The use of Thermo Scientific LTQ Orbitrap™ and Applied Biosystems Scex QTRAP® 5500 instruments were compared to study in vitro metabolism of stanozolol in the equine. Several major stanozolol metabolites were identified on both instruments, but the use of an elugated HPLC gradient and product ion scanning on the QTRAP® 5500 (not subject to the MS/MS low-mass cut-off of the LTQ Orbitrap™) allowed several additional isomers to be detected and their stereochemistry to be postulated.

Introduction

During the last decade, in vitro techniques to study drug metabolism have become routine in the pharmaceutical industry (1). In addition, in vitro metabolism studies are also expected to play an increasingly important role in supporting routine sports surveillance (2-4).

In vitro incubations are able to produce metabolites that can be used to confirm the presence of a metabolite where no reference standard or in vivo post administration sample is available. In addition, in vitro studies can be used to complement in vivo metabolite identification studies as experiments can be performed over much shorter time scales than animal administrations and they produce a cleaner, more concentrated extract for analysis.

Triple-quadrupole mass spectrometry has been established as a reliable analysis tool in the drug surveillance industry; however the use of a high resolution, accurate mass instrument was assessed to determine the potential benefits of such a system.

The methodology of the synthetic anabolic steroid; stanozolol (see Figure 1) was investigated in this study as an illustration of the future applicability of this technique.