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RESEARCH ARTICLE

Strategies for Head Space Gas Chromatographic Analysis of Residual Solvents

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ABSTRACT

Estimation of residual solvents for any drug substances before its marketing is a must requirement imposed by most of the regulatory bodies across the globe. Choice of analytical instrument (SHS-GC) remains same. A generic method was developed for the quantification of 38 residual solvents by SHS-GC, the response factors for the solvents were determined. Effect of chromatographic condition injection port temperature, carrier gas flow, detector temperature, incubation temperature of head space, incubation time and effect of matrix on the recoveries of the solvents was studied. Undertaken studies will be useful the researchers for the development of method for the estimation of residual solvents.

KEYWORDS

Residual solvents, HS-GC, ICH, Pharmacopoeia

INTRODUCTION

Residual solvents (RS) are volatile organic chemicals which are used in various stages of drug product or excipient manufacturing. It is very difficult to remove them completely from drug substance or drug product. As RS has no therapeutic value and many of them have toxic effect on human health, their level in drug product should be controlled to a safe level. Guidline Q3C of International conference on harmonization(ICH)[8] of technical Requirement for Registration of Pharmaceutical for Human Use classify RS on the basis of their toxicity as class-1: solvents to be avoided, class-2: solvents to be limited, class-3: solvents with low toxicity and should not be present more than 0.5%. Variours methods have been used[2,12,17,7,11,5,14,18] and reviewed[22,2] for the determination of RS, Static headspace gas chromatography (SHS-GC) with Flame Ionization Detector(FID) is most commonly used method[1,14,13,3,6,15,9] because of selectivity and reproducibility of results and most of pharmacopoeia endorse it[21]. From analytical view point using SHS-GC for analysis RS can be classified into two categories i.e. Category-1 low boiling solvents (solvents which can be analyzed with ease with SHS-GC), Category-2 High boiling solvents (solvents not suitable SHS-GC due to lower vapor pressure)[4]. Some of method describes[5] very detailed studies while some methods are for fast analysis, other address same class of solvents(polarity or boiling point)[10,16] biggest issue for the pharmaceutical manufacturer is selection of matrix[19,20,13] media for the sample preparation. Performance of the analytical method changes dramatically with change in matrix media used. With every new product chromatographic parameters needs to be evaluated. Presented work aims to identify crucial condition in quantification of residual solvents which can provide starting point in method development for new pharmaceutical drug and drug products. So study was conducted on pure solvents as aim was not to develop method specific to substance or matrix media for head space auto sampling.

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MATERIALS AND METHODS

Chemicals

Pentane, Diethylether, Cyclohexane, Benzene, 1,4-Dioxane, Xylene, Pyridine, Chlorobenzene, Tetraline, Hexane, n-Heptane, Methylcyclohexane, 1,2-Dimethodxyethane, Ethylacetate, Isopropyl alcohol, Acetonitrile, Toluene, Methylbutylketone, Acetone, Tetrahydrofuran, Methanol, Carbontetrachloride, Dichloromethane, Chloroform, 1,2-Dichloroethane, Ethanol, 1-Butanol, Nitromethane, Dimethylformamide, Acetic acid, N,N-dimethylacetamide, Dimethylsulfoxide, N-methylpyrrolidone, Formamide were procured form spectrochem pvt. ltd., (Mumbai, India).

Solution Preparation

Initially for the GC and HS parameters optimization of 34 solvents were classified into 4 categories as follows

Mixture-1 comprise of nine non-polar solvents namely Pentane, Diethylether, Cyclohexane, Benzene, 1,4-Dioxane, Xylene, Pyridine, Chlorobenzene, Tetraline.

Mixture-2 includes a blend of nine non -polar and mid polar solvents Hexane, n-Heptane, Methylcyclohexane, 1,2-Dimethodxyethane, Ethylacetate, Isopropyl alcohol, Acetonitrile, Toluene, Methylbutylketone.

Mixture-3 consist of seven solvents majority of which were chlorinated Acetone, Tetrahydrofuran, Methanol, Carbontetrachloride, Dichloromethane, Chloroform, 1, 2-Dichloroethane.

Mixture-4 consist of nine solvents majority of which were high boiling polar solvents Ethanol, 1-Butanol, Nitromethane, Dimethylformamide, Acetic acid, N,N-dimethylacetamide, Dimethylsulfoxide, N-methylpyrrolidone, Formamide.

The solvents were categories to make solution preparation facile, each mixture represents group of solvents with similar properties. Later on four solvents 1-Pentanol, 2-Ethoxyethanol, Ethylene glycol, Sulfolane were added to studies.

Mixture-1 was prepared by mixing 1 ml of Diethylether, Cyclohexane, Benzene, 1 ,4-Dioxane, Xylene, Pyridine, Chlorobenzene, Tetraline and 2 ml of Pentane to get 100 ppm solution of Diethylether, Cyclohexane, Benzene, 1,4-Dioxane, Xylene, Pyridine, Chlorobenzene, Tetraline each and 200 ppm solution of Pentane. Mixture-2 was prepared by mixing 1 ml of n-Heptane, Methylcyclohexane, 1,2-Dimethodxyethane, Ethylacetate, Isopropyl alcohol, Acetonitrile, Toluene, Methylbutylketone in 2 ml of Hexane to get 100 ppm solution of n-Heptane, Methylcyclohexane, 1,2Dimethodxyethane, Ethylacetate, Isopropyl alcohol, Acetonitrile, Toluene, Methyl butylketone and 200 ppm solution of Hexane. Mixture-3 was prepared by mixing 1 ml of Acetone, Tetrahydrofuran, Carbontetrachloride, Dichloromethane, Chloroform, 1,2-Dichloroethane in 3 ml of Methanol to get 100 ppm solution of Acetone, Tetrahydrofuran, Carbontetrachloride, Dichloromethane, Chloroform, 1,2-Dichloroethane each and 300 ppm solution of Methanol. Mixture-4 was prepared by mixing 1 ml of 1-Butanol, Nitromethane, Dimethylformamide, Acetic acid, N,N-dimethylacetamide, Dimethylsulfoxide, N-methylpyrrolidone, Formamide in 2 ml of Ethanol to get 100 ppm solution of 1-Butanol, Nitromethane, Dimethylformamide, Acetic acid, N,N-dimethylacetamide, Dimethylsulfoxide, N-methylpyrrolidone, Formamide each and 200 ppm solution of Ethanol.

Gases Heated Zones

Table 1: Instrument parameters for GC separation

Injection Mode	Split	Column Oven Temperature:	
Split Ratio	40:1	Initial	35 ˚C
Carrier Gas	N_2	Hold	4 min
Control	Pressure	Ramp1	10 ˚C min^{-1} to 190 ˚C
Flow	15 psi	Hold	1 min
Detector	Air +	Ramp2	10 ˚C

Gas	Hydrogen		min^{-1} to 220 ˚C
Ratio	450:45	Hold	1 min
Sampling Rate	12.5	Total Run Time Injector Temp	30.50 min
		Initial	100 ˚C Hold 5 min
		Ramp	10 ˚C min^{-1} to 210 ˚C
		Detector Temp	300 ˚C

Chromatographic Condition

All the chromatographic separation were achieved on Perkin Elmer Clarus 500 GC equipped with Turbomatrix 40 head space auto sampler, controlled by TotalChrome 6.3.1 and Turbomatrix software. Chromatographic data processing were also done using TotalChrome software. Two columns Rxi-1 (100% Dimethylpolysiloxane) 30 m * 0.25 mm * 0.25 µm and Rxi-Stabilwax (Polyethylene glycol) 30 m * 0.25 mm * 0.25 µm in parallel connection were used for the separation. Order of column connection was as followed sequence injection port Rxi-1 glass connector Rxi- Stabilwax detector. Parameters for GC separation are shown in Table 1. Head-Space operating condition is shown in Table 2 and Table 3.

Table 2: HS-Operating condition using PEG as matrix media

Vial oven	170 ˚C	Thermostate	30 min
Needle	175 ˚C	Injection	0.04
transfer line	180 ˚C	Pressurise	1 min
		Withdrawal	0.3 min
Injection mode	Time	Injection Pressure	15 psi

Table 3: HS-Operating condition using Paraffin as matrix media

Vial oven	150 ˚C	Thermostate	30 min
Needle	155 ˚C	Injection	0.04
transfer line	160 ˚C	Pressurise	1 min
		Withdrawal	0.3 min
Injection mode	Time	Injection Pressure	15 psi

Result and Discussion

Determination of Response Factor

Initially purity of the solvents was determined individually and purity obtained was considered as 100 %, instrument response factor was determined by replicated of two at three concentration level and instrument response factor was used in the recovery calculation in subsequent studies. Slope and intercept value along with retention time for 38 residual solvents are given in the Table 4.

Optimization of Gas Chromatographic condition

Column Selection

US pharmacopoeia stationary phase (Polyethylene glycol) and G46 (6 %Cyanopropylphenyl 94 % dimethylpolysiloxane) are very well studied for the RS estimation, to explore other possibilities a mixed stationary phase consist of completely non polar coating (Dimethylpolysiloxane) and highly polar coating (Polyethylene glycol) were used in the study. In the situation were estimation of residual solvents consist of mixture of polar and non-polar solvents like solvents form mixture-1.

Injection Port Temperature studies

Initial study showed that keeping injection port temperature between 100 ˚C to 150 ˚C gives good peak shapes and separation for mixture-1 and mixture-2 tailing was observed in mixture-3 and very broad peak were found in mixture-4,

Table 4: Retention time of solvents along with response factor or mixture-2 and mixture-4 mixed stationary phase can be harnessed for the better separation

Entry	Solvent	R.T.	Slop	Intercept	R_2
1	Pentane	6.15	92.34	-1000	0.997
2	Diethylether	6.34	55.345	-1827	0.987
3	Hexane	6.93	100.238	300	0.987
4	Cyclohexane	7.99	104.23	500.26	0.987
5	Acetone	8.04	4.567	-45.888	0.987
6	n Heptane	8.12	110.324	-2773.56	0.987
7	Methylcyclohexane	8.99	67.623	1222	0.987
8	1,2-dimethoxyethane	9.06	33.897	-2280	0.987
9	Tetrahydrofuran	9.24	32	1578	0.987
10	Ethylacetate	9.62	12.334	228.456	0.987
11	Methanol	9.66	0.488	2.553	0.987
12	Carbontetrachloride	9.97	4.02	-5.345	0.937
13	Dichloromethane	10.12	9.12	66.323	0.897
14	Isopropyl alcohol	10.43	4.668	99.673	0.987
15	Ethanol	10.49	0.678	12.55	0.987
16	Benzene	11.18	103.45	2248	0.987
17	Acetonitrile	12.11	23.089	-10.24	0.987
18	Chloroform	12.49	6.783	35.345	0.965
19	Methylisobutylketone	12.97	45.2	-223	0.987
20	Toluene	13.38	91.348	1500	0.987
21	1,2-Dichloroethane	13.66	15.45	-6.456	0.95
22	1,4-Dioxane	13.78	0.648	4.234	0.987
23	Methylbutylketone	14.77	12	1436	0.987
24	3-Xylene	15.75	3.4	-226	0.987
25	1-Butanol	15.99	2.987	38.334	0.987
26	Pyridine	16.38	17.68	12.56	0.987
27	Nitromethane	16.85	5	-667	0.965
28	Chorobenzene	17.13	2.68	897	0.987
29	1-Pentanol	17.4	45	-368	0.987
30	2-Ethoxyethanol	17.43	39	1786	0.987
31	Dimethylformamide	19.19	0.6	-2330	0.987
32	Acetic acid	20.47	1.45	1248.6	0.977
33	N,N-diethylacetamide	20.56	2.1	-206	0.965
34	Tetraline	23.06	78.6	1278	0.987
35	Dimethylsulfoxide	23.3	2.89	-557	0.987
36	Ethylene glycol	23.92	6.7	-987	0.988
37	N-Methylpyrrolidone	25.25	18.62	-1845	0.986
38	Formamide	26.74	0.467	2019	0.982

whereas temperature between 200 ˚C to 210 ˚C found better for mixture-3 and mixture-4. However higher injection port temperature when used alongside with head space auto sampler tends to distort peak shape and less resolution was obtained between mixture-1 and mixture-2 solvents. As depicted in Figure 1 injection port temp. 120 ˚C there is much spread in recovery of mixture-4 solvents whereas mixture-3 solvents show good to excellent recovery, whereas mixture-1 and mixture-2 solvents shows intermediate recovery, suggesting if solvents from mixture-3 are to be analysed, lower injection port temperature is advisable and for mixture-4 type solvents lower injection port temperature leads less recoveries. Injection port temperature between 150 ˚C to 180 ˚C is suitable for the quantification of solvents from mixture-1, mixture-2, mixture-3, as recovery between the temperature range is good and spread of recovery among solvents of same mixture around central recovery value is also less (see Figure 1 Injection port Temp. 150 ˚C and 180 ˚C). In situation where sample consist solvents from all categories higher injection port temperature 200 ˚C to 220˚C (see Figure 1 Injection Port Temp. 210 ˚C) recovery is good but a programmable temperature vaporizer (PTV) is advisable for resolution along with good recovery.

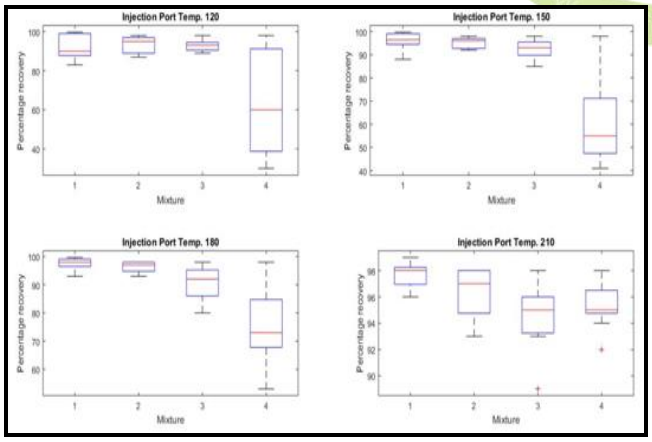

Figure 1: Boxplot showing recoveries of mixture-1, mixture-2, mixture-3, mixture-4 solvents at injection temperature

Carrier Gas effect

No significant effect of carrier gas velocity/pressure was observed on the recoveries of all the mixture however reproducibility changes dramatically if carrier gas pressure is kept below 7 psi or above 15 psi.

Detector Temperature Effect

For the reproducibility detector temperature was varied form 250 ˚C to 300 ˚C very minute difference was observed with varying temperature so detector temperature was kept at 300 ˚C, so as to prevent condensation of solvent in the detector to check non-reproducibility.

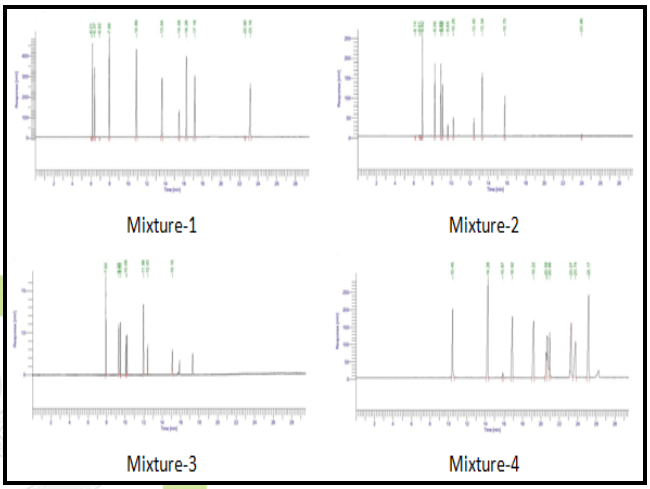

Figure 2: Chromatogram showing retention of mixture-1, mixture-2, mixture-3 and mixture-4 after optimized GC condition

Head Space Parameters Studies

Head space parameter rises are very crucial in residual solvents analysis. Initially thermostat temperature (incubation temperature) was studied, thermostat temperature was varied from 80 ˚C to 95 ˚C keeping incubation time 30 min for the separation of pure mixtures (without any matrix media) using chromatographic condition showed in Table 1. Mixture-1 and Mixture-2 showed good peak shape and separation at lower temperature but recovery of mixture-4 tends to decrease at lower incubation temperature. Higher incubation temperature showed good reproducibility for all the pure mixtures. Higher incubation temperature tends to reduce separation of mixture-1 and mixture-2 solvents. Reduced separation was caused by very high moving solvents moving from head space auto sampler into the column head. To achieve separation of mixture-1 and mixture-2 solvents molecules

kinetic energy(temperature) need to reduced so all the solvent molecules were focused on column head by reducing temperature of injection port at 100 ˚C and holding for 5 min and then increasing at 10 ˚Cmin^{-1} to 210 ˚C.

Sample Matrix

Sample matrix (solvent) used in quantification of residual solvent is one of the important factor as it control % recovery of the residual solvents from the drug products and drug substances. The most common samples matrix used are water (for water soluble substances), DMSO and DMF (for water insoluble samples). Recovery of the high boiling solvents (mixture-4) using water as matrix media was found very low, although intermediate to good recoveries were obtained using paraffin as matrix media for mixture-4 solvents but recoveries of mixture-1 declined with the use of the paraffin as matrix media, besides for practical use paraffin is not much use worthy as solvation properties of the paraffin is very poor. Other matrix medial used in the study are Polyethyleneglycol-400, found suitable for the estimation of mixture-4 and good to excellent recoveries were(see Figure 3) found for the mixture-1, mixture-2 and mixture-3. The major constrain using PEG as matrix media for residual solvents estimation is incubation temperature need to be as high as 150 ˚C for the incubation time of 40 min was needed.

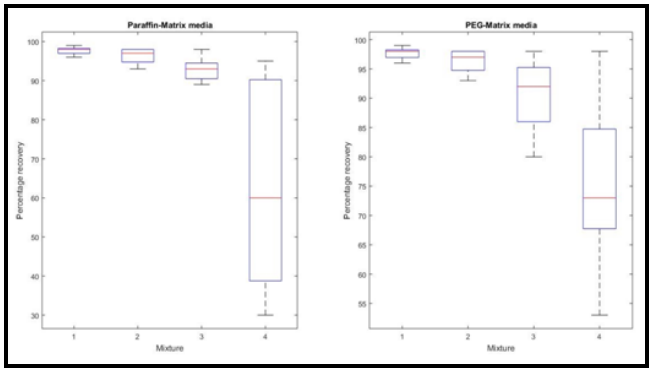

Figure 3: Boxplot showing recovery of mixture-1, mixture-2, mixture-3, mixture-4 effect of matrix media

CONCLUSION

Gas chromatographic and head space condition were optimised which enables simultaneous quantification of high boiling polar solvents along with low boiling non-polar solvents using either paraffin or PEG as matrix media. Although it is possible to quantify high boiling polar solvents along with low boiling non-polar solvents using paraffin or PEG as matrix media, optimized condition cannot be applied universally but it can reduce method development time and cost for the analysis of new drug product or drug substances as study provides insight of effect of various chromatographic condition on the separation and recoveries of the residual solvents.

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