Pitfalls associated with the use of a simple sample preparation in the analysis of saliva with LC-ESI-MS/MS.

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The fast analysis of biological fluids with LC-ESI-MS is often hampered by the presence of endogenous compounds. These matrix components restrict the use of a simple sample preparation and short chromatographic run times. Saliva is presented as a relatively clean matrix compared to other biological fluids such as plasma or serum. Indeed, typical matrix components like proteins and lipids are more than twenty times less concentrated.

Hence, a simple protein precipitation sample preparation method for LC-ESI-MS/MS analysis of saliva was developed, which would allow the simultaneous identification and quantification of amphetamines (amphetamine, methamphetamine, MDA, MDMA and MDEA), opiates (codeine, morphine and 6-monoacetylmorphine) and cocaine with its metabolite benzoylecgonine in one single experiment. To 200 µL of saliva, 100 µL of methanol (containing standards and internal standards) and formic acid were added, followed by vortex mixing and centrifugation at high speed. The supernatant was neutralised with ammonia and 50 µl was injected on a phenyl type column (100 x 2.1 mm) which was eluted with a mixture of water, methanol and ammonium formate in a gradient system. A validation of the method was performed, consisting of linearity evaluation, within-day and total-reproducibility, stability of the compounds during analysis, precision and absence of matrix suppression.

As judged by todays bioanalytical validation criteria, the method seemed successful, except for the presence of matrix suppression, which caused erroneous results when analysing the saliva of different individuals spiked with exactly the same quantity of the drugs. To ascertain this conclusion, matrix suppression was further investigated by the continuous postcolumn introduction of MDMA, during the analysis of a blank saliva sample. This allows a visualisation of the ionisation suppression by the matrix components, during the entire chromatographic run. It was seen that the ionisation was disturbed for almost the entire run. In order to avoid this, a more aggressive protein precipitation was developed. A high amount of acetonitrile (five times the sample volume) and methanolic hydrochloric acid were added to the saliva sample. After centrifugation an amount of supernatant was evaporated and reconstituted. In another experiment an extra ultrafiltration step was incorporated. However, both procedures still suffered from an extensive matrix suppressive effect. Therefore, it was concluded that the LC-ESI-MS/MS analysis of saliva, preceded by an aselective sample preparation, can seriously suffer from ionisation suppression by endogenous compounds. Our data clearly demonstrate the need to include evaluation of a suppressive effect by matrix constituents in a validation procedure.

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