Research Article

A New Validated GC-HS Method for the Determination of Residual Solvents in Famotidine using FID

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ABSTRACT

Residual solvents are used in the manufacturing of active pharmaceutical ingredients, excipients and film coating. These are toxic substances and not desirable in the final product because residual solvents effect the quality and stability of the drug. As the residual solvents cannot be removed completely, they should be within the acceptance limits published in regulatory guidelines such as ICH guidelines (Q3C). GCHS is the most commonly used technique for analysis of volatile solvents. So the aim of the present work is to develop a simple, specific GC-HS method for the determination of residual solvents in famotidine using nitrogen as the carrier gas at the rate of 2.5 ml/min with ZB-624 (30m × 0.53mm, 0.5µ) as column using FID as detector. The developed method was validated as per ICH guidelines and all the parameters are found to be within the limits.

Keywords: Famotidine, ZB-624 and FID

INTRODUCTION

Famotidine (Figure 1) is an anti-ulcer drug and chemically it is N' (aminosulfonyl)-3[[[2[(diamino methylene) amino]-4 thiazolyl] methyl] thio] propanimidamide.

It is a H_2-receptor antagonist which inhibits stomach acid production, and is used in the treatment of peptic ulcer disease.

From the scheme of synthesis of famotidine the volatile solvents used at various steps are methanol, acetone, IPA and toluene.

Residual solvents[1] are classified into 4 classes based on the toxicity and GMP demands control of residual solvents in the pharmaceutical products.

Literature survey revealed very few analytical methods for quantization of famotidine by HPLC[2,3] and SFC method but there is no single GC-HS method for the determination of the residual solvents[4] in famotidine.

So objective of the present study is to develop a simple GCHS method for identification and quantification of residual solvents in famotidine[5,6].

The residual solvents used in the manufacturing of famotidine[7,8] are methanol (class II), acetone (class III), IPA (class III) and toluene (class II).

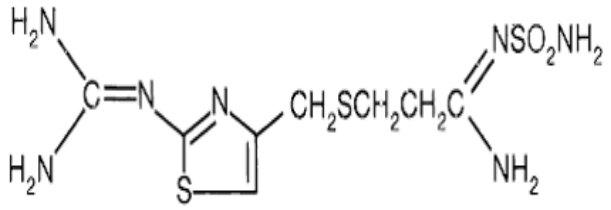

Figure 1: Chemical Structure of Famotidine

Table 1: Optimised Chromatographic Conditions

S. No.	Parameter	Values
1.	Column	ZB-624
2.	Dimension	30m × 0.53mm
3.	Detector	Flame ionization detector
4.	Detector temperature	260°C
5.	Injector temperature	220°C
6.	Injector volume	1mL vapor
7.	Temperature Conditions	350°C hold for 5 mins, rise at 20°C to 250°C hold for 5 mins.
8.	Runtime	21.75mins
9.	Split ratio	1:5
10.	Carrier gas	2.5 ML/min (Nitrogen)
11.	Makeup gas	25 mL/min (Nitrogen)
12.	Bath temperature	100°C
13.	Loop temperature	110°C
14.	Transfer line temperature	120°C
15.	Vial equilibration time	30 mins
16.	Pressurize time	0.5 mins
17.	Loop fill time	0.2 mins
18.	Loop equilibration time	0.2 mins
19.	Injection time	1.0 min
20.	Bath temperature	27 mins

MATERIALS AND METHODS

Head Space Gas Chromatography

Chromatography analysis was carried out by using Shimadzu GC – 2010 with a TELEDYNE TEKMAR head space sampler. Gas chromatograph was equipped with standard oven for temperature ramping, split injection

port and flame ionization detector. The analytes of interest were separated on a ZB-624 capillary column (30m x 0.53mm x 5µm film thickness) with nitrogen as carrier gas in the split mode by head space injection. The volume of 1.00 ml of standard and sample solution was injected in gas chromatograph injection port using the headspace sampler. The temperature of injection port was maintained at 220°C and split ratio 1:5, with Nitrogen as carrier gas. The column flow maintained at 2.5ml/min with constant mode. The temperature of detector was maintained at 260°C. The optimized chromatographic conditions are presented in Table 1.

Residual Standards Used

Methanol, IPA, acetone and toluene were obtained from Merck–Mumbai and used as such.

Preparation of Mixed Standard Solution

A standard stock solution was prepared such that the final conc. contains 6.3µl acetone, 6.4µl of IPA, 1.02µl of toluene and 3.7µl of methanol by using water as diluent. From this 5ml of solution was taken into head Space vial and sealed.

Blank Preparation

5ml of distilled water was transferred into a head space vial and sealed.

Sample Preparation

100mg of famotidine drug sample was weighed and transferred into a head space vial, dissolved in 5ml of distilled water and sealed.

Method Development Procedure

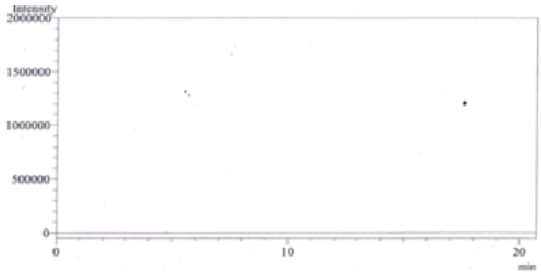

Figure 2: Chromatogram of Blank

A Method was developed by performing several trials and finally parameters were selected based on the acceptance limits of ICH. Then each 1ml of blank, standard and sample were injected into headspace and their chromatograms were recorded (Fig.2, Fig.3 and Fig.4).

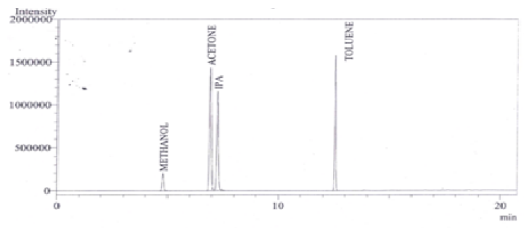

Figure 3: Chromatogram of Standard Solution

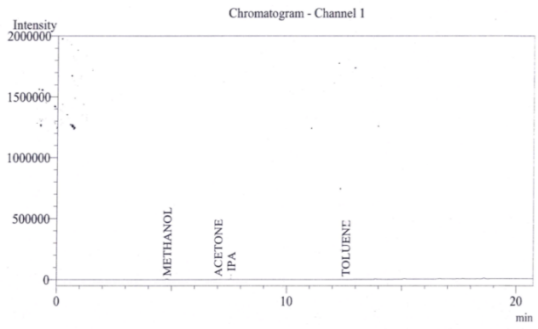

Figure 4: Chromatogram of Sample (Famotidine)

Method Validation

All the parameters are validated as per ICH guidelines.

System suitability

System suitability study of the method was carried out by injecting a blank i.e.; 5.0 ml of diluent (distilled water) and six replicate analysis of mixed standard solutions. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates and resolution between the peaks were determined.

Specificity

Specificity study of the method was carried out by injecting a blank i.e.; Diluent (Distilled water), a mixed standard solutions, the pure drug sample solution and individual solvents such as methanol, acetone, iso propyl alcohol(IPA), toluene into Gas chromatography and their respective retention times obtained.

Method Precision

Method Precision was carried out by injecting one batch of sample at 100% concentration 6 times into the head space sampler and % RSD values were calculated.

Linearity

Preparation of 150% Solution

Accurately 5.7 µl of Methanol, 9.3 µl of Acetone, 9.6 µl of Isopropyl alcohol, 1.53 µl of Toluene was transferred using a micro syringe into a 50ml volumetric flask containing 35 ml of Distilled water (diluent) and was mixed well. The volume was made up to level with Distilled water. From this 5ml of solution was pipetted into 3 head space vial fitted with septum and sealed with the sealer.

Preparation of 125% Solution

Accurately 41.6 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluent (Distilled water). From this 5ml of solution was pipetted into 3 head space vial fitted with septum and sealed with the sealer

Preparation of 100% Solution

Accurately 33.3 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluent (Distilled water). From this

5ml of solution was pipetted into 3 head space vial fitted with septum and sealed with the sealer.

Preparation of 75% Solution

Accurately 25.0 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluent (Distilled water). From this 5ml of solution was pipetted into 3 head space vial fitted with septum and sealed with the sealer.

Preparation of 50% Solution

Accurately 16.6 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluent (Distilled water). From this 5ml of solution was pipetted into 3 head space vial fitted with septum and sealed with the sealer.

Procedure

Linearity study of the method was carried out by injecting each 50%, 75%, 100%, 125% and 150% standard solution three times into the head space. 5 point Calibration curves were plotted by taking average areas on the y-axis and concentration on the x-axis. Linearity has been confirmed by statistical analysis and respective correlation coefficients and regression equations were calculated and the values are tabulated in Table 2.

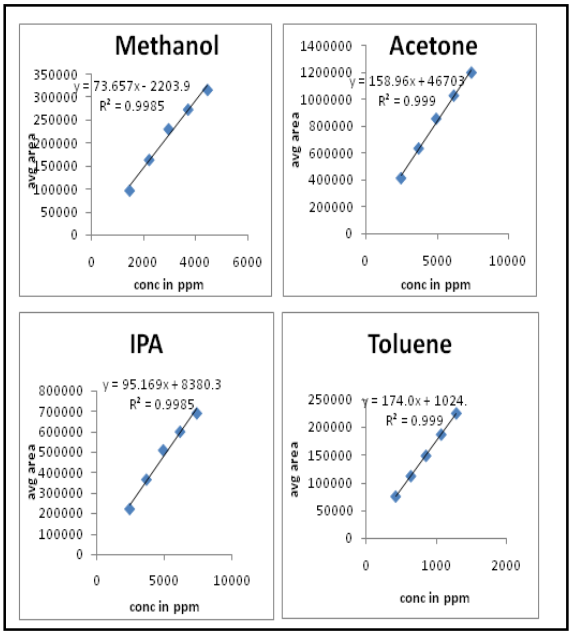

Figure 5: Linearity curves of the residual solvents

Table 2: Linearity Data

Solvent Name	Average Area					Correlation Coefficient	Regression Equation
	Concentration						
	50%	75%	100%	125%	150%		
METHANOL	461165.5	669663	888428	1060428	123242	0.998	y = 123.8x - 69578
ACETONE	3033257	422149	5458622	7233058	900748	0.999	y = 328.1x - 22343
IPA	2738686	383493	5029968	6245958	746194	0.998	y = 328.1x - 22343
TOLUENE	1614165	281940	4039897	4794522	554914	0.999	y = 75.20x - 6276

Table 3: Accuracy of the Proposed Method

Solvents	Level	Avg. Peak Area			% Recovery	Mean % Recovery
		Non Spiked Solution	Spiked Solution	Standard Solution		
Methanol	50%	46115	30599	461165	86.2	85.86
	100%	888428	61199	888428	86.4	
	150%	1232429	9179805	1232429	85.0	
Acetone	50%	3033257	35302	3033257	97.1	97.10
	100%	5458622	70604	5458622	97.2	
	150%	9007483	105907	9007483	97.01	
IPA	50%	2738686	1770	2738686	98.2	98.90
	100%	5029968	3541	5029968	99.1	
	150%	7461949	5311	7461949	99.4	
Toluene	50%	1614165	23475	1614165.5	97.1	97.10
	100%	4039897	46950	4039897	97.0	
	150%	5549148	70425	5549148	97.2	

Limit of Detection and Limit of Quantification

LOD and LOQ values were determined by signal-to-noise ratio (S/N) method by injecting each standard solution 6 times at its DL and QL concentration level.

Robustness

This study was performed by making small but deliberate variations in the method parameters and observing the changes. The effects of variation were ± 5°C change in the column oven temperature and ±2ml/min in the column flow. A blank (Distilled water), mixed standards and a pure drug sample solution was introduced into the head space sampler (n=6) and concentration of each solvent was calculated.

Ruggedness

Ruggedness study of the method was carried out by injecting one batch of sample solution by two different analysts on two different days and concentrations of four solvents were calculated.

Accuracy

Recovery studies was carried out by standard addition method at three different levels i.e. 50%,100% and 150%.The percentage recoveries of methanol, acetone, IPA and toluene in the sample mixture was determined. The results of mean percentage recoveries obtained by proposed method by statistical evaluation and they were given in Table 3.

Batch Analysis

Batch Analysis was carried out by injecting a pure drug sample solutions and a marketed formulation sample solution into the head space.

Preparation of Marketed Formulation

A weighed quantity equivalent to 100mg of famotidine marketed formulation was transferred into 20ml Headspace vial and 5ml of Distilled water (diluent) was added to the same vial fitted with septum and sealed.

RESULTS AND DISCUSSION

Method Development

Column Selection

The primary aim of the column selection was to resolve the four solvents (methanol, acetone, IPA, toluene) which are utilized in the process of synthesis of famotidine. Several trials were done with different wall-coated capillary columns having different stationary phases and dimensions in order to separate and quantify solvents present in famotidine.

For eg., DB-624 column (30m length,0.52mm i.d with a stationary phase of 6% cyano propyl phenyl & 94% Dimethyl poly siloxane film of 3.5µ), ZB-624 column (30m length, 0.52mm i.d with a stationary phase of 6% cyano propyl phenyl & 94% Dimethyl poly siloxane film of 5µ). Finally the ZB-624 column was found to be the best one for separation of all the 4 solvents in less time.

Thermal Programming

A linear thermal gradient was selected to provide elution of the solvent's peak during the chromatographic run for better resolution and quantification.

Several trials were performed by changing linear thermal gradient, among them an initial hold of 5min at 35°C and linear thermal gradient to 250°C at 20^0C/min was found to elute better peaks showing the resolution more than 2.

Headspace Method Optimization

The headspace method was finalized in such a way that 4 solvents present in the sample should vaporize for the detection. For this sample and standard vials were heated at 90°-85°-95°C for 30-25-35min with constant shaking. Among them a combination of sample vial heating at 85°C for 25mins shaking was found to suitable for getting better response.

Method Validation

System Suitability

System suitability parameters like asymmetry and resolution were calculated to evaluate the chromatographic parameters.

The number of theoretical plates for the six replicate injections of mixed standard solution was found to be more than 3000, tailing factor was found to be less than 2 and the resolution between any two adjacent peaks were more than 2.0.

The system suitability parameters were found to be in the acceptable range, which indicates suitability of system for the quantification of these 4 solvents by this method.

Specificity

The blank chromatogram did not show any interference with the solvent peaks. Rt of individual residual solvent are compared with Rt of the solvents peaks of the sample and Rt values for methanol, acetone, IPA, toluene were found to be 5.18min, 7.34min, 7.70min and 13.00min.

Method Precision

Method precision was done by injecting one batch of sample at 100% concentration for six times. For each solvent, from chromatogram peak areas % Relative standard deviation was calculated.

% Relative standard deviation for four solvents was found to be less than 15% hence the method is precise.

Linearity

Linearity is performed from 50-150% and graphs obtained from the linearity were observed to be linear and showing correlation coefficient R2 ≥ 0.999%.

Linearity range, correlation coefficient and slope values are tabulated in Table 2.

Detection (DL) and Quantization (QL) Limit

Solution containing individual solvent was prepared around its QL concentration and injected in six replicates. The DL and QL for all solvents were determined by signal-to-noise ratio (S/N) method. From these limits, it was observed that the minimum concentration (ppm) is at 3:1 S/N (for DL) and the quantification concentration is at 10:1 S/N (for QL) and the DL values for methanol, acetone, IPA, toluene were found to be 0.001887, 0.000779, 0.001542 and 0.003050 respectively and the QL values were found to be 0.0062271, 0.0025707, 0.0050886 and 0.010065 respectively.

Robustness

Robustness of the method was performed by making small variations in the optimized parameters. There were no marked changes in the %RSD of the areas of solvent peaks. Hence the method is said to be robust.

Ruggedness

Analysis was performed by different analyst on different days by injecting six replicates of the mixed standard solution into the optimized chromatographic system. %RSD was calculated from the data obtained and it was found that the %RSD values was less than 15% for all the 4 solvents although the analysis was performed on different days by different analysts hence the method is said to be rugged.

Accuracy

Accuracy of the method was done by recovery experiments by spiking known amount of each solvent at quantization limit, 50%, 100% and 150% of 5000 ppm to the test solution. Each preparation was analyzed in triplicate and percent recovery was calculated.

The recovery values were found to be between 85.86% and 98.90% and results obtained were within the limits and are summarized in the Table 3.

Batch Analysis

Batch analysis was performed by injecting test samples and a formulated product of a batch and whose results were found to be within the limits and the values for methanol, acetone, IPA, toluene were found to be 264.27ppm, 49.50ppm, 3.8ppm and 8.6ppm where as the acceptable limit is 3000ppm, 5000ppm, 5000ppm & 890ppm.

CONCLUSION

A single, renovative, simple and rapid GC-HS method is successfully developed for determination of residual solvents in Famotidine with FID detection. This method is very specific as the individual peaks of residual solvents were well separated on ZB-624 column with a chromatographic time course of 2.5ml/min and mobile phase of nitrogen. The method is validated for specificity, linearity, precision, batch analysis, system suitability, LOD and LOQ. All the validated parameters were found to be within the ICH limits.

Excellent results are obtained, within the globally accepted validation reference values. The suggested method can be successfully used to estimate the residual solvents present in the Famotidine pure drug and marketed formulation.

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