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Review

The coming of age of liquid chromatography coupled to tandem mass spectrometry in the endocrinology laboratory☆

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ABSTRACT

Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been rapidly incorporated in the routine of the endocrinology laboratory. Most endocrinologists are aware of the benefits afforded by this technique and tandem mass spectrometers are clearly no longer a mere research method but an important tool widely used for diagnosis. In the last 15 years, LC–MS/MS has replaced techniques such as immunoassay and HPLC for the analysis of hormones because it provides higher specificity and good sensitivity. Also, it permits simultaneous measurement of several analytes and sample preparation and acquisition are fast and simple. Although several strategies based on LC–MS/MS have been described in the last 15 years, there is still room for improvement. The impact of matrix effects and isobaric interferences have been addressed by only a few studies, and standardization with reference materials is available for a limited number of analytes. This review summarizes the application of LC–MS/MS in analyzing three classes of hormones: steroids, derivatives of the aromatic amino acids, and peptides and proteins. The benefits and current limitations of LC–MS/MS will be discussed for these hormone categories.

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1. Introduction

Laboratory testing plays a decisive role in the practice of endocrinology. The measurement of hormones and/or their metabolite levels in biological fluids such as blood and urine allows the clinician to determine in which gland(s) abnormal levels are being produced and trace a strategy for treatment. However, hormone quantification is among the most troublesome determinations in the clinical laboratory. One of the main reasons is the minute concentrations at which hormones are found in blood. For instance, important analytes such as parathyroid hormone, free thyroxine, and estradiol are present in the picomolar range (Fig. 1).

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic; APPI, atmospheric pressure photoionization; APCI, positive atmospheric pressure chemical ionization; DHEA, dehydroepiadrosterone; DHT, dihydrotestosterone; HAb, heterophilic antibodies; MRM, multiple reaction monitoring; PTH, parathyroid hormone; RIA, radioimmunoassay assays; T3, triiodothyronine or 3,3′,5-triiodo-L-thyronine; T4, thyroxine or 3,3′,5,5′-tetraiodo-L-thyronine.

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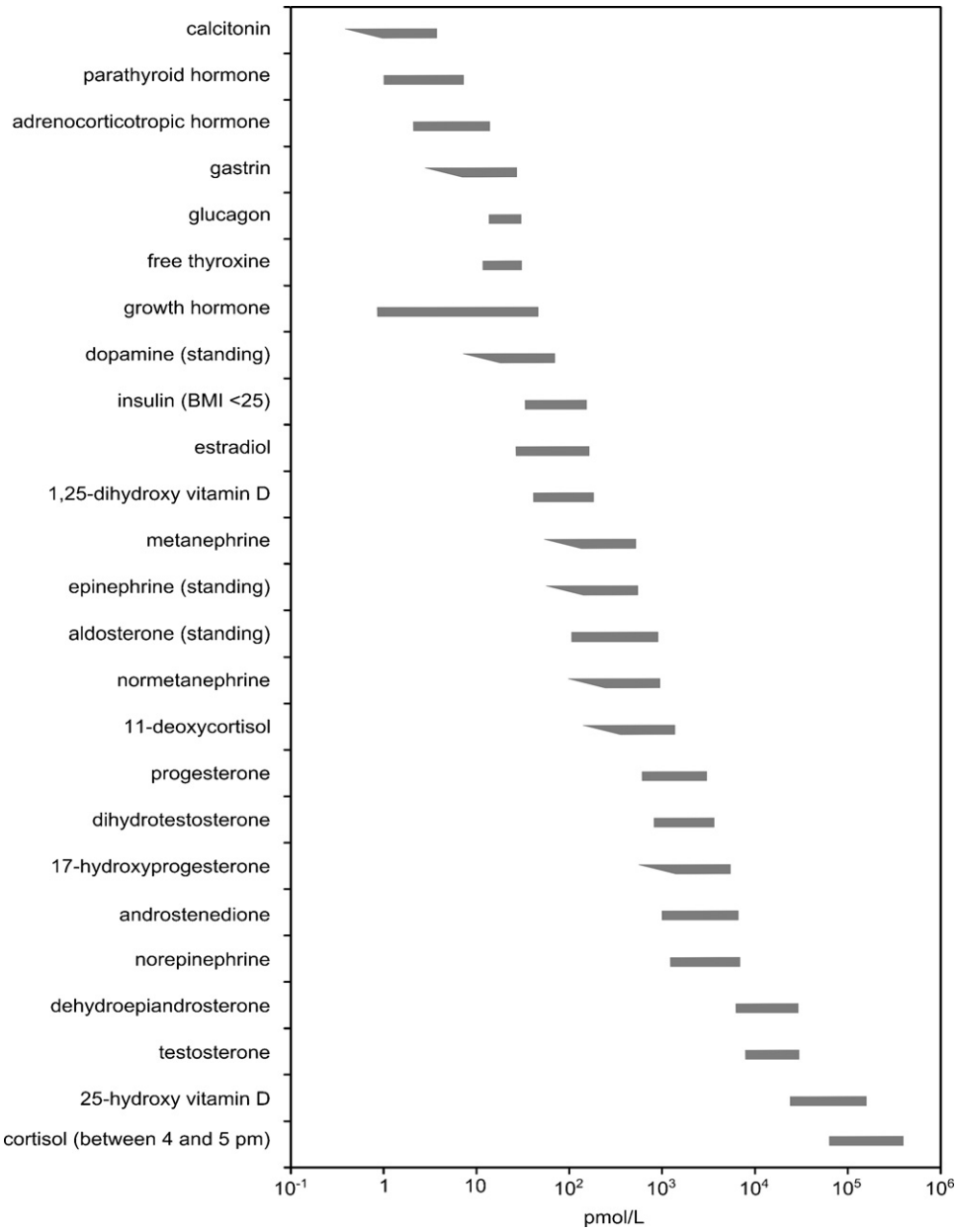

Fig. 1. Reference intervals for some human hormones in serum of adult males (based on Fleury Test Catalogue [159]). The left vertex indicates that the inferior limit is not defined or equals 0.

Detection of minute amounts within very complex matrices poses a tremendous analytical challenge.

The determination of low concentrations of hormones was made possible by the development of immunoassays. The basis of radioimmunoassay assays (RIA) postulated by Yalow and Berson in 1959 [1] turned endocrinology into a real quantitative science. Because antibodies can be produced to bind to virtually any analyte, RIA methods became highly successful as they allowed the determination of not only hormones but also a wide range of analytes of clinical importance. Still important for steroid determination, RIA produces reliable results when used in combination with previous extraction steps required for the elimination of interfering compounds [2,3]. However, laborious sample preparation, the need for radioactive reagents, and difficulties in achieving automation have limited its application in clinical laboratories.

In the late 1970s, the development of new approaches such as non-competitive assay [4], two antibody systems [5,6], chemiluminescent and fluorescent labels, as well as monoclonal antibodies [7] resulted in a new generation of immunoassays. These new techniques were successfully adapted into commercial kits to be used in automated platforms. Their low cost, simplicity, and speed led most clinical laboratories to acquire them.

However, recent studies have indicated that automated immunoassays are far from ideal and often fail to provide accurate results. For example, Taieb et al. [8] showed that among 10 commercially available immunoassays none was reliable enough for the investigation of testosterone levels usually found in children and women. The low accuracy obtained with the use of direct immunoassays could be explained by the limited specificity of the antibody, a frequent situation in the case of small molecules. Additionally, direct immunoassays overestimate the levels of estrone and estradiol in post-menopausal women due to cross-reaction with estrone sulfate, which is found in relatively higher levels [9].