IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2020 Vol.:18, Issue:1

Gas Chromatographic Method for the Estimation of Residual Solvents in Levonorgestrel and Ethinyl Estradiol Tablets

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Rajasekhara Reddy*[1], Satyavir[2], S.B.Puranik[3]

[1]*Research scholar OPJS University, Churu, Rajasthan, India*

[2]*Research Guide OPJS University, Churu, Rajasthan, India*

[3]*Drishti Institute of Distance Learning, Bangalore*

Submission: 22 March 2020
Accepted: 30 March 2020
Published: 30 April 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Gas chromatography, residual solvents, ICH, Analytical method, validation

ABSTRACT

Gas Chromatography (GC) is commonly used as an analytical technique in developing and validating Residual solvents methods for drug products and drug substances. Method validation provides documented evidence and a high degree of assurance that an analytical method employed for a specific test, is suitable for its intended use. Over recent years, regulatory authorities have become increasingly aware of the necessity of ensuring that the data submitted to them in applications for marketing approvals have been generated using the validated analytical methodology. The International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation. Both the United States Food and Drug Administration (USFDA), as well as United States Pharmacopoeia (USP), refer to ICH guidelines. All the validation criteria in the developed meet with ICH specifications.

INTRODUCTION

Gas Chromatography (GC) is commonly used as an analytical technique in developing and validating Residual solvents methods for drug products and drug substances (1). Method validation provides documented evidence and a high degree of assurance that an analytical method employed for a specific test, is suitable for its intended use. Over recent years, regulatory authorities have become increasingly aware of the necessity of ensuring that the data submitted to them in applications for marketing approvals have been generated using the validated analytical methodology. The International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation (2,3). Both United States Food and Drug Administration (USFDA), as well as United States Pharmacopoeia (USP), refer to ICH guidelines (4-7).

"It is indicated for fertility control in women and for the control of cases of dysfunctional uterine bleeding and symptomatic treatment of primary dysmenorrhea where contraception is also desired. Oral contraceptives of the combination type act by a multiplicity of mechanisms. Ovulation is inhibited by suppression of gonadotropin release, particularly the mid-cycle peaks and the viscosity of the cervical mucous is increased impairing sperm penetration, and an endometrium, less receptive for implantation is formed Literatureindicates that Levonorgestrel is rapidly and completely absorbed after oral administration(bioavailability nearly 100%) and is not subject to the first-pass metabolism. Ethinyl Estradiol is rapidly and almost completely absorbed from the gastrointestinal tract but due to the first-pass metabolism in gut mucosa and liver, the bioavailability of Ethinyl Estradiol is approximately 43%. Route of Administration Dosage Form / Strength All Non-Medicinal Ingredients Oral Tablet,150 mcg levonorgestrel and 20 mcg Ethinyl estradiol&150 mcg levonorgestrel and 30 mcg Ethinylestradiol. Each tablet contains croscarmellose sodium, FD&C Blue No. 1 aluminum lake (aluminum chloride, aluminum hydroxide, brilliant blue), FD&C (yellow No.6), D&C Yellow no. 10 lactose monohydrate, Corn Starch, magnesium stearate, microcrystalline cellulose, and povidone K25.

Levonorgestrel and Ethynyl Estradiol manufacturing Composition and Residual solvents:

Formulation Details

Sr. No.	Excipient / Ingredient name	mg/ tablets	
		0.15 mg/ 0.02 mg	**0.15 mg/ 0.03 mg**
	Intragranular		
1	Levonorgestrel (20mic)	0.15	0.15
2	Povidone K-25(Part A)	0.75	0.75
3	Lactose monohydrate (Pharmatose 200M)	76.05	76.32
4	Povidone K-25(Part B)	0.75	0.75
5	Chloroform#	·qs	qs
6	Dehydrated Alcohol#	qs	qs
7	Purified water#	qs	qs
	Extra granular		
8	Ethinylestradiol (20 mics)	0.02	0.03
9	Lactose monohydrate	15.00	15.00
10	Corn Starch(Maize starch extra white)	5.00	5.00
11	Magnesium stearate	1.00	1.00
12	D&C Yellow no. 10	0.0835	---
13	FD&C Blue no. 1	0.146	0.12
14	FD&C Yellow no. 6	0.05	0.01
Total weight of Tablet (mg)		**100.0**	**100.0**

Sr. No.	Excipient / Ingredient name	Qty. *I* Tab (mg)
1	Povidone K-25(Part A)	1.50
2	Lactose monohydrate (Pharmatose 200M)	92.05
3	Chloroform	qs
4	Dehydrated Alcohol	qs
5	Purified water	qs
6	Corn Starch(Maize starch extra white)	5.00
7	Magnesium stearate	1.00
8	D&C Yellow no. 10	0.0835
9	FD&C Blue no. 1	0.146
10	FD&C Yellow no. 6	0.05
Total weight of Tablet (mg)		**99.83**

Not present in final formulation except in traces.

Residual Solvents in the Product:

Sr. No.	Name of the solvents	In-House Limit (ppm)	ICH Limit (ppm)
1	Ethanol	2000	5000
2	Chloroform	60	60

Solubility Test for Levonorgestrel:

In water, it was observed: 2.055 mg/ml at Room temperature

The solubility of levonorgestrel is approximately 0.2 mg/ml in ethanol and approximately 5 mg/ml in DMSO and DMF. Levonorgestrel is sparingly soluble in **aqueous** buffers.

Solubility Test for Ethinyl Estradiol:

Solubility: 1 part in 6 of ethanol, 1 in 4 of ether, 1 in 5 of acetone, 1 in 4 of dioxane and 1 in 20 of chloroform. Soluble in vegetable oils, and solutions of fixed alkali hydroxides in water, 11.3 mg/L at 27 degrees Centigrade. And also soluble in Dimethylformamide

Observation while Sample preparation:

Weighed and transferred the tablet powder about 500.00mg into 20 ml headspace vial and added 2.00 mL of Dimethylformamide added. Tablet powder completely dispersed, it indicates solvents (Ethanol and Chloroform) are easily miscible with DMF.

Selection of Gases:

The choice of carrier gas determines the efficiency of chromatographic separation. The most widely used gases are hydrogen, helium, nitrogen, and argon. Requirements of carrier gas are inertness, suitable to the detector, high purity, easily available, cheap, less risk of explosion or fire hazards, should give best column performance consistent with the required speed of analysis Hydrogen is better thermal conductivity, low density. The disadvantage is that it is inflammable gas. It reacts with unsaturated compounds. So it cannot be used as the carrier gas. Nitrogen is an inert gas. Nitrogen also can use carrier gas in place of helium.

Selection of column

Choose the default column as DB-1 if column information is not available in the literature. Choose the bonding phase of the column based on the polarity of the stationary phase of the column. Silica-based columns with different cross linking's in the increasing order of polarity are as follows:

←------Non-polar--------moderately polar----------Polar----------→

DB-1< DB-5 < DB-624 < DB-1701< DB-WAX <DB-FFAP

Select the column which is based on the nature of the compounds. Whether the compound is acidic, basic, or neutral. If the compound is acidic, use more polar stationary phase like DB-WAX, DB-FFAP. For a basic compound, use non-polar or moderately polar like DB-1 or DB-5. For a neutral compound, use non-polar or moderately polar is suitable. The most recommended column for general residual solvents (methanol, ethanol, Isopropyl alcohol, acetone, etc) is DB-624 is equivalent to G43 for a list of columns phases.

Column: DB-624 (30 m, 0.530 mm ID, 3 µ)

Column oven temperature Program: 60°C for 10 minutes and then increase up to

225°c @ 46°C per minute and hold for 4 minutes.

All the peaks are well separated.

Remaining all other conditions are followed as mentioned in the final procedure.

The separation between Ethanol, Chloroform, and Diluent peak is very improved. And the baseline is satisfactory. An in-house Gas Chromatographic headspace method is developed for the determination of Residual solvent of Levonorgestrel and Ethinyl Estradiol in Levonorgestrel and Ethinyl Estradiol Tablets. The product is having two different strengths. The details of the same are given below.

Lower strength: Levonorgestrel and Ethinyl Estradiol tablets (0.15 mg/0.02 mg), which is having colour.

Higher-strength: Levonorgestrel and Ethinyl Estradiol tablets (0.15 mg/0.03 mg), which is without colour. Considering the worst-case scenario concerning drug to excipient ratio and color, validation was performed on lower strength. The same validation applied to higher strength also. The method is validated considering the validation challenges like Specificity, LOD-LOQ Precision, Linearity, Accuracy, Precision, Robustness and Stability of analytical solution. The validation study is intended to show that the method is suitable for the determination of Residual solvent of Levonorgestrel and Ethinyl Estradiol in Levonorgestrel and Ethinyl Estradiol Tablets (0.15 mg *I* 0.02 mg and 0.15 mg/ 0.03 mg).

METHODOLOGY FOR RESIDUAL SOLVENTS (By GC - HS):

Reagents and solvents:

1. Ethanol (HPLC Grade)

2. Chloroform (HPLC Grade)

Instrument: Perkin Elmer or equivalent

Column: DB-624 (30 m, 0.530 mm ID, 3 µ) or equivalent

Column oven temperature Program: 40°C for 10 minutes and then increase up to 225°c @ 46°C per minute and hold for 4 minutes.

Total run time: 20 minutes, Injector Temp.: 205ºC, Detector Temp.: 260°C,

Carrier Detector: Nitrogen, Range: 1, Attenuation: -5

Flow rate: 2.0ml/min, Split ratio: 1:10, Detector: FID

Headspace parameters:

Incubation Time: 15 minutes, Incubation Temp: 105ºC, Needle Temp: 110 ºC,

Transfer line

Temp: 115ºC

Pressurization time: 1minute

Withdrawal time: 0.2 minutes

Injection time: 0.1 minute

Cycle time: 30 minutes

Injection Volume: 0.2 mL by Headspace

Headspace pressure: 15 psi

Diluent:

Dimethylformamide

Blank:

Pipette out 2mL of Dimethylformamide in a headspace vial (20 mL) and seal it with a vial septa (PTFE - Silicon) and crimp tightly.

Preparation of Chloroform stock solution:

Weigh accurately about 300 mg of Chloroform in 200.00 mL volumetric flask containing about 100 mL diluent, mix and dilute up to the mark with the Diluent. Pipette out 5.00 mL of this solution into 200.00 mL volumetric flask and dilute with the mark with diluent.

Preparation of Standard:

Preparation of Standard - I:

Weigh accurately about 500.00 mg of Ethanol into 100.00 mL volumetric flask containing about 25 mL of diluent, dilute up to the mark with the Diluent and mix. Pipette out 10.00 mL of this solution in100.00 mL volumetric flask, add 10.00 mL of Solution B and dilute up to the mark with diluent. Further pipette out 2.00 ml of the resulting solution in six identical headspace vials (20 mL) and seal it with vial septa (PTFE - Silicon) and crimp tightly.

Preparation of Standard - II:

Prepare the same as the preparation of Standard - I.

Preparation of Sample solution:

Accurately weigh and transfer tablet powder about 500.00 mg into 20 ml headspace vial and add 2.00 mL diluent. Seal it with vial septa (PTFE - Silicon) and crimp tightly.

Procedure:

Inject equal and specified volumes of Blank (Dimethylformamide), system suitability solution, Standard solution-I (Six injections) and Standard solution-II (in duplicate) into a chromatograph. Record the peak responses due to Ethanol and Chloroform. Inject the sample solution and calculate the amount of Ethanol and chloroform in terms of ppm (µg per gm) by the following formula.

System Suitability Criteria:

1. The % RSD of peak area responses of Ethanol and Chloroform obtained from six injections of Standard solution should not be more than 15%.

2. The tailing factor for the peaks of Chloroform and Ethanol should not be more than 2 in the standard solution.

Note The similarity factor between standard solution I and standard solution II should be within 0.9 to 1.1.

Formula:

$$= \frac{\text{Average Area of standard I x Weight of standard II}}{\text{Average Area of standard II x Weight of standard I}}$$

Calculation:

Content of Chloroform in ppm (µg per gm):

$$\frac{\text{(Response - Blank) x Std Wt (gm) x 5 mL x 10 mL x 2 mL potency x } 10^6}{\text{(Response - Blank) x 200mL x 200mL x 100mL x Spl wt (gm) x 100}}$$

METHOD VALIDATION EXPERIMENTAL PLAN AND DATA EVALUATION FOR RESIDUAL SOLVENTS

Specificity

Interference and identification Study Prepared blank (diluent), standard solution, placebo solution, unspike sample solution and spike sample solution with all known solvents and inject as per methodology.

Data Evaluation:

Interference of blank, placebo solutions and co-elution of peaks at the retention time of Ethanol and the chloroform was checked.

Acceptance Criteria:

The difference in retention times of peak of all solvents obtained in spiked sample solution should be not more than ±10.0 %, with that obtained in the standard solution. (l st injection of standard solution injected for system suitability). The difference in the retention times of all known solvents in the spiked sample solution should be not more than± 10.0 % with that obtained in individual known standard solutions. Diluent and placebo should not show interference at the retention time of Ethanol and Chloroform. If negligible interference observed at the same retention time as that of the solvent under test, it shall be less than 2 % of the response of respective solvent from standard solution.

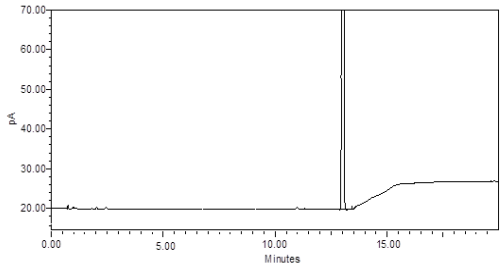
Observation

Diluent and placebo solution did not show interference at the retention time of Ethanol and Chloroform peak.

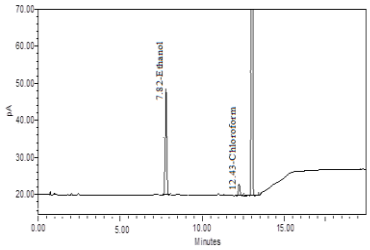
Interference Study

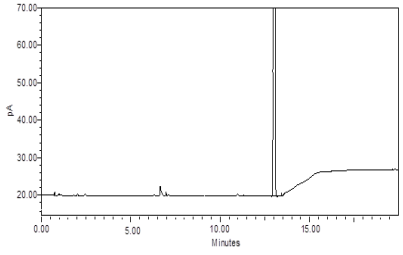
Solvents	Standard solution	Spiked Sample
	RT (in a minute)	RT (in a minute)
Ethanol	7.82	7.81
Chloroform	12.43	12.42

Chromatogram of Blank

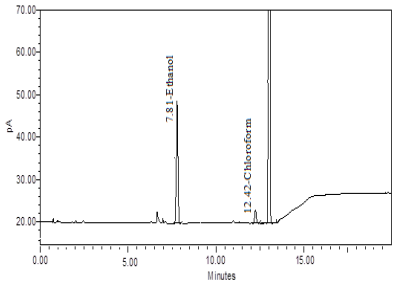


Chromatogram of Standard solution Chromatogram of Placebo solution





Chromatogram of the spiked sample



Conclusion

Method meets the acceptance criteria for Specificity. Hence the method is specific concerning the interference of blank, placebo solutions.

Limit of Detection (LOD) and Quantitation (LOQ)

Limit of Quantitation (LOQ) was established by injecting six replicates of a solution containing Ethanol and Chloroform.

Limit of Detection (LOD) was established by injecting six replicates of a solution containing Ethanol and chloroform.

Data Evaluation

% RSD (n=6) for the peak area counts of Ethanol and Chloroform in LOD and LOQ solution was determined.

Acceptance Criteria

For Limit of Detection (LOD)

% RSD (n = 6) of peak area counts of Ethanol and Chloroform from six replicate injections of LOD solution should be NMT 30.0.

For Limit of Quantitation (LOQ)

% RSD (n=6) of peak area counts of Ethanol and Chloroform from six replicate injections of LOQ solution should be NMT 15.0.

Observation:

% RSD (n=6) of peak area counts of Ethanol and Chloroform in LOD and LOQ solutions were found within acceptance criteria.

Limit of Detection for Ethanol and Chloroform

Sr. No.	Peak Area Counts	
	Ethanol	Chloroform
1	2955	500
2	2234	695
3	2351	455
4	2400	630
5	2321	525
6	3200	423
Mean	2577	538
SD	399.14	104.29
%RSD	15.5	19.4
Cone. (µg/mL)	1.5	2
Cone. wrt test (µg/g)	5	8

wrt = concerning.

Limit of Quantitation for Ethanol and Chloroform

Sr. No.	Peak Area Counts	
	Ethanol	Chloroform
1	4500	1893
2	4432	1802
3	4502	1797
4	4632	1822
5	4732	1856
6	4558	1801
Mean	4559.3	1828.5
SD	107.81	38.495
%RSD	2.4	2.1
Cone. (µg/mL)	2.5	4
Cone. wrt test (µg/g)	11	15

wrt = concerning test concentration.

Conclusion:

Acceptance criteria for Limit of Detection and Limit of Quantitation are met for LOD and LOQ values determined.

Linearity:

A series of solutions were prepared by quantitatively diluting the Chloroform stock the solution to obtain solutions at the level of LOQ to 150.0 % level of specification limit.

A series of solutions were prepared by quantitatively diluting the Ethanol stock solution to obtain solutions at the level of LOQ to the ICH limit.

Data Evaluation

A plot of concentration (concerning test) against area counts for Ethanol and Chloroform was plotted for each peak and c01Telation coefficient, slope and intercept were determined.

Acceptance Criteria

Correlation coefficient should be NLT 0.990.

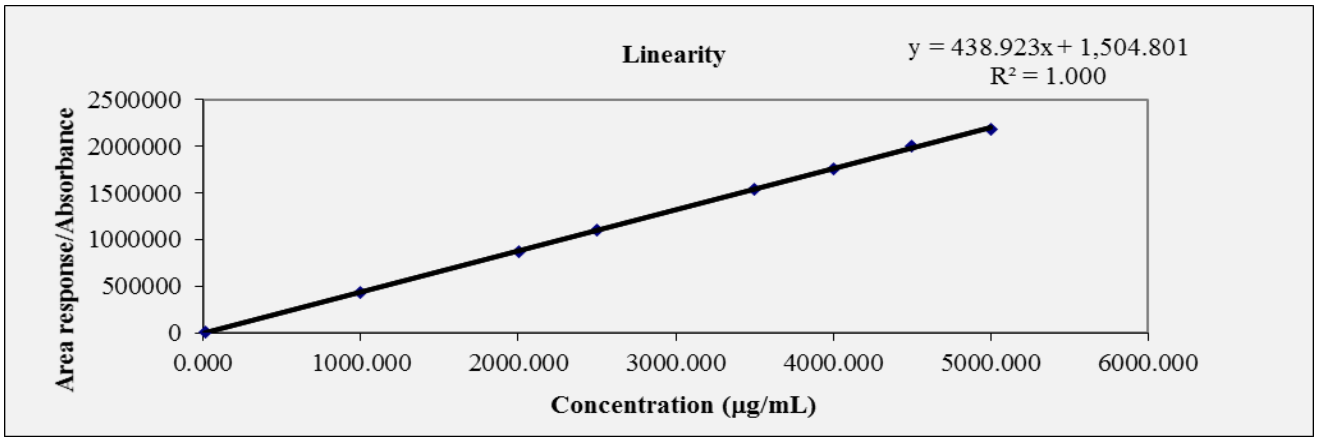
Observation

The correlation coefficient of Ethanol and Chloroform was found within acceptance criteria.

Linearity of Ethanol

Sr. No.	Linearity Level	Concentration		Area
		(µg/mL)	wrt test (µg/g)	
1	Linearity Level-I (LOQ)	2.61	10.55	4321
2	Linearity Level-2	250.1	1000.4	439967
3	Linearity Level-3	500.2	2000.8	871909
4	Linearity Level-4	625.2	2500.8	1108777
5	Linearity Level-5	875.3	3501.2	1441865
6	Linearity Level-6	1000.4	4001.6	1752722
7	Linearity Level-7	1125.5	4502.0	1999106
8	Linearity Level-8	1250.3	5001.2	2176819
		Correlation Coefficient (r)		0.9999
		Slope		438.923
		Intercept		1504.801

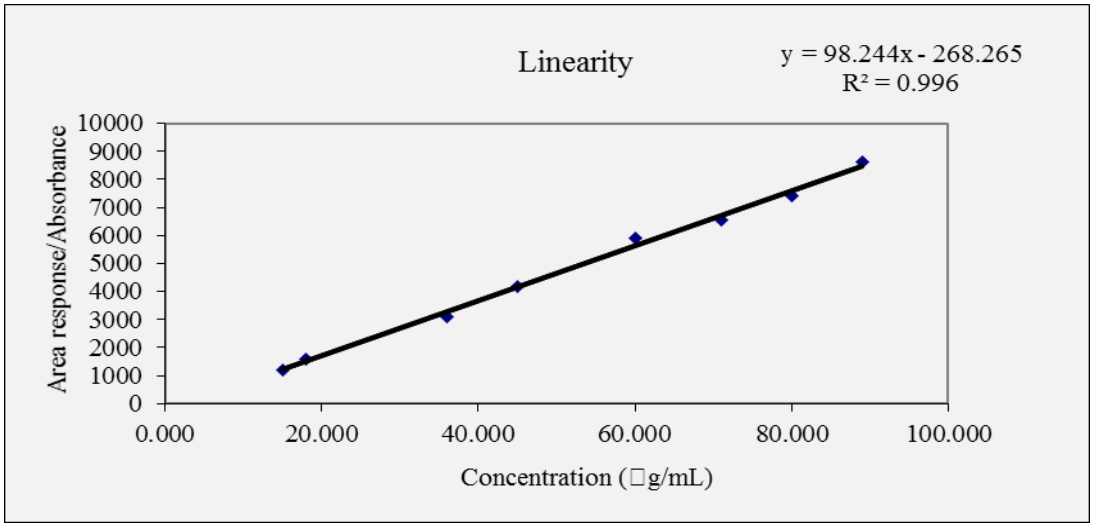
wrt = concerning.



Linearity of Chloroform

Sr. No.	Linearity Level	Concentration		Area
		(µg/mL)	wrt test (µg/g)	
1	Linearity Level- I (LOQ)	3.75	15.00	1236
2	Linearity Level-2	4.50	18.00	1550
3	Linearity Level-3	9.00	36.00	3196
4	Linearity Level-4	11.25	45.00	4247
5	Linearity Level-5	15.5	60.00	5801
6	Linearity Level-6	17.75	71.00	6621
7	Linearity Level-7	20.00	80.00	7515
8	Linearity Level-8	22.25	89.00	8630
		Correlation Coefficient (r)		0.9981
		Slope		98.2
		Intercept		-268.265

wrt = concerning.



Conclusion

The method meets the acceptance criteria of linearity for Ethanol and Chloroform. Hence the method is linear for Ethanol and Chloroform over the above-mentioned range.

Accuracy:

Prepared a series of accuracy preparations of Levonorgestrel and Ethinyl Estradiol Tablets in triplicate by spiking Chloroform at LOQ, 50 %, 100 %, and 150 % concerning specification limit and Ethanol at LOQ, 50 %, 100 % concerning specification limit and also at ICH limit.

Data Evaluation

The amount of each solvent recovered was calculated. From the amount added and amount recovered,

% recovery was calculated using the following formula.

% Recovery= Amount recovered x 100

Amount added

% Recovery at each level and overall ¾RSD for all levels (except LOQ) was also calculated.

Acceptance Criteria

For all levels individual and mean recovery should be between 80.0 % and 120.0 %.

Overall % RSD (n=12) of% recovery at all levels should be NMT 15.0.

Overall mean recovery should be between 80.0 % to 120.0 %.

Observation

For all levels individual and mean recovery was found within acceptance criteria. Overall%

RSD (n=12)of% recovery at all levels was found within acceptance criteria. Overall mean recovery found between 80.0 % to 120.0 %.

Accuracy of Methanol:

Level	Sample	Amount Added (ppm)	Amount Recovered (ppm)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
LOQ	1	10.511	10.678	101.6	103.1	1.60	1.6
	2	10.511	10.823	103.0			
	3	10.511	11.011	104.8			
50%	1	1000.222	1001.410	100.1	100.2	0.05	0.1
	2	1000.222	1002.111	100.2			
	3	1000.222	1001.945	100.2			
100%	1	2000.444	2005.232	100.2	100.4	0.15	0.2
	2	2000.444	2007.777	100.4			
	3	2000.444	2009.999	100.5			
ICH	1	5001.110	5006.660	100.1	100.4	0.30	0.3
	2	5001.110	5020.330	100.4			
	3	5001.110	5034.330	100.7			

Recovery of Chloroform

Level	Sample	Amount Added (ppm)	Amount Recovered (ppm)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
LOQ	1	15.100	16.023	106.1	105.4	0.61	0.6
	2	15.100	15.905	105.3			
	3	15.100	15.845	104.9			
50%	1	30.200	31.233	103.4	103.3	0.70	0.7
	2	30.200	30.998	102.6			
	3	30.200	31.398	104.0			
100%	1	60.400	62.344	103.2	104.2	0.90	0.9
	2	60.400	63.423	105.0			
	3	60.400	62.989	104.3			
150 %	1	90.600	96.789	106.8	106.6	0.72	0.7
	2	90.600	97.103	107.2			
	3	90.600	95.855	105.8			

Conclusion

The analytical method meets the acceptance criteria for recovery. Hence, it is concluded that the method is accurate with precision.

System Precision

Six replicate injections of the standard solution were injected into the chromatograph.

Data Evaluation

% RSD (n=6) for peak area counts of Ethanol and Chloroform from six replicate injections of the standard solution was calculated.

Acceptance Criteria

% RSD (n = 6) for peak area counts of Ethanol and Chlorofonn from six replicate injections of the standard solution should be NMT 15.0.

Observation

% RSD (11=6) for peak area counts of Ethanol and Chlorof01m from six replicate injections of the standard solution was found within acceptance criteria.

System Precision:

Injection	Peak Area counts	
	Ethanol	**Chloroform**
1	1127891	8211
2	1156533	8302
3	1146789	8003
4	1105671	7987
5	1129801	7999
6	1131908	7905
Mean	1133099	8068
SD	17483.03	153.18
% RSD	1.5	1.9

Conclusion:

The analytical method meets the acceptance criteria for system precision. Hence, the system is precise.

Method Precision:

Diluent, standard solution, three unspiked sample solutions were prepared as per the methodology and six sample solutions of Levonorgestrel and Ethinyl Estradiol Tablets (0.15 mg/ 0.02 mg) were prepared by spiking the Ethanol and Chloroform at the specification level and analyzed as per methodology.

Data Evaluation:

The content of each solvent in three sample solutions was determined.

Content (ppm) of each known solvents and % RSD (n=6) for the results of six spiked sample solutions was calculated.

Acceptance Criteria:

The content (ppm) of Ethanol and Chloroform in three sample solutions should comply the specification.

% RSD (n = 6) for the content (ppm) of Ethanol and Chloroform in six spiked sample solutions should be NMT 15.0.

Observation

The content of each solvent in three sample solutions was found within acceptance criteria. % SD (n=6) for the content of each solvent in six spiked sample solutions was found within acceptance criteria.

Method Precision

Sample	Ethanol (ppm)	Chloroform (ppm)
1	2011	65
2	1997	61
3	2004	66
4	1995	62
5	2008	67
6	2020	62
Mean	2006	64
SD	9.3	2.48
%RSD	0.5	3.9

Conclusion:

The analytical method meets the acceptance criteria for method precision. Hence, the method is precise for the estimation of residual solvents.

Intermediate Precision (Ruggedness)

Experiment

Diluent, standard solution, three unspiked sample solution were prepared as per the methodology and six sample solution was prepared by spiking Ethanol and Isopropyl alcohol at the specification level, from a single lot (same lot used for Method Precision) of Levonorgestrel and Ethinyl Estradiol Tablets (0.15 mg / 0.02 mg) *as per methodology* by a different analyst, on a different day, using different column of different make on a different instrument and inject.

Data Evaluation

The content of each solvent in three sample solutions was determined. Content (ppm) of each known solvents and % RSD (n=6) for the results of six spiked sample solutions was calculated.

Acceptance Criteria

The content (ppm) of Ethanol and Chloroform in three sample solutions should comply with the specification % RSD (n = 6) for content (ppm) of Ethanol and Chloroform in six spiked sample solutions should be NMT 15.0.

Overall % RSD (n = 6 + 6) for Ethanol and Chloroform content (ppm) in six spiked sample solutions from Method Precision and Intermediate Precision should be NMT 15.0.

Observation

The content of each solvent in three sample solutions was found within acceptance criteria

% RSD (n=6) for ppm of known solvents in six spiked sample solutions and overall

% RSD (n=12) for ppm of Ethanol and Chloroform from Method Precision and

Intermediate Precision results were found within acceptance criteria.

Variables used for Method Precision and Intermediate Precision

Parameter	Method Precision	Ruggedness
Analyst	Analyst-1	Analyst-2
HPLC Instrument ID No.	HPLC1	HPLC2
Column Name	RTX-624	DB-624
Column ID No.	Column1	Column2
Day	Day1	Day2

Intermediate Precision

Sample	Ethanol (ppm)	Chloroform (ppm)
1	2010	62
2	2022	66
3	2011	65
4	2007	60
5	2013	69
6	2019	62
Mean	2014	64
SD	5.71	3.28
%RSD	0.3	5.1

Cumulative results of Method and Intermediate Precision

Sample	Ethanol (ppm)		Chloroform (ppm)	
	MP	IP	MP	IP
1	2011	2010	65	62
2	1997	2022	61	66
3	2004	2011	66	65
4	1995	2007	62	60
5	2008	2013	67	69
6	2020	2019	62	62
Overall Mean (n=6+6)	2010		64	
SD (n=6+6)	8.41		2.77	
¾RSD (n=6+6)	0.4		4.3	

MP-Method precision; IP-Intermediate precision

Conclusion

The analytical method meets the acceptance criteria for the Intermediate Precision study. Hence, it is concluded that the method is precise and rugged.

Robustness:

To evaluate robustness, following small but deliberate variations were made in the analytical method parameters.

Change in the flow rate by ± 10% of 2.0 mL/min i.e. (2.2 mL/min; l .8mL/min).

Change in initial column oven temperature by ± 5° of 40°C. (45°C for the increase in temperature and 35°C for a decrease in temperature).

Change in incubation temperature by ± 5°C of 100°C. (105°C for an increase in incubation temperature and 95°C for the decrease in incubation temperature).

Diluent, system suitability solution, standard solution, and three preparations of sample solution spiked with known solvents at specification level were prepared and analyzed as per the methodology.

Data Evaluation:

System suitability was evaluated in each altered condition.

% cumulative RSD of results of solvents (ppm) from three results of each robustness condition was compared with that obtained in Method Precision.

Acceptance criteria

System suitability should pass for each altered condition.

RSD of results of Ethanol (ppm) and Chloroform (ppm) from three results of each ro should not be more than 15.0.

Abbreviations

Robustness Condition	Abbreviation	Instrumental Variation
Change in flow rate by ± 10 % of 2.0 mL.	-Flow	1.8 mL/min
	+Flow	2.2 mL/min
Change in initial column oven temperature by ± 5°C of 40°C.	-Temp.	35°C
	+Temp.	45°C
Change in incubation temperature by± 5°C of 100°C.	- Temp. (HS)	95°C
	+ Temp. (HS)	105ºC

Sr. No.	Method Precision	-Flow	-+Flow	-Temp.	+Temp.	-Temp. (HS)	+Temp. (HS)
1	2011	2022	1998	2045	2004	1996	2001
2	1997	2013	2007	2022	2007	1998	2004
3	2004	1999	2005	2011	2009	2003	2007
4	1995						
5	2008						
6	2020						
Overall Mean (n=6+3)		2008	2005	2013	2006	2004	2005
Overall SD (n=6+3)		9.74	7.81	15.19	7.45	8.29	7.54
Overall % RSD (n = 6+3)		0.5	0.4	0.8	0.4	0.4	0.4

Sr. No.	Method Precision	-Flow	+Flow	' .-Temp.	+Temp.	-Temp. (HS)	+Temp. (HS)
1	65	67	69	71	63	60	63
2	61	69	70	72	62	69	65
3	66	71	68	70	62	64	67
4	62						
5	67						
6	62						
Overall Mean (n=6+3)		66	66	66	63	64	64
Overall SD (n=6+3)		3.39	3.28	4.1	2.12	3.00	2.27
Overall % RSD (n = 6+3)		5.2	5.0	6.2	3.3	4.6875	3.5

Conclusion:

Method meets acceptance criteria for robustness and was robust concerning Change in flow rate

(± 10 %), change in initial column oven temperature (± 5°C) and Change in incubation temperature

(± 5°C). Hence, it is concluded that the method is robust.

Solution Stability:

Prepared standard solution, single unspiked sample solution, and single spiked sample solution by spiking, Ethanol, and Chloroform at the specification level to a single lot of Levonorgestrel and Ethinylestradiol \Tablets (0.15 mg / 0.02 mg) *as per methodology*. Injected six replicates of standard solution (initial-Ohr) and a single injection of unspiked sample and spike sample solution (initial-Ohr). Injected single injection of standard solution, unspiked sample solution, and spike sample solution at different time intervals up to 56 hours for standard and 52 hours for unspiked and 51 hours for spiked sample solution by storing the solutions at room temperature. For each sample solution results were calculated and compared with the initial.

RESULTS

Acceptance Criteria:

Standard Solution

The cumulative % RSD (n = 6 + 1) for peak area counts of Ethanol and Chloroform initially (n = 6) and peak area counts of Ethanol and Chloroform at each time interval (n = 1) should be NMT 15.0.

For Unspiked Sample Solution

The absolute value of % difference between ppm of each solvent in the initial unspiked sample and unspiked sample injected at each time interval concerning initial value should be NMT 15.0.

For Spiked Sample Solution

The absolute value of% difference between ppm of each solvent in the initial spiked sample and spiked sample injected at each time interval concerning initial value should be NMT 15.0.

Calculation of Absolute value of % difference

The absolute value of% difference for solvent peaks in the sample solution (unspiked and spiked) at each time point was calculated concerning the initial area as follows,

The absolute value of % difference=

(Results in ppm at respective time point)- (results in ppm at initial time point) x 100

Results in ppm at initial time point

Observation Standard Solution

The cumulative% RSD (n = 6 + 1) for peak area counts of Ethanol and Chloroform initially (n = 6) and peak area counts of Ethanol and Chlorofom1 at each time interval (n = 1) was found within the acceptance criteria up to 56 hours at room temperature.

For Unspiked sample:

The absolute value of% difference between each known solvents of Ethanol and Chloroform in initial unspiked sample and unspiked sample injected at each time interval concerning initial value was within the acceptance criteria up to 52 hours at room temperature.

For Spiked Sample Solution

The absolute value of the % difference between each known solvents of Ethanol and Chloroform in initial spiked sample and spiked sample injected at each time interval concerning initial the value was within the acceptance criteria up to 51 hours at room temperature.

Solution stability of Ethanol in standard solution

Area of Ethanol					
Sr. No.	**About 12 Hrs**	**About 24 Hrs**	**About 48 Hrs**	**About 60 Hrs**	**About 12 Hrs**
1	1115555	1115555	1115555	1115555	1115555
2	1127896	1127896	1127896	1127896	1127896
3	1123456	1123456	1123456	1123456	1123456
4	1118889	1118889	1118889	1118889	1118889
5	1123478	1123478	1123478	1123478	1123478
6	1122556	1122556	1122556	1122556	1122556
Time Interval		1119234	1108766	1111129	1143267
Mean	1121972	1121581	1120085	1120423	1125014
SD	4256.475	4021.029	6325.408	5647.367	8937.698
%RSD	0.4	0.4	0.6	0.5	0.8
The standard solution was found to be stable up to 56 hours at room temperature.					

Solution stability of Chloroform in standard solution

Area of Chloroform					
Sr. No.	**Initial**	**About 12 Hrs**	**About 24 Hrs**	**About 48 Hrs**	**About 60 Hrs**
1	8055	8055	8055	8055	8055
2	8023	8023	8023	8023	8023
3	8009	8009	8009	8009	8009
4	7998	7998	7998	7998	7998
5	7987	7987	7987	7987	7987
6	7990	7990	7990	7990	7990
Time Interval		7950	7662	7889	7777
Mean	8010	8001	7961	7993	7977
SD	25.56	32.62	133.70	51.45	91.22
%RSD	0.31	0.4	1.7	0.6	1.1
The standard solution was found to be stable up to 56 hours at room temperature.					

Results Table for Un-spiked Sample:

Time Interval	Ethanol		Chloroform	
	ppm	The absolute value of the % difference w.r.t. initial	ppm	Absolute value of % difference w.r.t. initial
Initial	ND	---	ND	---
10 Hrs.	ND	NA	ND	NA
24 Hrs.	ND	NA	ND	NA
38 Hrs.	ND	NA	ND	NA
52 Hrs.	ND	NA	ND	NA

Results Table for Spiked sample:

Time Interval	Ethanol		Chloroform	
	ppm	The absolute value of the % difference w.r.t. initial	ppm	Absolute value of % difference w.r.t. initial
Initial	2003	---	68	---
12 Hrs.	2002	0.05	67	1.5
24 Hrs.	2000	0.15	67	1.5
36 Hrs.	1998	0.25	66	3.0
48 Hrs.	1999	0.20	66	3.0

Conclusion:

Standard and sample solution is stable up to 60 hours, 48 hours respectively at room temperature.

For Residual solvents by GC

The test method for the determination of residual solvents by the Gas chromatographic method in Levonorgestrel and Ethinyl Estradiol tablets was developed and validated. The validation parameters ar like Specificity, LOD/LOQ, Linearity, Accuracy (Recovery), Precision, Robustness and Stability of Analytical Solution are verified. The method meets the acceptance criteria for all parameters. Hence, this method can be used for the determination

of residual solvents (Ethanol and Chlorofonn) in Levonorgestrel and Ethinylestradiol Tablets (0.15 mg *I* 0.02 mg and 0.15 mg *I* 0.03 mg) for QC release.

REFERENCES

1. International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," Federal Register 60(40), 11260–11262 (1995).
2. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register 62(96), 27463–27467 (1997).
3. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," Federal Register (Notices) 65(169), 52776–52777 (2000).
4. www.fda.gov/cder/guidance/cmc3.pdf
5. USP 25–NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopeial Convention, Rockville, Maryland, USA, 2002) p 2256.
6. ANALYTICAL METHODS FOR RESIDUAL SOLVENTS DETERMINATION IN PHARMACEUTICAL PRODUCTS KATARZYNA GRODOWSKA1,2* and ANDRZEJ PARCZEWSKI1 1 Jagiellonian University, Faculty of Chemistry, Department of Analytical Chemistry, Ingardena 3, 30-060 KrakÛw, Poland 2 Pliva KrakÛw S.A., Mogilska 80, 31-546 KrakÛw, Poland
7. Bauer M., BarthÈlÈmy C.: Handbook of solvents, Wypych G. Ed., 1st edn., p. 1129, ChemTec Publishing, Toronto, New York 2001.
8. http://www.pharmaquality.com/LabNotebook8. Htm
9. Scott R. P. W.: Gas chromatography, p. 45, Chrom-Ed Book Series.
10. Wittrig R. E., Dorman F. L., English C. M., Sacks R. D.: J. Chromatogr. A1027, 75 (2004)
11. Gorecki T., Harynuk J., PaniÊ O.: J. Sep. Sci. 27, 359 (2004)
12. http://www.pg.gda.pl/chem/CEEAM/Dokumenty/CEEAM_ksiazka/Chapters/chapter6.pdf
13. Avdovich H. W., Lebelle M. J., Savard C., Wilson W. L.: Forensic Sci. Int. 49, 225 (1991).
14. Thomasin C., Johansen P., Adler R., et al.: Eur. J. Pharm. Biopharm. 42, 16 (1996).
15. Osawa Z., Aiba M.: Polymer Photochem. 2, 339 (1982)
16. Weitkamp H., Barth R.: Bestimmung Kleiner Gehaltswerte nach dem Aufstockverfahren. Einfurungin die quantitative InfrarotSpektrophotometrie, Weitkamp H., Barth R. Eds., p. 58, Georg Thieme Verlag, Stuttgart 1976.
17. Benoit J. P., Courteille F., Thies C.: Int. J. Pharm. 29, 95 (1986)
18. Dubernet C., Rouland J. C., Benoit J. P.: Int. J. Pharm. 64, 99 (1990)
19. List P. H., Laun G.: Pharm. Ind. 42, 399 (1980)
20. http://www.ssi.shimadzu.com/products/detspecs.cfm
21. Westmorland D. G., Rhodes G. R.: Pure Appl. Chem. 61, 1148 (1989).
22. Fialkov A. B., Steiner U., Lehotav S. J., Amirav A.: Int. J. Mass Spectrom. 260, 31 (2007).
23. Witschi C., Doelker E.: Eur. J. Pharm. Biopharm. 43, 215 (1997).
24. Materials from the course: ìPractical aspects of gas chromatographyî, Analytical Chemistry Faculty, Chemistry Department, Politechnika GdaÒska, 02-06.07. 2007 GdaÒsk
25. ww.gerstelus.com/admin/prod_file.php?path= íramanaí&file=GC_Injection_Primer.pdf
26. http://www.restek.us/restek/images/external/59895A.pdf
27. Kolb B., Pospisil P.: Chromatographia 10, 705 (1977).
28. . Cramers C. A., Janssen J. G. M., Deursen M. M., van Beens J.: Gas chromatographic techniques and applications, Handley A. J., Adlard E. R. Ed., 1st edn. p. 207, CRC Press, Boca Raton 2001.
29. Jennings W.: Analytical Gas Chromatography, 2nd edn., p. 80, Academic Press, San Diego 1987.
30. http://www.chromatography-online.org/topics/capillary/column.html
31. http://www.chem.agilent.com/cag/cabu/capgccols.htm
32. USP<467>, General Chapter, Organic Volatile Impurities.
33. European Pharmacopoeia 6th Edition, 2.4.24. Identification and control of residual solvents

34. A technical guide for static headspace analysis using GC. http://restekcorp.us/restek/images/external/59895A.pdf