Cocaine Analysis on a Bonded Pentafluorophenyl Propyl High Performance Liquid Chromatography Stationary Phase Using Mass Spectrometry

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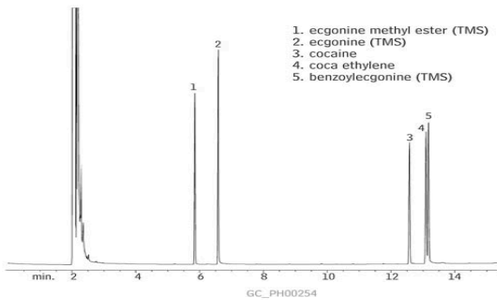
Introduction

When cocaine is introduced into the body, several main metabolites are produced: benzoylecgonine, ecgonine and ecognine methyl ester. To determine the presence of cocaine and its metabolites, urine samples are screened using enzyme immunoassay, and positive results are confirmed using GC/MS. Mass spectrometry is widely used for confirmation since MS data provides positive identification based on mass spectral information and can be used as confirming evidence in a court of law. Although GC/MS methods are well established and do provide excellent confirmational data, they can be time consuming due to long analysis times and multiple sample preparation steps, which include derivatization. Figure 1 shows the TMS derivatives of cocaine and its metabolites by GC with an analysis time of 14 minutes.

Figure 1



A second, less common, chromatographic method that can be used for identifying and quantifying cocaine and its metabolites is High Performance Liquid Chromatography (HPLC) coupled with mass spectrometry (MS). Since HPLC does not require derivatization of the compounds, sample preparation time is reduced making this technique more attractive to the analyst. And, combining the right stationary phase and mobile phase (high organic), allows for efficient desolvation and ionization in the MS. This combination can result in detection of cocaine and its metabolites at the low picogram levels.

Both GC/MS and HPLC/MS methodologies provide reproducible, reliable data that can be used in a court of law. However, the HPLC/MS method developed in this investigation not only provides a reduced analysis time, symmetrical peak shapes and excellent sensitivity, but it also eliminates sample preparation steps such as derivatization.

Experimental

For the purpose of this study, HPLC/TOF-MS data was obtained on a PFP Propyl and a C18 stationary phase. HPLC/MS/MS data was also reviewed and compared to the HPLC/TOF-MS data. To begin, chemical standards of cocaine, benzoylecgonine and ecgonine methylester were prepared at various concentrations in the mobile phase. Final on-column concentrations are listed in Table 1. Deuterated cocaine was used as the internal standard. HPLC methods were then optimized for each stationary phase and were evaluated for identification and quantification of each compound. Table 2 shows the optimized methods used for each stationary phase. Methods focused on choosing the best HPLC column stationary phase for maximizing sensitivity of all compounds while providing adequate analysis time and peak symmetry.

Table 1 Mean relative standard deviations for cocaine, benzoylecgonine, ecgonine methylester, and cocaine-d3 are substantially less than 10% for all on-column quantities but one, on a PFP Propyl column.

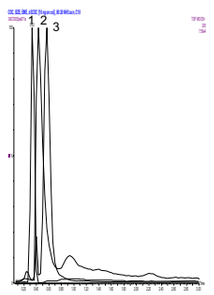
	Mean %RSD, n=7			
On-Column Conc.	COC	BZE	EME	Cd3
250ng	1.9	3.2	1.5	3.2
125ng	0.4	4.7	1.5	4.0
25ng	0.5	4.8	0.6	4.4
5ng	0.8	2.9	1.5	2.1
2.5ng	1.7	4.2	3.2	2.4
0.5ng	5.7	0.7	0.9	2.8
5pg	7.4	8.1	7.8	5.1
0.5pg	4.3	8.0	40.7	6.3

Table 2 HPLC/TOF-MS conditions for PFPP and C18 Columns

HPLC/TOF-MS Parameter	Column 1	Column 2
Column	PFP Propyl, 30mm X 2.1mmID, 5um particle size	C18, 30mm X 2.1mmID, 5um particle size
Mobile Phase Program	Isocratic	Isocratic
Mobile Phase	80:20, Acetonitrile:5mM Ammonium Formate in Water, pH=3.0	20:80, Acetonitrile:5mM Ammonium Formate in Water, pH=3.0
Column Flow	0.6 ml/min	0.6 ml/min
Column Temp.	25°C	25°C
MS	Micromass LCT Premier	Micromass LCT Premier
ESI	Positive	Positive
Capillary	3000V	3000V
Sample Cone	20V	20V
Desolvtion Temp.	250°C	250°C
Source Temp.	120°C	120°C

Cocaine, benzoylecgonine, and ecgonine methylester are hydrophilic, basic drugs with pKa values greater than 8 making them difficult to retain on a C18 column. Consequently, buffer salts or ion-pairing agents and a low-organic mobile phase are needed to ensure adequate retention on a typical C18 reversed phase column. Some retention can be achieved under these conditions (see **Figure 2**), but the highly aqueous mobile phase causes poor MS response due to inefficient desolvation, and the salts cause ion suppression during ESI. Under optimal screening conditions, limits of detection of 1ng/mL for cocaine and 5ng/mL for benzoylecgonine have been reported (10pg and 50pg on-column, respectively; 10µL injection) for the C18 stationary phase.

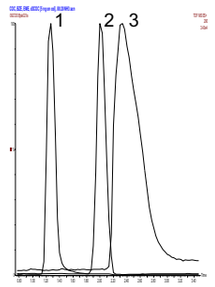
Figure 2 Extracted ion chromatograms of cocaine, benzoylecgonine and ecgonine methylester using HPLC/TOF-MS and a C18 column



Peak#	ID	Mass	RT (min.)
1	EME	200	0.33
2	COC	304	0.43
3	BZE	290	0.57

In contrast, the combination of a PFP Propyl column and a high-organic mobile phase (**Table 2**) provides not only adequate retention and short analysis times, but also excellent sensitivity without modifiers. All target compounds are eluted from the 30mm column within 3 minutes, with reliable reproducibility of responses (Figure 3). S/N:RMS values greater than 90 indicate excellent sensitivity at 5.0pg on-column for all compounds; values of 16 and greater indicate adequate sensitivity for most compounds at 0.5pg on-column. For each compound the relative standard deviation (%RSD) for intensity is below 10% across a broad concentration range, except for the 0.5pg value for metabolite ecgonine methylester (**Table 1**).

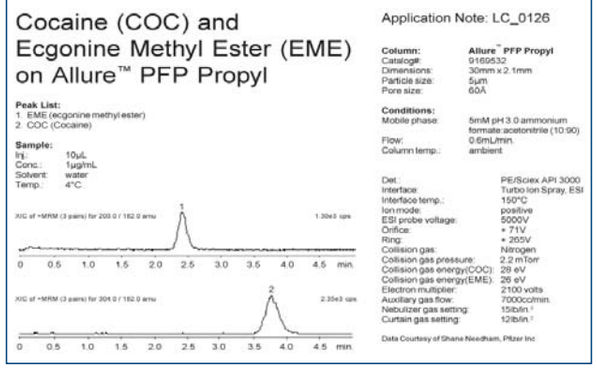
Figure 3 Extracted ion chromatograms of cocaine, benzoylecgonine and ecgonine methylester using HPLC/TOF-MS and a PFP Propyl column



Peak#	ID	Mass	RT (min.)
1	EME	200	1.27
2	BZE	290	2.00
3	COC	304	2.33

HPLC/ESI/MS/MS is also a viable method for cocaine and its metabolites. Not only does it reduce sample preparation time and analysis time, but it also provides reliable, reproducible results at the picogram level. Figure 4 shows cocaine and ecgonine methylester detected at 1.6pg and 2.8pg, respectively. Again, the PFP Propyl stationary phase combined with a highly organic mobile phase was required to achieve that limit of detection (pg). Analysis time is kept under 5 minutes with excellent peak shape.

Figure 4 Extracted ion chromatograms of cocaine and ecgonine methylester using HPLC/ESI/MS/MS and a PFPPropyl column



Conclusions

Even though cocaine and its metabolites are reliably and reproducibly detected using GC/MS, HPLC/TOF-MS and HPLC/MS/MS can provide alternate chromatographic confirmation methods. HPLC reduces sample preparation time by eliminating derivatization and reduces analysis times from 14 minutes (GC/MS) to less than 5 minutes (HPLC/MS/MS). The results demonstrated in this study show that the bonded pentafluorophenyl propyl stationary phase in combination with a highly organic mobile phase produces excellent sensitivity, symmetric peak shapes and adequate analysis times.

References

1. Jeanville, P.M., E.S. Estape, S.R. Needham, M.J. Cole, *J. Am. Soc. Mass Spectrom,* 11: 257-263 (2000).
2. Needham, S.R., P.M. Jeanville, P.R. Brown, E.S. Estape, *J. Chromatography B,* 748: 77-87 (2000).
3. Milner, C., R. Kinghorn, *Development of a Screening Analysis by LC Time-of-Flight MS for Drugs of Abuse,* Agilent Technologies, publication 5989-3157EN www.agilent.com/chem.