ESTIMATION AND VALIDATION OF RESIDUAL SOLVENT BY GC HEAD SPACE MASS CHROMATOGRAPHY OF COLCHICINE USP

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Abstract

The present paper deals with the estimation and validation of residual solvent by GC head space mass chromatography of the drug Colchicine USP. The various parameters were evaluated and the data were presented in the present communications which establish the estimation and validation of the drug colchicines USP.

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Introduction

The objective of validation of an analytical procedure is to demonstrate that it is suitable for the analysis of Colchicine for the determination of residual solvents/ Organic Volatile Impurities. The protocol is limited to validate the analytical procedure of colchicine for Organic Volatile Impurities only. The scope of this method validation protocol is to establish through documented evidence a high degree of assurance that an analytical method for organic volatile impurities will consistently yield results that accurately reflect the quality characteristics of the drug substance (Colchicine) tested. The description of product is given in table 1[1-2].

Table 1: Product description

1	Product	Colchicine USP
2	Batch Size	4.0 Kg
3	Mfg. License No.	6/UA/2009
4	Shelf Life	36 Months
5	Analysis Sample Qty.	7.0gm
6	Control Sample Qty.	21.0gm
7	Analysis reference (Test sample Colchicine)	USP

Material and Methods[3-10]

Method detail for standard and sample was presented in table 2. The instrumentation used in the present investigation is Gas chromatograph Agilent Technology-7890A, Headspace sampler (Agilent-7694E), Data handling system (MSD Chemstation, Version E.02.00.493), GC Column DB-624 (30 m× 0.53mm × 3.0µm)

Table 2: Standard and sample details

Sr. No.	Name	B. No. / Lot No.	Make	Purity (%)
1.	Methanol	95796912-3	Qualigens	99.8
2.	Ethanol	K40006483918	Merk	99.9
3.	Acetone	86426908-2	Qualigens	99.0
4.	Methylene dichloride	NL-2617-6402V	Qualigens	99.0
5.	Hexane	R144F09	Rankem	99.02
6.	Chloroform	89686912-2	Qualigens	99.5
7.	Sodium chloride	2944/1 6804-1	Qualigens	99.5
8.	Dimethyl formamide	8575-6907-2	Qualigens	99.0
9.	Water MILLI – Q	9109 6810-3	Qualigens	-
10.	Test Sample - COLCHICINE USP	M0250709	-	-

Preparation of Diluent: Use Dimethlformamide (DMF) and water mixture as diluent. (60:40)

Preparation of Blank: Transfer 5.0 ml of diluent in headspace vial containing 0.5gm sodium chloride and seal the vial immediately.

Standard Preparation:

Weigh accurately about 200 mg Ethanol, 200 mg Acetone, 11.6 mg Hexane, 4.0 mg Chloroform, 120 mg Methanol and 24 mg Methylene dichloride in 100ml Volumetric flask containing about 20 ml of diluent, make up to volume with diluent and shake well. Pipette out 10 ml of this solution in 100 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

Pipette 5 ml of above prepared solution in headspace vial containing 0.5 gm sodium chloride and seal the vial.

Test Sample Preparation:

Weigh accurately about 200 mg of test sample and transfer in headspace vial, add 5.0 ml of diluent in headspace vial containing 0.5 gm sodium chloride and seal the vial.

GC Parameter and Condition:

Initial Oven Temperature : 45^0C
Initial Hold Time : 5.0 min.
Ramp : 10.0^0C/min
Oven Temperature II : 90^0C
Hold Time II : 5.0 min.
Final Ramp : 20^0C
Final Oven Temperature : 200^0C
Final Hold Time : 5.0 min.
Carrier Gas : Nitrogen
Flow : 1.2 ml/min.
Split Ratio : 5:1

Injector Temperature	: 150^0C
Detector Temperature	: 250^0C
Head Space Parameters:	
Vial Temp.	: 80.0^0C
Loop Temp.	: 95.0^0C
Transfer line Temp.	: 105^0C
GC cycle Time	: 40 min.
Vial Equilibration Time	: 45 min.
Pressurization Time	: 0.5 min
Loop Fill Time	: 0.2 min.
Loop equilibration Time	: 0.05 min.
Shake	: 1 (Low)
Injection Time	: 1.0 min.
Withdrawal Time	: 0.5 min.
Thermostat Time	: 20 min.

Procedure:
Set the gas chromatograph and condition as mentioned above. After attaining the set temperature, ignite FID detector. Allow system to run for sometime to stabilize the baseline. It should not show any peak & drift. Inject blank preparation once, standard preparation in replicate and sample preparation in duplicate by taking separate weight.
The % RSD of area counts of replicate injections of all solvents standard is not more than 15.

Approximate RT and RRTs are given in table:

Name of the Peak	RT (min.)	RRT
Methanol	6.958	1.00
Ethanol	8.457	1.21
Acetone	9.323	1.34
MDC	10.254	1.47
Hexane	11.393	1.64
Chloroform	13.639	1.96

The calculation can be done by the following procedure

$$\text{Solvent Content } (\mu g/g) = \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{PF}{100} \times 1000000$$

Where, AT: Area of solvent in test preparation, AS: Average area of solvent in standard preparation, DS: Dilution factor for standard preparation, DT: Dilution factor for test preparation, PF: Purity Factor of standard

Specificity
Specificity was performed to demonstrate non-interference due to presence of other residual volatile impurities in the determinations of solvents (Methanol, Ethanol, Acetone, Methylene dichloride, Hexane, Chloroform, Ethyl acetate) in sample. The specificity has been performed by

injecting blank preparation, individual solvent standard preparations, solvents standard mixture and sample spiked with solvents standard mixture. The chromatograms of blank, solvents standards, and sample spiked with solvents standard are shown in Figure-I,II and III.

System Precision

System precision demonstrates that the analytical system gives precise measurements of the target value on replicate measurements.

The system precision has been performed by injecting standard preparation for six times using given chromatographic conditions and results are summarized in table-1. The representative chromatogram of system precision is shown in Figure-II. Results are summarized in table-4

Method precision

Method precision demonstrates that the analytical method gives precise measurements of the target value on replicate measurements. The method precision has been performed by preparing six different concentration of homogenous sample and injected.

Linearity

Linearity is the ability of the method to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

The linearity study was carried for solvents (Methanol, Ethanol, Acetone, Methylene dichloride, Hexane, Chloroform, Ethyl acetate) from LOQ concentration to 150% of specification level.

Determination of limit of detection and limit of quantitation and precision at LOQ level

LOD: To determine the lowest amount of analyte in a sample which the analytical method can Detect but not necessarily quantitate as an exact value (LOD)

LOQ: To determine the lowest amount of analyte in a sample which the analytical method can Quantitate with suitable precision and accuracy (LOQ).

The limit of quantitation and limit of detection for each individual solvent was determined by using the Standard deviation of response and the slope. The representative chromatograms of limit of detection and limit of quantification are shown in Figure-IV.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value.

Accuracy has been performed by addition of standard in the test sample at 50%, 100% and 150 % of specification level and then determining the content.

Robustness

The robustness of the analytical procedure is the measure of method capacity to remain unaffected by small, but deliberate, variations in method parameters are shown in this study. Robustness study has been established by varying initial oven temperature and flow.

Intermediate precision/ ruggedness

Ruggedness or intermediate precision expresses variations within laboratory like different analyst, different day.

Stability study of standard solution

The representative chromatograms of stability study are shown in Figure-IX

Results and discussion

The results for the validation are presented in table 3. Acceptance criteria: No peak should be observed at the RT of individual analyte peak. The data indicates that there is no interference observed at the retention time of individual analyte. The system precision data given above demonstrates that the % RSD of each individual solvent is well within the acceptance criteria, hence system is precise and suitable for analysis. The Percentage RSD of each individual solvent

area in standard injections should not be more than 15% (table 4). The representative chromatogram of method precision is shown in Figure-III. Results are summarized in table 5. The % RSD of content results of each individual solvent should not be more than 10. The method precision data given above demonstrates that the Percentage RSD for content result of each individual solvent in the six different preparations is well within the acceptance criteria and hence the method is precise. The results are tabulated in table-6 to table-11.The representative chromatograms of linearity are shown in Figure-IV. The linearity graph is drawn between % concentration of specification limit and area response. Linearity plots of each solvent are shown below the linearity table of respective solvent. R^2 should not be less than 0.98. The above linearity study establishes that the method is linear for each individual solvent from LOQ concentration to 150% of specification level**.** From chromatogram of standard preparation, Slope values had been calculated and are shown in table-12. Based on data of limit of quantitation and limit of detection values, standards were prepared to determine the precision (peak area) and respective data is given in table-10. The representative chromatogram of LOQ precision is shown in Figure-V. From Slope values and the residual standard deviation of individual solvent, the limit of detection and limit of quantitation values were determined. The LOD and LOQ values are summarized in table-13. The above data demonstrates that the method is precise for each individual solvent at LOQ level. The results were calculated in the form of % recovery and are summarized in the table-14 to table-16. The representative chromatograms of accuracy are shown in Figure-VI. The % recovery for each individual solvent should be between 80.0 to 120.0. The above data of recovery study of each individual solvent shows that the % recovery values are well within acceptance criteria and hence the method is accurate at 50%, 100% and 150% of specification level. The representative chromatograms of robustness are shown in Figure-VII. Observed data is summarized in table-17 to table-20. The overall %RSD of area of methods precision and robustness for each individual solvent should not be more than 15. The robustness study data shows that the resolution values and overall % RSD for each individual solvent are well within acceptance criteria and hence the method is robust. The ruggedness was performed by analyzing the sample on different day, by different analyst. The representative chromatograms of ruggedness are shown in Figure-VIII. The experimental details are given in table-21 and results are summarized in table-22. The Percentage RSD of ruggedness for each individual solvent content result should not be more than 10%. The overall Percentage RSD between method precision and ruggedness of content results for each individual solvent should not be more than 10%. The data of ruggedness study shows that overall %RSD values for each individual solvent are well within acceptance criteria and hence the method is rugged. The representative chromatograms of stability study are shown in Figure-IX . The experimental details are given in table-23 The % RSD of stability study for each individual solvent should not be more than 15. The data of stability study shows that overall %RSD values for each individual solvent are well within acceptance criteria and hence the standard solution stable upto 12-hr.

The validation data (table 24) proves that the method employed for determination of Methanol, Ethanol, Acetone, Methylene dichloride, Hexane, Chloroform as residual solvents in drug substance 'Colchicine' by headspace gas chromatography is specific, precise, linear, accurate, rugged and robust. The above GC method is therefore recommended to use for the routine analysis.

Table 3: Validation summary report

Parameter	Result		Acceptance Report
Specificity	No interference observed at the retention time of residual solvents		No interference observed at the retention time of residual solvents.
System precision	Name	%RSD	The Percentage RSD of each individual solvent area in standard injections comes with in 15%.
	Methanol	6.74	
	Ethanol	6.88	
	Acetone	5.33	
	Methylene dichloride	1.62	
	Hexane	5.91	
	Chloroform	2.19	
Linearity	Name	R^2	R^2 value of each solvent comes more than 0.98
	Methanol	0.9961	
	Ethanol	0.9962	
	Acetone	0..9983	
	Methylene dichloride	0.9929	
	Hexane	0.9960	
	Chloroform	0.9936	
Limit of Quantification (LOQ)	Name	ppm	Based on the standard deviation of the response and slope
	Methanol	24.72	
	Ethanol	42.14	
	Acetone	45.62	
	Methylene dichloride	10.15	
	Hexane	10.86	
	Chloroform	1.85	
Limit of Detection (LOD)	Methanol	8.16	Based on the standard deviation of the response and slope
	Ethanol	13.9	
	Acetone	15.06	
	Methylene	3.35	
	Hexane	3.58	
	Chloroform	0.61	
LOQ precision	Name	% RSD	The Percentage RSD of each individual solvent area at LOQ level comes with in 15%.
	Methanol	8.1	
	Ethanol	8.1	
	Acetone	5.1	

	Methylene	9.1	
	Hexane	13.4	
	Chloroform	9.8	
Method Precision	Name	% RSD	The Percentage RSD of content results of each individual solvent comes with in 10%.
	Methanol	Below LOD	
	Ethanol	8.46	
	Acetone	Below LOD	
	Methylene	7.29	
	Hexane	Below LOD	
	Chloroform	6.05	

Accuracy	Name	50%	100%	150%	The Percentage recovery for each individual solvent comes between 80.0% to 120.0%
	Methanol	98.07	98.52	99.14	
	Ethanol	104.74	105.33	107.13	
	Acetone	105.57	106.61	106.53	
	Methylenedichloride	91.82	86.67	85.43	
	Hexane	90.67	109.67	101.10	
	Chloroform	98.64	107.33	86.58	

Robustness	Name	Flow(±0.1ml/min)		Temperature(±2.0°C)		RSD of content of methods precision and robustness for each individual
	Methanol	Decreased	Increased	Decreased	Increased	
	Methanol	8.21	11.98	5.94	5.96	
	Ethanol	9.35	12.30	5.99	6.26	
	Acetone	8.12	6.98	5.67	5.96	
	Methylene	7.73	8.29	4.01	4.10	
	Hexane	5.76	10.45	7.46	14.22	
	Chloroform	4.15	4.80	2.04	2.49	

Rugged ness	Name	%RSD	%Overall RSD	The Percentage RSD of ruggedness for content results comes with in 10%. The overall Percentage RSD between method precision and
	Methanol	Below LOD	Below LOD	
	Ethanol	9.56	9.03	
	Acetone	Below LOD	Below LOD	
	Methylene dichloride	6.79	8.75	
	Hexane	Below LOD	Below LOD	

	Chloroform	1.29	5.34	ruggedness for content results comes with in 10%.

Stability of Solution	Name	% RSD	The Percentage RSD of area found in Stability of Solution comes with in 15%.
	Methanol	1.26	
	Ethanol	3.35	
	Acetone	4.17	
	Methylene dichloride	7.54	
	Hexane	8.19	
	Chloroform	8.05	

Table 4: System precision

Injection	Methanol	Ethanol	ACetone	Methylene dichloride	Hexane	Chloroform
	Peak area					
1	6610119	18977867	66121440	11060412	17016897	902659
2	7731552	21725978	72027205	10888002	15645839	888071
3	7876785	22628501	75322061	11276993	17341031	929934
4	7746032	21605334	71232787	10746065	16131653	874261
5	8026255	23078113	75853265	10970957	17424917	914808
6	7843031	22753090	76277578	10990060	18469179	895812
Mean	7638962.3	21794813.8	72805722.7	10988748.2	17004919.3	900924.2
STDEV	515122.2	1499067.4	3884149.9	177529.7	1004316.9	19695.7
%RSD	6.7	6.9	5.3	1.6	5.9	2.2

Table 5: Method precision

RSD of result method precision(ppm)						
	Methanol	**Ethanol**	**Acetone**	**Methylene dichloride**	**Hexane**	**Chloroform**
	76.12	487.09	11.35	98.36	6.43	97.35
	87.42	510.66	9.18	93.99	6.68	94.95
	83.13	480.88	8.06	98.76	6.69	94.34
	73.26	512.46	12.06	80.93	6.38	83.63
	79.44	463.61	11.32	96.28	7.15	94.05
	72.31	403.23	9.41	98.60	7.63	100.55
STDV	5.9	40.3	1.6	6.9	0.5	5.7
AVG	78.61	476.32	10.23	94.49	6.83	94.15
RSD	7.49	8.46	15.32	7.29	6.98	6.05
	Below LOD		Below LOD		Below LOD	

Table 6: Linearity of Methanol

Methanol	
Conc. (%)	Area
20	1972845
52	4017855
70	5683552
80	6489629
100	7731528
118	9537526
150	12231613
R^2	0.9961

Linearity plot of Methanol

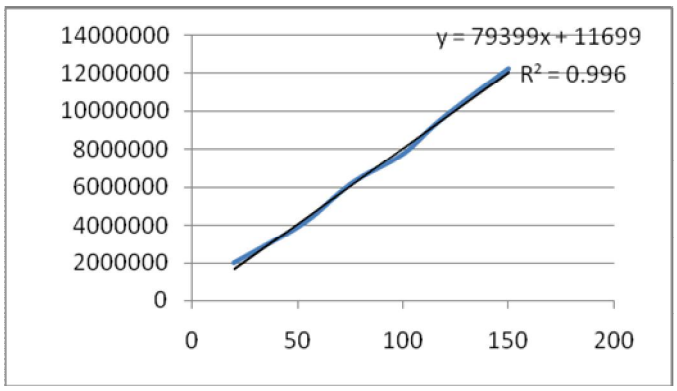

Table7: Linearity of Ethanol

Ethanol	
Conc. (%)	Area
19	3394846
52	9908955
71	14279743
77	16915956
101	20694001
120	24774857
152	32801544
R^2	0.9962

Linearity plot of Ethanol

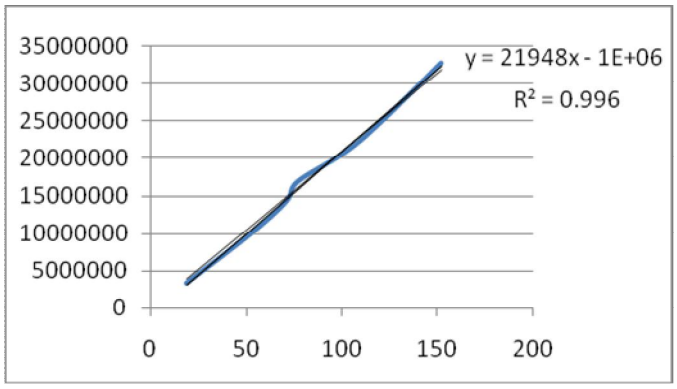

Table 8: Linearity of Acetone

Acetone	
Conc. (%)	Area
21	12094057
49	31434699
71	44181166
83	53583764
100	66554008
118	77286480
150	102294101
R^2	0.9983

Linearity plot of Acetone

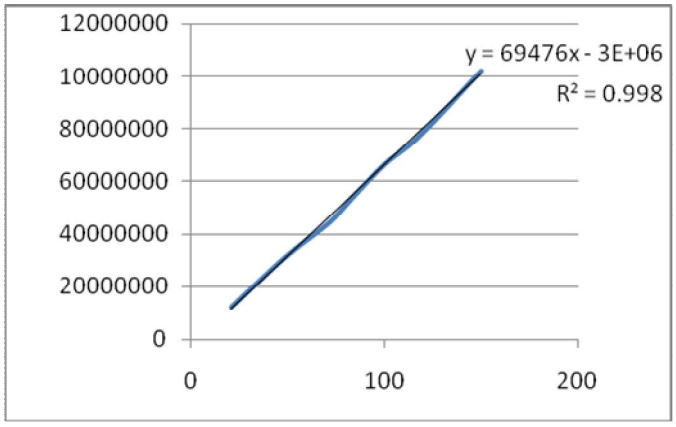

Table 9: Linearity of Methylene dichloride

Methylene dichloride	
Conc. (%)	Area
17	908133
51	5078542
68	6441442
82	7993224
105	9847709
118	10675659
151	13997553
R^2	0.9928

Linearity plot of Methylene dichloride

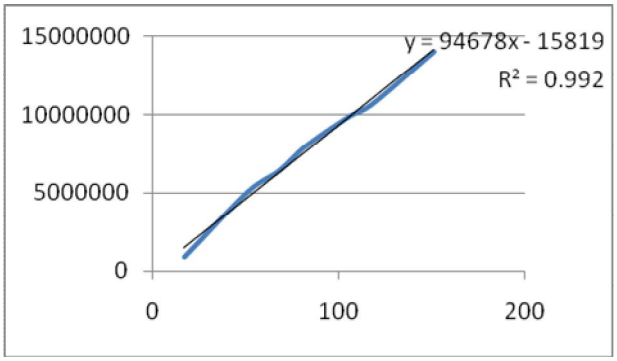

Table 10: Linearity of Hexane

Hexane	
Conc. (%)	Area
25	2736943.0
52	6124467
58	6794630
80	8836628
92	9913368
115	12367601
165	18685497
R^2	0.9960

Linearity plot of Hexane

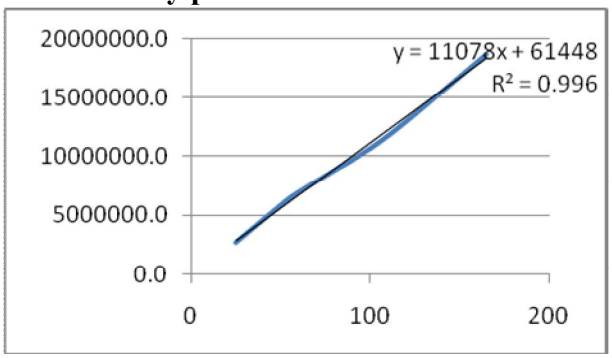

Table11: Linearity of Chloroform

Chloroform	
Conc. (%)	Area
20	320921
48	490049
65	630318
82	771367
100	944335
113	1008653
148	1333052
R^2	0.9936

Linearity plot of Chloroform

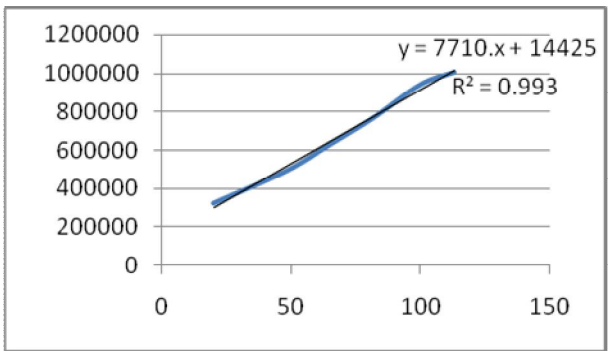

Table 12: Slope of standard

NAME	Methanol	Ethanol	Acetone	Methylene dichloride	Hexane	Chloroform
Slope	80854	224471	697444	88019	123426	8225

Table 10: Precision at LOQ level

Sr. No.	Methanol	Ethanol	Acetone	Methylene dichloride	Hexane	Chloroform
Area						
1	1853034	3314137	12382325	919009	2593576	335700
2	2158502	3478266	11209721	800677	2805381	280457
3	1907000	3392135	12690124	1004714	2811871	346605
4	2295590	4109498	11578492	794441	3049600	295095
5	1997812	3431815	11812087	849124	3522738	282366
6	1993463	3509923	12669322	899252	3591956	340215
Avg.	2034233.5	3539295.7	12057011.8	877869.5	3062520.3	313406.3
Stdev	164778.8	287618.8	614565.6	79980.6	410152.1	30667.0
%RSD	8.1	8.1	5.1	9.1	13.4	9.8

Table 13: Summary of LOQ & LOD in ppm

Components	LOQ	LOD
Methanol	24.72	8.16
Ethanol	42.14	13.9
Acetone	45.62	15.06
Methylene dichloride	10.15	3.35
Hexane	10.86	3.58
Chloroform	1.85	0.61

Table 14: Accuracy of solvents at 50%

Name of solvents	Sr. No	Added (ppm of spec. limit)	Theoritical area	Present (area)	Recovered (area)	% Recovery	Average (%)
Methanol	1	1508	4123017	225633	3884254	94.21	98.07
	2				4094514	99.31	
	3				4151003	100.68	
Ethanol	1	2502	10316785	2019021	10445439	101.25	104.74
	2				10786159	104.55	
	3				11185263	108.42	
Acetone	1	2552	33564888	126255	34792666	103.66	105.57
	2				35789824	106.63	
	3				35715863	106.41	
Methylene dichloride	1	285	5219950	1944136	4939710	94.63	91.82
	2				4962883	95.08	
	3				4475606	85.74	
Hexane	1	140	5218624	256232	4761497	91.24	90.67
	2				4984203	95.51	
	3				4449575	85.26	

Chloroform	1	52	588775	1148079	585053	99.37	98.64
	2				601631	102.18	
	3				555666	94.38	

Table 15: Accuracy of solvents at 100%

Name of solvents	Sr. No	Added (ppm of spec. limit)	Theoritical area	Present (area)	Recovered (area)	% Recovery	Average (%)
Methanol	1	3000	8202287	225633	8148374	99.34	98.52
	2				8061316	98.28	
	3				8033560	97.94	
Ethanol	1	5000	20617077	2019021	21651031	105.02	105.30
	2				22089276	107.14	
	3				21408993	103.84	
Acetone	1	5000	65761928	126255	70422202	107.09	106.61
	2				70605065	107.36	
	3				69302237	105.38	
Methylene dichloride	1	600	10989368	1944136	9716338	88.42	86.67
	2				9447849	85.97	
	3				9409831	85.63	
Hexane	1	352	13121111	256232	14351105	109.37	109.67
	2				14705663	112.08	
	3				14111771	107.55	
Chloroform	1	112	1268132	1148079	1387128	109.38	107.33
	2				1399654	110.37	
	3				1296557	102.24	

Table 16: Accuracy of solvents at 150%

Name of solvents	Sr. No	Added (ppm of spec. limit)	Theoritical area	Present (area)	Recovered (area)	% Recovery	Average (%)
Methanol	1	4503	12311633	225633	12370094	100.47	99.14
	2				12187799	98.99	
	3				12059704	97.95	
Ethanol	1	7525	31028700	2019021	33774385	108.85	107.13
	2				33043125	106.49	
	3				32904419	106.05	
Acetone	1	7515	98840178	126255	105878587	107.12	106.53
	2				105463152	106.70	
	3				104539975	105.77	
Methylene dichloride	1	860	15751428	1944136	13211102	83.87	85.43
	2				13881024	88.13	
	3				13278075	84.30	

Hexane	1	425	15842251	256232	15539739	98.09	101.10
	2				16247045	102.56	
	3				16263310	102.66	
Chloroform	1	148	1675745	1148079	1379355	82.31	86.58
	2				1510683	90.15	
	3				1462710	87.29	

Table 17: Robustness Study: Decreased flow (-0.1ml/min)

Injection	Methanol	Ethanol	Acetone	Methylene dichloride	Hexane	Chloroform
	Area					
1	8321256	21284550	68714359	12916439	15667492	979438
2	6667961	17443496	59551654	12832913	16872701	970396
System precision data						
1	6610119	18977867	66121440	11060412	17016897	902659
2	7731552	21725978	72027205	10888002	15645839	888071
3	7876785	22628501	75322061	11276993	17341031	929934
4	7746032	21605334	71232787	10746065	16131653	874261
5	8026255	23078113	75853265	10970957	17424917	914808
6	7843031	22753090	76277578	10990060	18469179	895812
Average	7602873	21187116	70637543	11460230	16821213	919422
STDEV	623893	1981196	5735038	886095	969497	38159
Overall %RSD	**8.21**	**9.35**	**8.12**	**7.73**	**5.76**	**4.15**

Table 18: Robustness Study: Increased flow(+0.1ml/min)

Injection	Methanol	Ethanol	Acetone	Methylene dichloride	Hexane	Chloroform
	Area					
1	5988835	17216212	65177513	13219388	20757772	986177
2	6053846	16897468	64475343	12794288	20442513	992943
Method precision data						
1	6610119	18977867	66121440	11060412	17016897	902659
2	7731552	21725978	72027205	10888002	15645839	888071
3	7876785	22628501	75322061	11276993	17341031	929934
4	7746032	21605334	71232787	10746065	16131653	874261
5	8026255	23078113	75853265	10970957	17424917	914808
6	7843031	22753090	76277578	10990060	18469179	895812
Average	7234556	20610320	70810899	11493270	17903725	923083

STDEV	866348	2534318	4945175	952962	1870118	44315
Overall %RSD	**11.98**	**12.30**	**6.98**	**8.29**	**10.45**	**4.80**

Table 19: Robustness Study: Decreased temperature (-2.0°C)

Injection	**Methanol**	**Ethanol**	**Acetone**	**Methylene dichloride**	**Hexane**	**Chloroform**
	Area					
1	8019192	21498297	67958354	11773152	14631497	889668
2	7601652	21017313	67375998	12012734	15715092	918232
Method precision data						
1	6610119	18977867	66121440	11060412	17016897	902659
2	7731552	21725978	72027205	10888002	15645839	888071
3	7876785	22628501	75322061	11276993	17341031	929934
4	7746032	21605334	71232787	10746065	16131653	874261
5	8026255	23078113	75853265	10970957	17424917	914808
6	7843031	22753090	76277578	10990060	18469179	895812
Average	7681827	21660561	71521086	11214796	16547013	901680
STDEV	456386	1297484	4056910	449227	1234192	18366
Overall %RSD	**5.94**	**5.99**	**5.67**	**4.01**	**7.46**	**2.04**

Table 20: Robustness Study: Increased temperature (+2.0°C)

Injection	**Methanol**	**Ethanol**	**Acetone**	**Methylene dichloride**	**Hexane**	**Chloroform**
	Area					
1	7700408	20459187	65367153	11681847	11661048	880885
2	8052386	21958473	69598911	12109797	13558119	935758
Method precision data						
1	6610119	18977867	66121440	11060412	17016897	902659
2	7731552	21725978	72027205	10888002	15645839	888071
3	7876785	22628501	75322061	11276993	17341031	929934
4	7746032	21605334	71232787	10746065	16131653	874261
5	8026255	23078113	75853265	10970957	17424917	914808
6	7843031	22753090	76277578	10990060	18469179	895812
Average	7698321	21648317	71475050	11215516	15906085	902773
STDEV	458765	1356203	4257489	460330	2262147	22447

Overall %RSD	**5.96**	**6.26**	**5.96**	**4.10**	**14.22**	**2.49**

Table 21: Experiment details of ruggedness Study

	Analyst-I	**Analyst-II**
Date of analysis	25.08.09	27.08.09
Column no.	GC-01-001/08	GC-01-001/08
Instrument make	Agilent	Agilent
Instrument no.	QC/GCHS/03	QC/GCHS/03

Table 22: Ruggedness Study

Average Result of Ruggedness(in ppm)						
	Methanol	**Ethanol**	**Acetone**	**Methylene dichloride**	**Hexane**	**Chloroform**
Analyst-I (25.08.09)	76.12	487.09	11.35	98.36	6.43	97.35
	87.42	510.66	9.18	93.99	6.68	94.95
Analyst-II (27.08.09)	81.91	482.70	16.83	89.05	6.91	97.43
	88.22	406.70	13.49	78.98	6.66	97.63
STDV	5.62	45.10	3.26	8.33	0.20	1.27
AVG	83.42	471.79	12.71	90.10	6.67	96.84
RSD	6.73	9.56	25.65	9.24	2.94	1.31
	Below LOD		Below LOD		Below LOD	

% RSD between method precision and Ruggedness (in ppm)						
	Methanol	**Ethanol**	**Acetone**	**Methylene dichloride**	**Hexane**	**Chloroform**
	76.12	487.09	11.35	98.36	6.43	97.35
	87.42	510.66	9.18	93.99	6.68	94.95
	83.13	480.88	8.06	98.76	6.69	94.3
	73.26	512.46	12.06	80.93	6.38	83.63
	79.44	463.61	11.32	96.28	7.15	94.05
	72.31	403.23	9.41	98.60	7.63	100.55
	81.91	482.70	16.83	89.05	6.91	97.43
	88.22	406.70	13.49	78.98	6.66	97.63
STDV	6.04	42.28	2.79	8.04	0.41	5.07
AVG	80.23	468.42	11.46	91.87	6.82	94.99
RSD	7.53	9.03	24.30	8.75	6.03	5.34
	Below LOD		Below LOD		Below LOD	

Table 23: Stability Study

	Methanol	Ethanol	Acetone	Methylene dichloride	Hexane	Chloroform
	Area					
0-Hr	8974661	2175685	6628859	9568095	14759769	665385
4-Hr	9200554	2332003	7255155	11116770	17915699	785939
8-Hr	9224002	2343547	7224368	10605983	15709073	745934
12-Hr	9182740	2283686	6938716	9586927	16190666	674761
Avg	9145489	2283730	7011774	10219443	16143801	718004
Stdev	115132	765550	2923422	770051	1322415	57832
%	**1.26**	**3.35**	**4.17**	**7.54**	**8.19**	**8.05**

Table 24: System Suitability study

System suitability Criteria	% RSD						Resolution between Ethanol and Acetone
Acceptance limit	**NMT 15%**						**NLT 2**
Solvent	**Methanol**	**Ethanol**	**Acetone**	**Methylene dichlorode**	**Hexane**	**chloroform**	
System precision	6.74	6.88	5.33	1.62	5.91	2.19	4.3
Method precision	1.73	2.08	2.41	2.04	6.65	1.85	3.9
Linearity	2.96	2.80	1.44	3.86	2.92	4.27	4.3
Robustness (At decreased flow)	8.21	9.35	8.12	7.73	5.76	4.15	4.4
ROBUSTNESS (At increased flow)	11.98	12.30	6.98	8.29	10.45	4.80	3.8
ROBUSTNESS (At decreased temp)	5.94	5.99	5.67	4.01	7.46	2.04	4.1
ROBUSTNESS (At increased temp)	5.96	6.26	5.96	4.10	7.46	2.04	4.0
LOQ Precision	3.83	4.66	5.28	8.34	11.25	10.98	3.7
Accuracy	1.73	2.08	2.41	2.04	6.65	1.85	3.9
Ruggedness	1.58	3.31	3.53	5.43	9.02	6.13	4.5

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