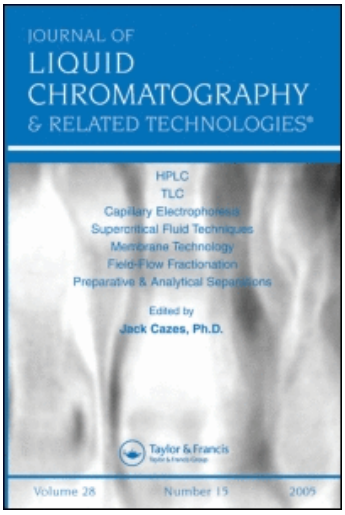

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VALIDATION OF AN HPLC METHOD FOR THE DETERMINATION OF SODIUM IN LY293111 SODIUM, A NOVEL LTB_4 RECEPTOR ANTAGONIST, USING EVAPORATIVE LIGHT SCATTERING DETECTION

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ABSTRACT

Analysis of inorganic ions such as sodium or chloride in pharmaceutical compounds has traditionally employed ion-chromatography (IC) with conductivity detection. A new quantitative method for the determination of sodium in LY293111 sodium, a novel LTB_4 receptor antagonist, using high performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) is discussed. The separation of sodium from other ions and interferences was achieved using a Zorbax 300 SCX cation-exchange column suitable for use with organic solvents. Acceptable levels of precision, linearity, recovery, selectivity and limit of detection were achieved during the validation of the method. The results of this method were within 99.8% agreement when compared to the theoretical amount of sodium in LY293111 sodium. HPLC coupled with evaporative light scattering detection offers a practical alternative to IC using conductivity detection in pharmaceutical compounds.

INTRODUCTION

Pharmaceutical drug candidates are often synthesized as salts in order to increase the aqueous solubility or stability of the drug. The most common pharmaceutical salt forms are sodium salts of acids and hydrochloride salts of amines.[1] Quantitation of inorganic counter-ions is necessary to determine the overall purity of a drug substance. Traditionally, the analysis of pharmaceutical counter-ions has been performed on ion-chromatography (IC) systems which employ conductivity detection. IC systems are generally dedicated for the analysis of ions and are not practical for use with other reversed-phase or normal phase pharmaceutical applications. This paper focuses on a new approach for the quantitation of inorganic counter-ions which utilizes existing HPLC systems common in the pharmaceutical laboratory with ELSD.

Recently, ELSD has been introduced commercially and has gained acceptance as a sensitive universal detector.[2-3] ELSD operates by nebulizing the volatile effluent from the HPLC column into a fine mist. The mist is carried through a heated drift tube which evaporates the mobile phase and leaves behind non-volatile solute particles. The fine cloud of solute particles is carried at a high speed through a beam of light where the scattered light is detected by a photomutiplier. The amount of light scattered is dependent upon the size and number of particles and therefore proportional to the concentration. The mobile phase used with ELSD must be free of non-volatiles which would be detected by the ELSD thus limiting its use for some traditional applications of ion-exchange/ion-pairing chromatography. However, we have developed a new quantitative HPLC method for the determination of sodium using ELSD with ammonium acetate, a volatile buffer salt.

ELSD has been shown in the literature to successfully detect phospholipids,[4-9] triglycerides, fats and fatty acid esters,[10-13] carbohydrates,[14-15] synthetic polymers,[16] and steroids.[17] Surprisingly, no information has been published on ELSD for the determination of inoganic salts. The aim of this paper is to show the applicability of an evaporative light scattering detector to accurately detect sodium in a pharmacuetical compound. In this study LY293111 sodium, a novel LTB_4 receptor antagonist, will be used to validate a method for

the quantitation of sodium. Experimental results will be compared to the theoretical sodium amount to verify the accuracy of the method.

MATERIALS AND METHODS

Chemicals

Sodium chloride A.R. and acetic acid A.R. were purchased from EM Science (Gibbstown, New Jersey). LY293111 Sodium lot 309MH3 was supplied by Eli Lilly and Company (Indianapolis, IN). HPLC grade methanol and ammonium acetate A.R. were purchased from Mallinckrodt (Paris, Kentucky). Water used was deionized and filtered through a Milli-Q water purification system (Millipore, New Bedford, MA). NF grade nitrogen was used for the ELSD.

Apparatus

The HPLC system used for this study consisted of a Shimadzu SCL-10A controller, LC-10AS pump, SIL-10A auto injector and a DGU-3A membrane degasser (Shimadzu, Kyoto, Japan). A Sedex 45 evaporative light scattering detector was used (Richard Scientific, Novato, CA). Sodium was separated on a 15 cm x 4.6 mm I.D. Zorbax 300 SCX cation-exchange column (Mac Mod Analytical, Chads Ford, PA). A guard column was not used.

Chromatographic Conditions

Operating conditions for the Sedex ELSD were optimized prior to the onset of the study. The conditions were optimized to obtain the greatest signal-to-noise ratio. The optimum conditions were: nitrogen pressure in the ELSD nebulizing chamber set at 14 psi and the temperature of the drift tube set at 25°C. The gain control for the ELSD was set at 5.

The mobile phase comprised of 50% methanol /50% aqueous buffer. The buffer was a 0.05 M ammonium acetate solution adjusted to pH 6.0 with acetic acid. The mobile phase flow rate was set at 1.5 mL/min. The column temperature for the Zorbax 300 SCX was ambient. Injection volume was 100 uL and the run time was 500 seconds. All sample preparations were sonicated for two minutes to completely dissolve the material.

RESULTS

The method for sodium determination was validated for the parameters of linearity, precision, recovery, selectivity and limit of detection. The validation of this method follows the general USP guidelines suggested for an HPLC method.[18]

Linearity

It is usual practice to perform linearity determinations over a wide range of sample concentrations to fully assess the linear dynamic range of the detection system. The linearity of the method was determined by injecting 22 samples which were serial dilutions from a stock solution of sodium chloride to locate the working range. The samples represented a range of 0.0001-1.0 mg/mL sodium concentration. The linear range of sodium was determined to be 0.1-1.0 mg/mL. This range included ten standards and resulted in a correlation coefficient of 0.9997. The linear range represents 20-200% of the nominal target concentration of 0.5 mg/mL.

Precision

The precision of this method was evaluated in two ways. First, ten replicate injections of the same sample were injected to determine the reproducibility of the method apart from analyst error. Second, ten separate preparations were injected singly to determine the overall precision of the method. 12.5 mg/mL solutions of LY293111 sodium diluted with mobile phase were prepared. LY293111 sodium contains approximately 4% sodium making the actual sodium concentration near the target nominal concentration of 0.5 mg/mL. The samples were then sonicated for 2 minutes until all the material was in solution. The samples were run against a five point standard curve (0.3-0.8 mg/mL) made from sodium chloride. The results indicated 1.5% RSD for ten replicate injections and 1.3% RSD for ten separate preparations. See Figure 1 for a typical sample chromatogram.

Selectivity

As part of the USP guidelines for validation, a method must be proven to be selective for the analyte of interest. For this method selectivity was assessed

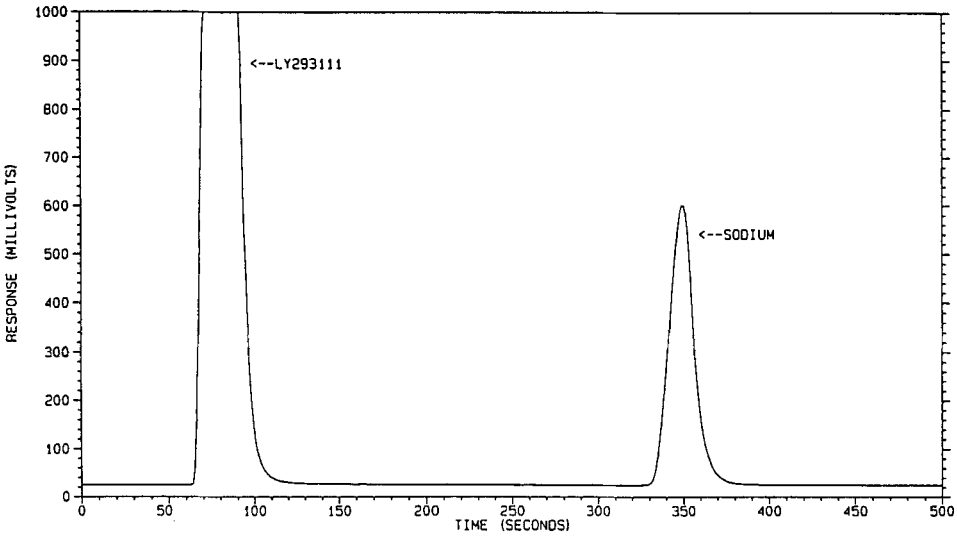

FIGURE 1. Typical sample chromatogram.

by separating sodium from all interfering peaks including LY293111 and similar monovalent ions such as potassium and lithium. The retention times of sodium, lithium, potassium, chloride and LY293111 are 350, 260, 440, 70, and 80 seconds respectively. Chromatograms of a blank, standard and sample are shown in Figure 2.

Recovery

Recovery was determined by a standard addition technique whereby ten separate preparations of LY293111 sodium containing approximately 0.4 mg/mL sodium were spiked with an additional 0.2 mg/mL sodium from a stock solution of sodium chloride. The ten samples were run against a standard curve consisting of five standard solutions in the range 0.3-0.8 mg/mL. The average percent recovery for the ten preparations was 104.5%.

Limit of Detection (LOD)

The LOD is defined as the lowest concentration of sample that can be clearly detected above the baseline noise. Typically this value is three times the

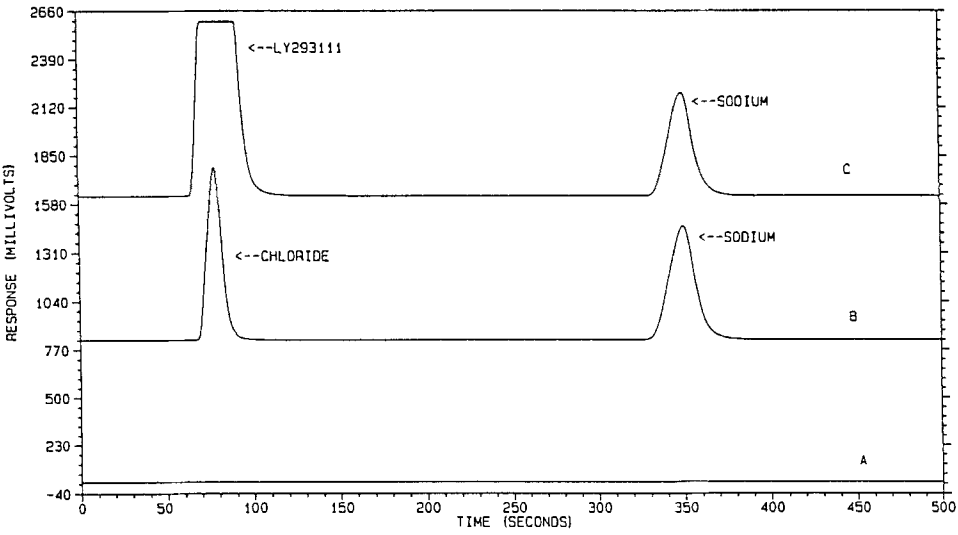

FIGURE 2. Chromatograms of a blank (A), standard (B) and a sample (C).

level of baseline noise. For this method the limit of detection was determined to be 0.0025 mg/mL. However, the limit of detection could be a much lower if a higher gain setting was used on the Sedex detector. The gain for this method is 5 and the gain can be adjusted to 12 for maximum sensitivity.

Theoretical Comparison

The results from ten separate preparations yielded a value of 4.05% sodium in LY293111 sodium. The theoretical amount of sodium in LY293111 sodium is 4.06%. Comparison of these results show the experimental results agree within 99.8% of the theoretical amount.

DISCUSSION

The applicability of a commercial evaporative light scattering detector for the analysis of sodium in a pharmaceutical compound has been demonstrated. The validation of this assay demonstrates that ELSD is an effective and practical

alternative to ion-chromatography with conductivity detection. The competitive nature of the pharmaceutical industry forces companies to analyze potential drug candidates in a quick, inexpensive manner in order to save time and conserve resources. ELSD provides a cost effective way of quickly determining the levels of ions in pharmaceutical compounds. Since ELSD is capable of detecting many types of solutes other than just ions, the versatility of an ELSD provides a practical tool for an analytical chemist . The cost savings of buying an ELSD and using existing HPLC equipment versus buying a dedicated IC system is substantial.

The practicality of this method should not be limited to only pharmaceutical compounds. This method has also been shown to determine sodium levels in various liquid forms such as beverages or other solutions. The intention of this paper is to propose the use of ELSD as an effective alternative for the determination of any inorganic salt for various applications. Our paper has shown the validity of ELSD for the determination of sodium in a pharmaceutical compound; however, other ions, such as chloride or other anions, may be quantitated with ELSD as well.

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