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Development and Validation of a GC-HS Method for the Estimation of Allylamine in Sevelamer Hydrochloride Tablets Dosage Form

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ABSTRACT

A sensitive static headspace gas chromatographic method using flame ionization detector was developed and validated for the estimation of Allylamine in Sevelamer hydrochloride tablet dosage form. The chromatographic separation was achieved on capillary column Rtx-5 Amine (30m×0.53mm×1.0µm) with 5% diphenyl/95% dimethyl polysiloxane stationary phase. The critical experimental parameters such as, diluents, columns and sample preparations were studied and optimized. The method was validated as per United States Pharmacopoeia (USP) and International Conference on Harmonization (ICH) guidelines in terms of detection limit, quantification limit, linearity, precision, accuracy, specificity, solution stability and robustness. The method was found to be linear in the range between 10 µg/mL and 75 µg/mL with a correlation coefficient (r2) of 0.99984. The detection and quantification limits obtained for Allylamine were 3.3 µg/mL and 10.0 µg/mL w.r.t sample concentration respectively. The recovery obtained for Allylamine was between 100.0% and 110.0%. The developed method was applied successfully for the estimation of Allylamine in Sevelamer hydrochloride tablet dosage form.

Keywords: GC-HS, Allylamine, Validation, Sevelamer hydrochloride tablets.

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INTRODUCTION

Sevelamer hydrochloride is known as poly (allylamine) cross-linked with epichlorohydrin. Sevelamer hydrochloride is hydrophilic and insoluble in water. It is chemically known as poly (allylamine-co-N,N'-diallyl 1,3-diamino-2-hydroxypropane) and exist in two salt form, Sevelamer hydrochloride (Figure-1) and Sevelamer carbonate [1]. Sevelamer hydrochloride is intended for oral administration in the treatment of hyperphosphatemia. Sevelamer acts as a phosphate binder and it has been shown to decrease serum phosphate concentrations in patients with chronic kidney disease [2]. Poly (allylamine hydrochloride) is an intermediate in the synthetic process of Sevelamer drug substance. In the preparation of poly(allylamine hydrochloride), Allylamine is used as a key starting material, which is an unsaturated aliphatic amine, a colorless liquid, and chemically known as 3-amino-1-propene (Figure-2).

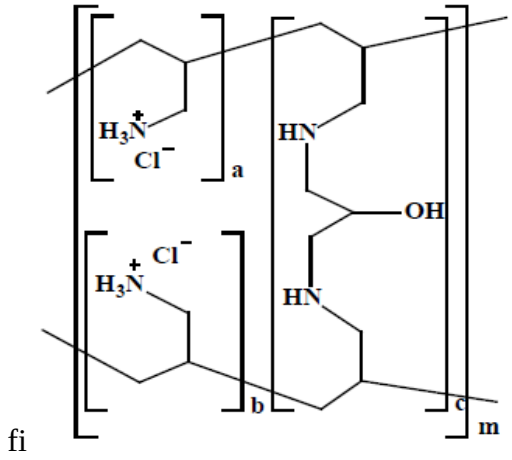

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Figure 1: Chemical structure of Sevelamer Hydrochloride

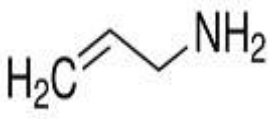

Figure 2: Chemical structure of Allylamine

Because of its known toxic nature, the presence of residual Allylamine in Sevelamer compounds should be controlled as per International Conference on Harmonization (ICH), Food and Drug Administration (FDA) and European Medicines Agency (EMEA) guidelines [3-5]. The general toxic effect of the Allylamine is primarily related to irritation of the mucous membranes, whereas the long history of experimental use of Allylamine has emphasized its extraordinarily deleterious effects on the heart and vascular tissue [6]. As per literature some analytical methods are already available for the determination of Allylamine. The United States Pharmacopeia has published a HPLC derivatization method using a fluorescence detector [7]. The

determination of Allylamine in Sevelamer hydrochloride and carbonate drug substance by using gas chromatography with flame ionization detector was reported by Raju V. S. N. Kadiyala [8]. As on date no GC-HS method has reported for determination of residual Allylamine in Sevelamer hydrochloride drug product. The present investigation was, therefore, initiated with the objective to develop a simple and sensitive analytical method for the determination of Allylamine in Sevelamer hydrochloride drug product using static headspace gas chromatography with flame ionization detector. Considering the maximum daily dosage of 7.2 g per day of Sevelamer hydrochloride, any impurity limit must be less than 0.05%, but 50 µg/g has been chosen as specification limit for the research work. The developed method was validated for specificity, sensitivity, linearity, precision, accuracy, solution stability and robustness as per ICH Q2 (R1) / United States Pharmacopeia (USP) guidelines [9-10].

MATERIALS AND METHOD

Chemicals and Reagents:

The Sevelamer hydrochloride drug substance and tablets were procured from the Emcure Pharmaceutical Limited (Pune, India). Allylamine standard was purchased from Avra Synthesis Limited (Hyderabad, India). Sodium hydroxide pellets were purchased from Merck Specialties private limited (Mumbai, India). 1-methyl-2-Pyrrolidinone GC grade was purchased from Spectrochem (Mumbai, India); Isopropyl alcohol was purchased from Avantor performance materials (Ankleshwar, India) and Methylene chloride was purchased from Sigma Aldrich (USA). Water was prepared by Milli-Q water purification system.

Chromatographic Conditions:

Table 1: Headspace conditions

HS Parameters	Perkin Elmer Clarus 680
Thermostat temperature	100°C
Needle temperature	110°C
Transfer line temperature	120°C
Thermostat time	20.0 minute
Pressurize time	3.0 minutes
Injection time	0.15 minutes
Withdraw time	0.5 minutes
Carrier pressure	21 psi
Shaker, vial vent	ON
GC cycle time	50 minutes

All experiments were performed on Perkin Elmer Clarus 680 Gas chromatograph equipped with Turbomatrix 40 headspace sampler, flame ionization detector and Totalchrom software

(PerkinElmer, Waltham, USA). Rtx – 5 Amine GC column, 30m length × 0.53mm ID × 1 µm film thickness was used (Restek, USA). The column oven temperature started at 35°C and held for 20 minutes and then it was raised to 250°C at the rate of 45°C/minute and held at 250°C for 12 minutes. Helium was used as a carrier gas with a constant pressure of 1.0 psi and split ratio 1:1. The injector temperature and the detector temperature were kept at 180°C and at 260°C, respectively. Headspace conditions were presented in Table. 1

PREPARATION OF SOLUTIONS

8% Sodium Hydroxide Solution:

Accurately weigh and transfer 8.0 g of sodium hydroxide pellets into a 100 mL volumetric flask containing about 50 mL of water, dissolve and then dilute to volume with water.

Diluent preparation:

Use 1-methyl-2-Pyrrolidinone (NMP) as diluent.

Diluent blank preparation:

Transfer 2 mL of diluent to HS vial, add 100 µL of 8% Sodium hydroxide solution, seal with septum and crimp cap.

Standard preparation:

Prepare the solution containing 12.5 µg/mL of allylamine in diluent. Transfer 2 mL of standard preparation to HS vial, add 100 µL of 8% Sodium hydroxide solution, seal with septum and crimp cap.

Sample preparation:

Crush 10 Sevelamer hydrochloride tablets to a fine powder. Weigh and transfer accurately about 500 mg of powder sample into headspace vial. Add 2 mL of diluent into the HS vial. Add 100 µL of 8% Sodium hydroxide solution and seal with septum and crimp cap. Further mix the content of vial on vortex mixer for about 3 minutes.

RESULTS AND DISCUSSION

Method development and optimization

The gas chromatograph with a flame ionization detector method was proposed as a suitable method for determination of Allylamine in Sevelamer hydrochloride tablets dosage form. Developments trials were initiated on the headspace technique using stationary phase 35% diphenyl, 65% dimethyl polysiloxane (Rtx-35 Amine; Make: Restek) as this column is an amine column specific for amines and other basic compounds. Finally the Rtx-5 Amine (30m length × 0.53mm ID × 1 µm film thickness) GC column was preferred, as Allylamine peak eluted with less

tailing and the column is more stable than Rtx-35 Amine. As Sevelamer hydrochloride is a known hydrophilic polymer having the tendency to swell with water, because of the poor solubility; preliminary experiments were conducted to optimize the suitable solvent for HS extraction. Initially Dimethyl sulphoxide was chosen as diluent, but some interference was observed at the retention time of Allylamine. Several commonly used organic solvents were studied, among the different organic solvents; 1-methyl-2-Pyrrolidinone was selected. The chromatographic conditions were optimized with different diluents, columns, flow rate, column oven temperature and headspace conditions. The sample preparation was optimized by using different sample concentration, concentration of Sodium hydroxide, use of shaker and vortex. Based on the results obtained from development activity a suitable method was developed and validated.

METHOD VALIDATION

The developed method was validated for specificity, detection limit (DL) & quantitation limit (QL), linearity, precision [system, method and intermediate precision], accuracy, solution stability and robustness as per ICH guideline Q2(R1) and USP<1225> [9-10].

Specificity:

The blank solution, individual injection of Allylamine standard, and all other known residual solvents (which are used in the manufacturing process of Sevelamer hydrochloride tablets i.e. Isopropyl alcohol and Methylene chloride), unspiked sample preparation and spiked sample preparation (sample spiked with Allylamine and all other known residual solvents) were prepared and injected into the GC system. From the observations, it was found that the Allylamine peak was separated from all other known residual solvents, indicating that the method is selective and specific for the estimation of Allylamine in Sevelamer hydrochloride tablet dosage form. Diluent blank, standard, unspiked sample and spiked sample chromatograms are presented in Figure 3, 4, 5 and 6 respectively.

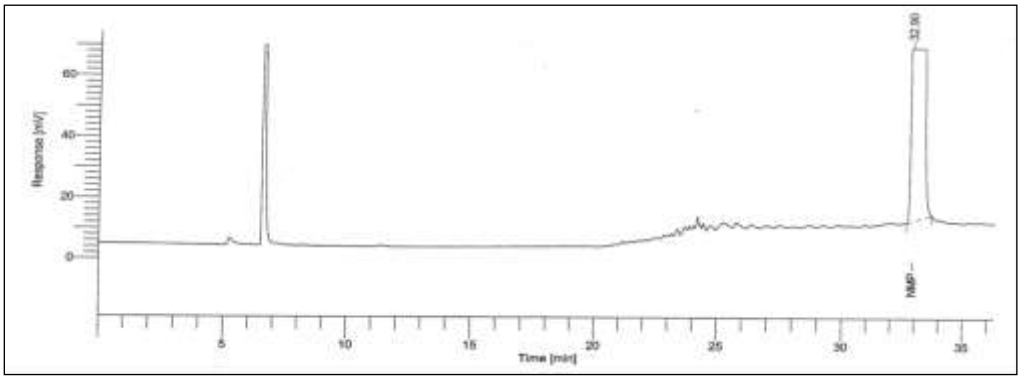

Figure 3: A Typical chromatogram of diluent blank preparation

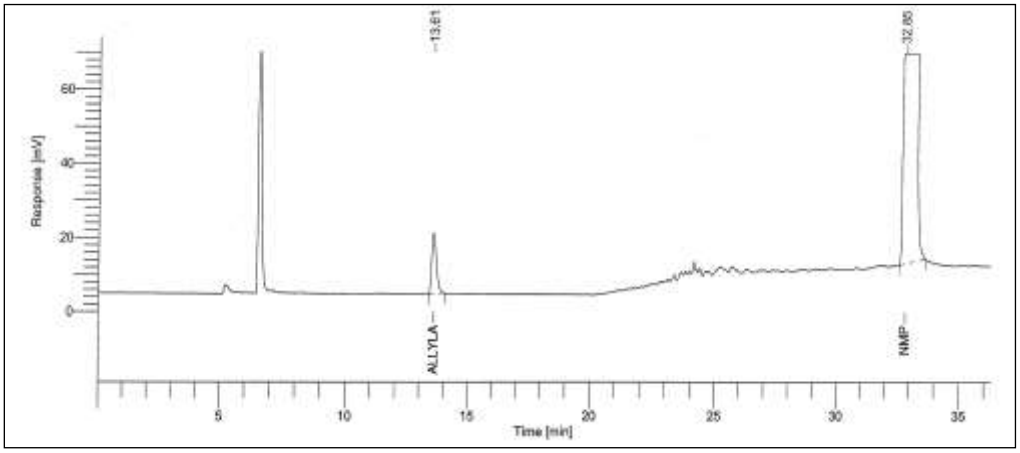

Figure 4: A Typical chromatogram of standard preparation

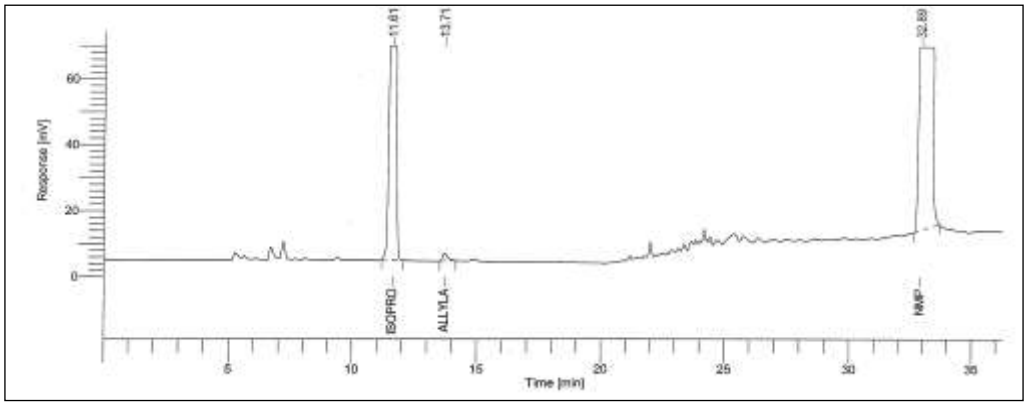

Figure 5: A Typical chromatogram of unspiked sample preparation

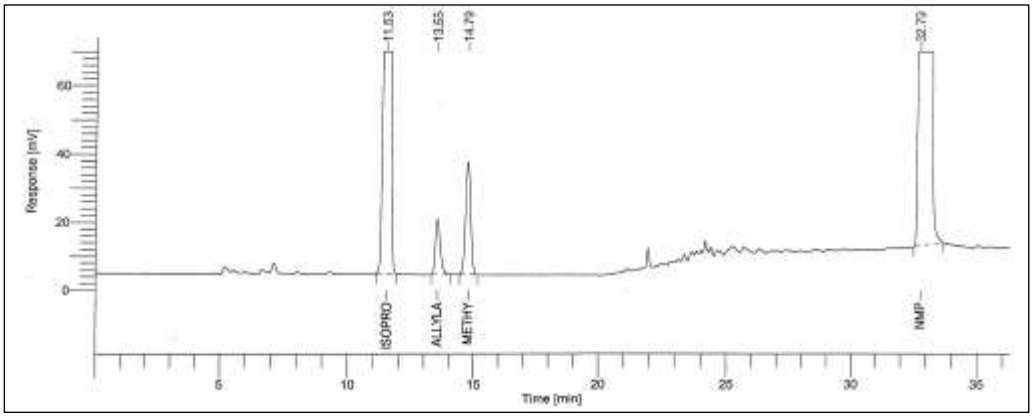

Figure 6: A Typical chromatogram of spiked sample preparation

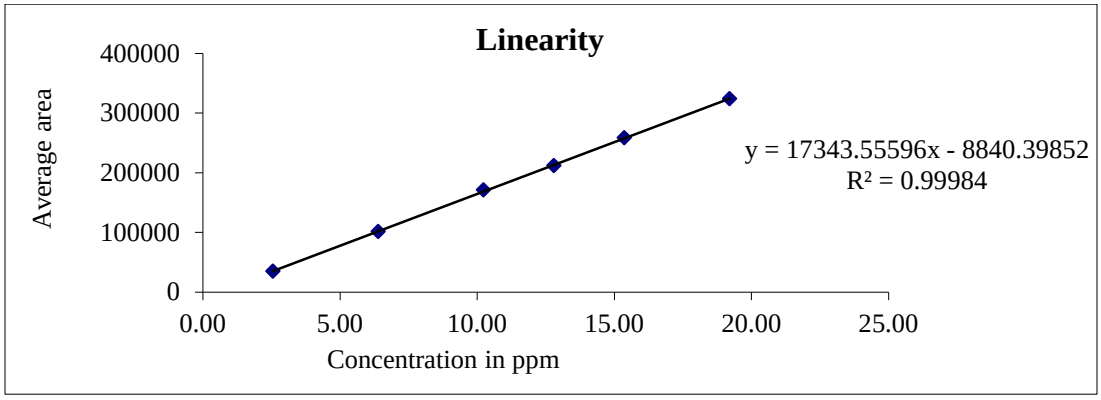

Figure 7: Linearity graph of Allylamine

Linearity:

The linearity was evaluated by measuring area response for Allylamine over the range of 10 to 75 µg/mL w.r.t. sample concentration (250 mg/mL). Six concentrations (n=6) were prepared across the range and injected each in triplicate. The mean (n=3) area calculated was plotted against the concentration. The correlation coefficient (r2) obtained for Allylamine was 0.99984. The linearity graph depicted in Figure 7

Detection Limit and Quantitation Limit:

The detection limit and quantification limit values for Allylamine were determined by signal-to-noise ratio (S/N) method. The minimum concentration at 3:1 S/N was considered as detection limit and the concentration at 10:1 S/N was established as quantification limit. The DL and QL values obtained for Allylamine was 3.3 and 10.0 µg/mL w.r.t. sample concentration (250 mg/mL). A solution containing Allylamine was prepared around its QL concentration and injected in six replicates. The RSD (n=6) value obtained for the area of Allylamine at QL was 4.42%. The results are tabulated in Table 2.

Table 2: Validation data of LOQ Precision

Injection	Allylamine Area	S/N ratio
Injection-1	33006	24
Injection-2	37767	28
Injection-3	34745	22
Injection-4	35425	21
Injection-5	35519	23
Injection-6	36116	20
Mean	35430	-
Standard Deviation	1567.489	-
% RSD	4.42	-

Precision:

The system precision was determined by injecting the standard preparation of Allylamine (n=6)

into GC system and calculating % RSD using the areas. The method precision was established by preparing six individual sample preparations by spiking known amount of Allylamine to sample preparation, injecting into the GC system, and calculating the Allylamine content. The ruggedness of the method was evaluated by preparing six individual sample preparations (the same samples which were used in the method precision experiment) by spiking Allylamine to sample preparation, injecting into the GC, and calculating the Allylamine content using two different columns, different instruments and by different analysts on different days. The RSD of the results (n = 6) obtained with different GC system were 1.70% and 1.08%, respectively. The overall RSD (n = 12) of the study was 1.54%. The achieved precision results are reported in Table 3.

Table 3: Validation data of Method and Intermediate precision

Injection	Allylamine (µg/mL)	
	Method precision	**Intermediate precision**
Injection-1	47	48
Injection-2	47	48
Injection-3	48	49
Injection-4	48	48
Injection-5	49	48
Injection-6	47	48
Mean (n=6)	48	48
% RSD (n=6)	1.70	1.08
% RSD (n=12)	1.54	

Accuracy:

Accuracy of the method was verified by performing recovery study by spiking known amounts of Allylamine at the LOQ level, 50%, 100%, and 150% of the specification level (i.e.250 µg/mL) to sample preparation and the obtained recovery results are tabulated in Table 4.

Table 4: Validation data of Accuracy

Recovery Levels	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Average Recovery (%)	SD	%RSD
LOQ	10	11	110.00	106.67	5.774	5.41
	10	10	100.00			
	11	10	110.00			
50%	27	26	103.85	102.57	2.223	2.17
	26	26	100.00			
	27	26	103.85			
100%	54	51	105.88	105.88	1.960	1.85
	53	51	103.92			
	55	51	107.84			
150%	80	77	103.90	103.47	0.751	0.73
	79	77	102.60			
	80	77	103.90			

Solution Stability:

The stability of Allylamine in standard and sample preparation was studied by measuring the area of standard and sample preparation (stored at 25 ± 2°C) injected over a period of 12 Hr. and 30 Hr. The cumulative % RSD of area for standard and sample preparation of Allylamine was 0.92 and 2.03 respectively. The achieved stability results are reported in Table 5.

Table 5: Validation data of solution stability

Stability parameters	Sample	Standard
Cumulative % RSD (fresh)	2.57	-
Cumulative % RSD (fresh & 12Hr)	2.22	1.05
Cumulative % RSD (fresh &12Hr & 30Hr)	2.03	0.92

Robustness:

This study was performed by making small but deliberate change in the method parameters. The effect of change in carrier gas flow and initial column oven temperature on the Allylamine determination was studied. The results including system suitability of standard preparation and Allylamine content in sample are presented in Table 6. Under all the variations, system suitability requirements (% RSD) were found to be well within the specified acceptance criteria.

Table 6: Validation data of Robustness

Variation	Retention time (min)	% RSD of Standard	Allylamine (µg/mL)
As per method	13.5	0.66	48
Flow rate (+10%)	12.5	1.19	52
Flow rate (-10%)	14.9	0.81	48
Column oven temperature (+3°C)	13.0	0.28	52
Column oven temperature (-3°C)	14.4	1.42	52
% RSD	-	-	4.35

CONCLUSION

The sensitive static headspace gas chromatographic method using flame ionization detector (GC-HS-FID) method described in this investigation was proved to be an ideal tool for the determination of Allylamine in Sevelamer hydrochloride tablet dosage form. The Method validation data demonstrated that the developed method is sensitive as well as accurate for the estimation of Allylamine. The method was found to be linear on the specified range, precise and robust. Hence, the proposed GC-HS-FID method can be used conveniently in the pharmaceutical laboratory for the routine analysis of Allylamine in Sevelamer hydrochloride tablet dosage form.

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