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Multi-Detection of Corticosteroids in Sports Doping and Veterinary Control using High Resolution Liquid Chromatography / Time-of-Flight Mass Spectrometry[1]

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Abstract

A liquid chromatography / time-of-flight mass spectrometry (LC/TOFMS) method was developed using the latest high-resolution LC column technology (UPLC^{TM}) and electrospray ionization (ESI) in the positive ion mode. Gradient UPLC separation conditions were optimized for a group of 22 analytes comprising 17 glucocorticosteroids, specific designer steroids such as tetrahydrogestrinone (THG) and specific $ß_2$-agonists such as formoterol. The UPLC/TOFMS separation obtained required 5.5 minutes only for all the substances tested. Even the critical pair of dexamethasone and betamethasone isomers was almost completely resolved. Thanks to the >10,000 (FWHM) mass resolution and high mass accuracy features of TOFMS 50 mDa window accurate mass chromatograms could be reconstructed for the individual analytes. Sensitive screening in human and calf urine samples samples fortified at the glucocorticosteroids MRPL of 30 µg L^{-1} (human urine, sports doping) and 2 µg L^{-1} (calf urine, veterinary control) could be obtained. The potential of UPLC/TOFMS for confirmatory analysis was shown by determining the accurate mass of all compounds and fragment ions upon in-source collision induced dissociation at different energies. The exact mass measurement errors for all (glucocortico)steroids were found to be within 6 ppm. Considering veterinary control, LOD and LOQ were determined for most of the analytes in calf urine and found to range from 0.1 to 3.3 and from 0.4 to 4.4 µg L^{-1}, respectively. The method can be easily extended with other banned substances of interest, as demonstrated by the addition of

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21 ß$_2$-agonists to the original analyte mixture in urine, without causing any interferences.

One specific pitfall should be considered when using reconstructed highly selective narrow window accurate mass chromatograms for the individual analytes. The accurate mass measured might have been shifted due to limitations in the linear dynamic range of the MS detector and/or due to co-elution with an isobaric mass from a different compound or from a different fragment ion. It can be calculated that co-elution of steroids (or fragments thereof) which differ only in one of the simple substructures C_2H_4, CO and N_2 can not be mass resolved by the current state-of-the-art mass resolution performance of (Q)TOFMS instruments [2]. A mass shifted outside the narrow window applied for the reconstructed chromatogram will yield undesirable false negative analysis results. Consequently accurate mass screening criteria were proposed in order to overcome this limitation [2].

References

[1] The full paper is published in *Analytica Chimica Acta* (2006) and available from the Internet at doi:10.1016/j.aca.2006.09.058.

[2] M.W.F. Nielen, M.C. van Engelen, R. Zuiderent, R. Ramaker, "Screening and confirmation criteria for hormone residue analysis using liquid chromatography accurate mass time-of-flight, FTICR and orbitrap mass spectrometry techniques", *Analytica Chimica Acta* (2006) available from the Internet at doi:10.1016/j.aca.2006.08.055.