Development and Validation of Static HS-GC Method for the Determination and Quantification of Residual Solvents of Bromhexine Hydrochloride (BHX)

PRAFULL MATHUR*, SUVIGYA MATHUR, ANAND KUMAR MISHRA and TANAJI KUNJIR

Analytical Development Laboratory, Research and Development Centre, Dr. Datsons Labs Ltd,K- 4/1, Additional MIDC, Mahad-402309, India

E-mail: prafull_mathur@yahoo.co.in

Abstract   The determination of residual solvents in drug substances is the mandatory requirement by various health authorities in the world. There are no analytical methods available in the literature that can simultaneously separate and quantitate residual solvents in bromohexine hydrochloride (BHX) active pharmaceutical ingredient (API). This paper describes the developmentand validation of a simple, efficient, accurate and robust static headspace gas chromatography method forthe determination of residual solvents, namely methanol, ethanol, ethyl acetate, monochlorobenzene and benzene, in BHXAPI.This new method has been demonstrated to beaccurate, linear, precise, reproducible, specific and robust for its intended purpose.The method give very good sensitivities viz. detection limits for benzene is 0.4 ppm, ethyl acetate 2 ppm and for others solvents 5 ppm and precision (below 9.0 % RSD) for all solvents. The results of this evaluation stronglyindicate that this method can be readily used to determine residual solvents in BHX.

Keywords: Residual solvents, Static Headspace Gas chromatography, Method Validation, Bromhexine hydrochloride, Active pharmaceutical ingredient (API)

1. INTRODUCTION

The residual solvents (RS) are potentially undesirable substances present in APIs. They either modify the properties of certain compounds and also hazardous to the health of the individual(Puranik *et al.* 2008;Guyot-Hermann, 1991;Devotta *et al.* 1995;Witschi & Doelker, 1997) mentioned thatthe presence of RS in an API affects the physiochemical properties (i.e., physicalforms) and or physical appearance and other characteristics(e.g., color, odor, etc.) of the bulk API. As such, appropriateattempts are always

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taken in the manufacturing of APIs (such asdrying) to eliminate and or minimize the presence of RS in the bulklots of APIs. However, depending on the characteristics of the API,RS, and drying conditions/parameters of the API, various levels ofRS can be retained in the final bulk lots of APIs. Hence evaluation of RS in drug substances, excipients or drug products is one of the most difficult and demanding analytical task in pharmaceutical industry.

RS are divided into four different classes according to the guidelines of International Conference on Harmonization(ICH, 1997 &2002), frommost toxic solvents to solvents with insignificant toxicologicaleffect on human health. Over, the last decade, gas chromatography have became the most practical and popular techniques to analyze the content of residual solvents in APIs (Pandey *et al.*2011).The sampling techniques usingstatic headspace gas chromatography (HSGC) gained preferenceand popularity over the direct injection GC because of various complicationsand disadvantages caused by the direct injection of theAPI into the GC system (B'Hymer, 2003). HSGC methods minimize any potentialinterference caused by non-volatile substances (or by the degradation/decomposition products of the non-volatile components) as aresult of direct injection into the GC system. Further, the directinjection method requires relatively high sample concentration,and this often leads to poor chromatography (for capillary columns)and limited injections of samples per sequence of sample analysis.Consequently, HSGC with FID detection has been widely used by different workers (Kolb& Ettre,1991;Mulligan&McCauley, 1995;Camarasu*et al.* 1998;QinL *et al.* 2004;Otero*et al.* 2004;Yarramraju*et al.* 2007;Laus*et al.*2009) forthe analysis of organic volatile ingredients present in API and drug products.

Bromhexine Hydrochloride (BHX) is a mucolytic agent used in the treatment of respiratory tract infections like acute tracheobronchitis, chronic bronchitis, bronchial asthma, and chronic sinusitis etc. It is secretolytic i.e. it increases the production of serous mucus in the respiratory tract and makes the phlegm thinner and less sticky. This contributes to a secretomotoric effect and helps the cilia (tiny hairs that line the respiratory tract) to transport the phlegm out of the lungs.The general procedure of Ph. Eur. and USP for RS determinationin pharmaceutical products includes analysis of many solvents andhence a longer GC cycle time. However, in the manufacturing of BHX only a handful of the solvents were used so our objective was to develop a simple, robust and efficientHSGC method that can accurately quantitate all the five RS present in commercial lots of BHX API.The present work describes the developmentand validation of an efficient, accurate, sensitive and rugged HSGC method for quantitationof RS present in commercial bulk BHX API.

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2. EXPERIMENTAL

2.1 Materials

The active substance – Bromhexine hydrochloride was synthesized in the R&D lab of Aanjaneya Lifecare. Ltd. Mahad, Maharashtra India. Analytical grade solvents viz., methanol, ethanol, ethyl acetate, benzene and monochlorobenzene were procured from Merck (Mumbai, India) and dimethylsulfoxide (DMSO) from Rankem (Delhi, India).

2.2 Instrumentation

Method development and validation was performed using a Shimadzu GC-2014 equipped with standard oven for temperature ramping, split/splitless injection ports and flame ionization detector (FID), a data system capable of performing data collection and integration and headspace auto sampler of Teledyne Tekmar capable of housing 10 ml GC headspace vials (versa).

2.3 Chromatographic conditions

Separation was performed on Rtx-1301, 30.0 m X 3.0 mm ID, 3.0 μm film thickness cross bonded (6% cyanopropyl phenyl and 94% dimethyl polysiloxane) column manufactured by Restek Bellefone, USA. The GC parameter, headspace parameter and temperature programming of the method are given in Table-1.

2.4 Preparation of standard stock solution

Standard stock solution of all five solvents viz., methanol, ethanol, ethyl acetate, benzene and monochlorobenzene was prepared in dimethylsulfoxide (DMSO) and was 200 ppm for benzene and 5000 ppm for the rest solvents.

2.5 Mixed standard preparation

Transferred 1.0 ml of each standard stock solution in to a 100.0 ml volumetric flask and diluted to volume with diluent. The prepared solution was equivalent to 2 ppm for benzene and 50 ppm for the rest solvents.

2.6 Sample Preparation

Weighed accurately about 1.0 gm of bromhexine hydrochloride and transferred into a vial, added 5 ml of diluent mixed well and sealed the vial with septum and metallic cap. Bromhexine hydrochloride dissolves completely at the sample oven temperature of 80 °C.

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Table 1. GC parameters, headspace parameters and temperature programming for GC column.

GC Parameters	
GC Column	RTX-1301, 30.0 m X 0.53 mm ID, 3.0 µm film thickness
Carrier gas	Nitrogen 3.0 ml/min. (constant flow)
Inlet Temperature	160 °C
Detector	Flame Ionization Detector (FID), 260 °C
Hydrogen	40 ml/min
Air 300 ml/min	
Make-up gas	40 ml/min
Inlet split ratio	20:1

Headspace Parameters	
GC Cycle time	38.00 min
Valve Oven temp.	80.0 °C
Transfer Line Temp.	85 °C
Platen/sample Temp.	80.0 °C
Platen Temp. Equil. Time	0.5 min
Sample Equil. Time	30.00 min
Mixer	On
Mixing Level	Medium
Mixing Time	1.00 min
Mixer Stabilize Time	0.20 min
Pressurize	15.0 psig
Pressurize Time	2.00 min
Pressurize Equil. Time	0.25 min
Loop Fill Pressure	5.0 psig
Loop Fill Time	2.00 min
Inject Time	1.00 min
Sample Loop Size	1.0 ml

Temperature programming for the GC column			
Temperature(°C)	Hold(min)	Ramp(°C/min)	
Initial Temperature	40.0 °C	7.0	15.0
Temperature I	230.0 °C	5.0	--

2.7 Validation Procedure

The validation parameters addressed were specificity, precision, linearity, and detectionlimit, quantitationlimit, robustness and system suitability. After equilibrating the column, system blank and blank solution (diluent) were run and correction made accordingly. The vials of standard and test solution were loaded into the headspace oven and heated for 10 minutes to ensure liquid-gas equilibrium of the residual solvents. The resulting samples were injected into the GC system via a 1-ml sample loop. The linearity study for all the five solvents was carried out both in the presence and absence of bromhexine hydrochloride. The linearity study in the absence of bromhexine hydrochloride was carried out from 1.6 to 2.4 ppm for benzene and from 40-60 ppm for the remaining residual solvents. The linearity/accuracy/precision study in the presence of bromhexine hydrochloride (spiked API samples) was carried out from1.6 to 2.4 ppm for benzene and from 40-60 ppm for the remaining residual solvents. RS spiked API samples were prepared by pipetting 1.0 ml of appropriate linearity standard solutions into 10.0 ml headspace vials containing 1.0 gm of bromhexine hydrochloride and 4.0 ml of DMSO. The detection limit (DL) was set at 0.2μg/ml (equivalent to 0.4 ppm) for benzene, 1 μg/ml (equivalent to 2 ppm) for ethyl acetate and 2.5μg/ml (equivalent to 5 ppm) for methanol, ethanol and monochlorobenzene. The quantitation limit (QL) was set at 75 μg/ml (equivalent to 150 ppm) for all RS. Robustness of the method was studied by deliberately varying GC parameters such as flow rate, inlet temperature and injector temperature. The method robustness was assessed by evaluating the system suitability criteria such as sample to noise (S/N) ratio of QL , resolution factor, tailing factor and % RSD in assay values compared to the procedural method (as-is) for each one of the RS.

2.8 Calculation

The statistical evaluation of the linearity experiments (correlation coefficient, y-intercept, slope of regression line and % of Y – intercept) are carried out by the below formula (Eq.-1,2,3&4) and represented in Table-2.

$$r = \frac{n(\Sigma_{xy}) - (\Sigma_x)(\Sigma_y)}{\sqrt{[n(\Sigma_{x^2}) - (\Sigma_x)^2 \left[n(\Sigma_{y^2}) - (\Sigma_y)^2 \right]}} \tag{1}$$

Where r is correlation coefficient, x is first score, y is second score, Σ_{xy} is sum of product of first and second score, Σ_x is sum of first score, Σ_y is sum of second score, Σ_{x^2} is sum of squire of first score, Σ_{y^2} is sum of squire of second

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score, is squire of sum of first score, is squire of sum of second score and n is the number of values or elements.

Y – Intercept has been calculated by –

$$a = \overline{y} - b\overline{x} \ or \ \overline{y} = b\overline{x} + a \tag{2}$$

Where is the intercept of the regression line, $\overline{y}$ is mean of the values at y, $\overline{x}$ is mean of the values at x and is slope and is calculated from:

$$b = \frac{\Sigma(x - \overline{x})(y - \overline{y})}{\Sigma(x - \overline{x})^2} \tag{3}$$

and % of y – intercept is calculated by

$$\frac{Y - intercept \ X100}{Mean \ Area \ of \ 100\%} \tag{4}$$

Standard deviation and relative standard deviation were calculated by the formula given below (Eq-5&6).

$$SD = \sqrt{S(x - \overline{x})/n - 1} \tag{5}$$

Where SD is standard deviation, x is each value in the sample, $\overline{x}$ is the mean of the values and n is the number of the values.

$$RSD = \frac{Average \ X \ 100}{SD} \tag{6}$$

Where SD is standard deviation RSD is relative standard deviation.

3. RESULTS AND DISCUSSION

3.1 Analytical method development

Critical elements of a new HSGC method development are: (i) identifying an appropriate diluent (ii) determining suitable headspace parameters (i.e., headspace temperature, vial equilibration time, vial pressurization),GC parameters (i.e., inlet split ratio, inlet temperature) andGC temperature programming to improve the sensitivity of themethod; (iii) determining the detection limit (DL) and quantitation limit (QL) levelsbased on the sensitivity of the method.

3.1.1 Selection of solvent for sample preparation

Many organic solvents were investigated, namely, N,N-Dimethylformamide, 1, 4-dioxane and dimethyl sulfoxide to identify the most suitable solvent (diluent)

for the intended purpose of this method. The initial GC oven temperature program used was 40 °C for 7 min, at 15°C/min ramp, then to 230 °C and hold for 5min. The headspace temperatures were set as 80 °C for oven, 80 °C for sample loop, and 85 °C for transfer line to aid the evaporation of the RS and to increase the sensitivity. Both N,N-Dimethylformamideand 1, 4-dioxane diluent peaks co-eluted with benzene and as such were unsuitable for this method. On the other hand, DMSO showed much cleaner chromatograms withinsignificant or no interfering peaks in the retention time window of the five RS in BHX. Hence DMSO was selected as the diluents for this method and all further studies were carried out with DMSO.

3.1.2 Selection of headspace oven temperature and other GC parameters

The headspace sample oven temperature has a profound effecton the sensitivity of the method because temperature has a directimpact on the equilibrium concentration of the RS in the headspaceof the sample vial (Witschi & Doelker, 1997). For these experiments, the transfer line was kept 5–10°C higher than the sample loop temperature (Yarramraju et al. 2007). Several sample oven temperatures were evaluated from 80 to 120 °C with the loop and transfer line temperatures changed accordingly but maintained the GC inlet temperature at160°C and the split ratio at 20:1. Under these conditions, all theRS were detected with an S/N much greater than 10.

3.1.3 Evaluation of Different GC Parameters

During the preliminary evaluations the GC parameters were set as: GC oven temperature was kept at 60°C, flow rate was adjusted to 5 ml/min and hold time was 4 min. The runs for this GC program exhibited overlapping for methanol and ethanol peaks. Hence a reliable and accurate change in GC parameters was extremely required for a good peak separation. To overcome this challenge the GC program was varied as oven temperature was set to 40°C, flow rate was decreased to 3ml/min and hold time was increased to 7 min. Under these conditions a good peak separation for RS methanol and ethanol was observed. Final GC and,headspace parameters for the presented HSGC method in this work are listedin Table 1.

3.2 Analytical method validation

Analytical method validation was carried out with respect to parameter such as specificity, accuracy, precision, linearity, DL, QL,robustness and system suitability.

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3.2.1 Linearity

The slope, y-intercept and coefficient of correlation were obtained from linear regression analysis. The peak area of each individual RS was plotted against corresponding theoretical concentrations obtained from each linearity solution. The results of statistical evaluation of the linearity experiments are calculated by the formula (Eq.1, 2, 3&4) and represented in Table-2. The obtained correlation coefficients of linear regression for all solvents were above 0.99. These indicate a linear relationship between the concentrations of analytes and the response of detector.

Table 2. Results of statistical evaluation of the linearity experiments

Sr. No.	Name of RS	Slope	Y-intercept	Correlation coefficient	% of Y-intercept
1.	Methanol	0.05	21.73	0.995	1.008
2.	Ethanol	0.04	24.82	0.996	1.010
3.	Ethyl acetate	0.01	98.21	0.999	1.000
4.	Benzene	0.10	10.38	0.996	1.000
5.	MCB	0.03	33.77	0.993	1.010

3.2.2 Method precision and ruggedness

The precision of the method was established as repeatability, system and intermediate precision. For the method precision six replicates of concentration of 50 ppm for methanol, ethanol, ethyl acetate, MCB and 2 ppm for benzene of mixed standard solution was analyzed. For each solvent, from chromatogram peak areas, standard deviation and relative standard deviation for system and intermediate precision were calculated by the formula (Eq- 5&6) and given in Table-3. For the precision of method and system the % RSD for five solvents complies with the acceptance criteria of not more than 15%, hence the method and system is said to be précised and has reproducibility.

3.2.3 Detection limit (DL) and quantitation limit (QL)

The sensitivity of the method was demonstrated by detection limit (DL). The DL calculated as the concentration, which generated a peak about 3 times as high as the height of noise and an average S/N was 47.33, 47.28, 47.27, 1.71 and 47.33 for methanol, ethanol, ethyl acetate, benzene and MCB. The quatitation limit (QL) calculated as the concentration, which generated peak about 10 times as high as the height of noise and the average S/N was 143.41,

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Table 3. Summary of system and intermediate precision

Sr. No.	RS	System Precision	Intermediate Precision (Day-I)	Intermediate Precision (Day-II)	
1.	Methanol	Mean	2191.0	2191.0	2229.1
		SD	51.65	51.65	64.97
		RSD	2.36	2.36	2.91
2.	Ethanol	Mean	2585.3	2585.3	2551.2
		SD	99.88	99.88	67.48
		RSD	3.86	3.86	2.65
3.	Ethyl acetate	Mean	10096.3	10096.3	10143.7
		SD	177.99	177.99	128.27
		RSD	1.76	1.76	1.26
4.	Benzene	Mean	1286.8	1286.8	1263.1
		SD	111.69	111.89	58.08
		RSD	8.86	8.86	2.91
5.	MCB	Mean	3367.5	3367.5	3457.2
		SD	79.15	79.15	117.11
		RSD	2.35	2.35	3.39

143.27, 143.24, 5.17 and 143.42 for methanol, ethanol, ethyl acetate, benzene and MCB.

3.2.4 Method specificity

The method specificity was demonstrated by injecting individual RS which showed good separation and resolution between the peaks of the five solvents viz., methanol, ethanol, ethyl acetate, benzene and MCB and also the diluents (DMSO). In addition, the diluents blank has no interfering peak that will affect the quantitation of the RS in the sample.

3.2.5 Accuracy and recovery

Accuracy was assessed on samples spiked with known amounts of each five solvent (from 80 % to 120 % of specified limit). The accuracy of the method was established by measuring nine sample solutions (triplicate preparations for solution of 80%, 100% & 120%) against standard solution. The acceptance criteria were set up as % RSD value below 10 % and recovery value 100 % ±10 %. The recovery results are presented in Table-4. The set up criteria were fulfilled, thus, the method is accurate.

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Table 4. Result of accuracy, recovery and RSD of RS

Sr. No RS	Concentration (%)	Accuracy (%)	Recovery (%)	RSD
1. Methanol	80	81.93	102.4	2.181
	100	97.73	97.73	2.770
	120	118.1	98.42	1.469
2. Ethanol	80	76.76	95.95	4.018
	100	90.77	90.77	3.362
	120	113.95	94.96	0.735
3. Ethyl acetate	80	85.32	106.6	1.367
	100	104.1	104.1	1.098
	120	125.0	104.2	1.319
4. Benzene	80	82.68	103.34	2.965
	100	100.39	100.4	9.942
	120	123.4	102.85	5.884
5. MCB	80	84.12	105.14	3.508
	100	99.36	99.34	1.927
	120	123.52	102.94	2.083

3.2.6 Method robustness: GC parameters variation

In order to evaluate robustness of the method, the influence of variations of method parameters such as column flow, injector temperature and column oven temperature were investigated. System suitability (SST) requirements were checked for variations of conditions, 3.5ml/min on the carrier gas flow, 35°C on the initial oven temperature and 180°C on the injector temperature. For each set of variation, six replicate injections of the standard solution were performed. The assay values at different variations were all within ±2% for all the five solvents and system suitability criteria were meeting for all the variations. The S/N ratios of QL were all above 10.The retention time of all RS obtained from parameter variations were within ±1 minute of the retention time obtained from the procedural conditions. The obtained results indicate that studied variations of GC conditions do not cause any significant changes in system suitability and the method is robust for quantitation analysis.

4. CONCLUSION

The HS-GC method developed for the identification and quantitationof residual methanol, ethanol, ethyl acetate, benzene and monochlorobenzene (MCB)

anddiluentDMSOin the samples of BHX API has been successfully validated. This method has been shown to have a high sensitivity since it has a low DL of 0.4 ppm for benzene, 2 ppm for ethyl acetate and 5 ppm for methanol, ethanol and MCB.The validation procedure, carried out according to ICH guidelines Q2A and Q2B, specificity, precision, accuracy, limits of detection and quantitation and robustness were evaluated. All set up criteria were fulfilled. The method is specific, accurate, and linear and shows a satisfactory level of precision. The developed and validation procedure shows that the method is suitable for its intended purpose.

5. ACKNOWLEDGEMENT

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