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Development of a new liquid chromatography-tandem mass spectrometry method for the determination of hormones in bovine muscle



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1. Introduction

Steroid hormones are a group of lipophilic, low-molecular-weight, and biologically active compounds [1]. The main compound from which all steroids are derived is cholesterol [2]. Natural and synthetic hormones have been widely used for many decades in animal husbandry to improve the rate of growth and the efficiency of feed conversion [3]. Nonetheless, some hormones might have a carcinogenic effect leading to breast cancer, ovarian cancer, and prostate cancer [4]. Moreover, some synthetic growth promoters have potential endocrine-disrupting properties causing behavioral disorders, decreased fertility, and birth malformations [5]. Consequently, the European Union's Scientific Committee on Veterinary Measures has banned the use of natural and artificial hormones in meat and meat production through the Council Directive 96/22/EC.

However, the evolution of the "black market" was limiting the efficiency of control of the residues of these substances in foods of animal origin. To ensure consumer's safety, and to detect their presence at very low levels in the food matrices, the development of sensitive, specific, and multi-residue analytical methods has become necessary and of great significance. For a long time, researchers used to work with gas chromatography (GC) as a tech-

nique for hormone analysis [6], but since hormones possess poor thermal stability and volatility, so a derivatization step, is required before GC analysis. Derivatization is a chemical reaction aiming to modify the compound' polarity and ionization by producing new derivatives easier to be analyzed by GC-MS. Despite that, the prederivatization processes are time-consuming [7], and difficult to be applied for all compound [8,9]. Indeed, liquid chromatographytandem mass spectrometry (LC-MS/MS) has gained popularity over the last decades for the detection of growth promoters in biologicals matrices [10]. Moreover, matrix complexity and the presence of hormones at an often low level, make residue analysis of animal matrices challenging. To detect residue low levels, sample preconcentration is needed aiming to decrease the interfering matrix contaminants. Thus, the extraction of analytes of interest is a very crucial step to be considered when working with complex samples containing very low concentrations of organic compounds [11]. Different extraction techniques have been used for veterinary drug analysis such as QuEChERS [12], Solid Phase Extraction (SPE) [13], Supercritical Fluid Extraction (SFE) [14], and Accelerated Solvent extraction (ASE) [15]. In this context, QuEChERS was the most applied technique used for the extraction of organic contaminants from animal tissues [16]. The major advantages that encouraged researchers to focus on this extraction method are related to its simplicity, its quick extraction, its high sample throughput, as well as the possibility of using small solvent volume.

In Lebanon, no restrictions were implemented yet to control the use of these hormones in animal husbandry. Until now, no data have been provided about the contamination level with hor-

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