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Review

Mass spectrometry theory and application to adrenal diseases

Kerry M. Wooding [a,b], Richard J. Auchus [c,*]

[a] Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO, United States
[b] Department of Pharmacology, University of Colorado Denver, Aurora, CO, United States
[c] Division of Metabolism, Endocrinology, & Diabetes, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, United States

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ABSTRACT

The diagnosis and management of adrenal diseases hinge upon accurate determination of hormone concentrations in blood and other body fluids. The advent of immunoassays for various steroid hormones has enabled the remarkable progress in adrenal disease over the last several decades, with some limitation. Sequential immunoassay of single analytes is a tedious process, which requires aliquots for each assay. In many complex adrenal diseases, including adrenal cancer and congenital adrenal hyperplasia, the patterns or ratios of multiple steroids rather than the value of any one steroid is more relevant. Although gas chromatography/mass spectrometry of urinary steroid metabolites has been employed to profile steroid production, throughput is slow, and availability is sparse. Recent generations of liquid chromatography–tandem mass spectrometry instruments (LC-MS/MS) provide the throughput and sensitivity required to measure many steroids simultaneously using small samples for commercial and research uses. Even in the best hands, however, LC-MS/MS suffers from limitations and requires diligent attention to detail during method development and implementation. This article reviews the theory, instrumentation principles and terminology, and practical application of mass spectrometry to clinical adrenal disorders.

Contents

Abbreviations: APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photochemical ionization; CDC, Center for Disease Control; CI, chemical ionization; CID, collision-induced dissociation; CRPC, castration-resistant prostate cancer; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; GC, gas chromatography; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LTQ, linear trapping quadrupole; MTBE, methyl *tert*-butyl ether; MRM, multiple reaction monitoring; MS^n, mass spectrometry to the *n*th, meaning *n* cycles of iterative mass spectrometry; Qn, quadrupole number *n*; RF, radio frequency; UPLC, ultra performance liquid chromatography.

* Corresponding author. Address: Division of Metabolism, Endocrinology, & Diabetes, Department of Internal Medicine, 5560A MSRBII, University of Michigan, 1150 West Medical Center Drive, Ann Arbor, MI 48109, United States. Tel.: +1 734 764 7764; fax: +1 734 936 6684.
E-mail address: rauchus@med.umich.edu (R.J. Auchus).

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1. Introduction

Steroid hormones derive from the coordinated regulation of multiple enzymes arranged in pathways to maximize final product formation (Miller and Auchus, 2011). Adrenal diseases occur when enzyme deficiencies disrupt this normal flow of precursors to product and lead to imbalances, with excess and deficiencies of particular steroids. Adrenal tumors often express steroidogenic enzymes via autonomous mechanisms and in aberrant proportions, leading to accumulation of precursors and mixed steroid products. Multiplexed steroid profiling with mass spectrometry is now available to guide diagnosis and treatment of these adrenal diseases, supplanting traditional immunoassays. The adrenal investigator, however, must understand the theory and limitations of mass spectrometry in order to use these techniques appropriately.

2. Mass spectrometer design

2.1. The triple quadrupole instrument

The most common tandem mass spectrometer used for quantitating steroids is the triple quadrupole because of its sensitivity and versatility. Triple quadrupole tandem mass spectrometers (the "back end") coupled with high performance liquid chromatography (HPLC[1], the "front end", making a LC-MS/MS system, Fig. 1A) can detect <1 pg per injection ("on column") and provide qualitative and quantitative data in the same analyte run; experiments can be multiplexed to detect and quantitate numerous compounds in the same sample. Instead of running numerous ELISA (enzyme-linked immunosorbent assay) plates to detect each steroid of interest, one sample is extracted, and all steroids are analyzed in the same experiment. Mass spectrometry yields primary data to unequivocally detect and confirm the presence of a steroid, whereas ELISA yields an indirect measurement, which can be confounded by cross-reactivity of similar compounds (Haisenleder et al., 2011).

2.2. Quadrupole mass analyzer and MS experiments

At the center of the triple quadrupole mass spectrometer is the quadrupole mass analyzer, which scans ions from the ion source to the detector. A quadrupole consists of four parallel cylindrical rods arranged at the corners of a square such that ions traverse through the area of the square between these rods. A superposition of direct current (DC) and radiofrequency (RF) voltage creates an electric field between the rods with opposite rods electrically paired. The

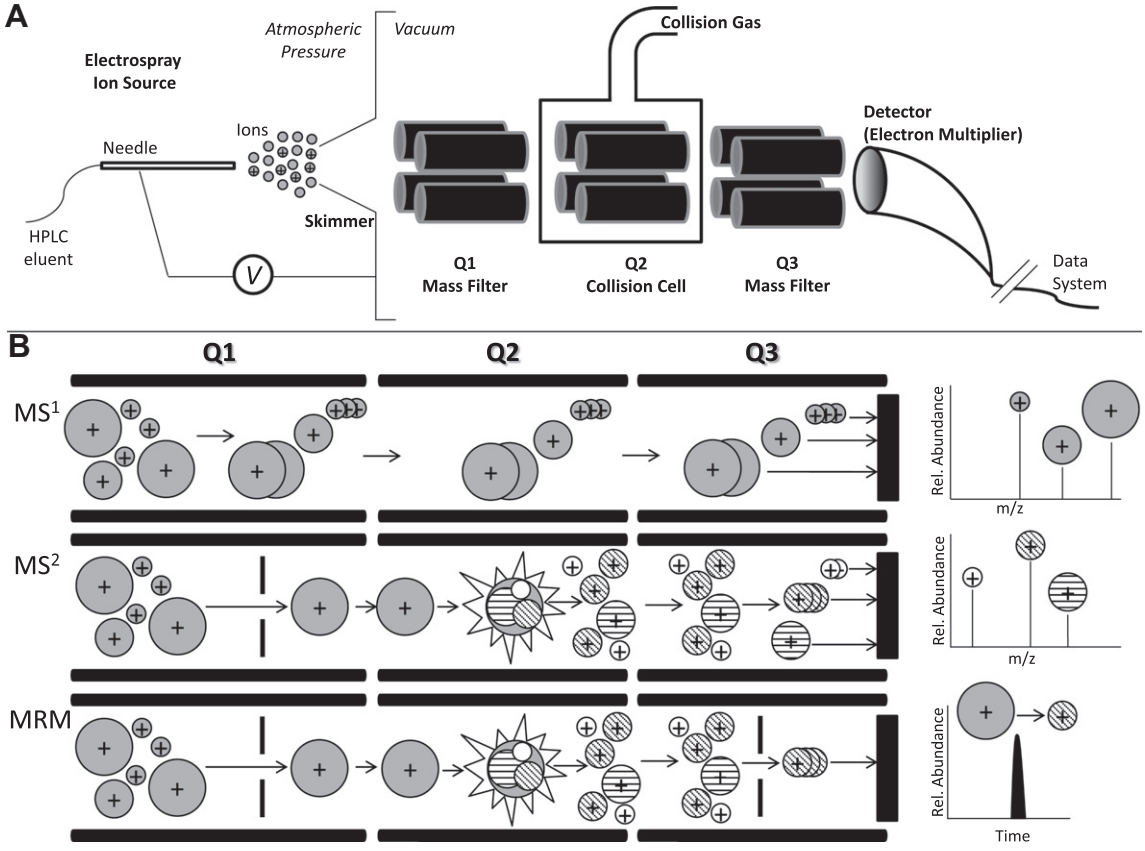

Fig. 1. Electrospray ionization (ESI) triple quadrupole mass spectrometer. (A) Schematic of an ESI source interfaced with a triple quadrupole mass spectrometer. After liquid chromatography separation, compounds elute into a high voltage needle, where a fraction exits as gas phase ions. The first and third quadrupoles (Q1 and Q3) function as mass analyzers whereas the second quadrupole (Q2) serves as a collision cell and ion guide. After ions pass through Q3, the ions collide with the detector, which measures ion abundance. The quadrupoles and detector function in conjunction to determine the mass of the ions as well as their abundance as described in B. (B) MS^1 experiments (top) are used to determine the mass spectrum of the ions in a sample. Q1 or Q3 scan the ions in a designated mass range and guide them to the detector; Q2 allows all ions in the designated mass range to pass through. MS^2 experiments (center) determine the collision-induced product ions of a designated precursor ion. Q1 selects and guides the precursor ion of interest to Q2, where it undergoes collision-induced dissociation with an inert gas to yield unique product ions. Q2 transmits all product ions to mass filter Q3, which sorts the ions and transmits them to the detector. MRM (multiple reaction monitoring, bottom) experiments are used to specifically quantitate multiple precursor ions using their characteristic product ions. Q1 selects the precursor ion, Q2 fragments it as in the MS^2 experiment, and Q3 transmits only a specific product ion to the detector. Multiple analytes can be quantitated in a single experiment using MRM.