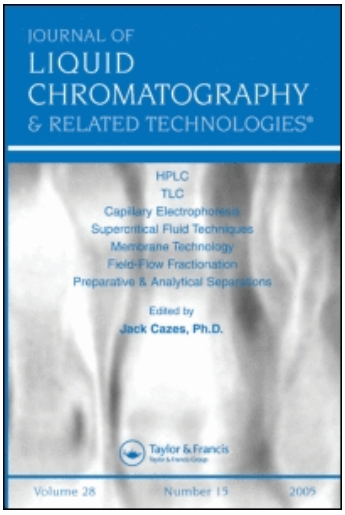

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Determination of Sulfonic Acids Using Anion-Exchange Chromatography with Suppressed Conductivity Detection

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Abstract: Accurate determination of low molecular weight sulfonic acids including cysteic acid, homocysteic acid, and taurine in aqueous solution is essential in many analyses of biological and clinical applications. These sulfonic acids are difficult to be separated and determined using reversed phase chromatography and cation-exchange chromatography. In this research, anion-exchange chromatography with suppressed conductivity detection is utilized in the separation and determination of these compounds. This technique has achieved excellent separation for these sulfonic acids. Among the three sulfonic acids, the calibration for taurine has demonstrated the poorest accuracy, the lowest sensitivity, narrowest linearity, and poorest limit of detection, as well as reproducibility. The inadequacy of this technique to quantify taurine is due to the low conductivity of taurine in aqueous solution. The characteristics of calibration curves for homocysteic acid and cysteic acid are exceptionally perfect. This technique has demonstrated to be excellent for accurately determining cysteic acid and homocysteic acid.

Keywords: Sulfonic acids, Anion-exchange chromatography, Suppressed conductivity detection, Limit of detection, Limit of quantitation, Linearity, Correlation coefficient, Sensitivity

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INTRODUCTION

Cysteic acid, homocysteic acid, and taurine have been shown to be toxic to human and rat neuronal cell lines.[1] These three sulfonic acids are found to be elevated in the plasma of patients with uremia[2] and migraine.[3] Cysteic acid and homocysteic acid have also demonstrated to be potent agonists of excitatory amino acid receptors in the mammalian central nervous system.[4] These two sulfonic acids are generated endogenously from oxidation of cysteine and homocysteine, respectively. The third sulfonic acid, taurine, is indispensable during mammalian development. However, the level of urinary taurine excretion has been also linked to ischemic heart disease,[5] as well as hepatic dysfunction and biochemical perturbation.[6] Taurine is mainly produced through human biosynthesis from methionine and cysteine.

Accurate determination of these low-molecular-weight sulfonic acids is important in many biological and clinical applications. The current methodologies for determining these sulfonic acids adopt the techniques being used for amino acid analysis. High performance liquid chromatography (HPLC) with pre-column derivatization and fluorescence detection,[7] HPLC coupled with electrochemical detection,[8] and ion-exchange HPLC with post-column derivatization and spectrometric detection[9] are used to determine the concentrations of amino acids. Gas chromatography-mass spectrometry[10] and ion-pair liquid chromatography combined with mass spectrometry[11] have also been used to analyze these three sulfonic acids in aqueous samples.

The authors have used ion-pair liquid chromatography and cation-exchange liquid chromatography with post-column derivatization to determine these three sulfonic acids in oxidized plasma samples.[12] However, a complete separation cannot be achieved due to the high and similar acidity of these three sulfonic acids. In this research, anion-exchange liquid chromatography with a suppressed conductivity detection, which serves as a universal detection mode for charged species, is utilized to separate and determine these three sulfonic acids. The processes to validate this analytical method including accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, sensitivity, and correlation coefficient of calibration curves for these three sulfonic acids are studied and compared.

EXPERIMENTAL

Chemicals and Reagents

The chemicals, including L-cysteic acid, DL-homocysteic acid, taurine, and sodium hydroide (NaOH, 50%) were obtained from Aldrich-Sigma

(St. Louis, Missouri). The eluent for anion-exchange chromatography was NaOH solutions with concentrations ranging from 15 to 50 mM, which were prepared from Aldrich-Sigma 50% NaOH solution. A series of standard solutions, which contain the three sulfonic acids ranging from 1 to 160 ppm were prepared into aqueous solution. The water used to prepare reagents was ultra pure water (18 mega-ohm/cm) produced from a Hydro's Picosystem (Levittown, Pennsylvania).

Chromatographic System

The system used for separating these sulfonic acids was a Dionex Bio LC system (Sunnyvale, California). A quantity of 25 μL sample solution was injected by a Dionex AS50 autosampler into the chromatographic system, which furnished an IonPac AG18 guard column (2 × 50 mm) and an IonPac AS18 analytical column (2 × 250 mm) isothermally controlled at 30°C in a column oven. The sulfonic acids were separated in the column and carried to a Dionex ASRS-ULTRA suppressed conductivity detector with a current setting at 50 mA for detection. The system was controlled and run by Dionex Chromeleon software (version 6.50). A variety of NaOH solutions with different concentrations were tested on the system to optimize the chromatographic parameters for separation and quntitation.

RESULTS AND DISCUSSION

The optimization of chromatographic parameters, including concentration and flow rate of eluent are studied. The baseline-separation between homocysteic acid and cysteic acid cannot be easily obtained when running the chromatograph at high concentration and high flow rate of eluent. On the other hand, the analysis time becomes too long when the concentration and flow rate of eluent are low. The peak shape starts to tail when the retention time is too long. A baseline-separation for the three sulfonic acids is achieved in a 10-minute run using 25 mM NaOH as eluent, running at a flow rate of 0.25 mL. Thus, the eluent of 25 mM NaOH and a flow rate of 0.25 mL/min are used in the determination of these sulfonic acids.

A typical chromatogram, which demonstrates an excellent separation for taurine, homocysteic acid, and cysteic acid, is illustrated in Figure 1. A water dip shown at a retention time of 2.90 minutes is common in ion-chromatographic analysis using conductivity detection. A peak at a retention time of 4.73 minutes has proven to be taurine. A weak peak at a retention time of 5.85 minutes is identified as carbonate, which is produced due to the absorption of carbon dioxide from air into the reagent solutions. Two adjacent peaks with baseline-separation at retention times of 7.22

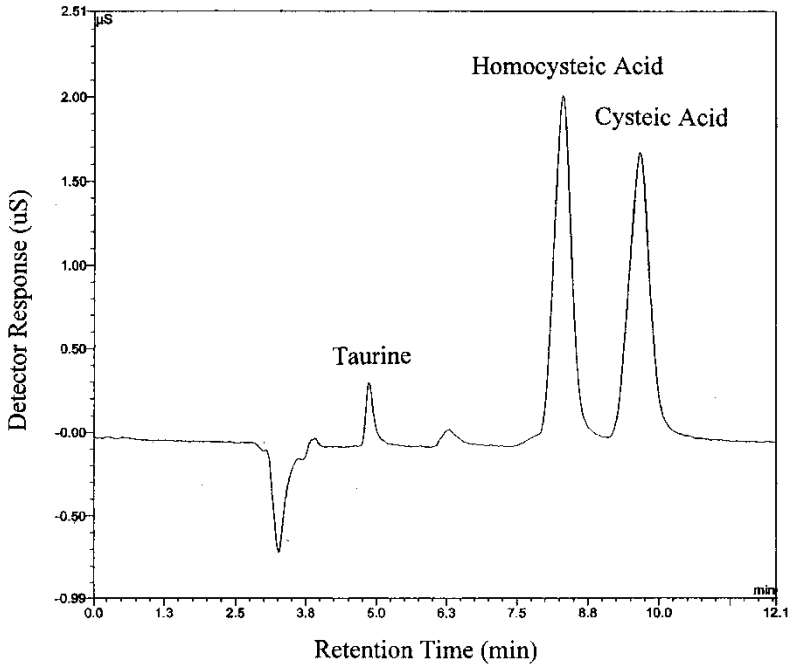

Figure 1. A typical chromatogram of separated taurine, homocysteic acid, and cysteic acid.

minutes and 8.26 minutes are identified as homocysteic acid and cysteic acid, respectively. The early elution of taurine is due to the weak affinity of the sulfonate ion of taurine and the stationary phase. In addition to sulfonate group, cysteic acid and homocysteic acid have a carboxylate group. They are eluted out later than taurine because of their high affinity to the stationary phase. The low intensity of taurine peak is caused by the low conductivity of

Table 1. The comparison of conductivity of cysteic acid, homocysteic acid, and taurine

	Conductivity, uS/cm		
Concentration, ppm	Cysteic acid	Homocysteic acid	Taurine
12.5	23.8	22.8	0.85
25.0	46.0	46.3	0.96
50.0	88.6	88.9	1.05
100	165.0	165.4	1.26
200	291.0	285.0	1.50

taurine in aqueous solution. The comparison of conductivity for the three sulfonic acids in aqueous solution is illustrated in Table 1.

The calibration curves of the three sulfonic acids are compared in Figures 2. The accuracy, LOD, LOQ, linearity, precision, sensitivity, and

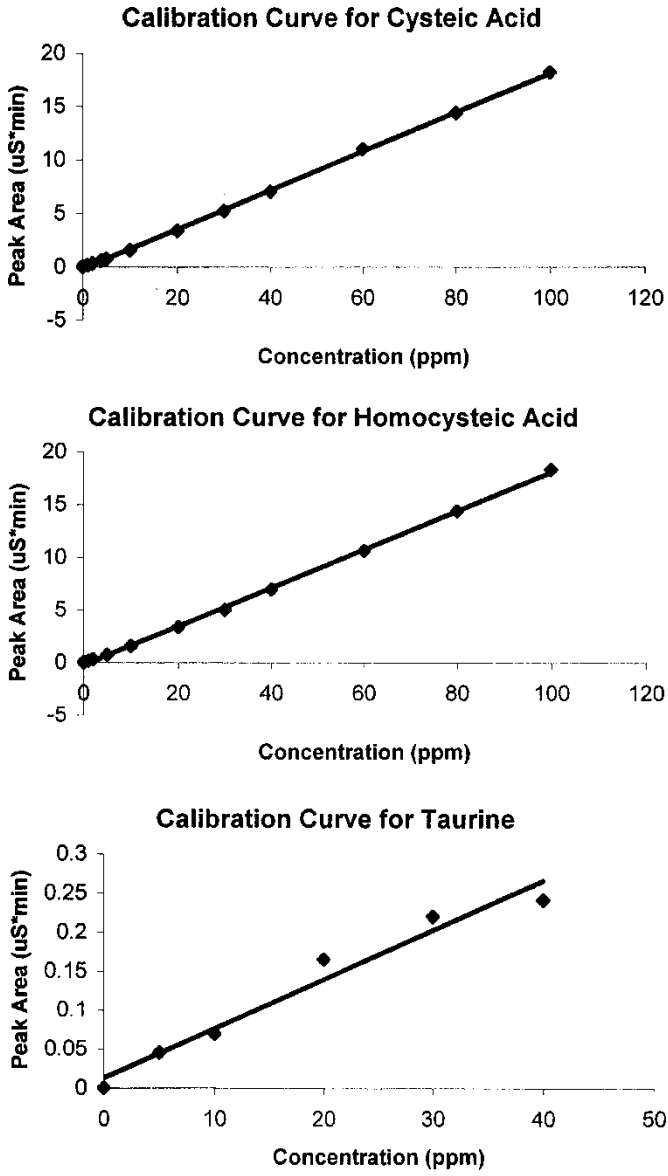

Figure 2. The comparison of calibration curves for cysteic acid, homocysteic acid, and taurine.

Table 2. The comparison of calibration characteristics for cysteic acid, homocysteic acid, taurine

Characteristic	Cysteic acid	Homocysteic acid	Taurine
Accuracy, % recovery	95–101	96–101	74–87
Limit of detection, ppm	0.05	0.05	1.25
Limit of quantitation, ppm	0.17	0.17	4.16
Linearity, ppm	0–120	0–120	0–30
Precision			
Reproducibility, %RSD	0.3–1.8	0.2–1.5	3.5–8.0
Repeatability, %RSD	0.4–2.7	0.4–2.6	5.6–9.8
Correlation coefficient	0.999	0.999	0.845
Sensitivity, uS*min/ppm	0.18	0.18	0.0068

correlation coefficient of calibration curves for the three sulfonic acids are summarized and compared in Table 2. The accuracy is determined by spiking three different concentrations, which cover the entire linear range of calibration curves, of the sulfonic acids into blank solution.[13] The spiked solutions are repeatedly run for five times. The average recoveries in percent (%) for the three concentrations of each sulfonic acid are calculated. The accuracy in terms of range of recovery is illustrated in Table 2. The LOD and LOQ in ppm are calculated using the standard deviation (SD) of detector baseline. Three SD's and ten SD's of the background are divided by sensitivity of the calibration curve to obtain LOD and LOQ,[13] respectively. The linearity is estimated using the portion of calibration curve which follows Beer's Law. Precision is expressed in terms of short-term precision—reproducibility and long-term precision—repeatability. The reproducibility is obtained by analyzing a series of standard solutions of the sulfonic acids 10 times for each. The relative standard deviation (RSD) of 10 integrated peak areas is then calculated for each sulfonic acid at different concentrations. High RSD's are normally obtained for analytes with low concentration and low RSD's for analytes with high concentration. The repeatability is obtained by analyzing a series of standard solutions of the sulfonic acids 16 times over a period of 4 weeks. The RSD of 16 integrated peak areas is calculated for each sulfonic acid at different concentrations. A range of RSD is again used to express the repeatability. The repeatability has a wider range than reproducibility in relation to the long-term stability of instrument and reagents. Sensitivity is defined as the slope of calibration curve. Among the three sulfonic acids, taurine has demonstrated the poorest accuracy, worst detection limit, narrowest linear range, worst precision, lowest correlation coefficient, as well as sensitivity. The inadequacy of this technique in quantifying taurine is mainly due to the low conductivity of taurine in aqueous solution. The calibration characteristics for cysteic acid

and homocysteic acid are exceptionally perfect. Both have excellent accuracy close to 98% recovery in average, LOD as low as 0.05 ppm, and correlation coefficient as high as 0.999. Their linear ranges (up to 120 ppm) are 3 times that of taurine. The sensitivity of detection for both is about 25 times better than that of taurine. The precision of measurement in terms of reproducibility is within 2% over their entire calibration ranges.

In conclusion, anion-exchange chromatography has proven to be an excellent separation technique for cysteic acid, homocysteic acid, and taurine. Although conductivity detection is not sensitive enough to quantify taurine, this technique has proven to be excellent for accurately determining cysteic acid and homocysteic acid.

FUTURE STUDY

Different detecting techniques will be experimented to enhance the determination of taurine. The sample preparation for human biological fluid, including plasma and urine, will be studied and evaluated before this technique is utilized to determine the content of sulfonic acids. The oxidized products of plasma, which contain cysteic acid, homocysteic acid, and taurine, will be analyzed by this technique.

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