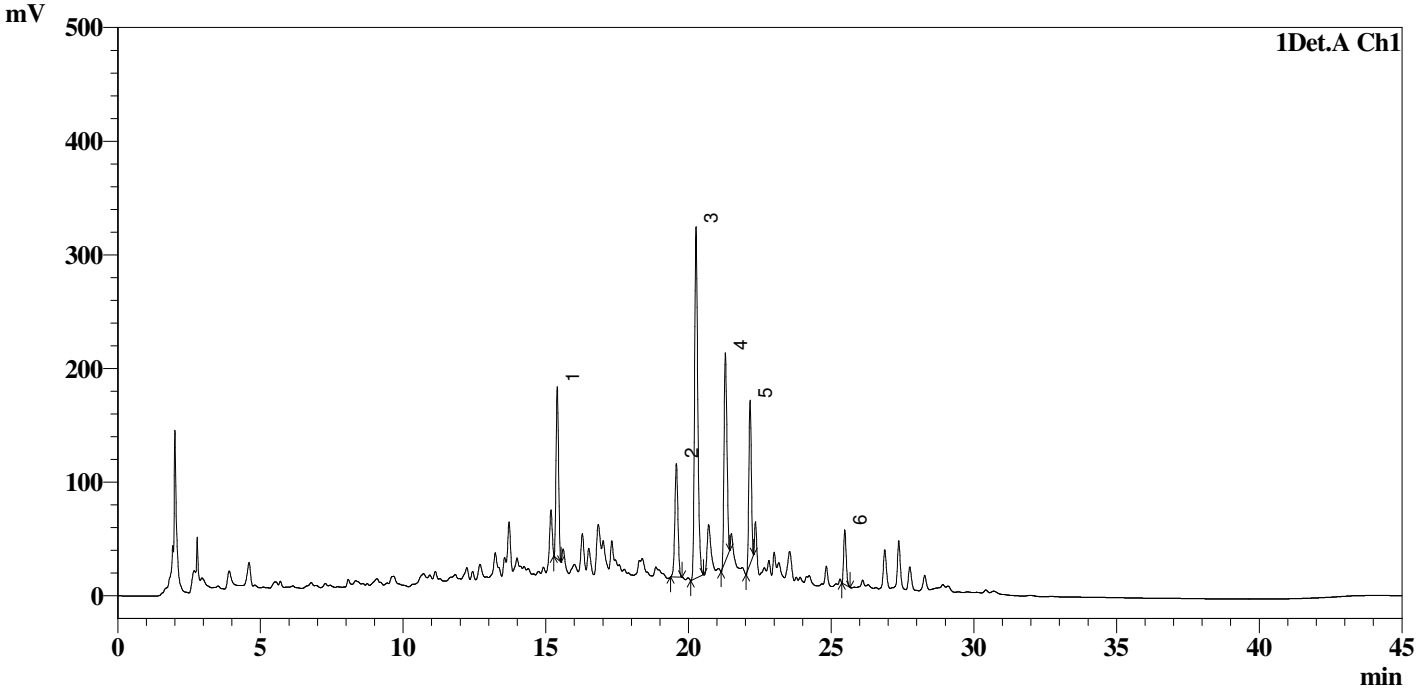
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.388	Withanoside IV	940690	14.082	155016	16.230
2	19.550	Withanoside V	759118	11.364	101878	10.667
3	20.237	Withaferin A	2448431	36.654	315482	33.031
4	21.269	12-Deoxywithastramonolide	1335639	19.995	186358	19.512
5	22.138	Withanolide A	896912	13.427	147509	15.444
6	25.463	Withanolide B	299140	4.478	48857	5.115
Total			6679931	100.000	955100	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1170-Rep-2**
Vail #	**: 5**
Injection Volume	**: 20 uL**
Data File Name	**: Sample011.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 4:47:54 AM**
Data Processed	**: 4/3/2007 9:54:05 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample011.lcd



1 Det.A Ch1/227nm

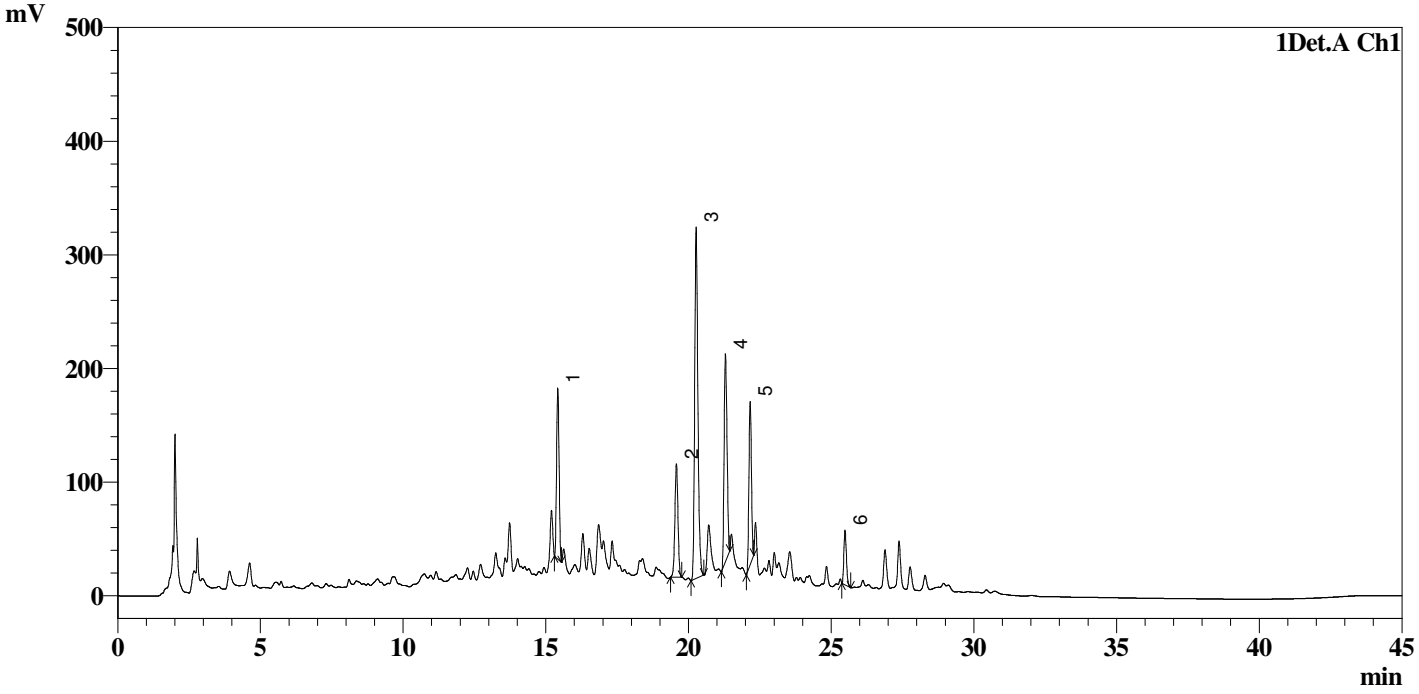
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.397	Withanoside IV	921873	14.084	151226	16.153
2	19.571	Withanoside V	737642	11.269	99713	10.651
3	20.259	Withaferin A	2408367	36.794	309147	33.022
4	21.287	12-Deoxywithastramonolide	1308480	19.990	183488	19.599
5	22.153	Withanolide A	874575	13.361	144423	15.427
6	25.472	Withanolide B	294693	4.502	48195	5.148
Total			6545629	100.000	936192	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1170-Rep-3**
Vail #	**: 5**
Injection Volume	**: 20 uL**
Data File Name	**: Sample012.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 5:33:40 AM**
Data Processed	**: 4/3/2007 9:55:31 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample012.lcd



1 Det.A Ch1/227nm

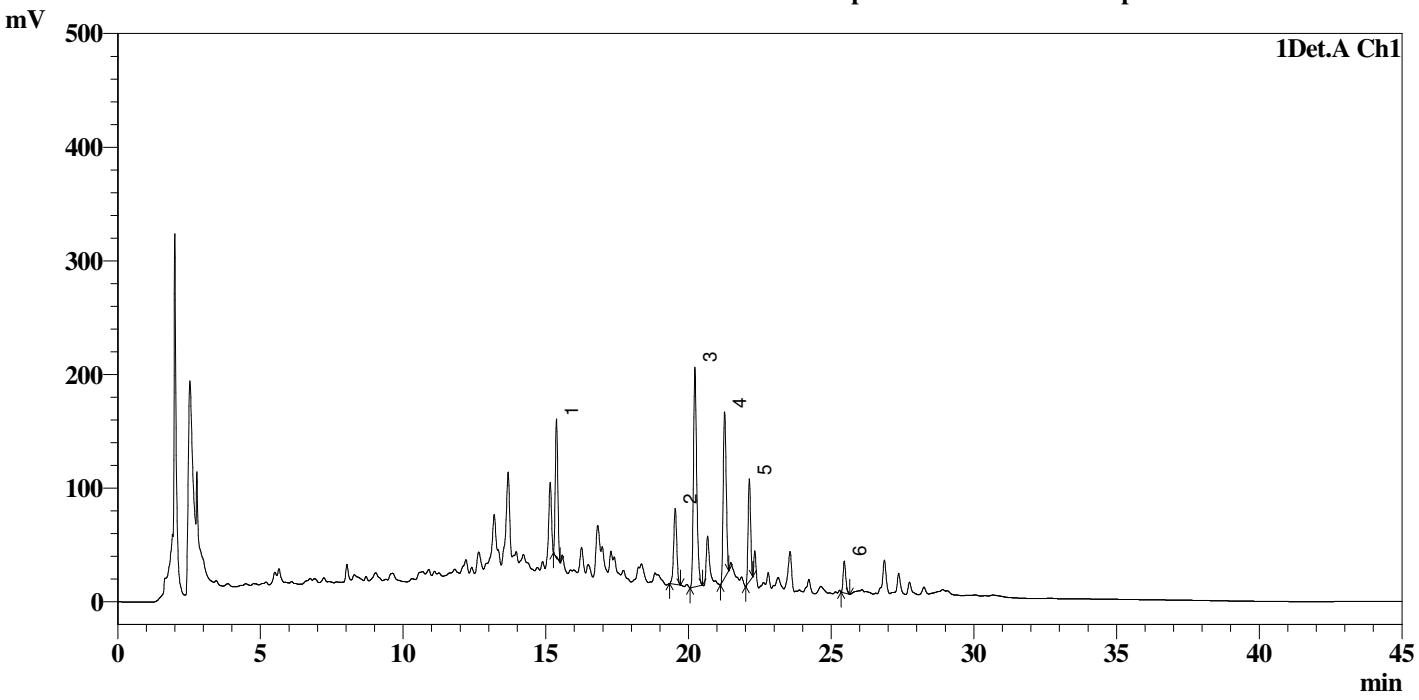
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.415	Withanoside IV	916504	14.030	150237	16.082
2	19.573	Withanoside V	738054	11.298	99686	10.671
3	20.263	Withaferin A	2401755	36.766	309264	33.106
4	21.290	12-Deoxywithastramonolide	1310057	20.054	183393	19.632
5	22.156	Withanolide A	872369	13.354	143701	15.383
6	25.480	Withanolide B	293807	4.498	47885	5.126
Total			6532546	100.000	934166	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1045-Rep-1**
Vail #	**: 4**
Injection Volume	**: 20 uL**
Data File Name	**: Sample007.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 1:44:59 AM**
Data Processed	**: 4/3/2007 9:48:54 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample007.lcd



1 Det.A Ch1/227nm

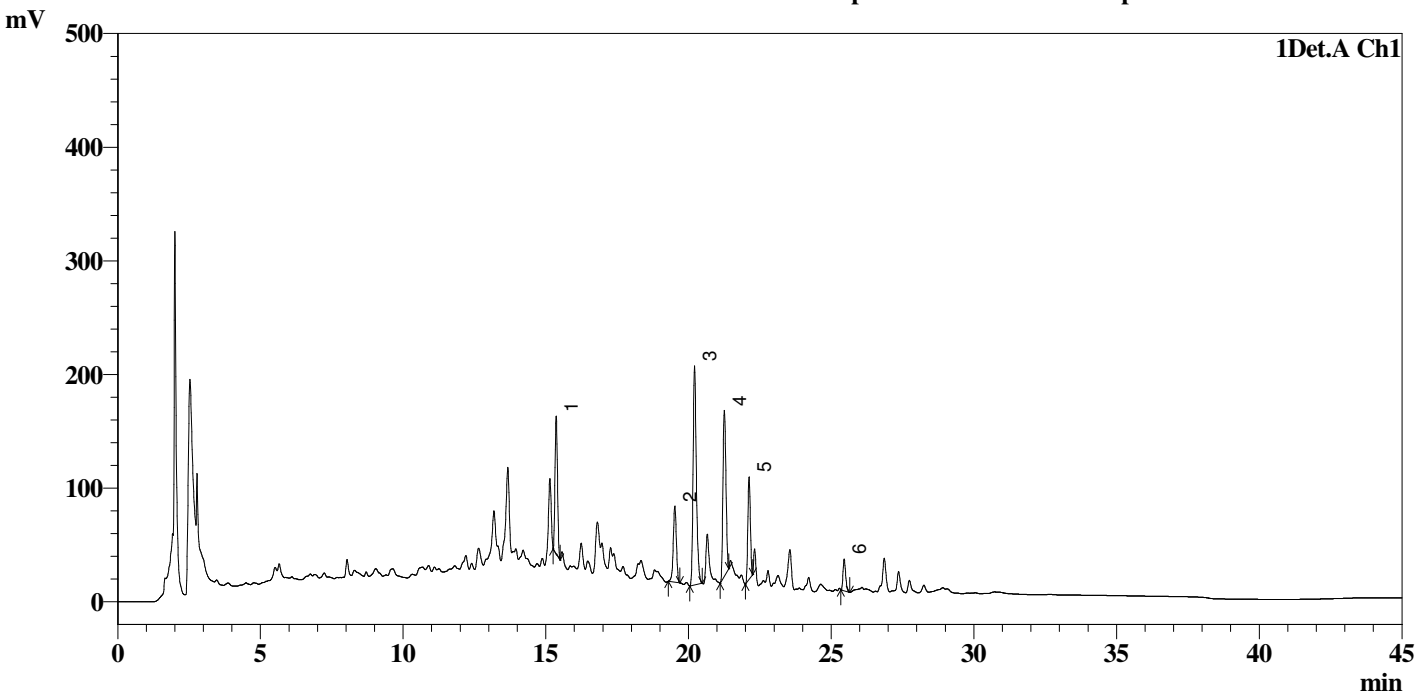
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.370	Withanoside IV	724917	15.941	121624	18.807
2	19.531	Withanoside V	497717	10.945	66647	10.306
3	20.221	Withaferin A	1518865	33.401	193343	29.897
4	21.259	12-Deoxywithastramonolide	1078101	23.708	146549	22.661
5	22.127	Withanolide A	549082	12.075	90425	13.983
6	25.455	Withanolide B	178746	3.931	28100	4.345
Total			4547427	100.000	646688	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1045-Rep-2**
Vail #	**: 4**
Injection Volume	**: 20 uL**
Data File Name	**: Sample008.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 2:30:41 AM**
Data Processed	**: 4/3/2007 9:50:01 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample008.lcd



1 Det.A Ch1/227nm

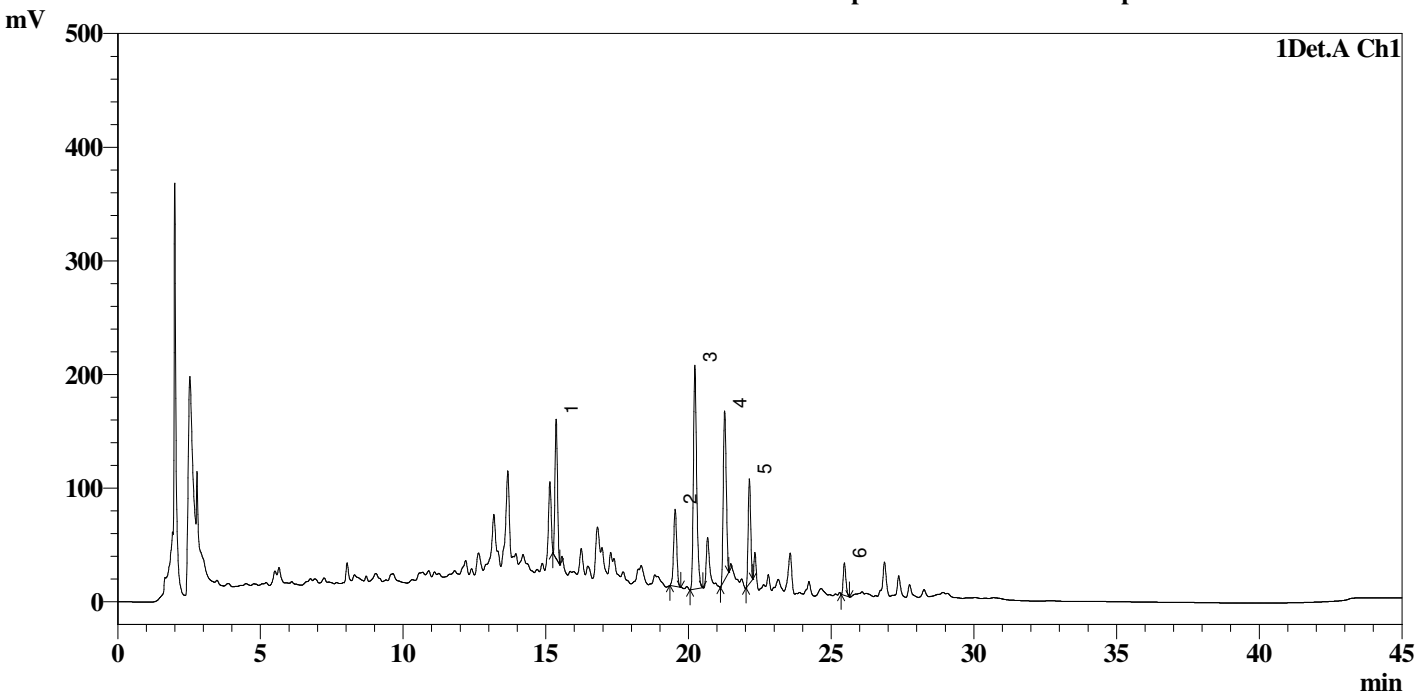
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.359	Withanoside IV	724690	15.910	120802	18.738
2	19.518	Withanoside V	504748	11.081	66864	10.372
3	20.208	Withaferin A	1520759	33.386	192663	29.885
4	21.250	12-Deoxywithastramonolide	1077210	23.649	145981	22.644
5	22.118	Withanolide A	549422	12.062	90379	14.019
6	25.450	Withanolide B	178247	3.913	27995	4.342
Total			4555075	100.000	644683	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera extract**
Sample ID	**: RD/1045-Rep-3**
Vail #	**: 4**
Injection Volume	**: 20 uL**
Data File Name	**: Sample009.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 3:16:25 AM**
Data Processed	**: 4/3/2007 9:51:26 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample009.lcd



1 Det.A Ch1/227nm

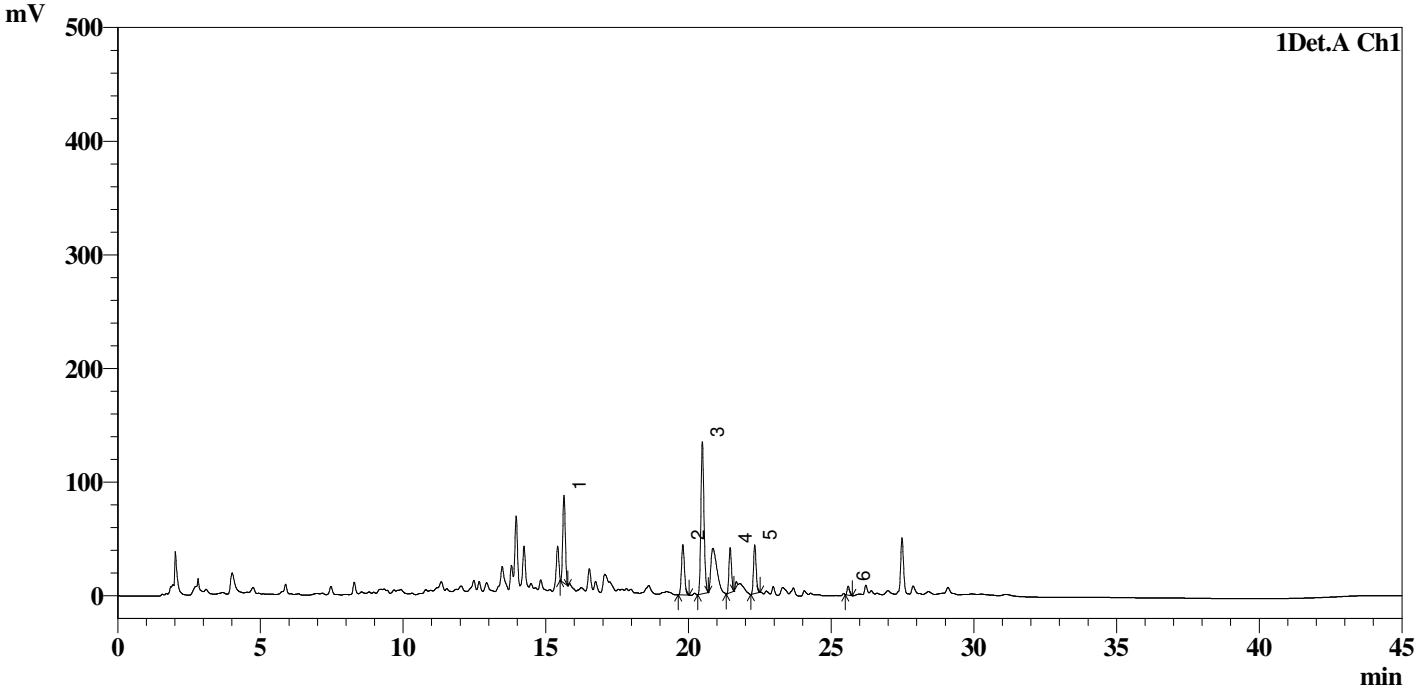
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.358	Withanoside IV	733458	15.865	122226	18.609
2	19.530	Withanoside V	504423	10.911	67752	10.315
3	20.219	Withaferin A	1548570	33.497	196992	29.993
4	21.261	12-Deoxywithastramonolide	1095396	23.694	149066	22.696
5	22.129	Withanolide A	559689	12.107	92307	14.054
6	25.459	Withanolide B	181483	3.926	28459	4.333
Total			4623020	100.000	656802	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: RD/1162-Rep-1**
Vail #	**: 8**
Injection Volume	**: 20 uL**
Data File Name	**: Sample019.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 10:53:55 AM**
Data Processed	**: 4/3/2007 1:05:38 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample019.lcd



1 Det.A Ch1/227nm

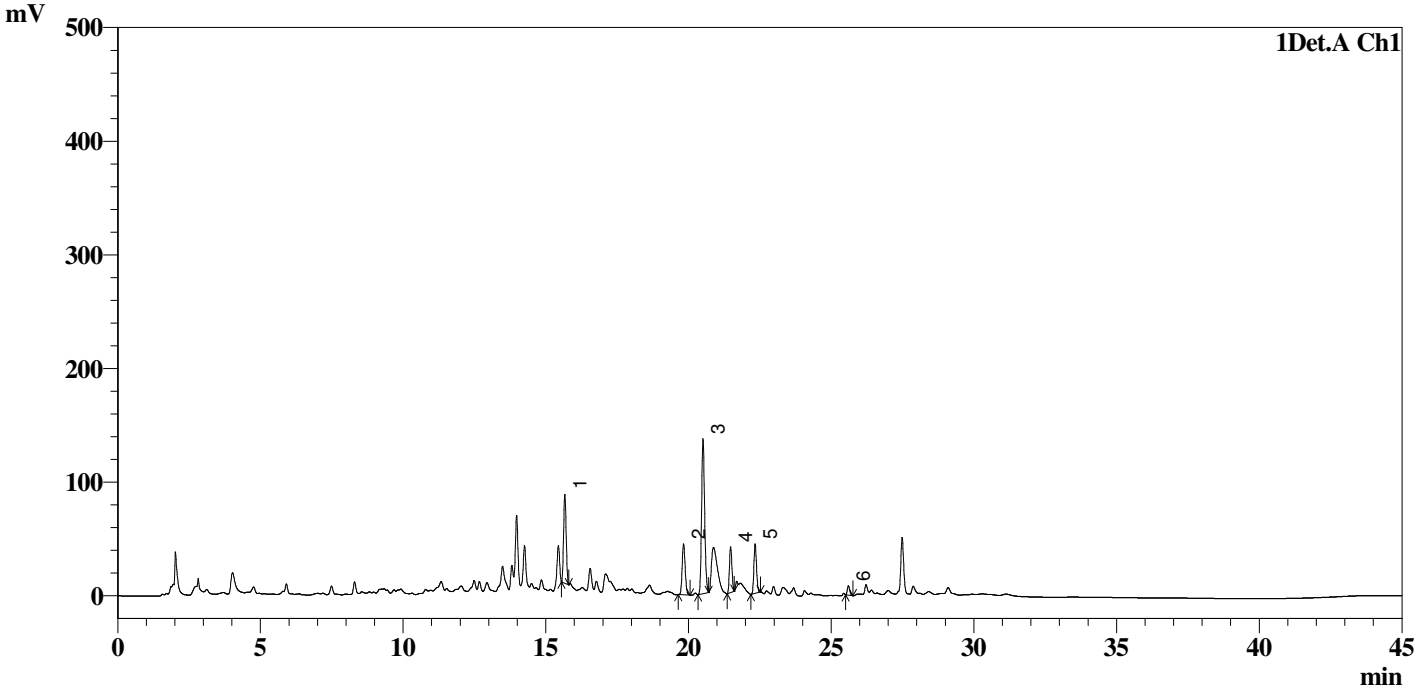
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.633	Withanoside IV	472663	20.155	77234	22.342
2	19.799	Withanoside V	322494	13.752	44080	12.751
3	20.482	Withaferin A	991174	42.266	133855	38.721
4	21.456	12-Deoxywithastramonolided	240327	10.248	39709	11.487
5	22.318	Withanolide A	271982	11.598	42925	12.417
6	25.592	Withanolide B	46463	1.981	7888	2.282
Total			2345102	100.000	345691	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: RD/1162-Rep-2**
Vail #	**: 8**
Injection Volume	**: 20 uL**
Data File Name	**: Sample020.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 11:39:39 AM**
Data Processed	**: 4/3/2007 1:07:06 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample020.lcd



1 Det.A Ch1/227nm

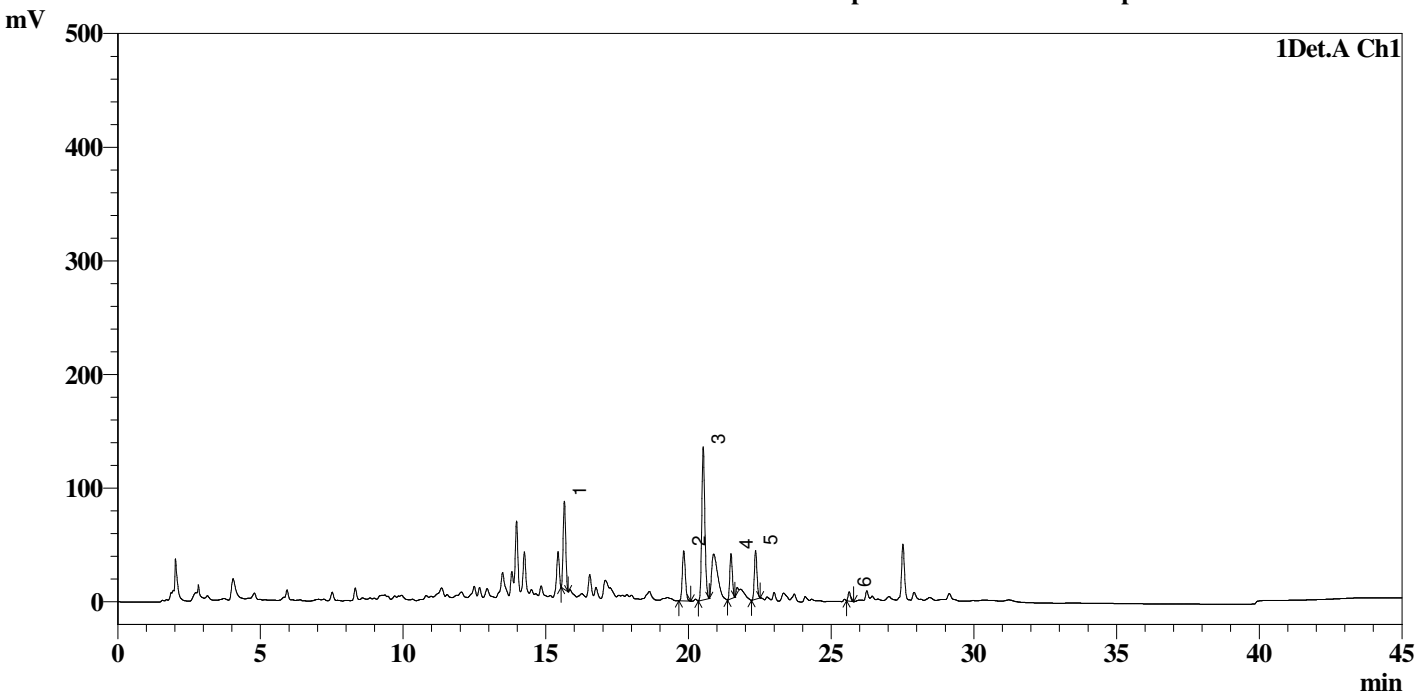
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.663	Withanoside IV	482797	20.270	78377	22.285
2	19.824	Withanoside V	327257	13.740	44710	12.712
3	20.505	Withaferin A	1004702	42.181	136637	38.849
4	21.472	12-Deoxywithastramonolided	244979	10.285	40310	11.461
5	22.331	Withanolide A	274521	11.525	43615	12.401
6	25.600	Withanolide B	47609	1.999	8061	2.292
Total			2381865	100.000	351711	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: RD/1162-Rep-3**
Vail #	**: 8**
Injection Volume	**: 20 uL**
Data File Name	**: Sample021.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 12:25:24 PM**
Data Processed	**: 4/3/2007 1:18:58 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample021.lcd



1 Det.A Ch1/227nm

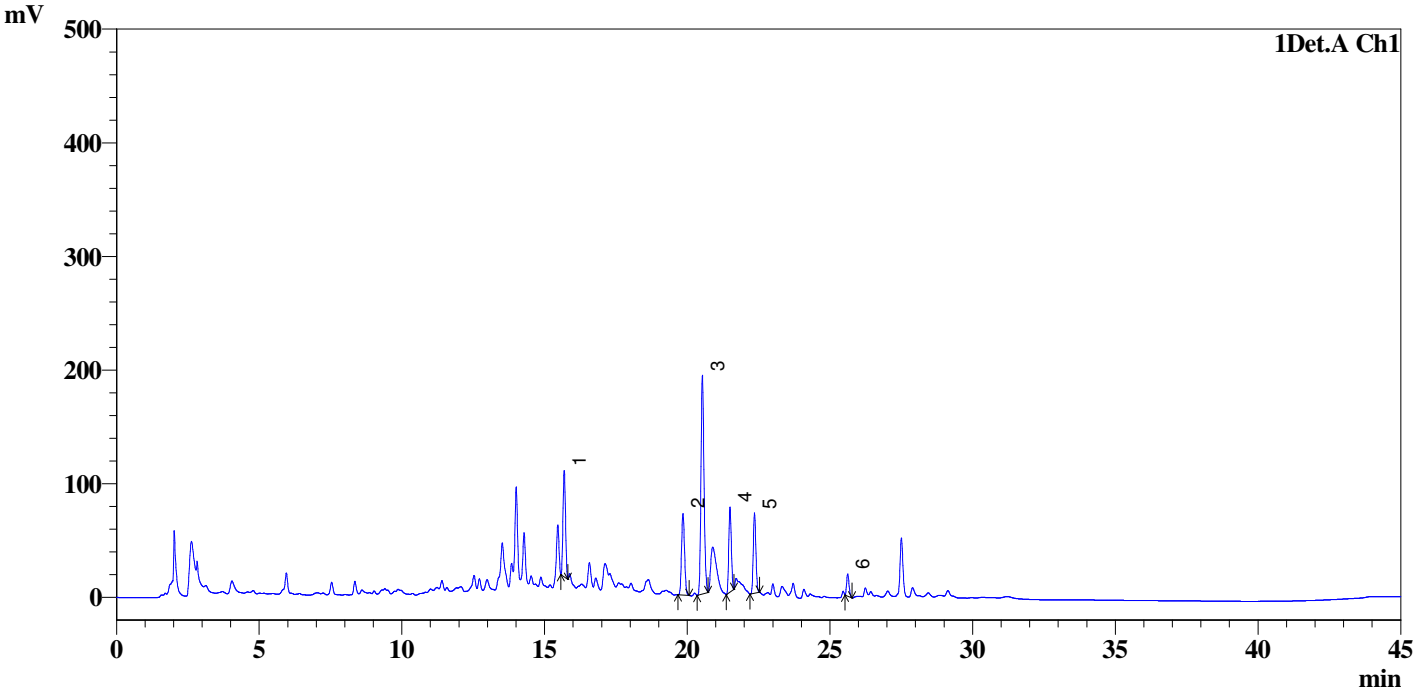
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.647	Withanoside IV	478280	20.332	77647	22.404
2	19.830	Withanoside V	324394	13.790	43988	12.692
3	20.512	Withaferin A	992429	42.189	134483	38.802
4	21.485	12-Deoxywithastramonolided	241618	10.271	39647	11.439
5	22.348	Withanolide A	268758	11.425	42854	12.365
6	25.627	Withanolide B	46860	1.992	7966	2.298
Total			2352339	100.000	346586	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: ERH/46-Rep-1**
Vail #	**: 9**
Injection Volume	**: 20 uL**
Data File Name	**: Sample022.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 1:11:17 PM**
Data Processed	**: 4/3/2007 4:06:57 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample022.lcd



1 Det.A Ch1/227nm

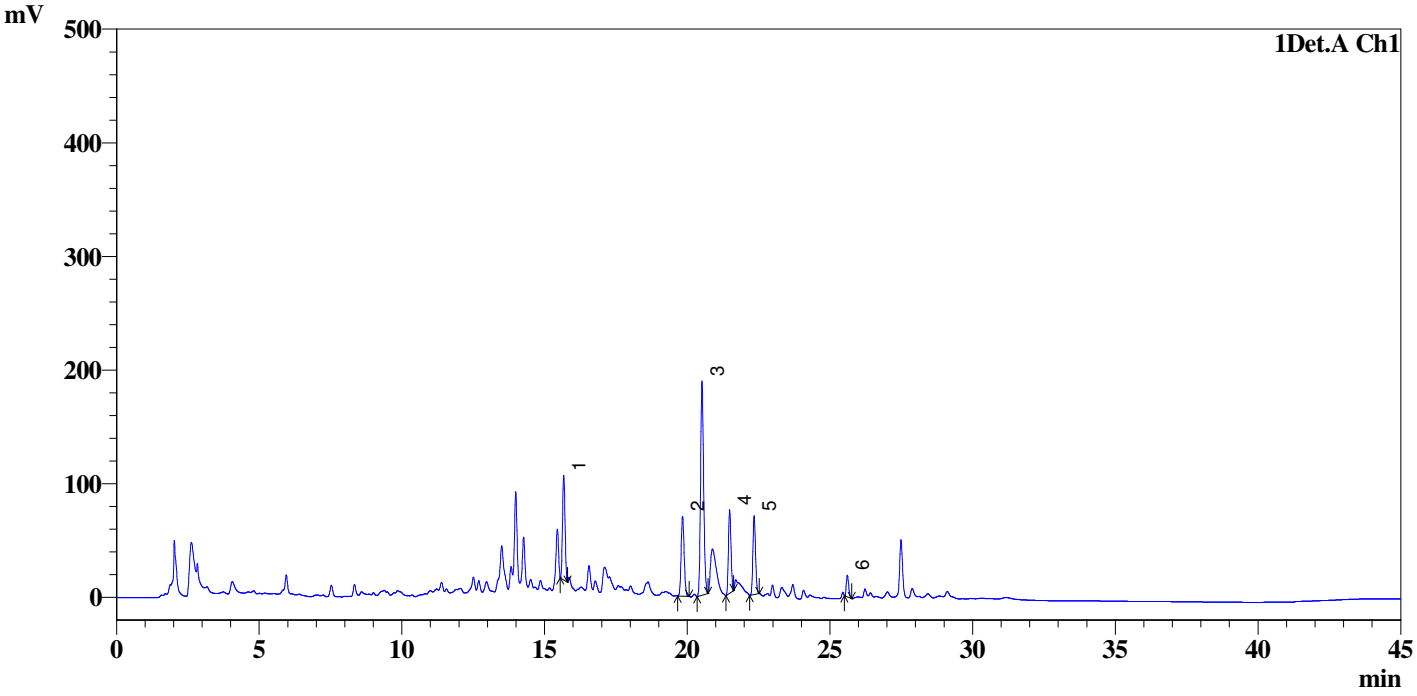
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.684	Withanoside IV	570039	16.120	93440	17.877
2	19.847	Withanoside V	525510	14.861	71709	13.719
3	20.526	Withaferin A	1419399	40.139	192581	36.844
4	21.493	12-Deoxywithastramonolided	461412	13.048	74799	14.310
5	22.352	Withanolide A	447130	12.644	70805	13.546
6	25.619	Withanolide B	112751	3.188	19361	3.704
Total			3536241	100.000	522697	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: ERH/46-Rep-2**
Vail #	**: 9**
Injection Volume	**: 20 uL**
Data File Name	**: Sample023.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 1:57:03 PM**
Data Processed	**: 4/3/2007 4:22:20 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample023.lcd



1 Det.A Ch1/227nm

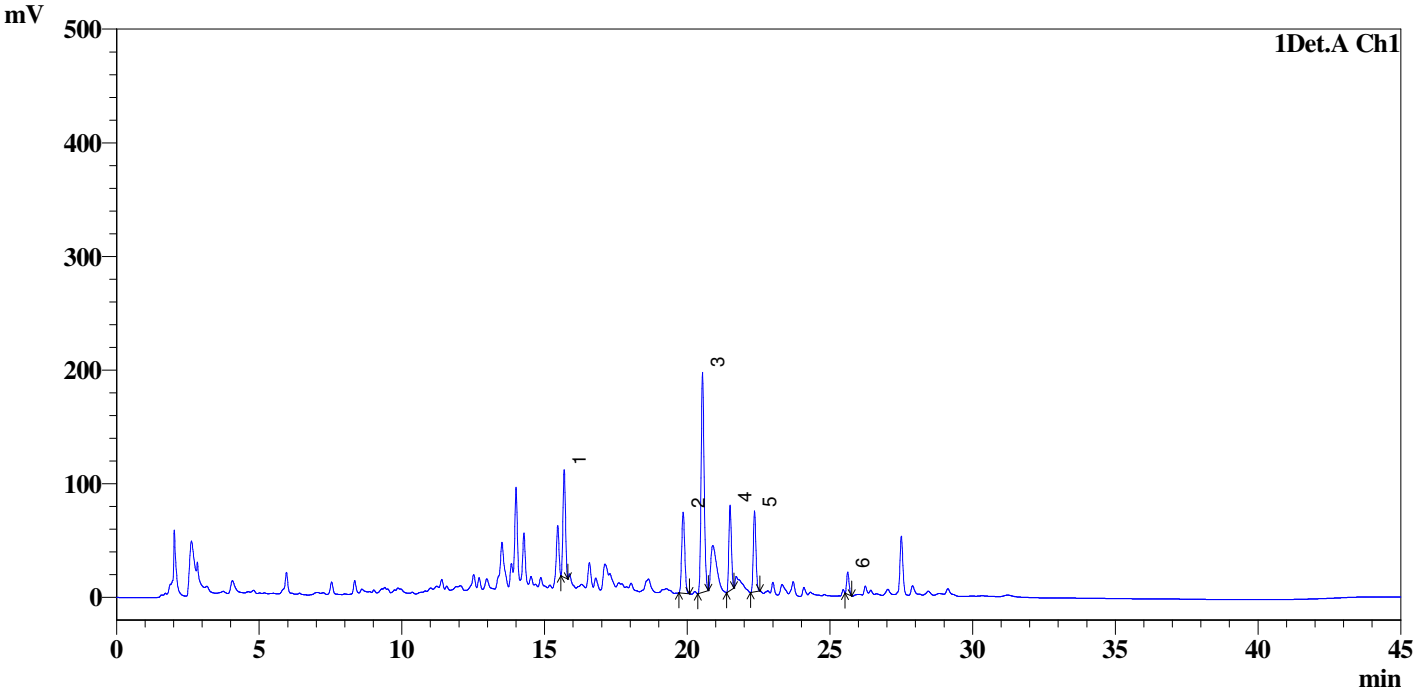
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.668	Withanoside IV	558638	16.083	92115	17.959
2	19.832	Withanoside V	516427	14.868	70260	13.698
3	20.513	Withaferin A	1395678	40.182	188665	36.782
4	21.481	12-Deoxy withastarmonolide	454409	13.083	73595	14.348
5	22.339	Withanolide A	438443	12.623	69373	13.525
6	25.607	Withanolide B	109782	3.161	18915	3.688
Total			3473378	100.000	512923	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera roots**
Sample ID	**: ERH/46-Rep-3**
Vail #	**: 9**
Injection Volume	**: 20 uL**
Data File Name	**: Sample024.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch-01.lcb**
Report File Name	**: DetA-Ch1_Ch2 - Dual.lcr**
Data Acquired	**: 4/3/2007 2:42:46 PM**
Data Processed	**: 4/3/2007 4:23:25 PM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\April 2007\02.04.2007\Sample024.lcd



1 Det.A Ch1/227nm

Detector A Ch1 227nm

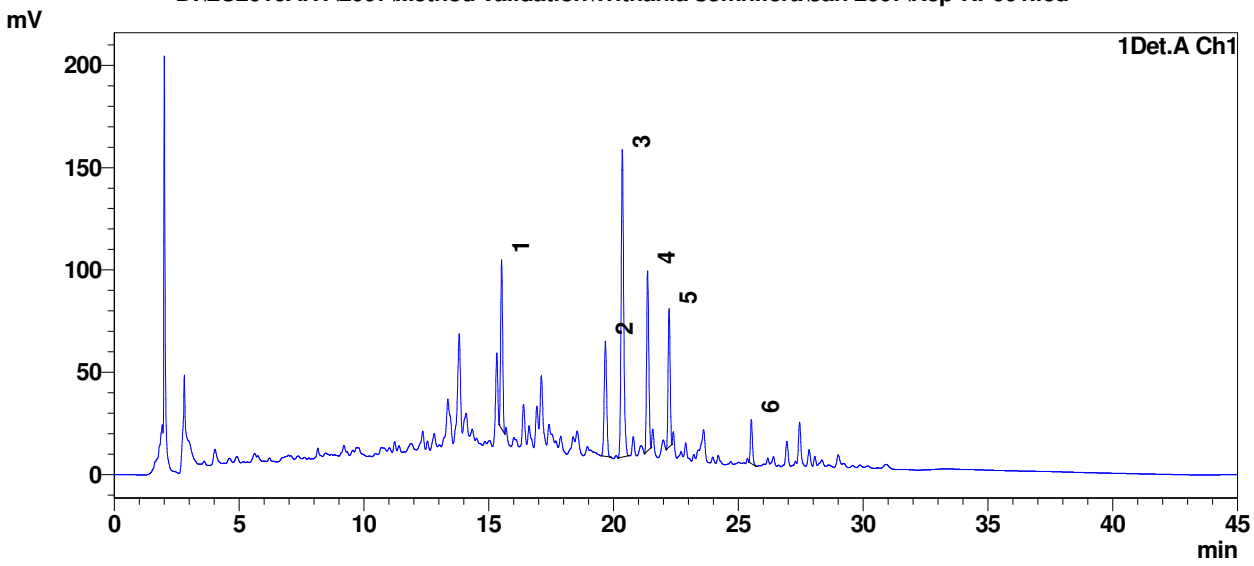
Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.684	Withanoside IV	571836	16.148	94296	17.964
2	19.853	Withanoside V	522247	14.747	71351	13.593
3	20.531	Withaferin A	1420722	40.119	193535	36.870
4	21.495	12-Deoxy withastarmonolide	464162	13.107	75230	14.332
5	22.353	Withanolide A	449853	12.703	71082	13.542
6	25.620	Withanolide B	112449	3.175	19415	3.699
Total			3541268	100.000	524908	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 1
Vail #	: 30
Injection Volume	: 20 uL
Data File Name	: Rep-RP001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 1:33:29 AM
Data Processed	: 1/23/2007 3:08:13 AM

Enclosure: 09

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Rep-RP001.lcd



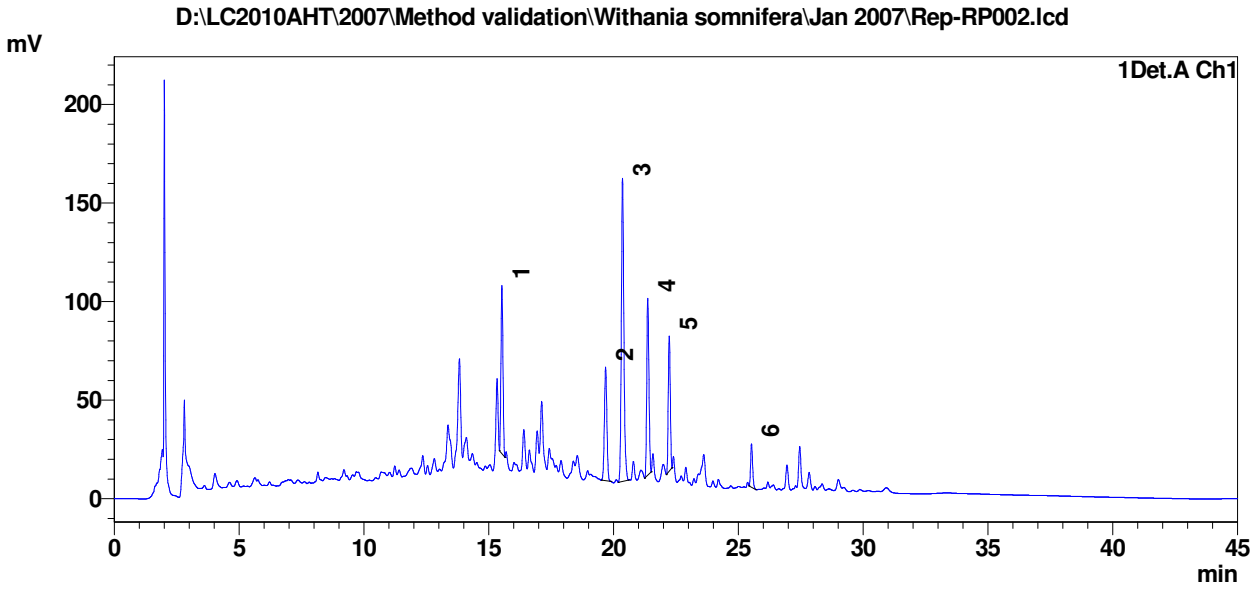
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.517	465764	82453	15.763	Withanoside IV
2	19.676	391249	56273	13.241	Withanoside V
3	20.354	1068054	150253	36.147	Withaferin A
4	21.366	533845	87827	18.067	12- Deoxy withastramonolide
5	22.228	376121	67629	12.729	Withanollide A
6	25.522	119712	21438	4.052	Withanolide B
Total		2954744	465874	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 2
Vail #	: 31
Injection Volume	: 20 uL
Data File Name	: Rep-RP002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 2:19:13 AM
Data Processed	: 1/23/2007 3:09:21 AM



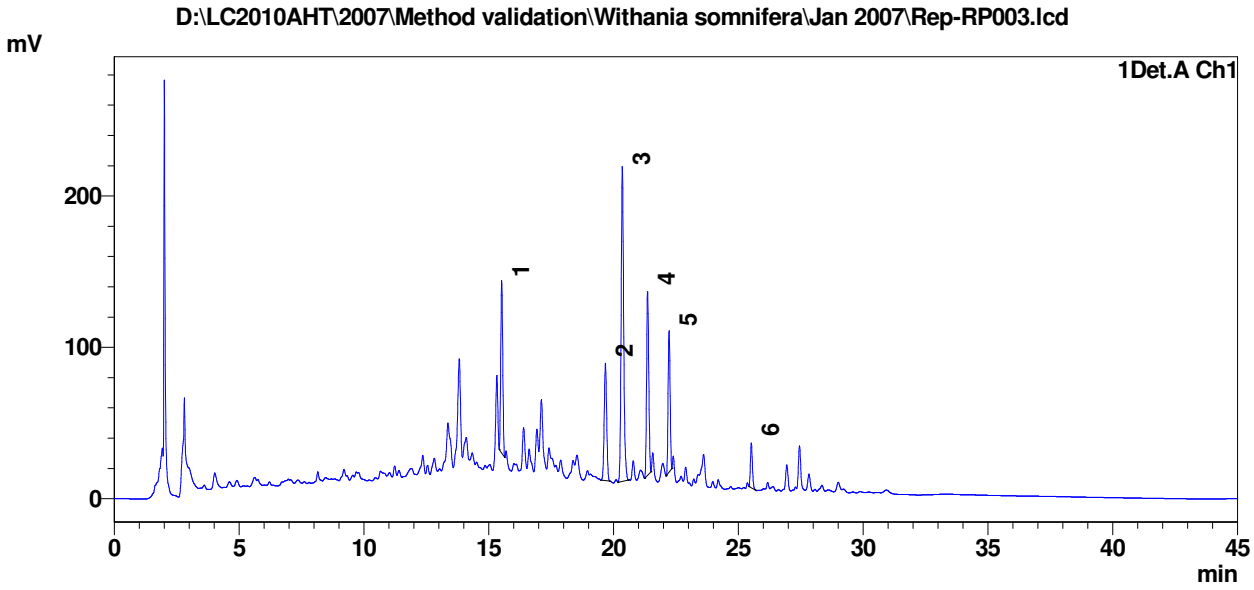
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.529	478028	85286	15.819	Withanoside IV
2	19.684	401702	57571	13.294	Withanoside V
3	20.362	1091994	153766	36.138	Withaferin A
4	21.373	545128	89813	18.040	12- Deoxy withastramonolide
5	22.234	382010	68586	12.642	Withanollide A
6	25.530	122902	21932	4.067	Withanolide B
Total		3021764	476953	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1000 mg / 100 ml : Trl 1
Vail #	: 32
Injection Volume	: 20 uL
Data File Name	: Rep-RP003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 3:04:58 AM
Data Processed	: 1/23/2007 3:10:30 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.521	644576	114102	15.768	Withanoside IV
2	19.676	539952	77457	13.208	Withanoside V
3	20.354	1477658	208029	36.147	Withaferin A
4	21.366	739704	121587	18.095	12- Deoxy withastramonolide
5	22.227	520470	93477	12.732	Withanollide A
6	25.522	165582	29605	4.050	Withanolide B
Total		4087942	644257	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1000 mg / 100 ml : Trl 2
Vail #	: 33
Injection Volume	: 20 uL
Data File Name	: Rep-RP004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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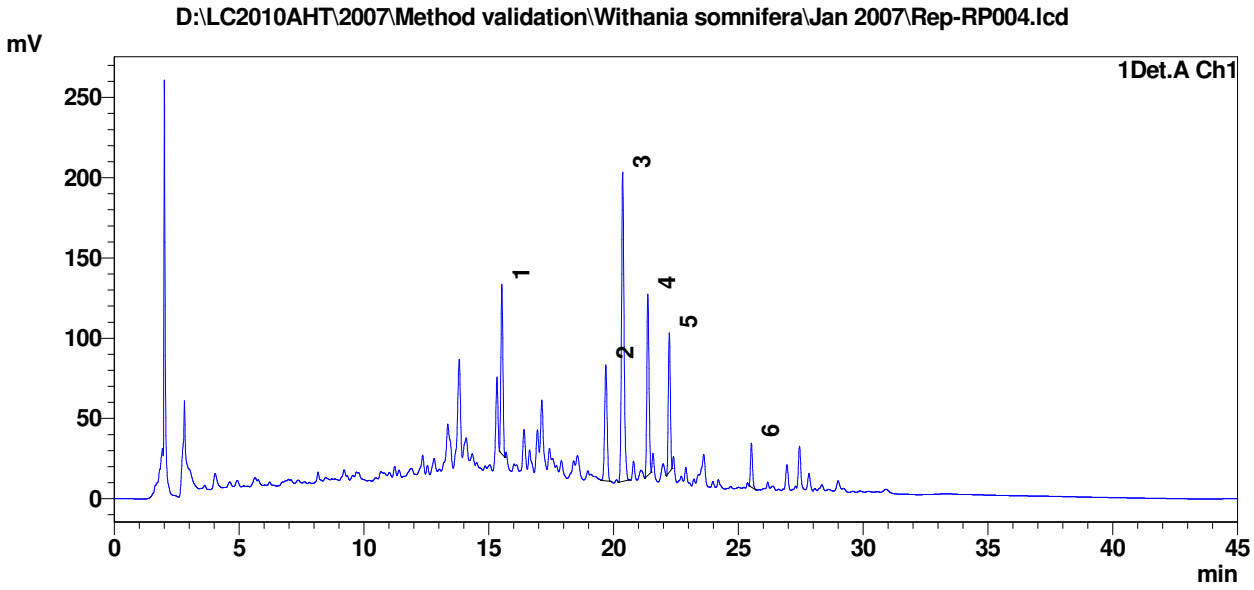

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.528	597742	105658	15.797	Withanoside IV
2	19.694	503383	72291	13.304	Withanoside V
3	20.369	1365742	192602	36.095	Withaferin A
4	21.377	683044	112832	18.052	12- Deoxy withastramonolide
5	22.236	481718	86757	12.731	Withanollide A
6	25.525	152164	27293	4.021	Withanolide B
Total		3783792	597433	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 1
Vail #	: 34
Injection Volume	: 20 uL
Data File Name	: Rep-RP005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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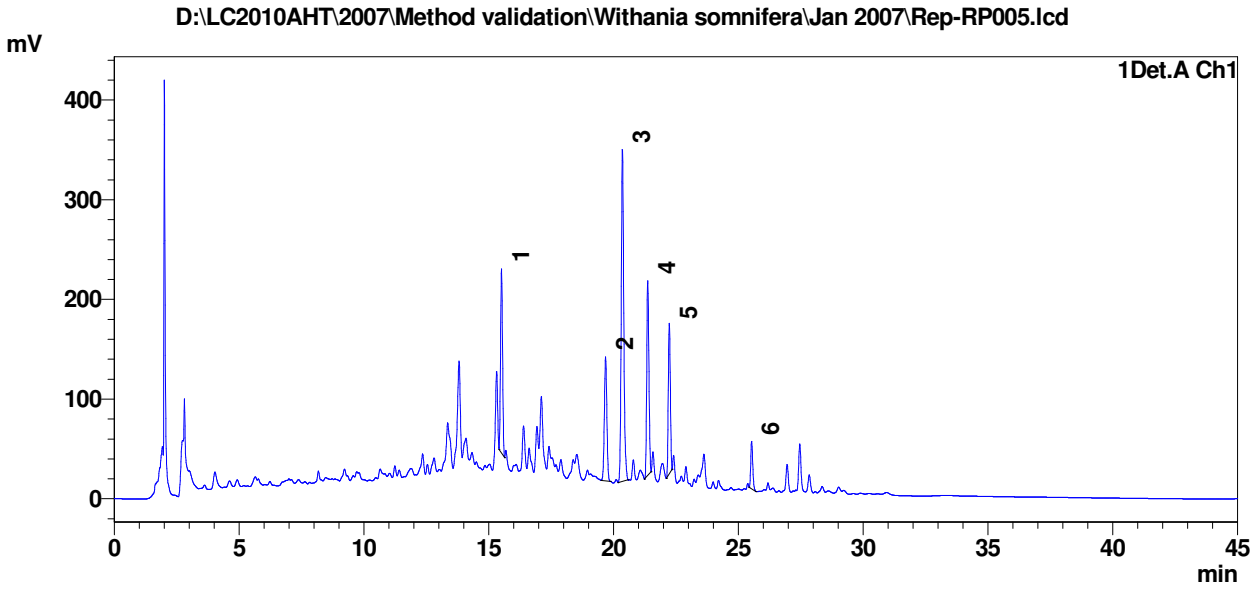
Data Processed	: 1/23/2007 3:13:11 AM



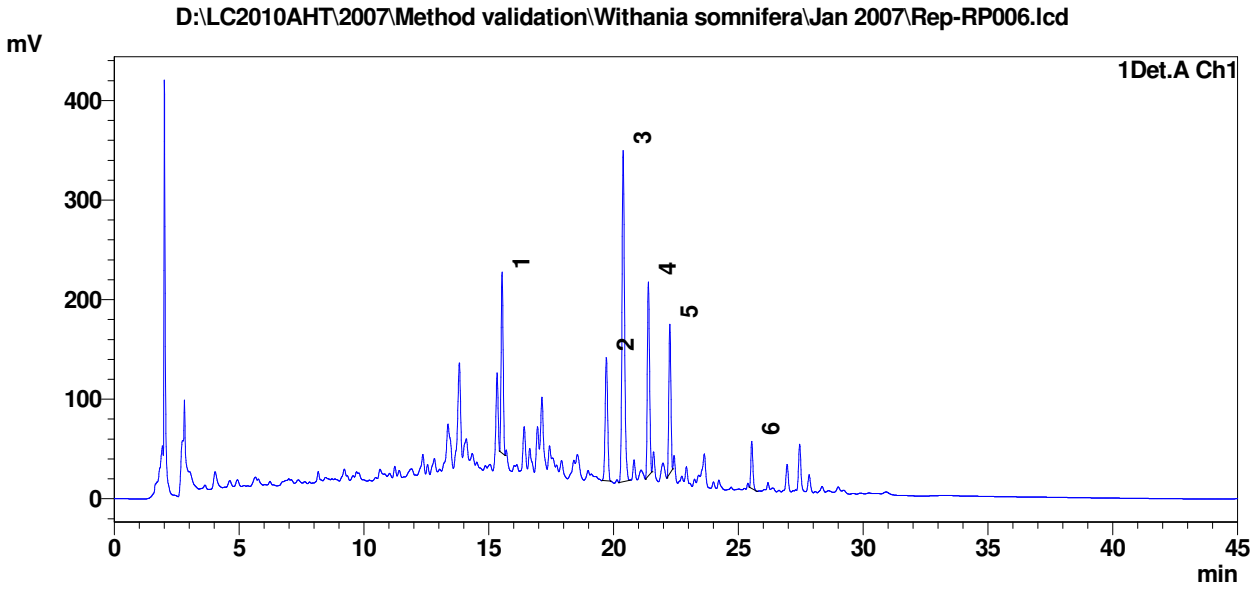
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.513	1042497	184367	15.831	Withanoside IV
2	19.683	869310	124386	13.201	Withanoside V
3	20.358	2368568	332886	35.969	Withaferin A
4	21.372	1186938	195375	18.025	12- Deoxy withastramonolide
5	22.236	851674	151330	12.933	Withanollide A
6	25.535	266074	47554	4.041	Withanolide B
Total		6585062	1035898	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 2
Vail #	: 35
Injection Volume	: 20 uL
Data File Name	: Rep-RP006.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 5:22:10 AM
Data Processed	: 1/23/2007 3:14:41 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.537	1029071	181569	15.729	Withanoside IV
2	19.715	860618	123678	13.154	Withanoside V
3	20.388	2362250	332899	36.107	Withaferin A
4	21.398	1182463	194673	18.074	12- Deoxy withastramonolide
5	22.257	843789	150165	12.897	Withanollide A
6	25.540	264250	47318	4.039	Withanolide B
Total		6542442	1030303	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 1
Vail #	: 36
Injection Volume	: 20 uL
Data File Name	: Rep-RP007.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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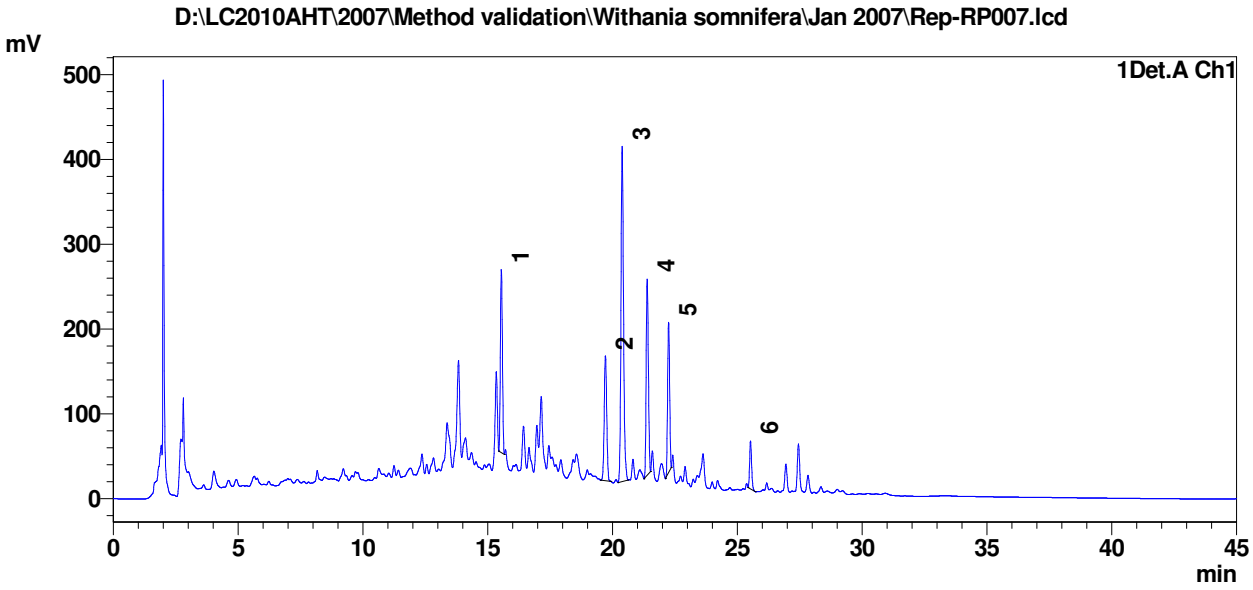
Data Processed	: 1/23/2007 3:15:45 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.548	1220186	215604	15.758	Withanoside IV
2	19.717	1028111	147371	13.277	Withanoside V
3	20.388	2795043	395277	36.096	Withaferin A
4	21.391	1401887	231397	18.105	12- Deoxy withastramonolide
5	22.248	983304	177119	12.699	Withanollide A
6	25.530	314770	56268	4.065	Withanolide B
Total		7743301	1223036	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 2
Vail #	: 37
Injection Volume	: 20 uL
Data File Name	: Rep-RP008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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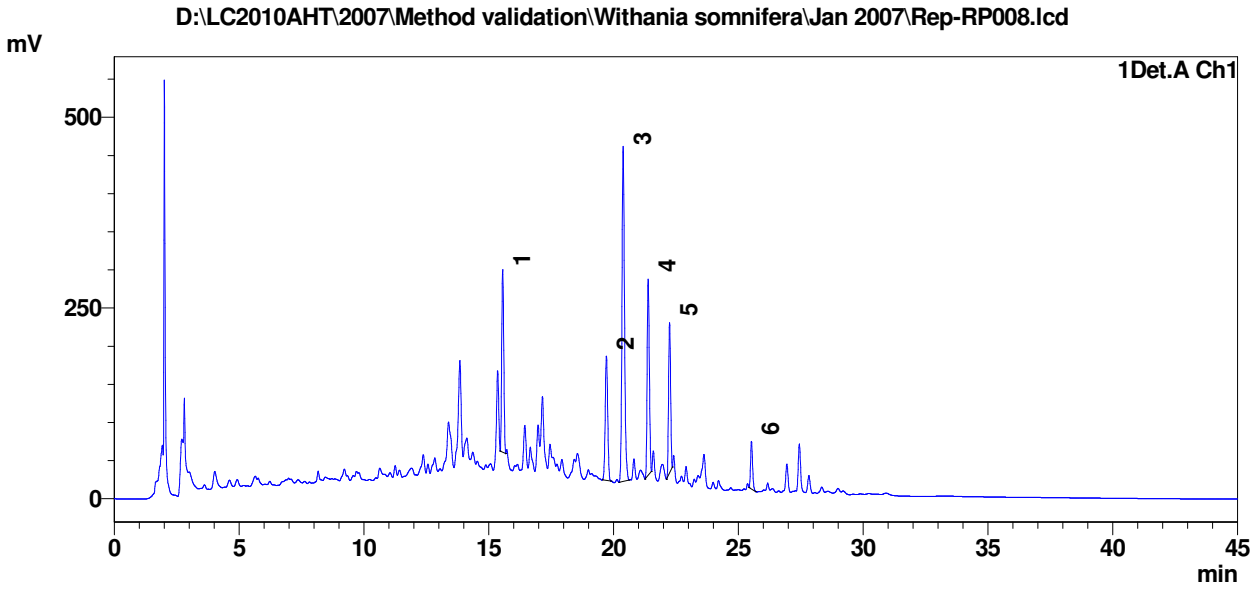
Data Processed	: 1/23/2007 3:16:49 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.561	1352797	239294	15.752	Withanoside IV
2	19.717	1130441	162804	13.163	Withanoside V
3	20.387	3101015	439045	36.108	Withaferin A
4	21.390	1556432	257206	18.123	12- Deoxy withastramonolide
5	22.247	1097660	197192	12.781	Withanollide A
6	25.528	349933	62551	4.075	Withanolide B
Total		8588278	1358092	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2500 mg / 100 ml : Trl 1
Vail #	: 38
Injection Volume	: 20 uL
Data File Name	: Rep-RP009.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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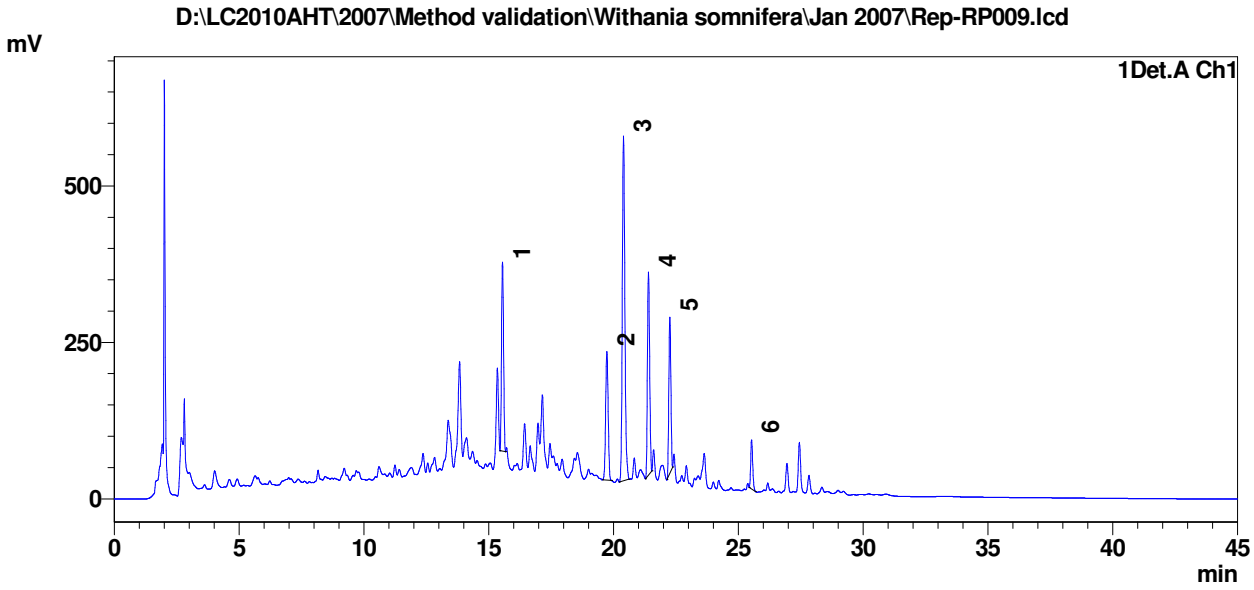

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.554	1707820	301723	15.750	Withanoside IV
2	19.735	1430482	205609	13.192	Withanoside V
3	20.402	3896838	550971	35.938	Withaferin A
4	21.401	1970705	324628	18.175	12- Deoxy withastramonolide
5	22.258	1397803	249659	12.891	Withanollide A
6	25.534	439557	78451	4.054	Withanolide B
Total		10843205	1711041	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2500 mg / 100 ml : Trl 2
Vail #	: 39
Injection Volume	: 20 uL
Data File Name	: Rep-RP010.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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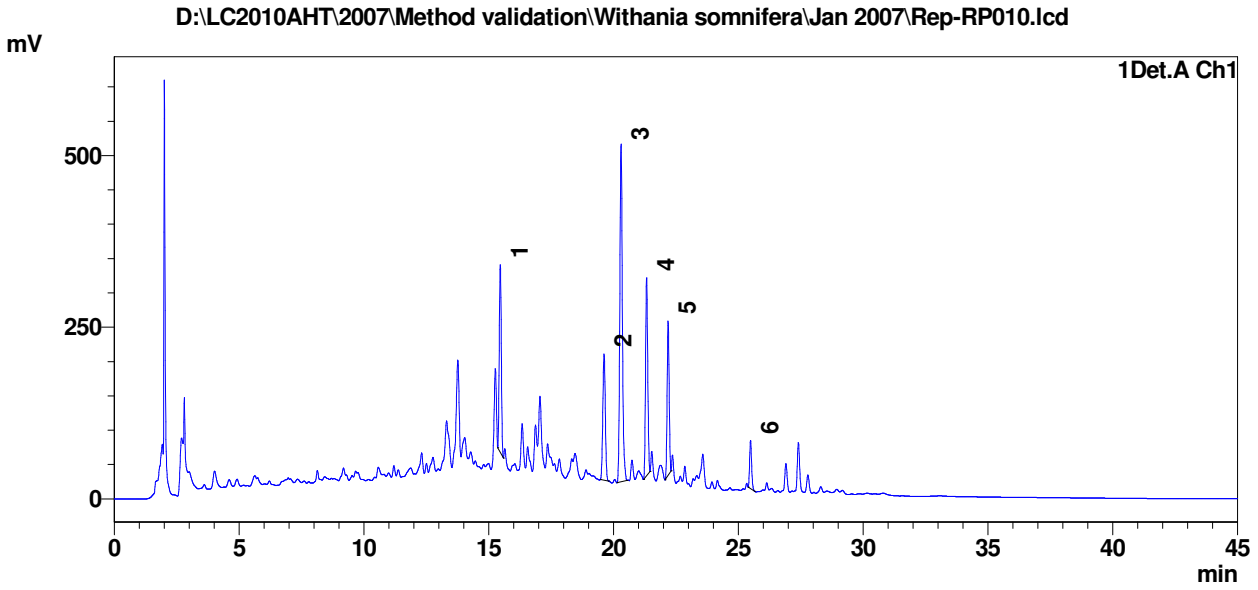

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.463	1546771	272883	15.837	Withanoside IV
2	19.621	1271665	183718	13.020	Withanoside V
3	20.302	3508268	491917	35.919	Withaferin A
4	21.325	1767374	288311	18.095	12- Deoxy withastramonolide
5	22.189	1279368	225079	13.099	Withanollide A
6	25.490	393606	70117	4.030	Withanolide B
Total		9767052	1532025	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 1
Vail #	: 43
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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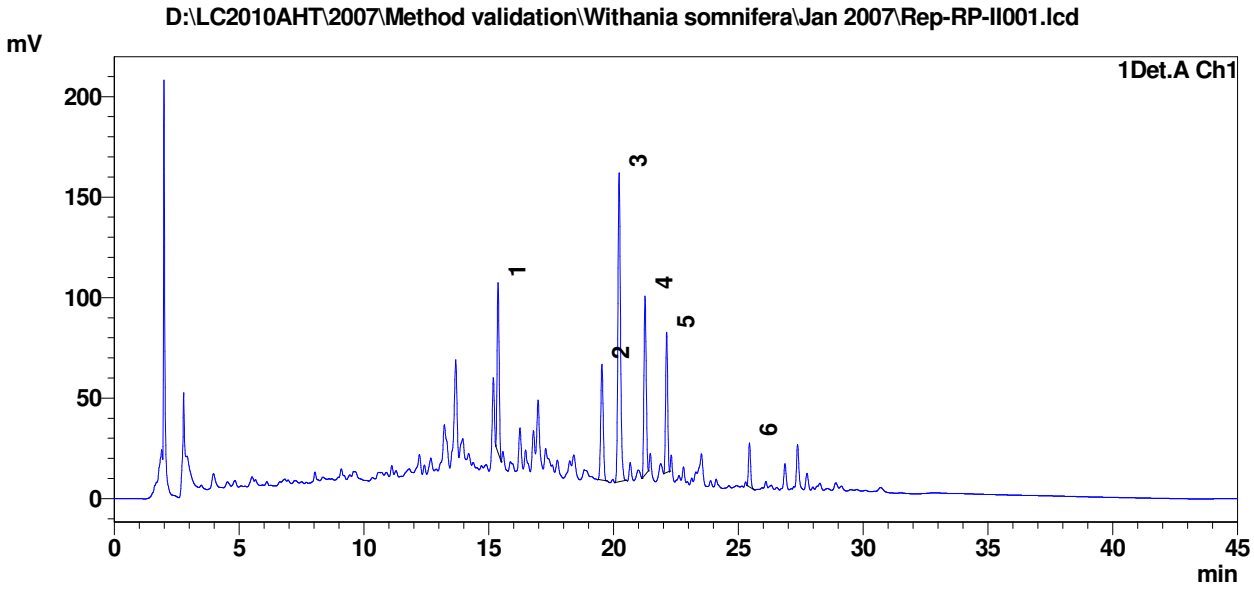

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.376	478462	84557	15.728	Withanoside IV
2	19.537	398109	57593	13.087	Withanoside V
3	20.226	1099263	153652	36.136	Withaferin A
4	21.263	548663	88994	18.036	12- Deoxy withastramonolide
5	22.128	394764	69732	12.977	Withanollide A
6	25.447	122771	21895	4.036	Withanolide B
Total		3042031	476423	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 750 mg / 100 ml :Trl 2
Vail #	: 44
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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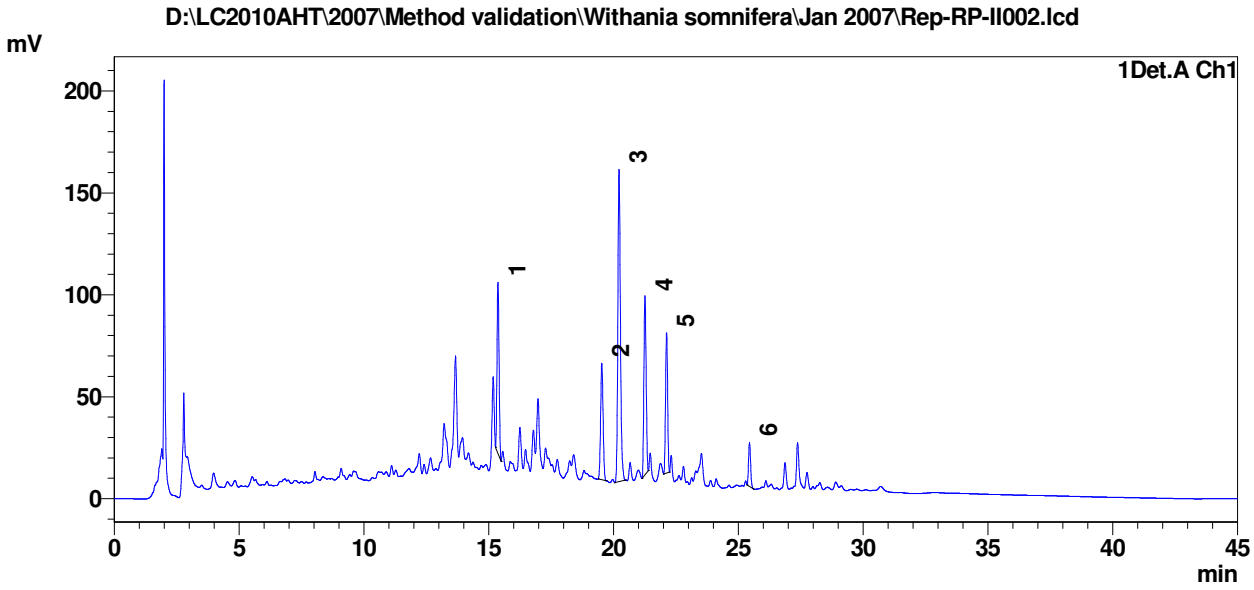
Data Processed	: 1/23/2007 2:57:48 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.372	479376	84003	15.865	Withanoside IV
2	19.532	394834	57054	13.067	Withanoside V
3	20.223	1091327	153105	36.119	Withaferin A
4	21.260	541636	87896	17.926	12- Deoxy withastramonolide
5	22.126	393611	68735	13.027	Withanollide A
6	25.448	120722	21488	3.995	Withanolide B
Total		3021506	472281	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1000 mg / 100 ml : Trl 1
Vail #	: 45
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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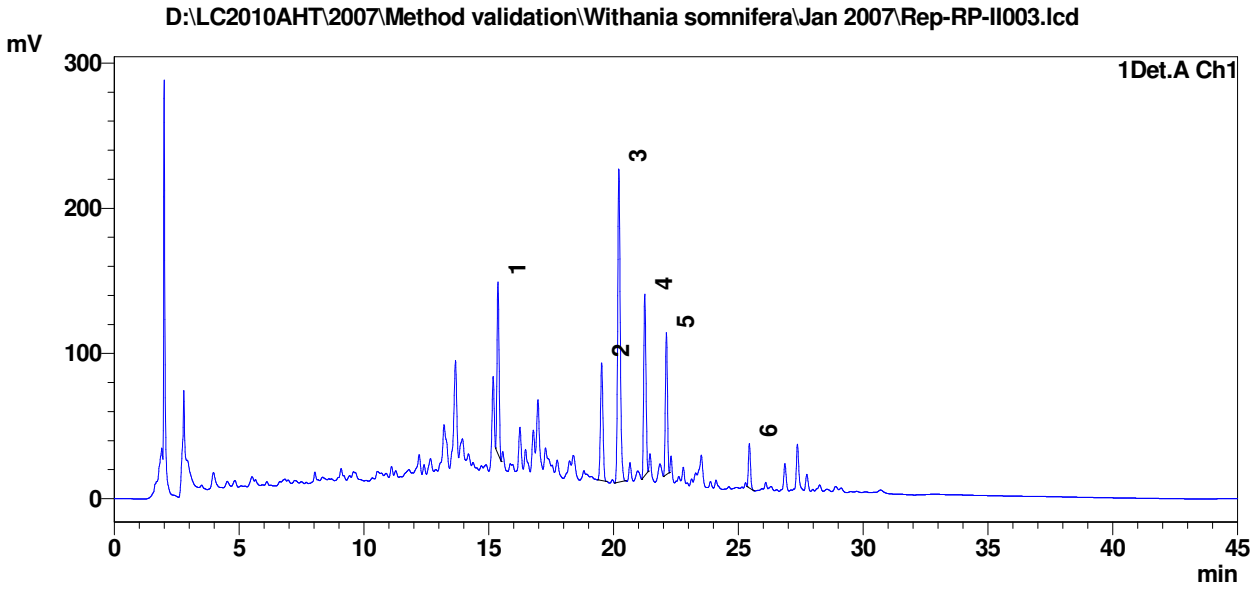
Data Processed	: 1/23/2007 2:58:55 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.372	677881	118436	15.841	Withanoside IV
2	19.526	560865	80925	13.106	Withanoside V
3	20.216	1542242	215590	36.039	Withaferin A
4	21.253	769019	125023	17.970	12- Deoxy withastramonolide
5	22.119	557605	97740	13.030	Withanollide A
6	25.444	171769	30570	4.014	Withanolide B
Total		4279382	668285	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1000 mg / 100 ml : Trl 2
Vail #	: 46
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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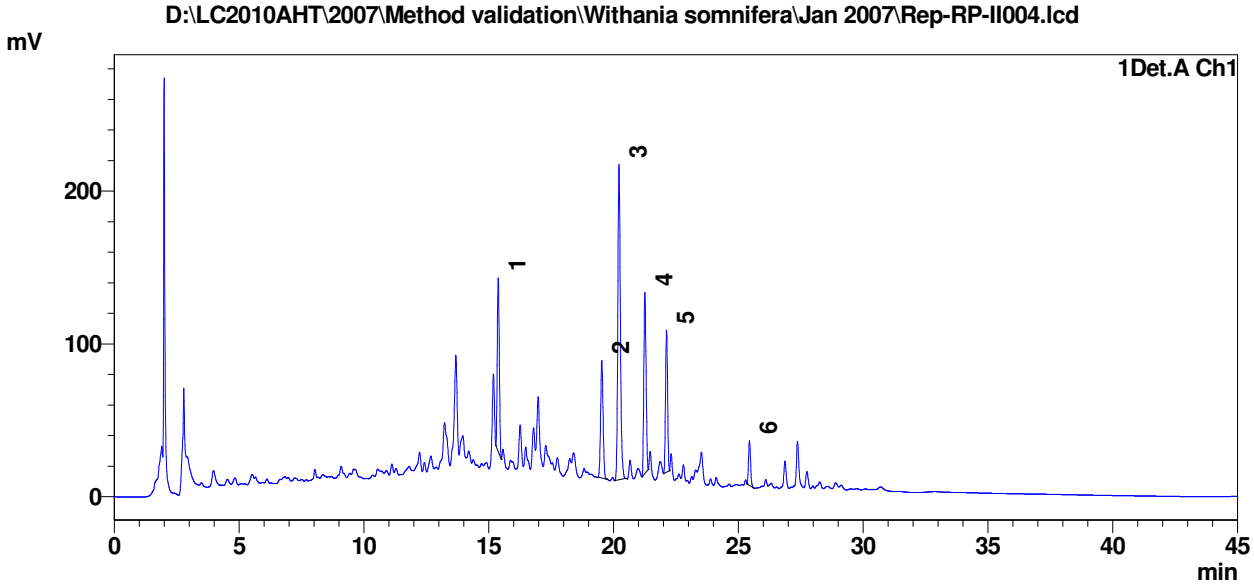
Data Processed	: 1/23/2007 3:01:02 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.382	645389	113325	15.853	Withanoside IV
2	19.532	530604	76793	13.034	Withanoside V
3	20.221	1468232	206318	36.066	Withaferin A
4	21.257	731496	118467	17.968	12- Deoxy withastramonolide
5	22.124	532618	92849	13.083	Withanollide A
6	25.447	162658	29130	3.996	Withanolide B
Total		4070996	636882	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 1
Vail #	: 47
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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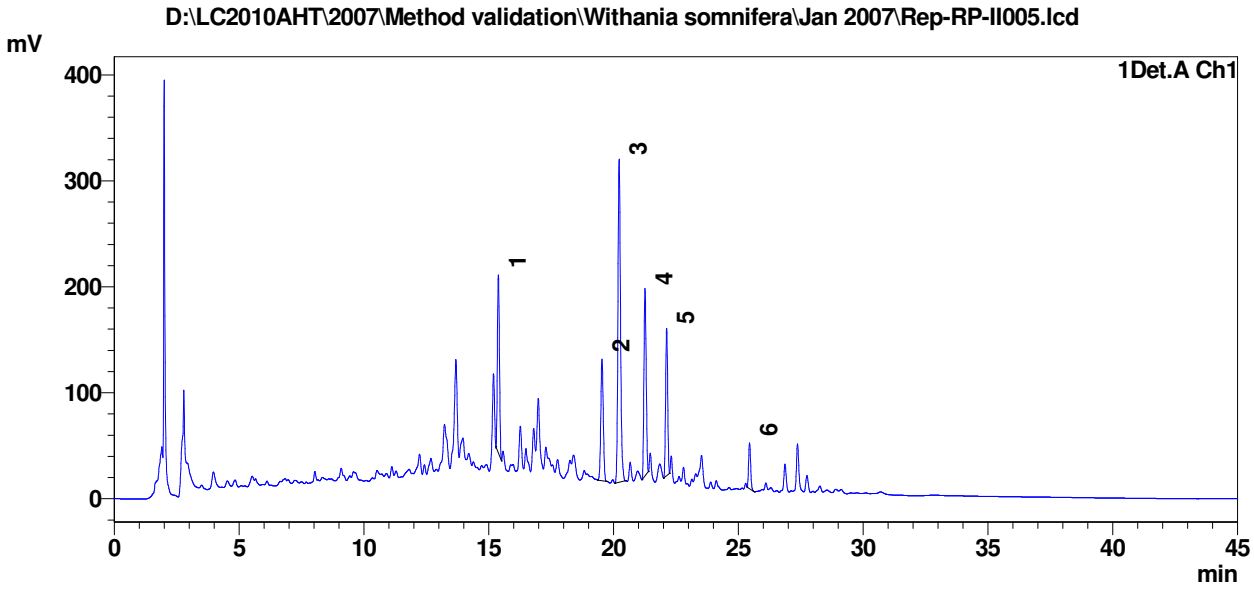
Data Processed	: 1/23/2007 3:02:09 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.388	949957	167823	15.716	Withanoside IV
2	19.540	793298	114653	13.124	Withanoside V
3	20.227	2173830	304832	35.963	Withaferin A
4	21.262	1088292	177034	18.004	12- Deoxy withastramonolide
5	22.128	797140	139023	13.187	Withanollide A
6	25.450	242191	43112	4.007	Withanolide B
Total		6044708	946478	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 1500 mg / 100 ml : Trl 2
Vail #	: 48
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II006.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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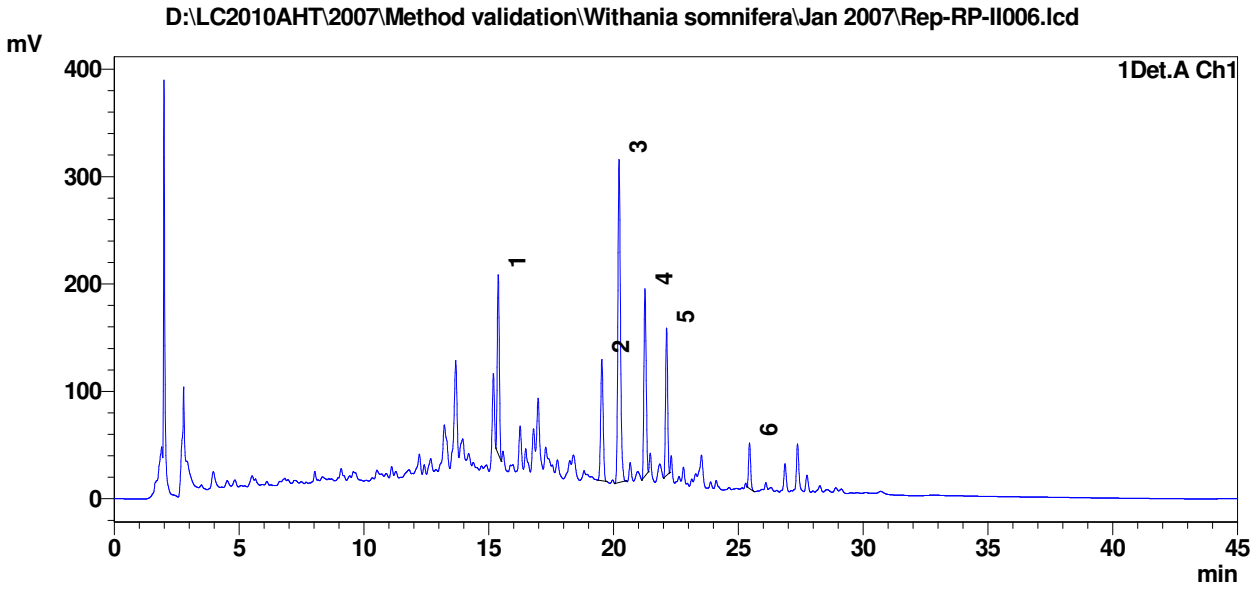
Data Processed	: 1/23/2007 3:03:19 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.383	949797	166616	15.870	Withanoside IV
2	19.534	783540	112934	13.092	Withanoside V
3	20.224	2152710	300796	35.970	Withaferin A
4	21.261	1075418	174559	17.969	12- Deoxy withastramonolide
5	22.126	784022	137155	13.100	Withanollide A
6	25.449	239205	42555	3.997	Withanolide B
Total		5984692	934615	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 1
Vail #	: 49
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II007.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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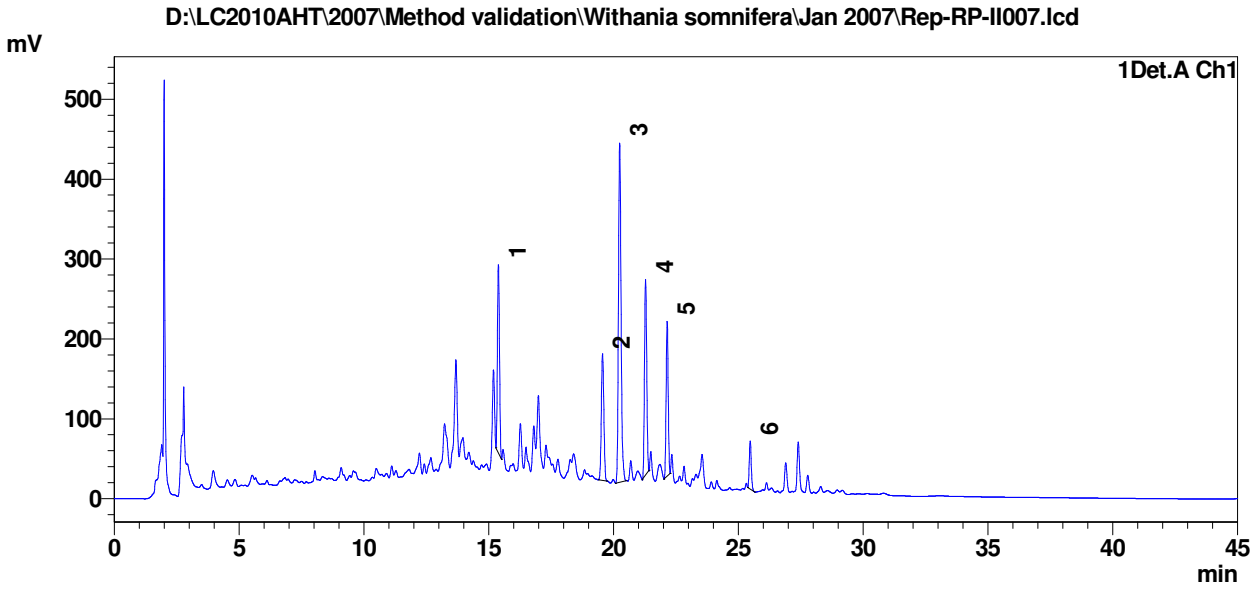
Data Processed	: 1/23/2007 3:04:28 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.390	1333648	234986	15.796	Withanoside IV
2	19.562	1102054	158403	13.053	Withanoside V
3	20.248	3036695	424260	35.968	Withaferin A
4	21.284	1511759	245224	17.906	12- Deoxy withastramonolide
5	22.149	1120023	193935	13.266	Withanollide A
6	25.476	338649	60102	4.011	Withanolide B
Total		8442826	1316911	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2000 mg / 100 ml : Trl 2
Vail #	: 50
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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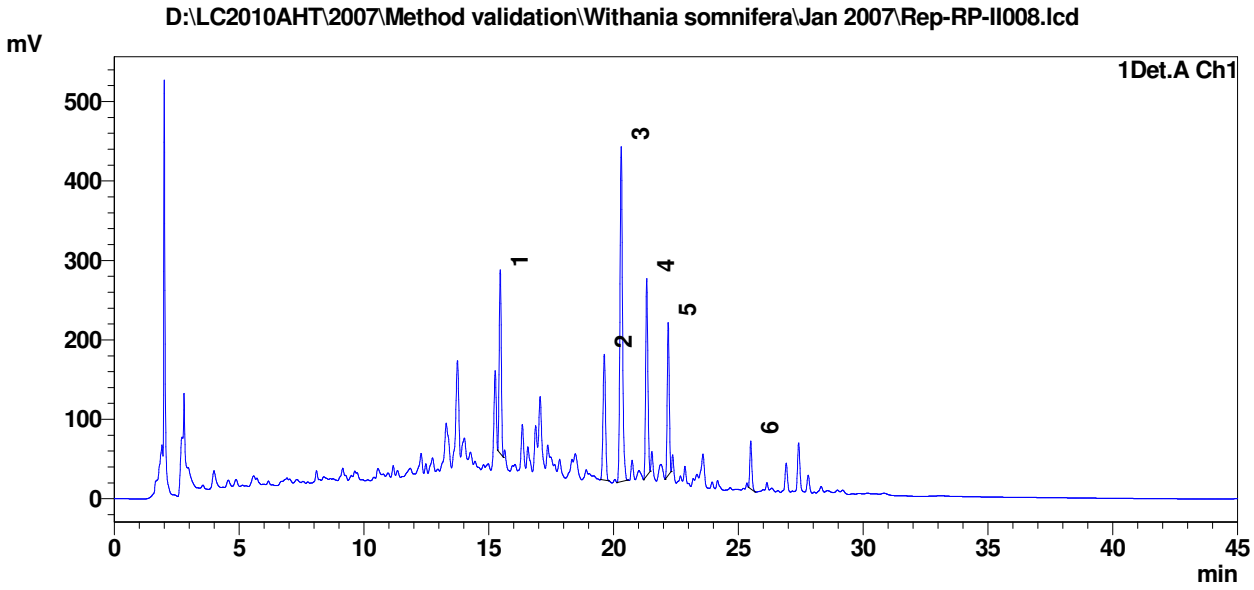

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.462	1320439	230805	15.782	Withanoside IV
2	19.630	1090118	158305	13.029	Withanoside V
3	20.308	3006238	421639	35.930	Withaferin A
4	21.330	1512807	248164	18.081	12- Deoxy withastramonolide
5	22.192	1101225	192750	13.162	Withanollide A
6	25.499	336094	60035	4.017	Withanolide B
Total		8366920	1311697	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera : WS / 06 lot 10
Sample ID	: 2500 mg / 100 ml : Trl 1
Vail #	: 51
Injection Volume	: 20 uL
Data File Name	: Rep-RP-II009.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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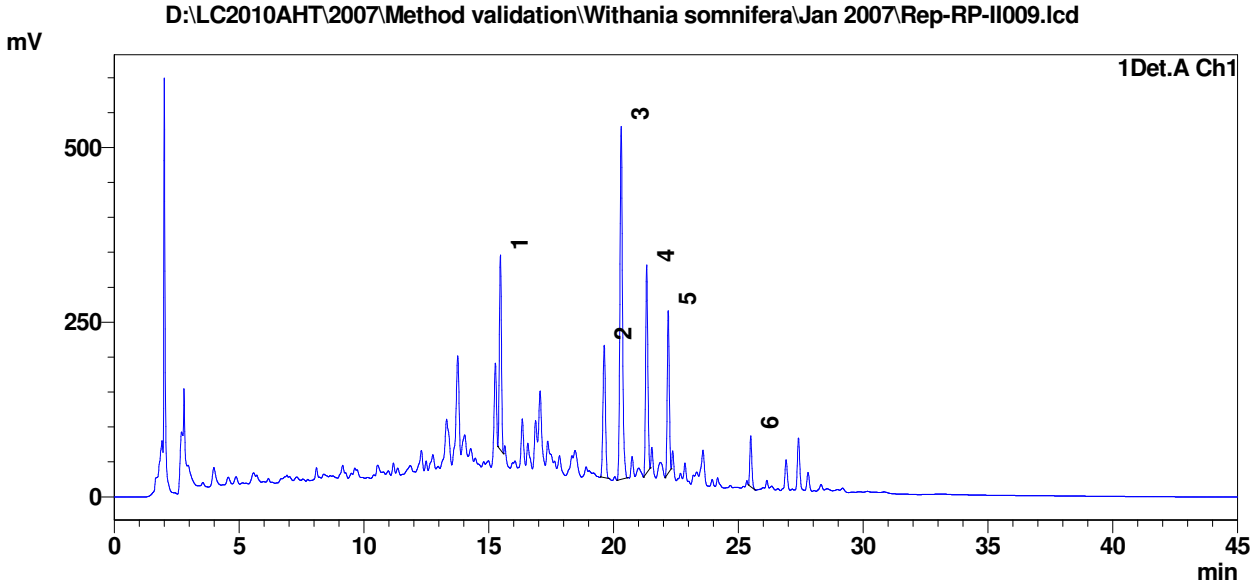

1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.468	1587176	278430	15.795	Withanoside IV
2	19.627	1303746	189091	12.974	Withanoside V
3	20.306	3606086	505110	35.887	Withaferin A
4	21.329	1816122	297967	18.073	12- Deoxy withastramonolide
5	22.193	1330784	232773	13.244	Withanollide A
6	25.498	404644	72505	4.027	Withanolide B
Total		10048558	1575875	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Enclosure: 10

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 06 LOT 08
Vail #	: 18
Injection Volume	: 20 uL
Data File Name	: Quan005.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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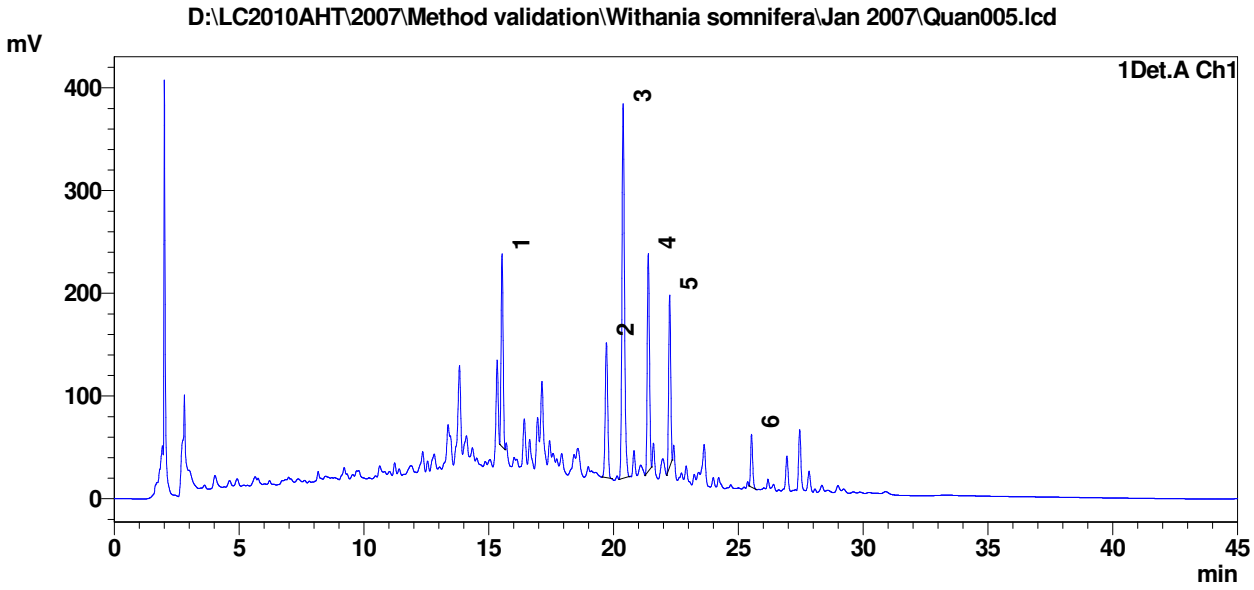
Data Processed	: 1/23/2007 2:51:10 AM



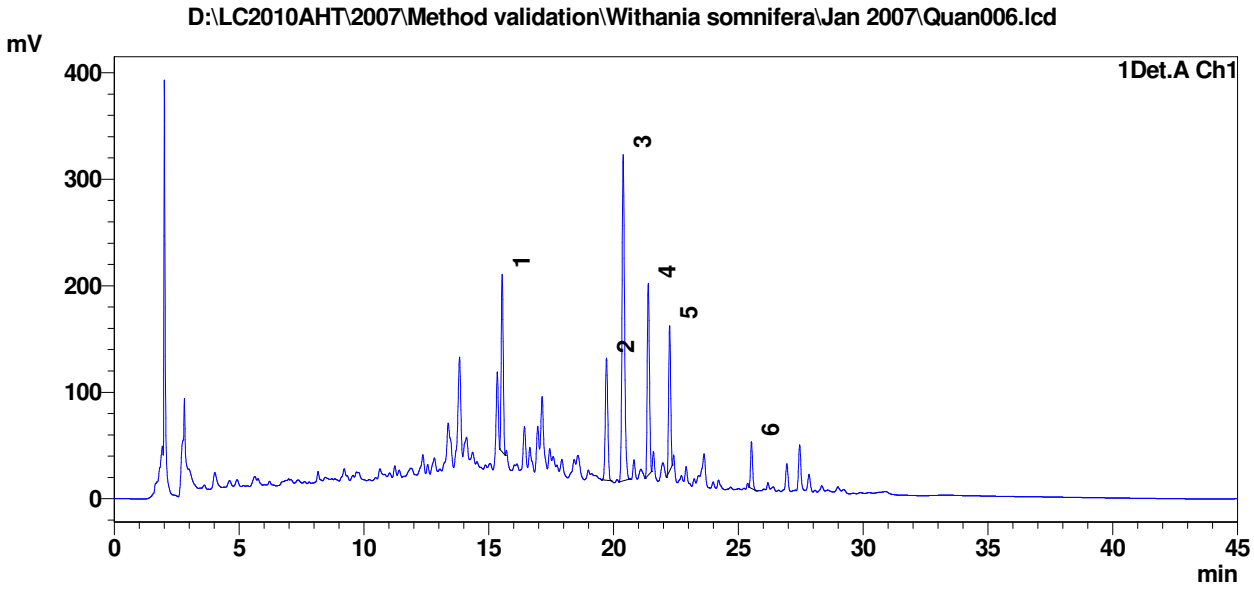
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.536	1057665	187666	14.949	Withanoside IV
2	19.718	918398	131432	12.981	Withanoside V
3	20.387	2591905	364794	36.635	Withaferin A
4	21.394	1290000	212280	18.233	12- Deoxy withastramonolide
5	22.251	929280	167586	13.135	Withanollide A
6	25.531	287702	51262	4.066	Withanolide B
Total		7074950	1115020	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 06 LOT 10
Vail #	: 19
Injection Volume	: 20 uL
Data File Name	: Quan006.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 11:49:50 AM
Data Processed	: 1/23/2007 2:52:16 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.540	944769	166740	15.760	Withanoside IV
2	19.720	795041	114533	13.262	Withanoside V
3	20.389	2165191	306133	36.117	Withaferin A
4	21.393	1091421	180160	18.206	12- Deoxy withastramonolide
5	22.250	755856	136295	12.608	Withanollide A
6	25.528	242624	43606	4.047	Withanolide B
Total		5994902	947467	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 05 LOT 20
Vail #	: 14
Injection Volume	: 20 uL
Data File Name	: Quan001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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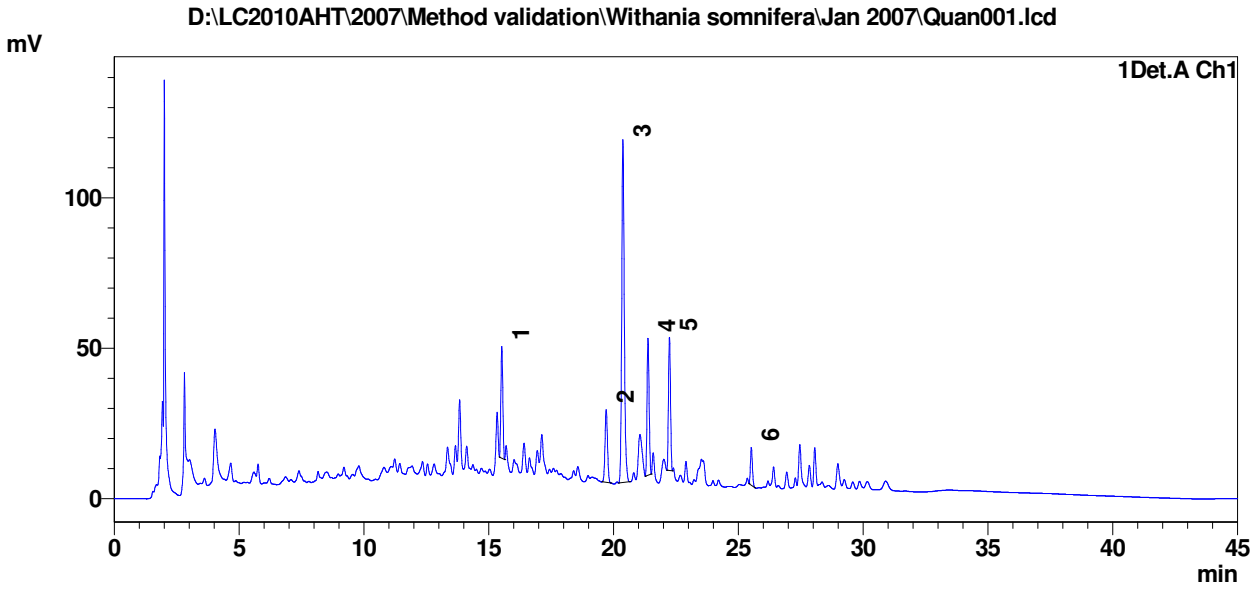
Data Processed	: 1/23/2007 2:45:58 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.525	205437	37048	11.645	Withanoside IV
2	19.708	168059	24112	9.526	Withanoside V
3	20.378	812277	113984	46.043	Withaferin A
4	21.383	263678	45550	14.946	12- Deoxy withastramonolide
5	22.242	246190	44160	13.955	Withanollide A
6	25.522	68520	12524	3.884	Withanolide B
Total		1764161	277378	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 05 LOT 21
Vail #	: 15
Injection Volume	: 20 uL
Data File Name	: Quan002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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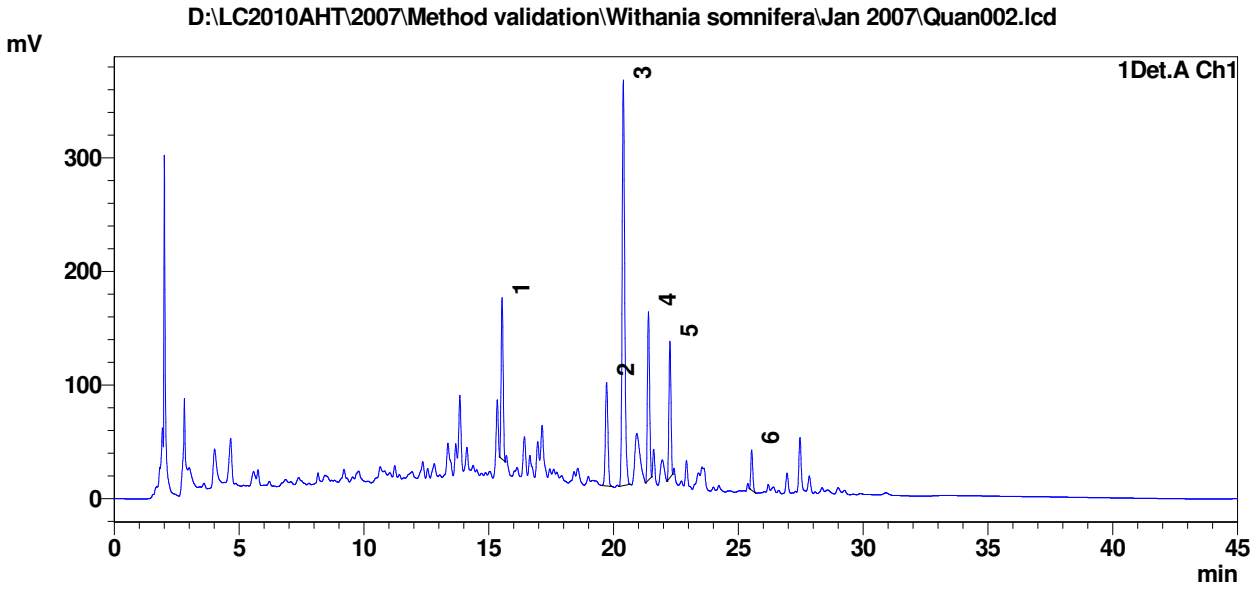
Data Processed	: 1/23/2007 2:47:29 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.537	806225	142188	14.131	Withanoside IV
2	19.725	637065	90668	11.166	Withanoside V
3	20.395	2532764	356934	44.392	Withaferin A
4	21.401	865621	148099	15.172	12- Deoxy withastramonolide
5	22.261	670597	120136	11.754	Withanollide A
6	25.537	193202	35413	3.386	Withanolide B
Total		5705473	893439	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - RD / 1170
Vail #	: 16
Injection Volume	: 20 uL
Data File Name	: Quan003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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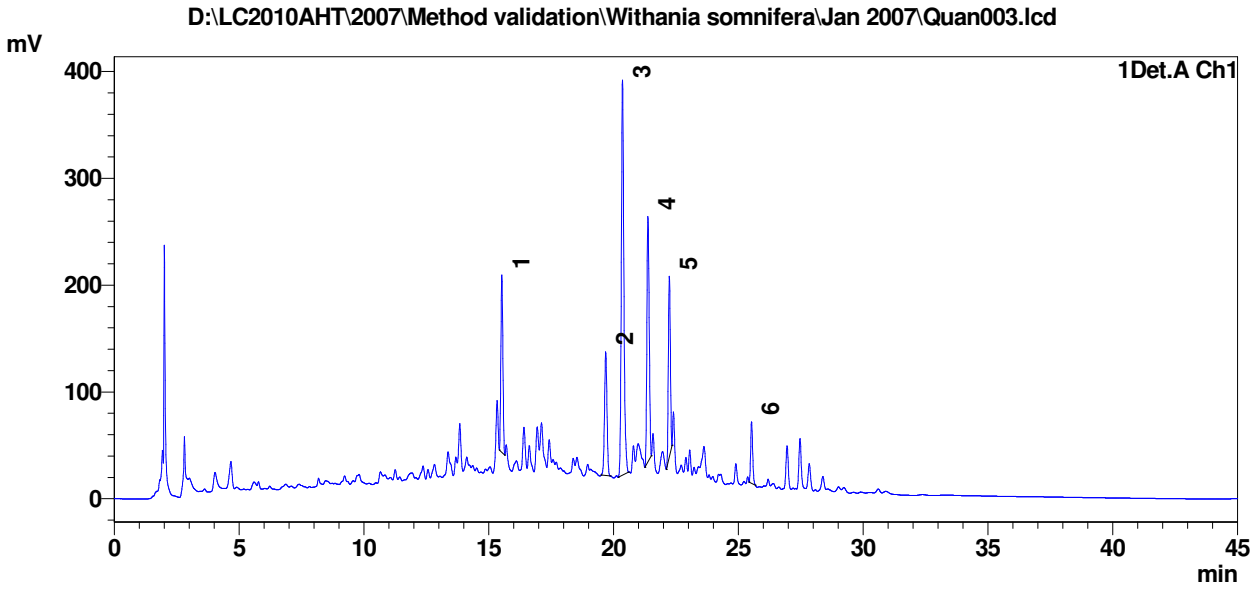
Data Processed	: 1/23/2007 2:48:46 AM



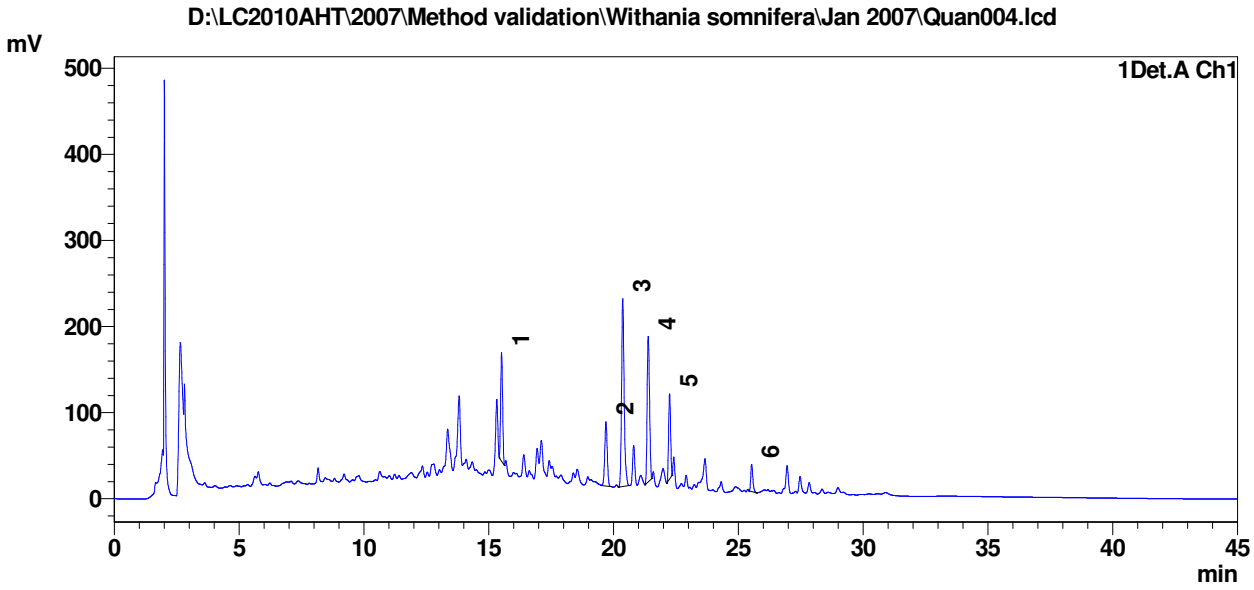
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.526	941120	166252	13.204	Withanoside IV
2	19.689	804822	115826	11.291	Withanoside V
3	20.361	2646818	369982	37.134	Withaferin A
4	21.381	1480793	230122	20.775	12- Deoxy withastramonolide
5	22.238	933574	169341	13.098	Withanollide A
6	25.532	320629	57353	4.498	Withanolide B
Total		7127757	1108877	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - RD / 1045
Vail #	: 17
Injection Volume	: 20 uL
Data File Name	: Quan004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 10:18:22 AM
Data Processed	: 1/23/2007 2:49:54 AM



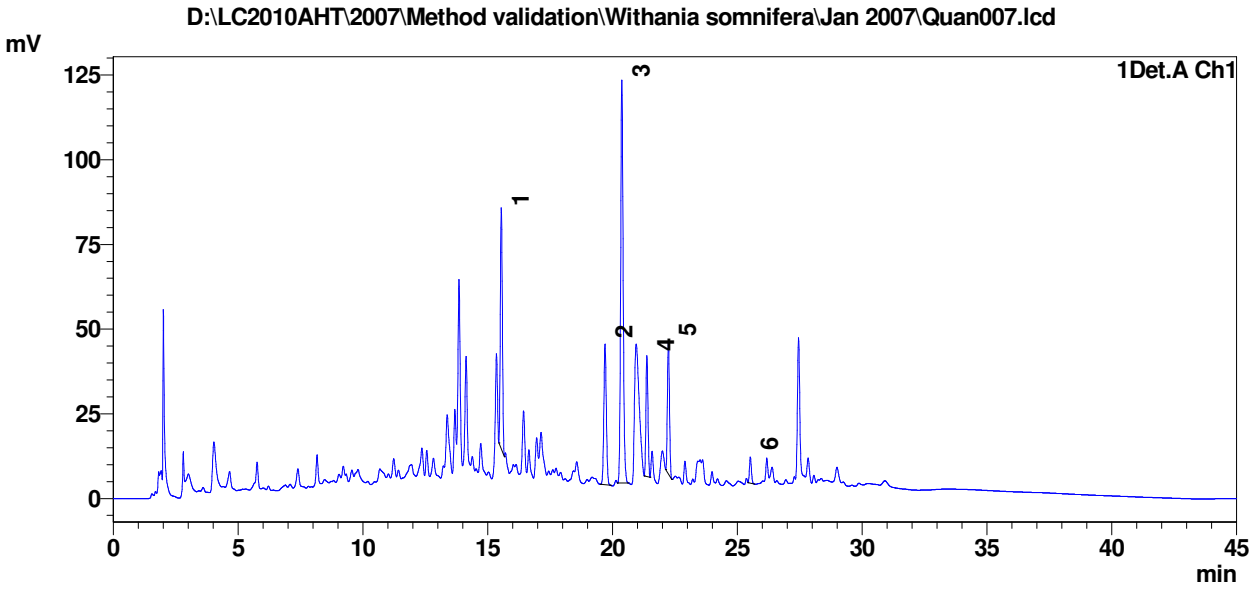
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.516	699116	126309	15.066	Withanoside IV
2	19.698	521948	74525	11.248	Withanoside V
3	20.371	1573294	218195	33.905	Withaferin A
4	21.392	1123326	169172	24.208	12- Deoxy withastramonolide
5	22.248	539632	98989	11.629	Withanollide A
6	25.536	183000	31526	3.944	Withanolide B
Total		4640315	718716	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: RM - RD / 1162
Vail #	: 20
Injection Volume	: 20 uL
Data File Name	: Quan007.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 12:35:34 PM
Data Processed	: 1/23/2007 2:53:34 AM



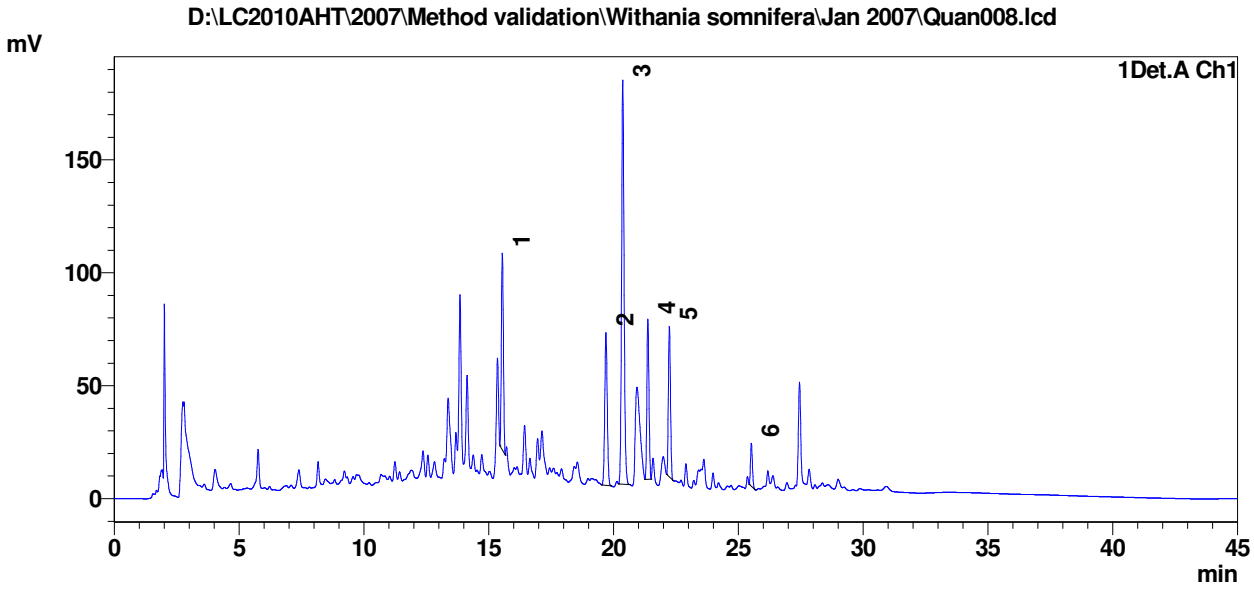
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.545	405115	71109	20.313	Withanoside IV
2	19.701	290787	41508	14.580	Withanoside V
3	20.375	833061	118930	41.770	Withaferin A
4	21.378	202935	35689	10.175	12- Deoxy withastramonolide
5	22.239	220542	38625	11.058	Withanollide A
6	25.523	41941	7689	2.103	Withanolide B
Total		1994381	313550	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: RM - ERH - 46
Vail #	: 21
Injection Volume	: 20 uL
Data File Name	: Quan008.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 1:21:18 PM
Data Processed	: 1/23/2007 2:54:40 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

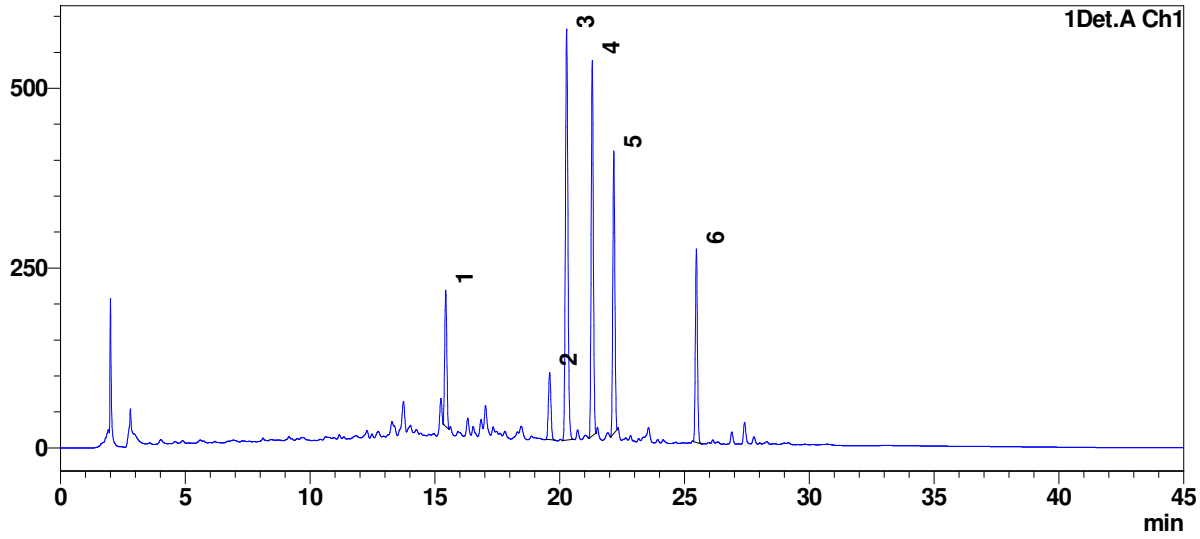
Peak #	Ret. Time	Area	Height	Area %	Name
1	15.547	491157	87210	15.779	Withanoside IV
2	19.698	473414	67676	15.209	Withanoside V
3	20.372	1250895	178900	40.186	Withaferin A
4	21.377	410796	70915	13.197	12- Deoxy withastramonolide
5	22.239	384409	66596	12.349	Withanollide A
6	25.523	102080	19017	3.279	Withanolide B
Total		3112751	490313	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 06 LOT 08
Vail #	: 27
Injection Volume	: 20 uL
Data File Name	: SSR002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 5:55:43 PM
Data Processed	: 1/23/2007 3:28:18 AM

Enclosure: 11

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SSR002.lcd



et.A Ch1/227nm

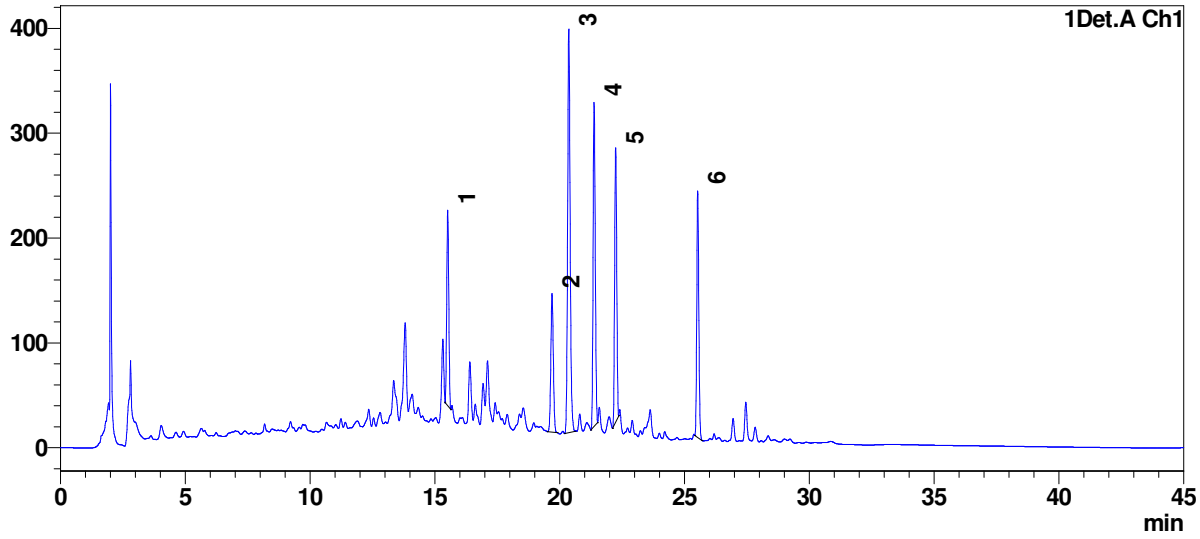
Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.435	1105227	189293	8.719	Withanoside IV
2	19.598	650581	93589	5.132	Withanoside V
3	20.278	3965398	571459	31.283	Withaferin A
4	21.305	3110148	521874	24.536	12- Deoxy withastramonolide
5	22.169	2296516	392754	18.117	Withanollide A
6	25.476	1547889	269117	12.211	Withanolide B
Total		12675760	2038086	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 06 LOT 10
Vail #	: 23
Injection Volume	: 20 uL
Data File Name	: SR002.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 2:52:46 PM
Data Processed	: 1/23/2007 3:23:04 AM

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SR002.lcd



et.A Ch1/227nm

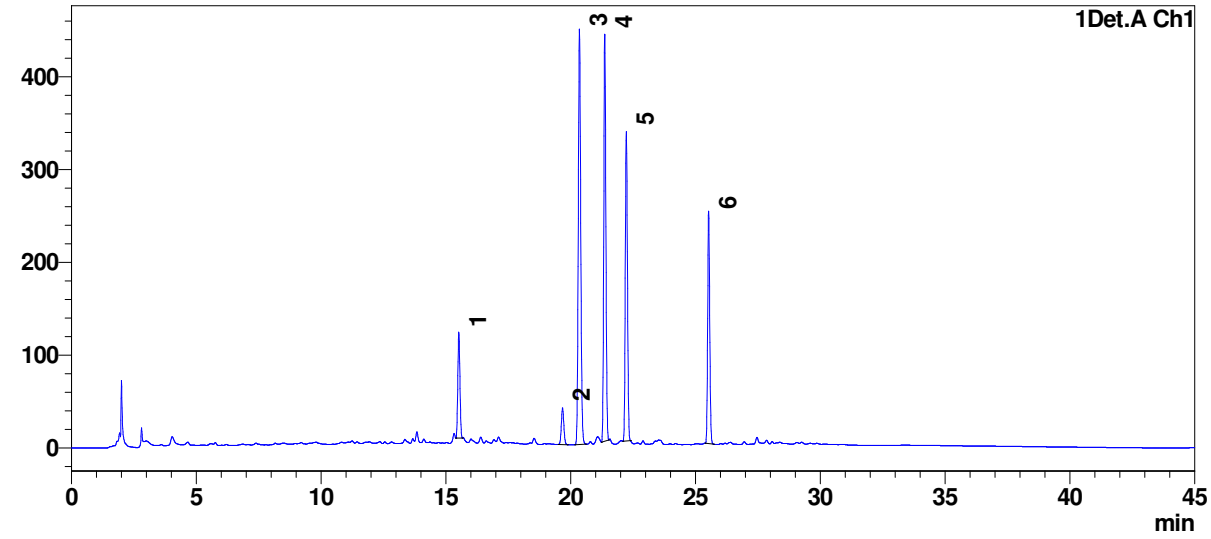
Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.515	1069212	186204	11.391	Withanoside IV
2	19.695	927543	132363	9.882	Withanoside V
3	20.367	2707641	384787	28.846	Withaferin A
4	21.381	1846124	308955	19.668	12- Deoxy withastramonolide
5	22.244	1500314	261841	15.984	Withanollide A
6	25.531	1335664	234969	14.230	Withanolide B
Total		9386498	1509118	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 05 LOT 20
Vail #	: 26
Injection Volume	: 20 uL
Data File Name	: SSR001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 5:10:00 PM
Data Processed	: 1/23/2007 3:26:52 AM

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SSR001.lcd



et.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.520	683387	114330	6.802	Withanoside IV
2	19.675	277532	39535	2.762	Withanoside V
3	20.352	3068219	447747	30.537	Withaferin A
4	21.366	2594887	438803	25.826	12- Deoxy withastramonolide
5	22.231	1985457	333647	19.761	Withanollide A
6	25.528	1438005	250635	14.312	Withanolide B
Total		10047486	1624696	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 05 LOT 21
Vail #	: 22
Injection Volume	: 20 uL
Data File Name	: SR001.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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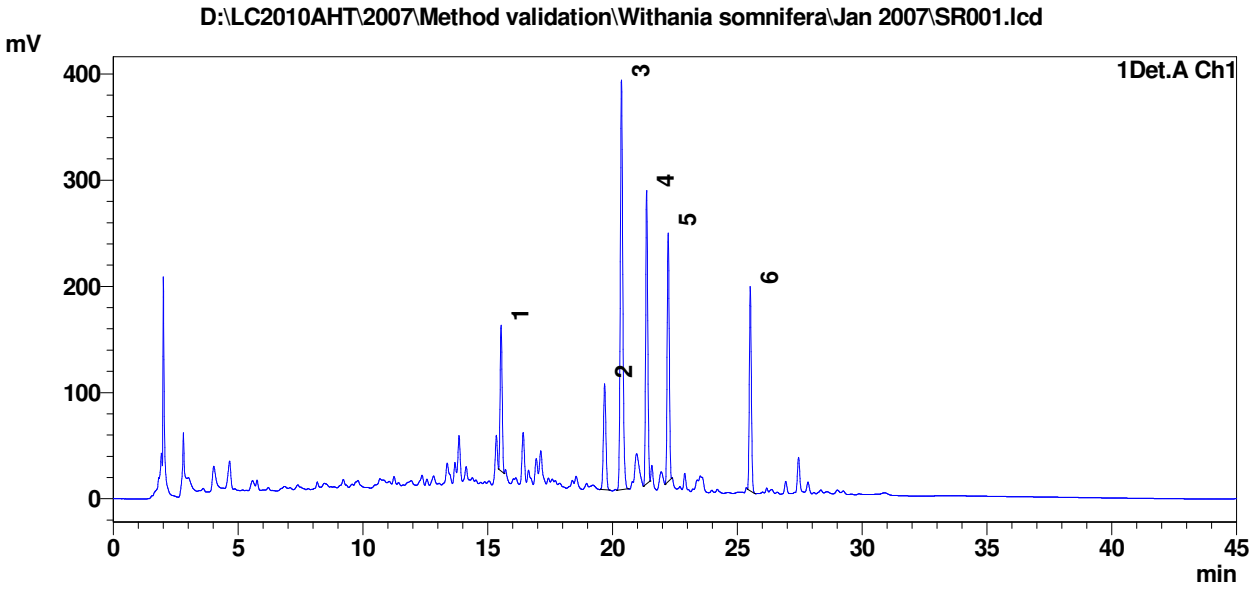
Data Processed	: 1/23/2007 3:21:56 AM



1 Det.A Ch1/227nm

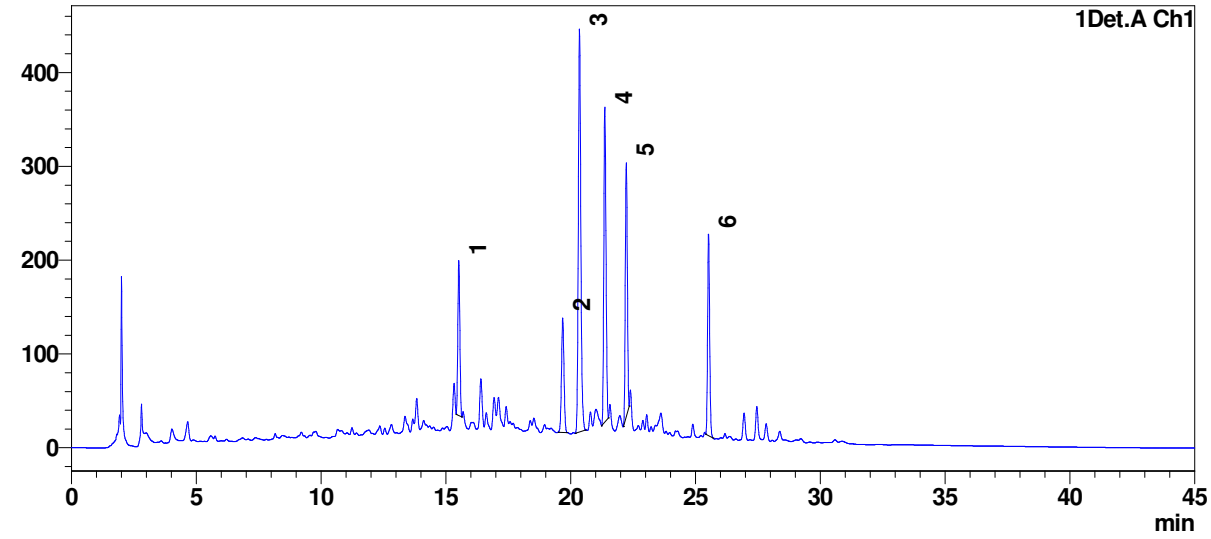
Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.537	783819	137203	9.524	Withanoside IV
2	19.685	696402	99709	8.462	Withanoside V
3	20.360	2695778	385557	32.756	Withaferin A
4	21.369	1614288	276366	19.615	12- Deoxy withastramonolide
5	22.232	1351576	233890	16.423	Withanollide A
6	25.520	1087909	192244	13.219	Withanolide B
Total		8229773	1324969	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - RD / 1170
Vail #	: 24
Injection Volume	: 20 uL
Data File Name	: SR003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 3:38:30 PM
Data Processed	: 1/23/2007 3:24:38 AM

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SR003.lcd



et.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.519	944658	165451	9.809	Withanoside IV
2	19.682	849034	121893	8.816	Withanoside V
3	20.354	3012010	429420	31.274	Withaferin A
4	21.369	2073431	335816	21.529	12- Deoxy withastramonolide
5	22.230	1525217	270242	15.837	Withanollide A
6	25.525	1226660	214413	12.737	Withanolide B
Total		9631011	1537235	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - RD / 1045
Vail #	: 28
Injection Volume	: 20 uL
Data File Name	: SSR003.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 6:41:31 PM
Data Processed	: 1/23/2007 3:29:31 AM

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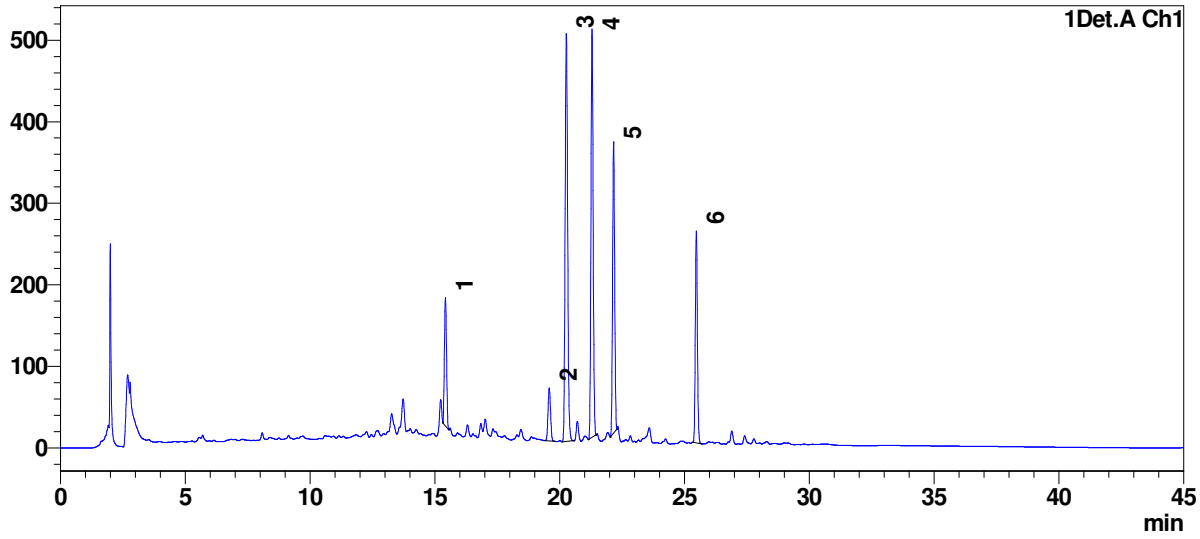

et.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.423	918311	157419	8.002	Withanoside IV
2	19.583	454914	64877	3.964	Withanoside V
3	20.267	3469556	500231	30.233	Withaferin A
4	21.295	3040941	500734	26.498	12- Deoxy withastramonolide
5	22.161	2085779	357485	18.175	Withanollide A
6	25.475	1506717	259646	13.129	Withanolide B
Total		11476218	1840392	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: RM - RD / 1162
Vail #	: 25
Injection Volume	: 20 uL
Data File Name	: SR004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
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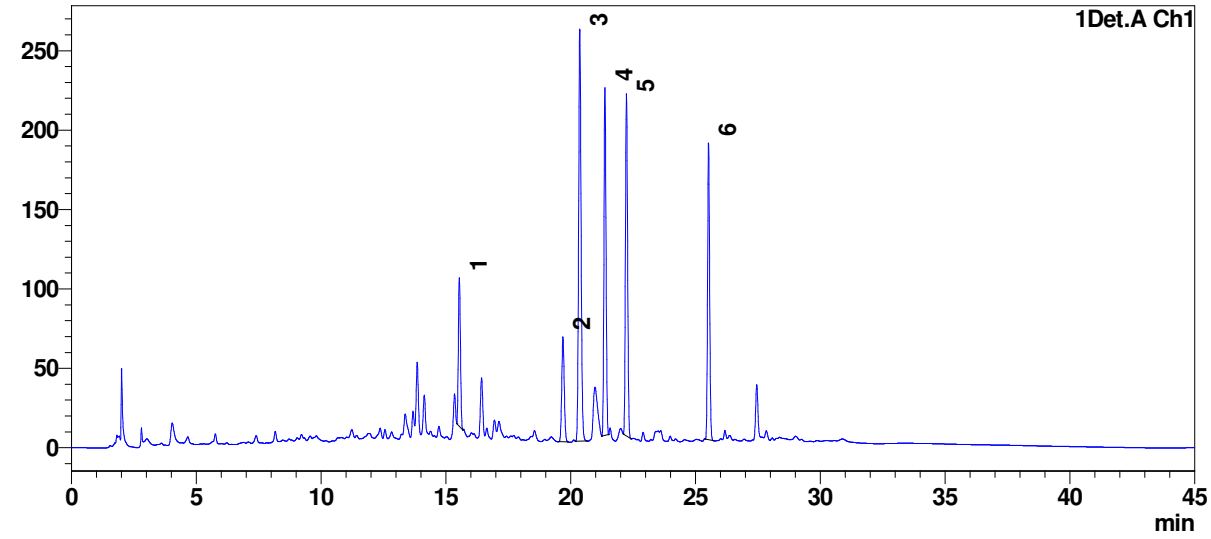
D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SR004.lcd



et.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.540	544320	93415	8.485	Withanoside IV
2	19.691	462387	66000	7.208	Withanoside V
3	20.366	1782419	259284	27.786	Withaferin A
4	21.374	1276721	218915	19.903	12- Deoxy withastramonolide
5	22.236	1277510	215493	19.915	Withanollide A
6	25.525	1071449	186725	16.703	Withanolide B
Total		6414805	1039832	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: RM - ERH - 46
Vail #	: 29
Injection Volume	: 20 uL
Data File Name	: SSR004.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 7:27:15 PM
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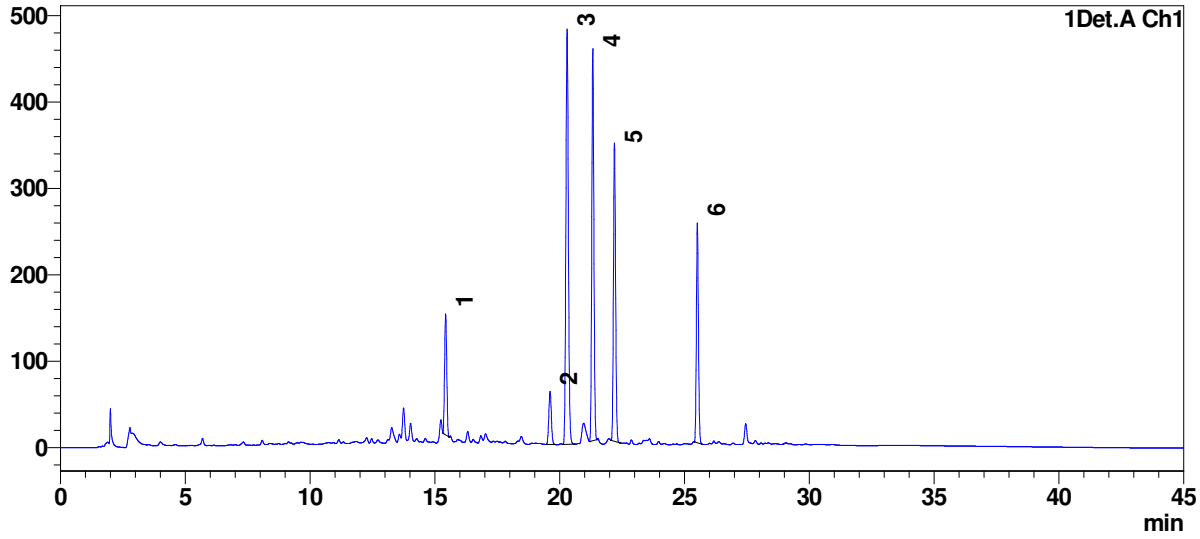
D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\SSR004.lcd



et.A Ch1/227nm

Detector A Ch1 227nm

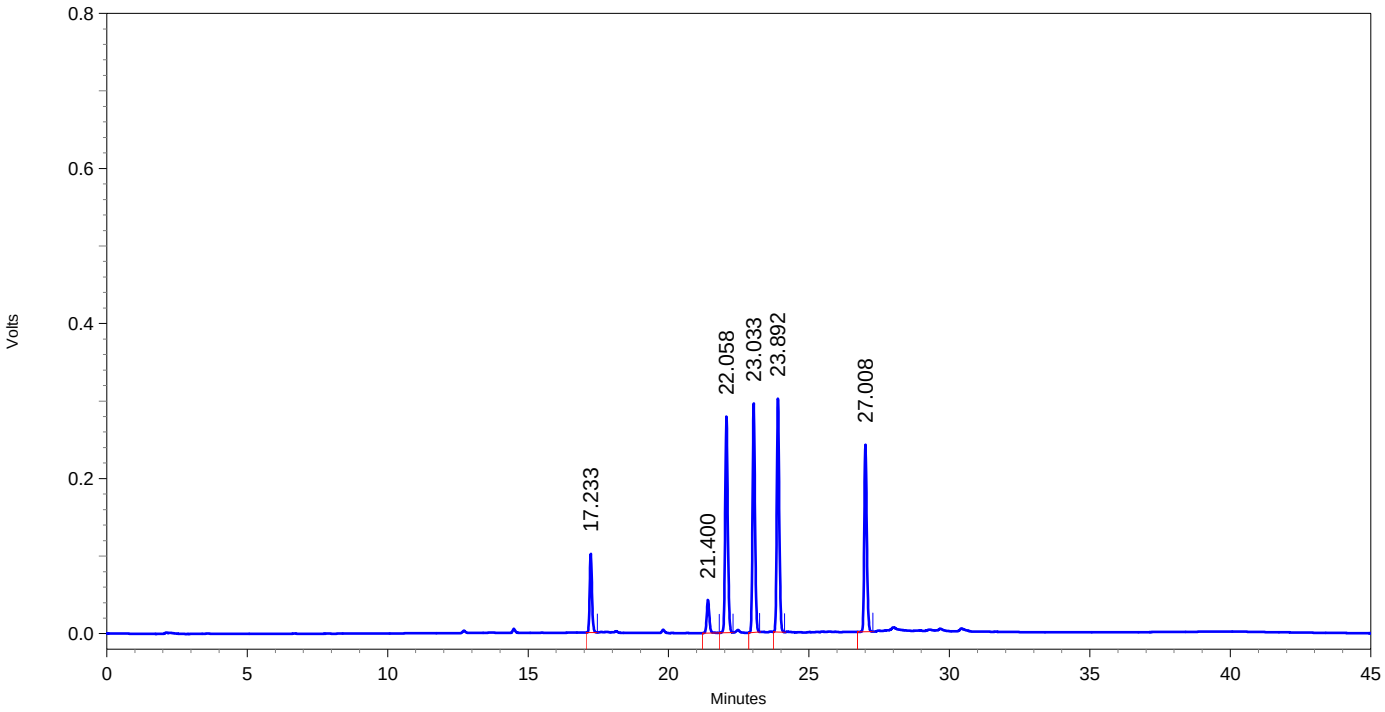
Peak #	Ret. Time	Area	Height	Area %	Name
1	15.431	828923	140122	7.668	Withanoside IV
2	19.614	432974	61422	4.005	Withanoside V
3	20.297	3319438	480548	30.705	Withaferin A
4	21.327	2686515	453987	24.850	12- Deoxy withastramonolide
5	22.196	2078342	345301	19.225	Withanollide A
6	25.516	1464553	254464	13.547	Withanolide B
Total		10810745	1735844	100.000	

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Enclosure: 12

Name of Sample : **Withanolides : std mix**
Method Name : **C:\Methods\WITHANIA.met**
Data File Name : **D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\SM-01-Rep1**
Vial No : **10001**
Injection Volume : **20**
Date & Time : **4/3/07 7:44:32 PM**



Detector A - 1 (227nm)

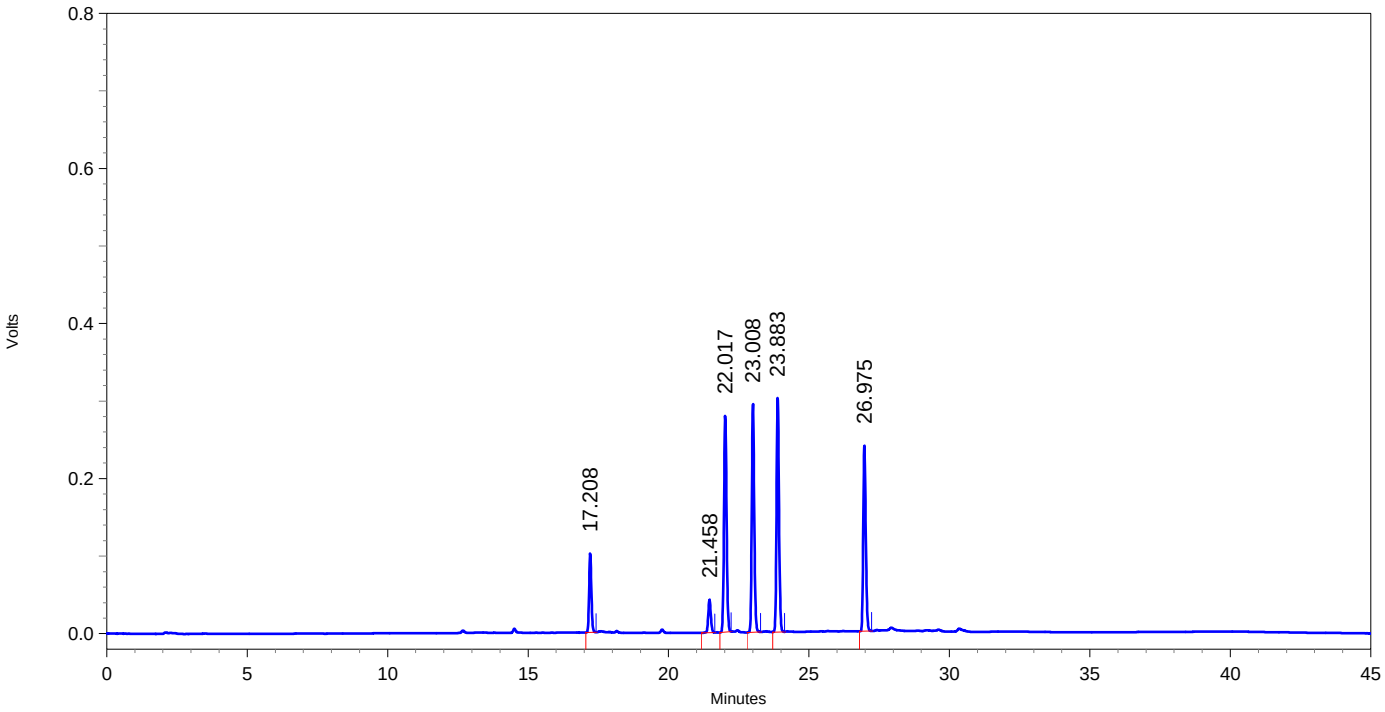
Pk #	Retention Time	Name	Area	Area Percent
1	17.233	Withanoside IV	575205	7.35
2	21.400	Withanoside V	274773	3.51
3	22.058	Withaferin A	1813207	23.18
4	23.033	Withastramonolide	1788787	22.86
5	23.892	Withanolide A	1849418	23.64
6	27.008	Withanolide B	1522047	19.45

Totals			7823437	100.00

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withanolides : std mix**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\SM-01-Rep2**
Vial No	**: 10001**
Injection Volume	**: 20**
Date & Time	**: 4/3/07 8:30:25 PM**



Detector A - 1 (227nm)

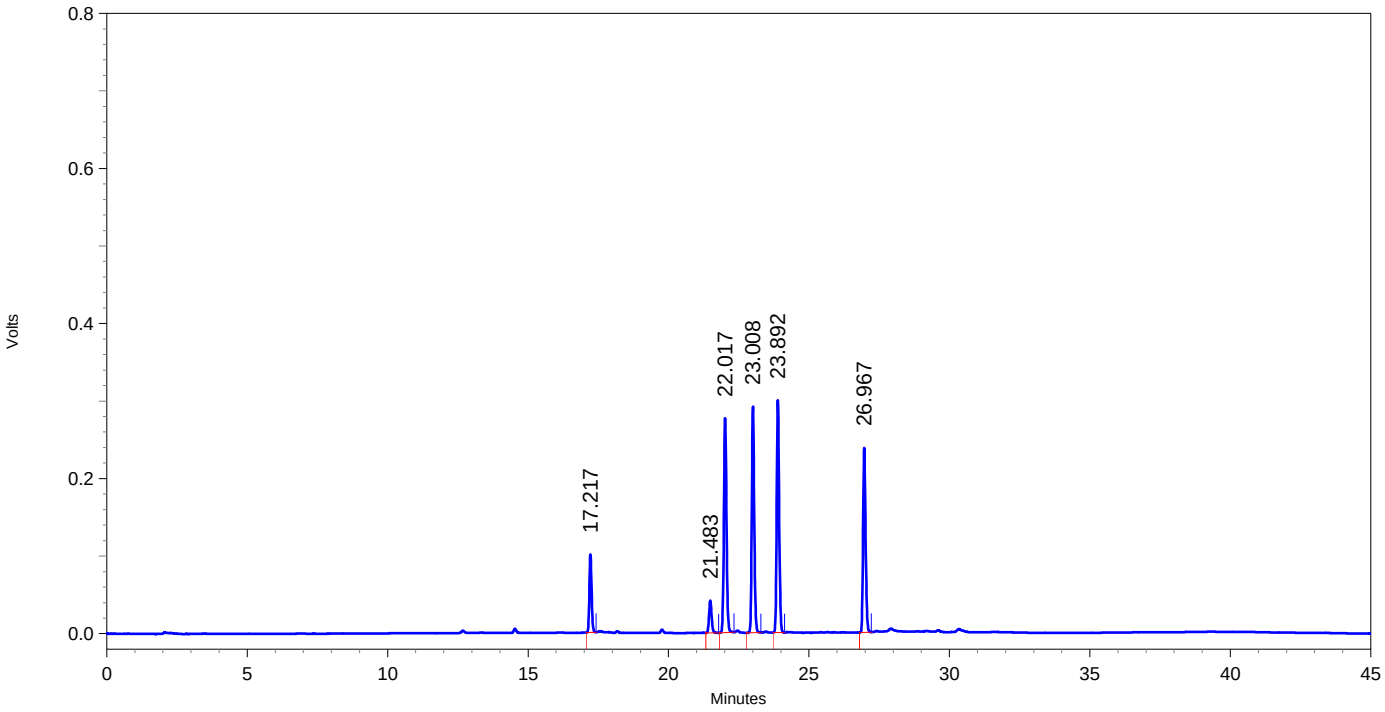
Pk #	Retention Time	Name	Area	Area Percent
1	**17.208**	**Withanoside IV**	**569898**	**7.32**
2	**21.458**	**Withanoside V**	**272130**	**3.50**
3	**22.017**	**Withaferin A**	**1797785**	**23.10**
4	**23.008**	**Withastramonolide**	**1787246**	**22.96**
5	**23.883**	**Withanolide A**	**1842576**	**23.67**
6	**26.975**	**Withanolide B**	**1513567**	**19.45**

Totals			**7783202**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withanolides : std mix**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\SM-01-Rep3**
Vial No	**: 10001**
Injection Volume	**: 20**
Date & Time	**: 4/3/07 9:16:17 PM**



Detector A - 1 (227nm)

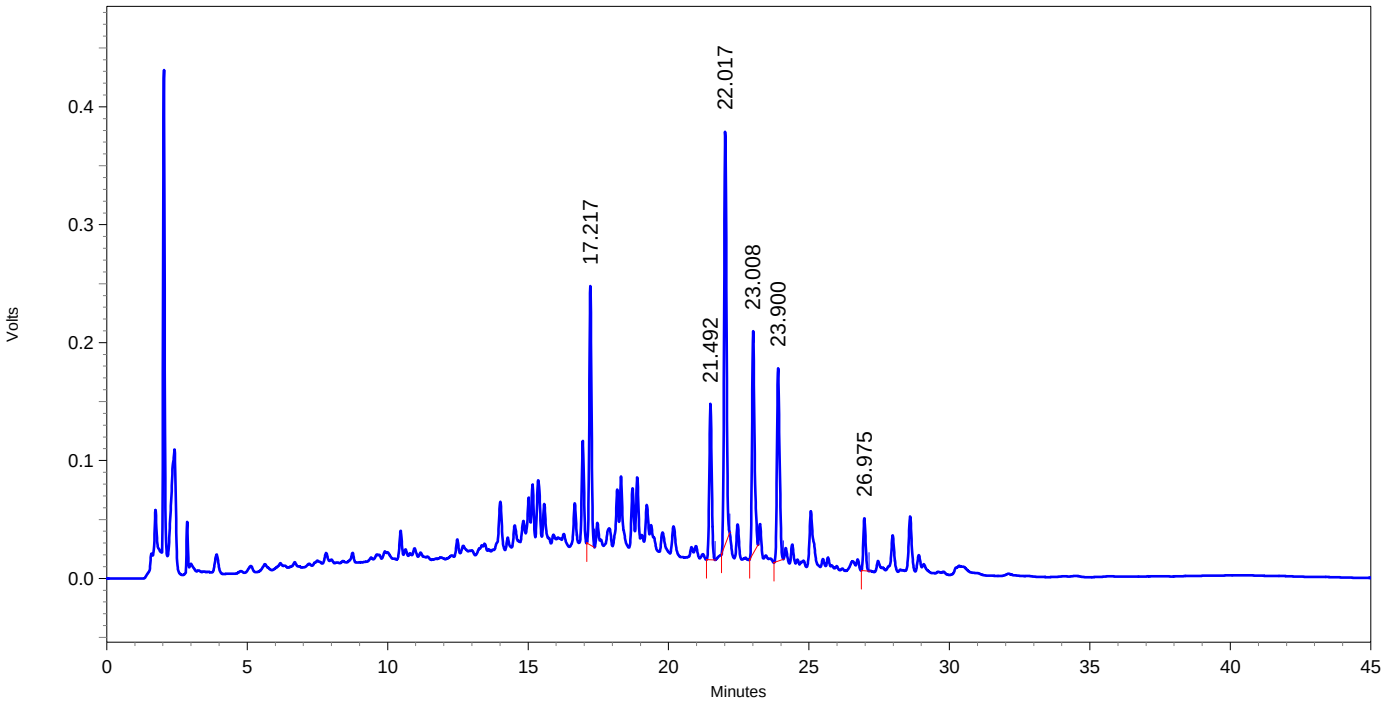
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**559628**	**7.30**
2	**21.483**	**Withanoside V**	**265630**	**3.46**
3	**22.017**	**Withaferin A**	**1788944**	**23.34**
4	**23.008**	**Withastramonolide**	**1756420**	**22.91**
5	**23.892**	**Withanolide A**	**1809961**	**23.61**
6	**26.967**	**Withanolide B**	**1485590**	**19.38**

Totals			**7666173**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 08**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S3-Rep1**
Vial No	**: 10004**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 4:55:00 AM**



Detector A - 1 (227nm)

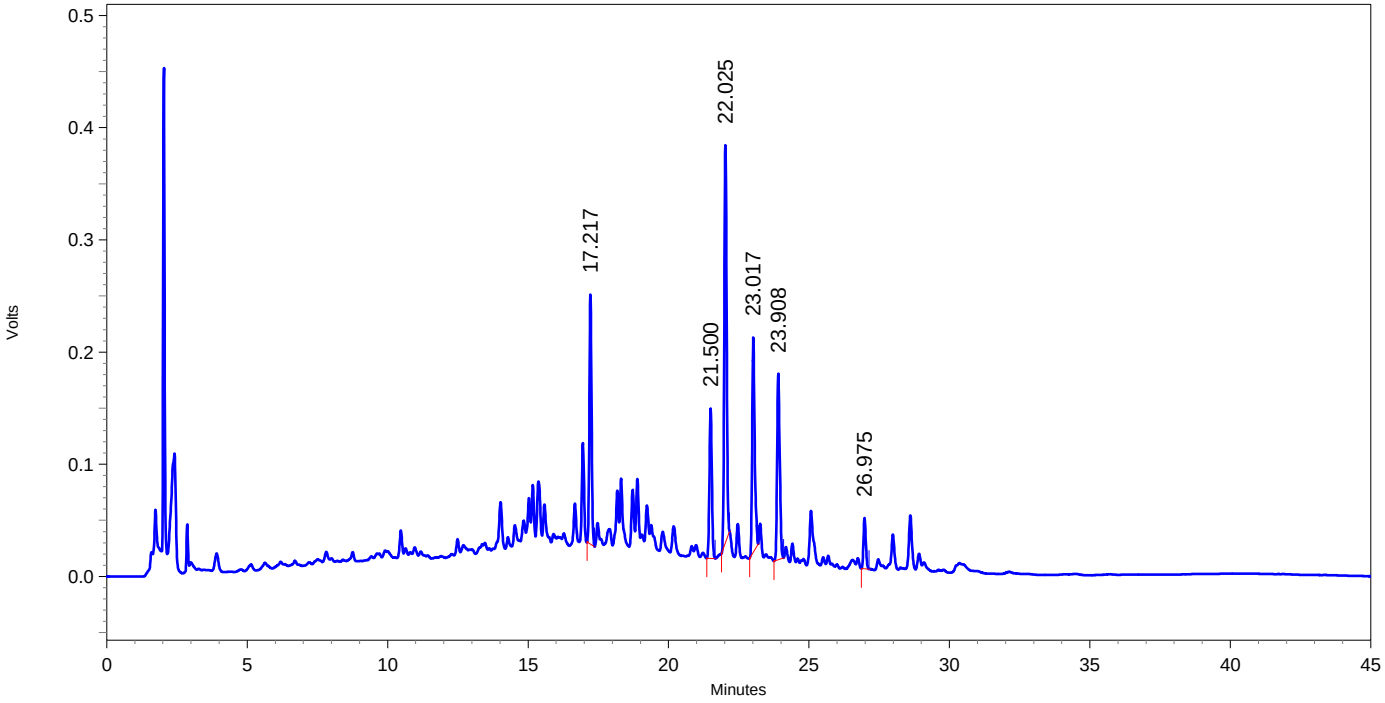
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**1195002**	**18.09**
2	**21.492**	**Withanoside V**	**802479**	**12.15**
3	**22.017**	**Withaferin A**	**2091436**	**31.67**
4	**23.008**	**Withastramonolide**	**1179907**	**17.87**
5	**23.900**	**Withanolide A**	**1073657**	**16.26**
6	**26.975**	**Withanolide B**	**261990**	**3.97**

Totals			**6604471**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 08**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S3-Rep2**
Vial No	**: 10004**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 5:40:51 AM**



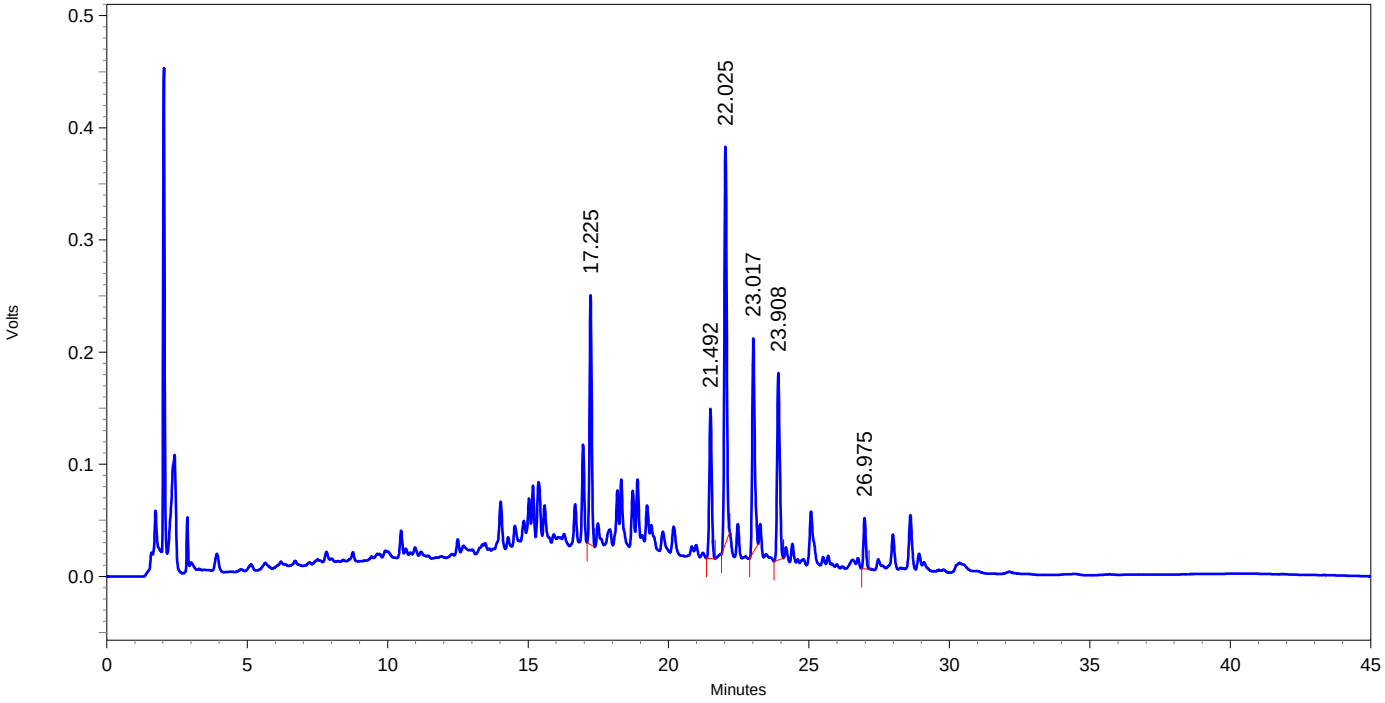
Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**1209175**	**18.11**
2	**21.500**	**Withanoside V**	**811366**	**12.15**
3	**22.025**	**Withaferin A**	**2109930**	**31.60**
4	**23.017**	**Withastramonolide**	**1191675**	**17.84**
5	**23.908**	**Withanolide A**	**1088863**	**16.31**
6	**26.975**	**Withanolide B**	**267000**	**4.00**
Totals			**6678009**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 08**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S3-Rep3**
Vial No	**: 10004**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 6:26:43 AM**



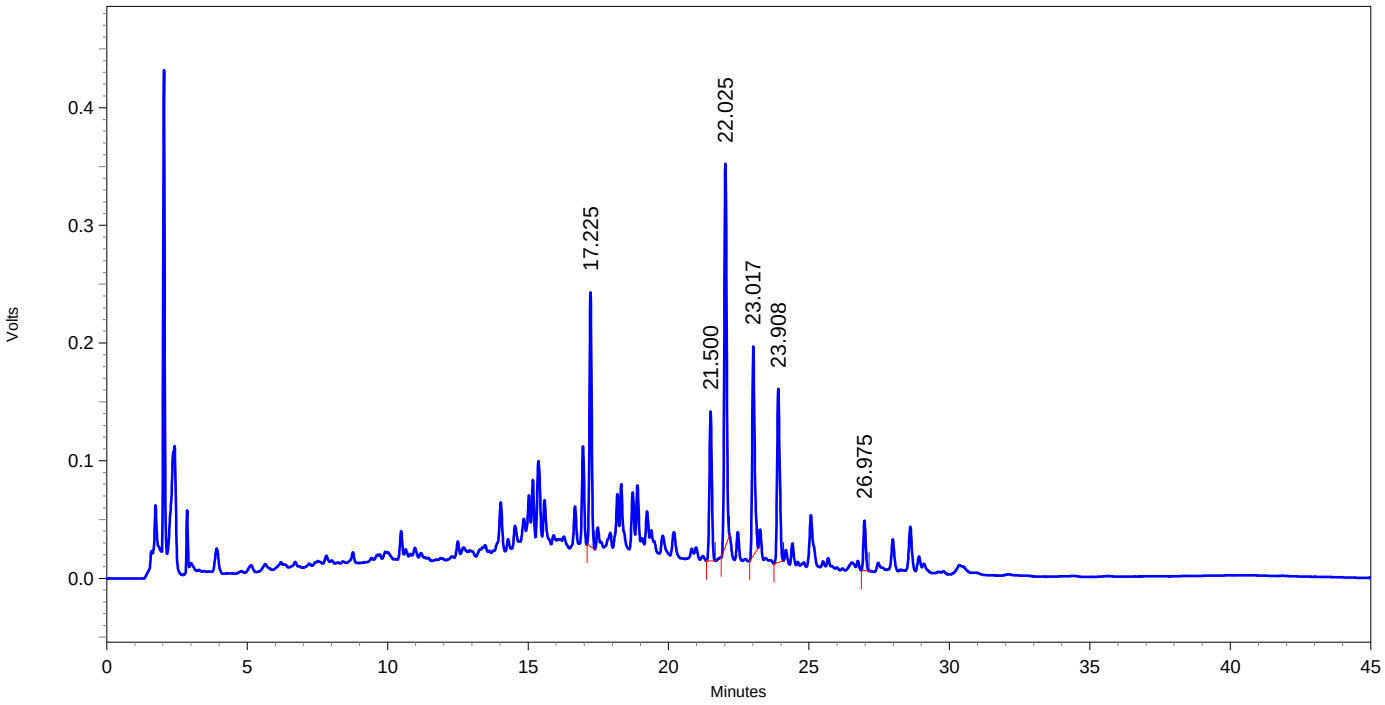
Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**1207529**	**18.11**
2	**21.492**	**Withanoside V**	**808773**	**12.13**
3	**22.025**	**Withaferin A**	**2109929**	**31.64**
4	**23.017**	**Withastramonolide**	**1186698**	**17.80**
5	**23.908**	**Withanolide A**	**1089796**	**16.34**
6	**26.975**	**Withanolide B**	**265589**	**3.98**
Totals			**6668314**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 10**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S4-Rep1**
Vial No	**: 10005**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 7:12:29 AM**



Detector A - 1 (227nm)

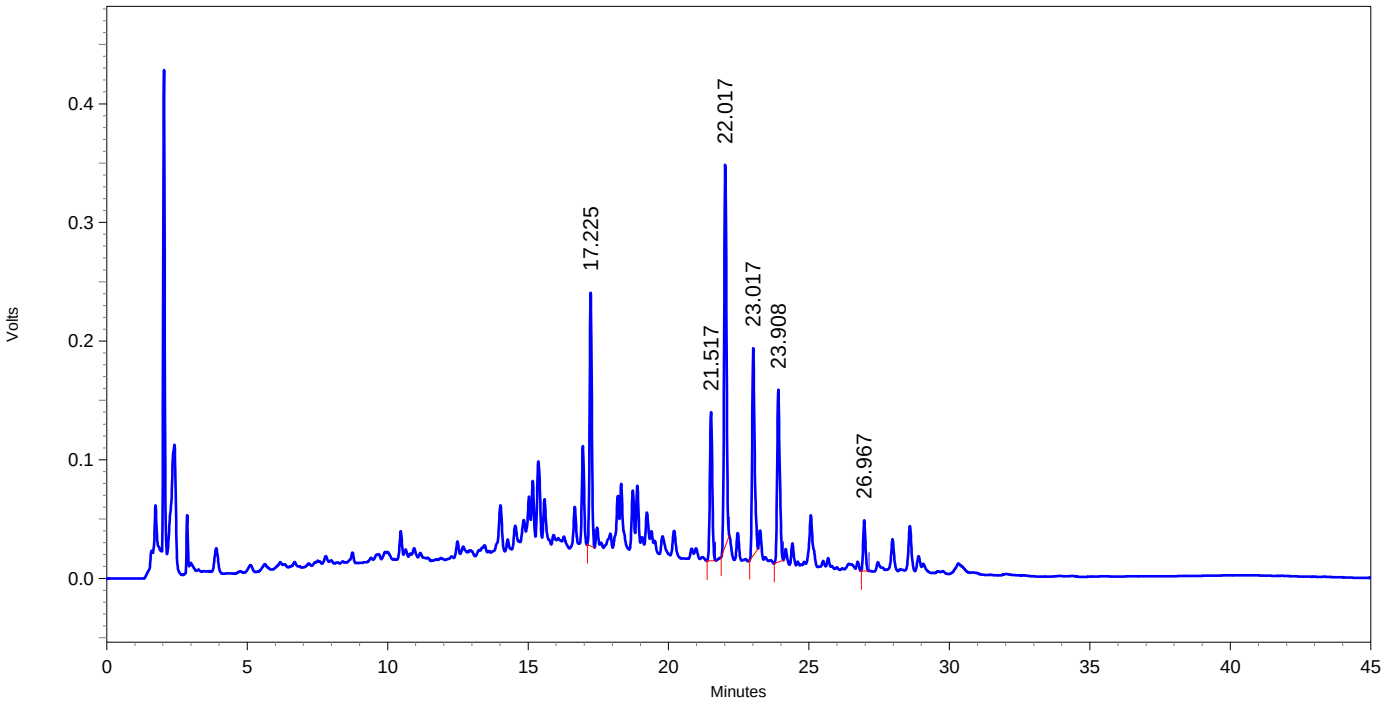
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**1178151**	**19.03**
2	**21.500**	**Withanoside V**	**770729**	**12.45**
3	**22.025**	**Withaferin A**	**1928284**	**31.15**
4	**23.017**	**Withastramonolide**	**1102671**	**17.81**
5	**23.908**	**Withanolide A**	**960958**	**15.52**
6	**26.975**	**Withanolide B**	**249257**	**4.03**

Totals			**6190050**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 10**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S4-Rep2**
Vial No	**: 10005**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 7:58:21 AM**



Detector A - 1 (227nm)

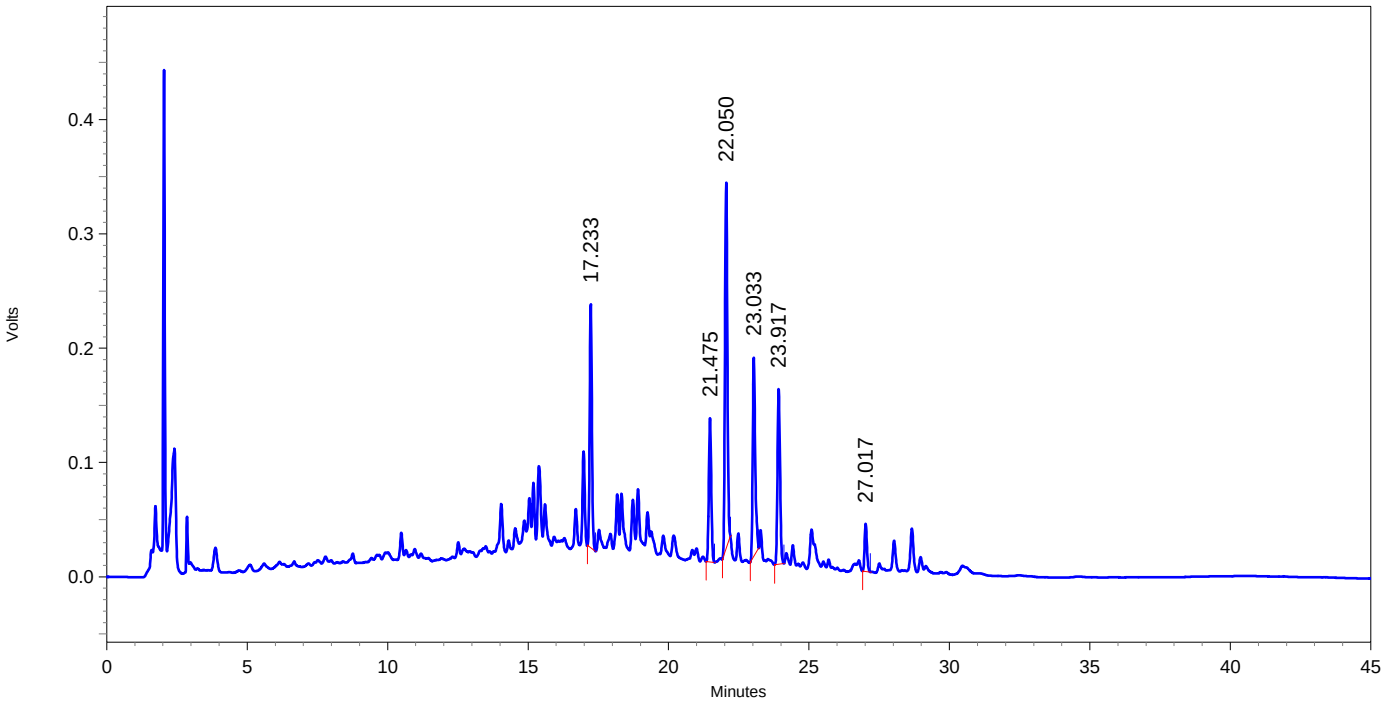
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**1150248**	**18.86**
2	**21.517**	**Withanoside V**	**757348**	**12.42**
3	**22.017**	**Withaferin A**	**1914204**	**31.38**
4	**23.017**	**Withastramonolide**	**1090591**	**17.88**
5	**23.908**	**Withanolide A**	**941431**	**15.43**
6	**26.967**	**Withanolide B**	**245613**	**4.03**

Totals			**6099435**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 06 lot 10**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S4-Rep3**
Vial No	**: 10005**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 8:44:13 AM**



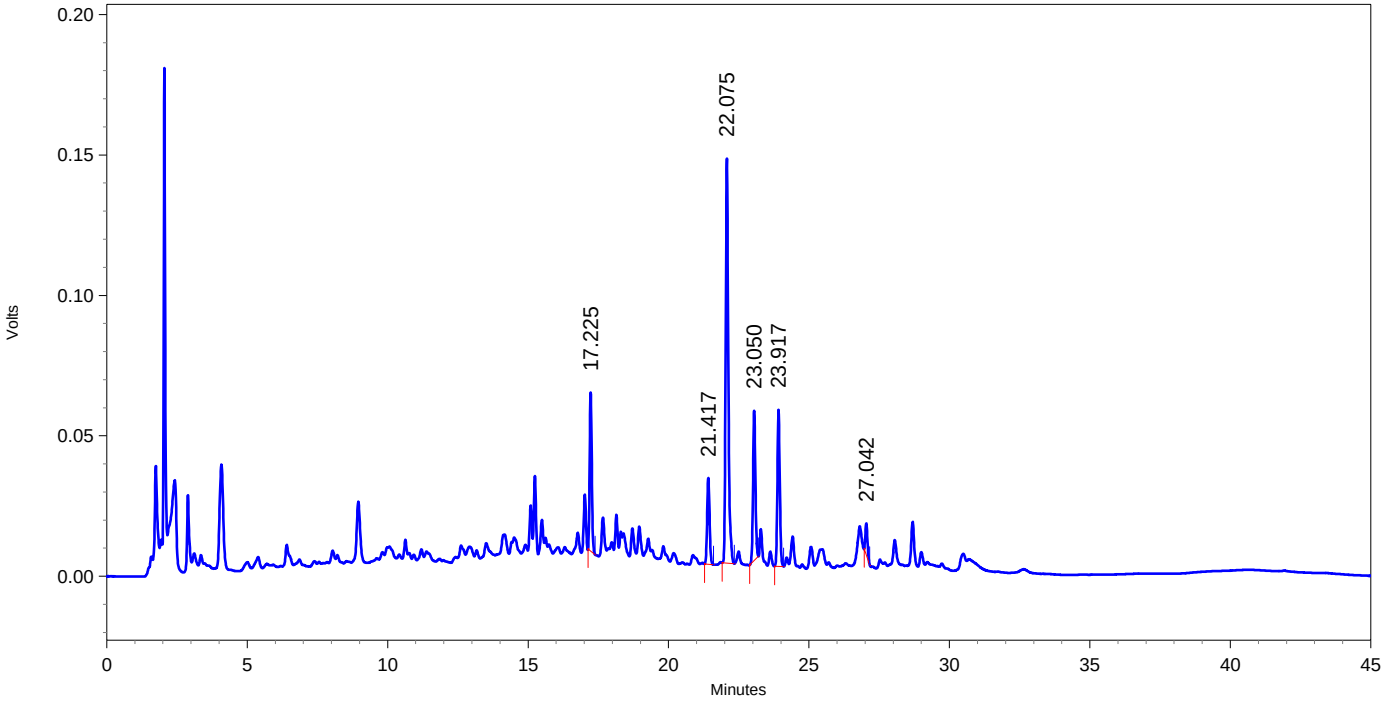
Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.233**	**Withanoside IV**	**1165114**	**18.99**
2	**21.475**	**Withanoside V**	**763045**	**12.44**
3	**22.050**	**Withaferin A**	**1917315**	**31.25**
4	**23.033**	**Withastramonolide**	**1075780**	**17.54**
5	**23.917**	**Withanolide A**	**968374**	**15.78**
6	**27.017**	**Withanolide B**	**245187**	**4.00**
Totals			**6134815**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 20**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S1-Rep1**
Vial No	**: 10002**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 9:30:05 AM**



Detector A - 1 (227nm)

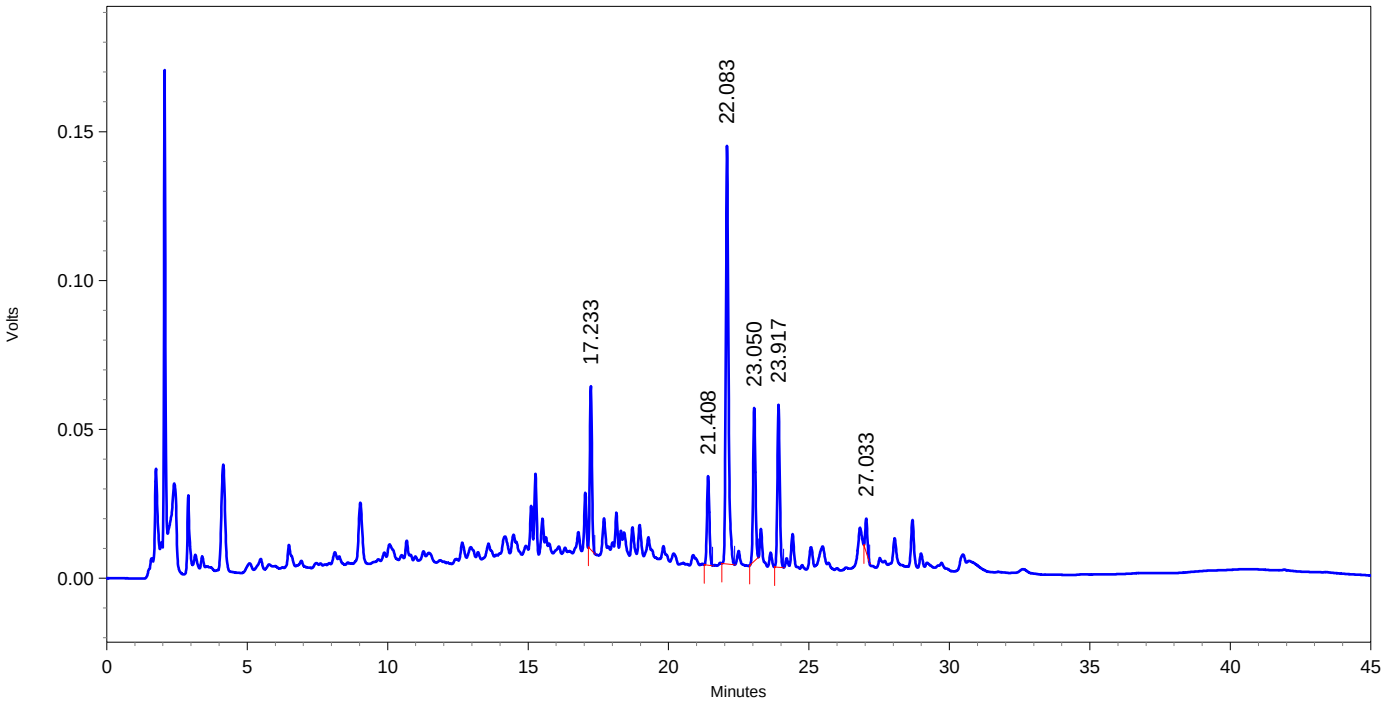
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**298076**	**13.93**
2	**21.417**	**Withanoside V**	**187974**	**8.79**
3	**22.075**	**Withaferin A**	**952512**	**44.52**
4	**23.050**	**Withastramonolide**	**311700**	**14.57**
5	**23.917**	**Withanolide A**	**330290**	**15.44**
6	**27.042**	**Withanolide B**	**58776**	**2.75**

Totals			**2139328**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 20**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S1-Rep2**
Vial No	**: 10002**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 10:15:57 AM**



Detector A - 1 (227nm)

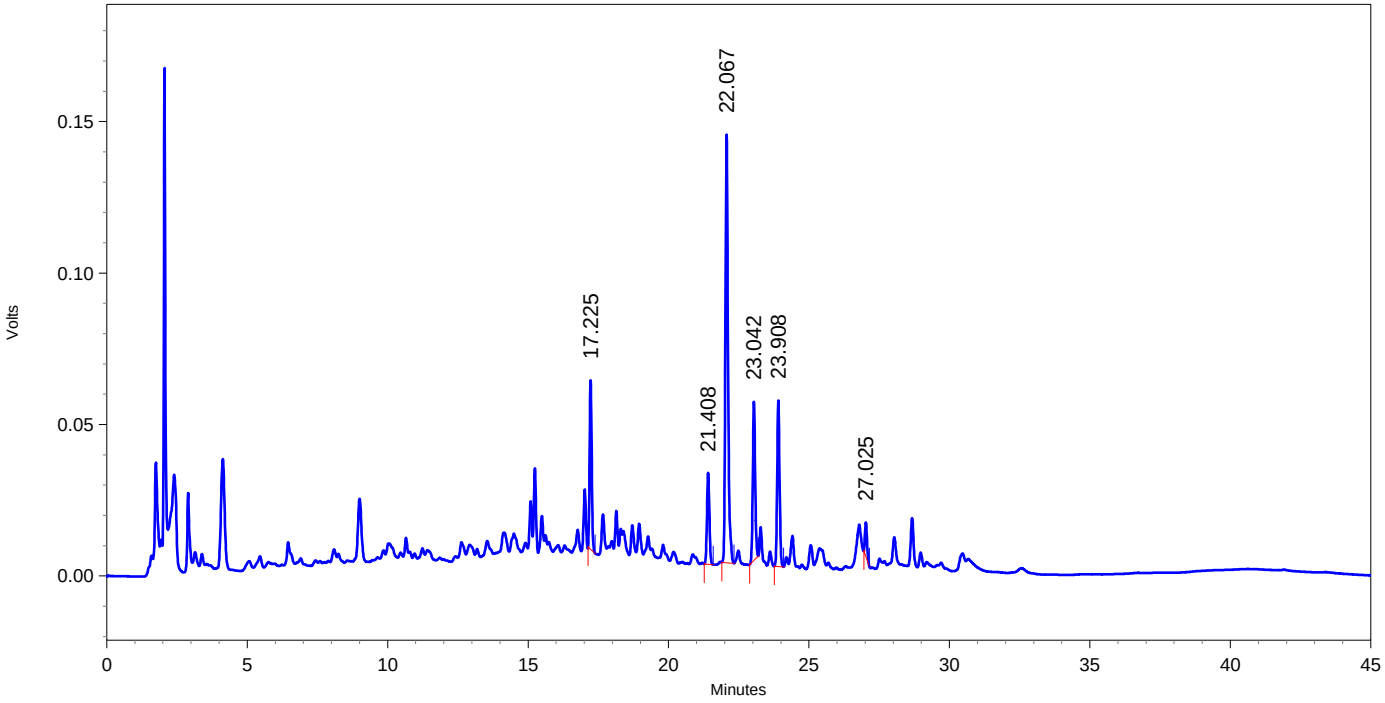
Pk #	Retention Time	Name	Area	Area Percent
1	**17.233**	**Withanoside IV**	**286199**	**13.79**
2	**21.408**	**Withanoside V**	**180229**	**8.69**
3	**22.083**	**Withaferin A**	**927184**	**44.69**
4	**23.050**	**Withastramonolide**	**300018**	**14.46**
5	**23.917**	**Withanolide A**	**321506**	**15.49**
6	**27.033**	**Withanolide B**	**59796**	**2.88**

Totals			**2074932**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 20**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S1-Rep3**
Vial No	**: 10002**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 11:01:48 AM**



Detector A - 1 (227nm)

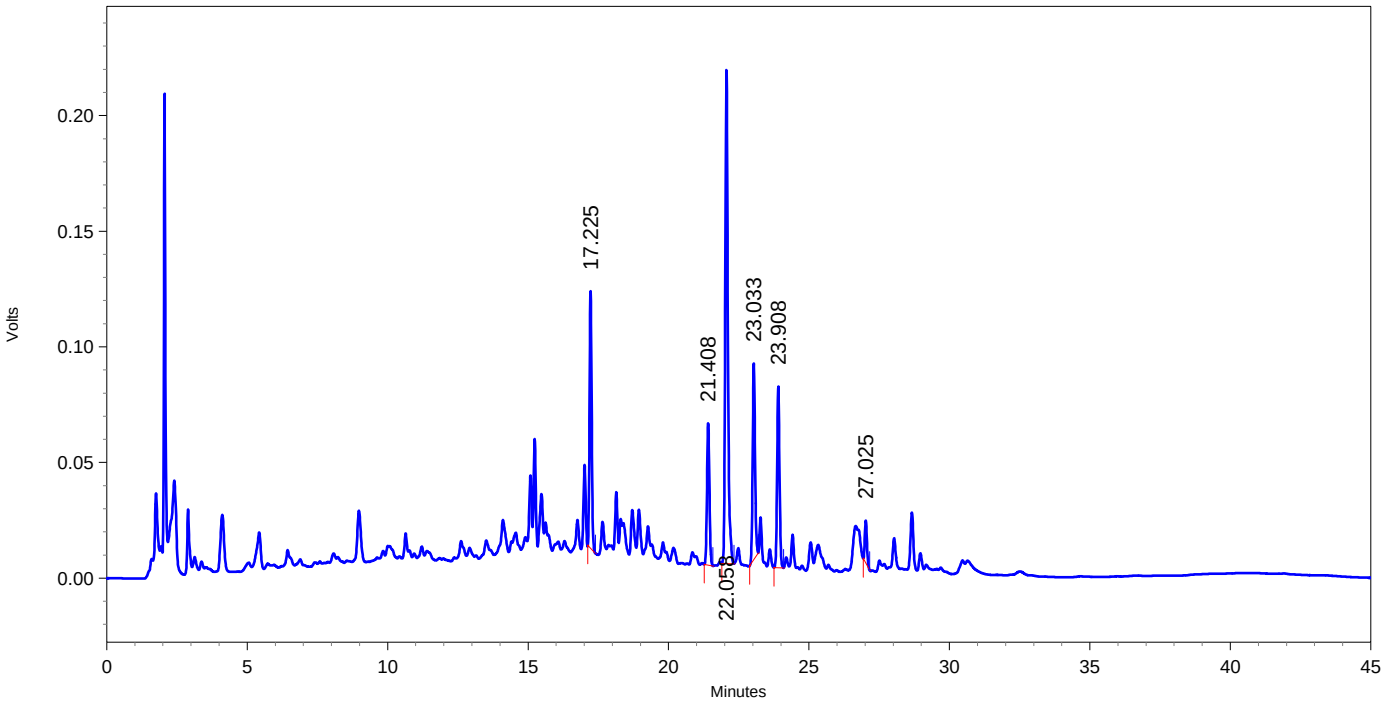
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**292499**	**13.99**
2	**21.408**	**Withanoside V**	**182887**	**8.75**
3	**22.067**	**Withaferin A**	**928052**	**44.39**
4	**23.042**	**Withastramonolide**	**304876**	**14.58**
5	**23.908**	**Withanolide A**	**323917**	**15.49**
6	**27.025**	**Withanolide B**	**58492**	**2.80**

Totals			**2090723**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 21**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S2-Rep1**
Vial No	**: 10003**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 11:47:39 AM**



Detector A - 1 (227nm)

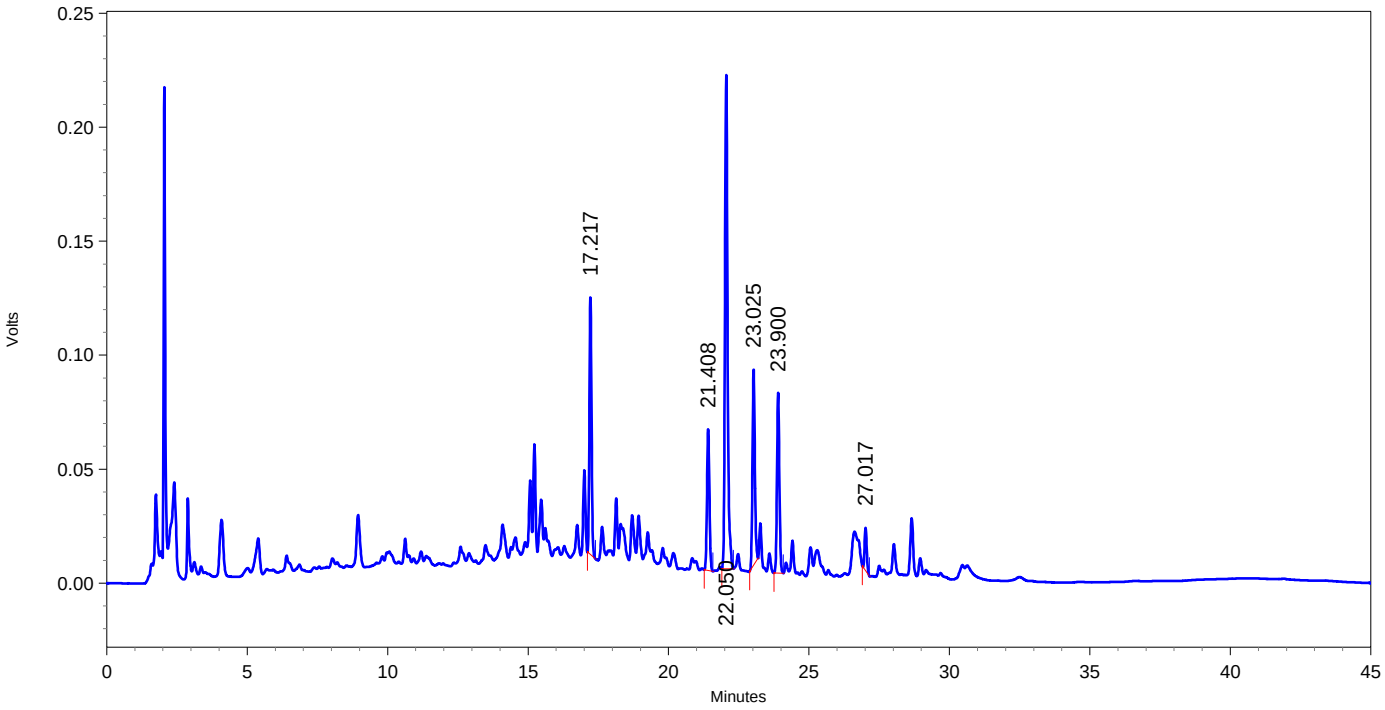
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**588934**	**17.19**
2	**21.408**	**Withanoside V**	**373582**	**10.90**
3	**22.058**	**Withaferin A**	**1403133**	**40.95**
4	**23.033**	**Withastramonolide**	**496352**	**14.49**
5	**23.908**	**Withanolide A**	**465701**	**13.59**
6	**27.025**	**Withanolide B**	**98415**	**2.87**

Totals			**3426117**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 21**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S2-Rep2**
Vial No	**: 10003**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 12:33:26 PM**



Detector A - 1 (227nm)

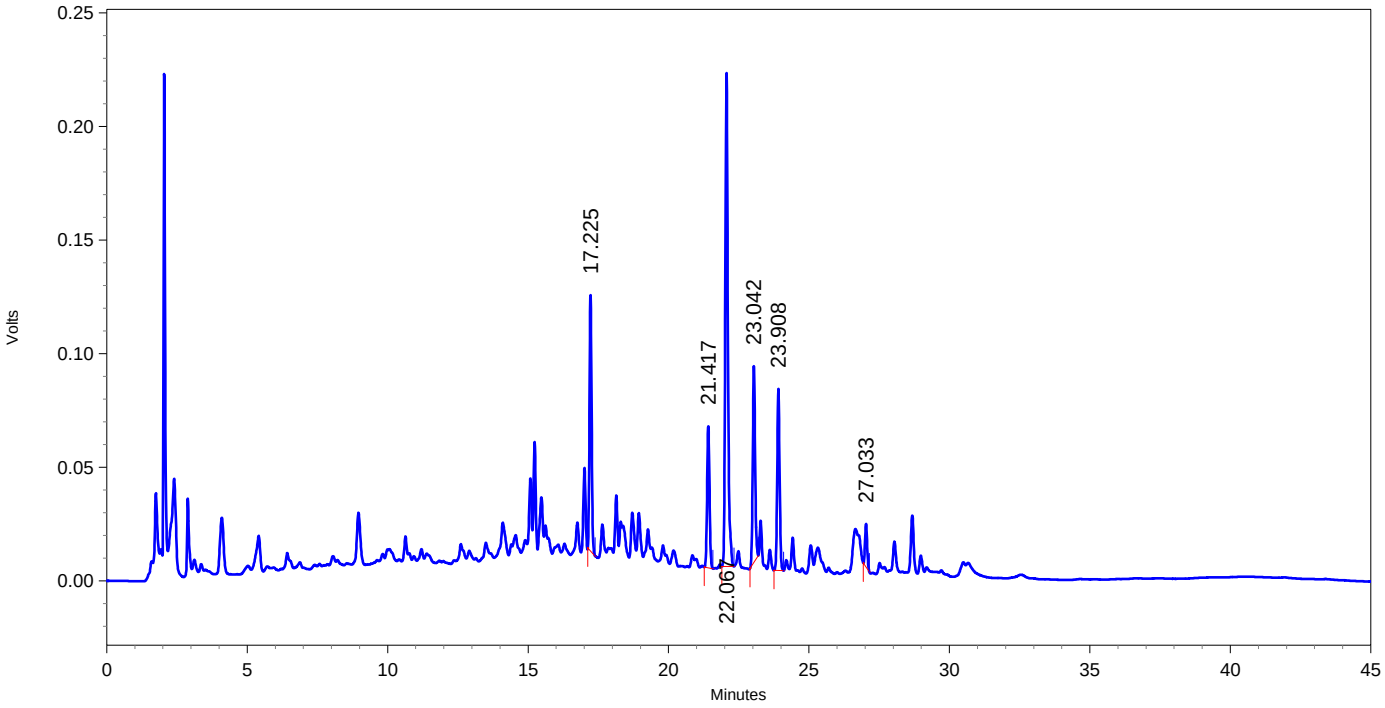
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**WIthanoside IV**	**599396**	**17.30**
2	**21.408**	**Withanoside V**	**377387**	**10.89**
3	**22.050**	**Withaferin A**	**1413249**	**40.80**
4	**23.025**	**Withastramonolide**	**502270**	**14.50**
5	**23.900**	**Withanolide A**	**471080**	**13.60**
6	**27.017**	**Withanolide B**	**100538**	**2.90**

Totals			**3463920**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : WS - 05 lot 21**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S2-Rep3**
Vial No	**: 10003**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 1:19:18 PM**



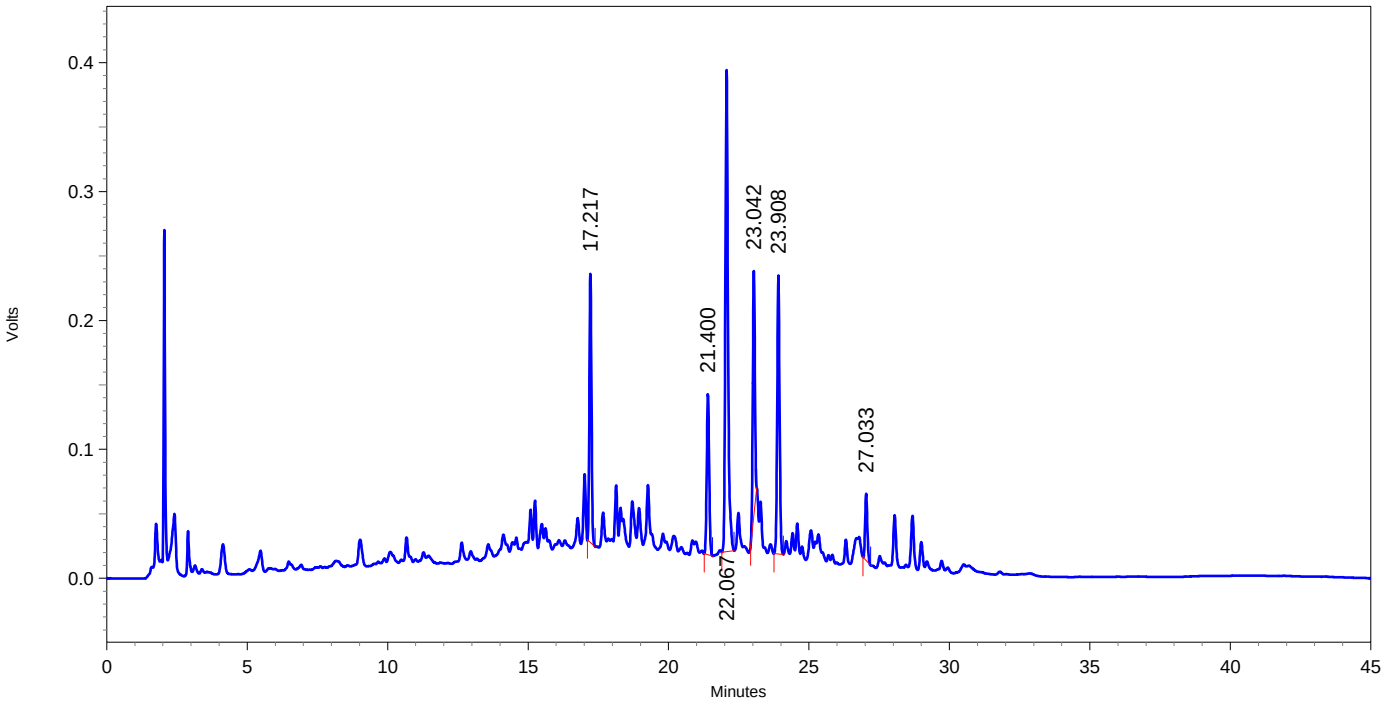
Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**WIthanoside IV**	**596170**	**17.16**
2	**21.417**	**Withanoside V**	**378348**	**10.89**
3	**22.067**	**Withaferin A**	**1422577**	**40.95**
4	**23.042**	**Withastramonolide**	**502808**	**14.47**
5	**23.908**	**Withanolide A**	**473580**	**13.63**
6	**27.033**	**Withanolide B**	**100847**	**2.90**
Totals			**3474330**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1170**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S6-Rep1**
Vial No	**: 10007**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 4:22:45 PM**



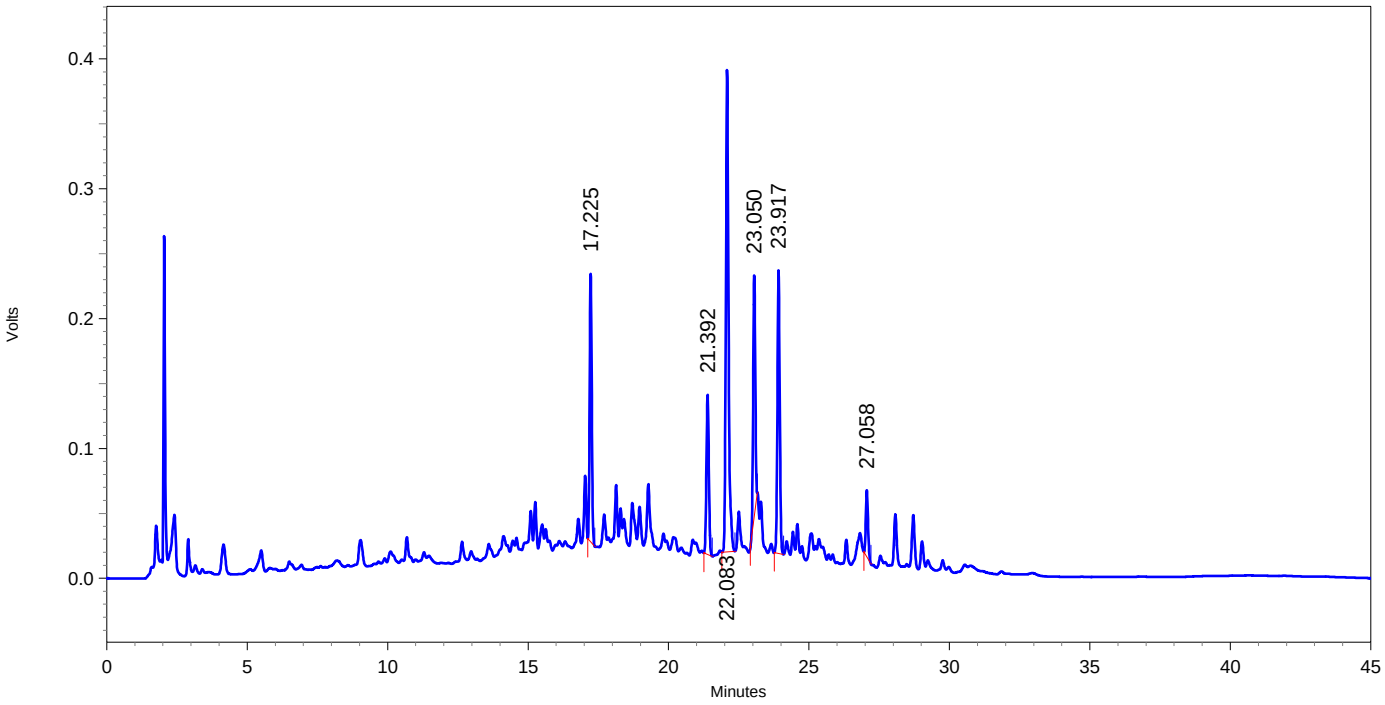
Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**WIthanoside IV**	**1093704**	**15.95**
2	**21.400**	**Withanoside V**	**749692**	**10.93**
3	**22.067**	**Withaferin A**	**2479748**	**36.17**
4	**23.042**	**Withastramonolide**	**972595**	**14.19**
5	**23.908**	**Withanolide A**	**1268626**	**18.50**
6	**27.033**	**Withanolide B**	**291589**	**4.25**
Totals			**6855954**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1170**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S6-Rep2**
Vial No	**: 10007**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 5:08:37 PM**



Detector A - 1 (227nm)

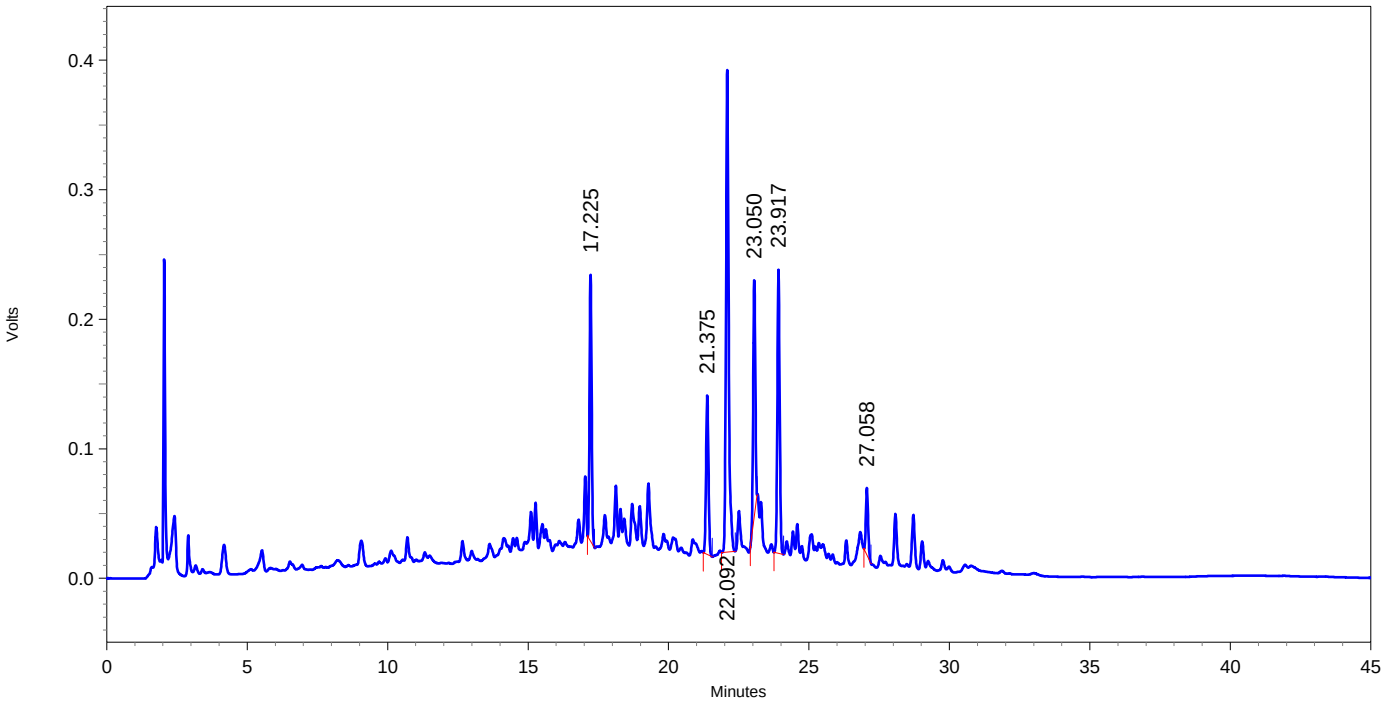
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**WIthanoside IV**	**1080249**	**15.83**
2	**21.392**	**Withanoside V**	**742686**	**10.88**
3	**22.083**	**Withaferin A**	**2475863**	**36.29**
4	**23.050**	**Withastramonolide**	**978751**	**14.34**
5	**23.917**	**Withanolide A**	**1255922**	**18.41**
6	**27.058**	**Withanolide B**	**289554**	**4.24**

Totals			**6823025**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1170**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S6-Rep3**
Vial No	**: 10007**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 5:54:29 PM**



Detector A - 1 (227nm)

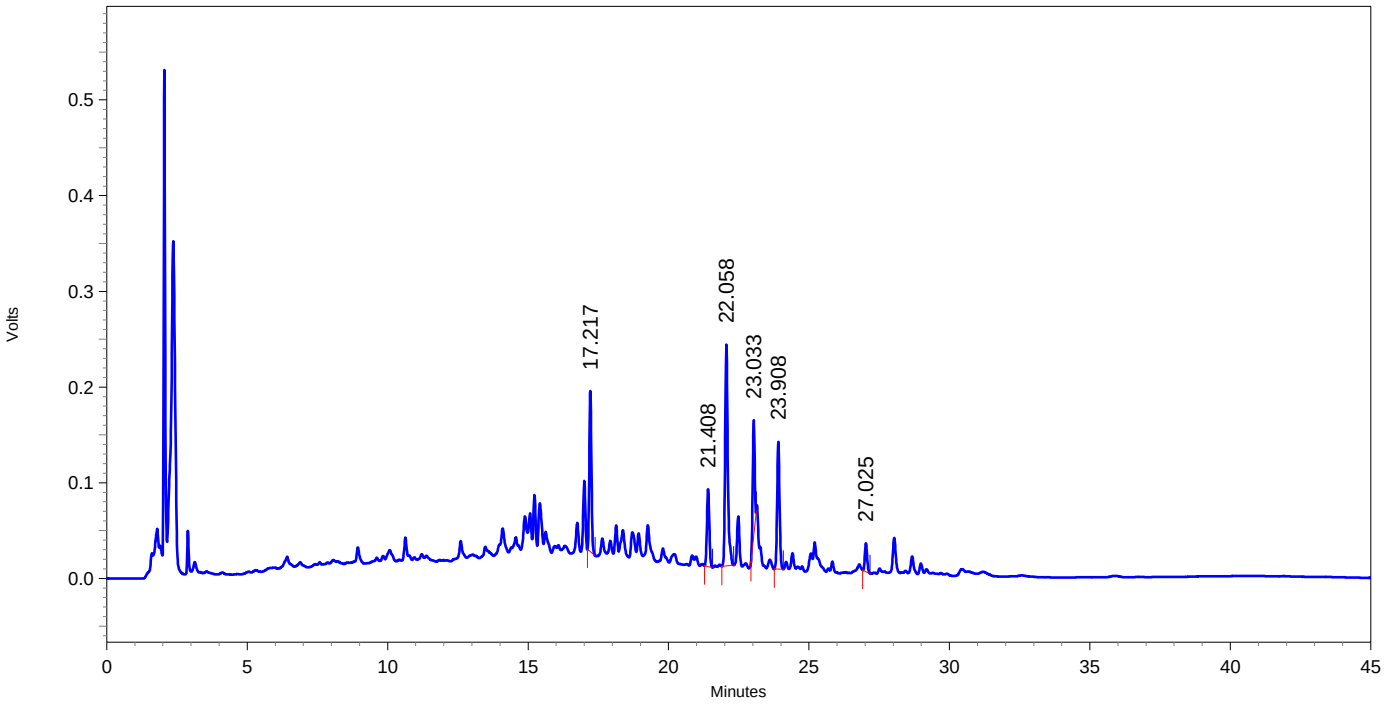
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**WIthanoside IV**	**1075861**	**15.78**
2	**21.375**	**Withanoside V**	**740290**	**10.86**
3	**22.092**	**Withaferin A**	**2484320**	**36.43**
4	**23.050**	**Withastramonolide**	**965956**	**14.17**
5	**23.917**	**Withanolide A**	**1260234**	**18.48**
6	**27.058**	**Withanolide B**	**292481**	**4.29**

Totals			**6819142**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1045**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S5-Rep1**
Vial No	**: 10006**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 2:05:10 PM**



Detector A - 1 (227nm)

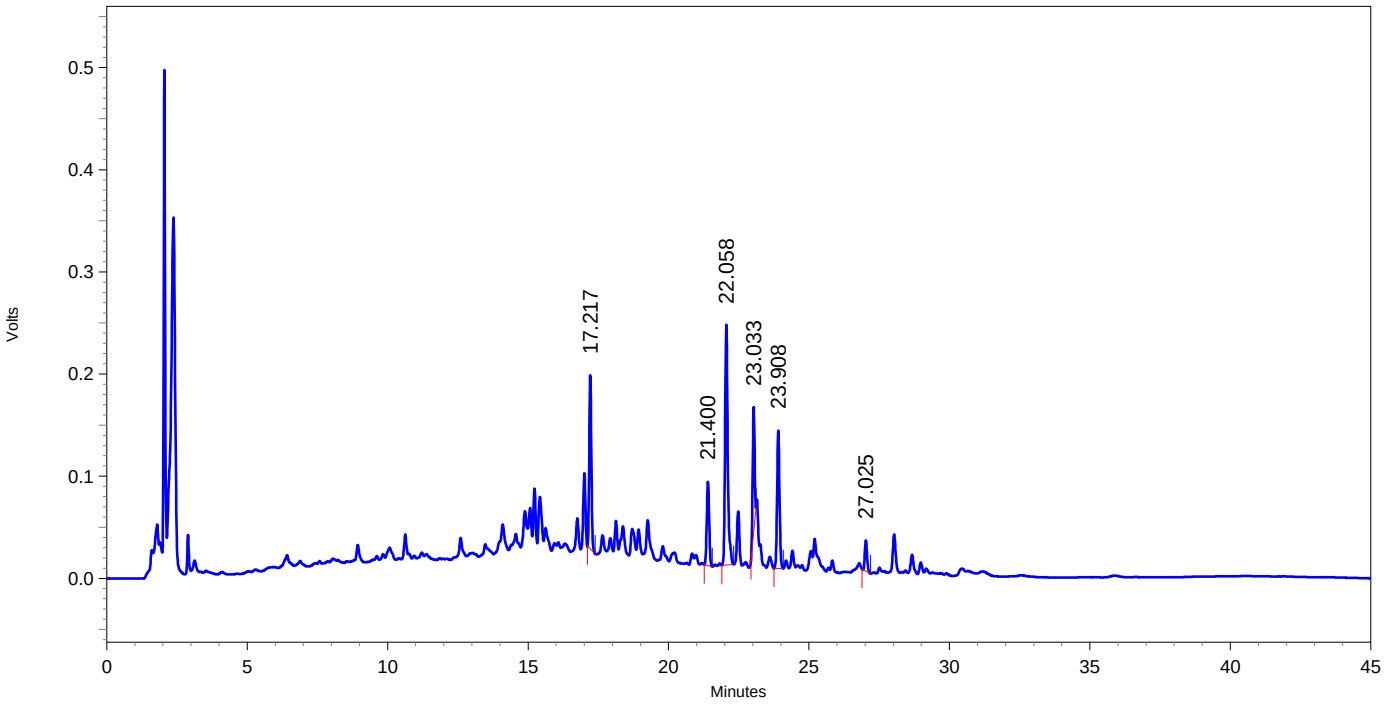
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**WIthanoside IV**	**891560**	**20.04**
2	**21.408**	**Withanoside V**	**482933**	**10.86**
3	**22.058**	**Withaferin A**	**1539138**	**34.60**
4	**23.033**	**Withastramonolide**	**572491**	**12.87**
5	**23.908**	**Withanolide A**	**793283**	**17.83**
6	**27.025**	**Withanolide B**	**168573**	**3.79**

Totals			**4447978**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1045**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S5-Rep2**
Vial No	**: 10006**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 2:51:02 PM**



Detector A - 1 (227nm)

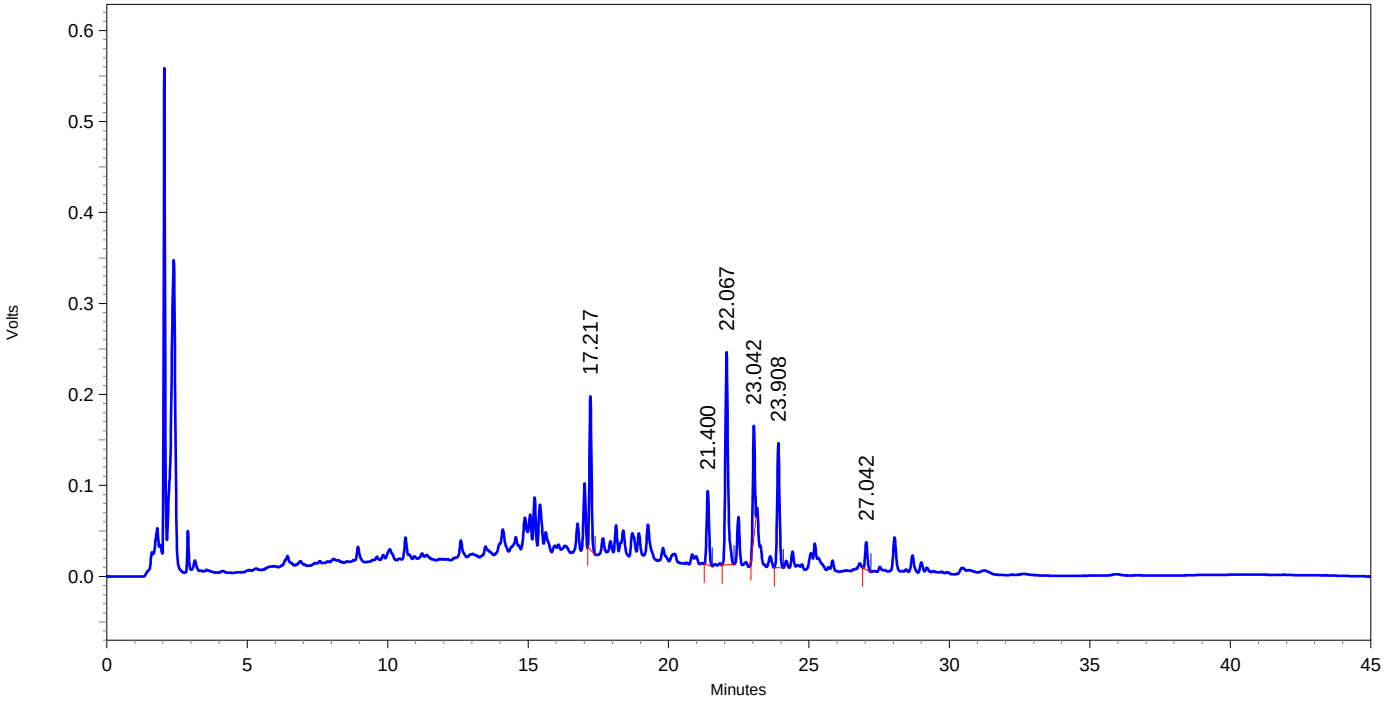
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**WIthanoside IV**	**898123**	**19.87**
2	**21.400**	**Withanoside V**	**492508**	**10.90**
3	**22.058**	**Withaferin A**	**1567533**	**34.68**
4	**23.033**	**Withastramonolide**	**584106**	**12.92**
5	**23.908**	**Withanolide A**	**807866**	**17.87**
6	**27.025**	**Withanolide B**	**169826**	**3.76**

Totals			**4519962**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1045**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S5-Rep3**
Vial No	**: 10006**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 3:36:53 PM**



Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**WIthanoside IV**	**883242**	**19.68**
2	**21.400**	**Withanoside V**	**487860**	**10.87**
3	**22.067**	**Withaferin A**	**1557209**	**34.71**
4	**23.042**	**Withastramonolide**	**585455**	**13.05**
5	**23.908**	**Withanolide A**	**802542**	**17.89**
6	**27.042**	**Withanolide B**	**170641**	**3.80**

Totals			**4486949**	**100.00**

NATURAL REMEDIES
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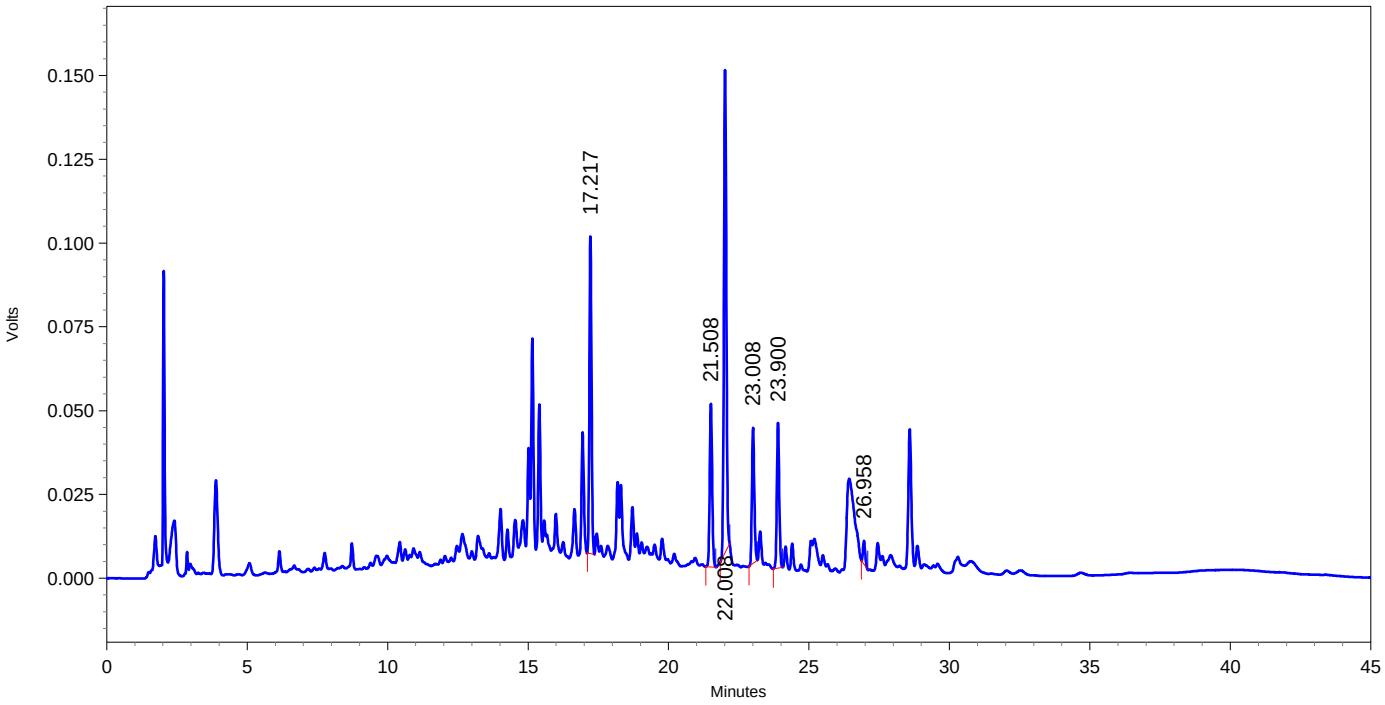
QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1162 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S7-Rep1**
Vial No	**: 10008**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 12:19:51 AM**



Detector A - 1 (227nm)

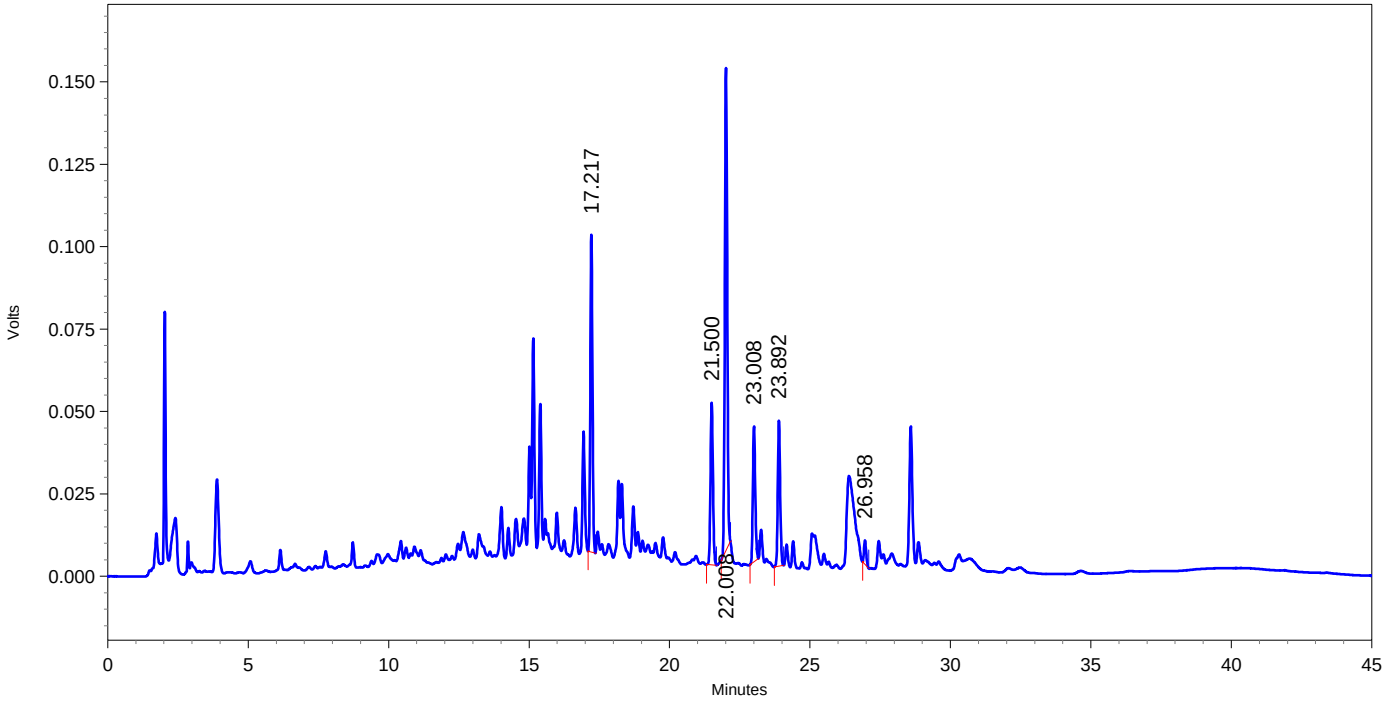
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**505117**	**22.86**
2	**21.508**	**Withanoside V**	**298751**	**13.52**
3	**22.008**	**Withaferin A**	**872090**	**39.47**
4	**23.008**	**Withastramonolide**	**239104**	**10.82**
5	**23.900**	**Withanolide A**	**255962**	**11.59**
6	**26.958**	**Withanolide B**	**38216**	**1.73**

Totals			**2209240**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1162 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S7-Rep2**
Vial No	**: 10008**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 1:05:44 AM**



Detector A - 1 (227nm)

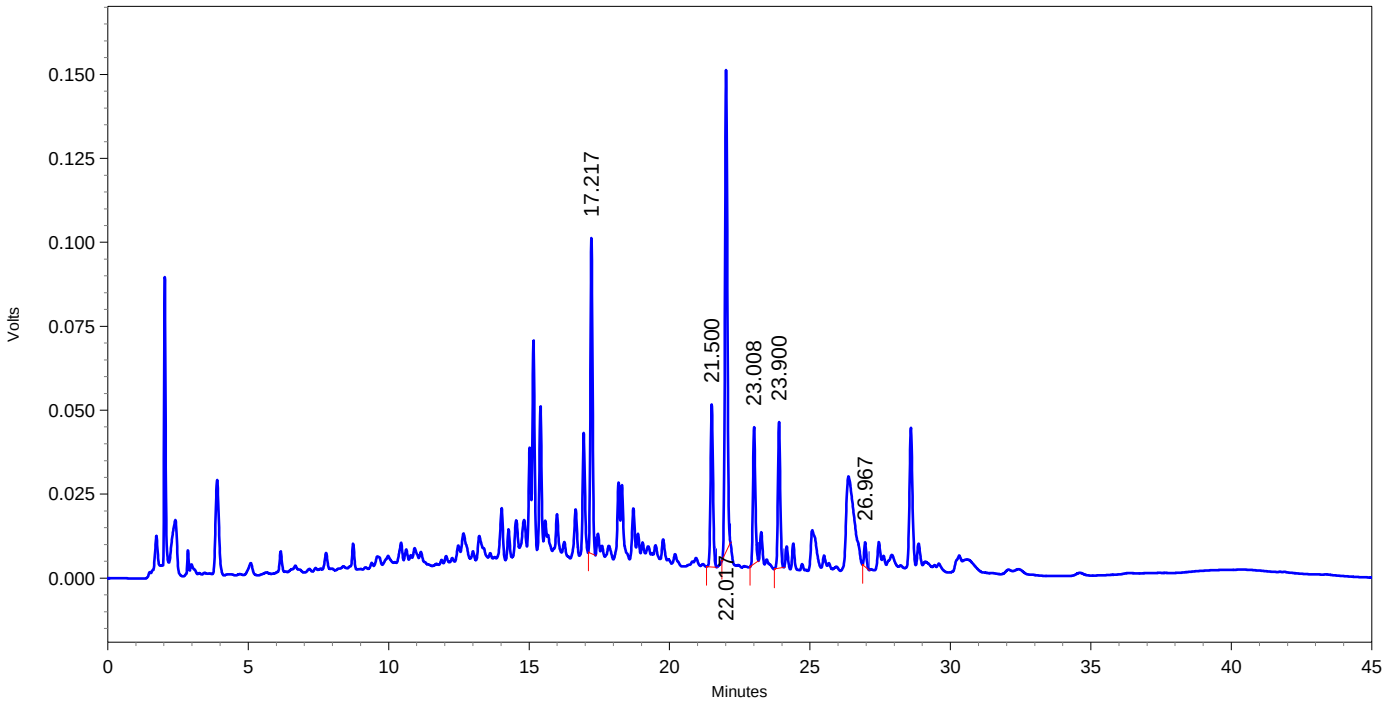
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**512636**	**22.91**
2	**21.500**	**Withanoside V**	**301908**	**13.49**
3	**22.008**	**Withaferin A**	**880299**	**39.35**
4	**23.008**	**Withastramonolide**	**243613**	**10.89**
5	**23.892**	**Withanolide A**	**259210**	**11.59**
6	**26.958**	**Withanolide B**	**39607**	**1.77**

Totals			**2237273**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : RD - 1162 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S7-Rep3**
Vial No	**: 10008**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 1:51:37 AM**



Detector A - 1 (227nm)

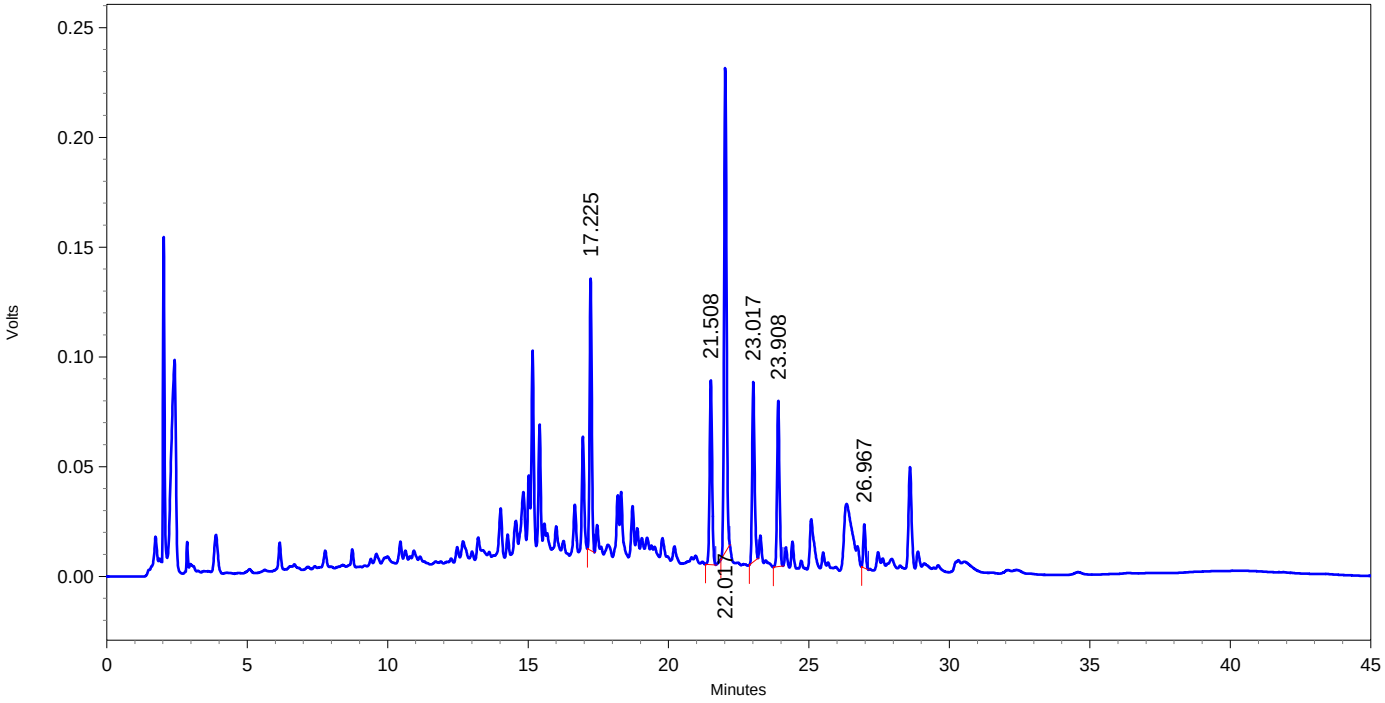
Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**503502**	**22.88**
2	**21.500**	**Withanoside V**	**296939**	**13.49**
3	**22.017**	**Withaferin A**	**863184**	**39.23**
4	**23.008**	**Withastramonolide**	**241291**	**10.97**
5	**23.900**	**Withanolide A**	**255090**	**11.59**
6	**26.967**	**Withanolide B**	**40438**	**1.84**

Totals			**2200444**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : ERH - 046 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S8-Rep1**
Vial No	**: 10009**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 2:37:28 AM**



Detector A - 1 (227nm)

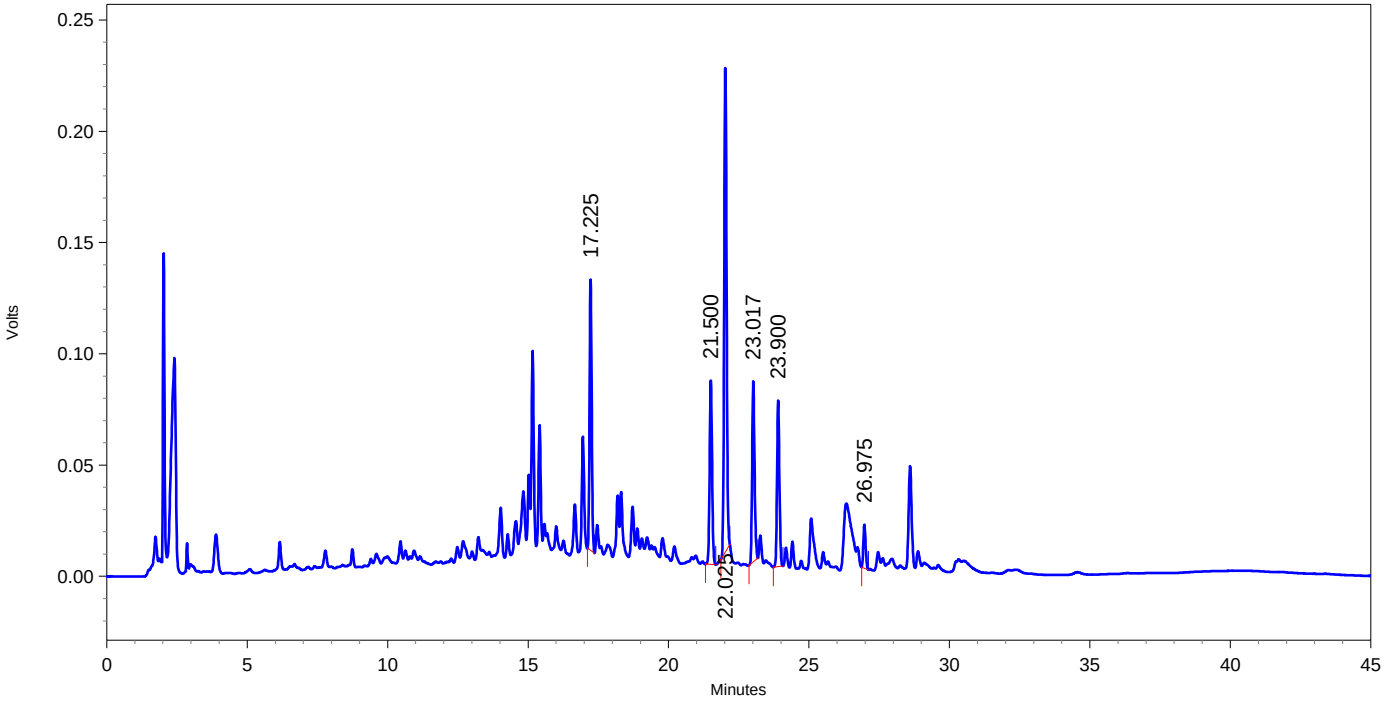
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**657778**	**18.46**
2	**21.508**	**Withanoside V**	**517598**	**14.52**
3	**22.017**	**Withaferin A**	**1338712**	**37.56**
4	**23.017**	**Withastramonolide**	**492426**	**13.82**
5	**23.908**	**Withanolide A**	**444196**	**12.46**
6	**26.967**	**Withanolide B**	**113041**	**3.17**

Totals			**3563751**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : ERH - 046 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S8-Rep2**
Vial No	**: 10009**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 3:23:20 AM**



Detector A - 1 (227nm)

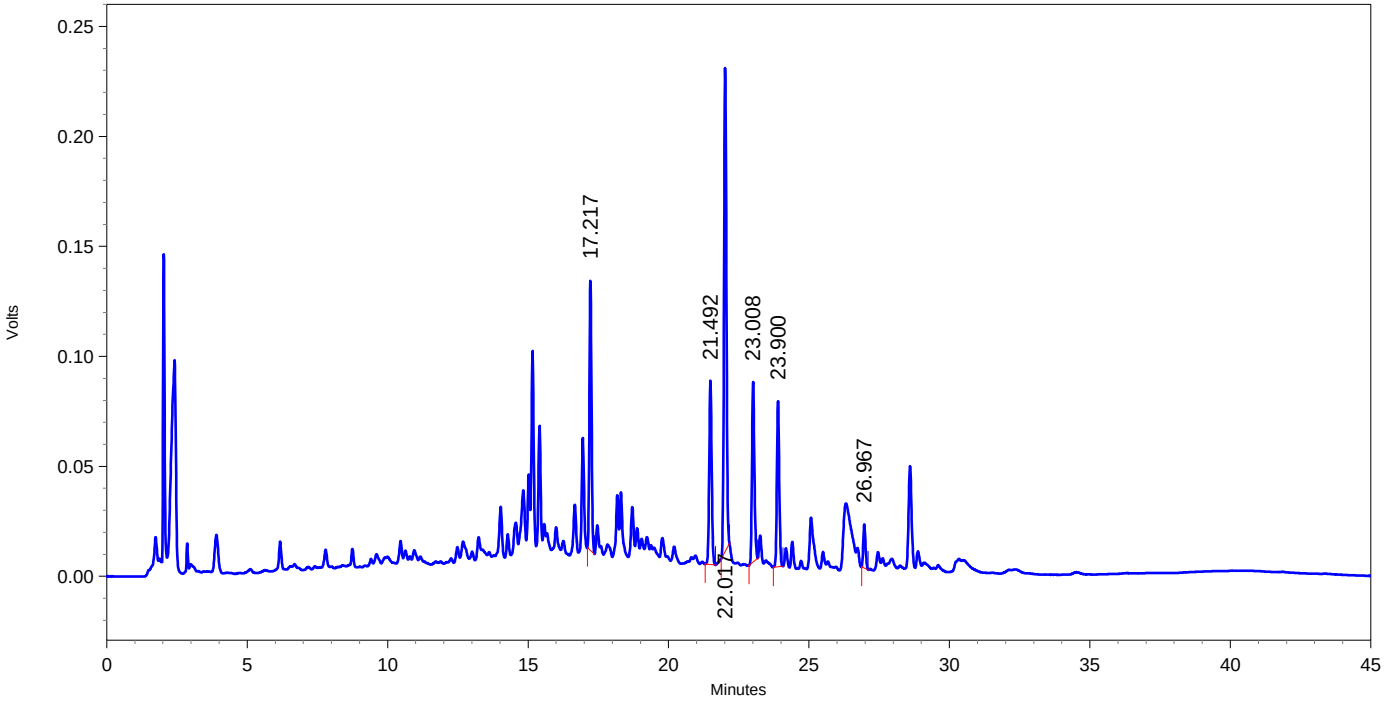
Pk #	Retention Time	Name	Area	Area Percent
1	**17.225**	**Withanoside IV**	**646445**	**18.42**
2	**21.500**	**Withanoside V**	**509223**	**14.51**
3	**22.025**	**Withaferin A**	**1318826**	**37.57**
4	**23.017**	**Withastramonolide**	**485660**	**13.84**
5	**23.900**	**Withanolide A**	**438254**	**12.48**
6	**26.975**	**Withanolide B**	**111940**	**3.19**

Totals			**3510348**	**100.00**

NATURAL REMEDIES
PRIVATE LIMITED

QUALITY CONTROL DEPARTMENT

Name of Sample	**: Withania somnifera : ERH - 046 : RM**
Method Name	**: C:\Methods\WITHANIA.met**
Data File Name	**: D:\LC10-ATvp\2007\Method validation\Apr 2007\020407\S8-Rep3**
Vial No	**: 10009**
Injection Volume	**: 20**
Date & Time	**: 4/4/07 4:09:12 AM**



Detector A - 1 (227nm)

Pk #	Retention Time	Name	Area	Area Percent
1	**17.217**	**Withanoside IV**	**654341**	**18.51**
2	**21.492**	**Withanoside V**	**513695**	**14.53**
3	**22.017**	**Withaferin A**	**1323105**	**37.42**
4	**23.008**	**Withastramonolide**	**490071**	**13.86**
5	**23.900**	**Withanolide A**	**442221**	**12.51**
6	**26.967**	**Withanolide B**	**112454**	**3.18**

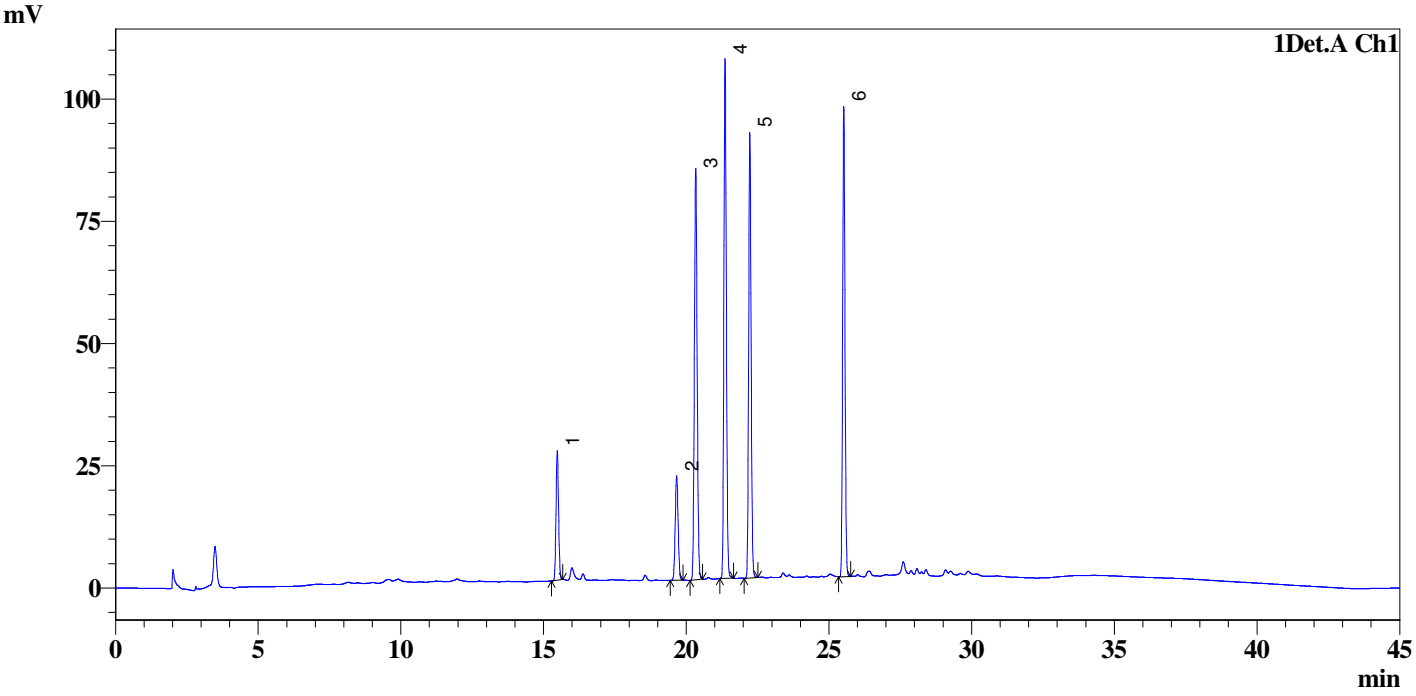
Totals			**3535887**	**100.00**

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania : std mix**
Sample ID	**: Dilution : VI ; 0 hrs solution stability**
Vail #	**: 7**
Injection Volume	**: 20 uL**
Data File Name	**: Lin003.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch 01.lcb**
Report File Name	**: Calibration.lcr**
Data Acquired	**: 1/14/2007 2:08:21 AM**
Data Processed	**: 3/17/2007 3:36:01 PM**

Enclosure: 13

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Lin003.lcd



1 Det.A Ch1/227nm

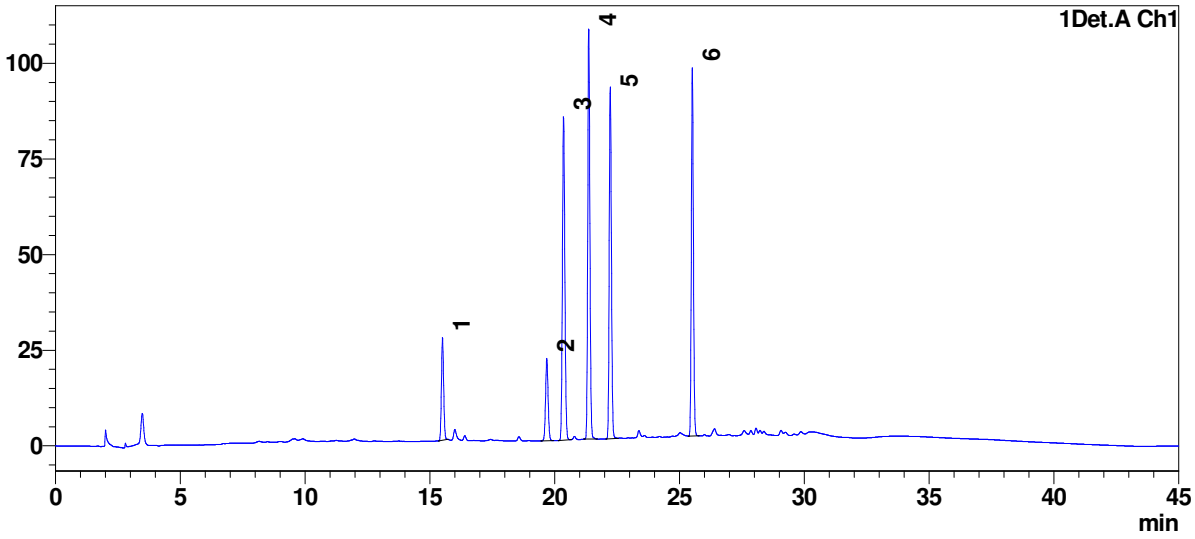
Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.475	Withanoside IV	165895	6.311	26495	6.226
2	19.658	Withanoside V	151156	5.751	21357	5.019
3	20.327	Withaferin A	573021	21.800	84126	19.769
4	21.357	12- Deoxy withastramonolide	634904	24.155	106253	24.969
5	22.224	Withanollide A	550930	20.960	91150	21.420
6	25.515	Withanolide B	552601	21.023	96154	22.596
Total			2628507	100.000	425535	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania : std mix
Sample ID	: Dilution : VI : Soln stab:24hrs
Vail #	: 7
Injection Volume	: 20 uL
Data File Name	: Std-ss01.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/15/2007 3:26:39 AM
Data Processed	: 1/20/2007 5:04:14 PM

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Std-ss01.lcd



et.A Ch1/227nm

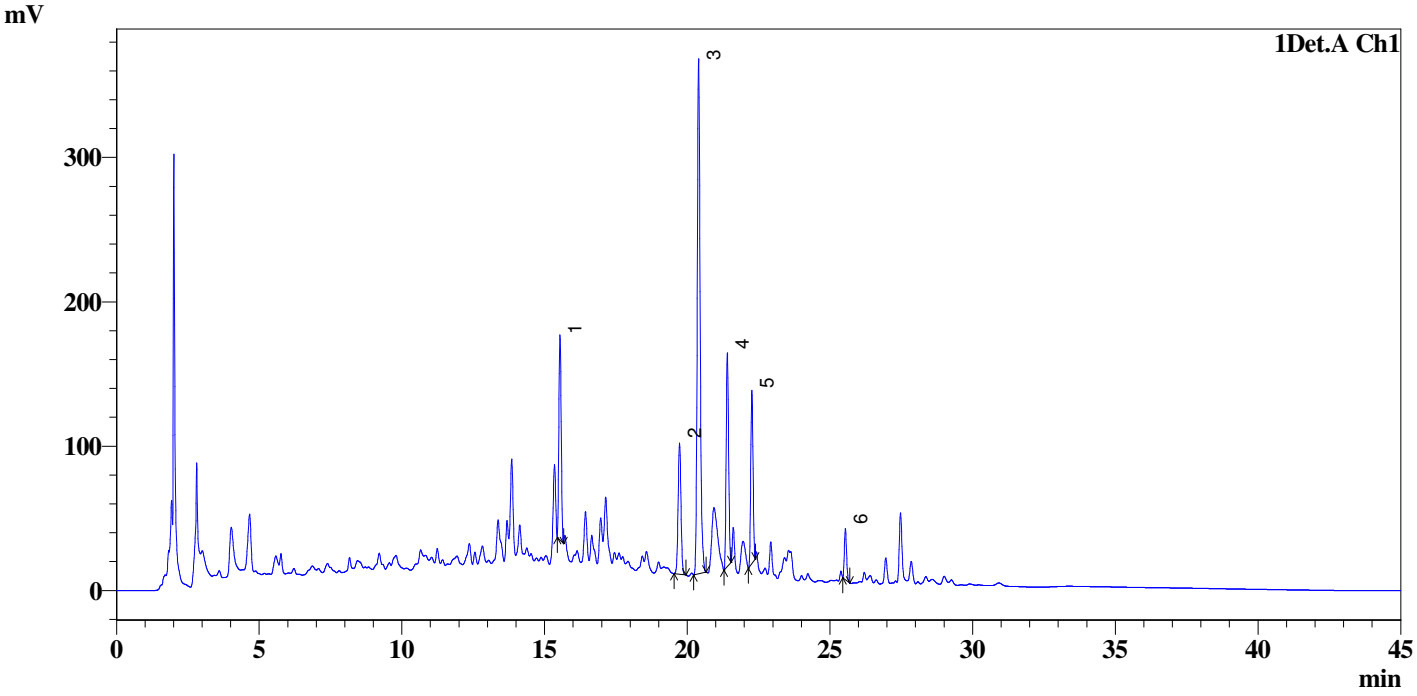
Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.506	167920	26838	6.329	Withanoside IV
2	19.685	152720	21510	5.756	Withanoside V
3	20.357	576614	84572	21.734	Withaferin A
4	21.366	642763	107132	24.228	12- Deoxy withastramonolide
5	22.228	555790	91881	20.949	Withanollide A
6	25.515	557192	96219	21.002	Withanolide B
Total		2653000	428151	100.000	

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	**: Admin**
Sample Name	**: Withania somnifera ext**
Sample ID	**: WS - 05 lot 21 : 0hrs - Solution stability**
Vail #	**: 15**
Injection Volume	**: 20 uL**
Data File Name	**: Quan002.lcd**
Method File Name	**: Withaina.met.lcm**
Batch File Name	**: Batch 01.lcb**
Report File Name	**: Calibration.lcr**
Data Acquired	**: 1/15/2007 8:46:51 AM**
Data Processed	**: 1/23/2007 2:47:29 AM**

D:\LC2010AHT\2007\Method validation\Withania somnifera\Jan 2007\Quan002.lcd



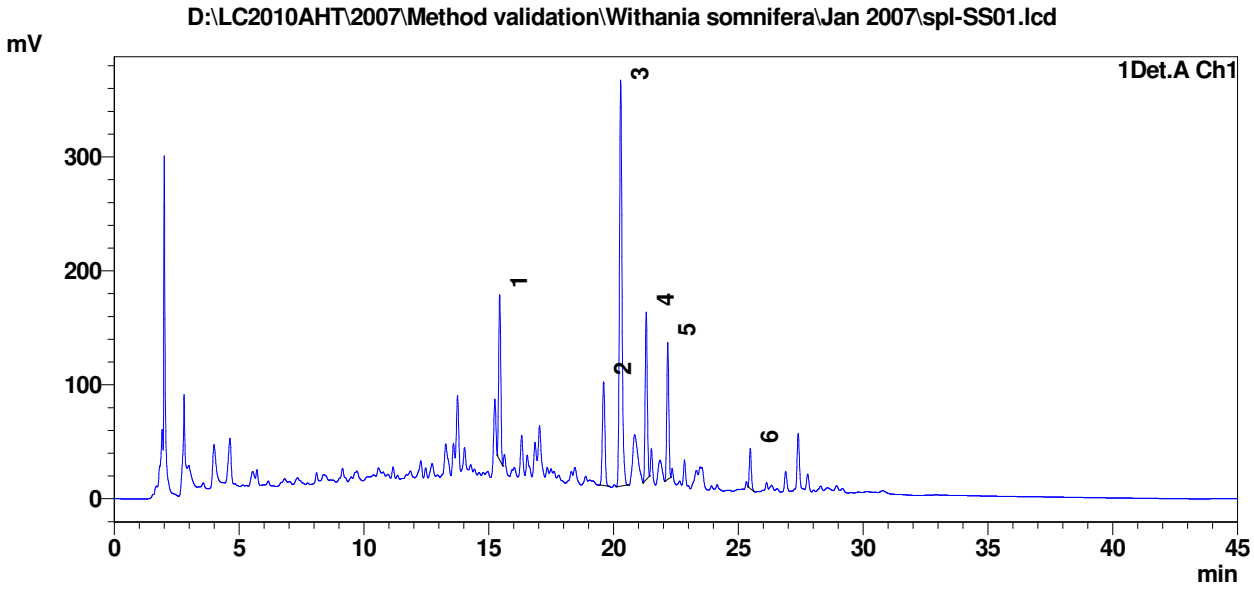
1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Height	Height %
1	15.537	Withanoside IV	806225	14.131	142188	15.915
2	19.725	Withanoside V	637065	11.166	90668	10.148
3	20.395	Withaferin A	2532764	44.392	356934	39.951
4	21.401	12- Deoxy withastramonolide	865621	15.172	148099	16.576
5	22.261	Withanollide A	670597	11.754	120136	13.446
6	25.537	Withanolide B	193202	3.386	35413	3.964
Total			5705473	100.000	893439	100.000

NATURAL REMEDIES PVT LIMITED
QUALITY CONTROL DEPARTMENT

Acquired by	: Admin
Sample Name	: Withania somnifera ext
Sample ID	: WS - 05 LOT 21 :Soln stab
Vail #	: 15
Injection Volume	: 20 uL
Data File Name	: spl-SS01.lcd
Method File Name	: Withaina.met.lcm
Batch File Name	: Batch 01.lcb
Report File Name	: Calibration.lcr
Data Acquired	: 1/16/2007 9:10:52 AM
Data Processed	: 1/23/2007 3:20:28 AM



1 Det.A Ch1/227nm

Detector A Ch1 227nm

Peak #	Ret. Time	Area	Height	Area %	Name
1	15.440	824770	144847	14.362	Withanoside IV
2	19.605	629870	90882	10.968	Withanoside V
3	20.287	2544347	356312	44.307	Withaferin A
4	21.310	863998	147275	15.045	12- Deoxy withastramonolide
5	22.174	687063	120359	11.964	Withanollide A
6	25.479	192537	35181	3.353	Withanolide B
Total		5742585	894855	100.000	

NATURAL REMEDIES
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QUALITY CONTROL DEPARTMENT

HPLC METHOD VALIDATION REPORT

NR/QCD/MVR/11

ESTIMATION OF WITHANOLIDES (WITHANOSIDE IV, WITHANOSIDE V, WITHAFERIN A, 12-DEOXY WITHASTROMONOLIDE, WITHANOLIDE A, WITHANOLIDE B) IN *WITHANIA SOMNIFERA*

[NR/QCD/APM04 WI(17)]

Method validation carried out by	**:**	**Mr.Rojison Koshy – Sr. Research Officer**
Report Verified by	**:**	**Mr. M. S.Anand – Dy. Manager - QC**
Approved by	**:**	**Mr. B. Murali - AGM (QC/QA)**

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HPLC METHOD VALIDATION
ESTIMATION OF Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B *WITHANIA SOMNIFERA* (ROOTS) AND ITS EXTRACT

1.0] SUMMARY:

1.1] Introduction: The validation of an analytical procedure is the process of confirming the performance of the analytical procedure employed for the test. The performance of the analytical procedure is established by various kinds of validation characteristics such as Specificity, Linearity, Range of quantification, Accuracy, precision, Repeatability & System suitability. The validity of a proposed analytical procedure is shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standards set up according to the acceptable limits of testing.

An HPLC method [NR/QCD/APM04 WI(17)] for estimation of Withanolides (sum of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B) in *Withania somnifera* and its extract was validated using Shimadzu High Performance Liquid Chromatography system, model: LC 2010A with system controller unit, degasser unit, low pressure gradient unit up to 4 solvents, pump unit with seal unit mechanism mixer, auto sampler, column oven, UV-VIS detector, 220V 50Hz with LC solution software and another Shimadzu LC10A system equipped with binary LC10ADVP pump, with SIL10AD*vp* auto sampler, SPD-M 10A*vp* Photo Diode Array Detector, SCL 10AVP system controller, CTO 10AVP column oven in combination with Class VP software version 6.03.

Column containing octadecylsilane C18, 5µ particle size was used to separate Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B from the compounds present in *Withania somnifera*. A gradient mixture of HPLC phosphate buffer and Acetonitrile (55 : 45) were used as mobile phase to elute the compounds. Chromatograms were recorded at 227nm using UV and SPD-M 10A*vp* Photo diode Array Detector.

Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B were separated from the other secondary metabolites present in the plant indicating specificity. Solutions of five different concentrations of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide-B were prepared and injected to find out the linearity and reproducibility. Different samples containing different concentrations of Withanoside IV, Withanoside V, Withaferin A, 12-

Deoxy withastramonolide, Withanolide A and Withanolide-B were injected to determine the precision. Spike recovery study was conducted by adding known quantity of pure compounds to the extract and to find the recovery. Lowest concentration of standard mix solution was prepared and injected to find out the detection limit**.** The results are tabulated below.

1.2] SUMMARY REPORT OF VALIDATION:

Specificity

Very specific for Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy Withastramonolide, Withanolide A & Withanolide B with no interference from other compounds. Confirmed by the PDA spectra and the peak purity.

Peak purity > 0.98

Ruggedness

One sample, 2 different HPLC columns, 2 different conditions and 2 different instruments

Acceptance criterion: RSD = < 6.0%

Actual RSD% = 5.0%

Accuracy

Acceptance criterion for recovery:
75 to 125% for assay > 0.1 and < 0.5%.
85 to 120% for assay > 0.1 and < 0.5%.
70 to 130% for assay < 0.1%.

Compound	Recovery
Withanoside IV	94.6 to 101.8%
Withanoside V	95.3 to 101.2%
Withaferin A RSD	95.9 to 100.7%
12-Deoxy Withastramonolide	91.2 to 101.6%
Withanolide A	93.7 to 99.6%
Withanolide B	74.0 to 106.0%

Precision (of Concentration, Retention time and Reproducibility)

7 concentrations of each, 5 replicates of each

Acceptance criterion: RSD = < 2.5%

Compound	RSD	
	Retention time	Peak area
Withanoside IV	0.62	0.5%
Withanoside V	0.55	0.86%
Withaferin A	0.49	0.5%
12-Deoxy Withastramonolide	0.38	0.6%
Withanolide A	0.34	1.5%
Withanolide B	0.21	0.9%

Range of quantification

The following limits are considered as a better working range based on linearity and closeness (within ± 5%) to mean response factor.

Compound	Concentration (mcg/ml)
Withanoside IV	20.64 – 330.3
Withanoside V	18.76 – 300.15
Withaferin A	23.05 – 184.39
12-Deoxy Withastramonolide	22.21 – 177.65
Withanolide A	21.59 – 172.76
Withanolide B	21.09 – 168.79

Stability of Sample & Standard Solutions

Sample solution tested after 24 hrs. The variation in assay found to be + 3.2%

The solution is stable for 24 hours.
Standard solution is also stable for 24 hours.

Linearity

7 concentrations of each, 5 replicates of each

Acceptance criterion: Correlation coefficient (r^2) for all compounds to be >0.98

Compound	r^2	Concentration range(mcg/ml)
Withanoside IV	0.999	20.0 to 1321
Withanoside V	0.999	18.7 to 1200
Withaferin A	0.996	23.0 to 737
12-Deoxy Withastramonolide	0.990	22.0 to 355
Withanolide A	0.992	21.0 to 345
Withanolide B	0.984	21.0 to 337

System suitability

1) The resolution is >3 for Withanoside V and Withaferin A in the standard mix.
2) The asymmetric factor (tailing factor) should be < 1.5
3) The precision of injection (with minimum of 3 replicates) RSD should be < 2.5%.
4) Relative retention time of

Withanoside IV	-	0.7
Withanoside V	-	0.89
Withaferin A	-	0.92
12-Deoxy Withastramonolide	-	0.96
Withanolide A	-	1.0
Withanolide B	-	1.15

Conclusion: The HPLC method [NR/QCD/APM04 WI(17)] for the estimation of *Withania somnifera* is validated.

2.0] Introduction

2.1) Objective: To ensure the method [(NR/QCD/APM04 WI(17) - estimation of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide-B in *Withania somnifera*)] developed by Natural Remedies Private Limited provides high degree of assurance that it will consistently produce result which is accurate, precise, reproducible and robust when performed at Natural Remedies Private Limited or at any other laboratory by competent analyst using appropriate equipment, chemicals and reference standards.

2.2) Scope: This method of estimation of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B, and total Withanolides using Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B as reference standards applicable to *Withania somnifera* roots (raw material) and its extract. These compounds are ranging from 0.01 to 10%. It is applicable to all material having matrix similar to that of *Withania somnifera* roots (raw material) and extracts / products containing *Withania somnifera* extracts blended with excipients which are insoluble in methanol and acetonitrile.

2.3) Purpose and application: *Withania somnifera* extract is prepared by extracting *Withania somnifera* roots with solvents like methanol, water etc. It is standardized to 1.5%, 2.5%, 3%, 5% Total withanolides. In general the content of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B would be 20 to 30% of Total withanolides. It is important to estimate the Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide- B contents to ensure the product meets the specification before its release to the market. Also the raw material used for extraction to be estimated for withanolides content as the product quality depends on raw material used for extraction. Withanolides (Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide- B) can be estimated only after clearly separating the various secondary metabolites present in the raw material or in the extract along with the withanolides. This could be achieved only by employing chromatographic techniques. Hplc is more suitable for this purpose.

The purpose of this method validation is to establish documental evidence which provides adequate assurance that the method comply with the requirements of validation characteristics suggested in the protocol which was prepared based on international guidelines. The purpose is also to demonstrate that the method performs in accordance to the claims made.

2.4) Procedure: The method is validated as per the protocol number NR/QCD/PRT/001 and standard operating procedure (SOP No. NR/QCD/SOP/072/00).

2.5) HPLC method

Purpose: To estimate the content of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B in *Withania somnifera* by HPLC.

Analysis:

Chromatographic system: Shimadzu High Performance Liquid Chromatographic system equipped with LC10A pump with SPD-M 10A*vp* Photo diode Array Detector or UV detector in combination with Class-VP software or LC 2010 A and LC 2010HT integrated system equipped with Quaternary gradient, auto injector in combination with Lab solution software or any other suitable HPLC system with similar configuration can be used.

Chromatographic conditions:

Mobile phase :

1) Dissolve 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 ml of HPLC grade water (obtained from Millipore, Milli-Q Water purification system) and add 0.5ml of orthophosphoric acid. Make upto 1000 ml with water, filter through 0.45 µ membrane and degas in a sonicator for 3 minutes **(Solvent A).**

2) Acetonitrile **(Solvent B)**

Time (min)	Buffer	Value	Acetonitrile	Value
0.01	A.Conc	95.0	B.Conc	5
18.0	A.Conc	55.0	B.Conc	45
25.0	A.Conc	20.0	B.Conc	80
28.0	A.Conc	20.0	B.Conc	80
35.0	A.Conc	55.0	B.Conc	45
40.0	A.Conc	95.0	B.Conc	5
45.0	A.Conc	95.0	B.Conc	5

Column : i) Col No: LiChrosphere RP-18e Catalogue No:1.50995
ii) Phenomenex- Luna 5 µ C-18(2) Size: 250x4.60mm, S No.:417653

Column oven Temperature : Maintained at a constant temperature between 20 to 30°C(preferably 27°C)

Detector : SPD-M 10A*vp* Photo diode array detector or UV Detector

Wave length : 227 nm

Flow rate : 1.5ml/ min

Run time : 45minutes

Injection volume: 20 µl

Standard Preparation:

- Weigh accurately each 5mg of Withanoside IV, Withanoside V Withaferin A, 12 Deoxy Withastramonolide, Withanolide-A and Withanolide-B reference standards to a separate 10 ml volumetric flask.
- Dissolve in 10ml of methanol gently heating and cool then make up to 50ml with methanol.

Sample preparation:
Extract:

- Weigh accurately a sample quantity of *Withania somnifera* extract equivalent 10mg (about 5 g will be sufficient) of Withanoside IV, Withanoside V, Withaferin A, 12 Deoxy Withastramonolide, Withanolide A and Withanolide B in a 250ml beaker,
- Extract with 100 ml of methanol boiling on water bath for 10-15minutes and repeat the procedure 3 - 4 times till the raw material is completely extracted or till the extracts turn colorless.
- Combine all the fractions, concentrate and make up the volume to 50ml with methanol. Filter through 0.45microns membrane filter paper.

Procedure: Inject three times the standard preparation and calculate the mean area and the RSD. The RSD should not be more than 2%. Inject 20µl of sample preparation and record the chromatogram at 227nm. Calculate the percentage of Withanoside IV, Withanoside V, Withaferin A, 12 Deoxy Withastramonolide, Withanolide A and Withanolide B content from the peak areas using the formula:

Peak integration **:** Base to base

$$\frac{\text{Area of the sample}}{\text{Area of the standard}} \times \frac{\text{Weight of standard in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \text{Purity of standard}$$

3.0] Materials and Method:

3.1) Solvents:

Solvent Name	Grade	Product No.	Batch No.	Supplier
Acetonitrile	HPLC	A0755	R014F06	Rankem
Methanol	HPLC	M0275	R246A07 R189A07 R180M06	Rankem
Water	Ultra pure Grade	From Sartorius water purification system.		

3.2) Chemicals and their details

3.2.1) Reference Standard: Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy Withastramonolide, Withanolide A, Withanolide B Reference standard with % purity >90 %.

Reference standard	Lot No.	% Purity	Supplier
Withanoside IV	T6B107	90% w/w	Natural Remedies Private Limited, Bangalore
Withanoside V	T5A096	90% w/w	
Withaferin A	T7A047	99% w/w	
12- Deoxy withastramonolide	T7A099	95% w/w	
Withanolide A	T6K051	99% w/w	
Withanolide B	T6A088	99% w/w	

3.2.2) Other sources of reference standard: M/s. Chromadex and M/s. Extrasynthese

3.2.3) Sample and their details:

Sample	Standardized to	Batch No
Raw material	Total withanolides >0.2% by HPLC	RD/1162
Raw material	Total withanolides >0.2% by HPLC	ERH-046
Water extract	Total withanolides >0.15% by HPLC	WS–05Lot20
Hydro-alcoholic extract	Total withanolides >1.0% by HPLC	WS–05Lot21
Methanolic extract	Total withanolides >1.5% by HPLC	RD–1045
Methanolic extract	Total withanolides >2.5% by HPLC	WS–06Lot08
Methanolic extract	Total withanolides >2.5% by HPLC	WS–06Lot10
Methanolic extract	Total withanolides >2.5% by HPLC	RD–1170

QC Check sample: The QC check sample is the reference extract of *Withania somnifera* extract. It is prepared out of botanically authenticated *Withania somnifera* and confirmed for the presence of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B using the respective reference standards.

Possible matrix: Other secondary metabolites like alkaloids, phytosterols, proteins, carbohydrate, and excipients like Colloidal silicon dioxide, Light magnesium carbonate or any other inorganic salts**.**

3.3) Equipments:

3.3.1) Analytical Weighing Balance:

Balance Name	Instrument Code	Description
Sartorius-BP-211D	QCD/CIF/EB/01	Weighing capacity 1mg to 210gm (max) with 0.01mg accuracy
Sartorius-CP-245D	QCD/AHP/EB/01	Weighing capacity 1mg to 220gm (max) with 0.1mg accuracy

3.3.2) HPLC:

System	Instrumental code	Description
HPLC	QCD/CIF/HPLC/03	SHIMADZU LC 2010 A with system controller unit, degasser unit, low pressure gradient unit up to 4 solvents, pump unit with seal unit mechanism, mixer, auto sampler, column oven, UV-VIS detector, LC solution
HPLC	QCD/CIF/HPLC/02	Shimadzu LC10A system equipped with binary LC10ADVP pump, with SIL10AD*vp* auto sampler, SPD-M 10A*vp* Photo Diode Array Detector, SCL 10AVP system controller, CTO 10AVP column oven in combination with Class VP software version 6.03

3.3.3) Column:

Column	Column code	Column No.	Description
ODS C_{18} Silica	QC-18-59	625381	Merck kGaA, Hibar ® RT250-4.9; Prepacked Column; Cat1.01886; Lichrospher® 100; RP-18(5µm); Lot L56139833; No. 625381
C_{18}	QC-18-57	359208-12	Phenomenex, Luna 5 µ C_{18} (2)100 A, 250 X 4.60 mm, 5 microns Part No. :00G-4252- E0 No. 359208-12
C_{18}	QC-18-61	S.N.0170100159	Grace Smart RP 18 5µ Lot No.39/085 Col. No. S.N.0170100159 PN 5138810

3.3.4) Sonicator:

System	Instrument Code	Description
SIDILU UTRASONICS SONICATOR	QCD/AHP/SON/01	50 frequency/sec, Ultrasonic frequency 37±3,230 Volt, adjust for 1-9 min
BANDELIN SONOREX	QCD/CIF/SON/01	230Volt ≈ 50/80Hz, HF-frequency 35KHz,adjust for 1-15 min

3.3.5) Water purification system:

System	Instrument Code	Description
Sartorius, arium 611 UV purifier	QCD/CIF/WP/01	Sartorius,arium 611 UV,100Psi/6,9 bar max

3.3.6) Vacuum filter pump:

System	Description
Aue-DLF Vacuum filter pump	aue-LF,1/4HP,230Volt, 1PH,50Hz,1440rpm

3.3.7) Glass vacuum mobile phase system:

System	Description
Glass vacuum mobile phase system	Made up of borosilicate glass having filter size 47 mm diameter

3.3.8) Water bath:

Apparatus	Description
Water bath	Equitron make stirred water bath, having inner chamber (LxBxDepth) 30x15x15 cm, 5 ltr

3.3.9) Sample filtration assembly (Syringe filtration assembly):

System	Description
Sample filtration assembly	Syringe filter 1-5 ml at high flow rate and higher filtration speed with a diameter 25 mm

3.4) Miscellaneous:

3.4.1) Glassware:

Glassware	Capacity in ml	Class
Volumetric flask	10ml,100ml	B
Pipettes	1ml, 2ml, 5ml	A
Beakers	10ml, 250ml, 500ml	
Measuring cylinder	100ml, 1000ml	
Funnel		

3.4.2) Membrane Filter:

Filtration type	Filter	Description
For mobile phase filtration	Nylon	Ultipor® N_{66} Nylon 6,6 membrane (0.45µm), 47mm P/N NX047100
Sample filtration	Supor-450	Supor-450 [Poly ether sulphonate (PES)] membrane (0.45µm), 25 mm

3.4.3) Safety precautions:

i) Exercise due care. Refer material safety data sheet for details.

ii) Solvents are toxic, flammable exercise due care while handling.

iii) Read “safety manual” before analysis for safety precautions. Strictly adhere to the requirements.

4.0] STEP-1: VALIDATION OF INSTRUMENTAL PARAMETERS

4.1) Specificity:

4.1.1) Definition: The specificity is the ability of an analytical procedure to measure accurately an analyte in the presence of components that may be expected to be present in the sample matrix.

4.1.2) Assessment: It was confirmed by spectral similarity, determining the resolution between each peak, by checking the peak purity using PDA detector.

4.1.3) Acceptance Criteria: The compound of interest should be well separated and resolved from the rest. The resolution should be > 2.0. PDA spectrum of sample peak should be similar to the standard peak; peak purity should be > 0.98.

4.1.4) Experiment:

a) Standard solution preparation: The following concentrations of withanolides were prepared in methanol.

Table-1

Sl. No.	Reference standard	Concentrations (mcg/ml)	
		UV detector	PDA detector
1.	Withanoside IV	330.30	310.0
2.	Withanoside V	300.15	360.0
3.	Withaferin A	368.78	210.0
4.	12-Deoxy withastramonolide	355.30	300.0
5.	Withanolide A	345.51	240.0
6.	Withanolide B	337.59	350.0

b) Sample preparation: The sample solution was prepared by weighing the quantity shown in the **Table-1** in a 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

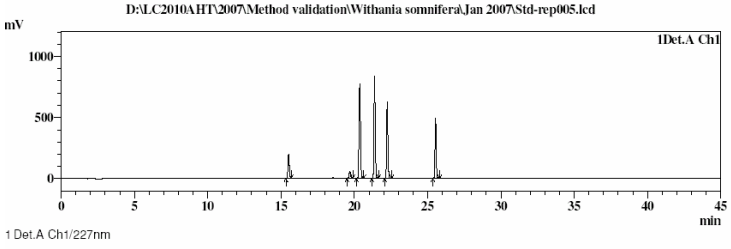
Table-2

Batch No	Quantity (mg)	
	UV detector	PDA detector
WS/06Lot10	750 mg	1520.4 mg
ERH-46	4091mg	4091.0 mg

c) **Procedure:** 20µl of each individual standard solution, mixed standard solutions, sample solutions were injected into two different HPLC's [(i) LC2010A equipped with UV detector and (ii) LC10ADVP equipped with PDA detector)] on different date and time and the chromatograms were recorded. Relative retention time of different withanolides were calculated with respect to Withanolide A**.** .

4.1.5) Observation: Retention time of Withanoside IV is about 15.5 minutes, Withanoside V is about 19.7 minutes, Withaferin A is about 20.3 minutes, 12-Deoxy withastramonolide is about 21.4 minutes, Withanolide A is about 22.2 minutes and Withanolide B is about 25.5 minutes (Refer chromatogram-1). In the sample chromatogram all the individual withanolides are well resolved. (Refer chromatogram-2).

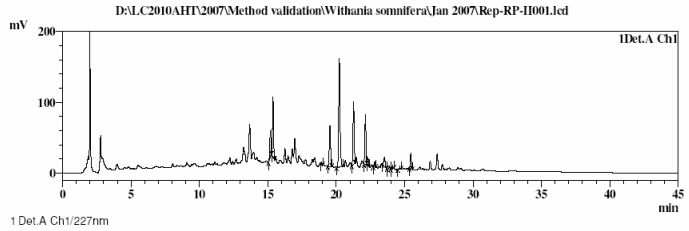
Chromatogram – 1: Standard mix chromatogram



Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.516	Withanoside IV	1234072	6.645	0.000
2	19.692	Withanoside V	391438	2.108	22.163
3	20.366	Withaferin A	5318748	28.640	3.454
4	21.379	12- Deoxy withastramonolide	5001196	26.930	5.576
5	22.242	Withanolide A	3786871	20.391	5.030
6	25.539	Withanolide B	2839005	15.287	19.518
Total			1857330	100.000	

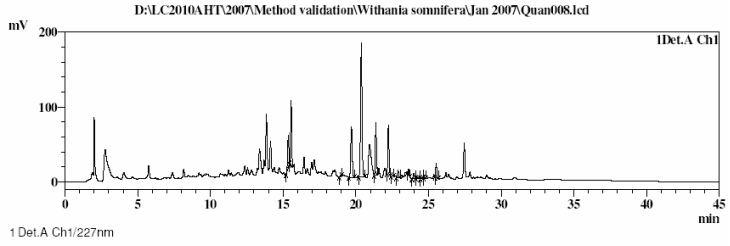
Chromatogram – 2: Sample chromatogram



Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.187	RT15.187	225004	6.435	0.000
2	15.376	Withanoside IV	478462	13.685	1.135
3	18.909	RT18.909	3921	0.112	23.432
4	19.537	Withanoside V	400277	11.448	3.776
5	20.226	Withaferin A	1097206	31.381	3.516
6	21.263	Withastramonolide	548663	15.692	5.550
7	22.128	Withanolide A	398098	11.386	5.034
8	22.311	RT22.311	43911	1.256	1.219
9	22.804	RT22.804	36992	1.058	3.406
10	23.524	RT23.524	100800	2.883	3.854
11	23.891	RT23.891	15874	0.454	1.951
12	24.112	RT24.112	17872	0.511	1.423
13	24.625	RT24.625	6589	0.188	3.252
14	25.447	Withanolide B	122702	3.509	5.045
Total			3496369	100.000	

Chromatogram – 3: Raw material chromatogram

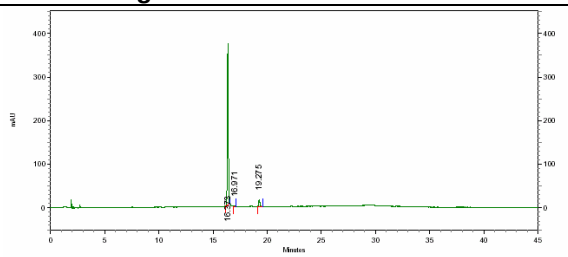


Detector A Ch1 227nm

Peak #	Ret. Time	Name	Area	Area %	Resolution
1	15.352	RT15.352	265649	7.501	0.000
2	15.547	Withanoside-IV	491157	13.869	1.158
3	18.980	RT18.980	8341	0.236	21.729
4	19.698	Withanoside V	473414	13.368	4.128
5	20.372	Withaferin A	1250895	35.323	3.455
6	21.377	Withastramonolide	410796	11.600	5.561
7	22.239	Withanolide A	384409	10.855	5.143
8	22.491	RT22.491	1440	0.041	1.589
9	22.903	RT22.903	54369	1.535	2.662
10	23.619	RT23.619	36688	1.036	4.654
11	23.987	RT23.987	38306	1.082	2.373
12	24.208	RT24.208	14767	0.417	1.282
13	24.567	RT24.567	4931	0.139	2.088
14	24.709	RT24.709	4074	0.115	0.994
15	25.523	Withanolide B	102080	2.883	5.790
Total			3541316	100.000	

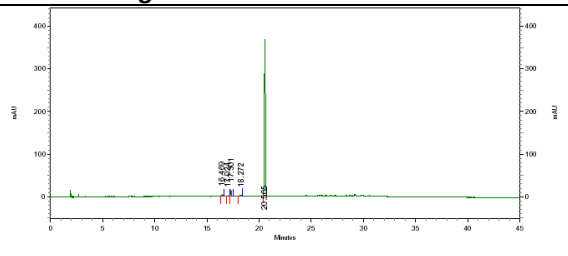
When analysed using PDA detector the retention times of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B were about 16.373, 20.565, 21.376, 22.368, 23.211 and 26.475 respectively (Refer chromatogram-4,5,6,7,8,9,10 & 11). The PDA spectra of all the withanolides peaks in the sample are identical to that of the respective standards. The purity of peaks due to all the withanolides in the sample as well as standard are >0.98. The sample chromatogram has many other peaks than six withanolides.

Chromatogram – 4: Standard Withanoside IV



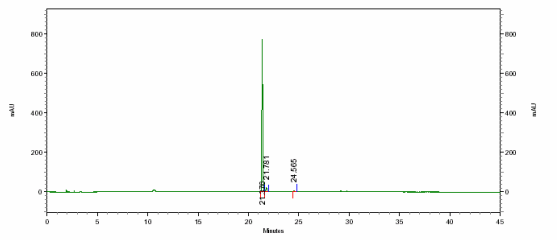
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.373	2346882	94.68	0.00	1.10	138825.47	-0.05	0.37	0.18	369468
16.971	13273	0.54	3.32	0.00	135239.38	-0.02	0.22	0.18	2153
19.275	118703	4.79	12.20	1.23	159399.92	0.12	0.53	0.19	16352
Totals	2478858	100.00							387973

Chromatogram – 5: Standard Withanoside V



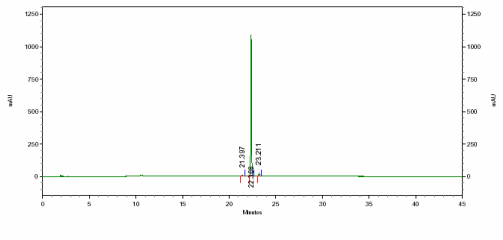
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.469	23248	0.87	0.00	1.07	126689.00	-0.05	0.34	0.19	3544
17.024	11761	0.44	3.01	0.97	137036.11	-0.01	0.30	0.18	1619
17.301	94069	3.53	1.52	1.13	146967.23	0.00	0.33	0.18	14261
18.272	15926	0.60	4.98	0.74	122179.84	0.06	0.39	0.21	1877
20.565	2522780	94.56	11.48	1.05	186867.95	0.19	0.35	0.19	366195
Totals	2667784	100.00							387496

Chromatogram – 6: Standard Withaferin A



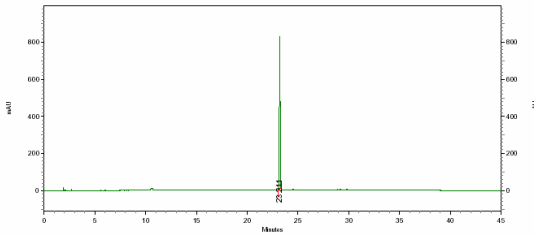
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
21.376	5088622	97.13	0.00	1.11	221434.52	0.24	0.45	0.18	769328
21.781	134321	2.56	2.25	1.16	235949.08	0.26	0.39	0.18	20879
24.565	15855	0.30	15.94	1.56	334448.38	0.42	0.39	0.17	2343
Totals	5238798	100.00							792550

Chromatogram – 7: Standard 12-Deoxy withastamonolide



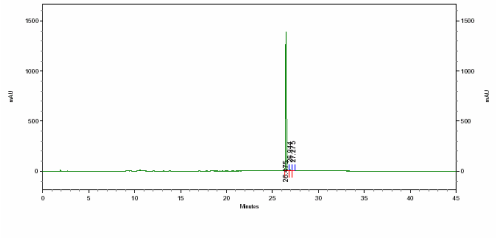
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
21.397	47071	0.70	0.00	1.14	218371.22	0.24	0.41	0.18	6927
22.368	6561291	97.03	5.56	1.03	290232.75	0.30	0.43	0.17	108561
23.211	153754	2.27	5.02	1.14	298543.09	0.35	0.42	0.17	25530
Totals	6762116	100.00							111806

Chromatogram – 8: Standard Withanolide A



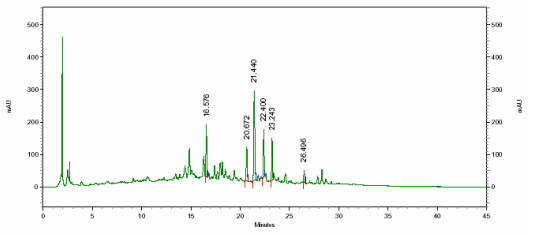
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
23.211	4806845	100.00	0.00	1.10	315411.41	0.35	0.26	0.17	822204
Totals	4806845	100.00							822204

Chromatogram – 9: Standard Withanolide B



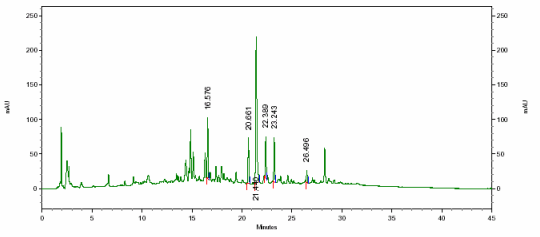
Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
26.475	8139020	99.20	0.00	1.13	413752.75	0.53	0.49	0.16	138429
26.944	40938	0.50	2.81	1.23	407283.75	0.56	0.34	0.17	6620
27.275	25063	0.31	1.87	1.12	349493.94	0.58	0.33	0.18	3846
Totals	8205021	100.00							139475

Chromatogram – 10: Extract sample



Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.576	998797	17.55	0.00	1.04	137409.30	-0.04	0.24	0.18	159064
20.672	745691	13.11	21.84	1.02	177685.13	0.20	0.30	0.20	104250
21.440	2024836	35.59	3.87	1.13	182807.92	0.24	0.34	0.20	275305
22.400	940711	16.53	5.02	1.04	243337.77	0.30	0.25	0.18	146645
23.243	757964	13.32	4.78	1.02	296866.34	0.35	0.22	0.17	127311
26.496	221905	3.90	19.32	1.19	406992.84	0.54	0.22	0.17	37559
Totals	5689904	100.00							850134

Chromatogram – 11: Raw material

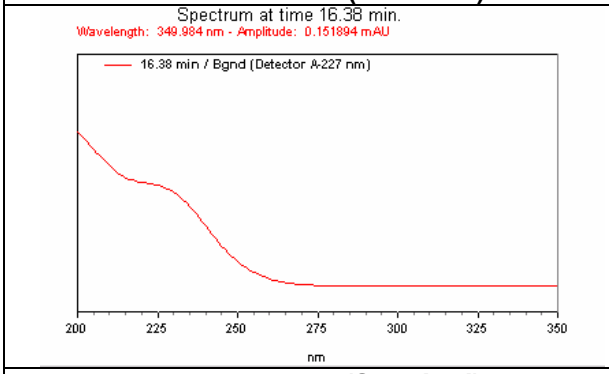
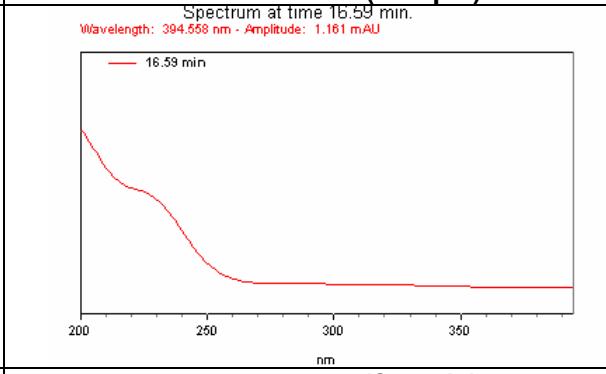
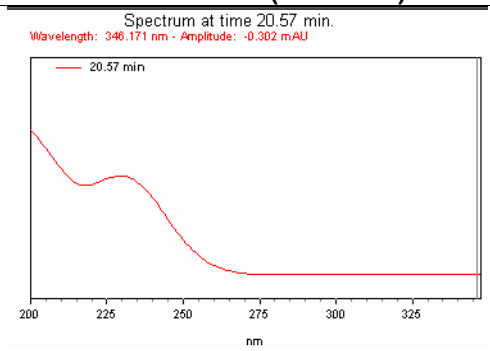
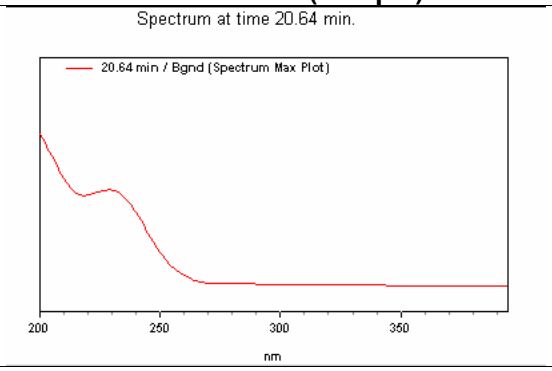
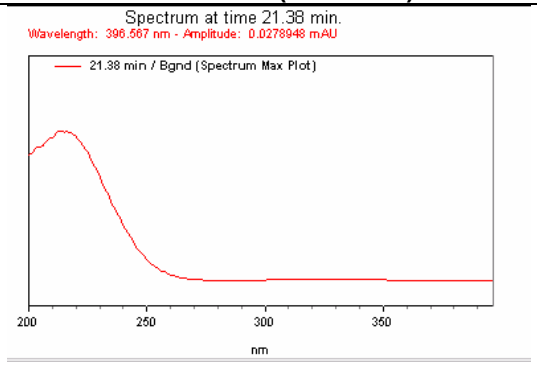
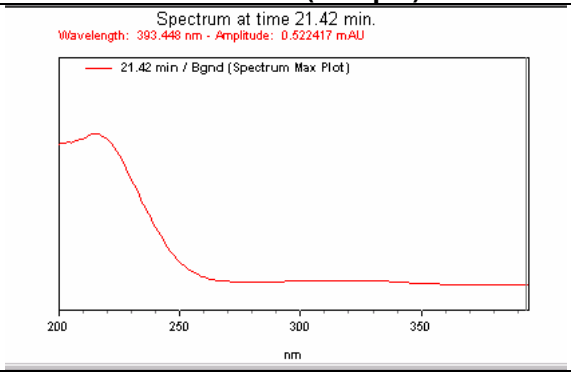
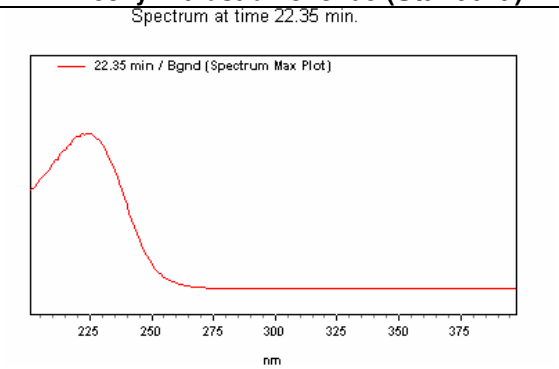
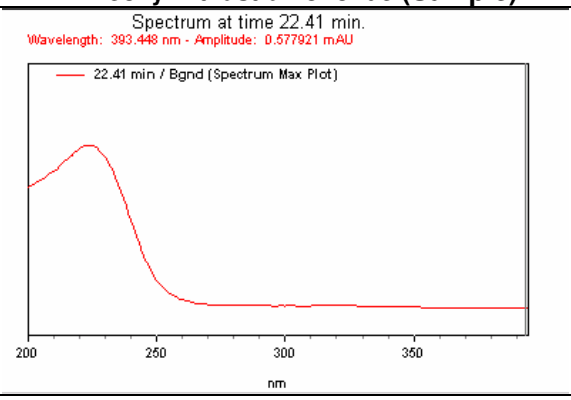


Detector A-227 nm Retention Time	Area	Area Percent	Resolution	Asymmetry	Theoretical plates	Capacity factor	Width	USP Width	Height
16.576	537308	15.13	0.00	1.08	145898.22	-0.04	0.24	0.17	87496
20.661	453713	12.78	22.24	1.01	181706.16	0.20	0.29	0.19	65400
21.440	1723297	48.54	3.82	1.39	161359.55	0.24	0.48	0.21	211277
22.389	357061	10.06	5.00	1.02	291271.69	0.30	0.24	0.17	59849
23.243	380726	10.72	5.14	1.04	314259.59	0.35	0.26	0.17	64237
26.496	98366	2.77	20.08	1.23	449097.84	0.54	0.21	0.16	17761
Totals	3550471	100.00							506020

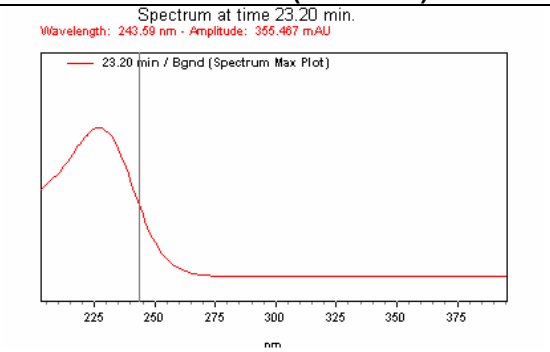
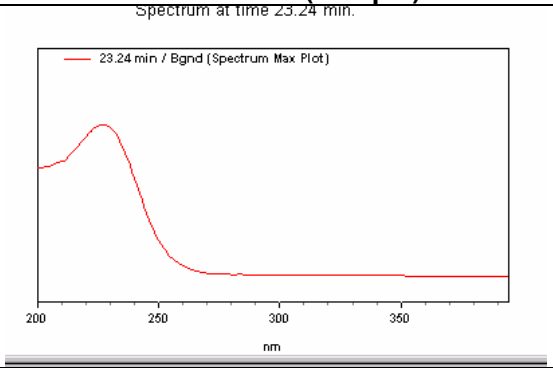
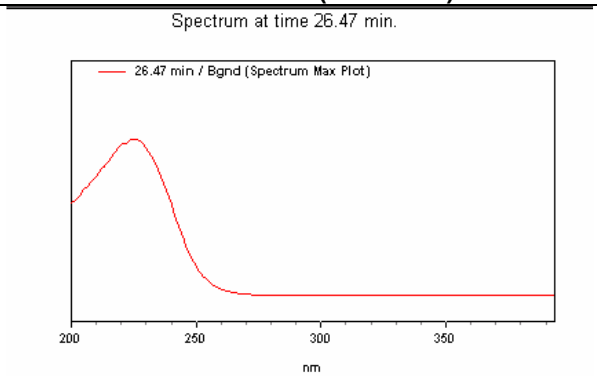
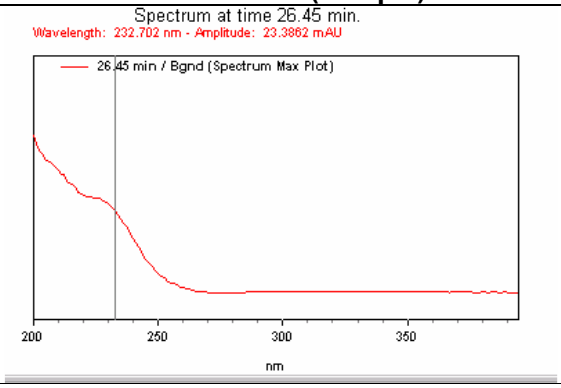
Table-3: Data from sample chromatogram

Substance	Compound	UV detector		PDA detector	
		Retention time (minutes)	Relative retention time	Retention time (minutes)	Relative retention time
Standard mix	Withanoside IV	15.516	0.698	16.373	0.705
	Withanoside V	19.692	0.885	20.565	0.886
	Withaferin A	20.366	0.916	21.376	0.921
	12-Deoxy withastramonolide	21.379	0.961	22.368	0.964
	Withanolide A	22.242	1.0	23.211	1.0
	Withanolide B	25.539	1.148	26.475	1.141
Extract	Withanoside IV	15.376	0.695	16.576	0.713
	Withanoside V	19.537	0.883	20.672	0.889
	Withaferin A	20.226	0.914	21.440	0.922
	12-Deoxy withastramonolide	21.263	0.961	22.400	0.964
	Withanolide A	22.128	1.0	23.243	1.0
	Withanolide B	25.447	1.149	26.496	1.140
Raw material	Withanoside IV	15.547	0.699	16.576	0.713
	Withanoside V	19.698	0.886	20.661	0.889
	Withaferin A	20.372	0.916	21.440	0.922
	12-Deoxy withastramonolide	21.377	0.961	22.389	0.963
	Withanolide A	22.239	1.0	23.243	1.0
	Withanolide B	25.523	1.148	26.496	1.140

PDA SPECTRUM

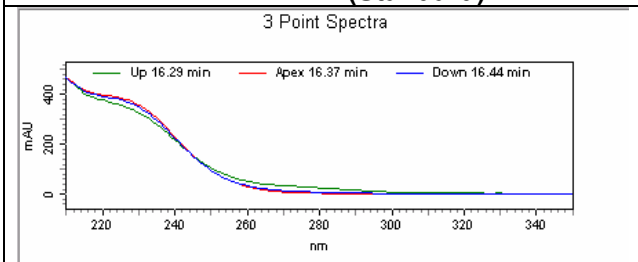
Withanoside IV (Standard)	Withanoside IV (Sample)
	
Withanoside V (Standard)	**Withanoside V (Sample)**
	
Withaferin A (Standard)	**Withaferin A (Sample)**
	
12-Deoxy withastramonolide (Standard)	**12-Deoxy withastramonolide (Sample)**
	

PDA SPECTRUM

Withanolide A (Standard)	Withanolide A (Sample)
	
Withanolide B (Standard)	**Withanolide B (Sample)**
	

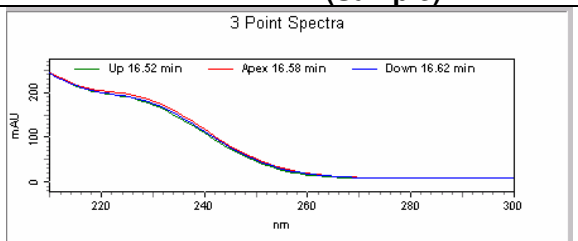
PEAK PURITY SPECTRUM

Withanoside IV (Standard)



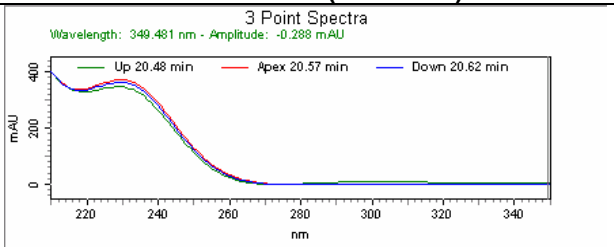
Up slope similarity: 0.996791 (Threshold = 0.874408) (Index = 0.12238
Down slope similarity: 0.999891 (Threshold = 0.985453) (Index = 0.01443
3 point peak purity: 0.998341 (Threshold = 0.929931) (Index = 0.06841

Withanoside IV (Sample)



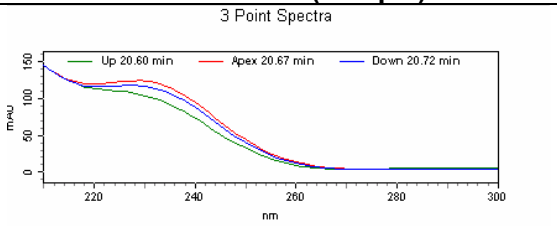
Up slope similarity: 0.998907 (Threshold = 0.937049) (Index = 0.06185
Down slope similarity: 0.999764 (Threshold = 0.946012) (Index = 0.05375
3 point peak purity: 0.999336 (Threshold = 0.941530) (Index = 0.05780

Withanoside V (Standard)



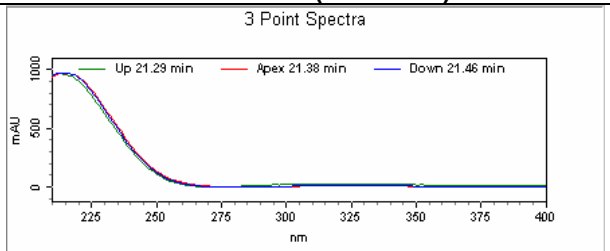
Up slope similarity: 0.998560 (Threshold = 0.999964) (Index = -0.00140
Down slope similarity: 0.999818 (Threshold = 0.999986) (Index = -0.00016
3 point peak purity: 0.999189 (Threshold = 0.999975) (Index = -0.00078

Withanoside V (Sample)



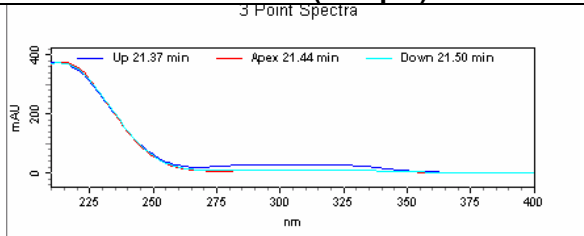
Up slope similarity: 0.994789 (Threshold = 0.824520) (Index = 0.17026
Down slope similarity: 0.999574 (Threshold = 0.897342) (Index = 0.10223
3 point peak purity: 0.997182 (Threshold = 0.860931) (Index = 0.13625

Withaferin A (Standard)



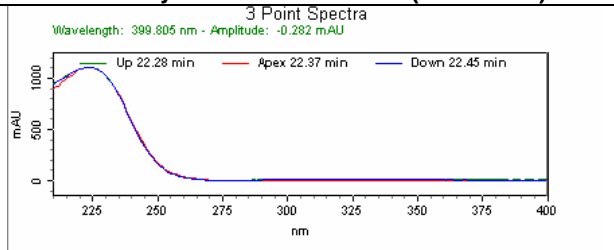
Up slope similarity: 0.997839 (Threshold = 0.999918) (Index = -0.00207
Down slope similarity: 0.999792 (Threshold = 0.999975) (Index = -0.00018
3 point peak purity: 0.998815 (Threshold = 0.999946) (Index = -0.00113

Withaferin A (Sample)



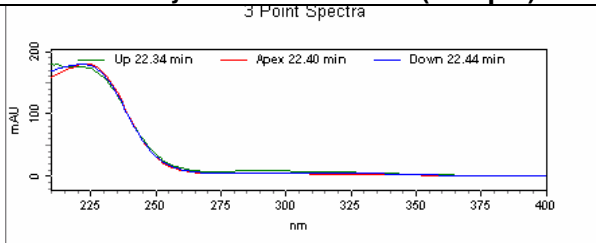
Up slope similarity: 0.995696 (Threshold = 0.935925) (Index = 0.05977
Down slope similarity: 0.999862 (Threshold = 0.955335) (Index = 0.04452
3 point peak purity: 0.997779 (Threshold = 0.945630) (Index = 0.0521

12-Deoxy withastramonolide (Standard)



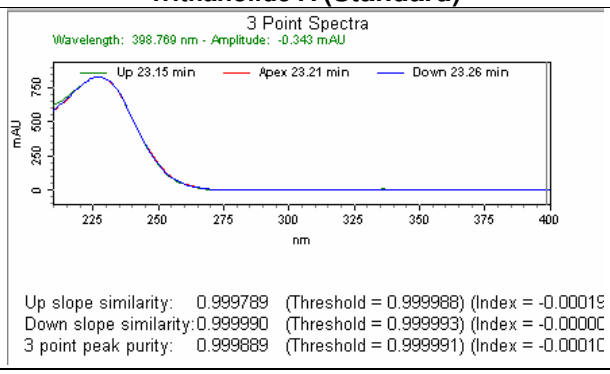
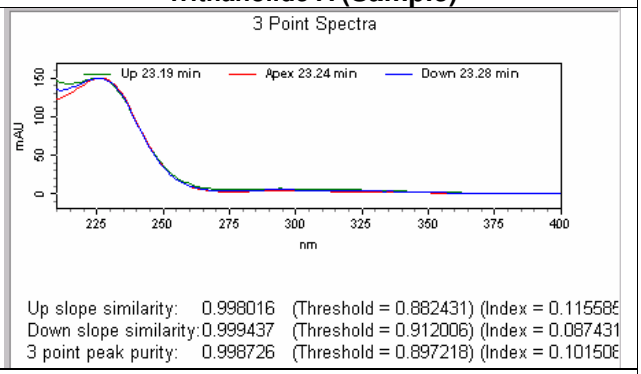
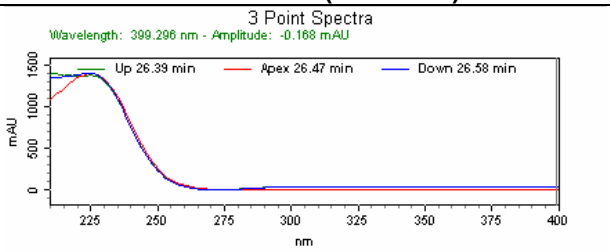
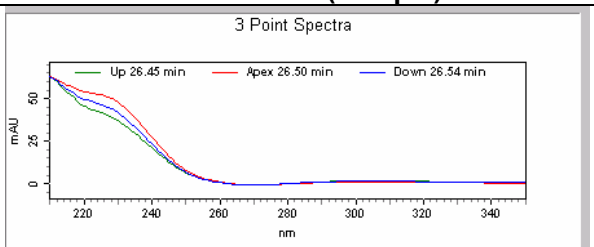
Up slope similarity: 0.999497 (Threshold = 0.999961) (Index = -0.00046
Down slope similarity: 0.999519 (Threshold = 0.999965) (Index = -0.00044
3 point peak purity: 0.999508 (Threshold = 0.999963) (Index = -0.00045

12-Deoxy withastramonolide (Sample)



Up slope similarity: 0.997838 (Threshold = 0.888872) (Index = 0.10896
Down slope similarity: 0.999535 (Threshold = 0.935487) (Index = 0.06404
3 point peak purity: 0.998687 (Threshold = 0.912179) (Index = 0.08650

PEAK PURITY SPECTRUM

Withanolide A (Standard)	Withanolide A (Sample)
 Up slope similarity: 0.999789 (Threshold = 0.999988) (Index = -0.00019 Down slope similarity: 0.999990 (Threshold = 0.999993) (Index = -0.00000 3 point peak purity: 0.999889 (Threshold = 0.999991) (Index = -0.0001C	 Up slope similarity: 0.998016 (Threshold = 0.882431) (Index = 0.11558 Down slope similarity: 0.999437 (Threshold = 0.912006) (Index = 0.087431 3 point peak purity: 0.998726 (Threshold = 0.897218) (Index = 0.10150
Withanolide B (Standard)	**Withanolide B (Sample)**
 Up slope similarity: 0.995169 (Threshold = 0.999969) (Index = -0.0048 Down slope similarity: 0.996248 (Threshold = 0.999972) (Index = -0.0037 3 point peak purity: 0.995708 (Threshold = 0.999970) (Index = -0.0042	 Up slope similarity: 0.995211 (Threshold = 0.480453) (Index = 0.51475 Down slope similarity: 0.998584 (Threshold = 0.564319) (Index = 0.43426 3 point peak purity: 0.996897 (Threshold = 0.522386) (Index = 0.47451

4.1.6) Conclusion: The difference in the retention time of withanolides indicates the method is capable of separating the individual withanolides from the other.

The similarity in the PDA spectra of the peaks of withanolides obtained with the sample and the standard indicate the peak purity.

From the above data it can be concluded that the method is very specific for the estimation of withanolides and there is no interference from any other compounds present in the extract.

Enclosure-1: Individual chromatogram of standards, standard mix chromatogram and chromatogram of methanol (**8 pages)**
Enclosure-2: Sample chromatograms **(2 pages)**
Enclosure-3: Chromatogram of individual standard in a PDA detector **(6 pages)** and Sample chromatograms for extract and raw material **(2 pages)**
Enclosure-4: PDA spectrum and Peak purity spectrum **(6 pages)**

4.2) Linearity:

4.2.1) Definition: The linearity is ability of an analytical procedure to elicit response linearity related to the amount or concentration within the specified range.

4.2.2) Assessment: By plotting a graph using concentrations on X-axis and the respective peak areas on Y-axis. Correlation co-efficient r^2 is calculated.

4.2.3) Acceptance Criterion: co-efficient r^2 should be > 0.98.

4.2.4) Experiment:

a) **Standard Preparation:** 6.60 mg of Withanoside IV, 6.0 mg of Withanoside V, 7.38 mg of Withaferin A, 7.11 mg of 12-Deoxy withastramonolide, 6.91 mg of Withanolide A and 6.75 mg of Withanolide B were dissolved in 10ml methanol by gently heating, cooled and made up to 50ml with methanol. **Stock solution.**

The concentration of each analyte in 5 ml is;

$$\text{Withanoside IV} = 7.34 \times \frac{90}{100} = 6.606 \text{ mg}$$

$$\text{Withanoside V} = 6.67 \times \frac{90}{100} = 6.003 \text{ mg}$$

$$\text{Withaferin A} = 7.45 \times \frac{99}{100} = 7.3755 \text{ mg}$$

$$\text{12-Deoxy withastramonolide} = 7.48 \times \frac{95}{100} = 7.106 \text{ mg}$$

$$\text{Withanolide A} = 6.98 \times \frac{99}{100} = 6.9102 \text{ mg}$$

$$\text{Withanolide B} = 6.82 \times \frac{99}{100} = 6.7518 \text{ mg}$$

Table-4: Stock solution

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	1321.20
2.	Withanoside V	1200.60
3.	Withaferin A	1475.10
4.	12-Deoxy withastramonolide	1421.20
5.	Withanolide A	1382.04
6.	Withanolide B	1350.36

Dilution-1: 5ml of stock solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-5

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	660.60
2.	Withanoside V	600.30
3.	Withaferin A	737.55
4.	12-Deoxy withastramonolide	710.60
5.	Withanolide A	691.02
6.	Withanolide B	675.18

Dilution-2: 5ml of Dilution-1 solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-6

Sl. No.	Reference standard	Concentrations (mcg/ml)
7.	Withanoside IV	330.30
8.	Withanoside V	300.15
9.	Withaferin A	368.78
10.	12-Deoxy withastramonolide	355.30
11.	Withanolide A	345.51
12.	Withanolide B	337.59

Dilution-3: 5ml of Dilution-2 solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-7

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	165.15
2.	Withanoside V	150.08
3.	Withaferin A	184.39
4.	12-Deoxy withastramonolide	177.65
5.	Withanolide A	172.76
6.	Withanolide B	168.79

Dilution-4: 5ml of Dilution-3 solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-8

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	82.58
2.	Withanoside V	75.04
3.	Withaferin A	92.19
4.	12-Deoxy withastramonolide	88.83
5.	Withanolide A	86.38
6.	Withanolide B	84.39

Dilution-5: 5ml of Dilution-4 solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-9

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	41.29
2.	Withanoside V	37.52
3.	Withaferin A	46.09
4.	12-Deoxy withastramonolide	44.41
5.	Withanolide A	43.19
6.	Withanolide B	42.19

Dilution-6: 5ml of Dilution-5 solution was pipetted to10ml volumetric flask and made up to 10ml with acetonitrile.

Table-10

Sl. No.	Reference standard	Concentrations (mcg/ml)
1.	Withanoside IV	20.64
2.	Withanoside V	18.76
3.	Withaferin A	23.05
4.	12-Deoxy withastramonolide	22.21
5.	Withanolide A	21.59
6.	Withanolide B	21.09

b) **Procedure:** All the above 1, 2, 3, 4, 5 and 6 dilutions were injected five times each and chromatograms were recorded. A graph was plotted using concentrations on X-axis and the respective peak areas on Y-axis. Correlation co-efficient r^2 was calculated.

4.2.5) Observation: Correlation co-efficient r^2 for

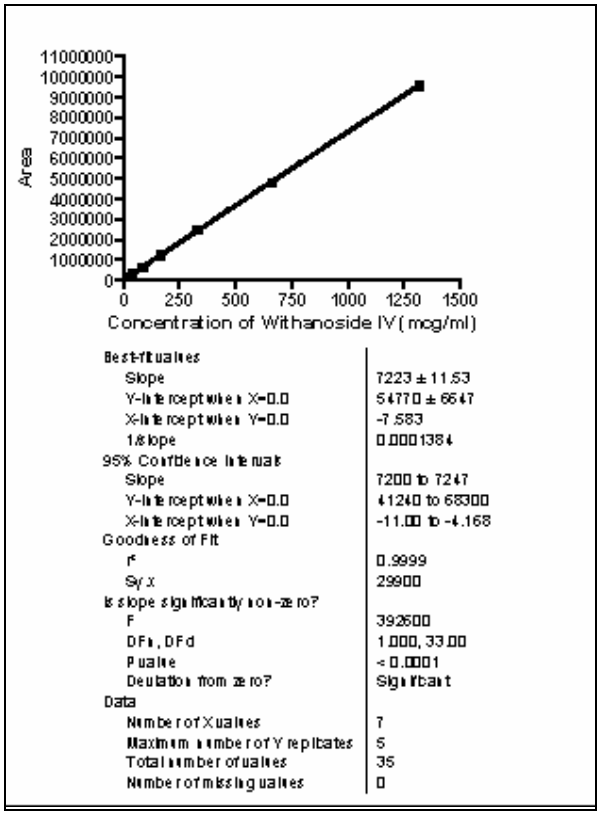
Compound	r^2	Concentration range(mcg/ml)
Withanoside IV	0.999	20.0 to 1321
Withanoside V	0.999	18.7 to 1200
Withaferin A	0.996	23.0 to 737
12-Deoxy Withastramonolide	0.990	22.0 to 355
Withanolide A	0.992	21.0 to 345
Withanolide B	0.984	21.0 to 337

PEAK AREA OF WITHANOSIDE IV

Table-11

Withanoside IV (mcg/ml)	Different Concentrations						
	Dilution -6 (20.64)	Dilution -5 (41.29)	Dilution -4 (82.58)	Dilution -3 (165.15)	Dilution -2 (330.30)	Dilution -1 (660.60)	S. solution (1321.20)
Replicate – 1	166047	329878	651382	1275513	2493967	4815460	9597190
Replicate –2	166038	330019	651009	1278440	2493747	4811411	9587035
Replicate –3	166015	329943	651630	1269142	2491031	4819826	9584608
Replicate –4	166025	329827	649769	1278566	2492809	4815725	9588582
Replicate – 5	165993	329140	651869	1279224	2497552	4818317	9579369
Mean	166024	329761	651132	1276177	2493821	4816148	9587357
Response factor Area / concentration	8042.32	7986.95	7885.34	7727.38	7550.17	7290.57	7256.55
Mean (Dilution 2 to 6)	7838.43						
RSD (Dilution 2 to 6)	2.29						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION

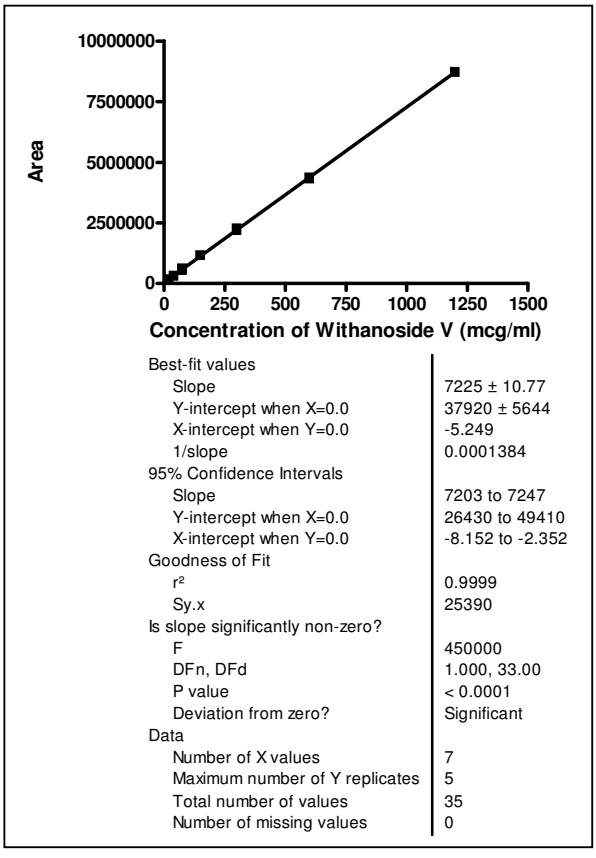


PEAK AREA OF WITHANOSIDE V

Table-12

Withanoside V (mcg/ml)	Different Concentrations						
	Dilution -6 (18.76)	Dilution -5 (37.52)	Dilution -4 (75.04)	Dilution -3 (150.08)	Dilution -2 (300.15)	Dilution -1 (600.30)	S. solution (1200.60)
Replicate – 1	151294	298008	586819	1146136	2238822	4334899	8731747
Replicate –2	151121	297953	585559	1146843	2237319	4327804	8721728
Replicate –3	151156	298036	585906	1141122	2243282	4330341	8719358
Replicate –4	151277	297352	581818	1152990	2237337	4338646	8718285
Replicate – 5	150985	297517	584964	1151745	2248262	4345473	8714428
Mean	151167	297773	585013	1147767	2241004	4335433	8721109
Response factor Area / concentration	8058.19	7936.65	7796.28	7647.96	7466.28	7222.11	7263.96
Mean (Dilution 2 to 6)	7781.072						
RSD (Dilution 2 to 6)	2.68						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION

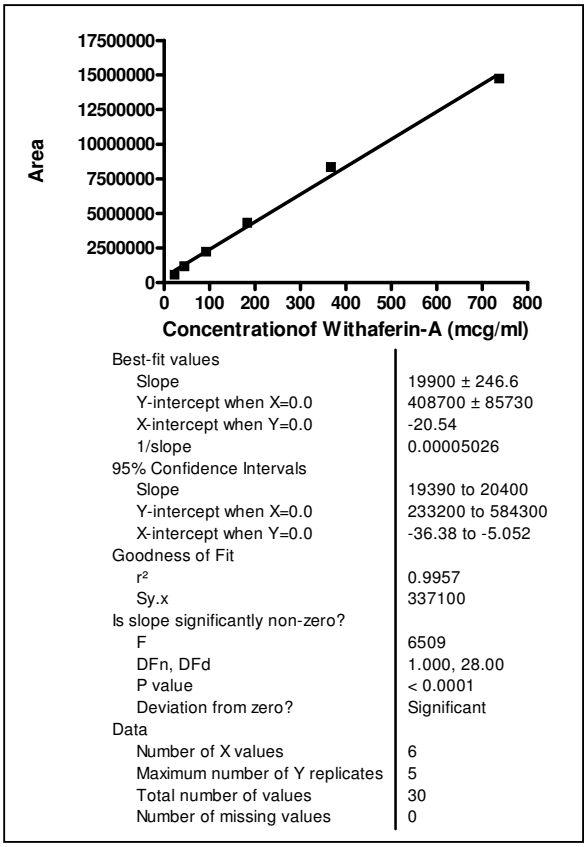


PEAK AREA OF WITHAFERIN A

Table-13

Withaferin A (mcg/ml)	Different Concentrations						
	Dilution -6 (23.05)	Dilution -5 (46.09)	Dilution -4 (92.19)	Dilution -3 (184.39)	Dilution -2 (368.78)	Dilution -1 (737.55)	S. solution (1475.10)
Replicate – 1	573810	1134850	2231492	4332623	8325259	14666731	21469032
Replicate –2	573224	1134742	2227105	4337796	8322031	14756049	21438480
Replicate –3	573669	1134589	2228288	4310236	8317825	14770374	21482780
Replicate –4	573097	1134172	2211324	4335168	8328440	14788044	21438918
Replicate – 5	572942	1131663	2224755	4340882	8338317	14782253	21442185
Mean	573348	1134003	2224593	4331341	8326374	14752690	21454279
Response factor Area / concentration	24875.80	24600.44	24129.54	23490.43	22578.47	20002.29	14544.29
Mean (Dilution 3 to 6)	24274.053						
RSD (Dilution 3 to 6)	2.16						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION

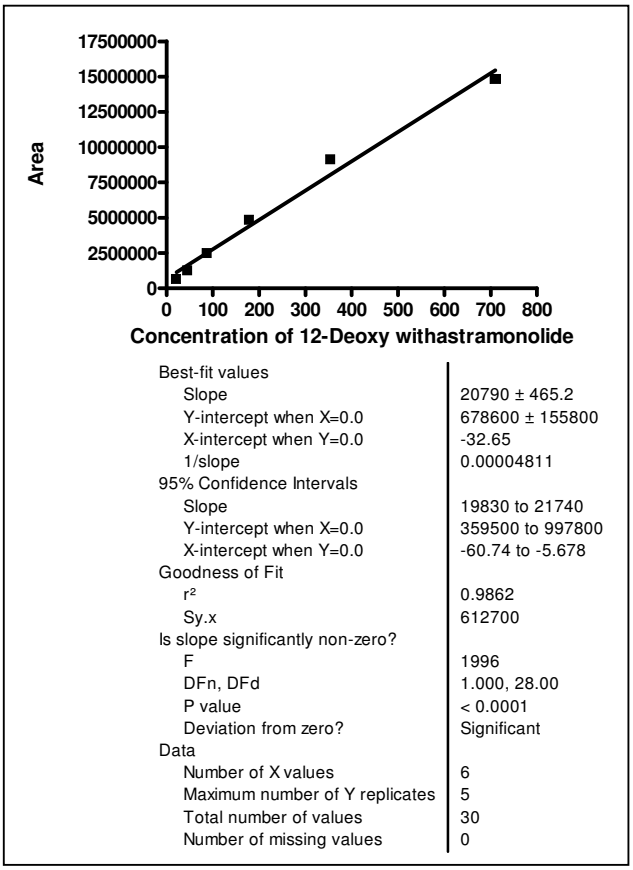


PEAK AREA OF 12-DEOXY WITHASTRAMONOLIDE

Table-14

12-Deoxy withastramonolide (mcg/ml)	Different Concentrations						
	Dilution -6 (22.21)	Dilution -5 (44.41)	Dilution -4 (88.83)	Dilution -3 (177.65)	Dilution -2 (355.30)	Dilution -1 (710.60)	S. solution (1421.20)
Replicate – 1	**635487**	**1256078**	**2472835**	**4800626**	**9145644**	**14809446**	**18080882**
Replicate –2	**634565**	**1254992**	**2469751**	**4809678**	**9138372**	**14845283**	**18085009**
Replicate –3	**634904**	**1256714**	**2471386**	**4778467**	**9140140**	**14833377**	**18068450**
Replicate –4	**634821**	**1256803**	**2448908**	**4808531**	**9151223**	**14874117**	**18045786**
Replicate – 5	**634615**	**1254538**	**2471248**	**4813165**	**9156566**	**14859008**	**17998894**
Mean	**634878**	**1255825**	**2466826**	**4802093**	**9146389**	**14844246**	**18055804**
Response factor Area / concentration	**28590.08**	**28276.39**	**27771.75**	**27031.20**	25742.72	20889.74	12704.62
Mean (Dilution 3 to 6)	**27917.36**						
RSD (Dilution 3 to 6)	**2.11**						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION

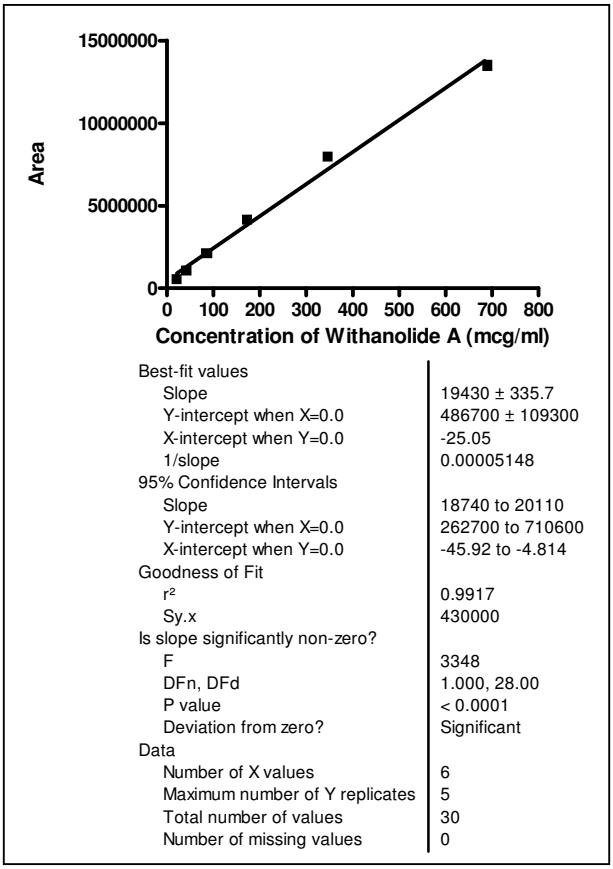


PEAK AREA OF WITHANOLIDE A

Table-15

Withanolide A (mcg/ml)	Different Concentrations						
	Dilution -6 (21.59)	Dilution -5 (43.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (691.02)	S. solution (1382.04)
Replicate – 1	551317	1084798	2130939	4137256	7955602	13463661	13878628
Replicate –2	550721	1084624	2130222	4140860	7957220	13489137	13809664
Replicate –3	550930	1084955	2130126	4118314	7958121	13484780	13766317
Replicate –4	550882	1084918	2112327	4144205	7963123	13502916	13721170
Replicate – 5	552430	1082926	2131439	4149692	7973789	13491381	13664575
Mean	551256	1084444	2127011	4138065	7961571	13486375	13768071
Response factor Area / concentration	25527.76	25109.41	24624.59	23953.38	23042.95	19516.62	9962.14
Mean (Dilution 3 to 6)	24803.79						
RSD (Dilution 3 to 6)	2.36						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION

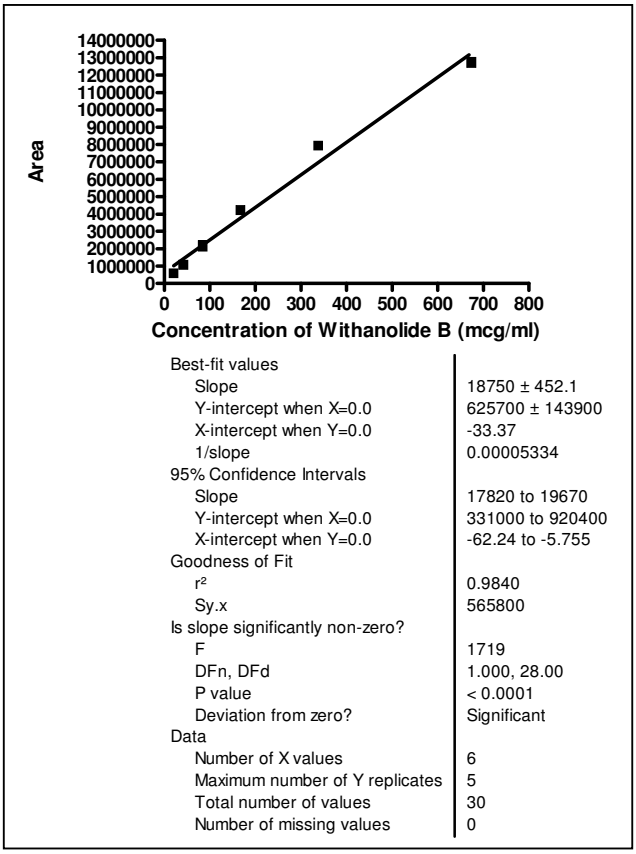


PEAK AREA OF WITHANOLIDE B

Table-16

Withanolide B (mcg/ml)	Different Concentrations						
	Dilution -6 (21.09)	Dilution -5 (42.19)	Dilution -4 (84.39)	Dilution -3 (168.79)	Dilution -2 (337.59)	Dilution -1 (675.18)	S. solution (1350.36)
Replicate – 1	553596	1095189	2157826	4196971	7938851	12728719	12264511
Replicate –2	552800	1094573	2156794	4205282	7947620	12721830	12206630
Replicate –3	552601	1096209	2156648	4174239	7944196	12736068	12166317
Replicate –4	552865	1094840	2139922	4205370	7953428	12723957	12126384
Replicate – 5	552430	1093649	2158084	4205743	7963457	12720153	12051739
Mean	552858	1094892	2153855	4197521	7949510	12726145	12163116
Response factor Area / concentration	26202.60	25946.08	25520.36	24867.57	23547.83	18848.52	9007.31
Mean (Dilution 3 to 6)	25634.15						
RSD (Dilution 3 to 6)	1.97						

LINEARITY GRAPH PEAK AREA Vs CONCENTRATION



4.2.6) Conclusion:

Correlation Coefficient (r^2) for Withanoside IV was found to be 0.9999

Correlation Coefficient (r^2) for Withanoside V was found to be 0.9999

Correlation Coefficient (r^2) for Withaferin A was found to be 0.9957

Correlation Coefficient (r^2) for 12 Deoxy Withastramonolide was found to be 0.9862

Correlation Coefficient (r^2) for Withanolide A was found to be 0.9917

Correlation Coefficient (r^2) for Withanolide B was found to be 0.9840

Correlation coefficient (r^2) found to be >0.99 for both the analytes indicating the linearity and the method is linear between the below concentrations.

Withanoside IV – 20. 6 and 330.0 mcg

Withanoside V – 18.7 and 300.15 mcg

Withaferin A – 23.0 and 184.0 mcg

12 Deoxy Withastramonolide – 22.0 and 177.0mcg

Withanolide A – 21.0 and 172.0 mcg

Withanolide B – 21.0 and 169.0 mcg

Enclosure-5: Refer standard chromatograms **(35 Pages)**

4.3) Precision:

4.3.1) Definition: The precision is a measure of the closeness of agreement between observed values obtained independently from multiple samplings of a homogenous sample and is expressed as the variance, standard deviation or relative standard deviation (coefficient of variation) of observed values.

4.3.2) Assessment for Area: Determined by injecting 5 different concentrations of standards 5 times each and 7 different samples 2 times each and calculating the relative standard deviation (RSD).

4.3.3) Assessment for Retention Time: Checking the relative standard deviation of retention time of the peaks obtained using the above solutions.

4.3.4) Assessment for reproducibility of result: By analyzing the same sample in duplicate and finding the relative standard deviation of the assay.

4.3.5) Acceptance Criteria: The Relative standard deviation to be less than 2.5% for all the above.

4.3.6) Precision for peak areas:

Experiment:

a) **Standard preparation: Prepared as mentioned in linearity 4.2.4 (a).**
b) **Sample preparation:** The sample solution was prepared by weighing the quantity of the sample shown in the **Table** in a 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

Table -17

Batch No.	Quantity (mg)
WS/06Lot10	753.6
	1503.2
	2096

c) **Procedure:** 20µl of each of the standard solutions were injected two times and 20µl of each of the sample solutions were injected two times and Chromatograms were recorded. There are various late eluting compounds and hence run time of the sample chromatograms were at least for 60 minutes. From the peak area Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A, and Withanolide B were calculated. RSD for the peak area, retention time and content of all the withanolides were calculated.

4.3.7) Calculation:

Calculation for individual withanolides

$$\frac{\text{Areas of Withanoside IV in the sample}}{\text{Area of the Withanoside IV RS}} \times \frac{\text{Weight of Withanoside IV RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

$$\frac{\text{Areas of Withanoside V in the sample}}{\text{Area of the Withanoside V RS}} \times \frac{\text{Weight of Withanoside V RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

$$\frac{\text{Areas of Withaferin A in the sample}}{\text{Area of the Withaferin A RS}} \times \frac{\text{Weight of Withaferin-A RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

$$\frac{\text{Areas of 12-deoxywithastramonolide in the sample}}{\text{Area of the 12-Deoxystramonolide RS}} \times \frac{\text{Weight of 12-deoxywithastramonolide RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

$$\frac{\text{Areas of Withanolide A in the sample}}{\text{Area of the Withanolide A RS}} \times \frac{\text{Weight of Withanolide A RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

$$\frac{\text{Areas of Withanolide B in the sample}}{\text{Area of the Withanolide B RS}} \times \frac{\text{Weight of Withanolide B RS in mg}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight in mg}} \times \frac{\text{Purity of standard}}{100} \times 100$$

4.3.8) Observation:

Table-18: Peak area of Withanoside IV

Withanoside IV (mcg/ml)	Different Concentrations						
	Dilution -6 (20.64)	Dilution -5 (41.29)	Dilution -4 (82.58)	Dilution -3 (165.15)	Dilution -2 (330.30)	Dilution -1 (660.60)	S. solution (1321.20)
Replicate – 1	166047	329878	651382	1275513	2493967	4815460	9597190
Replicate – 2	166038	330019	651009	1278440	2493747	4811411	9587035
Replicate – 3	166015	329943	651630	1269142	2491031	4819826	9584608
Replicate – 4	166025	329827	649769	1278566	2492809	4815725	9588582
Replicate – 5	165993	329140	651869	1279224	2497552	4818317	9579369
Mean	166024	329761	651132	1276177	2493821	4816148	9587357
RSD	0.01	0.11	0.13	0.33	0.10	0.07	0.07

Table-19: Peak area of Withanoside IV in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate – 1	478028	949957	1320439
Replicate - 2	479376	949797	1333648
Mean	478702	949877	1327044
RSD	0.14	0.01	0.50

Table-20: Peak area of Withanoside V

Withanoside V (mcg/ml)	Different Concentrations						
	Dilution -6 (18.76)	Dilution -5 (37.52)	Dilution -4 (75.04)	Dilution -3 (150.08)	Dilution -2 (300.15)	Dilution -1 (600.30)	S. solution (1200.60)
Replicate – 1	151294	298008	586819	1146136	2238822	4334899	8731747
Replicate – 2	151121	297953	585559	1146843	2237319	4327804	8721728
Replicate – 3	151156	298036	585906	1141122	2243282	4330341	8719358
Replicate – 4	151277	297352	581818	1152990	2237337	4338646	8718285
Replicate – 5	150985	297517	584964	1151745	2248262	4345473	8714428
Mean	151167	297773	585013	1147767	2241004	4335433	8721109
RSD	0.08	0.11	0.33	0.42	0.21	0.16	0.07

Table-21: Peak area of Withanoside V in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	401702	793298	1090118
Replicate - 2	394834	783540	1102054
Mean	398268	788419	1096086
RSD	0.86	0.62	0.54

Table-22: Peak area of Withaferin A

Withaferin A (mcg/ml)	Different Concentrations						
	Dilution -6 (23.05)	Dilution -5 (46.09)	Dilution -4 (92.19)	Dilution -3 (184.39)	Dilution -2 (368.78)	Dilution -1 (737.55)	S. solution (1475.10)
Replicate – 1	573810	1134850	2231492	4332623	8325259	14666731	21469032
Replicate – 2	573224	1134742	2227105	4337796	8322031	14756049	21438480
Replicate – 3	573669	1134589	2228288	4310236	8317825	14770374	21482780
Replicate – 4	573097	1134172	2211324	4335168	8328440	14788044	21438918
Replicate – 5	572942	1131663	2224755	4340882	8338317	14782253	21442185
Mean	573348	1134003	2224593	4331341	8326374	14752690	21454279
RSD	0.07	0.05	0.03	0.06	0.06	0.34	0.10

Table-23: Peak area of Withaferin A in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	1091994	2173830	3006238
Replicate - 2	1091327	2152710	3036695
Mean	1091661	2163270	3021467
RSD	0.03	0.49	0.50

Table-24: Peak area of 12-Deoxy Withastramonolide

12-Deoxy withastramonolide (mcg/ml)	Different Concentrations						
	Dilution -6 (22.21)	Dilution -5 (44.41)	Dilution -4 (88.83)	Dilution -3 (177.65)	Dilution -2 (355.30)	Dilution -1 (710.60)	S. solution (1421.20)
Replicate – 1	635487	1256078	2472835	4800626	9145644	14809446	18080882
Replicate – 2	634565	1254992	2469751	4809678	9138372	14845283	18085009
Replicate – 3	634904	1256714	2471386	4778467	9140140	14833377	18068450
Replicate – 4	634821	1256803	2448908	4808531	9151223	14874117	18045786
Replicate – 5	634615	1254538	2471248	4813165	9156566	14859008	17998894
Mean	634878	1255825	2466826	4802093	9146389	14844246	18055804
RSD	0.06	0.08	0.41	0.29	0.08	0.17	0.20

Table-25: Peak area of 12-Deoxy withastramonolide in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	545128	1088292	1512807
Replicate - 2	541636	1075418	1511759
Mean	543382	1081855	1512283
RSD	0.32	0.59	0.04

Table-26: Peak area of Withanolide A

Withanolide A (mcg/ml)	Different Concentrations						
	Dilution -6 (21.59)	Dilution -5 (43.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (691.02)	S. solution (1382.04)
Replicate – 1	551317	1084798	2130939	4137256	7955602	13463661	13878628
Replicate – 2	550721	1084624	2130222	4140860	7957220	13489137	13809664
Replicate – 3	550930	1084955	2130126	4118314	7958121	13484780	13766317
Replicate – 4	550882	1084918	2112327	4144205	7963123	13502916	13721170
Replicate – 5	552430	1082926	2131439	4149692	7973789	13491381	13664575
Mean	551256	1084444	2127011	4138065	7961571	13486375	13768071
RSD	0.13	0.08	0.39	0.29	0.09	0.11	0.60

Table-27: Peak area of Withanolide A in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	382010	797140	1101225
Replicate - 2	393611	784022	1120023
Mean	387810.5	790581	1110624
RSD	1.50	0.83	0.85

Table-28: Peak area of Withanolide B

Withanolide B (mcg/ml)	Different Concentrations						
	Dilution -6 (21.09)	Dilution -5 (42.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (675.18)	S. solution (1350.36)
Replicate – 1	553596	1095189	2157826	4196971	7938851	12728719	12264511
Replicate – 2	552800	1094573	2156794	4205282	7947620	12721830	12206630
Replicate – 3	552601	1096209	2156648	4174239	7944196	12736068	12166317
Replicate – 4	552865	1094840	2139922	4205370	7953428	12723957	12126384
Replicate – 5	552430	1093649	2158084	4205743	7963457	12720153	12051739
Mean	552858	1094892	2153855	4197521	7949510	12726145	12163116
RSD	0.08	0.09	0.36	0.32	0.12	0.05	0.66

Table-29: Peak area of Withanolide B in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	122902	242191	336094
Replicate - 2	120722	239205	338649
Mean	121812	240698	337371.5
RSD	0.89	0.62	0.38

4.3.9) Conclusion:

From the above analytical data it is observed that the related standard deviation of peak areas of different concentrations of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A, Withanolide B in sample as well as standard is <2.0%.

Precision of Retention time:

4.3.10) Observation

Table-30: Retention time of Withanoside IV

Withanoside IV (mcg/ml)	Different Concentrations						
	Dilution -6 (20.64)	Dilution -5 (41.29)	Dilution -4 (82.58)	Dilution -3 (165.15)	Dilution -2 (330.30)	Dilution -1 (660.60)	S. solution (1321.20)
Replicate – 1	15.466	15.499	15.523	15.539	15.489	15.732	15.513
Replicate – 2	15.481	15.479	15.533	15.539	15.5	15.53	15.516
Replicate – 3	15.474	15.511	15.512	15.504	15.485	15.502	15.517
Replicate – 4	15.467	15.494	15.513	15.534	15.497	15.519	15.513
Replicate – 5	15.483	15.509	15.52	15.502	15.68	15.515	15.511
Mean	15.474	15.498	15.520	15.524	15.530	15.559	15.514
RSD	0.05	0.08	0.05	0.12	0.54	0.62	0.02

Table-31: Retention time of Withanoside IV in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	15.529	15.383	15.462
Replicate - 2	15.372	15.388	15.390
Mean	15.451	15.386	15.426
RSD	0.51	0.02	0.23

Table-32: Retention time of Withanoside V

Withanoside V (mcg/ml)	Different Concentrations						
	Dilution -6 (18.76)	Dilution -5 (37.52)	Dilution -4 (75.04)	Dilution -3 (150.08)	Dilution -2 (300.15)	Dilution -1 (600.30)	S. solution (1200.60)
Replicate – 1	19.651	19.657	19.708	19.721	19.676	19.926	19.688
Replicate – 2	19.641	19.664	19.862	19.686	19.689	19.689	19.695
Replicate – 3	19.658	19.67	19.694	19.691	19.648	19.687	19.702
Replicate – 4	19.635	19.668	19.674	19.691	19.697	19.684	19.706
Replicate – 5	19.665	19.664	19.68	19.688	19.877	19.676	19.704
Mean	19.650	19.665	19.724	19.695	19.717	19.732	19.699
RSD	0.06	0.03	0.40	0.07	0.46	0.55	0.04

Table-33: Retention time of Withanoside V in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	19.684	19.534	19.630
Replicate - 2	19.532	19.540	19.562
Mean	19.608	19.537	19.596
RSD	0.39	0.02	0.17

Table-34: Retention time of Withaferin A

Withaferin A (mcg/ml)	Different Concentrations						
	Dilution -6 (23.05)	Dilution -5 (46.09)	Dilution -4 (92.19)	Dilution -3 (184.39)	Dilution -2 (368.78)	Dilution -1 (737.55)	S. solution (1475.10)
Replicate – 1	20.32	20.328	20.374	20.383	20.337	20.563	20.337
Replicate – 2	20.311	20.332	20.351	20.353	20.349	20.347	20.344
Replicate – 3	20.327	20.341	20.362	20.355	20.314	20.344	20.352
Replicate – 4	20.306	20.339	20.345	20.357	20.361	20.342	20.352
Replicate – 5	20.333	20.336	20.349	20.355	20.525	20.335	20.352
Mean	20.319	20.335	20.356	20.361	20.377	20.386	20.347
RSD	0.05	0.03	0.06	0.06	0.41	0.49	0.03

Table-35: Retention time of Withaferin A in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	20.362	20.224	20.308
Replicate - 2	20.223	20.227	20.248
Mean	20.293	20.226	20.278
RSD	0.48	0.01	0.15

Table-36: Retention time of 12-Deoxy Withastramonolide

12-Deoxy withastramonolide (mcg/ml)	Different Concentrations						
	Dilution -6 (22.21)	Dilution -5 (44.41)	Dilution -4 (88.83)	Dilution -3 (177.65)	Dilution -2 (355.30)	Dilution -1 (710.60)	S. solution (1421.20)
Replicate – 1	21.351	21.351	21.391	21.394	21.36	21.542	21.36
Replicate – 2	21.341	21.358	21.367	21.367	21.366	21.366	21.365
Replicate – 3	21.357	21.361	21.38	21.376	21.341	21.363	21.373
Replicate – 4	21.338	21.362	21.365	21.367	21.382	21.362	21.37
Replicate – 5	21.356	21.358	21.37	21.376	21.51	21.353	21.372
Mean	21.349	21.358	21.375	21.376	21.392	21.397	21.368
RSD	0.04	0.02	0.05	0.05	0.32	0.38	0.03

Table-37: Retention time of 12-Deoxy Withastramonolide in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	21.378	21.261	21.330
Replicate - 2	21.260	21.262	21.284
Mean	21.319	21.262	21.307
RSD	0.28	0.002	0.11

Table-38: Retention time of Withanolide A

Withanolide A (mcg/ml)	Different Concentrations						
	Dilution -6 (21.59)	Dilution -5 (43.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (691.02)	S. solution (1382.04)
Replicate – 1	22.219	22.216	22.255	22.253	22.225	22.391	22.219
Replicate – 2	22.209	22.226	22.23	22.23	22.225	22.227	22.224
Replicate – 3	22.224	22.225	22.245	22.241	22.206	22.221	22.229
Replicate – 4	22.206	22.229	22.231	22.227	22.247	22.223	22.224
Replicate – 5	22.221	22.224	22.235	22.241	22.364	22.213	22.228
Mean	22.216	22.224	22.239	22.062	22.253	22.255	22.225
RSD	0.04	0.02	0.05	0.05	0.29	0.34	0.02

Table-39: Retention time of Withanolide A in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	22.234	22.126	22.192
Replicate - 2	22.126	22.128	22.149
Mean	22.180	22.127	22.171
RSD	0.24	0.005	0.097

PEAK AREA OF WITHANOLIDE B

Table-40: Retention time of Withanolide B

Withanolide B (mcg/ml)	Different Concentrations						
	Dilution -6 (21.09)	Dilution -5 (42.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (675.18)	S. solution (1350.36)
Replicate – 1	25.517	25.509	25.544	25.53	25.514	25.639	25.521
Replicate – 2	25.511	25.526	25.52	25.521	25.514	25.525	25.527
Replicate – 3	25.515	25.516	25.538	25.536	25.512	25.516	25.522
Replicate – 4	25.51	25.526	25.529	25.514	25.551	25.521	25.516
Replicate – 5	25.509	25.52	25.53	25.529	25.625	25.513	25.52
Mean	25.512	25.519	25.532	25.526	25.543	25.543	25.521
RSD	0.01	0.03	0.04	0.03	0.19	0.21	0.02

Table-41: Retention time of Withanolide B in sample (B. No. WS/06Lot10)

Replicate	Conc-1 (753.6 mg/100ml)	Conc-2 (1503.2 mg/100ml)	Conc-3 (2096.0 mg/100ml)
Replicate - 1	25.530	25.449	25.499
Replicate - 2	25.448	25.450	25.476
Mean	25.489	25.450	25.488
RSD	0.16	0.0020	0.06

4.3.10) Conclusion:

1) Relative standard deviation for the mean values of retention times for Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B in standard mix dilution is < 0.1%.

2) Relative standard deviation for the mean values of retention times for Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B in sample is < 0.1%.

For Standard chromatograms refer **Enclosure-5**

Enclosure – 6: Sample chromatograms **(6 Pages)**

4.4) Reproducibility of Results:

4.4.1) Experiment

Eight different samples containing various percentages were analysed in triplicate and Withanolide content of each injection was calculated, the mean of three values and the RSD was determined.

a) **Standard preparation:** 2.18mg of Withanoside IV (Purity 90%), 0.91mg of Withanoside V (Purity90%), 2.04mg of Withaferin A (Purity 99%), 1.86mg of 12-Deoxy withastramonolide (Purity 95%), 1.92mg of Withanolide A (Purity 99%), 1.58mg of Withanolide B (Purity 99%) was dissolved in 5ml of methanol.

b) **Sample preparation:** The sample solution was prepared by weighing the quantity of the sample shown in the **Table** in a 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

Table-42

Name of the sample	Sample weight
WS/06Lot08	1551.9
WS/06Lot10	1535.8
WS/05Lot20	5047.6
WS/05Lot21	1527.0
RD/1170	1503.3
RD/1045	1653.2
RD/1162	4335.7
ERH-46	4228.6

c) **Procedure:** 20µl of standard solutions and sample solutions were injected in triplicate and the chromatograms were recorded. From the mean peak area of standard and samples Withanolide content is calculated.

Table-43: B. No. WS/06Lot08

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.908	0.638	0.652	0.281	0.223	0.066	2.768
Replicate - 2	0.921	0.654	0.663	0.286	0.227	0.067	2.818
Replicate - 3	0.921	0.658	0.662	0.285	0.226	0.066	2.819
Mean	0.917	0.650	0.659	0.284	0.225	0.066	2.802
RSD	0.85	1.61	0.89	0.93	0.86	0.71	1.03

Table-44: B. No.WS/06Lot10

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.909	0.615	0.615	0.267	0.205	0.063	2.673
Replicate - 2	0.912	0.627	0.616	0.267	0.205	0.062	2.690
Replicate - 3	0.911	0.621	0.615	0.266	0.206	0.062	2.682
Mean	0.911	0.621	0.616	0.267	0.205	0.062	2.681
RSD	0.21	0.97	0.09	0.20	0.29	0.31	0.32

Table-45: B. No.WS/05Lot20

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.070	0.046	0.085	0.024	0.022	0.006	0.253
Replicate - 2	0.067	0.044	0.080	0.022	0.021	0.005	0.239
Replicate - 3	0.064	0.042	0.077	0.021	0.020	0.005	0.231
Mean	0.067	0.044	0.081	0.022	0.021	0.005	0.241
RSD	4.50	3.92	4.68	5.13	5.46	3.68	4.56

Table-46: B. No.WS/05Lot21

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.477	0.307	0.410	0.125	0.102	0.029	1.450
Replicate - 2	0.478	0.311	0.410	0.125	0.102	0.029	1.455
Replicate - 3	0.479	0.311	0.411	0.124	0.102	0.029	1.456
Mean	0.478	0.310	0.410	0.125	0.102	0.029	1.453
RSD	0.22	0.71	0.15	0.46	0.21	0.49	0.23

Table-47: B. No.RD/1170

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.861	0.605	0.708	0.337	0.233	0.077	2.821
Replicate - 2	0.844	0.588	0.697	0.330	0.227	0.076	2.762
Replicate - 3	0.839	0.588	0.695	0.330	0.227	0.076	2.755
Mean	0.848	0.594	0.700	0.332	0.229	0.076	2.779
RSD	1.37	1.65	1.04	1.16	1.54	0.97	1.32

Table-48: B. No.RD/1045

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.603	0.361	0.399	0.247	0.130	0.042	1.782
Replicate - 2	0.603	0.366	0.400	0.247	0.130	0.042	1.788
Replicate - 3	0.610	0.365	0.407	0.251	0.132	0.043	1.809
Mean	0.606	0.364	0.402	0.248	0.131	0.042	1.793
RSD	0.68	0.79	1.09	0.95	1.09	0.97	0.80

Table-49: B. No.RD/1162

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.150	0.089	0.099	0.021	0.025	0.004	0.388
Replicate - 2	0.153	0.090	0.101	0.021	0.025	0.004	0.395
Replicate - 3	0.152	0.090	0.100	0.020	0.024	0.004	0.390
Mean	0.152	0.090	0.100	0.021	0.025	0.004	0.391
RSD	1.06	0.74	0.75	1.0	1.06	1.24	0.86

Table-50: B. No.ERH/46

Replicate	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Replicate - 1	0.185	0.149	0.146	0.041	0.041	0.010	0.573
Replicate - 2	0.182	0.146	0.144	0.041	0.041	0.010	0.563
Replicate - 3	0.186	0.148	0.146	0.042	0.042	0.010	0.574
Mean	0.184	0.148	0.145	0.041	0.041	0.010	0.570
RSD	1.26	0.88	1.0	1.09	1.34	1.46	1.07

4.4.2) Conclusion:

1) Relative standard deviation for the mean values of assay of Withanoside IV in the samples is <2.5% except WS/05Lot20 where it is 4.5% because the contents are very low.
2) Relative standard deviation for the mean values of assay of Withanoside V in sample is <2.5% except WS/05Lot20 where it is 3.92% because the contents are very low.
3) Relative standard deviation for the mean values of assay of Withaferin A in sample is <2.5% except WS/05Lot20 where it is 4.68% because the contents are very low.
4) Relative standard deviation for the mean values of assay of 12-Deoxy withastramonolide in <2.5% except WS/05Lot20 where it is 5.13% because the contents are very low.
5) Relative standard deviation for the mean values of assay of Withanolide A in sample is <2.5% except WS/05Lot20 where it is 5.46% because the contents are very low.
6) Relative standard deviation for the mean values of assay of Withanolide B in sample is <2.5% except WS/05Lot20 where it is 3.68% because the contents are very low.

Enclosure-7: Standard chromatograms **(3 Pages)**

Enclosure-8: Sample chromatograms **(24 pages).**

4.4.3) Reproducibility of results by analyzing same sample at various concentrations:

The total withanolides contents were estimated using the same sample at different concentration. The contests were calculated by using only one standard concentration. The results are tabulated.

4.4.4) Experiment

a) Standard preparation: Refer 4.2.4 – Dilution-3.

b) Sample preparation: The sample solution was prepared by weighing the quantity of the sample shown in the **Table** in a 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

Table-51:

Concentration of the extract		Trial-1 (mg/100ml)	Trial-2 (mg/100ml)	Trial-3 (mg/100ml)	Trial-4 (mg/100ml)
Concentration-1	7.5mg/ml	751.1	753.6	747.4	755.3
Concentration-2	10.0mg/ml	1032.2	935.9	1072.5	1010.6
Concentration-3	15.0mg/ml	1659.6	1627.7	1503.2	1497.2
Concentration-4	20.0mg/ml	1959.2	2158.8	2100.3	2096.0
Concentration-5	25.0mg/ml	2762.4	2449.0	2509.0	-

c) Procedure: 20µl of each of the standard solution, 20µl of each concentration of the sample solutions were injected and Chromatograms were recorded. There are various late eluting compounds and hence run time of the sample chromatograms were at least for 60 minutes. From the peak area withanolides were calculated. RSD was determined.

Table-52: Assay content of withanolides in sample: WS/06Lot10

Concentration		W –IV	W –V	WF-A	12-D	W –A	W –B	Sum
Concentration-1 (7.5mg/ml)	Trial -1	0.802	0.681	0.605	0.263	0.209	0.064	2.625
	Trial -2	0.821	0.697	0.617	0.268	0.212	0.066	2.680
	Trial -3	0.828	0.696	0.626	0.272	0.221	0.066	2.709
	Trial -4	0.821	0.684	0.615	0.265	0.218	0.064	2.667
Concentration-2 (10.0mg/ml)	Trial -1	0.808	0.684	0.609	0.265	0.211	0.065	2.642
	Trial -2	0.827	0.703	0.621	0.270	0.215	0.065	2.701
	Trial -3	0.818	0.684	0.612	0.265	0.217	0.064	2.661
	Trial -4	0.826	0.687	0.618	0.268	0.220	0.065	2.684
Concentration-3 (15.0mg/ml)	Trial -1	0.813	0.685	0.608	0.265	0.214	0.064	2.649
	Trial -2	0.818	0.691	0.618	0.269	0.216	0.065	2.678
	Trial -3	0.818	0.690	0.616	0.268	0.221	0.065	2.677
	Trial -4	0.821	0.684	0.612	0.266	0.219	0.064	2.666
Concentration-4 (20.0mg/ml)	Trial -1	0.806	0.686	0.607	0.265	0.210	0.065	2.638
	Trial -2	0.811	0.685	0.612	0.267	0.212	0.065	2.651
	Trial -3	0.822	0.686	0.616	0.266	0.223	0.065	2.677
	Trial -4	0.815	0.68	0.611	0.267	0.219	0.064	2.657
Concentration-5 (25.0mg/ml)	Trial -1	0.800	0.677	0.601	0.264	0.211	0.064	2.617
	Trial -2	0.817	0.679	0.610	0.267	0.218	0.065	2.656
	Trial -3	0.818	0.679	0.612	0.268	0.221	0.065	2.663
Mean		**0.816**	**0.686**	**0.613**	**0.267**	**0.216**	**0.065**	**2.663**
RSD		**0.98**	**0.98**	**0.96**	**0.83**	**2.01**	**1.01**	**0.88**

W-IV: Withanoside IV
W-V: Withanoside V
WF-A: Withaferin-A
12-D: 12-Deoxy Withastramonolide
W-A: Withanolide A
W-B: Withanolide B

4.4.5) Conclusion: Relative standard deviation of assay of sum of withanolides is < 1.0%
The above results indicate the method is precise and reproducible irrespective of the sample concentration taken for analysis.

For standard chromatograms Enclosure – 5

Enclosure-9: Sample chromatograms **(19 Pages).**

4.5) Accuracy:

4.5.1) Definition: The accuracy is expressed as the difference between the average value obtained from large series of observed value and the true value.

4.5.2) Assessment: Accuracy is determined by Spike recovery method. A known quantity of standards is added to the sample and the sample is re-analyzed using the same method which is to be validated.

4.5.3) Four different batches of *Withania somnifera* extracts were analyzed to find out actual content of withanolides. All markers were calculated using standard dilution–III. The same sample were spiked with different quantities of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy Withastramonolide, Withanolide A and Withanolide B and analysed for their total content.

4.5.4) Acceptance Criteria:

1) For Assay of >10 and < 95% recovery should be 95.0 -110%.
2) For Assay of >7.5 and < 10% recovery should be 90-115%.
3) For the Assay > 0.5 and < 7.5% recovery should be 85-120%.
4) For the Assay > 0.1 and < 0.5% recovery should be 75-125%.
5) For the Assay of < 0.1% recovery should be 70 to 130%.

4.5.5) Experiment:

a) Standard preparation: Refer 4.2.4 – Dilution-3.

b) Sample preparation: The sample solution was prepared by weighing the quantity of the sample shown in the **Table** in a 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

Table-53:

Batch No.	Quantity (mg)
WS/06Lot08	1714.4
WS/06Lot10	1520.4
WS/05Lot20	5029.7
WS/05Lot21	2612.5
RD/1170	1632.9
RD/1045	1640.2
RD/1162 RM	4003.8
ERH-46 RM	4091

d) Procedure: 20µl of each of the standard solution, 20µl of each concentration of the sample solutions were injected and Chromatograms were recorded. From the peak area individual withanolides content were calculated. The results are given below: -

Table-54:

Batch No.	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
WS/06Lot08	0.798	0.700	0.644	0.278	0.226	0.067	2.715
WS/06Lot10	0.804	0.684	0.606	0.266	0.208	0.064	2.631
WS/05Lot20	0.053	0.044	0.069	0.019	0.020	0.005	0.211
WS/05Lot21	0.399	0.319	0.413	0.123	0.107	0.030	1.390
RD/1170	0.746	0.644	0.690	0.335	0.239	0.079	2.733
RD/1045	0.552	0.416	0.408	0.253	0.137	0.045	1.812
RD/1162 RM	0.131	0.095	0.089	0.019	0.023	0.004	0.360
ERH-46 RM	0.155	0.151	0.130	0.037	0.039	0.010	0.523

For standard chromatograms Enclosure – 5

Enclosure-10: Initial analysis sample chromatograms **(8 Pages).**

Spike recovery studies:

a) Sample Preparation for Spike recovery:

1) Samples of WS/05Lot21 (453.70 mg) was weighed in to 250 ml beaker and 0.91mg of Withanoside IV, 2.53mg of Withanoside V, 1.03mg of Withaferin A, 1.09mg of 12-Doxy withastramonolide, 1.03mg of Withanolide A and 0.90mg of Withanolide B were added to the same beaker. The mixture was extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper. 20µl of standard solution dilution-3 (Refer 4.2.4) and sample solutions were injected and chromatograms were recorded. From the obtained chromatograms individual withanolides content were calculated.

2) Samples of WS/06Lot10 (328.90mg) was weighed in to 250 ml beaker and 1.08mg of Withanoside IV, 2.38mg of Withanoside V, 1.00mg of Withaferin A, 1.00mg of 12-Doxy withastramonolide, 0.91mg of Withanolide A and 1.15mg of Withanolide B were added to the same beaker. The mixture was extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane

filter paper. 20µl of standard solution dilution-3 (Refer 4.2.4) and sample solutions were injected and chromatograms were recorded. From the obtained chromatograms individual withanolides content were calculated.

3) Samples of RD/1170 (313.9mg) was weighed in to 250 ml beaker and 0.94mg of Withanoside IV, 2.43mg of Withanoside V, 1.14mg of Withaferin A, 0.97mg of 12-Doxy withastramonolide, 0.91mg of Withanolide A and 1.02mg of Withanolide B were added to the same beaker. The mixture was extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper. 20µl of standard solution dilution-3 (Refer 4.2.4) and sample solutions were injected and chromatograms were recorded. From the obtained chromatograms individual withanolides content were calculated.

4) Samples of RD/1162-RM (755.9mg) was weighed in to 250 ml beaker and 0.96mg of Withanoside IV, 2.43mg of Withanoside V, 1.26mg of Withaferin A, 1.22mg of 12-Doxy withastramonolide, 1.25mg of Withanolide A and 1.07mg of Withanolide B were added to the same beaker. The mixture was extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper. 20µl of standard solution dilution-3 (Refer 4.2.4) and sample solutions were injected and chromatograms were recorded. From the obtained chromatograms individual withanolides content were calculated.

5) Samples of WS/06Lot08 (1714.4mg), WS/05Lot20 (5029.7mg), RD/1045 (1640.2mg), ERH-46 (4071mg) were weighed in to a separate 250 ml beaker and was extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 100ml with methanol.

1ml each of these sample solutions were mixed with 1ml of standard solution containing 0.164mg of Withanoside IV, 0.056mg of Withanoside V, 0.23mg of Withaferin A, 0.19mg of 12-Doxy withastramonolide, 0.16mg of Withanolide A and 0.156mg of Withanolide B. The resultant solution was filtered through 0.45 microns membrane filter paper.

20µl of standard solution dilution-3 (Refer 4.2.4) and sample solutions were injected and chromatograms were recorded. From the obtained chromatograms individual withanolides content were calculated.

b) Standard preparation for Spike recovery: Refer section 4.2.4 (Dilution-4)

Table-55: WS/05Lot21: Weight of sample: 453.70 mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken	Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
W-IV	0.399	453.7	1.81	0.91	90	2.629	0.570	0.550	96.45
W-V	0.319		1.447	2.53	90	2.358	0.511	0.494	96.54
WF-A	0.413		1.874	1.03	99	2.893	0.627	0.622	99.15
12-D	0.123		0.558	1.09	95	1.594	0.346	0.324	93.69
W –A	0.107		0.485	1.03	99	1.505	0.326	0.306	93.72
W –B	0.030		0.136	0.90	99	1.027	0.223	0.237	106.48

Table-56: WS/06LoT10: Weight of sample: 328.90mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken	Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
W-IV	0.804	328.90	2.684	1.08	90	3.656	1.087	1.028	94.62
W-V	0.684		2.256	2.38	90	3.113	0.925	0.901	97.40
WF-A	0.606		2.016	1.00	99	3.006	0.894	0.857	95.86
12-D	0.266		0.878	1.00	95	1.828	0.543	0.508	93.39
W –A	0.208		0.710	0.91	99	1.611	0.479	0.465	97.18
W –B	0.064		0.214	1.15	99	1.352	0.402	0.399	99.30

Table-57: RD/1170: Weight of sample: 313.9mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken	Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
W-IV	0.746	313.9	2.342	0.94	90	3.188	0.992	0.951	95.88
W-V	0.644		2.022	2.43	90	2.896	0.901	0.864	95.82
WF-A	0.690		2.166	1.14	99	3.295	1.025	0.998	97.30
12-D	0.335		1.052	0.97	95	1.973	0.614	0.597	97.19
W –A	0.239		0.750	0.91	99	1.651	0.514	0.495	96.41
W –B	0.079		0.248	1.02	99	1.258	0.391	0.384	98.05

Table-58: RD/1162-RM: Weight of sample: 755.9mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken	Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
W-IV	0.121	755.9	0.915	0.96	90	1.779	0.233	0.230	99.01
W-V	0.094		0.711	2.43	90	1.585	0.207	0.198	95.34
WF-A	0.089		0.673	1.26	99	1.920	0.251	0.248	98.79
12-D	0.018		0.136	1.22	95	1.295	0.169	0.155	91.18
W –A	0.023		0.174	1.25	99	1.411	0.185	0.174	94.47
W –B	0.006		0.045	1.07	99	1.105	0.145	0.141	97.51

Table-59: WS/06LOT08: Weight of sample: 1714.4mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken		Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
			100 ml	1 ml						
W-IV	0.798	1714.4	13.7	0.137	0.164	90	0.284	1.571	1.580	100.58
W-V	0.700		12.0	0.120	0.056	90	0.170	0.941	0.940	99.84
WF-A	0.644		11.0	0.110	0.230	99	0.338	1.868	1.865	99.86
12-D	0.278		4.8	0.048	0.190	95	0.228	1.261	1.271	100.86
W –A	0.226		3.9	0.039	0.160	99	0.197	1.089	1.059	97.26
W –B	0.067		1.1	0.011	0.156	99	0.166	0.917	0.688	75.03

Table-601: WS/05LOT20: Weight of sample: 5029.7mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken		Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
			100 ml	1 ml						
W-IV	0.053	5029.7	2.7	0.027	0.164	90	0.174	0.340	0.345	101.50
W-V	0.044		2.2	0.022	0.056	90	0.073	0.142	0.142	100.06
WF-A	0.069		3.5	0.035	0.230	99	0.262	0.512	0.510	99.55
12-D	0.019		1.0	0.010	0.190	95	0.190	0.371	0.375	101.02
W –A	0.020		1.0	0.010	0.160	99	0.168	0.329	0.323	98.41
W –B	0.005		0.3	0.003	0.156	99	0.157	0.306	0.226	73.69

Table-61: RD/1045: Weight of sample: 1640.2mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken		Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
			100 ml	1 ml						
W-IV	0.552	1640.2	9.1	0.091	0.164	90	0.238	1.372	1.369	99.81
W-V	0.416		6.8	0.068	0.056	90	0.119	0.683	0.685	100.28
WF-A	0.408		6.7	0.067	0.230	99	0.295	1.697	1.702	100.27
12-D	0.253		4.1	0.041	0.190	95	0.222	1.279	1.296	101.35
W –A	0.137		2.2	0.022	0.160	99	0.181	1.042	1.003	96.29
W –B	0.045		0.7	0.007	0.156	99	0.162	0.932	0.698	74.88

Table-62: ERH-46: Weight of sample: 4071mg - 100ml with methanol

Marker	Actual content (% w/w)	Weight of sample (mg)	Actual quantity in the weight taken		Quantity added (mg)	Purity	Total Quantity (mg)	Calculated Values (%w/w)	Observed Value (%w/w)	Recovery (%w/w)
			100 ml	1 ml						
W-IV	0.155	4071	6.3	0.063	0.164	90	0.211	0.506	0.515	101.82
W-V	0.151		6.1	0.061	0.056	90	0.112	0.268	0.272	101.21
WF-A	0.130		5.3	0.053	0.230	99	0.281	0.674	0.678	100.71
12-D	0.037		1.5	0.015	0.190	95	0.196	0.469	0.477	101.64
W –A	0.039		1.6	0.016	0.160	99	0.174	0.418	0.416	99.57
W –B	0.010		0.4	0.004	0.156	99	0.159	0.380	0.283	74.31

4.5.6) Conclusion:

Table-63:

Batch No.	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B
WS/06Lot08	100.6	99.8	99.9	100.9	97.3	75.0
WS/06Lot10	94.6	97.4	95.9	93.4	97.2	99.3
WS/05Lot20	101.5	100.1	99.6	101.0	98.4	73.7
WS/05Lot21	96.5	96.5	99.2	93.7	93.7	106.5
RD/1170	95.9	95.8	97.3	97.2	96.4	98.1
RD/1045	99.8	100.3	100.3	101.4	96.3	74.9
RD/1162 RM	99.0	95.3	98.8	91.2	94.5	97.5
ERH-46 RM	101.8	101.2	100.7	101.6	99.6	74.3

Spike recovery of Total withanolides is within the acceptance criteria indicating the method is accurate.

Enclosure-11: Sample chromatograms **(8 Pages).**

4.6) Ruggedness

4.6.1) Definition: Ruggedness of an analytical method is a measure of degree of reproducibility of results obtained under different environmental conditions such as different laboratory, different chemist and different instrument.

Purpose of ensuring the method under validation provides the same results when analysed in a different HPLC, different brand column and different conditions.

4.6.2) Assessment: Withanoside IV, Withanoside V, Withaferin A, 12-Doxy withastramonolide, Withanolide A and Withanolide B contents in extracts sample and raw material were analysed by two different analysts at different dates using different instruments and C18 column from a different manufacturer. The results were compared and RSD was calculated.

Table-64:

Name of the instrument	Condition–1	Condition-2
HPLC	LC-2010A	LC10ATvp
C18 Column	Lichrospher 100 RP-18e(5µm) Col. No. 625364 Make: Merck (Hiber)	Phenomenex, Luna 5 µ C_{18} (2)100 A, 250 X 4.60 mm, 5 microns Part No. :00G-4252- E0 No. 359208-12
Column oven temperature	25°C	30°C
Concentration of H_3PO_4	0.5ml in 1000ml	1.5ml in 1000ml

4.6.3) Acceptance criteria: The RSD between the two results obtained two different analysts using two different instruments on different date should be less than 6.0%.

4.6.4) Experiment:

Experiment-1 (Condition - 1)

a) Standard / sample preparation: Refer 4.3.11

Table-65:

Batch No.	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastra monolide	Withanolide A	Withanolide B	Sum
WS/06Lot08	0.917	0.650	0.659	0.284	0.225	0.066	2.802
WS/06Lot10	0.911	0.621	0.616	0.267	0.205	0.062	2.681
WS/05Lot20	0.067	0.044	0.081	0.022	0.021	0.005	0.241
WS/05Lot21	0.478	0.310	0.410	0.125	0.102	0.029	1.453
RD/1170	0.848	0.594	0.700	0.332	0.229	0.076	2.779
RD/1045	0.606	0.364	0.402	0.248	0.131	0.042	1.793
RD/1162	0.152	0.090	0.100	0.021	0.025	0.004	0.391
ERH-46	0.184	0.148	0.145	0.041	0.041	0.010	0.570

Experiment-2 (Condition - 2)

b) Standard / sample preparation: Refer 4.3.11

Table-66:

Batch No.	Withanoside IV	Withanoside V	Withaferin A	12-Deoxy withastra monolide	Withanolide A	Withanolide B	Sum
WS/06Lot08	1.071	0.629	0.608	0.304	0.290	0.071	2.974
WS/06Lot10	1.047	0.601	0.561	0.282	0.258	0.067	2.817
WS/05Lot20	0.080	0.044	0.083	0.024	0.027	0.0005	0.263
WS/05Lot21	0.538	0.298	0.415	0.130	0.128	0.027	1.537
RD/1170	0.995	0.599	0.740	0.257	0.348	0.080	3.020
RD/1045	0.744	0.357	0.422	0.140	0.201	0.043	1.906
RD/1162	0.162	0.083	0.090	0.022	0.025	0.0004	0.386
ERH-46	0.213	0.147	0.141	0.046	0.403	0.011	0.601

Table-67:

Batch No.	Condition	Sum
WS/06Lot08	1	2.974
	2	2.802
	RSD	**2.977839**
WS/06Lot10	1	2.817
	2	2.681
	RSD	**2.473627**
WS/05Lot20	1	0.263
	2	0.241
	RSD	**4.365079**
WS/05Lot21	1	1.537
	2	1.453
	RSD	**2.809365**
RD/1170	1	3.020
	2	2.779
	RSD	**4.155889**
RD/1045	1	1.906
	2	1.793
	RSD	**3.05488**
RD/1162	1	0.386
	2	0.391
	RSD	**0.643501**
ERH-46	1	0.601
	2	0.570
	RSD	**2.64731**

4.6.5) Conclusion: Relative standard deviations for same batch sample when analysed in two different instruments found to be < 6.0% indicating the method is very rugged.

Enclosure 12: Standard and sample chromatograms **(27 pages)**

4.7) Range of quantification:

4.7.1) Definition: The range of the method is the interval between the lower and upper concentration level that have been demonstrated to be linear.

4.7.2) Assessment: It was calculated from the graph. A graph is plotted using the concentration on the X-axis and response factor on Y-axis. Response factor is the ratio between the area and the concentration in mcg/ml.

4.7.3) Acceptance Criteria: Response factors of concentrations fall within the horizontal zone ±**7.5%**.

4.7.4) Experiment:

a) Procedure: 20µl of each individual standard solution (refer section 4.2.4) were injected in replicates and the chromatograms were recorded. Mean area was determined. Response factors were calculated for all the Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy withastramonolide, Withanolide A and Withanolide B by dividing the mean area with the concentration.

The mean response factor for Withanoside IV between the concentration 20.64 to 330.3 mcg/ml is 7838.43

Table-68:

Withanoside IV (mcg/ml)	**Different Concentrations**						
	Dilution -6 (20.64)	**Dilution -5 (41.29)**	**Dilution -4 (82.58)**	**Dilution -3 (165.15)**	**Dilution -2 (330.30)**	**Dilution -1 (660.60)**	**S. solution (1321.20)**
Mean	**166024**	**329761**	**651132**	**1276177**	**2493821**	**4816148**	**9587357**
Response factor Area / concentration	**8042.32**	**7986.95**	**7885.34**	**7727.38**	**7550.17**	7290.57	7256.55
Mean (Dilution 2 to 6)	**7838.43**						

± 5.0% of 7838 is 7446 to 8229

The mean response factor for Withanoside V between the concentration 18.76 to 300.15 mcg/ml is 7781.07.

Table-69:

Withanoside V (mcg/ml)	Different Concentrations						
	Dilution -6 (18.76)	Dilution -5 (37.52)	Dilution -4 (75.04)	Dilution -3 (150.08)	Dilution -2 (300.15)	Dilution -1 (600.30)	S. solution (1200.60)
Mean	**151167**	**297773**	**585013**	**1147767**	**2241004**	**4335433**	**8721109**
Response factor Area / concentration	**8058.19**	**7936.65**	**7796.28**	**7647.96**	**7466.28**	7222.11	7263.96
Mean (Dilution 2 to 6)	**7781.072**						

±5.0% of 7781 is7391 to 8170

The mean response factor for Withaferin A between the concentration 23.05 to 184.39 mcg/ml is 24274.

Table-70:

Withaferin A (mcg/ml)	Different Concentrations						
	Dilution -6 (23.05)	Dilution -5 (46.09)	Dilution -4 (92.19)	Dilution -3 (184.39)	Dilution -2 (368.78)	Dilution -1 (737.55)	S. solution (1475.10)
Mean	**573348**	**1134003**	**2224593**	**4331341**	**8326374**	**14752690**	**21454279**
Response factor Area / concentration	**24875.80**	**24600.44**	**24129.54**	**23490.43**	22578.47	20002.29	14544.29
Mean (Dilution 3 to 6)	**24274.053**						

±5.0% of 24274 is 23060 to 25487

The mean response factor for 12-Deoxy withastramonolide between the concentration 22.21 to 177.65 mcg/ml is 27917.

Table-71:

12-Deoxy withastramonolide (mcg/ml)	Different Concentrations						
	Dilution -6 (22.21)	Dilution -5 (44.41)	Dilution -4 (88.83)	Dilution -3 (177.65)	Dilution -2 (355.30)	Dilution -1 (710.60)	S. solution (1421.20)
Mean	**634878**	**1255825**	**2466826**	**4802093**	**9146389**	**14844246**	**18055804**
Response factor Area / concentration	**28590.08**	**28276.39**	**27771.75**	**27031.20**	25742.72	20889.74	12704.62
Mean (Dilution 3 to 6)	**27917.36**						

±5.0% of 27917 is 26521 to 29312

The mean response factor for Withanolide A between the concentration 21.59 to 172.76 mcg/ml is 24803.79.

Table-72:

Withanolide A (mcg/ml)	Different Concentrations						
	Dilution -6 (21.59)	Dilution -5 (43.19)	Dilution -4 (86.38)	Dilution -3 (172.76)	Dilution -2 (345.51)	Dilution -1 (691.02)	S. solution (1382.04)
Mean	**551256**	**1084444**	**2127011**	**4138065**	**7961571**	**13486375**	**13768071**
Response factor Area / concentration	**25527.76**	**25109.41**	**24624.59**	**23953.38**	23042.95	19516.62	9962.14
Mean (Dilution 3 to 6)	**24803.79**						

±5.0% of 24803 is 23562 to 26043

The mean response factor for Withanolide B between the concentration 21.09 to 168.79 mcg/ml is 25634.15.

Table-73:

Withanolide B (mcg/ml)	Different Concentrations						
	Dilution -6 (21.09)	Dilution -5 (42.19)	Dilution -4 (84.39)	Dilution -3 (168.79)	Dilution -2 (337.59)	Dilution -1 (675.18)	S. solution (1350.36)
Mean	**552858**	**1094892**	**2153855**	**4197521**	**7949510**	**12726145**	**12163116**
Response factor Area / concentration	**26202.60**	**25946.08**	**25520.36**	**24867.57**	23547.83	18848.52	9007.31
Mean (Dilution 3 to 6)	**25634.15**						

±5.0% of 25634 is 24352 to 26915

Though the linearity is observed through out the six concentrations ranging from 20 to 600mcg. The following limits are considered as a better working range based on linearity and closeness (within ± 5%) to mean response factor.

Table-74:

Name of the analyte	Concentration (mcg/ml)
Withanoside IV	20.64 to 330.3
Withanoside V	18.76 to 300.15
Withaferin A	23.05 to 184.39
12-Deoxy withastramonolide	22.21 to 177.65
Withanolide A	21.59 to 172.76
Withanolide B	21.09 to 168.79

Table-75:

Name of the analyte	Mean response factor	Relative response factor in comparison with Withaferin A	Multiplication factor
Withanoside IV	7838	0.322	3.1
Withanoside V	7781	0.321	3.1
Withaferin A	24274	1	1
12-Deoxy withastramonolide	27917	1.15	0.87
Withanolide A	24803	1.02	0.98
Withanolide B	25634	1.056	0.947

4.8) System suitability:

4.8.1) Definition: System suitability tests are integral part of chromatographic methods and they are used to verify the performance of the chromatographic system. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such.

4.8.2) Assessment: To ascertain the effectiveness of the final operating system, it is subjected to suitability testing. Replicate injections of the standard preparation are made to demonstrate adequate system precision. It is performed before the injection of samples.

The resolution is checked to verify the resolution power of the column and to ensure that closely eluting compounds are resolved from each other. Column efficiency is further checked based on theoretical plates and asymmetry factor. No sample analysis is acceptable unless the requirements of system suitability have been met.

4.8.3) Acceptance criteria: To be determined.

4.8.4) Experiment: Refer 4.1

4.8.5) Observation: Refer **4.1**

4.8.6) Conclusion: The following system suitability parameters are determined for this method: -

1) Standard solution and reference extract solution to be injected
2) Peaks of all the withanolides are well separated and the resolution is >3 for Withanoside & Withaferin A in the standard solution.
3) The asymmetric factor (tailing factor) for Withanolides should be < 1.5.
4) The relative retention time of Withanoside IV is about 0.70, Withanoside V is about 0.89, Withaferin A is about 0.92, 12-Deoxy withastramonolide is about 0.96 and Withanolide B is about 1.15.
5) The precision of injection (with minimum of 3 replicates) RSD should be <1.0% for standard and sample solution.
6) Retention time window ± 3σ of mean retention time of the standard or reference extract.
7) Precision in result (The result of QC check sample (reference extract) shall be within ±10% of the labelled result).

If the system suitability parameters are not met, achieve the same by changing the composition of the mobile phase within permissible range or by using new column. The analysis is to be proceeded further only after the system suitability parameters are met.

5.0] STEP-2: STABILITY OF SAMPLE SOLUTIONS

5.1) Assessment: Stability of in solution was determined by checking the assay of a sample using freshly prepared solution of that sample and comparing it with the assay after 24 hours of preparing the solution.

5.2) Acceptance Criteria: The longest duration at which the assay shows variation ± 4.0%.

5.3) Experiment: Batch No. WS/05Lot21 prepared and injected to the HPLC immediately after the preparation. The sample solution was left in the auto injector vial and again injected exactly after 24 hours. The assays were calculated in 0, 6, and 24 hours.

a) Standard solution preparation: Refer 4.2.4 – Dilution-6.

b) Sample preparation: Sample of WS/05Lot021 (2612.50mg) was weighed in 250 ml beaker, extracted with 100ml of methanol, boiling on water bath for 10-15 minutes and repeated the procedure 3-4 times till the material is completely extracted or till the extracts turn colourless. Combined all the fractions, concentrated and made up the volume to 50ml with methanol. Filtered through 0.45 microns membrane filter paper.

5.4) Procedure: 20µl of each standard solution was injected and chromatogram was recorded. 20µl of sample solution at 0 and 24 hours were injected and chromatograms were recorded. Withanolides content were calculated using the peak area, sample and standard.

Table-76: Withanolides content at various intervals

Duration	Withanoside IV (%w/w)	Withanoside V (%w/w)	Withaferin A	12-Deoxy withastramonolide	Withanolide A	Withanolide B	Sum
Initial result	0.399	0.319	0.413	0.123	0.107	0.030	1.390
After 24 hours	0.409	0.350	0.415	0.122	0.110	0.030	1.435

The percentage variation is calculated using the following formula: -

$$\frac{\text{The difference between two results} \times 100}{\text{The initial result}} = \%$$

$$\frac{1.435 - 1.390 \times 100}{1.390} = 3.237\%$$

Conclusion: The solution is stable for 24 hours.

Enclosure-13: Standard and sample chromatograms **(4 Pages)**

6.0] VALIDATION RESULT (CONCLUSIONS):

6.1) HPLC consisting of the following modules are least required for the performing the analysis.

a) Pump
b) UV / PDA detector (227nm ± 3)
c) Auto injector
d) Column oven
e) Data processor / Integrator / Control software

Any Octa Decyl Siliane (ODS) column 250 x 4.0 mm, 5µ particle size, 100 A° pore size with carbon content >10% and end capped is suitable. Particle size up to 3µ and dimension up to 15 cms and diameter up to 4.6mm can also be used. In most of the C18 column a peak which is eluting just before Withanoside IV may merge with it leading to higher content of Withanoside IV. Hence Merck Hibar Lichrosphere, Phenomenex, Luna are the validated columns for this estimation.

6.2) **Specificity:** PDA spectra and the peak purity of the Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy Withastramonolide, Withanolide A, Withanolide B indicate the method is very specific for withanolides and there is no interference from any other derivatives present in the extract which is confirmed by the resolution of Withanoside IV, Withanoside V, Withaferin A, 12-Deoxy Withastramonolide, Withanolide A, Withanolide B.

Permissible variation allowed in the mobile phase concentration is ± 3% of each solvent. The column to be maintained constant temperature (a temperature of 20 – 35) is suitable.

6.3) **Linearity:** The Correlation Coefficient (r^2) for withanolides was found to be > 0.98 indicating the method is linear.
Though the method is linear up to concentration 1000mcg, it is advisable to operate within 20-200mcg where the linearity is the best.

6.4) **Precision:** The RSD was found to be within the specified limits for area, retention time and for the precision of assay indicating the method is precise and reproducible.

The method is precise. The variation in the retention time is found to be within 1% RSD. Hence the variation of the retention time is to be ± 3σ of the mean of the retention time of standard and reference extract. Number of injections of standard or reference extract used for calculation of RT window should be ≥ 3 and to be done initially and at the end of the

analysis. If the variation in retention time is more than the limit, the result should not be reported.

The highest variation observed in the reproducibility in the area is 1.5%. Hence mean of duplicate injection of sample and mean of triplicate injection of standard is recommended for calculation. The variation found in reproducibility of result for total withanolides is RSD <1.5%. Hence duplicate result obtained with two trials of sample with same concentration should be < 2% otherwise the analysis need to be repeated.

6.5) System suitability: The following system suitability parameters are determined for this method: -

1) Standard solution and reference extract solution to be injected
2) Peaks of all the withanolides are well separated and the resolution is >3 for Withanoside & Withaferin A in the standard solution.
3) The asymmetric factor (tailing factor) for Withanolides should be < 1.5.
4) The relative retention time of Withanoside IV is about 0.70, Withanoside V is about 0.89, Withaferin A is about 0.92, 12-Deoxy withastramonolide is about 0.96 and Withanolide B is about 1.15.
5) The precision of injection (with minimum of 3 replicates) RSD should be <1.0% for standard and sample solution.
6) Retention time window $\pm 3\sigma$ of mean retention time of the standard or reference extract.
7) Precision in result (The result of QC check sample (reference extract) shall be within ±10% of the labelled result).

If the system suitability parameters are not met, achieve the same by changing the composition of the mobile phase within permissible range or by using new column. The analysis is to be proceeded further only after the system suitability parameters are met.

6.6) Accuracy: Spike recovery is >90% indicating the method is accurate.

6.7) Ruggedness: Relative standard deviations for same batch sample when analysed in two different instruments found to be < 5.0% indicating the method is rugged.

6.8) Final Conclusion: On the basis of above results the HPLC method [NR/QCD/APM04 WI(17)] for the estimation of ***Withania somnifera*** is considered as validated.

7.0] REVALIDATION CRITERIA: The above HPLC method need to be revalidated if the

1) Sample matrix is different (for example *Withania somnifera* extract is mixed with other botanical extracts or excipients).
2) If there is any change in the column packing material other than C18 or the dimension of the column or the particle size are very different from the one used for the validation (example instead of column with dimension 250mm x 4.6mm, a column 5 mm x 4 or 15 x 2.0 mm are used).
3) An instrument with different characteristic is introduced like change in the detector (example instead of a UV detector an ELSD etc.)
4) If sample solution results or QC sample analysis are out of specification and the source of error cannot be tracked back. However, it needs to be identified.

The extent of revalidation and the method characteristic selected solely depends on need and lead to the choice of the in-charge.

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