Pharmacognosy Magazine Vol 4, Issue 18, Apr-Jun, 2009 Page 139-144
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 6 October, 2008
Modified: 4 December, 2008
Accepted: 29 January, 2009

PHCOG MAG.: Research Article

Determination of Organic Volatile Impurities in Herbal Formulations and Extracts

S. B. Puranik*, P. N. Sanjay Pai, G. K.Rao

Al-Ameen College of Pharmacy, Near Lalbagh Main Gate, Bangalore-560027, India
Author for correspondence:*sangpur@rediffmail.com*

Abstract
Organic volatile impurities (OVIs), residual solvents in herbal formulations and extracts were monitored using gas chromatography (GC) with Flame Ionisation detector (FID). As per GMP, measuring residual solvents is mandatory. It is now possible to take advantage of GC equipment with faster temperature ramping capabilities, in combination with shorter capillary GC columns, to achieve considerable gain in efficiency and reduction in analysis time. In the present study Gas chromatographic method for the determination of methanol, ethanol and isopropyl alcohol at residual levels in herbal formulations and extracts was developed using a flame ionization detector and the separation was carried out on BP 624 column, using GC 17 A shimadzu, with nitrogen as carrier gas in the split mode by direct injection method. The retention time for standard methanol, ethanol and isopropyl alcohol was found to be 3.72, 5.26 and 6.28 min respectively. The linearity for methanol, ethanol and isopropyl alcohol (IPA) was found to be in the range of 10-400 µL mL^{-1}, 10 - 500 µL mL^{-1} and 1 -240 µL mL^{-1} respectively. The method was validated according to ICH guidelines. The method described is simple, sensitive, rugged, reliable and reproducible for the quantitation of methanol, ethanol and isopropyl levels from herbal formulations and extracts and their levels are found to be within the ICH limits.
Key words: Herbal formulations, extracts, OVIs, solvents.

INTRODUCTION

Organic solvents are entrapped within the formulation either during the course of manufacture of active pharmaceutical ingredients or during coating of formulation. Residual levels of these organic solvents in tablet cores and film coats are critical as beyond permissible limits they are likely to cause undesirable side effects or alter some kind of physicochemical property of the active pharmaceutical ingredients (3-5). Hence it becomes necessary to limit the amount of these residual solvents, which can be called as organic volatile impurities, to a certain levels within the ICH prescribed limits. Herbal extract (1) is a liquid extract of herbs, dried or fresh herbs are combined with solvent, and then the solid matter is removed leaving only the required active constituents of the herbs mixed in the solvent. Herbal extracts are sold as dietary supplements and alternative medicine. The active constituents in the herbs are extracted by using various solvents methanol, isopropyl alcohol, acetone, toluene, butanol, dichloromethane etc. These solvents cannot be completely removed by practical processes such as freeze drying and drying at higher temperature under vaccum. The fraction of solvents always remains with the extract and are referred as residual solvents or organic volatile impurities. The residual solvents have no therapeutic benefits and are toxic and hazardous to human health. ICH has prescribed the limits for the solvents in herbal extracts and formulations (2). The content of residual solvents in herbal extracts is routinely measured by gas chromatography. Routine GC applications include analysis of herbal extracts to comply to good laboratory and good manufacturing practices as well as in process testing of residual solvents to optimize drying procedure (3). Over the last decade, several GC methods to monitor residual solvents have been reported in the literature (4, 5, 6, 7, 8). The detection limits were determined as 3ppm for methanol, 2 ppm for ethanol and isopropyl alcohol and 1 ppm for acetone and ethyl acetate. An ICH Class 3 solvent, 1-

propanol has been determined in propylgallate sample (80 µg/g ppm) with Agillent (Wilmington, DE, USA) GC, over 60m X 0.53mm id RTX column has been recorded with carrier gas system of helium with FID, ethyl acetate has been determined in carbomer (0.155 µg/g ppm) using same system. A module drug powder soluble (9) in water was chosen and residues of four solvents ethanol, cyclohexane triethylamine and pyridine were investigated were all involved in the synthesis of drug. Ethanol was the purification/crystallization solvent, cyclohexane was used to denature ethanol, triethylamine was synthesis reactant and pyridine was the extracting solvents .The above solvents were determined in drug powder (product) with Varian 3800 CX system connected to Varian 8200 CX auto sampler for SPME over CP-select 624 CB column (Chrompack, Les Vils, France) 30mX 0.25mm id, thickness of 1.8µm with retention time 4.9 min, 9.6 min,11.5 min and 15 min for ethanol, cyclohexane, triethylamine and pyridine respectively using FID with helium as carrier gas system(10). The residual solvents testing in samples of drug substance intermediates were determined by using Rapid GC and Flash GC methods. Rapid GC was performed on Agilent (Alto, CA) Models 5890 series II and 6890 were used over the column ARTX 502.2 column with a 1.4µm film of diphenyl/dimethyl polysiloxane stationary phase 30m x 0.25mm id, with FID, static headspace injection was performed via a model 7694 headspace sampler from Agilent technologies. A Flash GC method was derived from thermo Orion application note (11) with narrow bore column 10mX0.1mm id, with a 0.4 µm film thickness, 6% cyanopropyl, 94% polydimethyl siloxane. In the reported method by using Rapid GC, sample A consists of methanol (0.34%w/w), ethanol (2.64%w/w), and THF (0.21%W/W), sample B consists of ethanol (0.04%w/w), sample C consists of dichloromethane (0.05%w/w) and toluene (0.06%w/w), sample D consists of toluene (0.03%w/w) and p- xylene (0.26%w/w). The same samples analysed in EZ Flash GC. The results were as follows, sample A consists of methanol (0.35%w/w), ethanol (2.64%w/w), and THF (0.26%w/w), sample B consists of ethanol (0.04%w/w), sample C consists of dichloromethane (0.04%w/w) and toluene (0.07%w/w) and sample D consists of toluene (0.03%w/w) and p- xylene (0.27%w/w).

The author (12) reported the GC separation was carried on Agilent DB-wax column with a dimension of 30m x 0.53mm and film thickness of 1µm. Helium was used as carrier gas at a flow rate of 5ml/min with FID. The samples were injected with the Agilent 6890 series auto sampler in splitless mode. The detection limit (LOD) of the method for the mesylate esters was estimated from a chromatogram of a solution containing about 0.04µg/ml each of the esters, signal to noise ratio of 16, 25 and 29 for Methyl methane sulfonate (MMM), Ethyl methyl sulfonate (EMS) and Isopropyl methane sulfonate (IPMS), in pharmaceuticals where mesylate esters are known to be potent mutagenic, carcinogenic and teratogenic compounds LOQ was determined to be less than or equal to 5µg/g (5ppm) for MMS, EMS or IPMS based on the precision and accuracy data. Authors has (13) developed head space SPME method developed for the analysis of volatile polar residual solvents by GC-MS. The GC-ion trap mass spectrometer (GC-MS) was used with 30m x 0.25mm id. SPB-1 column coated with 1µm thickness (Supleco Park Bellefoute PA, USA) using helium as carrier gas system, the external electron ionization ion source was operated at an electron energy of 70ev and the filament emission was set at 200mA. SPME method has been developed and optimized for the polar residual solvent determination in pharmaceutical products. Five different polymers were investigated and the caboxen/polydimethylsiloxane was found to be the most sensitive for all compounds. Two head space SPME methods were developed and optimized one for the extraction from aqueous solutions and the other for the extraction from organic solvents N, N-dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). It was found that the addition of 100ml DMSO or DMF to 50mg drug substance and slightly pressurizing the head space vial gives good results in terms of sensitivity and reproducibility. The detection limit were between 0.4 and 200 ng/ml of relative standard deviate on data were between 2 and 9 % the head space SPME from aqueous solution was found to be 10 times more sensitive than immersion SPME and head space SPME from organic solutions. The author (14) has developed HS-GC method for the quantitative determination of residual solvents in a drug substance has been developed according to the European Pharmacopoeia general procedure. A 6890 series Hewlett pack and GC system with a FID system (Waldbronn, Germany) and a 7496 H-P head space auto sampler equipped with a 1ml sample loop were used. An OVI-G43capillary column (30m × 0.53mm id) and 3µm film thickness (Supleco) was used. Sample solvent water- DMF (3:2) was selected to obtain good recoveries for ethanol, tetrahydrofuran, same sample dilution sample was adopted to detect all class 1 and 2 solvent are the ICH recommended levels. With FID (except 1, 1, 1) the

trichloro ethane found to be at 10 ppm instead of 500 ppm (ICH limit). In present method has been developed and validated using BP-624 column , in the split mode with FID and nitrogen as the carrier gas system for detection and quantification of residual solvents methanol, ethanol and isopropyl alcohol in herbal formulations Viz; pigmento, pilex, alserex, pileum, livotrit, gerifort, gasex, neo, mystol forte, liv-52. Herbal extracts of *Momordica charentia, Morinda Citrifolia, Rosemary, Curcumin, Green tea, Gymnema, Phyllantus niruri*.

Experimental Method

Instruments and Materials

Gas chromatograph was equipped with standard oven option for temperature ramping, split/splitless injection ports and flame ionisation detector. BP 624 column (30m X 0.53mm i.d. X 0.25µm coating thickness, 4% cynaopropyl phenyl and 94% dimethyl poysiloxane stationary phase), with nitrogen as carrier gas in the split mode by direct injection method was used. Analytical grade solvents methanol, ethanol, isopropyl alcohol and dimethyl sulphoxide (DMSO) were purchased from Thomas Baker, Mumbai, India. Herbal formulations pigmento, pilex, alserex, pileum, livotrit, gerifort, gasex, neo, mystol forte, liv-52. were procured from the market and herbal extracts of *Momordica charentia, Morinda Citrifolia, Rosemary, Curcumin, Green tea, Gymnema, Phyllantus niruri* were obtained as gift samples from Phytotech Herbal Extracts Pvt Ltd. Bangalore, India.

Preparation of standard

Dimethyl sulphoxide (DMSO) was selected as the standard and sample diluent, based on its ability to dissolve wide variety of substances (7, 15, 16, and 17). Also DMSO is a solvent with high boiling point that does not interfere with more volatile solvents tested by GC for the method involving analysis of high boiling point solvents. Standard stock of methanol and isopropyl alcohol were prepared by diluting with DMSO in 10 mL volumetric flask to get concentration of 1000µLmL^{-1}. From these stocks 8 serial working standard solutions were prepared to obtain concentrations ranging from 10-400µLmL^{-1} ,10 - 400 µL mL^{-1} and 1 -240 µLmL^{-1} for methanol, ethanol and isopropyl alcohol respectively, volume is made with DMSO. 1µL of working standards were injected in to gas chromatograph and standard calibration curves were obtained for methanol and isopropyl alcohol.

Preparation of Sample

Accurately weighed 1g powdered tablets of pigmento, pilex, alserex, pileum, livotrit, gerifort, gasex, neo, mystol forte, liv-52 extracts of *Momordica charantia, Morinda citrifolia, Rosemary, Curcumin, Green tea, Gymnema, Phyllantus niruri* the rationale behind the selection of these formulations was that, all these formulations were film coated, some organic solvents were used for film coating, these were dissolved and sonicated with DMSO (7, 15, 16, 17) filtered through whatman filter paper No 1 and volume made up to 10mL with DMSO, in a separate volumetric flask. From these samples 1µL samples were injected and concentrations of methanol and isopropyl alcohol in sample were calculated by interpolating standard calibration curve.

Gas chromatographic conditions

The experimental conditions were used; 1 µL volume of either standard or sample solutions was injected in GC injection port. The injection port maintained at temperature 35 ^{0}C with a split ratio 1:10. Nitrogen used as a carrier gas with pressure 16 kpa for an expected flow of 3.5 mL min^{-1}. Temperature of the detector was set at 250^{0}C. Temperature gradient maintained at 35^{0}C for five min and then increased at a rate of 10^0 C min^{-1} to 55^{0}C and maintained for 10min, finally increased at the rate of 25^0 Cmin^{-1} to reach the final temperature of 200^0 C and maintained for 2 min.

Method Validation

The analytical method validation was carried out as per ICH method validation guidelines [9]. The validation parameters addressed were specificity, precision, linearity, and limit of detection, limit of quantitation, ruggedness and system suitability. Standard plots were constructed for methanol, ethanol and isopropyl alcohol in the range of 10–400 µL mL,$^{-1}$ 10–400 µL mL^{-1} and 1-240 µL mL^{-1} respectively. Precision of the instrument has been carried out for methanol, ethanol and isopropyl alcohol of concentrations 200 µLmL $^{-1}$, 200 µLmL and 120 µL mL^{-1} respectively. From this stock five successive injections of 1µL were injected, from the area recorded in the chromatograms of methanol, ethanol and isopropyl alcohol standard deviation and relative standard deviation (%RSD) were calculated.

RESULTS AND DISCUSSION

Development of method

Gas chromatographic method for the determination of methanol, ethanol and isopropyl alcohol residual levels in herbal formulations and extracts was developed. The column used was BP624 capillary column, with flow rate 3.2 mL min^{-1}, linear velocity 22 cm sec^{-1} and column pressure 14 kpa with total flow of 116 mL min^{-1} in the split mode. The retention time for standard

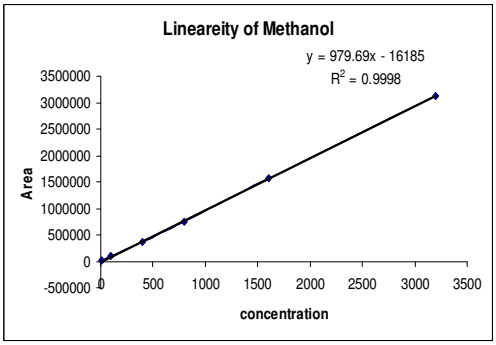

Fig 1 : Linearity graph of Methanol

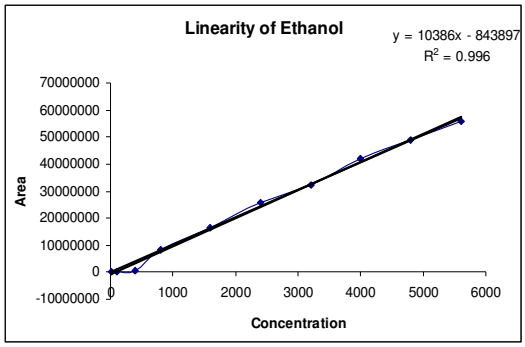

Fig 2: Linearity graph of Ethanol

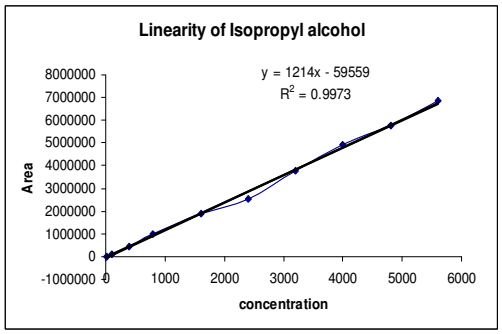

Fig 3: Linearity graph of isopropyl alcohol

Table 1: Amount of Organic Volatile Impurities Present in Herbal Formulations and Extracts.

Herbal Formulation Quantity (1gm powder)	Methanol (ppm)	Ethanol (ppm)	IPA (ppm)
Pigmento	00	25	00
Pilex	00	00	00
Alsarex	34	00	00
Pileum	59	43	00
Livotrit	87	00	63
Gerifort	00	00	00
Gasxex	00	00	00
Neo	00	00	21
Mystol forte	2	00	4
Liv- 52	8	1	28

Herbal Extracts	Methanol (ppm)	Ethanol (ppm)	IPA (ppm)
Morinda Citrifolia	32	00	9
Momardica charentia	92	00	98
Rosemary	563	00	3.15
Curcumin	540	00	5.7
Green tea	231	00	2.45
Gymnema	87	00	131
Phyllantus niruri	231	00	4.8

methanol, ethanol and isopropyl alcohol was found to be 3.72, 5.26 and 6.28 min respectively. The presence of methanol, ethanol and isopropyl alcohol in different concentrations were presented in (Table 1).

Validation of method

Specificity

The specificity of analytical method was determined by injecting a blank solution, pure dimethyl sulphoxide solution under the same experimental conditions. No peak was observed from the chromatogram obtained by injecting 1µL of DMSO as a blank.

Precision

The precision of method is the extent to which the individual test results of multiple injections of the series of standard agree. Precision of the analytical method usually expressed in standard deviation and relative standard deviation (coefficient of variance). Standard deviation and %RSD for methanol found to be 505.09, 0.2585 for ethanol 605.09, 0.356 and for isopropyl alcohol 959.32, 0.6213 respectively.

Linearity

The calibration curves were obtained in a concentration range 79.2 to 3168 µg/ml, 90.0 to 3600 µg/ml and 78.8 to 3152 µg/ml for methanol, ethanol and isopropyl alcohol Calibration curves were presented in Fig 1, 2and 3

Limit of Detection (LOD) and limit of Quantitation (LOQ)

The Limit of Detection for methanol, ethanol and isopropyl alcohol was found to be 1 µL mL^{-1}. Limit of Quantitation for methanol, ethanol and isopropyl alcohol was found to be 2 µL mL^{-1}.

Ruggedness

The ruggedness was established by determining methanol and isopropyl alcohol using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision. Additionally, good separations were achieved, which suggested that the method was selective for all components under the test.

System suitability

A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, asymmetry of the peak) before the validation runs. Three replicate injections of the standard solution and three injections of the solution prepared for the specificity procedure were used. The retention time of methanol, ethanol and isopropyl alcohol was found to be 3.095, 2.897 and 4.097 min respectively. HETP and No. of theoretical plates for methanol found to be 2.26, 1326, ethanol found to be 3.36, 1426 and for isopropyl alcohol 1.29, 2324 respectively.

CONCLUSION

This study presents a simple and validated Gas Chromatographic method for estimation of residual solvents methanol and isopropyl alcohol in herbal extracts. The developed method is specific, accurate, precise and rugged. The amounts of organic volatile impurities present in the herbal formulations and extracts were found to be within the ICH limits.

ACKNOWLEDGEMENT

Highly thankful to Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy, Bangalore, for facility and encouragement, Phytotech Herbal Extracts Pvt Ltd. Bangalore, India. for providing the gift samples, and to all my research colleagues for their support in the work.

REFERENCES

1. P. K. Mukherjee. *Quality Control of Herbal Drugs*, 1st Edition, Bussiness Horizons, New Delhi, 380-395, (2002).
2. Impurities in New drug Substances International Conference on Harmonization. Available from: http://www.ich,org. (1997).
3. S. B. Puranik, P. S. Pai and G. K. Rao. Organic Volatile Impurities in Pharmaceuticals. *Indian Journal of Pharmaceutical Sciences* **69:** 353 -59, (2007).
4. A. M. Diwedi. Residual Solvent Analysis in Pharmaceuticals. *Pharma. Technol*. **26:**42-46 (2002).
5. T. K. Chen, W. Moeckel and H. L. Superhant. Modifications of the Europiean Pharmacopoeias Proposal on Residual Solvents. *Pharmeurope* **4:**62 -7, (1992).
6. Z .Li, Y. H. Han and G. Zhuang. Static Head Space Gas Chromatographic Analysis of Residual Solvents in Gel Extrusion Model Tablet Formulations. *J. Pharm. Biomed. Anal.* **28:**673- 82, (2002).
7. C. C. Camarasu. Head Space SPME Method Development for the Analysis of Volatile Polar Residual Solvents by GC-MS. J. Pharm. Biomed. Anal. **23:**197- 210,(2000).
8. S. C. Rastogi. Gas Chromatographic Analysis of Organic solvents Mixtures and Capillary Columns of Different Polarity. *Chromatographia* **37:**211-4, (1993).
9. Impurities in New drug Substances International Conference on Harmonization. Available from: http://www.ich,org. (2002)
10. D. A. Lambropoulou, I. K. Konstantinou and T. A. Albanis. Recent developments in headspace microextraction techniques for the analysis of environmental contaminants in different matrices. *J. Chromatogr. A* 1150: 70-5, (2007).
11. A. Kurganov. Mass-balanced definition of corrected retention volume in gas Chromatography. *J. Chromatogr. A* **1150:** 100-10, (2007).
12. A. lvaro, G. Lopez, Vanesa, Esquiu, Maria and T Teresa. Comparison of three gas chromatography methods for the determination of slip agents in polyethylene films. *J.Chromatogr. A* **1150:** 178 -86, (2007).
13. M. Rocheleau, M. Titley1 and J. Bolduc Measuring residual solvents in pharmaceutical samples Using fast gas

chromatography techniques. *J. Chromatogr. B* **805:** 77-88, (2004).

14. Z. Li, Y. H. Han and G. Zhuang.static headspace gas chromatographic analysis of the residual solvents in gel extrusion module tablet formulations. *J. Pharm. Biomed. Anal*. **28:** 673-82, (2002).
15. A.R. Raghani. High speed gas chromatographic analysis of solvents in pharmaceuticals using solid phase microextraction. *J. Pharm. Biomed. Anal*. **29:** 507-518, (2002).
16. K.Filszar, J.M.Wiggins, C.M. Pignoli, G.P.Martin and Z.Li. Analysis of organic volatile impurities in pharmaceutical Excipients by static headspace capillary gas chromatography. *J. Chromatogr. A*. **1027:** 83-91, (2004).
17. K.Urakami, A. Higashi, K.Umemoto, M. Gogo, Matrix media selection for the determination of residual solvents in pharmaceuticals by static headspace gas chromatography. *J. Chromatogr. A*. **1057:** 203-10, (2004).

****