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Simultaneous Estimation of Residual Solvents (Ethanol, Acetone, Dichloromethane and Ethyl Acetate) in Dosage Form by GC-HS-FID

PRAVEEN KUMAR BALIYAN*, R.P. SINGH† and SAURABH ARORA
Analytical Division, Arbro Pharmaceuticals Ltd.,
4/9, Kirti Nagar Industrial Area, Delhi-110 015, India
E-mail:praveenbaliyanraj@yahoo.co.in

A simple and sensitive method for the determination of ethanol, acetone, dichloromethane and ethyl acetate as residual solvent was developed and validated on gas liquid chromatography with headspace sampler fitted with flame ionization detector (GC-HS-FID). The carrier gas was helium and separation was carried out on Elite-624 (30 meter, 0.53 mm ID, 3µm df) capillary column consisting of 6 % cyanopropyl-phenyl-94 % dimethyl polysiloxane stationary phase. The retention time for ethanol, acetone, dichloromethane and ethyl acetate were 11.4, 12.7, 14.0 and 16.9 min, respectively.

Key Words: Ethanol, Acetone, Dichloromethane, Ethyl acetate, GC-HS-FID.

INTRODUCTION

Residual solvent specifications limits, set in accordance with the toxicity of solvents, vary from a few ppm to thousands of ppm. Static headspace gas chromatographic determination of residual solvents is nowadays a mature technique[1-4], well established in pharmaceutical analysis[5-7]. Usually the technique is applied to drugs soluble in water[5-10]. Residual solvent determination from drug products only slightly soluble or even insoluble in water[11,12] is still an analytical challenge.

Organic volatile impurities (OVIs)[13] are residual solvents that are used in and are produced during the synthesis of drug substances, or in excipients used in the production of drug formulations. Many of these residual solvents generally cannot be completely removed by standard manufacturing processes or techniques and are left behind, preferably at low levels. These impurities encounter during manufacture, storage of active pharmaceutical ingredients, excipients and drug products and moreover, residual solvents in the active pharmaceutical ingredients or from other drug manufacturing processes can be harmful for the human health, if they exceed a certain level. ICH[14] has given limits for the presence of OVIs in active pharmaceutical ingredients[15].

†Department of Chemistry, D.A.V. College, Muzaffarnagar-251 001, India.

The method for the determination of four residual solvents *viz.*, ethanol, acetone, dichloromethane and ethyl acetate simultaneously by gas chromatography with headspace sampler fitted with flame ionization detector was proposed. This method is very simple, accurate and precise.

EXPERIMENTAL

Working standard: Dichloromethane, acetone, ethyl acetate and ethanol manufactured by MERCK, were used.

Instrumentation: Gas chromatography Claus 500 and headspace sampler TurboMetrix 16 Perkin-Elmer with capillary column Elite-624 consisting of 6 % cyanopropylphenyl and 94 % dimethyl polysiloxane stationary phase with 0.53 mm internal diameter, 30 meter length and film thickness of 3 µm were used.

Chromatographic condition: Column: Elite-624 (30 meter, 0.53 mm ID, 3 µm df) (6 % cyanopropylphenyl-94 % dimethyl polysiloxane); carrier gas: helium; flow rate: 1.0 mL/min; injector temperature: 180 ºC; split ratio: 1:100; oven program: initial 50 ºC hold for 10 min, increase @ 10 ºC/min up to 200 ºC, hold for 5 min; detector temperature: 270 ºC; air gas flow: 450 mL/min; hydrogen gas flow: 45 mL/min; run time: 20 min.

Headspace sampler condition: Oven temperature: 75 ºC; needle temperature: 95 ºC; transfer line temperature: 100 ºC; thermostat period: 30 min; pressurize time: 1.0 min; inject time: 0.1 min; withdraw time: 0.5 min; GC cycle time: 40 min; capillary pressure: 15 psi.

Preparation of standard stock solution: Accurately weigh and transfer about 0.06 g of dichloromethane, 0.5 g of acetone, 0.2 g of ethyl acetate and 0.5 g of ethanol to a 50 mL volumetric flask containing about 20 mL of dimethyl formamide and make up the volume with dimethyl formamide. Further dilute 1 mL of this to 10 mL with dimethyl formamide.

Preparation of standard solution: Take 1.0 mL of standard stock solution in a headspace vial and seal.

Test preparation: Accurately weigh and transfer about 0.2 g of cefoperazone powder for injection in headspace vial, add 1 mL of dimethyl formamide and seal.

Blank preparation: Take 1.0 mL of dimethyl formamide in a headspace vial and seal.

Procedure: Inject blank preparation in single, standard preparation in triplicates and test preparation in duplicates on a GC-HS-FID system and record the chromatograms.

Calculations:

$$\text{Concentration of (acetone) (\% w/w)} = \frac{AT \times WT \times 1 \times 1 \times 1 \times P \times 100}{AS \times 50 \times 10 \times 1 \times ST \times 100}$$

$$\text{Concentration of (dichloromethane) (\% w/w)} = \frac{AT \times WT \times 1 \times 1 \times 1 \times P \times 100}{AS \times 50 \times 10 \times 1 \times ST \times 100}$$

$$\text{Concentration of (ethanol) (\% w/w)} = \frac{AT \times WT \times 1 \times 1 \times 1 \times P \times 100}{AS \times 50 \times 10 \times 1 \times ST \times 100}$$

$$\text{Concentration of (ethyl acetate) (\% w/w)} = \frac{AT \times WT \times 1 \times 1 \times 1 \times P \times 100}{AS \times 50 \times 10 \times 1 \times ST \times 100}$$

where, AT = Mean peak area counts of respective solvent in the chromatogram of the sample solution. AS = Mean peak area counts of respective solvent in the chromatogram of the standard solution. WT = Weight of respective solvent in gram. ST = Weight of sample in gram. P = Purity of respective standard solvent used (in per cent).

RESULTS AND DISCUSSION

Specificity: Specificity is the ability of method to confirm the analyte identity from other interferences. Specificity has been established by injections of ethanol, acetone, ethyl acetate and dichloromethane individually. The resolution obtained between the peaks was > 5. No peaks were observed in blank injection.

System precision: The system precision of this method is expressed in the term of % RSD of the data. System precision has been demonstrated by six replicate injections of standard solutions. The RSD was found out to be less than 10 %. All values are listed in Table-1.

TABLE-1
SYSTEM PRECISION

Sr. No.	Compound			
	Ethanol area	Acetone area	Ethyl acetate area	Dichloromethane area
1	16782.52	58780.73	14685.01	1701.59
2	16666.51	58249.46	14555.33	1695.92
3	17098.09	59405.82	14914.64	1671.15
4	17386.02	60241.95	15091.73	1697.24
5	16911.10	58240.22	14578.80	1627.83
6	17259.02	59384.83	14918.00	1656.96
Mean	17017.21	59050.50	14790.59	1675.12
Std Dev.	279.41	778.28	216.17	29.00
% RSD	1.64	1.32	1.46	1.73

Method precision: The method precision of the proposed method is expressed in the term of % RSD of the data. Method precision has been demonstrated by separately analyzing one batch of sample six times (as per the method). RSD was found to be less than 15 % (calculated only for residual solvent). All values are listed in Table-2.

Linearity: The method has been shown to be linear by a plot of min. 5 points in the range LOQ-120 % of specification limits. This has been confirmed by appropriate statistical methods. Correlation coefficient for each residual solvent was found to be more than 0.98 (Table-3).

TABLE-2
METHOD PRECISION

Sr. No.	Acetone area	Ethyl acetate area
1	169060.90	7885.94
2	173347.86	7569.78
3	178615.36	7999.45
4	191040.44	8432.55
5	158747.00	7027.50
6	182085.69	8248.93
Mean	175482.88	7860.69
Standard deviation	11147.53	505.27
% RSD	6.35	6.43

TABLE-3
LINEARITY AND RANGE

Compound	Linearity range (µg)	Regression coefficient (R^2)	Retention time (min)
Ethanol	500-1200	0.9991	11.40
Acetone	500-1200	0.9962	12.78
Dichloromethane	60-145	0.9983	14.01
Ethyl acetate	200-480	0.9991	16.91

Ruggedness: Ruggedness is the ability of a chemical measurement process to resist changes in the test results when subjected to minor changes in environmental and method procedural variables, laboratories, personnel, *etc*. Ruggedness has been established by separate six analyses of single batch of sample, prepared by two different analysts on different days. Overall RSD of residual solvents were found out to be less than 15 %. All values are listed in Table-4.

TABLE-4
RUGGEDNESS

Compound	Sr.No.	Acetone	Ethyl acetate
		Test Area	Test Area
Ist Day	1	187154.24	8308.07
	2	162704.64	6906.64
	3	192785.99	8442.63
	4	149483.83	6563.21
	5	160494.12	7082.32
	6	157695.95	7146.76
IInd Day	1	176214.61	8001.75
	2	162344.79	7863.70
	3	181980.84	8079.29
	4	172631.79	8051.18
	5	183764.55	8212.03
	6	158370.79	7473.56
	Mean	170468.85	7677.60
	Standard deviation	13814.06	619.61
	% RSD	8.10	8.07

Accuracy: For accuracy studies, known amount of residual solvent standards were spiked into the placebo at about 50, 100 and 150 % of specification limits in triplicate. Recovery represents the ability of method to estimate the analyte from the matrix interference at lowest concentration. Recoveries of the analyte at fortification level were determined by comparing the peak area obtained from sample spiked with known concentration of analyte. The recoveries have been calculated as given in the Table-5. Average recoveries were calculated to be 106.51, 102.51, 101.41 and 104.39 % for ethanol, acetone, dichloromethane and ethyl acetate, respectively. So, it may be concluded that the method is accurate (Table-5).

TABLE-5
RECOVERY (%)

Compound	Level of spiking (% w/w)	Spiked amount (% w/w)	Recovered amount (% w/w)	Recovery (%)	Average recovery (%)
Ethanol	0.25	0.25	0.257137958	98.97	106.51
	0.25	0.25	0.267017297	102.53	
	0.25	0.25	0.255460983	99.47	
	0.50	0.50	0.532687565	101.51	
	0.50	0.50	0.526901818	99.05	
	0.50	0.50	0.529500126	100.98	
	0.75	0.75	0.81203652	106.20	
	0.75	0.75	0.815982692	106.67	
	0.75	0.75	0.811797207	106.32	
Acetone	0.25	0.25	0.247417788	85.95	102.51
	0.25	0.25	0.256325752	89.74	
	0.25	0.25	0.248663274	81.49	
	0.50	0.50	0.507541266	99.14	
	0.50	0.50	0.495230056	104.88	
	0.50	0.50	0.504881431	107.82	
	0.75	0.75	0.796534285	87.28	
	0.75	0.75	0.800008619	95.89	
	0.75	0.75	0.797372245	91.59	
Dichloromethane	0.03	0.03	0.029161614	97.21	101.41
	0.03	0.03	0.031538566	105.13	
	0.03	0.03	0.028773416	95.91	
	0.06	0.06	0.063583380	105.97	
	0.06	0.06	0.061442240	102.40	
	0.06	0.06	0.063274329	105.46	
	0.09	0.09	0.09016225	100.18	
	0.09	0.09	0.090429381	100.48	
	0.09	0.09	0.090116144	100.13	
Ethyl acetate	0.1	0.1	0.100955688	100.96	104.39
	0.1	0.1	0.105080535	105.08	
	0.1	0.1	0.101410240	101.41	
	0.2	0.2	0.207062529	103.53	
	0.2	0.2	0.202424314	101.21	
	0.2	0.2	0.206512087	103.26	
	0.3	0.3	0.324494505	108.16	
	0.3	0.3	0.324890328	108.30	
	0.3	0.3	0.322740541	107.58	

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection is the smallest concentration where the analyte can be identified. The limit of quantification is the smallest concentration where the analyte can be quantified with acceptable precision and accuracy. The limit of quantification is usually higher than a limit of detection, higher enough where a quantitative value can be obtained. LOQ is declared by establishing the minimum concentration of analyte, at which the analyte can be reliably quantified. LOD and LOQ have been established on the bases of S/N ratio (signal to noise ratio) by six injections at LOD level and six injections at LOQ level. The S/N ratio was found to be more than 3 for LOD and more than 10 for LOQ. The RSD was found out to be less than 15 %. The limits of detection for ethanol, acetone, dichloromethane and ethyl acetate of the proposed method are 0.15, 0.15, 0.018 and 0.06 % w/w, respectively. The limits of quantification for ethanol, acetone, dichloromethane and ethyl acetate are 0.25, 0.25, 0.03 and 0.1 % w/w, respectively (Table-6).

TABLE-6
LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Compound	LOD		LOQ	
	Standard (µg)	RSD (%)	Standard (µg)	RSD (%)
Ethanol	300	2.26	500	2.41
Acetone	300	1.83	500	2.40
Ethyl acetate	120	1.91	200	2.44
Dichloromethane	36	6.63	60	3.38

Robustness: Robustness has been established by analyzing sample in triplicate as per the proposed method and by changing the carrier gas flow rate by ± 10 % of the original value. Overall RSD calculated only for residual solvents were found to be less than 15 % (Table-7).

TABLE-7
ROBUSTNESS

Sr. No.	Acetone Area	Ethyl acetate Area
1	208071.22	6683.03
2	204946.83	6482.86
3	170126.50	6354.14
1	149835.21	6683.04
2	160834.53	7202.88
3	158013.63	7264.37
Mean	175304.65	6778.39
Standard deviation	25044.67	374.67
% RSD	14.29	5.53

System suitability: System Suitability has been demonstrated by analyzed standard solution during validation study. The system performance was checked by the resolution, tailing factor, theoretical plates and % RSD (Table-8).

TABLE-8
SYSTEM SUITABILITY

Compound	Retention time	Resolution	Tailing factor	No. of theoretical plates	RSD (%)
Ethanol	11.46	–	1.08	27018	1.64
Acetone	12.84	5.1	1.06	40424	1.32
Dichloromethane	14.06	5.0	1.03	58656	1.46
Ethyl acetate	16.95	13.6	1.02	123361	1.73

Conclusion

A simple, accurate and highly precise method for the determination of ethanol, acetone, dichloromethane and ethyl acetate in dosage form was developed. This method is a fine example of the advantage of GC-HS-FID. The method was subsequently validated in accordance with current ICH guidelines. This method will undoubtedly prove to be suitable for the identification and quantification of residual solvents (ethanol, acetone, dichloromethane and ethyl acetate).

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