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Short Communication

Separation and purification of azetidinyl methyl sulfinates using preparative high-performance liquid chromatography

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ABSTRACT

An improved method for the separation and purification of azetidinyl methyl sulfinates is described. This method utilizes a Waters preparative high-performance liquid chromatographic set-up. The methyl sulfinate isomers are isolated in high purity and no double bond isomerization is observed to occur. The diastereomers eluted from both the analytical and preparative columns at approximately the same retention times under the conditions used in the method.

INTRODUCTION

In our laboratories a method for determining the purity of azetidinyl sulfinyl chloride (**1**) involves quenching it with methyl alcohol to yield ($\alpha\beta$,2β,3β)-2-(methoxysulfinyl)-α(1-methylethenyl)-4-oxo-3-(phenoxyacetylamino)-1-azetidineacetic acid, (4-nitrophenyl)methyl esters **2** and **3**[a] which are then quantitated by high-performance liquid chromatography (HPLC). The success of this method depends on having azetidinyl methyl sulfinates **2,3** of known purity for use as reference standards. Being able to separate and purify each diastereomer is also desirable so that the UV response of each isomer can be measured and compared.

Previous workers have attemped to purify **2,3** using flash chromatography [1] and preparative HPLC [2]. However these procedures did not separate the isomers or completely remove all of the impurities. Furthermore both of these methods utilized columns containing normal-phase silica, which caused partial isomerization of the isopropenyl bond of **2,3** to give **4,5** (refer to Fig. 1). To further complicate matters the double bond isomers were found to coelute with **2,3** under their separation conditions. These results prompted us to investigate the application of a preparative HPLC method utilizing a reversed-phase column for separating and purifying **2,3**.

EXPERIMENTAL

Materials

The acetonitrile, methyl alcohol, and tetrahydrofuran were HPLC grade. The water was deionized and all other chemicals were reagent grade.

[a] Azetidinyl sulfinyl chloride **1** is a single diastereomer whose configuration about the sulfur atom is unknown. The methyl sulfinates **2,3** are a pair of diastereomers that are epimeric at the sulfur atom.

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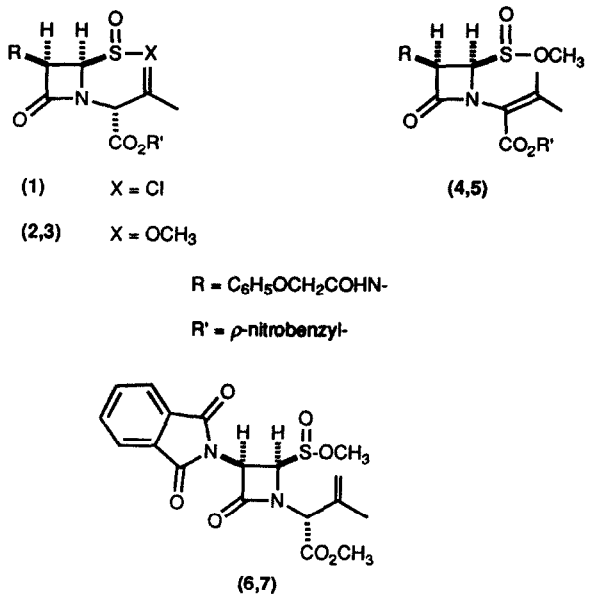

Fig. 1. Structures of azetidinyl sulfinyl chloride and azetidinyl methyl sulfinates.

Analytical HPLC

The analytical HPLC system was composed of a Waters 600 multi-solvent delivery system (with 225-μl pump heads), a Rheodyne Model 7125 valve, a Kratos Spectroflow 757 absorbance detector, and a Hitachi Model D-2000 chromato-integrator. The system was operated at room temperature at a flow-rate of 3 ml/min and the peaks were monitored at 300 nm. The mobile phase was a mixture of acetonitrile–0.2% (v/v) glacial acetic acid in water (45:55). A Prep Nova-Pak HR C_{18} cartridge column (100 mm × 8 mm I.D., 6 μm particle size) was used.

The following system was used to determine the purity of the azetidinyl methyl sulfinates after purification: Waters 600 multi-solvent delivery system, Rheodyne Model 7125 valve, Kratos Spectroflow 757 absorbance detector, and Hitachi Model D-2000 chromato-integrator. The mobile phase was a mixture of methyl alcohol–tetrahydrofuran–0.02 % trifluoroacetic acid in water (50:5:45). The flow-rate was 1.0 ml/min and the column temperature was 50°C. The peaks were monitored at 260 nm.

Preparative HPLC

The same Waters 600 multi-solvent delivery system as described above was used except that all sections of tubing between the pump and the detector outflow were replaced with 16 mm O.D., 1.02 mm I.D. stainless-steel tubing. The L-Series flowcell, 12-μl volume in the Kratos Spectroflow 757, was replaced with an L-Series flowcell of 2.5 μl and 3 mm pathlength. A Prep Nova-Pak HR C_{18} cartridge column (100 mm × 25 mm I.D., 6 μm particle size) was used. The flow-rate was 30 ml/min. A 10.0-ml sample loop (16 mm O.D., 1.02 mm I.D. stainless-steel tubing) was used. The mobile phase and detector wavelength were the same as those for the analytical set-up.

Purification procedure

Methyl alcohol was added to a toluene solution of the azetidinyl sulfinyl chloride [3] and the solvent was evaporated under reduced pressure to afford an orange, gummy solid. This solid was then dissolved in the mobile phase and injected into the preparative HPLC system at a 50 to 100-mg loading level. The collected fractions were concentrated under reduced pressure to remove acetonitrile and then extracted with methylene chloride. The methylene chloride extracts were washed with saturated brine and then dried by stirring over magnesium sulfate. The fractions were then evaporated under reduced pressure to afford colorless, amorphous solids.

RESULTS AND DISCUSSION

*Purification of **2,3** via preparative HPLC*

After failing to purify and separate **2,3** *via* flash chromatography or a Waters Prep 500, we turned our attention to an alternative means of purification. An analytical HPLC method was developed for separating **2,3** *via* a Waters Prep Nova-Pak HR C_{18} column. This method provided good peak shapes and allowed for a good separation of **2** from **3** and from the other impurities present in the sample. However, our main interest in this method was to employ it as a basis for developing a preparative-scale method for separating and isolating **2,3**.

The preparative-scale method was easily accomplished by making some minor modifications of the equipment and substituting a larger, preparative-scale column for the analytical one. Thus, the original pump and detector could be utilized for both analytical and preparative purposes. In this method

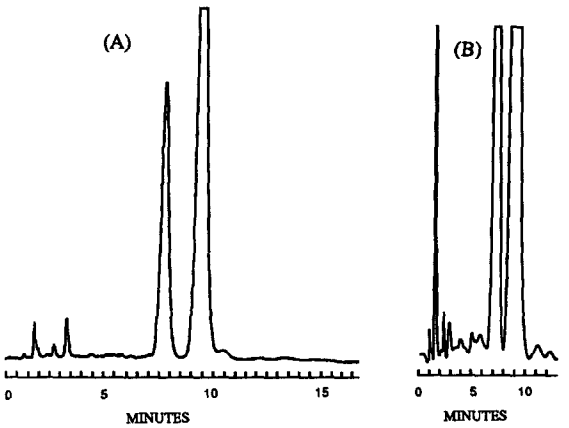

Fig. 2. HPLC of crude methyl sulfinates **2,3** on the analytical column (A) and the preparative column (B). The diastereomers **2,3** eluted at 7.68 and 9.45 min on the analytical column (column: 100 mm × 8 mm I.D., Prep Nova-Pak HR C_{18}; sample: 1 mg/ml, 20-μl injection; flow-rate: 3 ml/min) and 7.19 and 8.91 min on the preparative column (column: 100 mm × 25 mm I.D., Prep Nova-Pak HR C_{18}; sample: 100 mg/ml, 1-ml injection; flow-rate: 30 ml/min).

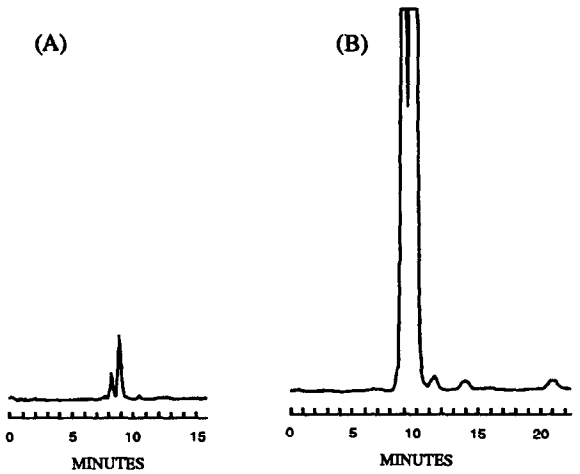

Fig. 3. HPLC of crude methyl sulfinates **6,7** on the analytical column (A) and the preparative column (B). The diastereomers **6,7** eluted at 8.14 and 8.79 min on the analytical column (column: 100 mm × 8 mm I.D., Prep Nova-Pak HR C_{18}; sample: 1mg/ml, 20-μl injection; flow-rate: 3 ml/min) and 8.62 and 9.91 min on the preparative column (column: 100 mm × 25 mm I.D., Prep Nova-Pak HR C_{18}; sample: 50 mg/ml, 1-ml injection; flow-rate: 30 ml/min).

the larger column allowed for a ten-fold increase in the flow-rate. At this higher flow-rate the sample components eluted at 7.19 and 8.91 min on the preparative column and 7.68 and 9.45 min on the analytical column (see Fig. 2). Another advantage of this method was that it utilized a column containing reversed-phase silica that was less likely to cause double bond isomerization of **2,3**, because of the non-polar nature of this stationary phase.

In a typical preparative run, 50–100 mg of the **2,3** reaction mixture was loaded onto the column. At this loading rate excellent separation of the desired components was effected. As can be seen in Table I, the methyl sulfinates **2,3** were separated and recovered in purities exceeding 94%. We saw no evidence of double bond isomerization occurring during the run. Also, HPLC showed that the purified methyl sulfinates did not contain the double bond isomers **4,5**.

No attempt was made to optimize the loading rate of **2,3** on the column. Our interest was in obtaining highly purified methyl sulfinates for use as analytical reference standards. However, because a

TABLE I

PURIFICATION OF METHYL SULFINATES

Substrate	Amount purified[a] (g)	Amount recovered (mg)		Total recovery (%)	Purity[b] (%)	
		4 or **8**[c]	**5** or **9**		**4** or **8**[c]	**5** or **9**
4,5	1.0	195	570	76.5	97.6	97.6
4,5	1.5	220	620	56.0	94.9	95.1
8,9	1.2	320	523	70.2	95.6	95.5

[a] Weights are pooled amounts from 50–100-mg injections.
[b] Determined by HPLC.
[c] Early-eluting component arbitrarily assigned as **4** or **8**.

typical preparative run lasted only 10–15 min, it was possible to accumulate quantities of material in a reasonable amount of time by making multiple injections of the reaction mixture.

The purified diastereomers were successfully employed as reference standards in an HPLC procedure developed to quantitate the yield of azetidinyl sulfinyl chloride **1**.

Purification of ***6,7***

The Waters preparative method was extended to the separation and purification of methyl sulfinates **6,7** [3]. Because **6,7** are less lipophilic than **2,3** the solvent composition had to be modified so that a good separation of the components could be effected[a]. Even though the two components were only slightly separated (see Fig. 3) purified samples of each component were obtained (see Table I). As with the purification of **2,3** no evidence of double bond isomerization was observed during the purification of **6,7** when the column containing reversed-phase silica was utilized.

[a] The mobile phase used in this separation was acetonitrile–0.2% (v/v) glacial acetic acid in water (30:70).

CONCLUSIONS

An improved method for the separation and purification of azetidinyl methyl sulfinates has been developed. This method utilizes a Waters 600 multisolvent delivery system that can be easily modified to perform both analytical and preparative-scale HPLC runs. The methyl sulfinate distereomers can be separated with good resolution in a single run using the method. No double bond isomerization is observed to occur and the diastereomers are isolated in high purity. The components of the crude mixture have approximately the same retention time on both the analytical and preparative columns.

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