METHOD FOR THE ANALYSIS AND QUANTITATION OF PHARMACEUTICAL COUNTERIONS UTILIZING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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INTRODUCTION

Over 50% of drugs on the market today are prescribed in the salt form[1]. Some popular active pharmaceutical ingredients (APIs) lack aqueous solubility[2,3]. Therefore insoluble APIs are combined with soluble salts to increase the bioavailability. Since the salt from of the drug is more effective, the identification and quantification of APIs and their counterions is necessary for quality control testing[4]. Given the chemical differences between APIs and their salts, multiple methods such as reversed phase and ion-exchange would need be to used to identify and quantify a final drug product. This can be time consuming and expensive. Here, we offer a single HILIC method that can separate and retain not only the API but also the pharmaceutical counterions.

This method specifically uses Z-HILIC, or zwitterionic hydrophilic interaction liquid chromatography. The zwitterionic HILIC stationary phase contains a sulfobetaine functional group that features a negatively charged sulfonate group and a positively charged quaternary ammonium group[5]. This will allow the retention and separation of cations and anions in the same method.

Further challenges of pharmaceutical counterions analysis involves the lack of chromophores in ions. In this method we utilize an evaporative light scattering detector (ELSD) to mitigate this hurdle. ELSD works by nebulizing mobile phase with analytes leaving the column. The volitive mobile phase will evaporate leaving the dry solute particles behind. These dry particles flow into the ELS detector and scatter a beam of light. The amount of scattered light is measured and correlated to the concentration of eluting material[6].

This powerful combination of Z-HILIC and ELSD provides a strategic approach to testing pharmaceutical counterions and their associated APIs.

METHODS

LC Conditions

ACQUITY™ Arc™ Premier LC System
Column: Atlantis™ Premier BEH Z-HILIC Column 4.6 x 100 mm, 2.5 µm heated to 40ºC
Flow rate: 1.4 mL/min
Injection vol: 10.0 µL
Run Time: 10 minutes

Mobile phases used in Gradient Mode:
A: Acetonitrile B: DI Water
C: 200mM Ammonium Formate D: 2% Formic Acid
Gradient Table:

Time (min)	Flow (mL/min)	%A	%B	%C	%D	Curve
Initial	1.4	80.0	10.0	5.0	5.0	initial
10.00	1.4	10.0	80.0	5.0	5.0	6
12.00	1.4	10.0	80.0	5.0	5.0	6
12.10	1.4	80.0	10.0	5.0	5.0	6
16.00	1.4	80.0	10.0	5.0	5.0	6

ELSD Conditions

Waters™ 2424 Evaporative Light Scattering Detector
Gas Pressure: 40psi Nebulizer: OFF
Drift Tube Temp: 50°C Detector Gain: 75

Sample Preparation

The salts used in this study were potassium chloride, sodium sulfate, calcium nitrate, potassium phosphate, and magnesium chloride were purchased from Sigma-Aldrich (Allentown, PA).

These various salts were made as separate stock solutions at a 1mg/mL concentration in 50% acetonitrile. Then, the stock salt solutions were combined and diluted to a 0.1mg/mL concentration using 60% acetonitrile to make a dissolved counterions standard.

The salts used in the linearity portion of this study were sodium sulfate, magnesium chloride, and potassium phosphate were purchased from Sigma-Aldrich (Allentown, PA). These various salts were made as separate stock solutions at a 100mM concentrations in 60% acetonitrile. Then, the stock salt solutions were individually diluted into various calibration standards, dependent on the salt, ranging from 1 to 100mM using 60% acetonitrile to make three different linear curves.

All solutions were stored at 2°C -8°C and allowed to warm up to ambient room temperature before making dilutions or injecting as samples

RESULTS AND DISCUSSION

Method Of Separation

The method developed separated a variety of different counterions with good retention, resolution, and reproducibility. Over the course of 10 injections of the counterions standard %RSDs were ≤6% for both area and retention time (Figure 1).

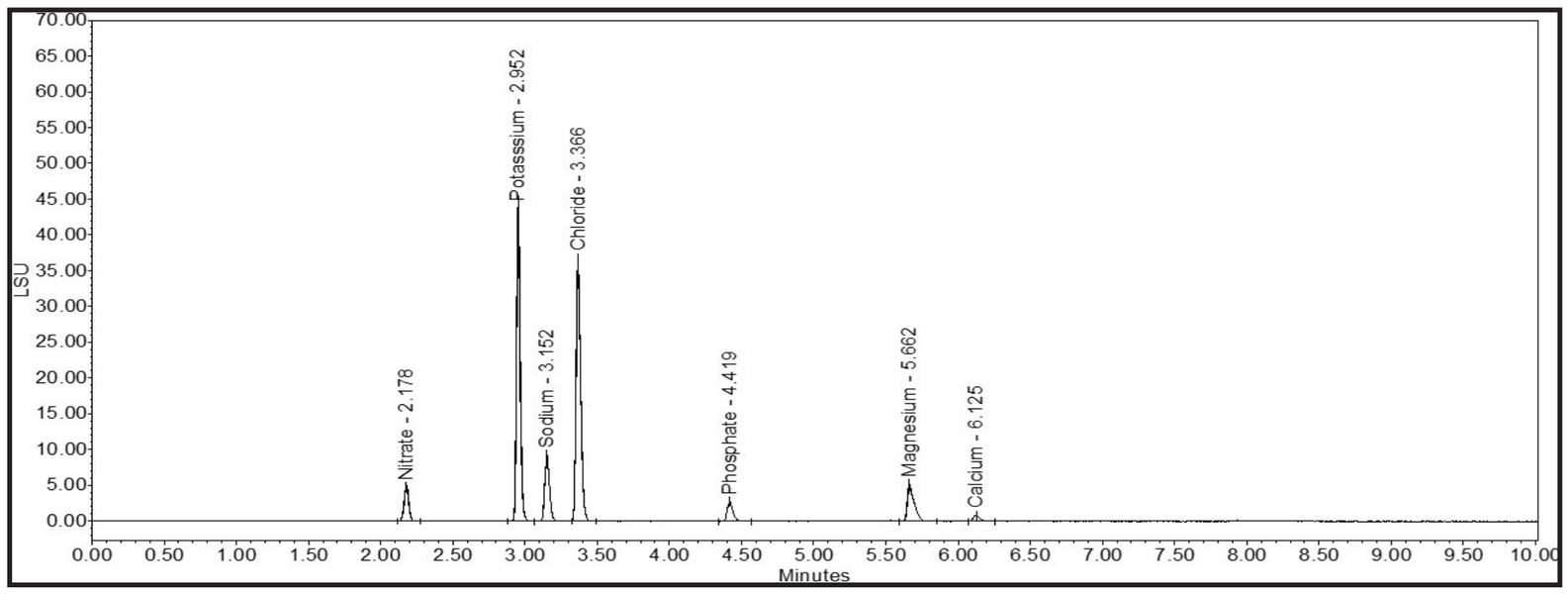

Figure 1: Overlay of 10 ELSD Chromatograms for the counterions standard mixture , demonstrating the reproducibility of this method.

Method Linearity

Dynamic linearity was achieved for three commonly used salts: potassium, sodium, and chloride. (Figure 2). The R^2 for all curves was ≥0.997 using a linear log/log fit as recommended by the ELSD owner's manual.

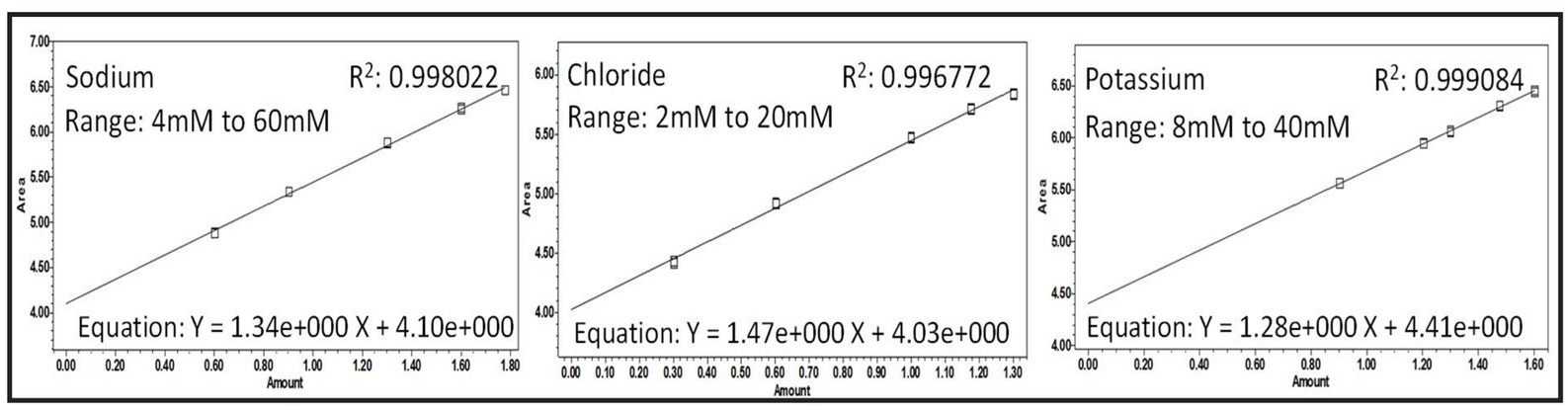

Figure 2. The calibration curves for sodium, chloride, and potassium with their respective ranges, linear regression coefficients, and equations. These R^2 values offer confidence in the quantitative usability of this method.

Drug Formulation Quantitation

This method was shown to be capable of separating the counterions, but it also can separate the API quantify the counterions in drug substances. Naproxen sodium, metformin hydrochloride, and losartan potassium were prepared at various concentrations in 60% acetonitrile. The peaks were aligned against the counterions standard to identify the separated ion from the API of the drug salt (Figure 2A through 2D). The linear curves achieved were then used to quantitate the amount of salt in pharmaceutical drug salt formulation.

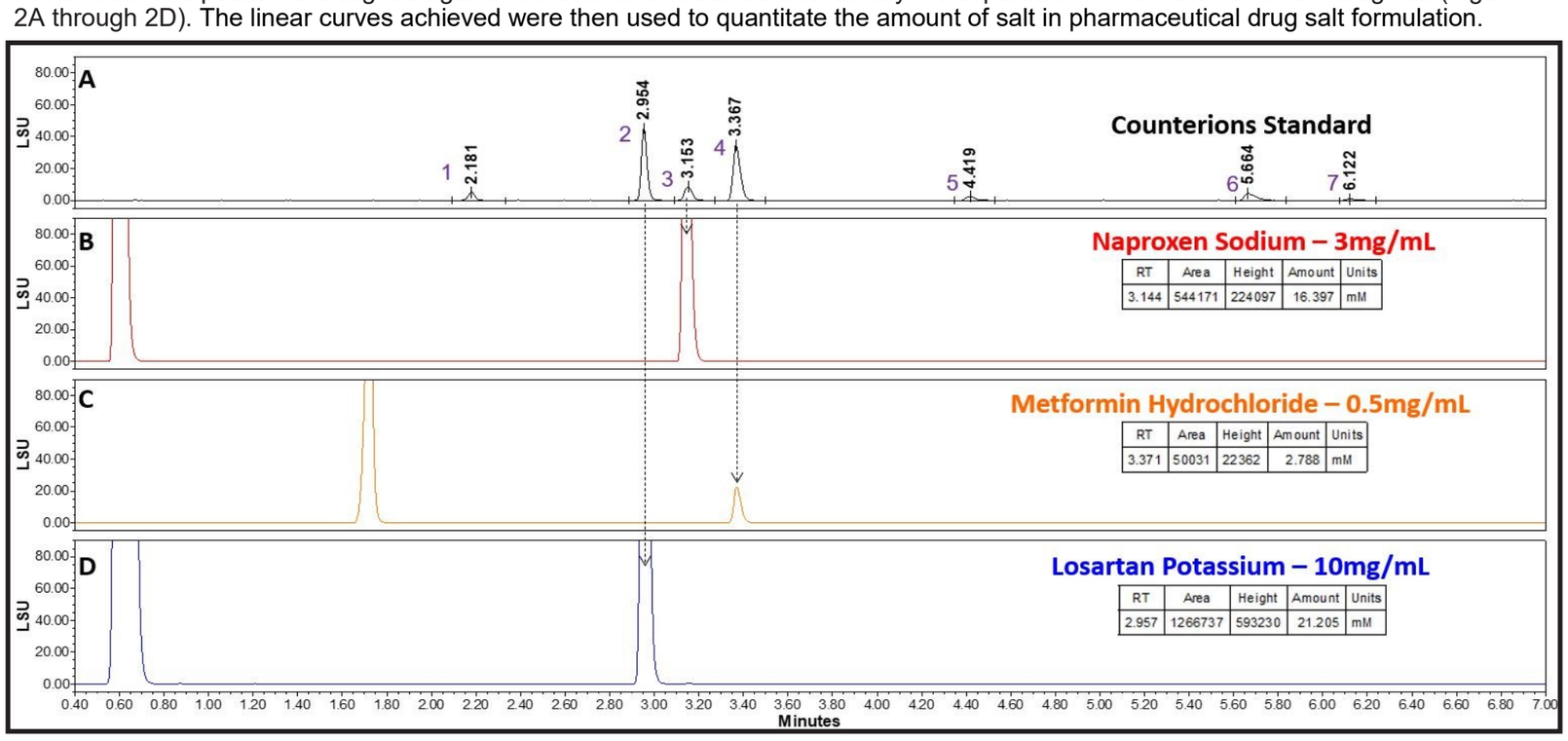

Figure 3A. ELSD Chromatogram of the counterions standard (black). The specific peaks in the chromatogram are Nitrate (1), Potassium (2), Sodium (3), Chloride (4), Phosphate (5), Magnesium (6), and Calcium (7).

Figure 3B. An ELSD chromatogram of the drug Naproxen Sodium prepared at 3mg/mL (red).
Figure 3C. An ELSD chromatogram of the drug Metformin Hydrochloride prepared at 0.5mg/mL (orange).
Figure 3D. An ELSD chromatogram of the drug Losartan Potassium prepared at 10mg/mL (blue).

CONCLUSION

- **Accurate, precise, and reproducible linear data for quantitative applications.**
- **Visibility to not only the API but chromophore lacking ions through the use of the ELSD.**
- **Separation and retention of APIs and their associated ions in a single method, which can cut costs and time on QC testing.**
- **Ability to separate both anions and cations to support the challenges associated with combination drug formulation testing.**

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