Systematic toxicological analysis of xenobiotics – The LC/MS approach

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GOALS: The systematic toxicological analysis of xenobiotics is usually performed by gas chromatography coupled to mass spectrometry (GC/MS) and/or by liquid chromatography coupled either to a UV diode array detector (LC/DAD) or to a mass spectrometer (LC/MS). A preliminary extraction step is required before chromatographic analysis. Although this discriminating step is fundamental, existing commercial libraries currently do not provide information on the extraction protocol or on the estimation of the limits molecule identification. In this work, we present the compilation of a library, based on in-source fragmentation, for > 500 molecules, the development of a generic solid-phase extraction (SPE) method and an evaluation of the utility of the recently introduced ChromaLynxTM deconvolution software, for the identification of these xenobiotics in serum and urine.

MATERIALS AND METHODS: Reference mass spectra were obtained from solutions of the pure standards injected at known concentrations. Liquid chromatography was performed using an XTerra column (150 × 2.1 mm, 3.5 µm) protected by a column (10 × 2.1 mm) with a gradient elution at 0.2 mL/min using acetonitrile and 5 mM ammonium formate buffer, pH3.0, increased from 5 to 90% in 26 min. The column was connected to a Waters ZQ mass spectrometer operated in electrospray (ESI) mode. Data was acquired in full scan mode (100 to 650 m/z) using 7 different acquisition channels set at different cone voltages and polarity (1 channel in negative ESI, and 6 in positive ESI). In a second step, we optimized the SPE method on Waters MCX cartridges, using a saline solution of molecules selected for their very different chemical properties. Lastly, we constituted pools of 15 to 20 analytes in serum (with concentrations in the high therapeutic range (c.a. mg/L)) and urine matrices. The chromatograms were then automatically analyzed by the Chromalynx™ software to generate a summary table of the identified molecules with an identification confidence factor.

RESULTS AND DISCUSSION: Different in-source collision induced dissociation (CID) mass spectra were obtained depending on the applied cone voltages. Low value cone voltages provided the molecular weight information (protonated or deprotonated molecules). Higher cone voltages values provided fragmentation spectra. The identification of the xenobiotics was improved when taking in consideration the spectra obtained at the different cone voltages in addition to chromatographic retention time. The optimized SPE method demonstrated good recoveries, however it was noted that the background noise was higher in comparison to a previously used liquid-liquid extraction protocol. This is likely to be as a result of a less discriminating protocol.

The ChromaLynx™ software examines every scan and extracts the most abundant ions (up to 8 ions) on all acquisition channels (different cone voltages). The result (identified molecule) from each channel is compared to the results of the other channels, thus, identified molecules are provided with an average similarity index.

CONSLUSION: We have optimized the analytical conditions and obtained in-source-CID spectra for more than 500 molecules (equivalent to >2500 spectra) with corresponding retention times. The generic SPE method was been evaluated on all molecules, with better results than liquid liquid extraction in serum and urine and in the future could be automated. A large proportion of the molecules were identified automatically at their high therapeutic level. The complete evaluation of all the molecules of this library will give a better knowledge of the possibilities and limitation of this analytical approach for the systematic toxicological analysis of xenobiotics in urine and serum.

KEYWORDS: *Systematic toxicological analysis, LC/MS, solid-phase extraction*

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