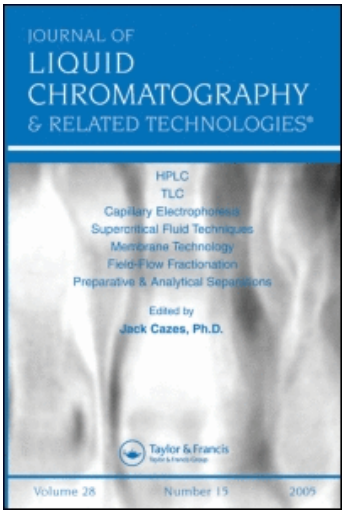

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Enhancing the Determination of Sulfonic Acids using Anion-Exchange Chromatography with Post-Column Derivatization and Spectrometric Detection

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Enhancing the Determination of Sulfonic Acids using Anion-Exchange Chromatography with Post-Column Derivatization and Spectrometric Detection

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Abstract: Accurate determination of 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, an accurate and sensitive determination for the three sulfonic acids has been achieved using anion-exchange chromatography with post-column derivatization and spectrometric detection. This technique has outperformed the technique using suppressed conductivity detection. It has enormously enhanced the accuracy, detection limit, sensitivity, and linearity in the determination of these three sulfonic acids. This technique has demonstrated to be excellent for simultaneously determining cysteic acid, homocysteic acid, and taurine.

Keywords: Sulfonic acids, Anion-exchange chromatography, Post-column derivatization, Accuracy, Precision, Limit of detection, Limit of quantitation, Linearity, Correlation coefficient, Sensitivity

INTRODUCTION

Cysteic acid, homocysteic acid, and taurine have 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 migraines.[3] Cysteic acid and homocysteic acid have also demonstrated to be potent agonists of

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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. Taurine, the third sulfonic acid in the group, 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 analyses of 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 cation-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 high and similar acidity of these three sulfonic acids.

The authors have switched to anion-exchange liquid chromatography with suppressed conductivity detection to analyze these sulfonic acids.[13] An excellent separation for the three sulfonic acids has been achieved. However, the quantification for taurine has suffered low sensitivity, poor detection limit, narrow linear range, and poor accuracy when the suppressed conductivity detection is used. In this research, the anion-exchange chromatography with post-column derivatization and spectrometric detection is used to enhance the detection for these three sulfonic acids. The characteristics of calibration, including accuracy, sensitivity, limit of detection, limit of quantitation, linearity, and correlation coefficient of calibration curve have been enhanced immensely. This technique has been proven to be an excellent method for the simultaneous determination of these three sulfonic acids.

EXPERIMENTAL

Chemicals and Reagents

The chemicals including L-cysteic acid, DL-homocysteic acid, taurine, ninhydrin, hydrindantin, tetramethylene sulfone, sodium acetate, acetic acid, and sodium hydroxide (NaOH, 50%) were obtained from Aldrich-Sigma (St. Louis, Missouri). The eluent for anion-exchange chromatography was

20 mM NaOH solution, which was prepared from Aldrich-Sigma 50% NaOH solution. The post-column derivatizing reagent, ninhydrin solution, was prepared by dissolving 20.00 g ninhydrin and 0.19 g hydrindantin in 160 mL tetramethylene sulfone. This ninhydrin in solfone solution was then thoroughly mixed with a 500 mL buffer solution, which was prepared by dissolving 41.00 g sodium acetate and 6.00 g acetic acid into a total volume of 500 mL aqueous solution with a pH equal to 5.5, to form a homogeneous solution. This homogeneous solution was then filtered through filtering paper (Schleicher & Schuell #560 pre-pleated filters) to remove particulate material in the solution. A series of standard solutions, which contain the three sulfonic acids ranging from 0.05 to 500 ppm, were prepared in 20 mM NaOH solution. The water used to prepare reagents was ultra pure water (18 megaohm/cm) produced from a Hydro's Picosystem (Levittown, Pennsylvania).

Chromatographic System

The equipment used to analyze samples was a system which combined a HP 1050 high performance liquid chromatograph (HPLC) (Hewlett-Packard Corporation, Palo Alto, California) and a Pickering PCX3100 post-column derivatization unit (Pickering Laboratories, Sunnydale, California). The detector was a HP 1050 variable wavelength detector. The samples were injected into HPLC through a HP 1050 autosampler. The system was controlled and run by HP Chemstation (3.1 Version).

The chromatographic parameters were as follows: A portion of 20 μL sample solution was automatically injected into the chromatograph. The sample was carried through a Dionex (Sunnyvale, California) IonPac AG18 guard column (2 × 50 mm) and an IonPac AS18 analytical column (2 × 250 mm) by the eluent of 20 mM NaOH solution, with a flow rate at 0.3 mL/min. The guard and analytical columns were isothermally controlled at 30°C in a column oven. The analytes were separated in the column and directed to a 130°C reactor where a 0.3 mL/min flow of the post-column derivatizing reagent, ninhydrin solution, was pumped through to react with the separated analytes. The formed Ruhemann's purple was spectrometrically detected at a wavelength set at 570 nm.

RESULTS AND DISCUSSION

A chromatogram which demonstrates an excellent separation and detection for taurine, homocysteic acid, and cysteic acid is illustrated in Figure 1. In this chromatogram, all of the three sulfonic acids have a concentration of 5 ppm in the same solution. Unlike that observed in the chromatogram obtained using the suppressed conductivity detection,[13] the baseline of the chromatogram is extremely smooth. The peak at a retention time of 3.75 minutes is

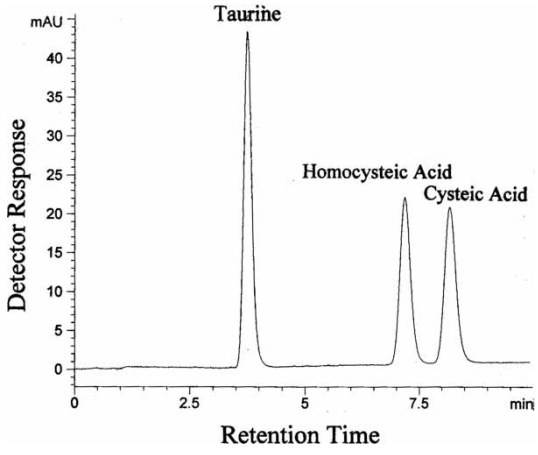

Figure 1. Chromatogram of separated taurine, homocysteic acid, and cysteic acid.

proven to be taurine. Two adjacent peaks with baseline separation at retention times of 7.18 minutes and 8.20 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 the sulfonate group, both cysteic acid and homocysteic acid have a carboxylate group to interact with anion-exchange resin. They are, therefore, eluted out later than taurine because of their high affinity to the stationary phase. The immense intensity of the taurine peak among these three sulfonic acids is mainly due to the fact that there are more molecules of taurine than those of cysteic acid or homocysteic acid in the 5 ppm solution.

The calibration curves of the three sulfonic acids are compared in Figure 2. The accuracy, LOD, LOQ, linearity, precision, sensitivity, and correlation coefficient of calibration curves for the three sulfonic acids are summarized and compared in Table 1. In addition to the data obtained using the derivatization/spectrometric detection, the data obtained using the suppressed conductivity detection in a previous publication[13] are also included in the parentheses of Table 1 for comparison. 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.[14] The spiked solutions are repeatedly analyzed for five times. The average recoveries in percent (%) for the three concentrations of each sulfonic acid are calculated. The accuracy in terms of % recovery is used. The LOD and LOQ in ppm are calculated using the standard deviation (SD) of the detector baseline. Three SD's and ten SD's of the background are divided by the sensitivity of the calibration curve to obtain LOD and LOQ,[14] respectively. The linearity is estimated by using the linear range of the 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

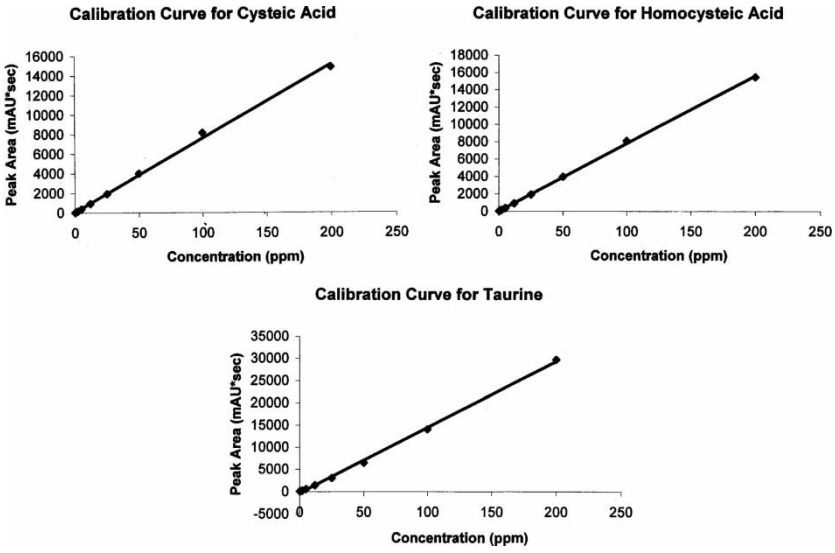

Figure 2. Comparison of calibration curves for cysteic acid, homocysteic acid, and taurine.

Table 1. The comparison of calibration characteristics between derivatization/spectrometric detection and suppressed conductivity detection for cysteic acid, homocysteic acid, and taurine

Characteristic	Cysteic acid	Homocysteic acid	Taurine
Accuracy, % recovery	98–101 (95 –101)	98–101 (96–101)	98–100 (74–87)
Limit of detection, ppm	0.02 (0.05)	0.02 (0.05)	0.01 (1.25)
Limit of quantitation, ppm	0.06 (0.17)	0.06 (0.17)	0.04 (4.16)
Linearity, ppm	0–200 (0–120)	0–200 (0–120)	0–200 (0–30)
Precision Reproducibility, %RSD	0.3–4.1 (0.3–1.8)	0.3–4.5 (0.2–1.5)	0.2–3.9 (3.5–8.0)
Repeatability, %RSD	15–30 (0.4–2.7)	16–28 (0.4–2.6)	13–29 (5.6–9.8)
Correlation coefficient	0.997 (0.999)	0.998 (0.999)	0.998 (0.845)
Sensitivity, mAU*s/ppm uS*min/ppm	75.8 (0.18)	77.9 (0.18)	145.6 (0.0068)

The data in the parentheses were obtained from suppressed conductivity detection.[13]

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 used to express the precision.

The derivatization/spectrometric detection has outperformed the suppressed conductivity detection in the calibration characteristics including accuracy, LOD, LOQ, linearity, and sensitivity, based on the data illustrated in Table 1. The derivatization/spectrometric detection has enormously increased the sensitivity for determining these sulfonic acids. If the sensitivity is compared in terms of compatible terms, mAU*s/ppm and μS*s/ppm, the derivatization/spectrometric detection has enhanced the sensitivity of the determination more than 300-fold for taurine and about 7-fold for cysteic acid, as well as homocysteic acid. The reproducibility for determining cysteic acid and homocysteic acid using the suppressed conductivity detection is better than that obtained using the derivatization/spectrometric detection. However, the derivatization/spectrometric detection has a better reproducibility than the suppressed detection for taurine determination. As for repeatability, the suppressed conductivity detection is significantly better than the derivatization/spectrometric detection. This can be explained by the complexity and the stability of the ninhydrin solution. Because of the relative low solubility of ninhydrin and hydrindantin in sulfone and water, the variation in preparing this five compound solution is experienced. Also, the effectiveness of the ninhydrin solution is gradually decreased when exposed to air.[15] This has contributed to the poor long term precision. However, if the calibrated concentrations, instead of peak areas, of the standards are used in the calculation of the repeatability, the repeatability obtained using the derivatization/spectrometric detection can be brought to the level of precision obtained for the reproducibility illustrated in Table 1. Therefore, it is recommended that the solutions should be freshly prepared and the ninhydrin solution should be blanketed with helium or nitrogen during the analysis in order to minimize the data fluctuation caused by the reagent variation.

In conclusion, anion-exchange chromatography has proven to be an effective separation technique for cysteic acid, homocysteic acid, and taurine. When this separation technique and the derivatization/spectrometric detection are used to analyze the sulfonic acids, the accuracy, LOD, LOQ, linearity, and sensitivity of the determination can be enhanced enormously. Therefore, anion-exchange chromatography with post-column derivatization and spectrometric detection has demonstrated to be excellent for simultaneously determining cysteic acid, homocysteic acid, and taurine.

Future Study

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 the three sulfonic acids. The oxidized products of plasma, which contain cysteic acid, homocysteic acid, and taurine will also be analyzed by this technique to assess the possibility of determining total plasma homocysteine as homocysteic acid.

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