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Short communication

Development and validation of rapid ion-chromatographic method with conductivity detection for trace level determination of allylamine in sevelamer drug substances

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ABSTRACT

A sensitive and rapid ion chromatography (IC) method was developed for the low level determination of allylamine (AAM) in sevelamer (SVM) drug substances, i.e., sevelamer hydrochloride (SVH) and sevelamer carbonate (SVC). This method utilized a Dionex Ion Pack CS14 IC column, a mobile phase of 10 mM methane sulfonic acid with conductivity detection. The total chromatographic run time was as short as 8 min. The various factors involved in the sample preparation such as, extraction solvent, extraction time and stirrer speed were evaluated. This method was validated as per United States Pharmacopoeia (USP) and International Conference on Harmonization (ICH) guidelines in terms of detection limit, quantitation limit, linearity, precision, accuracy, specificity and robustness. Linearity of the method was very good over the concentration range of 9–750 μg/mL with the coefficient of determination (r^2) 0.999. The detection and quantitation limit of AAM were 2.7 and 9.0 μg/mL, respectively. The recovery data obtained for AAM were between 97% and 109%. Also, the specificity of the method was proved through IC coupled with mass spectrometer (IC–MS). The developed method was found to be robust and applied successfully to determine the content of AAM in Sevelamer bulk drugs.

1. Introduction

Sevelamer (SVM) is known chemically as poly(allylamine-co-N,N′-diallyl-1,3-diamino-2-hydroxypropane) and it exists in two salt forms, sevelamer hydrochloride (SVH) and sevelamer carbonate (SVC) [1]. While the counter ions differ for the two salts, the active polymer moiety is the same. SVM drug substances are intended for oral administration in the treatment of hyperphosphatemia. SVM acts as a phosphate binder and it has been shown to decrease serum phosphorus concentrations in patients with end-stage renal disease. SVM is poly(allylamine) cross-linked with epichlorohydrin in which 40% of the amines are protonated. The primary amine groups in the structure (Fig. 1) are derived directly from poly(allylamine), and the crosslinking groups consist of two secondary amine groups derived from poly(allylamine) and one molecule of epichlorohydrin. Poly(allylamine), a key starting material/precursor and polymeric backbone of SVM is produced by the polymerization of allylamine (AAM) monomer. AAM, an unsaturated aliphatic amine chemically described as 3-amino-1-propene (Fig. 1), is potentially toxic. Its acute toxicity is believed to involve metabolism of AAM to highly reactive acrolein [2,3]. Because of its known toxic nature, the presence of residual AAM in SVM compounds should be controlled as per International Conference on Harmonization (ICH), Food and Drug Administration (FDA) and European Medicines Agency (EMEA) guidelines [4–6]. Considering the recommended starting dose of 2.4 g per day and a maximum daily dosage of 7.2 g per day, any impurity in SVM drug substances must be limited to less than 0.05% (500 μg/g). Since, AAM is susceptible to undergo polymerization and metabolize to acrolein [2], its presence in the drug substances should be as minimum as possible. Hence, a sensitive and robust analytical method is required to detect and quantitate AAM in SVM.

SVM is not official in any pharmacopoeia; however, few reports are available for aliphatic amines [7–10] and AAM [11,12] determination. The volatile aliphatic amines in air were determined by solid-phase micro extraction (SPME) coupled with gas chromatography-flame ionization detection (GC-FID) [7]. HPLC method with chemiluminescence detection was reported for the determination of aliphatic amines as their dansyl derivatives in water samples [8]. A GC head space (HS) microextraction method for the estimation of aliphatic amines in aqueous solution was

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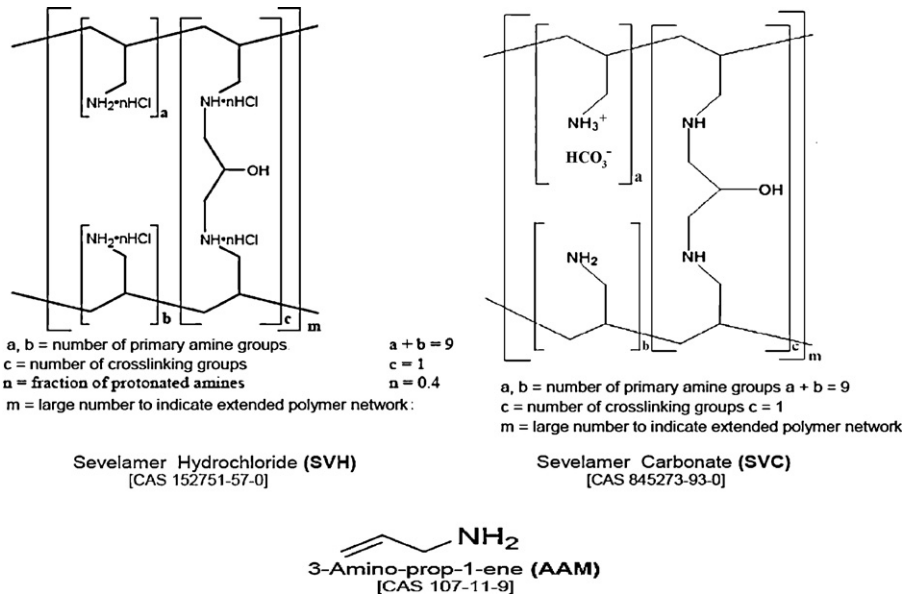

Fig. 1. Chemical structure of sevelamer hydrochloride (SVH), sevelamer carbonate (SVC) and allylamine (AAM)

demonstrated [9]. Also, the development of a GC head space method was published to determine the residual aliphatic amines in oligonucleotides [10]. Determination of aliphatic amines including AAM in air by reverse phase HPLC with UV detection using 1-naphthyl isocyanate derivative was reported [11]. A thin layer chromatographic–fluorometric method was developed for the determination of AAM in the form of the dansylamide derivative [12]. Thus, even though different methods were reported in the literature for the determination of aliphatic amines [7–10] and AAM [11,12] in different sample matrices viz., water, air and oligonucleotides, no analytical method was published so far, for the estimation AAM in SVM polymeric drug substances utilizing ion chromatography (IC).

The estimation methods reported for AAM [11,12] involve complex derivatization procedures in chromatography followed by fluorometric and UV detection. But, most of the derivatives are often unstable and thereby generating unknown peaks in chromatography. Also, the compatibility of the derivatization reagents with the sample matrix, completion of derivatization reaction and quenching of excess reagent to suppress the side reaction, are the major concerns in the reported methods. Hence, the present investigation was initiated with the objective to develop a method for the direct estimation (i.e., without derivatization) of AAM in SVM, utilizing IC with conductivity detection (CD). An important and critical aspect about SVM is related to its solubility. Being a polymeric material, SVM is poorly soluble in most of the commonly used organic and aqueous solvents and hence trace level estimation of AAM in SVM samples by a direct and conventional GC or LC method is practically difficult. However, the possibility of head space extraction followed by GC analysis was attempted in this study but it was found unsuitable for AAM determination. Thus, the more selective and sensitive IC method [13] which is widely used in the analysis of amines (as their protonated organic cation) was utilized in this investigation. Additionally, the availability of high level automation and suppression techniques in IC has extended its application potentiality for AAM determination. The concentration of the extraction solvent, mobile phase, extraction time and stirrer speed were studied to develop a robust method. The proposed IC method with conductivity detection was validated using ICH and USP [14,15] guidelines as references.

2. Experimental

2.1. Chemicals and reagents

Methanesulfonic acid (≥99.5%) and AAM (98+%) were purchased from Sigma–Aldrich® (Steinheim, Germany). Analytical grade hydrochloric acid was obtained from Qualigens Fine Chemicals (Mumbai, India). The water used was from a Milli-Q™ purification system, Millipore® (Bedford, USA). SVH and SVC drug substances were obtained from Shasun® Chemicals and Drugs Ltd. (Chennai, India).

2.2. Chromatographic conditions

All experiments were performed on Dionex® ICS 3000 IC system equipped with conductivity detector and autosampler (Sunnyvale, CA). Chromeleon® version 6.8 Chromatography data system (Build 2212, Dionex®) was used for the data acquisition and processing. The peak purity studies were carried out utilizing a Thermo Finnigan® LCQ™ DECA XP Plus ion-trap mass spectrometer with Xcaliber® software for the MS data acquisition and analysis (San Jose, USA). Magnetic stirrer from Deepali United (Mumbai, India) and nylon membrane syringe filter (0.2 μm) from Sartorius Stedim™ (Goettingen, Germany) were used. Dionex® Ion Pac® CS14 IC column, a medium low-capacity carboxylate-functionalized cation exchange resin cross-linked with 55% divinylbenzene copolymer (250 mm length × 4.0 mm id) with guard column CG-14 (50 mm length × 4.0 mm id) was utilized (Sunnyvale, CA). The cation self-regenerating suppressor CSRS® 300 (4 mm) from Dionex® (Sunnyvale, CA) was used in recycle mode. The column and the cell heater were maintained at (30 ± 2) and (35 ± 2) °C, respectively. The mobile phase was 10 mM methanesulfonic acid (MSA). The injection volume was 50 μL and the flow rate was 1.0 mL/min. The total run time was 8 min. 10 mM hydrochloric acid was used as diluent for sample and standard preparations.

2.3. Solution preparation

Standard stock solution was prepared by dissolving 50 mg of AAM into a 100 mL volumetric flask containing 50 mL of diluent

then diluted to volume (0.5 mg/mL). Standard solution was prepared by diluting 1.0 mL of standard stock solution into a 100 mL volumetric flask containing 50 mL of diluent and then diluted to volume (0.005 mg/mL). The relative standard deviation (% RSD) for the area of AAM peak from six replicate injections of standard solution was evaluated as system suitability with the acceptance criteria of not more than 5.0%. 100 mg of sample was transferred into a 10 mL volumetric flask containing 5 mL of diluent which was then made up to the volume (10 mg/mL). This preparation was kept for stirring for about 30 min, filtered and the resultant filtrate (extract) was used for analysis.

3. Results and discussion

3.1. Development of chromatographic method

Because of the poor UV absorptivity of AAM, development of HPLC method with UV detection for the direct estimation of AAM was not considered in this study. The possibility of developing a GC method with static HS extraction [16] was investigated first. Since SVM compounds are amine salts, AAM also would exist as its corresponding salt. To get extracted in the gaseous form into HS during GCHS analysis, AAM salt was treated with sodium hydroxide in dimethyl sulfoxide medium followed by HS incubation to generate AAM free base. This technique, as expected, generated free AAM but the peak shape was not good. Different types of GC columns, such as, DB624 (6% cyanopropylphenyl-94% dimethyl polysiloxane), DB1 (100% dimethyl polysiloxane) and DBWAX (polyethylene glycol) were studied to improve the peak shape. However, little improvement in the peak shape was found only with DB1 column, but the linearity of area response versus concentration of AAM was inconsistent. Also, the peak observed at lower levels was very broad which resulted in poor signal to noise ratio. These observations suggested to look for a robust and sensitive method, and hence, IC with conductivity detection [13] was selected as an ideal tool.

In IC, since the organic amines could be detected in their protonated form directly, SVM was treated with HCl to extract AAM in its soluble HCl form, and the filtrate collected was subjected to IC analysis. The MSA was selected as mobile phase and Ion Pac CS14 cation column was utilized for better separation. A cation self-regenerating suppressor (CSRS) in recycle mode was used for a better sensitivity of AAM. From the investigations carried out, mobile phase composition was found to be very critical. When 5 mM MSA was used, the retention of AAM was found to be 10.2 min and the peak shape was very broad; but with 15 and 20 mM MSA, the retention time was 5.0 and 4.4 min, respectively. At lower retention times, 5.0 and 4.4 min, however, the interference from blank and sample matrix was high. Interestingly, 10 mM MSA as mobile phase yielded good peak shape with optimum retention of about 6.6 min without any interference. As a part of method development, various experimental parameters of sample preparations such as, extraction time, concentration of extraction solvent (HCl), and stirrer speed were studied to achieve high sensitivity and good precision (Table 1), and the experimental results showing the effect of variations in these parameters on the area response of AAM are represented graphically in Fig. 2. From the results obtained, optimum chromatographic and extraction conditions were finalized for rapid determination (30 min extraction time, 10 mM extraction solvent, 700 rpm stirrer speed). Also, different sample sizes from 5 to 100 mg/mL were studied, and 10 mg/mL was finalized to have consistency/uniformity in extraction, better sensitivity and to avoid filtration issues. As a part of robustness study, flow rate and column oven temperature were investigated, and minor variations in these parameters had not influenced significantly the area response and results. Since, the AAM content in SVM drug substances was less than the detection limit (<2.7 μg/mL), SVM sample spiked with AAM at 0.05% level, relative to the sample concentration (10 mg/mL), was used in all the developmental and validation studies. Typical retention time of AAM was 6.6 min.

Table 1
Extraction conditions used in the variation studies.

Variation	Parameters		
	Extraction time (min)	Concentration of extraction solvent (mM)	Stirrer speed (rpm)
No variation	30	10	700
Extraction time (min)			
Experiment 1	15	10	700
Experiment 2	30	10	700
Experiment 3	60	10	700
Experiment 4	120	10	700
Experiment 5	180	10	700
Extraction solvent (mM)			
Experiment 1	30	5	700
Experiment 2	30	10	700
Experiment 3	30	15	700
Experiment 4	30	20	700
Stirrer speed (rpm)			
Experiment 1	30	10	400
Experiment 2	30	10	600
Experiment 3	30	10	700
Experiment 4	30	10	800
Experiment 5	30	10	1000

3.2. Method validation

To establish the specificity of the method, AAM spiked with common inorganic (viz., sodium, potassium, ammonium) and organic

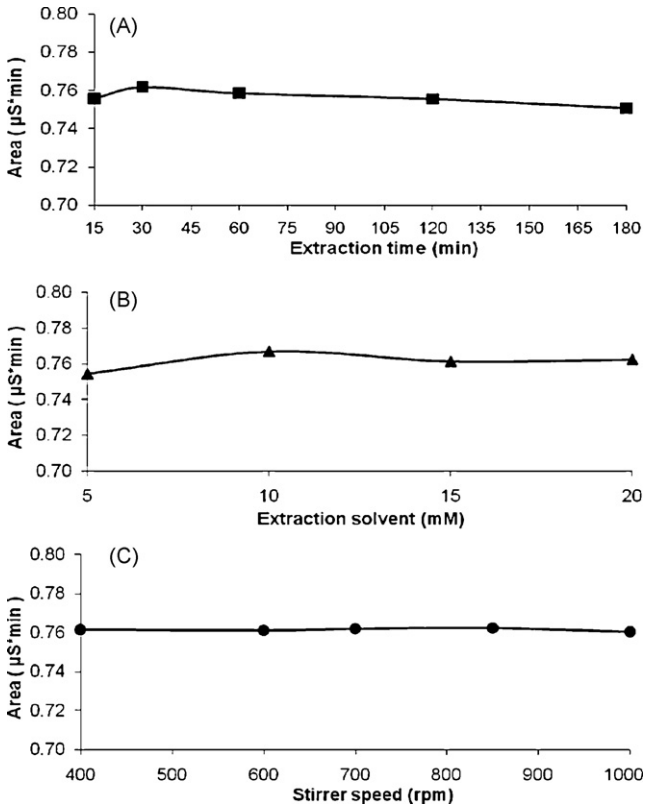

Fig. 2. Influence of variation in extraction time (A), extraction solvent (B), and stirrer speed (C) on the area response of AAM

Table 2
Robustness for SVH and SVC.

Variation	SVH			SVC		
	RT	RSD (%)	AAM[a] (%)	RT	RSD (%)	AAM[a] (%)
No variation	6.660	0.27	0.051	6.530	2.01	0.052
Flow rate (+10%)	5.603	0.41	0.050	5.494	1.10	0.050
Flow rate (−10%)	7.502	0.25	0.051	7.383	1.31	0.048
Column oven temperature (+5 °C)	6.562	0.49	0.051	6.436	1.12	0.050
Column oven temperature (−5 °C)	6.854	1.21	0.050	6.720	1.35	0.050
Mobile phase concentration[b]	6.389	0.23	0.051	6.005	0.88	0.050
Mobile phase concentration[c]	7.316	0.36	0.051	7.057	0.35	0.050
RSD (%)			0.96			2.31

[a] Relative to 10 mg/mL of sample.
[b] 11 mM MSA.
[c] 9 mM MSA.

(viz., triethylamine, propylamine, butylamine) cations was studied. AAM was well resolved from all the above specified cations as illustrated in Fig. 3. Moreover, it is significant to note that no interference from blank and reagents was found at the retention time of AAM. Since peak purity determination by conductivity detection is not feasible, it was demonstrated through mass spectral studies by utilizing IC coupled with mass spectrometer (IC–MS). Hence, mass spectra were collected at upslope and down slope of the AAM peak and compared with the peak apex spectrum [17]. The mass spectra of AAM showed a parent peak at m/z 58 $[M+H]^+$, in the protonated form, corresponding to the molecular formula C_3H_7N, and no significant change in mass spectra was found across AAM peak which proved the homogeneity of the peak. From the IC–MS and IC–CD data, it could be concluded that the proposed method is highly specific. Repeatability was evaluated by injecting AAM standard preparation (0.005 mg/mL) in six replicates. The RSD ($n = 6$) value obtained for the area response and retention time of AAM were 0.27% and 0.03%, respectively. The linearity was performed by measuring area response for AAM over the range of 9 to 750 μg/mL, relative to sample concentration (10 mg/mL). Eight different concentrations were prepared across the range and injected each in triplicate. The mean ($n = 3$) area calculated was plotted against the concentration. The coefficient of determination (r^2) obtained was 0.999. The slope of regression line and y-intercept were 0.00149 and 0.01024, respectively. Accuracy of the method was validated through recovery experiments by spiking known amount of AAM at 0.025%, 0.05% and 0.06% with SVM relative to sample concentration (10 mg/mL). Each preparation was analyzed in triplicate ($n = 3$) and percent recovery was calculated. The recovery was found to be between 100 and 109% with the RSD ($n = 9$) of 2.9 for SVH, and between 97% and 106% with the RSD ($n = 9$) of 3.2 for SVC. The detection (DL) and quantitation (QL) limit for AAM were determined by signal to noise ratio (S/N) method. The DL and QL obtained for AAM were 2.7 and 9.0 μg/mL, respectively, relative to sample concentration (10 mg/mL). A solution containing AAM was prepared around its QL concentration and injected in six replicates. The RSD ($n = 6$) value obtained for the area of AAM at QL was 3.3%. The stability of AAM in solution was studied by measuring the area response of standard preparation (stored at (25 ± 2) °C) injected over a period of 24 h. The RSD ($n = 13$) value obtained for the area response of AAM was 0.5%. The ruggedness of the method was evaluated by performing the analysis of SVM samples in six replicates using two different columns, different analysts on different days. The overall RSD ($n = 6$) obtained for AAM content in SVH and SVC were 1.7 and 2.4%, respectively. To demonstrate the robustness of the method the effect of small but deliberate variations in flow rate, mobile phase composition and column oven temperature were studied. The results, including system suitability test for both SVC and SVH are presented in Table 2. Under all the variations, system suitability requirements were found to be well within the acceptance criteria.

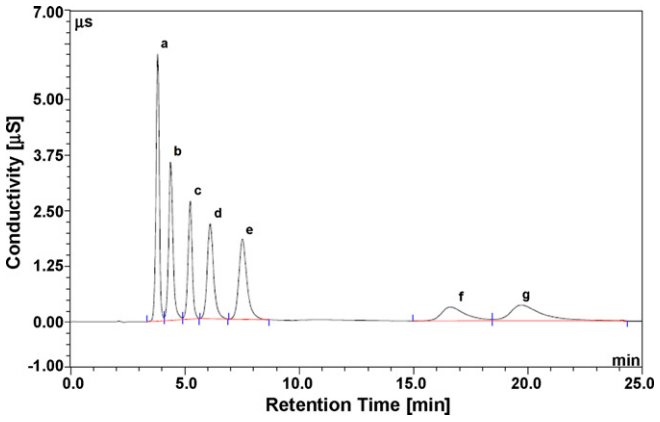

Fig. 3. Chromatogram showing the separation of allylamine (d) from sodium (a), ammonium (b), potassium (c), propylamine (e), triethylamine (f), and butylamine (g).

3.3. Discussion

The IC method with conductivity detection was selected in this work because of the poor UV absorptivity of AAM and inconsistency issues in HS extraction for GC analysis. Previously reported methods [11,12] for AAM determination involved derivatization and thin layer chromatography (TLC) procedures but the proposed IC method with conductivity detection involves direct estimation of AAM without derivatization, and thus it could overcome the difficulties associated with earlier methods. It is significant to note that AAM peak was well separated and its specificity was proved by peak purity studies through IC–MS. Moreover, the presently developed method is rapid with the run time of less than 8 min and robust.

4. Conclusion

The IC–CD method described in this paper was proved to be an ideal tool for the direct estimation of AAM in SVM drug substances at trace levels to comply with the ICH/FDA/EMEA regulatory requirement. Method validation data demonstrated that the developed method is sensitive and accurate for the determination of AAM and robust to minor variations in the chromatographic parameters. The identity and specificity of the AAM peak was well established by IC–MS detection. This method is rapid and simple to adopt. Hence, the proposed IC–CD method can be used conveniently for the routine quality control of AAM in SVM drug substances.

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