Comparative study of simplified sample preparation on ionization efficiency of ESI and APCI and development of a sensitive LC-MS/MS method for the analysis of multiple drugs of abuse in biological fluids

Dams R.\textsuperscript{1,2}, Murphy C.\textsuperscript{1}, Choo R.\textsuperscript{1}, Lambert W.\textsuperscript{2}, and Huestis M.\textsuperscript{1}

\textsuperscript{1} National Institute on Drug Abuse, 5500 Nathan Shock Drive, Baltimore, MD, 21224, USA, \textsuperscript{2} Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium.

Liquid chromatography (LC) combined with atmospheric pressure mass spectrometry (MS) is a very powerful technique suitable for the analysis of biological fluids. Both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are compatible with on-line analysis of LC effluent. One advantage of LC-ESI-MS and LC-APCI-MS over the more traditional GC-MS, is that the LC-MS techniques do not require derivatization of compounds prior to ionization. Furthermore, LC-MS provides for a specific, sensitive analysis with minimal sample preparation. By minimizing sample handling and clean up prior to the quantitative analysis we also reduce sample analysis time and the risk of additional sample loss. However, matrix suppression due to the presence of endogenous matrix compounds has been noted in ESI. The purpose of the present work was to simplify the sample handling and clean-up of biological fluids (plasma, urine, and saliva) prior to LC-MS analysis of a large range of drugs of abuse, i.e. opioids, cocaine, methadone and their metabolites. Four different sample preparation techniques were investigated, i.e. solid-phase extraction, protein precipitation, dilute-and-inject and direct injection. We evaluated the influence of residual endogenous matrix components after sample handling on the ionization efficiency of ESI and APCI for 7 major compounds, namely morphine, codeine, 6-acetylmorphine, cocaine, cocaethylene, propoxyphene, and methadone.

All LC-MS experiments were carried out on an LCQ Deca XP Ion Trap Mass Spectrometer interfaced to a Surveyor HPLC system (ThermoFinnigan, CA). The instrument could be fitted with either an ESI or APCI source, both operated in positive ion mode. Chromatographic separation was performed on a Synergi Polar RP column (150 x 2.0 mm, 4\textmu m) (Phenomenex, CA). Gradient elution with (A) 10 mM ammonium formate formate in water, 0.001% formic acid (pH=4.5) and (B) acetonitrile, at a flow rate of 300\mu l/min was applied. The initial gradient conditions were 5% B, increased to 26% B in 13 min, with a final composition of 90% B in 9 min. The column was flushed for 2 min at 90% B. The initial gradient conditions were reestablished in 3 min and the column was equilibrated for an additional 7 min. Identification and quantitation were performed by single, and multiple ion reaction monitoring (SRM,MRM).

Electrospray ionization proved to be more susceptible to the presence of matrix compounds than APCI. Conversely, the presence of matrix compounds had little to no effect on APCI. Subsequently, a quantitative LC-APCI-MS/MS method for a large number of drugs of abuse, namely opioids, cocaine, methadone, and their metabolites was developed and validated. The following preliminary data, on a limited number of compounds, were obtained. Calibration, using deuterated internal standards, was done by
linear regression analysis. Linearity was obtained with an average correlation coefficient ($r^2$) >0.991. Intra-day reproducibility of the method was evaluated at 10 ng on column and proved to be less than 8% (%RSD) for all compounds. Limits of detection (LOD, with S/N ≥3) and quantitation (LOQ, with S/N ≥10) were established between 20-100 pg on column and 50-300 pg on column, respectively. A range of LODs and LOQs was noted for the various sample preparation procedures; all were clinically relevant. LC-APCI-MS/MS provided a fast, efficient method for the quantitation of a wide variety of illicit drugs from a number of different biological matrices. Finally, the method will be applied in a controlled in-utero study.