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Review

Role of mass spectrometry in steroid assays

Apport de la spectrométrie de masse au dosage des stéroïdes

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Abstract

In addition to protein hormones, steroids measurement constitutes the basis of modern endocrinology. Immunoassays have shown their limits in this field. In contrast, mass spectrometry shows an excellent sensitivity and specificity that make it the method of choice for steroids assays. The recent introduction of UHPLC-MS is a major advance which reinforces this position. In fact, mass spectrometry provides a lot of advantages such as determination of certain steroids in saliva, diagnosis of enzyme deficiencies, or measurement of molecules previously inaccessible like aldosterone. However, standardization is still needed to ensure good comparability of results between laboratories. In the future, mass spectrometry should not replace the immunoassays but rather complement it.

Keywords: Mass spectrometry; Steroids; Estrogens; 17-hydroxyprogesterone; Testosterone

Résumé

À côté des hormones protéiques, le dosage des stéroïdes est à la base de l'endocrinologie moderne. Les techniques immunologiques ont montré leurs limites dans ce domaine. Au contraire, l'excellente sensibilité et spécificité de la spectrométrie de masse en font la méthode de choix et l'introduction récente de l'UHPLC-MS constitue une avancée majeure qui conforte cette position. En effet, la spectrométrie de masse apporte beaucoup d'avantages comme le dosage de certains stéroïdes dans la salive, le diagnostic des déficits génétiques en enzymes de la biosynthèse de ces hormones, ou encore le dosage de molécules autrefois difficilement accessibles comme l'aldostérone. Toutefois, la standardisation reste nécessaire pour permettre une bonne comparabilité des résultats entre les laboratoires. Dans l'avenir, la spectrométrie de masse ne devrait pas remplacer l'immunoanalyse, mais plutôt lui être complémentaire.

Mots clés : Spectrométrie de masse ; Stéroïdes ; Estrogènes ; 17-hydroxyprogestérone ; Testostérone

1. Introduction

Chromatography coupled to mass spectrometry is a very useful method for the measurement of steroids. These methods are characterized by high sensitivity and selectivity, while using *samples with small volume* [1]. The most often, the developed methods associate the power of chromatographic separation with the sensitivity of mass spectrometry. Different mass analyzers can be used like *time* of *flight mass spectrometry* (TOF-MS), high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), and *gas chromatography mass spectrometry* (GC-MS). Mass spectrometry has known in last few *years* a huge progress in medical biology. Thus, these techniques have been used in various applications such as the identification of germs in bacteriology, neonatal screening for inborn errors of metabolism, proteomics and hormonology. The aim of this article is to treat the recent advances regarding steroid assays using mass spectrometry.

2. Steroid assays history

In addition to protein hormones, the determination of steroids constitutes the basis of modern endocrinology. The use of mass

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spectrometry for steroids measurement started with analysis of these hormones in urine by GC-MS, that was described for the first time by Horning and Sweeley in 1960 [2]. Immunoassays have been a significant development in the 1960s. The first steroid measurement by radioimmunoassay (RIA) dates back to 1969 with the determination of estradiol (E2). Then, RIA has been quickly adapted to other steroids such as testosterone and progesterone. The sensitivity, accuracy and reproducibility of RIA made that it was the reference method whether for research or clinical practice. In the late 1970s, others immunoassays were developed based on enzymometry, fluorimetry or chemiluminescence. Like RIA, these methods can require pretreatment with a chemical extraction or chromatography, depending on antibody specificity. But, automation made them much faster and simple to use, which contributed to their generalization use in daily practice of clinical laboratories. In recent years, mass spectrometry has experienced major technological advance and has become the method of choice for steroids measurement [3].

3. Mass spectrometry

Mass spectrometry is an analysis method used to quantify, identify compounds, and elucidate the structure and chemical properties of different molecules (M). The process of analysis by mass spectrometry involves the ionization of molecules and conversion into a gas phase to allow analysis. This step can be performed with or without fragmentation. Thereafter, the separation is done as a function of mass to charge ratio (m/z). We are treating here only the main methodological aspects of mass spectrometry, essential to understand steroid assays. For readers interested in learning more, we recommend the review of Menet [4].

3.1. Ionization

3.1.1. Atmospheric pressure chemical ionization (APCI)

Liquid effluent is introduced directly into the ionization source through a probe. The sample solution undergoes a nebulization to form an aerosol spray of fine droplets and is rapidly heated in a stream of nitrogen gas. The mixture of the nebulizing gas and those from evaporation is driven to a corona discharge, where acid-base reactions occur. In general, proton transfer occurs in the positive or negative ion mode to yield $[M\text{-}H]^+$ or $[M\text{-}H]^-$ ions, respectively.

3.1.2. Atmospheric pressure photoionization (APPI)

APPI is the last arrival in the family of atmospheric pressure ionization methods to couple mass spectrometry to liquid-phase separation techniques [5]. This method is used for low to moderately polar compounds. The samples are ionized by using UV light; the molecules interact with photon of UV light and vapors of nebulizer liquid solution. The analyte molecules absorb a photon (hv) and become electronically excited molecules. If the ionization energy of molecules is lower than that of photons, molecules lose an electron to form cations.

3.1.3. Desorption electrospray ionization (DESI)

This method has high sensitivity, is virtually instantaneous in response time, and is applicable to small-molecule organic compounds as well as to biological macromolecules [6].

Ions are generated from the sample surface by way of bombardment with high velocity, charged micro-droplets through the atmosphere. The spray impact causes the formation of microscopic liquid layers on the sample surface in which the condensed-phase analyte dissolves. This process is followed by desorption via momentum transfer when additional droplets collide with the liquid layer forcing the dissolved analyte into the gas (atmospheric air) phase in the form of micron-sized droplets [7].

3.1.4. Atmospheric pressure photoionization (DAPPI)

DAPPI is an ambient ionization technique for mass spectrometry; it relies on a heated nebulizer microchip delivering a heated jet of vaporized solvent and a photoionization lamp emitting 10-eV photons. The solvent jet is directed toward sample spots on a surface, causing the desorption of analytes from the surface. The photons emitted by the lamp ionize the analytes, which are then directed into the mass spectrometer [8].

3.1.5. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI)

They allowed analysis of non-volatile and thermolabile compounds under adequate experimental conditions, with minimal fragmentation. These two techniques can be easily coupled with liquid chromatography systems. ESI can proceed via different mechanisms. Low molecular weight analytes follow the ion evaporation model, whereas the charged residue model applies to large globular species [9].

In MALDI analyses, the sample must be mixed with matrix and spotted in a stainless steel plate prior the analysis in the mass spectrometer. The sample is co-crystallized with the matrix, which has an essential function in MALDI. The co-crystallized sample is ionized by short laser pulses [10].

3.2. Analyzers

The analyzers are usually composed of several quadrupoles (generally three). Each quadrupole includes four electrodes that apply two electric fields: constant and alternative. In the quadrupoles, the trajectory of an ion subjected to these electric fields depends on both its mass and its load.

In case of tandem experiments, parent ions are separated from most others of the mixture in the first quadrupole. Then, parent ions are fragmented in the second quadrupole and analyzed in the third one. The combination of two or more analyzers in the same mass spectrometer yielded the high performance and resolution of the nowadays equipments. In tandem mass spectrometry, simple reaction monitoring (SRM) and multiple reactions monitoring (MRM) are used in quantitative analysis. In SRM, one of the fragment ions is analyzed, whereas multiple fragments ions are monitored and quantified in MRM [11]. The SRM gives a more important signal than MRM, but the background noise of