

Positive Ion Chemical Ionization and Gas Chromatography/Quadrupole Mass Spectrometry for Confirmation and Quantitation of Benzoyllecgonine in Oral Fluid

Trisa Robarge, Eric Phillips, James Edwards, William Seiter, Thermo Fisher Scientific, Austin, TX, USA
David Hollaway, Lance Presley, LabOne Inc., Lenexa, KS, USA

Overview

Purpose

Develop an analytical method for identification and quantification of benzoyllecgonine, a primary metabolite of cocaine, in oral fluids utilizing positive chemical ionization with a single-stage quadrupole mass spectrometer and gas chromatograph. Provide sensitivity, reproducibility, and precision to ensure applicability to a high-throughput laboratory environment. Demonstrate robustness and method transferability for use on several instruments performing the same analysis.

Methods

All calibrators, controls, and unknown samples were prepared utilizing a solid phase extraction technique. The resulting extracts were derivatized and reconstituted according to laboratory protocol. A MS method was developed that optimized ionization to provide both an $[M+H]^+$ and at least one confirming ion by comparing ionization efficiencies of methane, isobutane and ammonia as reagent gases. Following determination of optimal reagent gas type and flow rate, a MS method using selected ion monitoring (SIM) for collection of data for BE and its deuterated internal standard was developed. Chromatography was optimized to ensure adequate separation of the target components from matrix peaks. The method was validated according to laboratory protocol, which included determination of linearity, precision, and parallel studies.

Results

Use of methane reagent gas and positive chemical ionization resulted in an analytical method that satisfies the performance and reliability needs of the laboratory. The method's linear range of 0.75 ng/mL to 10 ng/mL and limit of detection of 0.5 ng/mL were determined experimentally. Through a series of replicate injections made on two separate runs, precision was determined to be 6.0% (CV) at low and high QC levels. The method was then placed on four different instruments with no modifications, and performance was similar across all four, demonstrating transferability of the method.

Introduction

The use of oral fluid to test for illicit drug use has gained widespread acceptance in recent years. Oral fluid is a suitable matrix for this detection due to its ease of collection and greater protection of subject privacy¹. However, drug levels in oral fluid are typically present at levels that present analytical challenges, due to the low concentration and

small sample size. The concentration of drugs, or their metabolites, in oral fluid is generally an order of magnitude or more lower than those found in urine, which is another common specimen for this type of testing. Furthermore, the matrix itself presents analytical challenges.

A method has been developed to determine benzoyllecgonine (BE), a major metabolite of cocaine, in oral fluids using gas chromatography and positive ion chemical ionization (PCI) with a single-stage quadrupole mass spectrometer. GC/MS was used due to current widespread implementation of this methodology in many laboratories for routine confirmation of the presence of common drugs of abuse in urine samples. Though this analysis may be conducted using conventional electron impact (EI) ionization, the sensitivity and selectivity of the analytical method can be improved through the use of chemical ionization (CI).

Methods

Instrumentation

A DSQ™ single quadrupole mass spectrometer system configured with a 250 L/s turbo pump and chemical ionization was used for sample acquisition. A TRACE GC Ultra™ gas chromatograph provided for sample introduction, using a 15 m x 0.25 mm id x 0.25 μm 5% phenyl phase column. Helium carrier gas flow was programmed across the run to aid in chromatography. The GC was equipped with a split/splitless injection port and a 5 mm id single gooseneck liner. A 2 μL injection volume with a 1.0 minute splitless injection duration was used for sample introduction (Table 1).

The DSQ was operated in both electron impact (EI) and positive ion chemical ionization (PCI). To switch between ionization modes, a vacuum interlock system allowed changing of the ion volume without breaking system vacuum. For method development, a high concentration derivatized standard was used. Methane, ammonia, and isobutane were evaluated for ease of use and ionization efficiencies. The DSQ was tuned and calibrated in both EI and PCI using standard automatic tuning algorithms. The PCI tune allows users to select masses according to target analyte mass ranges; however, the default ions in the PCI tune were sufficient for this analysis.

Key Words

- Benzoyllecgonine
- Chemical Ionization
- Drugs of Abuse
- DSQ Series GC/MS
- Oral Fluid Analysis

Results

Method Development Results

Initial method development focused on an EI-SIM method for the analysis of BE in oral fluids. By using a deuterated internal standard and monitoring two masses each for BE-HFIP (m/z 318 and 439) and BE-D3-HFIP (m/z 321 and 442), good quantitative results were obtained and chromatography was acceptable. Ion ratio confirmation, which compares the area of a qualifier mass to the quantification mass, was calculated through the use of ToxLab™ 2.0 intelligent sequencing software. Through ion ratio confirmation and calibration at a single point, the EI-SIM method was linear from the 50% control (1 ng/mL, equivalent to 8 pg on column) through the high control (4.0 ng/mL). However, the signal to noise on the qualifier ion (m/z 439) for the 1.0 ng/mL control was 17:1 using RMS signal to noise calculation (Figure 2). Thus, chemical ionization was pursued to improve the signal to noise and enhance the linear range of the method.

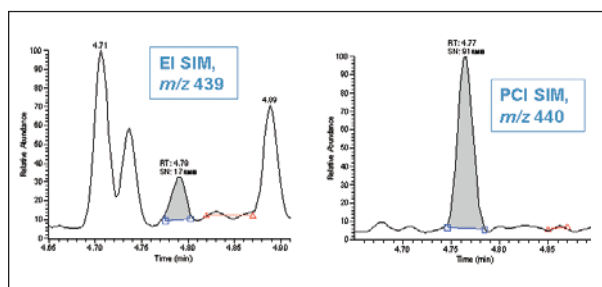


Figure 2: Comparison of signal to noise, m/z 439 and 440, using EI and PCI with methane. In EI, the signal to noise for m/z 439 (RMS) is 17. In PCI, at the same concentration, the RMS S/N ratio for m/z 440 is 91:1.

Three reagent gases were studied, using positive ionization. To utilize PCI, the EI ion volume was exchanged for a CI ion volume using a vacuum interlock system that allows for this change without venting the instrument. The DSQ was autotuned in PCI using default positive ions for mass calibration, prefilter and ion offset voltages, and resolution.

Figure 3 shows the full scan spectra resulting from the analysis of BE-HFIP using PCI with different reagent gases. In addition, the full scan spectra in EI is also shown to demonstrate the formation of the $[M+H]^+$ ion using PCI. PCI with methane yields $[M+H]^+$ (m/z 440), along with a base peak of m/z 318.

Ammonia PCI generated primarily $[M+H]^+$ at a flow rate of 1.5 mL/min, along with m/z 318 at a low abundance. The proton affinity of ammonia, being close to that of the derivatized molecules, imparts a relatively low amount of supplemental energy beyond that necessary for target molecule protonation. This reduces production of fragmentation ions via fragmentation pathways. However, because of the analytical requirement for confirmatory ions, the formation of largely $[M+H]^+$ ion is not desirable for this analysis. The concern is that at low concentrations, the confirming ion will drop out. For this reason, ammonia as a reagent gas was deemed unsuitable for this assay.

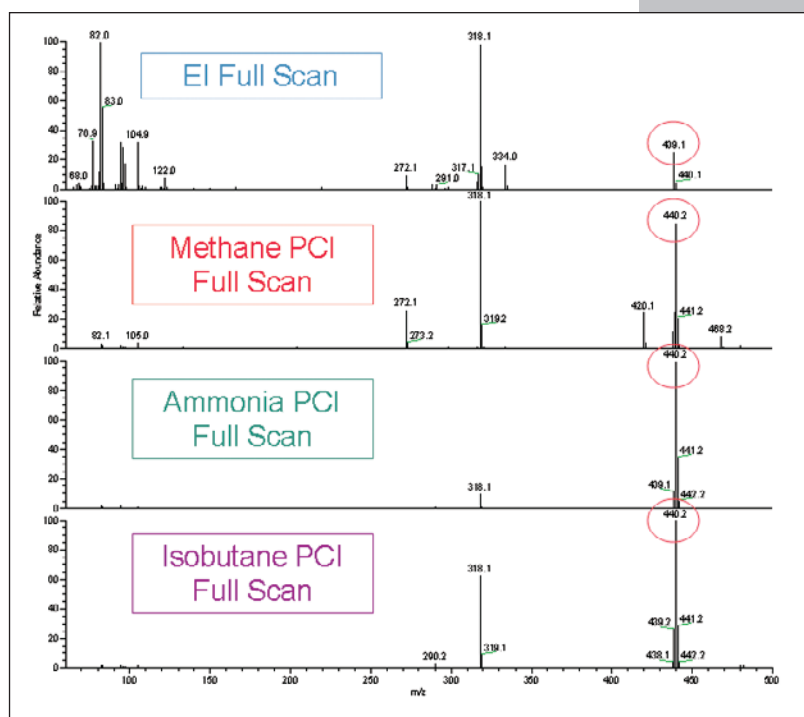


Figure 3: Comparison of full scan spectra in EI and PCI. For all spectra, the scan range was from 50-650 amu at 1197 amu/sec. PCI spectra for each reagent gas are shown at a 1.5 mL/min flow rate.

Isobutane at 1.0 mL/min offered a spectrum with complexity similar to that of methane. However, isobutane was ruled out as a reagent gas due to susceptibility to contamination. Because of the needs for a robust method suitable for high sample throughput, methane was chosen as the reagent gas for this assay.

Ion ratio stability over different flow rates was assessed for both methane and isobutane. For greater suitability for method development and transferability, the reagent gas that provided the most stable ion ratios over a range of flow rates would be best suited for this analysis. Figure 4 shows the ion ratio percent (Area of m/z 440/Area of m/z 318 \times 100) stability for three different flow rates of methane and isobutane. Methane flow rates of 1.5, 2.5 and 4.9 mL/min were assessed, with ion ratio variations of 7.4% RSD. Isobutane at 1.0, 1.5 and 2.5 mL/min gave an ion ratio variation of 37.6% RSD.

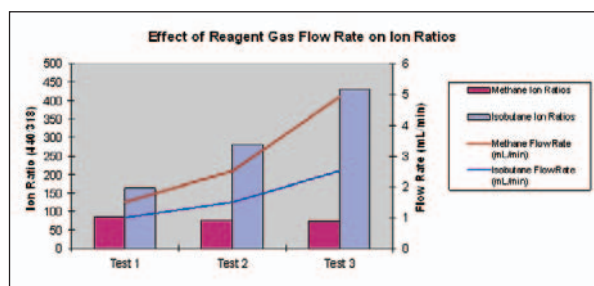


Figure 4: Comparison of the effect of reagent gas flow rates on ion ratios using methane and isobutane as reagent gases. Using digital flow control, ion ratios were evaluated at three different flow rates. Ion ratios using methane as reagent gas were stable across flow rates (7.4% RSD), while those using isobutane varied considerably (37.6% RSD).

