Original Article

HS-GC Method Validation for Determination of Residual Solvents In Herbal Extracts

Datendra Nath Tripathi[1], Monica Kachroo[1], B. Murali[2]

[1] Al- Ameen College of Pharmacy, Near Lalbagh Main Gate, Bangalore-560027
[2] Natural Remedies Private limited, Veersandra Indl. Area, Hosur Road, Bangalore

ABSTRACT:

Residual solvent in herbal extracts were monitored using head space gas chromatograph (HS-GC) with flame ionization detector (FID) and headspace AOC 5000 auto sampler. The separation of the residual solvents was achieved on a Zebron (ZB-624) column (30 m X 0.25 mm i.d. X 0.25 µm coating thickness) using GC 2010 Shimadzu, with nitrogen as a carrier gas in the split mode by headspace method. Fast separation of 7 commonly used solvents (methanol, ethanol, acetone, n-hexane, ethyl acetate, ethylene dichloride and toluene) was achieved in a single analysis of 9.19 min run time with 1.95 ml/min flow rate. The oven and detector temperature was 34°C and 255°C respectively using N,N'-Dimethylformamide(DMF) as a diluent.

The method was successfully validated for linearity, specificity, precision, accuracy, and range with good recoveries. The linear range for standard mix solvent was 10 to 200 ppm.

Keywords: Herbal extract, residual solvents, gas chromatography, Flame ionization detector

INTRODUCTION

A range of organic solvents are used for manufacturing herbal medicines, and can be detected as residue of such processing in herbal extracts and finished herbal products. [14–17] They should be controlled through Good manufacturing practice (GMP) and quality control. Solvents like methanol, ethanol, isopropyl alcohol, acetone, toluene, dichloromethane etc are used for isolation of the active constituents in herbs. These solvents cannot be completely removed by any practical processes, the fraction of the solvent always remains referred as residual solvents.[1,2] These residual solvents in herbal extracts possess toxicological effects, so International Conference on Harmonisation(ICH) has prescribed acceptable limits for residual solvents in active pharmaceutical ingredients (APIs).[2] Hence evaluation of residual solvent is considered as an important tool in the quality control of pharmaceuticals.[3-9]

Presently in the pharmaceutical industries, special importance is given for residual solvent testing. The content of residual solvents in APIs is analyzed by gas chromatography.[10-13,16-19] Over the last decade; several GC methods used to monitor residual solvents have been reported in the literature. Accordingly, the method has been developed and validated for detection and quantification of residual solvents like methanol, ethanol, acetone, n-hexane, ethyl acetate, ethylene dichloride, and toluene in herbal extracts using head space gas chromatography. Acceptable levels of these solvents are included in guideline Q3C (R5) issued by ICH and must not exceed: methanol 3000 ppm, acetone, ethanol, ethyl acetate 5000 ppm, n-hexane 290 ppm, toluene 890 ppm.

MATERIALS AND METHODS

Gas Chromatograph Shimadzu 2010 was used in the development of GC method.

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Address for correspondence
Dr. Monica Kachroo
HOD & Professor,
Department of pharmaceutical Chemistry, Al-Ameen College of pharmacy,
Opp. Lalbagh Main Gate,
Hosur Road,
Bangalore 560027,
Karnataka (India),
Ph: 011-91-9916973586,
E-mail: monicakachroo@gmail.com

www.rjps.in

Gas chromatograph was equipped with standard oven for temperature ramping, split injection ports and flame ionization detector. ZB 624 column (30 m × 0.53 mm i.d. × 0.25 µm coating thickness, 4% cyanopropyl phenyl and 96% dimethylpolysiloxane stationary phase), with nitrogen as carrier gas in the split mode. HPLC and analytical grade solvents methanol, ethanol, acetone, n-hexane, ethylacetate, ethylene dichloride, toluene and DMF were purchased from Thomas Baker, Mumbai, India.

Preparation of standard

Standard solvent mix

DMF was selected as the diluents, 500 mg each of methanol,ethanol, acetone,n-hexane, ethyl acetate, ethylene dichloride, and toluene was taken in 50 ml standard volumetric flask containing 5 to 10 ml DMF and made up the volume with proper mixing using DMF as a diluent. [19] (weight taken in mg for the uniformity of dilution).

Solvent standard

10 mg of each solvent methanol,ethanol, acetone,n-hexane, ethyl acetate, ethylene dichloride isopropyl alcohol, acetonitrile, dichloromethane, carbon tetrachloride, and benzene were taken separately in10 ml standard volumetric flask which were previously filled with DMF.

Sample preparation

100 mg (±15 mg) of *Andrographis paniculata* extract was taken in clean head space vials. 5 ml imethyformamide was added to the headspace vials with 5 ml glass pipette and capped the vial air tight.[2]

Gas chromatographic conditions

The experimental conditions were used, 1 mL volume of either standard or sample solutions was injected in GC injection port. The injection port was maintained at temperature 250°C with split ratio 1:5. Nitrogen was used as a carrier gas with pressure 130 kpa for an expected flow of 1.95 ml/min. Temperature of detector was set at 250°C with total run time 9.19 min. temperature gradient maintained at 34°C increased at a rate of 15°C min^{-1} to 100°C further increased at a rate of 30°C min^{-1} to 180°C and finally increased at the rate of 40°C min^{-1} to 225°C and maintained for 1 min.

Method validation

The method validation was carried out as per ICH method validation guideline and USP pharmacopeia. The validation parameters addressed were specificity, precision, linearity accuracy, limit of detection, limit of quantification and range.

RESULT AND DISCUSSION

Development of method

Gas chromatographic method for the determination of residual solvent in herbal extract was developed. The column used was ZB-624 capillary column, with flow 1.95 ml/min. column pressure 130 kpa with split ratio 1:5. In prescribe method all seven solvents eluted with in 9.19 min (Figure 1), the retention time for solvent are mentioned in Table 1.

Validation of method

Specificity

The specificity of the gas chromatographic method was determined by comparing the retention time of the standard solvent mix peak with that to the individual standard solvent peak and checking resolution of the individual standard solvent peak and spike sample with standard solvent mix peak. There is no interference from any other solvent present in the standard mix solvent.

Linearity

Determined by a series of 3 to 5 injections of 5 or more standard concentration. The correlation coefficient (R^2) for standard mix solvent (Figure 2) was found to be 0.997–0.999, indicating the linearity of the method. The % RSD for standard mix solvent was found to be 1.5 to 1.6. indicating the linearity of the method (Table 2).

Precision

5 ml of Standard mix solvent was injected. It was repeated for 5 times for all the 6 dilutions. The mean area and standard deviation, and relative standard deviations were calculated. Relative standard deviation (RSD) was found to be <2.5% and <0.5 for peak area and retention time respectively for residual solvent in standard solvent mix dilutions (Table 2).

Accuracy

Andrographis paniculata extract was analyzed for the actual content of residual solvent in it. Different concentration of samples was spiked into different concentration of Standard Solvent Mix and the recovery was calculated. Three concentration of 10, 50 and 250 ppm of the standard mix solvent was spiked in *Andrographis paniculata* extract. Each concentration were triplicated and assessed for recovery. Spike recovery was observed between 89 to 119% indicating the method is accurate (Table 2).

Range

The specific range derived from the linearity studies was calculated from graph.

A graph is plotted using the concentration on the x-axis and response factor on the y-axis. The method was

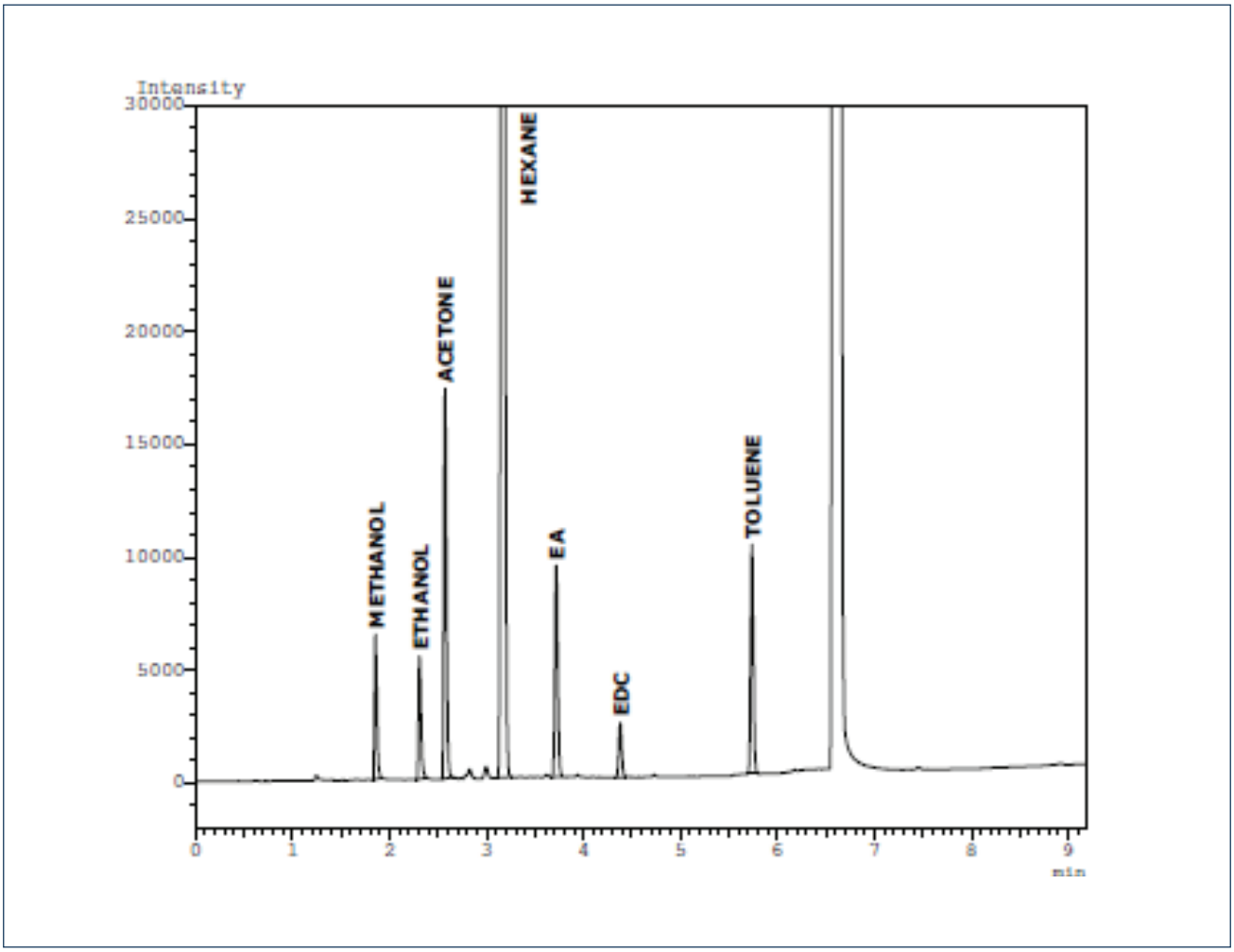

Figure1: Chromatogram of different solvents in standard solvent mix.

Table 1: Retention time and area of standard solvent

Solvent	Retention time	Area
Methanol	1.854	10969
Ethanol	2.308	9578
Acetone	2.571	23710
N-Hexane	3.166	73544
Ethyl acetate	3.719	15164
Ethylene dichloride	4.379	5564
Toluene	5.738	16388

accurate, linear and specific for standard solvent mix to the following range 10 to 200 ppm.

Limit of detection and Limit of quantification

The limit of quantification and detection were calculated by the instrumental method. For the instrumental method LOD and LOQ is determined by signal to noise. For Limit of detection and limit of quantification signal to noise ration should be 3:1 and 10:1. The value for the LOD and LOQ for methanol, ethanol, acetone, n-hexane, ethyl acetate, ethylene dichloride and toluene are mentioned in Table 2.

The results obtained for the validation parameters were within the proposed limits given by ICH guidelines USP34. The parametes evaluated for the extract of *Andrographis paniculata* exhibited the values within the range of ICH and USP34 guidelines.

CONCLUSION

On the basis of the validation parameters obtained the proposed gas chromatographic method can be provided for the quantitative and qualitative estimation of residual solvents in herbal extracts as it was found to be linear, accurate, precise, and within the range. Hence this method guarantees the level of residual solvent to be present below the permissible level as per the ICH guidelines or USP 34. This method can be extended for the estimation of residual solvents in herbal formulations, which can be further standardized and validated.

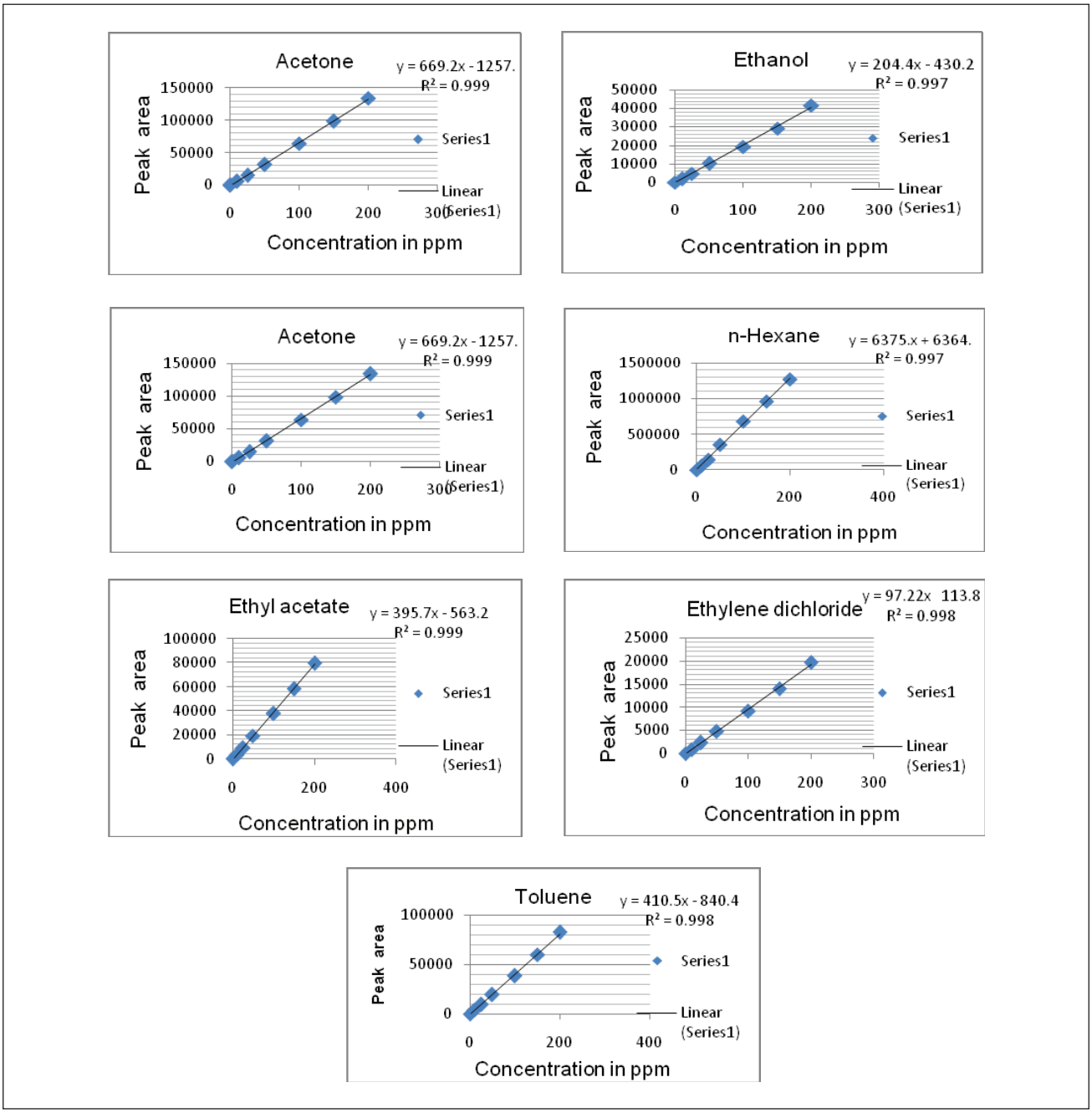

Figure 2: Linearity graph for the standard solvent mix.

Table 2: Validation parameters

Name of solvents	Linearity			Precision		ACCURACY (%Recovery)			LOD (ppm)	LOQ (ppm)
				Assessment of Area	Assessment of Retention time					
	R^2	Slope	RSD	RSD	RSD	10%	50%	250%		
Methanol	0.998	404.0	0.9022	1.5141	0.0219	99.91	99.05	102.3	0.75	50
Ethanol	0.997	430.0	0.9654	1.5371	0.0190	116.5	101.1	111.1	1.5	50
Acetone	0.999	1257	0.6835	0.8245	0.0135	95.95	97.46	111.8	0.75	50
n- Hexane	0.997	6364	1.246	1.4248	0.0028	87.34	91.04	99.28	0.375	50
Ethyl acetate	0.999	563.2	0.6833	1.1035	0.0060	107.0	104.8	116.0	0.75	50
Ethylene dichloride	0.998	113.8	1.6812	1.9312	0.0063	100.0	103.2	109.4	1.5	50
Toluene	0.998	840.4	1.1825	1.5178	0.0204	106.0	106.6	119.7	0.75	50

R^2- Co-relation co-efficient, RSD- Relative standard deviation, LOD- Limit of detection, LOQ- Limit of quantification.

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