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Investigation of electrosray photoionization (ESPI) as new 'universal' ionization method for determination of doping agents by HPLC/Orbitrap mass spectrometry

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Abstract

In this work we describe results based on the combination of APPI with ESI. The main purpose of combining two sources is to extend the range of compounds that can be simultaneously analyzed. This configuration minimally affects the performance of either ionizer relative to the standard single sources. However, it is observed that the operation of both ionizers together does not typically give the sum signal from either source operating alone.

Introduction

Owing to the advantages of omitting the derivatization step, the number of LC-MS methods for the determination of doping agents in human urine has increased in the past years [1]. Unfortunately, these methods normally deal with a limited range of compounds. This is a direct consequence of the unique ionization mechanism associated with ESI, APCI and APPI. Given the diverse ionization mechanism of these methods, it is logical to conclude that there are scenarios in which one source performs better than another, and indeed a combined dual source would be ideal. Undoubtedly, the coverage of doping agents will be dependent on the ability of the ion source to ionize the compounds.

Recently, Short and colleagues demonstrated the advantages of using ESPI source for the detection of several compounds [2]. The ESPI source is probably the most adequate combination of two ion sources, spanning the greatest range of compounds on the polarity scale. However, the ESPI source has required additional research to improve and validate this method in terms of doping control. The goal of this study is to present ESPI-MS as a new 'universal' ionization method for a wide range detection of doping agents by HPLC/Orbitrap mass spectrometry.

Regardless of the fact that Orbitrap has been used for doping control in terms of screening in biological matrices [3], this recently introduced technique has not been used with ESPI. The performance of the ESPI was evaluated against exogenous anabolic steroids.

Materials and Methods

Compounds were more mostly purchased from LGC Standards. The HPLC grade acetonitrile are obtained from Merck (Darmstadt, Germany). The stock solutions of each analyte at a concentration of 1 mg/mL were prepared. The experiments were performed using Accela HPLC system interfaced to an Exactive mass spectrometer (Bremen, Germany). The Kr lamp (Syagen PhotoMate) is mounted in the ESI/APPI source. The ESI/APPI source does not use an APCI nebulizer/vaporizer unit, but rather relies on the vaporization of analyte achieved by HESI-II from ThermoScientific. The mass spectrometer was operated in the positive mode. The desolvation temperature was 450^{o}C. The instrument was calibrated daily using the manufacturer's calibration mixture that consisted of caffeine, the tetrapeptide MRFA and Ultramark to reach mass accuracies in the sub-ppm range. The mass spectrometer was operated at a resolution of 50000 @ *m/z* 200 (FWHM). Data were acquired in the full scan mode from 100 to 650 Da, with a scan time 1.2 s, and processed using Xcalibur software (ThermoScientific, Bremen, Germany). Nitrogen produced by a Peak nitrogen generator system (Peak Scientific, Billerica, MA) was used as the nebulizing gas, the flow rate was 1 L/min. Capillary temperature was 275^{o}C.

The HPLC separation was carried out at ambient temperature on an ACE C_{18} analytical column (50×1mm, 3 µm particle size) from ACE (Aberdeen, Scotland). Solvent A was 0.2 % formic acid and for B acetonitrile was used. The solvent composition started at 30 % A (1 min), was raised to 100 % in 10 min, held for 5 min and re-equilibrated for 5 min at the starting conditions. The flow rate was 100 µL/min.

Results and Discussion

Fig. 1 illustrates the proposed ion source configuration. In this arrangement, the HESI source is liquid-ionization technique used to deliver and vaporize the analyte. APPI is a gas-ionization technique, thus a heated sheath flow of nitrogen provides the evaporative assistance. During ESPI experiments the spray voltage was reduced from 5000 V to 2500 V to achieve the optimum ion abundance. Optimum ion abundance for the ESPI source was observed when the lamp was aligned orthogonal to the plane defined by the MS inlet axis and the nebulizer/vaporizer axis. Method development for ESPI focused on ion source parameters,

mobile phase composition and LC flow rate, since they are important for successful ionization by ESPI. High nebulizing temperatures and lower HPLC flow rates increased ion intensity. With regard to performance as function of flow rate, the ESPI sensitivity (defined as signal per unit mass) was optimal in the range of 100-170 µL/min.

We compared the ESPI, APPI and ESI LC-MS results for anabolic steroids. The ESI results presented here are for the ESPI source with the lamp off so that the only functioning ionization mechanism was ESI. As an example, **Fig.2** compares the sensitivity for 3'-hydroxystanozolol and 5α-estrane-3α-ol-17-one (nandrolone metabolite). Fig.2 shows an example where APPI is the preferred ionizer for 5α-estrane-3α-ol-17-one, whereas, ESI provides better signal than APPI for 3'-hydroxystanozolol. Consequently the combination of ESI and APPI (ESPI) offers simultaneous detection of these two anabolic steroids. This configuration minimally affects the performance of either ionizer relative to the standard single source. However, it is observed that the operation of both ionizers together does not typically give the sum signal from either source operating alone.

Acknowledgements

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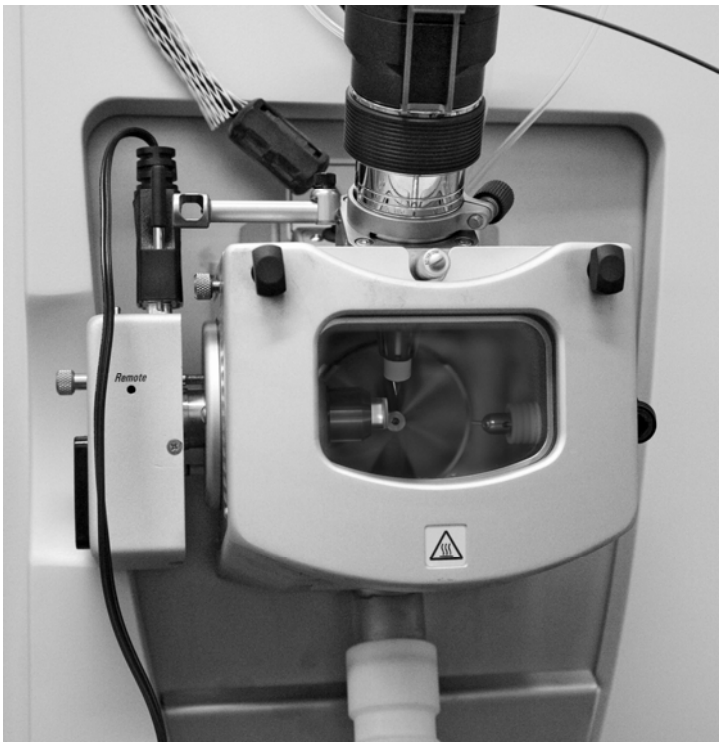

Fig.1. Dual ESI/APPI source configuration for the Exactive mass spectrometer

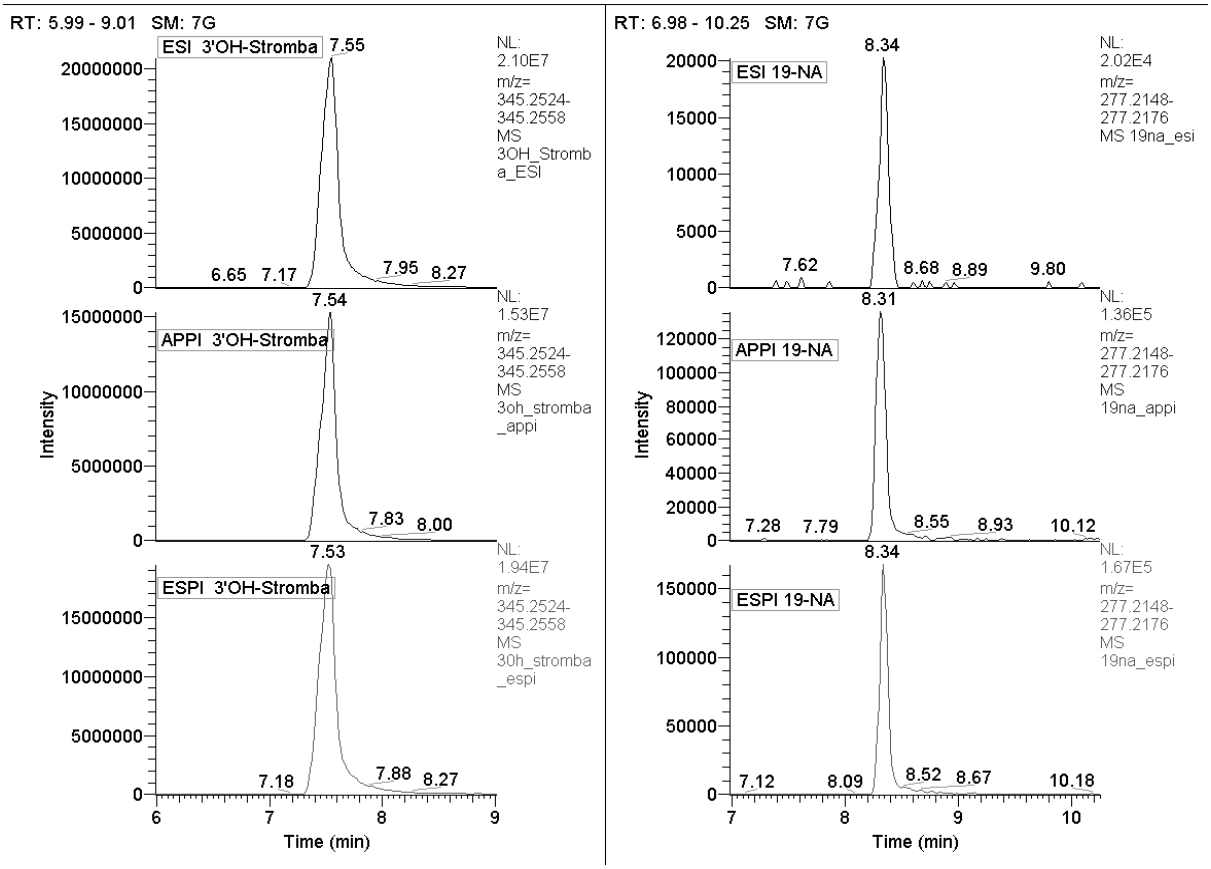

Fig.2. Extracted ion chromatograms for the injection of 3'-hydroxystanozolol (*m/z* 345.2537) and 5α-estrane-3α-ol-17-one (nandrolone metabolite, *m/z* 277.2167) obtained by ESI, APPI and ESPI (20 ng injected)