K6 An LC-Electrospray Tandem Mass Spectrometric Method for Identification and Quantitation of Cocaine and Key Metabolites in Biological Matrices

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After attending this presentation, attendees will understand a highly sensitive and specific method for analysis of cocaine and its primary metabolites in blood and urine

Since cocaine is one of the most widely abused illegal drugs, providing a robust and reliable new method for the analysis of this compound and its metabolites will likely prove valuable to many practitioners in the fields of criminal forensic toxicology, workplace and sports drug testing, and postmortem toxicology. Many features of the reported method help to ensure that accurate testing results are obtained rapidly with a high degree of analytical confidence.

This poster will present a recently developed method for the simultaneous analysis of cocaine, benzoylecgonine, methylecgonine, and cocaethylene in biological matrices. Both validation data and data from several cases illustrative of the analytical power of the method will be presented.

The presented LC-ESI-MS/MS analytical method was developed to replace an existing GC-MS (CI) method that relied upon derivatization of the extracted analytes to achieve sufficient component volatility. Prior experience had revealed several limitations to this method, including poor long-term stability of the derivatives, poor derivatization efficiency in some putrefied samples, and compromised chromatography in many putrid or highly concentrated specimens. Additionally, the derivatizing reagents for the GC-MS method were costly, toxic, and had a relatively short shelf life, making their elimination highly desirable.

The solid phase extraction of specimens from the prior method was retained essentially intact, with only a change in one of the internal standard compounds. The new internal standard for benzoylecgonine was the phenyl-d5 isotopomer, which, in combination with the use of d3cocaine, provides a built-in check for any in-assay hydrolysis. In the new method, dried extracts were reconstituted in unbuffered mobile phase and analyzed directly by LC-ESI-MS/MS, using a column and isocratic mobile phase from a specialty method already in use for trace-level quantitation of benzoylecgonine in solid tissues.

The new analytical system was validated on a series of blood calibration curves over a two order of magnitude concentration range for each component. Curves were extracted on three separate days and analyzed in duplicate, with one curve also rerun a day later to check for compound stability. Analytical run time was 15 min, comparable to the 11.5 min run time from the prior GC method. Validation results were generally very good, with excellent lower limits of detection and quantitation, good between day reproducibility, wide linear ranges, and negligible carryover. Best quantitative results were obtained by measurement of RIC traces for the pseudomolecular ions for the analytes and internal standards in full scan MS mode, while tandem mass spectrometry provided unambiguous identification of all analytes. Analysis was performed to a data-dependant scanning mode to allow collection of both types of data in a single analytical run. One interesting cautionary observation emerges from these data. With the chosen chromatographic system, methylecgonine is separated from its N-methyl-d3 isotopomer by almost one minute.While not unheard of, this is a very rare observation, and one that many forensic scientists may not typically have encountered in practice.This peak separation does somewhat compromise the quantitative analysis of methylecgonine, which shows the smallest linear range of the four targeted compounds.

Several of the case specimens analyzed since the development and adoption of this new method illustrate its great power and stability. To date, no sample has been able to “break” the method from the standpoints of putrefaction, interferences, carryover, or failed recovery of internal standards. The only cases requiring reanalysis have resulted from analyte concentrations higher than the chosen calibration range.

The authors feel that this reliable and robust analytical method will be of value to many forensic toxicology laboratories, and points towards a scheme for potential improvements in many other targeted toxicological analyses.

Cocaine, Liquid Chromatography, Mass Spectrometry

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