Determination of Organic Volatile Impurities in Active Pharmaceutical Ingredients

S Puranik, P Sanjay Pai, G Rao

Citation

Abstract
Organic solvents such as acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene frequently used in pharmaceutical industry for the manufacturing of Active Pharmaceutical ingredients (APIs). GMP conditions commands to control adequately the quality of APIs. A selective Gas Chromatographic (GC) method has been developed and validated as per ICH guidelines for residual solvent analysis in 16 different APIs. Residual solvents in APIs were monitored using gas chromatography (GC) with Flame Ionisation detector (FID). The separation was carried out on BP 624 column (30m X 0.53mm i.d. X 0.25mm coating thickness), using GC 17 A Shimadzu, with nitrogen as carrier gas in the split mode by direct injection method. The method described is simple, sensitive, rugged, reliable and reproducible for the quantitation of acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene at residual level from intermediates and APIs.

INTRODUCTION
Organic Volatile impurities (OVIs), 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 API, excipients and drug products. ICH has given limits for the presence of OVIs in APIs. In manufacturing drug substances, organic volatile impurities (OVI’s) arising from the final purification by recrystallization, and also from one or more steps of the synthetic process, can be retained in the end products. Very often these solvents, referred to as residual solvents, are carried to the pharmaceutical preparation concerned, making their determination mandatory. During the manufacturing process, certain types of formulations like Gel extrusion module tablet (GEM) is exposed to several organic volatile chemicals. Ethanol, isopropyl alcohol and acetone are commonly used during granulation process. Methanol may be present in certain grades of ethanol. Ethanol and acetone are also used in the preparation of the polymeric coating of GEM tablets. Isopropyl alcohol may be used in the crystallization of the active ingredient while ethyl acetate is a process solvent for the gel forming polymer. Drug product even after drying process. OVIs, not only affect physiochemical properties of a drug, such as particle size, dissolution rate and stability, but also can present a serious potential health hazard. Methods for the quantification of OVI’s are reported by the United States Pharmacopoeia (USP), and European Pharmacopoeia (EP), where the wide spread but relatively expensive automated head space gas chromatography (HS-GC) instrument is used. With these methods relative standard deviations RSDs as high as 15% are allowed. However the methods mentioned in the USP and EP are dedicated to the determination of residual solvents in drug substances, excipients or drug products. The content of residual organic solvents in pharmaceuticals is routinely measured by gas chromatography (GC). Routine GC applications include the analysis of samples of active pharmaceutical ingredients and their intermediates to comply with good laboratory practices (GLP) and good manufacturing practices (GMP), as well as in- process testing for residual solvents to optimize the drying process. Over the last decade, several GC methods to monitor residual solvents in pharmaceutical samples have been reported in the literature. Several of these GC methods tend to have long run times and are very specific for a limited number of OVI’s. There are several methods reported for the determination of residual solvents. Residual solvents are detected using Hewlett Packard (Agilent, Wilmington), MODEL 6890 over column a 60m X0.53mm...
RTX-1701 column with thickness 1.0µm with FID using carrier gas system of helium. The detection limit were determined as 3 ppm 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 Agilent (Wilmington, DE, USA) GC, over 60m X 0.53mm id RTX column with carrier gas system of helium with FID. With the same system ethyl acetate has been determined in carboxer (0.155 µg/g ppm). A module drug powder soluble in water was chosen and residues of four solvents ethanol, cyclohexane triethylamine and pyridine were investigated. Ethanol was the purification/crystallization solvent, cyclohexane was used to denature ethanol, triethylamine was reactant and pyridine was the extracting solvent. 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. 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) modes 5890 series II and 6890 column ARTX 502.2, 1.4µm film of diphenyl/dimethyl polysiloxane stationary phase 30 m 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 with narrow bore column 10mX0.1mm id, with a 0.4 µm film thickness, 6% cyanopropyl, 94% polydimethyl siloxane [9]. In the reported method using rapid GC, sample A consisting of methanol (0.34%w/w), ethanol (2.64%w/w), and THF (0.21%W/W), sample B consisting of ethanol (0.04%w/w), sample C consisting of dichloromethane (0.05%w/w) and toluene (0.06%w/w), sample D consisting of toluene (0.03%w/w) and p-xylene (0.26%w/w) were determined accurately with good sensitivity. The residual solvents were determined in selected drug substances, 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 presion and accuracy data.

Costing C Camarasu developed headspace SPME method 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 (supelco 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 100m DMSO or DMF to 50 mg drug substance and slightly pressurizing the head space vial gave good results in terms of sensitivity & reproducibility. The detection limit were between 0.4 & 200 ng/ml of RSD on data were between 2 & 9 %. The headspace SPME from aqueous solution was found to be 10 times more sensitive than immersion SPME and head space SPME from organic solutions. Raquel Otero. et al. have developed HS-GC method for the quantitative determination of residual solvents in a drug substance according to the European Pharmacopoeia method. A 6890 series Hewlett pack and GC system with a FID system (Waldbronn, Germany) and a 7496 HP headspace auto sampler equipped with a 1mL sample loop were used. An OVI-G43capillary column (30m x 0.53mm id) and 3µm film thickness (Supelco) was used. 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 at ICH recommended levels. By FID except 1, 1, 1-trichloroethane found to at 10 ppm instead of 500 ppm (ICH limit). Koji Urakami reported the influence of the matrix media used for the determination of residual solvents in pharmaceuticals by static headspace gas chromatography for water soluble samples. Water is the matrix medium of...
Determination of Organic Volatile Impurities in Active Pharmaceutical Ingredients

choice, for water insoluble samples, dimethyl sulfoxide (DMSO), N-dimethyl formamide (DMF), N,N-dimethyl acetamide (DMA), benzyl alcohol (BA), and 1,3-dimethyl-2-imidazolidine (DMI) are recommended by many authors. The author has also given some information about the formation of artifacts arising from the matrix medium heating the sample solution in a HS sampling apparatus or from ultrasonication of the sample solution used to increase sample solution. A Shimadzu GC-2010 gas chromatography equipped with FID and Perkin Elmer HS-40 headspace injector was used, with column 30x 0.53 mm id, fused silica capillary coated with 3.0µm film thickness of 6% cyanopropylphenyl 94% dimethyl polysiloxane (OV1-G43, Spleco Co.Ltd.Bellefonte,PA,USA) with heium as carrier gas system. Yunfei sha reported a head space solid-phase microextraction (HS-SPME) coupled to GC-MS for the determination of volatile compounds in a famous Chinese medicinal prescription, Xiao- Cheng –Qi –Tang. Some parameters affecting the extraction efficiency such as stirring, extraction temperature, fiber exposer time and desorption time were optimized. The results were obtained using a 100µm poly dimethyl siloxane (PDMS), during headspace extraction at 100 rpm for 20 min. Twenty seven compounds were identified in Xiao Cheng-Qi-Tang including some main compounds such as d-Limonene and linalol. Analysis was carried out on HP 6890 GC system, coupled with a HP MD 5973 quadrupole mass spectrometer, using HP-5MS capillary column (30m x 0.25mm id x 0.25µm film thickness), with helium as the carrier gas system at flow rate of 1ml/min. Saeed Nojavan reported the relationship between residual solvents concentration and ampicillin trihydrate crystals stability. The amount of residual solvents determied by GC, x-ray powder diffraction (XRPD) and fourier transform Infrared spectroscopy (FT-IR) were used for characterization of solid state. The results have good relationship between concentration of methylene chloride (as a critical residual solvent) and degree of ampicillin trihydrate crystallinity with the increasing methylene chloride concentration in the sample. Analysis of the residual solvents was carried out on a Varian Modle 3600 GC, (Varian Iberica, Madrid, Spain) with FID using CP-Sil-5 capillary column (25m x 0.32mm id x 0.53µm film thickness), helium was the carrier gas system at a flow rate of 1ml/min. J. P. Guzowski has developed GC method for measuring hydroxylamine (HA) in a variety of sample matrix including pharmaceutical formulations. This article relies on converting HA into nitrous oxide (N₂O) which a single step reaction carried out directly in a heated headspace vail. The gaseous products were then analysed by headspace capillary GC, HP 5890, equipped with GC-Q megabore column (30m x0.53 mm id, P/N 1153432) with helium as carrier gas system. The electron capture detector (ECD) provided the best sensitivity (4 ppb). HA was efficiently recovered (98%) from a sample matrix that contained only the drug substance, however, a lower recovery was achieved (83%), when excipients were present. Mary-Annede Barrio reported the simultaneous determination of formic acid and formaldehyde in pharmaceutical excipients using Agilent model 6890N GC equipped with a model 5973 N quadrupole mass selective detector (MSD) and Model 7694 headspace auto sampling unit with column Phenomenex ZB-WAX column (100% polyethylene glycol), 30m long with 0.32mm id and 0.5µm film thickness, with helium as the carrier gas system. The amount of formic acid and formaldehyde present in various excipients were lactose, formicacid (1.0ppm) and formaldehyde (< 0.2ppm), powdered cellullose, formic acid (2.9ppm) and formaldehyde (0.4ppm), mannitol, formic acid (0.0ppm) and formaldehyde (0.0ppm), microcrystalline cellulose 50µm, formic acid (9.3ppm) and formaldehyde (<0.2ppm), starch 1500 formic acid (3.0ppm) and formaldehyde (<0.2ppm) and magnesium sterate, formic acid (2.1ppm) and formaldehyde (0.9ppm). In present article GC 17 A Shimadzu was used with Flame Ionization detector (FID). The separation was carried out on BP 624 column (30m X 0.53mm i.d. X 0.25µm coating thickness), with nitrogen as carrier gas in the split mode by direct injection method. The method described was simple, sensitive, rugged, reliable and reproducible for the quantitation of acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and tolouene at residual level from intermediates and APIs.

EXPERIMENTAL METHOD

INSTRUMENTS AND MATERIALS

Gas Chromatograph Shimadzu 17A version 3 was used in the development and validation of GC method. Gas chromatograph was equipped with standard oven for temperature programming, split/splitless injection ports and Flame Ionisation detector. BP 624 column (30m X 0.53mm i.d. X 0.25µm coating thickness, 4% cyanopropyl phenyl and 96% dimethyl polysiloxane stationary phase), with nitrogen as carrier gas in the split mode by direct injection method was used. Analytical grade solvents acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and tolouene and dimethyl sulphoxide (DMSO) were purchased from Thomas...
Baker, Mumbai, India. Sample APIs were obtained as gift samples from R. L. Fine Chemicals 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 and high boiling point that does not interfere with more volatile solvents analyzed by GC. Standard stock of each solvent acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene was prepared by diluting with DMSO. Working standard of each solvent ranging from concentration 100ppb to 4500ppm was prepared with DMSO in 10 mL volumetric flasks. 1µL of each working standard was injected in to gas chromatograph and standard calibration curve was obtained for each solvent, acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene.

**PREPARATION OF SAMPLE**

Accurately weighed 1g sample of each APIs was dissolved and sonicated with DMSO in separate 10 mL volumetric flask, filtered through whatman filter paper No 1 and volume made up to 10mL with DMSO, in separate 10mL volumetric flask. From each these samples 1µL sample was injected and concentration of solvents acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene in all samples were calculated by interpolating standard calibration curve.

**GAS CHROMATOGRAPHIC CONDITIONS**

The volume of 1 µL standard and sample solution was injected in GC injection port. The temperature of injection port was maintained at 230 ° C, split ratio 1:15, with nitrogen as a carrier gas. The pressure of 14 kpa with flow of 3.2 mL min⁻¹ was maintained. The temperature of the detector was set at 250 ° C. Temperature was maintained at 40 ° C for five min and then increased at a rate of 10 ° C min⁻¹ to 55 ° C min⁻¹ and maintained for 5min, finally increased at the rate of 10 ° C min⁻¹ to reach the final temperature of 200 ° C and maintained for 5 min.

**METHOD VALIDATION**

The analytical method validation was carried out as per ICH method validation guidelines [2]. The validation parameters addressed were specificity, precision, linearity, limit of detection, limit of quantitation, ruggedness and system suitability.

---

### Table 1: Report of standard solvent chromatograms

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Retention time</th>
<th>Area</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>5.02</td>
<td>93091</td>
<td>95.02</td>
</tr>
<tr>
<td>Acetone</td>
<td>4.61</td>
<td>206433</td>
<td>94.52</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>4.77</td>
<td>114320</td>
<td>95.59</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>5.44</td>
<td>77557</td>
<td>104.19</td>
</tr>
<tr>
<td>Toluene</td>
<td>15.99</td>
<td>319412</td>
<td>98.09</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>12.21</td>
<td>998520</td>
<td>94.42</td>
</tr>
</tbody>
</table>

---

**RESULTS AND DISCUSSION**

**DEVELOPMENT OF METHOD**

Gas chromatographic method for the determination of residual solvents in API was developed. The column used was BP624 capillary column, flow rate 3.2 mL min⁻¹, linear velocity 22 cm sec⁻¹ and column pressure 14kpa with total flow of 116mL min⁻¹ in the split mode. In the prescribed method all six solvents eluted within 16 min (Fig. 1). The retention time for solvents is given in Table 1. The APIs have shown presence of various residual solvents in different concentrations (Table 2).
**VALIDATION OF METHOD**

**SPECIFICITY**

The specificity of the analytical method was determined by injecting blank solution of 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**

Precision of the method and system are expressed in terms of standard deviation and relative standard deviation. For the precision of method and system the %RSD for six solvents complies with the acceptance criteria of less than 2% (Table 3.1). Hence the method and system is said to be precise.
**LINEARITY**

All six solvents showed broad range of linearity (Fig.2). The correlation coefficient calculated for all six solvents lies in the acceptance criteria of more than 0.99 (Table 3.2).

**LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)**

The LOD and LOQ were calculated by statistical methods. For the instrumental method LOD is determined as the lowest amount for detection and LOQ is the lowest amount to quantifiable by the detector. LOD and sLOQ were determined by statistical formula.

\[
\text{LOD} = 3.3\text{SD/slop} \quad \text{LOQ} = 10\text{SD/slope}, \quad \text{Where, SD is standard deviation}
\]

The values for the Limit of Detection and Limit of Quantification for acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene are mentioned in Table 3.
RUGGEDNESS
The ruggedness of method was established by performing the experiments on same chromatographic system with the same column by two analysts on a different day. The assay results of ruggedness studies were in the range of 90-105% v/v (Table 3.3). Additionally, good separation between the peaks of standard was achieved, which indicated that the method was selective for all components under the test.

CONCLUSION
This study presents a simple and validated Gas Chromatographic method for estimation of residual solvents in intermediates and APIs. The method developed is specific, accurate, precise and rugged. The amount of organic volatile impurities present in the intermediates and APIs 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, R. L. Fine Chemicals Pvt Ltd. Bangalore, India for providing the gift samples and to all my research colleagues for their support in the work.

CORRESPONDENCE TO
S. B. Puranik Department of Quality Assurance, Al Ameen College of Pharmacy, Opp. Lalbagh Main Gate, Hosur Road, Bangalore-560 027. Karnataka (India) Phone no. 011-91-9980231925 Fax no. 011-91+ 80 22225834/22278464 Email: sangpur@rediffmail.com

References
1. Dmorcillo Y, Cai Y, Bayona JM. Rapid determination of
methylene compounds in aqueous sample using solid phase microextraction and capillary gas chromatography following in –situ derivation with sodium tetraethyl borate. J. High Resol Chromatogr 1995; 18: 776
Author Information

S.B. Puranik
Al-Ameen College of Pharmacy

P.N. Sanjay Pai
Al-Ameen College of Pharmacy

G.K. Rao
Al-Ameen College of Pharmacy