Chromatography
Separation Techniques

Bunch and Wang, J Chromatograph Separat Techniq 2014, 5:4
DOI: 10.4172/2157-7064.1000e122

Research Article Open Access

Steroid Analysis by Liquid Chromatography-Mass Spectrometry: Derivatization Consideration

Bunch DR and Wang S*

Department of Clinical Pathology, Cleveland Clinic, Cleveland, OH 44195, USA

Steroids are small non-polar cholesterol derived hormones that regulate metabolic processes through the endocrine systems [1], and are essential to proper physical development, sexual differentiation and maturation, and metabolic homeostasis. Abnormal steroid hormone production can indicate various human diseases such as congenital metabolic defects, hyperplasia, and cancer. Accurate steroid assessment in circulation is essential for determining pathophysiological conditions and forming treatment strategies.

Steroid measurement was first achieved through radioimmunoassay (RIA) and later by direct immunoassays (IA). RIA requires sample pre-treatment to produce reliable results. Direct IAs are quick and require no pre-extraction steps and are currently the most commonly used steroid assays within clinical laboratories [1]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) leverages specificity for steroid analysis to eliminate the accuracy issues associated with IAs, which are commonly due to interfering substances such as heterophilic antibodies that tend to produce false positives [2] or negatives [3] and limited antibody specificity, which cross react with other steroids [4]. LC-MS/MS is quickly becoming the preferred technology for steroid determination.

The major analytical issues with LC-MS/MS are ionization efficiency, matrix effects, and isobaric interferences [4]. Some steroids such as pregnenolone, 17-hydroxypregnenolone, and dehydroepiandro stenedione are particularly difficult to ionize using normal LC-MS/MS ionization techniques, electrospray or atmospheric chemical ionization. One method for addressing this is chemical derivation. For steroids, these chemical derivations usually utilize the keto or hydroxyl groups to add chargeable moieties allowing for improved ionization [5]. Keto group derivation is usually performed with a Schiff base type reaction to attach a new moiety. These moieties can carry charged groups (nitrogen-Girard P and T) or groups that are easily ionized such as hydroxyl (hydroxylamine), carboxylic acid (carboxymethoxylamine), or sulfur groups (*p*-toluenesulfonyl hydrazone). The compounds capable of derivatizing the hydroxyl groups (2-fluoro-1-methylpyridinium p-toluenesulfonate and dansyl chloride) have similar motifs [5]. In addition to the improved ionization, these chemical additions can improve chromatographic separation of isobaric interferences and substances causing matrix effects. The additional moieties can also change the parent ion fragmentation pattern yielding high abundance product ions that distinguish between isobars. The disadvantages of the derivatization strategies are three folds: 1) extra labor time is needed to perform the assays; 2) precision may be compromised dues to extra steps introduced; 3) accuracy could be reduced due to the possible hydrolysis of conjugates under the derivatization conditions [6]. One should consider the steroid panel components and the clinical needs to determine if derivatization is necessary.

References

1. Kulle AE, Welzel M, Holterhus PM, Riepe FG (2011) Principles and clinical applications of liquid chromatography - tandem mass spectrometry for the determination of adrenal and gonadal steroid hormones. J Endocrinol Invest 34: 702-708.
2. Fingerhut R (2009) False positive rate in newborn screening for congenital adrenal hyperplasia (CAH)-ether extraction reveals two distinct reasons for elevated 17alpha-hydroxyprogesterone (17-OHP) values. Steroids 74: 662-665.
3. Carvalho VM (2012) The coming of age of liquid chromatography coupled to tandem mass spectrometry in the endocrinology laboratory. J Chromatogr B Analyt Technol Biomed Life Sci 883-884: 50-58.
4. Kushnir MM, Rockwood AL, Roberts WL, Yue B, Bergquist J, et al. (2011) Liquid chromatography tandem mass spectrometry for analysis of steroids in clinical laboratories. Clin Biochem 44: 77-88.
5. Higashi T, Shimada K (2004) Derivatization of neutral steroids to enhance their detection characteristics in liquid chromatography-mass spectrometry. Anal Bioanal Chem 378: 875-882.
6. Soldin SJ, Soldin OP (2009) Steroid hormone analysis by tandem mass spectrometry. Clin Chem 55: 1061-1066.

***Corresponding author:** Wang S Department of Clinical Pathology LL3-1409500 Euclid Ave, leveland, OH 44195, Tel: 216-445-2634; Fax: 216-445-0212; Email: wangs2@ccf.org

Received July 27, 2014; **Accepted** July 28, 2014; **Published** September 01, 2014

Citation: Bunch DR, Wang S (2014) Steroid Analysis by Liquid Chromatography-Mass Spectrometry: Derivatization Consideration. J Chromatograph Separat Techniq 5: e122. doi:10.4172/2157-7064.1000e122