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Method for the Analysis and Quantitation of Pharmaceutical Counterions Utilizing Hydrophilic Interaction Liquid Chromatography

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PURPOSE

Background

More than 50% of drugs manufactured today are produced in the drug salt form. Active Pharmaceutical Ingredients (APIs) are attached to salts to increase the physiochemical properties and bioavailability of drugs. The identification and quantification of APIs and salt counterions is crucial for quality control (QC) testing in the pharmaceutical industry.

Challenges in Counterions Analysis

1. QC Testing for pharmaceutical counterions typically **requires multiple methods** of analysis.
2. Due to the different chemical properties of APIs and their counterions limits the visibility of both components in a single method.

OBJECTIVES

1. To support QC testing by creating a single HILIC method in which pharmaceutical anions and cations are **consistently** identified and quantitated.
2. To create a method in which APIs and counterions are visible in **a single method**.

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

Mobile phases used in Gradient Mode:

A: Acetonitrile B: DI Water

C: 200mM Ammonium Formate D: 2% Formic Acid

MPA decreased from 80% to 10% and is replaced by MPB over the course of a 10-minute run time.

MPC and MPD were mixed at 5% consistently throughout the run.

ELSD Conditions

Waters™ 2424 Evaporative Light Scattering Detector

Gas Pressure: 40psi Nebulizer: OFF

Drift Tube Temp: 50°C Detector Gain: 75

RESULTS

Method Reproducibility

A counterions standard of 7 different ions was successfully separated. Over the course of 10 injections, the **%RSDs were ≤5% and ≤6% for area and retention time**, respectively (Fig. 1). This standard was used as an identification tool for ions in drug substances (Fig 3).

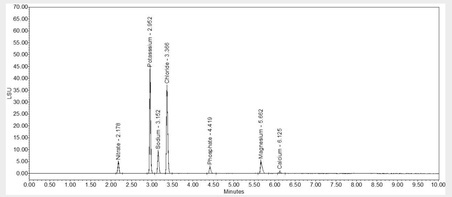

Figure 1. An overlay of 10 Chromatograms for the counterions standard mixture.

Method Linearity

Dynamic linearity was achieved for three commonly used salts: potassium, sodium, and chloride. (Fig. 2).

The R^2 for all curves was **≥0.997** using **a linear log/log fit** as recommended by the ELSD owner's manual.

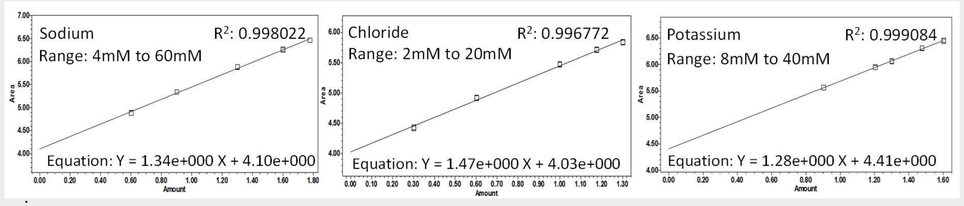

Figure 2. The calibration curves for sodium, chloride, and potassium with their respective ranges, linear regression coefficients, and equations.

Drug Formulation Quantitation

The linear curves achieved were then used to quantitate the amount of salt in pharmaceutical drug salt formulations.

Naproxen sodium, **Metformin hydrochloride**, and **Losartan potassium** were selected and quantitated.

The **sodium**, **chloride**, and **potassium ions** in the formulations were **quantitated** at **16.4mM**, **2.79mM**, and **2.96mM**, respectively.

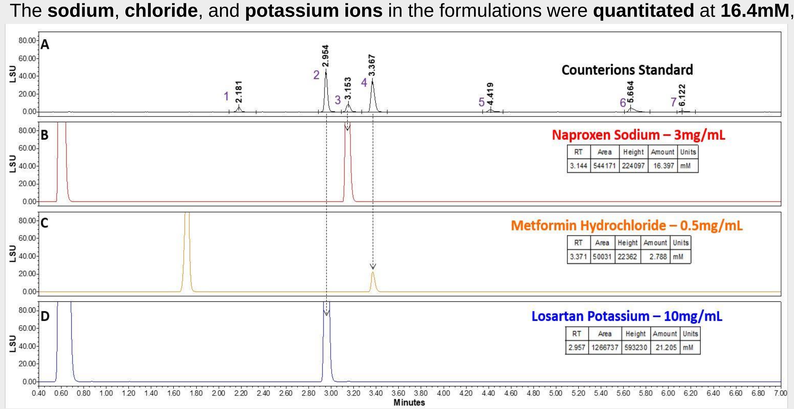

Figure 3A. ELSD Chromatogram of the counterions standard (black). The specific peaks in the chromatogram are Nitrate (1), Potassium (2), Sodium (3), Chloride (4), Phosphate (5), Magnesium (6), and Calcium (7).
Figure 3B. An ELSD chromatogram of the drug Naproxen Sodium prepared at 3mg/mL (red).
Figure 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).

CONCLUSIONS

Method Reproducibility

The RSDs indicate this method produced accurate, precise, and reproducible data.

Method Linearity

Linear regression coefficients of ≥0.997 demonstrates this method can be used for accurate quantitation over a dynamic linear range.

Drug Formulation Quantitation

This method provides accurate quantitation for a variety pf pharmaceutical drugs salts. Further, visibility is given to both the pharmaceutical APIs and counterions.

Also, some APIs had considerable retention, such as Metformin hydrochloride. The method provides flexibility and has the potential to be used with combination drugs.

Ultimately, the method developed offers a unique solution to the challenges associated with pharmaceutical drug salt analysis.

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