RESIDUAL SOLVENT ANALYSIS IN HYDROCHLORIDE SALTS OF ACTIVE PHARMACEUTICAL INGREDIENTS

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ABSTRACT
GMP conditions commands to control adequately the quality of APIs by checking the levels of residual solvents. 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). A selective Gas Chromatographic (GC) method has been developed and validated as per ICH guidelines for residual solvent analysis in 10 different hydro chloride salts of 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 0.25µm 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 hydrochloride chloride salts of APIs.

Keywords: Hydrochloride salts, API, residual solvents, gas chromatography.

INTRODUCTION

Organic impurities (http://www.ich,org. 2002; Puranik *et al.,* 2007; Chen *et al.,* 1992) may arise during the manufacture of new substance. They may be identified or unidentified, volatile or non volatile; include starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts. The residual solvents are potentially undesirable substances, they either modify the properties of certain compounds and also hazardous to the health of the individual. OVI's also affect physico- chemical properties like crystallinity (Puranik SB *et al.,* 2008) of the bulk drug, as a difference in the crystal structure may lead to change in dissolution properties and problems with formulations of the finished product. Also residual solvents may create odour problem and colour change in the finished products. The safety of the drug is determined by its pharmacological, toxicological profile and adverse effects. The residual solvents in APIs possess toxicological effects, so ICH has prescribed acceptable limits for residual solvents in APIs (http://www.ich,org., 2002) Hence evaluation of organic volatile impurities (OVI's) is considered as an important tool in (http://www.ich,org. 1997) the quality control of pharmaceuticals. Presently in the pharmaceutical industries, special importance given for residual solvent testing. The content of residual solvents in APIs analysed by gas chromatography (Pai *et al*., 2006. Over the last decade, several GC methods to monitor residual solvents have been reported in the literature (Kevin *et al.,* 2006; Costin *et al.,* 1998; Kalchenko *et al.,* 1995; Clayton BH *et al.,* 2003). The APIs viz; Emipramine HCl, Desipramine HCl, Clomipramine HCl, Doxepine HCl, Pitofenone HCl, Pargiverine HCl, Amitriptyline HCl and Ambroxyl hydrochloride HCL stage II,III and IV have been selected for residual solvent analysis. For synthesis of these APIs various solvents such as acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene have been used. Accordingly, the method has been developed and validated for detection and quantification of residual solvents acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene in 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 ramping, 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 toluene 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

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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, tetra-hydrofuran and toluene was prepared by diluting with DMSO. Working standard of each solvent ranging from concentration 100ppb to 5600 ppm 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 mixed standard

Accurately weighed 1g sample of each APIs dissolved with DMSO in different 10 ml volumetric flask and filtered through whatman filter paper No.1. The volume was made up to 10mL with DMSO. From each sample 1µL was injected into chromatograph and chromatogram was recorded. Concentrations of solvents acetone, ethyl acetate, isopropyl alcohol, methanol, tetrahydrofuran and toluene in all samples were calculated from the peak areas obtained cin chromatogram.

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^{o}C and split ratio 1:15, with nitrogen as a carrier gas. The pressure maintained at 14 kpa with flow of 3.2 mL min^{-1}. The temperature of the detector was set at 250^{o}C. Temperature gradient maintained at 40^{o}C for five min and then increased at a rate of 10^{o}Cmin^{-1} to 55^{o}C min-^{1} and maintained for 5min, finally increased at the rate of 10^{0}Cmin^{-1} to reach the final temperature of 200^{o}C and maintained for 5 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, limit of detection, limit of quantitation, ruggedness and system suitability.

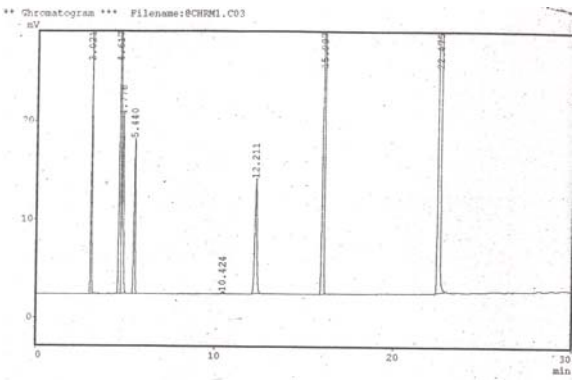
RESULTS AND DISCUSSION

Development of method

Gas chromatographic method for the determination of residual solvents in API was developed. The column used was BP624 capillary column, with flow rate 3.2mLmin^{-1}, linear velocity 22cmsec^{-1} and column pressure 14kpa with total flow of 116mLmin^{-1} in the split mode. In the prescribed method all six solvents eluted within 16 min (fig. 1), the retention time for solvents mentioned in table 1. The APIs have shown presence of various solvents in different concentrations (table 2). The chromatograms for APIs have been mentioned in fig. 2.

Table 1: Report of standard solvent chromatograms

Solvents	Retention time	Area	% Recovered
Methanol	3.02	93091	95.02
Acetone	4.61	206433	94.52
Isopropyl alcohol	4.77	114320	92.59
Ethyl acetate	5.44	77557	117.31
Toluene	15.99	319412	98.09
Tetrahydrofuran	12.21	998520	94.42



RT of the above solvents: Methanol 3.02, Acetone 4.61, IPA 4.77, EA 5.44, THF 12.21 and Toluene 15.99.

Fig. 1: Standard chromatogram of solvents

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

For the method precision six replicates of concentration of 100 ppm of mixed standard solution 1µL was injected into the chromatograph for each solvent from chromatogram peak areas standard deviation and relative standard deviation were calculated. For the precision of method and system the %RSD for six solvents complies with the acceptance criteria of less than 2% (table 3), hence the method and system is said to be précised.

Linearity

All six solvents showed broad range of linearity (fig. 3). The correlation coefficient calculated for all six solvents lies in the acceptance criteria of more than 0.99 (table 3).

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

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method LOD is determined as the lowest amount to detect and LOQ is the

Table 2: Determination of residual solvents in APIs

Name of the API	Peaks detected (RT)	Methanol	Acetone	Isopropyl alcohol	Ethyl acetate	Toluene	Tetrahydro-furan
Imipramine HCL	4.80, 4.98	---	2434 1.36	3247 1.86	---	---	---
Desipramine HCL	No peaks detected	---	---	---	---	---	---
Colmipramine HCl	4.905	---	---	2468 1.37	---	---	---
Doxepin HCL	5.411, 15.637	---	---	---	66384 100	2446 1.36	---
Pitofenone HCL	No peaks detected	---	---	---	---	---	---
Pargiverine HCL	5.254, 15.475	---	---	---	34100 50	26244 29	---
Amitriptyline HCL	5.14	---	---	---	2170 7.05	---	---
VB- HCL II	3.15	1110 0.61	---	---	---	---	---
VB- HCL III	3.25	3371 1.86	---	---	---	---	---
VB- HCL -IV	5.14	---	---	---	2170 7.05	---	---

Table 3: Data of linearity Precision LOD and LOQ

Name of solvents	Linearity			Precision				LOD (ppm)		LOQ (ppm)		Ruggedness	
				System		Method						Intra-day	Inter-day
	Range (ppm)	Slope	R^2	SD	% RSD	SD	% RSD	Instru-mental	Stat.	Instru-mental	Stat.		
Methanol	10-3200	972.42	0.9997	1253.31	1.35	1.27	1.35	70	42.5	100	128	94.65	93.67
Acetone	10-5600	2108.8	0.9952	4543.16	2.25	2.08	2.25	100	71.0	300	215	92.12	89.57
Isopropyl alcohol	10-5600	1198.8	0.9971	9833.57	8.94	7.96	8.94	200	270	300	820	89.03	92.73
Ethyl acetate	100-5600	670.35	0.9963	1050.26	0.61	1.58	1.37	70	51.7	100	156	115.7	105.6
Toluene	10-1600	3228.1	0.9995	7459.72	2.39	2.29	2.39	80	76.2	150	231	95.66	95.87
Tetrahy-drofuran	10-5600	10170	0.9952	11547.01	10.84	1.09	10.8	60	37.4	100	113	100.7	95.45

lowest amount to quantify by the detector. For statistical method LOD and LOQ determined by statistical formula.

LOD= 3.3 SD/ Slope LOQ= 10 SD/Slope
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.

Table 4: Data of system suitability for six solvents

Solvents	N/m	N/column	HETP
Methanol	8864	265920	0.0033845
Acetone	5323.94	159718.2	0.0056349
Iso propyl alcohol	6033.06	180991.8	0.0049726
Ethyl acetate	7179.84	215395.2	0.0041784
Toluene	3545.6	106368	0.0008461
Tetra hydrofuran	2396.84	71905.2	0.0125165

Ruggedness

The ruggedness of method was established by performing same chromatographic system and the same column by two analysts on a different day. The standard mixture of solvents of concentration 100ppm was used for ruggedness study. The assay results of ruggedness studies were in the range of 90-105% v/v (table 3). Additionally, good separation between the peaks of standard was achieved, which indicated that the method was selective for all components under the test.

System suitability

A system suitability parameters number of theoretical plates, HETP, asymmetry and resolution was calculated to evaluate the chromatographic parameters. For all six solvents. The system suitability parameters found in the acceptable range, which indicates the efficiency of the method (table 4).

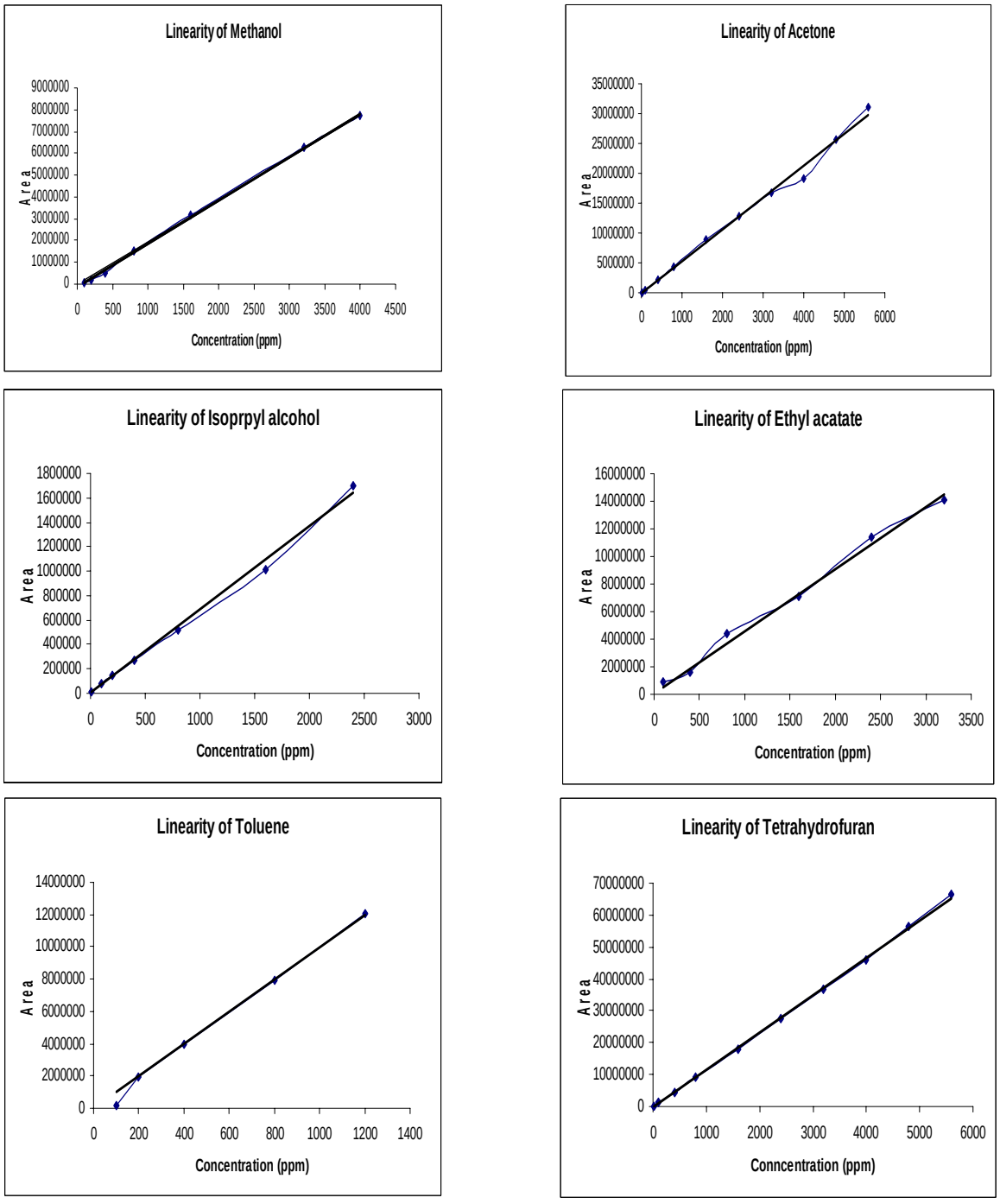


Fig. 2: Standard calibration curves for six solvents

CONCLUSION

This study presents a simple and validated Gas Chromatographic method for estimation of residual solvents in intermediates and APIs. The developed method is specific, accurate, precise and rugged. Acetone ane IPA was found in impramine HCl, in colmipramine HCl only IPA was detected, ethyl acetate and toluene were detected in Doxepin HCl and Pargiverine HCl, inamitriptyline HCl and VB HCl–IV ethyl acetate was detected, in VB-HCL II and III methanol was detected. No solvents were identified in Desipramine HCl and Pitofenone HCl. The amounts of organic volatile impurities present in the intermediates and APIs were found to be within the ICH limits.

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