ELSEVIER

Journal of Chromatography A, 724 (1996) 199–206

JOURNAL OF CHROMATOGRAPHY A

Determination of alkyl sulfonic acids in pharmaceuticals by ion chromatography

P.L. Annable

Marion Merrell Dow Inc., Marion Park Drive, Park B, Kansas City, MO 64137, USA

First received 23 May 1995; revised manuscript received 2 August 1995; accepted 22 August 1995

Abstract

Ion chromatography (IC) using chemically suppressed conductivity detection was developed to separate and detect methane- through octanesulfonic acids. Using a gradient borate–acetonitrile mobile phase system on a Dionex PAX-500 column, one is able to resolve the alkylsulfonic acids from most matrix interferents. Resolution was determined for all acid pairs from methane- through octanesulfonic acid. A specific application is described using a SARASEP AN1 column with a borate mobile phase for the assay of methanesulfonic acid (MSA) in a sulfated sugar compound under pharmaceutical investigation. MSA is one constituent used in the synthesis of this sulfated sugar compound. The average method recovery for MSA over the six levels investigated was 102.9% and the average method recovery for sulfate was 92.3%. Method reproducibility was determined over a two-day period with a single sample to be 7.5% and 14% for MSA and sulfate, respectively. The IC method was determined to have a linear response for MSA and sulfate over the working range investigated. The limit of quantification was 0.004% and 0.02% (w/w) for MSA and sulfate, respectively. The limit of detection was 0.0005% and 0.001% for MSA and sulfate, respectively.

Keywords: Alkylsulfonic acids; Methanesulfonic acid

1. Introduction

Alkylsulfonic acids are typically used as catalysts, solvents and blocking agents in the synthesis of organic compounds. When used in the synthesis of potential pharmaceutical drug compounds, it is important to monitor the residual levels of these sulfonic acids in the final bulk drug material. In most synthesis cases, only a single alkylsulfonic acid is the subject of analytical concern. Preliminary analytical work was focused on the homologous series of alkylsulfonic acids [methane-(MSA) through octane sulfonic acid (OSA)] in the hope of developing a more universal method. Ideally, the final method would be adaptable in its ability to assay specific sulfonic acids in a given drug matrix. By altering an eluent concentration or adjusting a gradient profile, a specific sulfonic acid could be resolved from any potential matrix interferent such as a common anion or co-sulfonic acid.

In addition to the general separation of the sulfonic acids which was investigated, a specific application was developed for the determination of MSA in a bulk drug material. MSA is used in the synthesis of a sulfated sugar compound under

0021-9673/96/$15.00
SSDI 0021-9673(95)00923-X

investigation as a drug candidate. Monitoring of residual MSA levels in the final bulk material ensures the purity of the drug compound.

Numerous methods utilizing a variety of techniques have been developed to determine MSA levels in various sample matrices. In part, these methods include the following: ion exchange [1], liquid chromatography (reversed-phase (RP) with indirect detection) [2], titration (alkalimetric with color indicator) [3] and laser microprobe mass analysis (LAMMA) [4]. Each method has its merits given the sample type and matrix involved; however, none addressed the specific homologous series investigated in this work.

A simple chromatographic method was desired that would assay the level of MSA in a pharmaceutical bulk drug material, while also allowing the determination of sulfate present in the same sample. If a general IC gradient method existed which addressed the separation of the sulfonic acid series from MSA to OSA, then method development of any specific sulfonic acid would be expedited. This "general IC gradient" method would also be adaptable to synthesis route changes during the drug development process. Therefore, a general gradient method that demonstrated selectivity for the alkylsulfonic acid homologous series was the initial goal of this work.

In summary, methodology is presented which is capable of separating the homologous series of alkylsulfonic acids (MSA through OSA). Also, a specific application of the proposed IC gradient method is presented for determining both MSA and sulfate in a bulk drug material in a single injection. Method validation data are also presented.

2. Experimental

2.1. Instrumental

All IC work was performed on a Dionex BioLC ion chromatograph. The pump system employed for the isocratic and gradient work was a dual-head microprocessor controlled gradient pump module (GPM) from Dionex. A Dionex pulsed electrochemical detector (PED) in the conductivity mode was used to measure analyte conductivity after chemical suppression of the background signal. Two different columns were utilized. A Dionex PAX-500 was selected for the general gradient work and a SaraSep AN1 was used for the specific assay of MSA in the sulfated sugar. Chromatographic system I (Table 1) was employed for the final MSA/sulfate method validation, while chromatographic system II was utilized for all general gradient work.

Both carbonate and borate salt gradient eluent systems were employed with the PAX-500 column (chromatographic system II). With the exception of MSA and ethanesulfonic acid (ESA), either mobile phase is capable of separating the entire sulfonic acid series (MSA through OSA) in approximately 15 min. Additional work was completed to optimize the separation of MSA and ESA.

2.2. Reagents

All reagents were employed as received from the various manufacturers. MSA, ESA, OSA and sulfate standards were prepared from the sodium salts (Fluka). Propane-, butane-, pentane-, hexane- and heptanesulfonic acids were also prepared from their sodium salts (Aldrich). Stock solutions were prepared in polypropylene volumetric flasks and were found to be stable for a minimum of 1 week. Serial dilutions of the stock solutions at appropriate levels were made to obtain working standard solutions. A system suitability standard containing MSA and chloride was used to monitor injection precision and resolution prior to sample analyses.

2.3. Sample preparation

Sample preparation consisted of extracting the MSA and sulfate from the bulk drug material using distilled, deionized (DDI) water. A horizontal shaker was found to give the best solution/suspension agitation during the extraction step of the method. The low solubility of the sulfated sugar compound in the DDI water versus the high solubility of the MSA and sulfate

Table 1
Chromatographic systems I and II

Parameter	System I		System II
Ion chromatograph		Dionex BioLC or equivalent system (capable of operating a binary gradient program)	
Separator column	SaraSep AN1		Dionex PAX-500
Supressor column		Dionex anion micromembrane suppressor	
Detection		Chemically suppressed conductivity	
Flow-rate		1.0 ml/min	
Temperature		Ambient	
Eluent system	(A) 17 m*M* $Na_2B_4O_7$		(A) 5 m*M* $Na_2B_4O_7$–5% acetonitrile
	(B) 30 m*M* $Na_2B_4O_7$		(B) 20 m*M* $Na_2B_4O_7$–40% acetonitrile
Gradient profile	Time	A (%)	B (%)
	0	100	0
	10	0	100
	15	0	100
Injection volume		20 μl	
Regenerative solution		12.5 m*M* sulfuric acid	

permitted simple extraction. All samples were filtered through a 0.45-μm filter prior to injection into the IC system.

2.4. Data analysis

All chromatographic data collection and analysis were accomplished through the Dionex application software. All figures presented here were obtained by exporting ASCII data files from the Dionex software into Orgin, a graphics application.

3. Results and discussion

3.1. Alkylsulfonic acids

IC was selected as the technique of choice for assaying MSA and sulfate in the bulk drug material for various reasons. MSA and sulfate will exist as anions at pH > 7 and have aqueous solubility. Since the sulfated sugar compound had limited water solubility, the MSA and sulfate could be extracted into an aqueous phase and injected directly into an anion-exchange chromatographic system. MSA and sulfate have greatly different retentions as they are mono- and divalent anions, respectively.

Isocratic separations using 0.5–2 m*M* carbonate and 1–5% acetonitrile were investigated on a mixed-mode (ion-exchange/reversed-phase) column. These systems either did not resolve fluoride, MSA and chloride, or sulfate eluted too late to be analytically useful. The required eluent strengths to elute sulfate in a reasonable period of time made it impossible to resolve the early-eluting species, including MSA. Gradient ion chromatography allowed the elution of sulfate in less than 20 min while also achieving baseline resolution of MSA from the early-eluting common anions such as chloride.

The Dionex columns, PAX-500 and the AS4A, and a SaraSep AN1 column were evaluated. The columns are made with polystyrene–divinylbenzene (PSDVB) supports, with the active sites on the Dionex columns being aminated latex beads, while the SaraSep column possesses an alkyldimethylethanolammonium functionality. The differences in the two Dionex columns are primarily due to the degree and type of cross-linking of the PSDVB supports. The PAX-500 column is solvent compatible, which was found to be beneficial to elute quickly alkylsulfonic acids with carbon chains longer than three. The primary use of the PAX-500 column is for mixed-mode separations, or separations based on both reversed-phase and ion-exchange mechanisms. The AS4A and the AN1 columns possess very similar anion-exchange characteristics.

Initial gradient work was performed using the more traditional IC carbonate eluent system. MSA and ESA could not be resolved using this eluent system and therefore the focus of the work was switched to the borate eluent system.

Fig. 1 is a representative chromatogram of the separation of MSA through OSA on a PAX-500 column. The eluent system used for this separation utilized a carbonate and acetonitrile gradient. It should be noted that numerous variations of this eluent system were attempted, including 0.5–1 m*M* carbonate and 1–40% acetonitrile, in efforts to resolve MSA from ESA. All were unsuccessful, and a weaker initial ion-exchange eluent was required.

A similar elution order and resolution as seen in Fig. 1 were obtained on the PAX-500 column using a borate eluent system. Using chromatographic system II, a resolution table (Table 2) was created for the various sulfonic acid pairs. Excluding MSA and ESA, all the remaining sulfonic acids studied were fairly well resolved as defined by having a resolution factor greater than 1.5. In addition, this chromatographic system could separate the sulfonic acids (MSA through OSA) from several of the common anions such as fluoride, chloride, nitrate and sulfate. Unsuccessful attempts were made with this system to resolve MSA and ESA while still eluting OSA acid in a reasonable time.

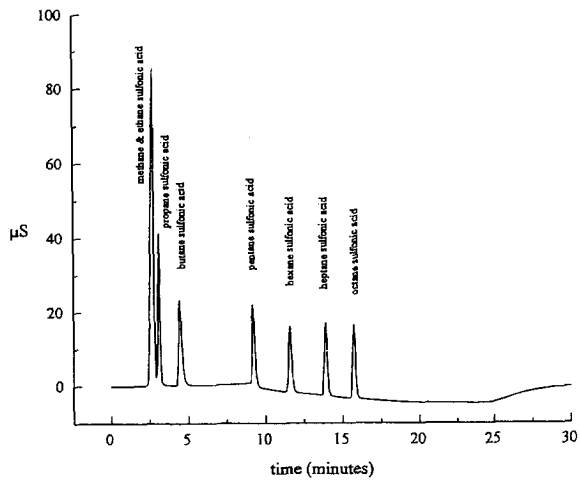

Fig. 1. Separation of alkylsulfonic acids. Conditions: ion chromatograph, Dionex BioLC; Columns: separator, Dionex PAX-500; suppressor, Diones anion micromembrane suppressor; detection, chemically suppressed conductivity; flow, 1.0 ml/min; temperature, ambient; eluent: A, 0.5 m*M* sodium carbonate, 1% ACN; B, 10 m*M* sodium carbonate, 40% ACN; gradient profile:

time	A%	B%
0	100	0
15	0	100
20	0	100

Injection, 20 μl; regenerative solution, 25 m*M* sulfuric acid.

Table 2
Resolution of sulfonic acid pairs (chromatographic system II)

Sulfonic acid	k'	R_s
Methanesulfonic acid	2.3	Not resolved
Ethanesulfonic acid	2.3	1.7
Propanesulfonic acid	2.9	4.7
Butanesulfonic acid	3.8	3.7
Pentanesulfonic acid	4.8	3.9
Hexanesulfonic acid	5.6	3.1
Heptanesulfonic acid	6.1	2.2
Octanesulfonic acid	–	–

3.2. Methane- and ethanesulfonic acids

By focusing solely on the MSA–ESA pair, separation was achieved using a very mild borate–acetonitrile gradient system. Fig. 2 shows a resolution map for the separation of MSA and ESA. It can be seen that the optimum resolution was found with ESA having a k' of ca. 15. This corresponds to an eluent composition of ca. 2.5 mM tetraborate and 10% (v/v) acetonitrile on the PAX-500 column at a flow-rate of 1 ml/min. These chromatographic conditions were found to be very useful for the separation of MSA and ESA, but were not useful for the later eluting sulfonic acids. The stronger previously described gradients should be employed for the separation of propane-through octanesulfonic acids.

Fig. 3 is a representative chromatogram for the optimum separation of MSA and ESA on the PAX-500 column. It can be seen that the elution order is reversed. This elution order "reversal" with MSA and ESA is attributed to competition in the reversed-phase mode of the mixed-mode mechanism of the PAX-500 column. Size exclusion may also be a contributing factor in the overall mechanism. Additional work exploring stationary phases with different pore sizes, solvent systems with different selectivities and non-solvent-based eluent systems (100% aqueous) are planned to explain this phenomenon further.

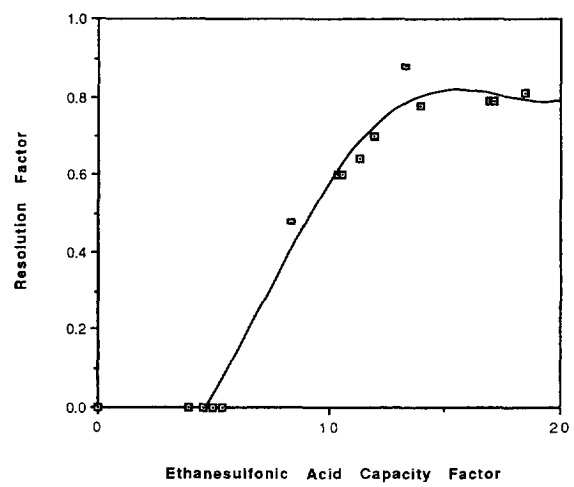

Fig. 2. Resolution map for MSA and ESA. Chromatographic System II.

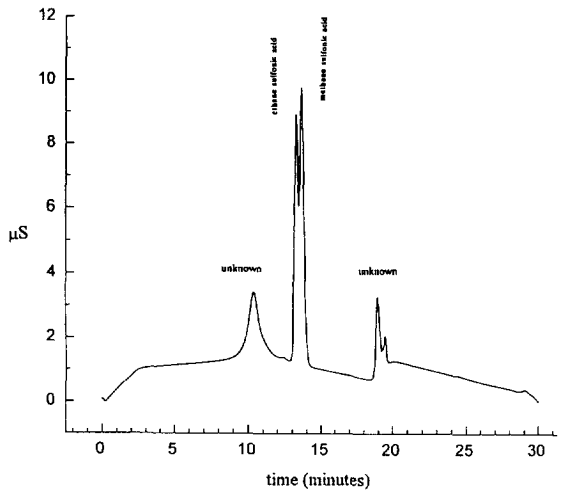

Fig. 3. Separation of MSA and ESA. Conditions: ion chromatograph, Dionex BioLC; columns: separator, Dionex PAX-500; suppressor, Dionex anion micromembrane suppressor; detection, chemically suppressed conductivity; flow, 1.0 ml/min; temperature, ambient; eluent: A, 0.1 mM sodium tetraborate, 5% ACN; B, 10 mM sodium tetraborate, 20% ACN; gradient profile:

time	A%	B%
0	99	1
25	1	99

Injection, 20 μl; regenative solution, 25 mM sulfuric acid.

3.3. Method validation results

Initially, the typical eluent system (carbonate–hydrogen carbonate) used with chemically suppressed conductivity detection anion-exchange systems was tested. Even with large dilutions (less than 1 mM carbonate–hydrogencarbonate), this eluent system did not efficiently provide the required resolution of chloride and MSA. An eluent with a weaker elution strength was required to separate the early-eluting species (monovalent anions). A tetraborate eluent system at low concentrations has been shown [5] to be an excellent eluent for resolution of the early-eluting anions when using the types of columns described. Two columns were investigated with

the borate eluent systems, the Dionex AS4A and the SaraSep AN1. Initial gradient conditions of 10–20 m*M* tetraborate enabled chloride to be baseline ($R_S = 2$) resolved from MSA, while still eluting them in under 15 min. A higher tetraborate concentration (>20 m*M*) is required to elute sulfate in less than 20 min. Analysis times >20 min are generally not acceptable for methods that are transferred to the Quality Assurance (QA) Department owing to the large number of samples assayed daily.

Transferring a method to the QA Department requires an analytical validation of the IC method. The validated IC system consisted of a SaraSep AN1 column with a salt gradient profile of 17 to 30 m*M* tetraborate over a 10-min period followed by holding the eluent concentration constant at 30 m*M* tetraborate for an additional 5 min at a flow-rate of 1 ml/min (see system I). Acceptable parameters were measured to ensure an accurate and reproducible analytical method.

System suitability requirements consisted of determining the injection precision ($n = 5$) of MSA and sulfate at levels equivalent to the proposed specification limits, and also in the system suitability was a chloride standard which gave a peak height response approximately equivalent to that of the MSA standard. By including the chloride in the system suitability standard, one is able to determine the resolution of factor (R_S) for chloride and MSA, and the injection precision. A minimum resolution factor of $R_S = 2$ is obtainable using the described chromatography system. The peak area R.S.D.s were 5% and 10% for MSA and sulfate, respectively. Fig. 4 is an example chromatogram of a MSA and sulfate standard from which the resolution of MSA–chloride can also be seen. An actual chromatogram from a sulfated sugar sample is shown in Fig. 5.

Linearity plots were constructed for both MSA and sulfate dissolved in water. In both cases over the concentration ranges investigated the peak area responses were found to be linear with correlation coefficients of 0.99999 for MSA and 0.9999 for sulfate. For MSA and sulfate determinations in the sulfated sugar within the ranges investigated, a single-point calibration is possible.

From calibration plots, limits of detection (LOD) and limits of quantification (LOQ) can be calculated. For each standard ($n = 4$), the R.S.D. of the resulting peak areas was calculated and plotted against the standard concentration, creating a parabolic limit of quantification plot. The LOQs were calculated from the intersection of tangents drawn from the LOQ plot. The LOD was calculated as the equivalent analyte concentration peak height at twice the signal-to-noise ratio. LODs for MSA and sulfate were 5 and 10 ppm (in sample), respectively, and LOQs were 40 and 200 ppm (in sample), respectively.

Method reproducibility was determined over a two-day period by assaying a single sulfated sugar raw material batch for MSA and sulfate. In addition to the day-to-day variance studied, two different analytical columns and mobile phase solutions were used to ensure reproducibility of the final method. On each day four separate samples were assayed and the R.S.D.s determined for the final MSA and sulfate concentrations. Average R.S.D.s were 7.5% and 14% for MSA and sulfate, respectively, over the two-

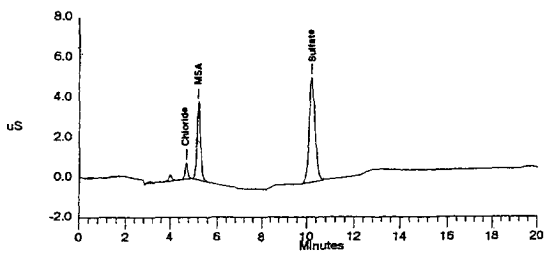

Fig. 4. Chromatographic separation of MSA standard (10.3 ppm) and sulfate standard (10.5 ppm) (working concentrations). Chromatographic system I.

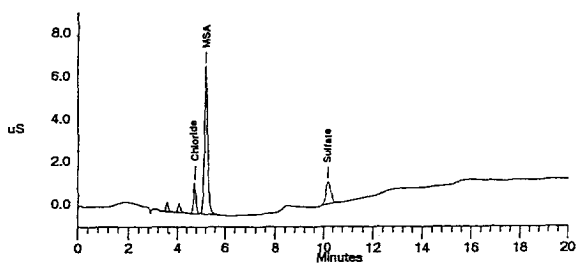

Fig. 5. Chromatogram of 0.01 g/ml sulfate sugar sample extracted with mobile phase. Chromatographic system I.

day period. Given the working levels of the MSA and sulfate in these samples, ca. 20 and 2 ppm, respectively, the determined R.S.D.s of their concentration over the two-day period are reasonable.

As part of the method validation procedure, experiments were performed to determine the most efficient mechanical action (horizontal shaker or vortex mixer) to extract the analytes from the sulfated sugar matrix. Results based on analyte recovery showed that the horizontal shaker was the more efficient. Studies also indicated that samples must be shaken on the horizontal shaker for a minimum of 1 h (see Fig. 6). This part of the method is only time and not labor intensive. All samples were filtered through a 0.45-μm filter prior to injection.

Additional efforts were made to eliminate chloride from the sample matrix through sample preparation to shorten the overall method run time. Experiments were performed using a solid-phase extraction (SPE) column (silver form, Dionex) to remove selectively all chloride from sample solutions prior to injection into the IC system. The sample chromatograms obtained after the AgSPE pretreatment showed complete removal of the chloride typically present in the sulfated sugar samples, but a large sulfate contamination occurred. It was assumed that sulfate was being leached from the SPE column. This was not acceptable for the final quantification of sulfate in the sulfated sugar samples. If these experiments had been successful then perhaps an isocratic system could have been employed. This not being the case, a gradient profile was selected which would resolve chloride from MSA while still eluting sulfate in less than 20 min.

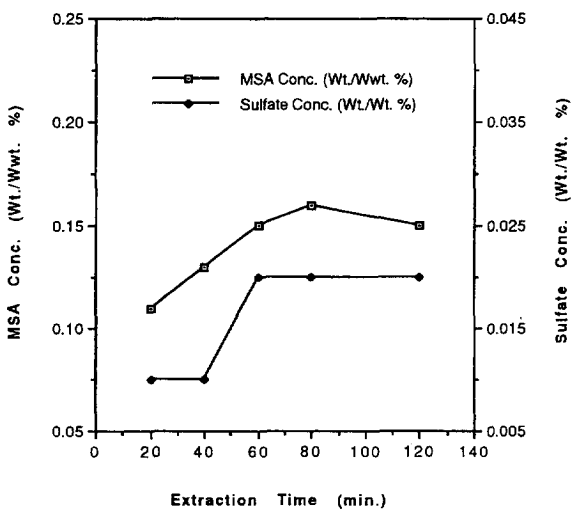

Fig. 6. Extraction profile of (□) MSA and (◆) sulfate using a horizontal shaker. Sample concentration, 0.01 g/ml sulfated sugar.

Method recovery experiments were performed for both MSA and sulfate. Samples were spiked with aliquots from stock solutions of the analytes of interest and assayed, which enabled the analyte recovery from the bulk drug sample matrix to be determined. Gross inconsistencies in analyte recoveries would indicate possible analyte–sample interactions. The samples were spiked at levels which would be the equivalent of 80%, 100% and 120% of a specified allowable limit. The average method recovery for MSA over the six levels investigated was 102.9% and the average method recovery for sulfate determined in a similar fashion over the three levels investigated was 92.3%.

4. Conclusion

MSA is a potential synthesis impurity in a sulfated sugar bulk raw material under pharmaceutical investigation, and a method was desired to monitor the level of MSA in the raw material. An IC method has been developed which has been shown to be precise, accurate and durable for determining MSA in the raw material. In addition to the MSA concerns, sulfate has been used as a control parameter over the quality of the sulfated sugar raw material. In an effort to minimize the analysis time, a gradient IC method has been developed which is capable of determining MSA and sulfate in bulk drug material in a single injection.

Validation data have been presented which demonstrate the integrity of the proposed IC method. Method linearity, reproducibility and accuracy were determined using a sample lot of the bulk drug. Sample preparation was optimized by investigating the extraction efficiencies of

several methods of extraction. The solid–liquid extraction technique proposed has been shown to be both reproducible and complete. Method ruggedness has been determined by the use of two different columns and mobile phase preparations incorporated into the method validation experiments.

The use of gradient IC to assay two differently charged ions (monovalent MSA and divalent sulfate) has proved to be a good method. The method should be incorporated into the testing scheme of the sulfated sugar raw material.

For general information on this subject see Refs. [6–8].

References

[1] V. Cheam, J. Chromatogr., 482 (1989) 381–392.
[2] D.J. Pietrzyk, P.G. Rigas and D. Yuan, J. Chromatogr. Sci., 27 (1989) 485–490.
[3] V. Zaharans, V. Dudins and A. Avots, Latv. PSR Zinat. Akad. Vestis, Kim. Ser., (1988) 706–711.
[4] L.N. Kolaitis, F.J. Bruynseels, R.E. Van Grieken and M.O. Andreae, Environ. Sci. Technol., 23 (1989) 236–240.
[5] P.L. Annable and L.A. Sly, J. Chromatogr., 546 (1991) 325–334.
[6] H. Small, T.S. Stevens and W.C. Bauman, Anal. Chem., 47 (1975) 1801.
[7] A. Rychtman, LC·GC, 7 (1989) 508.
[8] R. Williams, J. Chromatogr. Sci., 20 (1982) 560.