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アプリケーションノート

LC-MS Analysis of Aldosterone in Plasma

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates efficient analysis of aldosterone in plasma by LC-MS.

Benefits

Efficient measurement of aldosterone with LC-MS in plasma.

Introduction

Aldosterone (a steroid hormone made by the adrenal gland) helps regulate sodium and potassium levels in the body to exert hormonal control on blood pressure and the balance of fluids and electrolytes in the blood.

Accurate, low level measurement of steroid hormones, like aldosterone, in plasma has proven to be difficult. Many existing assays suffer from a lack of specificity due to antibody cross reactivity with other closely structurally related steroids, and also tend to be highly variable at lower steroid concentrations. Other methods typically require extensive and time consuming extraction and purification steps, followed by derivitization for analysis. As a result of these issues with older methods, many laboratories are turning to LC-MS to provide the sensitivity and specificity required to measure steroid hormones. Even when utilizing LC-MS, the measurement of aldosterone is challenging as the compound is often found in very low levels in plasma and poorly ionizes in a mass spectrometer, making it particularly difficult to analyze.

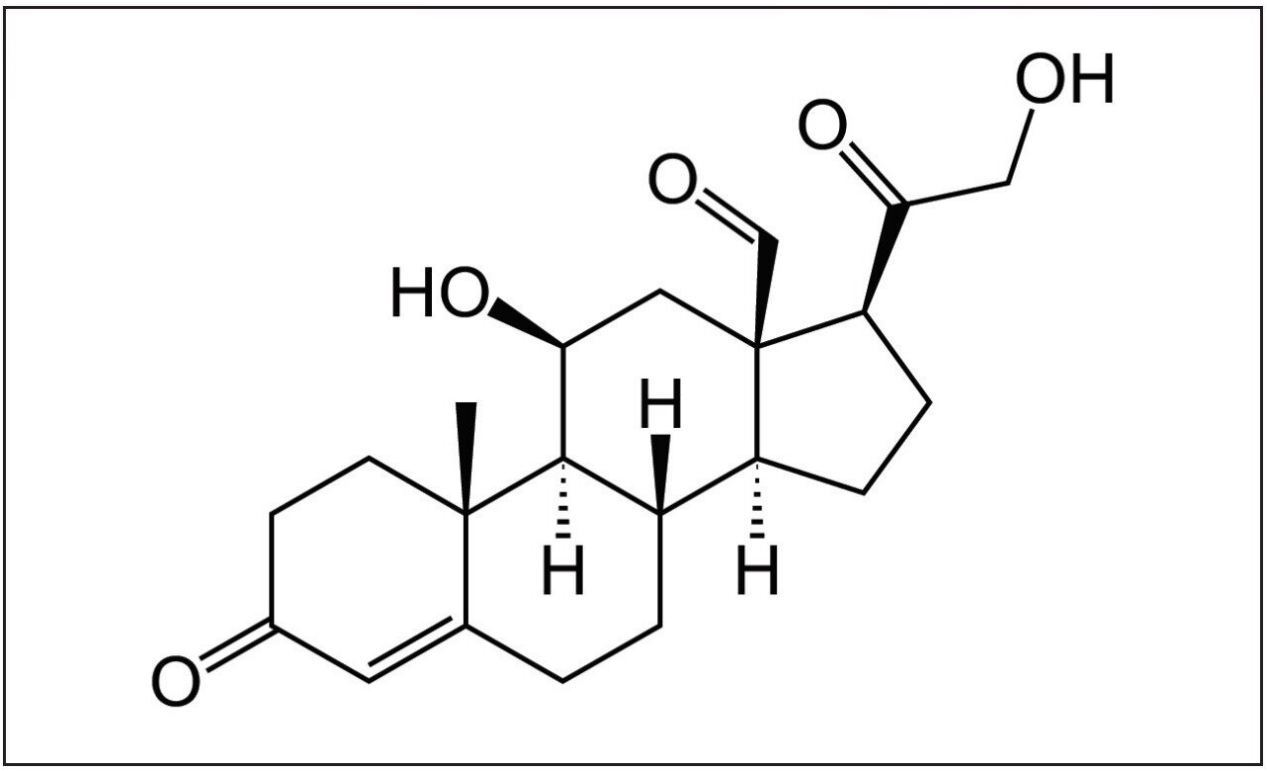

Figure 1: Structure of aldosterone.

Experimental

Sample Preparation Method

Plasma samples were diluted 4:1 with $ZnSO_4$ to precipitate protein and centrifuged. After protein removal, a 25 µL aliquot of sample was injected into an LC-MS system comprised of a classic ACQUITY UPLC with a Xevo TQ-S Mass Spectrometer equipped with an Online SPE Manager (OSM). SPE was performed as follows:

Step	Solvent	Volume (µL)
Cartridge conditioning	Methanol	1000
Cartridge conditioning	Magic Mix	1000
Cartridge equilibration	Water	1000
Sample load	Water	2000
Cartridge wash	15% methanol + 0.1% ammonia	500
Cartridge wash 2	10% methanol + 0.1% formic acid	250
Cartridge wash 3	10% methanol	250
Clamp flush	Methanol	1000
Clamp flush 1	Magic Mix	1000
Clamp flush 2	Water	1000
Clamp flush 3	10% MeOH	500

Table 1. Conditions for Online Solid Phase Extraction. Magic Mix = MeOH/ACN/IPA/H_2O + 0.2% formic acid.

After SPE, samples were analyzed by LC-MS using the following gradient conditions: 15% water/MeOH + 0.01% ammonia (100 - >10%; 3 min) on an ACQUITY UPLC BEH C_{18} Column, 130Å, 1.7 µm, 2.1 mm X 50 mm.

Results and Discussion

In this technology brief, a clinical research method for analyzing aldosterone from plasma was developed. An online SPE system was incorporated into the LC-MS system used for aldosterone measurement. This sample preparation module allows for full integration and automation of sample prep with LC-MS analysis.

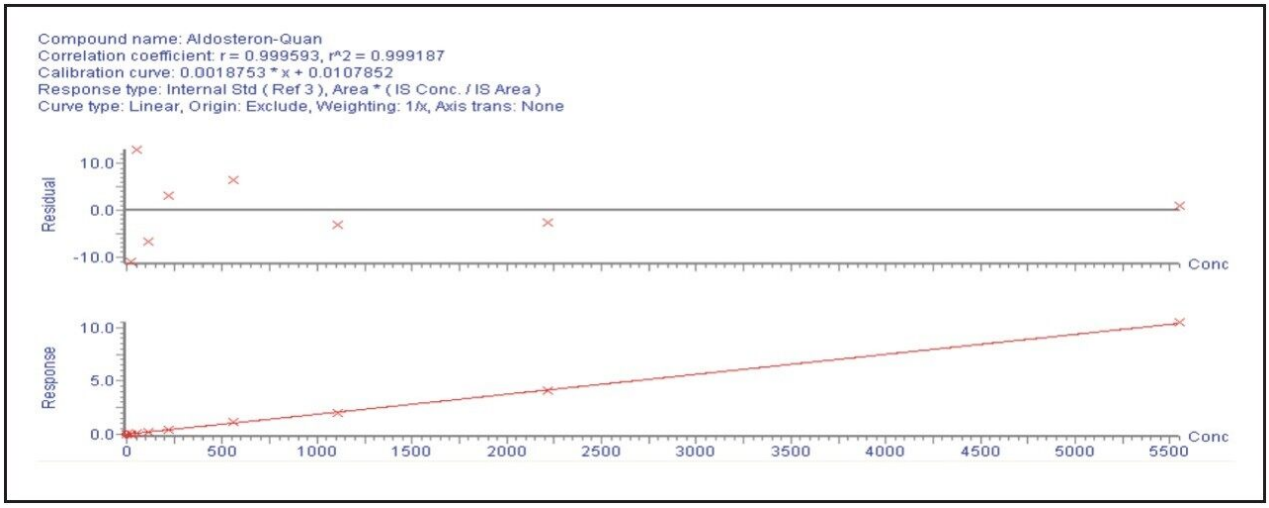

Figure 1: Calibration curve of aldosterone standards. The calibration curve of aldosterone ranges from 22 pM to 5550 pM.

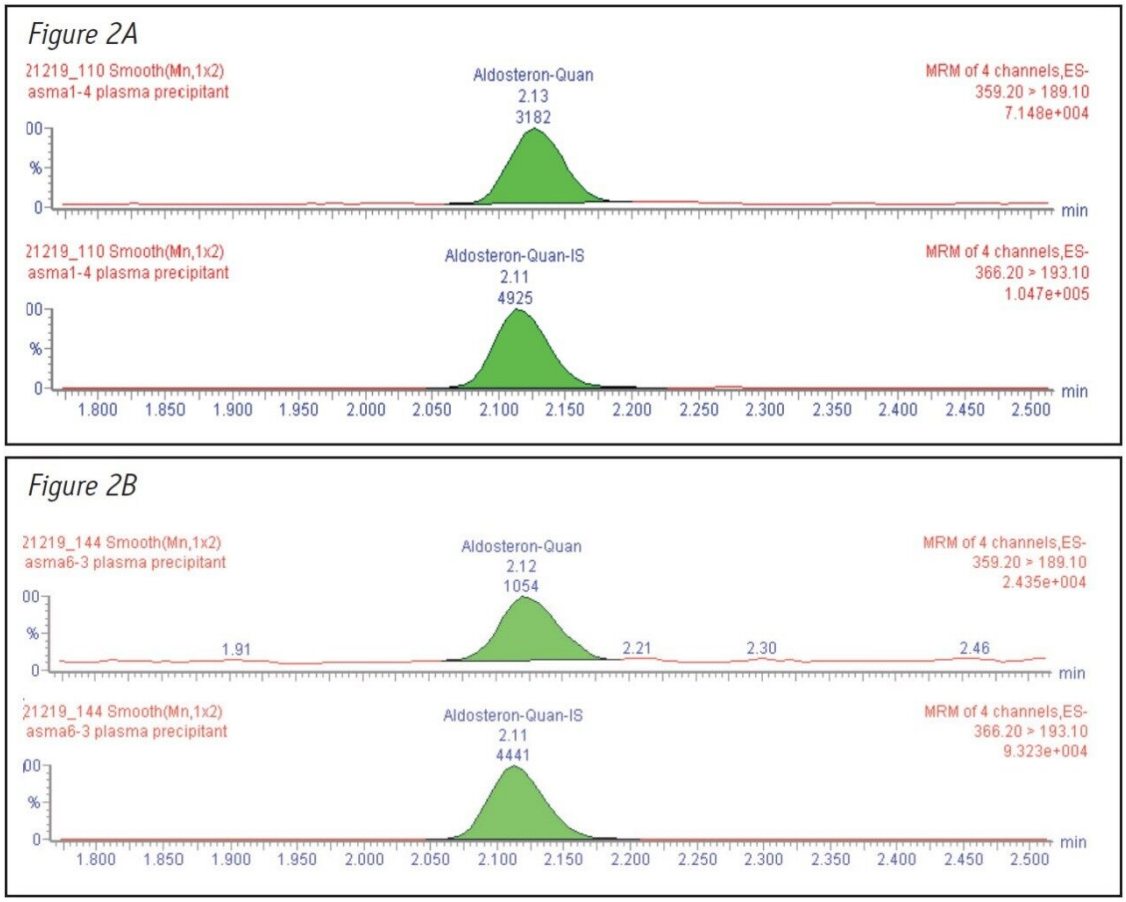

Figure 2: Measurement of aldosterone in plasma. In Figure 2A, 346 pM aldosterone was measured in plasma. The S/N ratio for this level is 100 with a CV of 2%. In Figure 2B, 124 pM of aldosterone was measured in plasma. The S/N ratio was 43, with a CV of 11%. The overall LLOQ of the method was determined to be ~60 pM with S/N > 10 and CV < 20%.

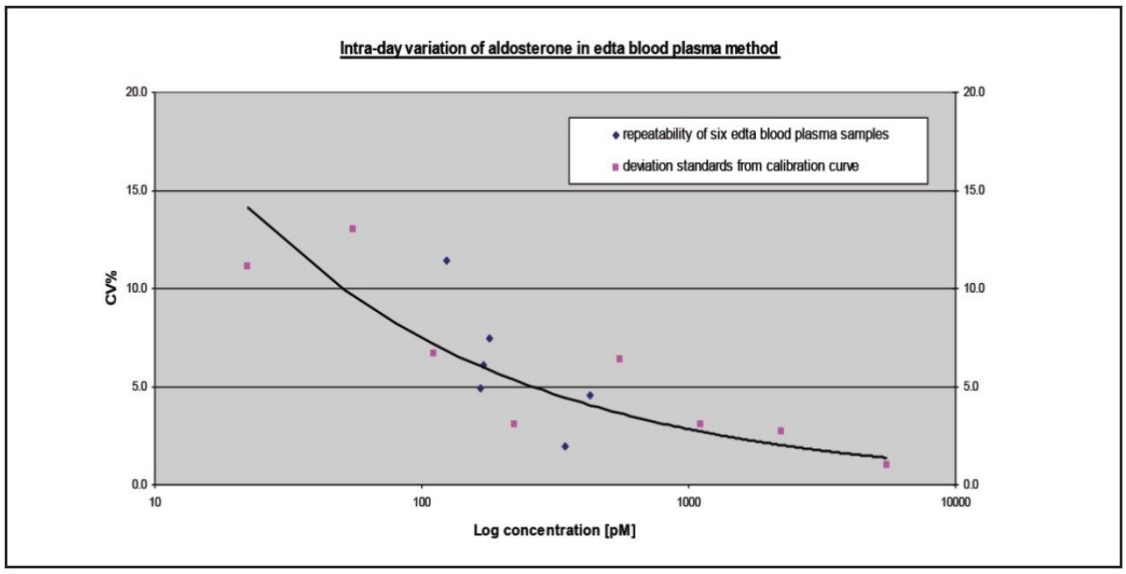

Figure 3: Intra-day variability of aldosterone measurement in plasma. This figure compares the %CV with the concentration of aldosterone in six plasma samples containing aldosterone as well as the aldosterone standards. The overall reproducibility of the method was < 11%.

The reproducibility and stability of the method over time was also tested by injecting samples from three different plasma pools containing three different levels of aldosterone (100, 700 and 2000 pM). Each sample was injected 20 times to determine if the peak areas remained constant. Results for these experiments are shown in Figure 4.

Aldosterone Conc	%CV
100 pM	12%
700 pM	6.5%
2100 pM	2.9%

Figure 4. Method Reproducibility Determination.

Conclusion

In this work, a very efficient method for clinical research has been developed for the measurement of aldosterone from plasma. The method utilizes LC-MS and an online SPE system. This combination of SPE sample preparation coupled with the analytical power of LC-MS provides the necessary sensitivity and productivity required to routinely measure aldosterone in plasma for this important steroid.

The method developed here provides:

- Analytically sensitive analysis of aldosterone in plasma (LLOQ ~60 pM)
- Effective separation of aldosterone to avoid interferences and overestimation
- Highly-efficient SPE sample preparation integrated with LC-MS
- Very good reproducibility and assay variation across a wide range of aldosterone concentrations

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