0.5pg Limit of Detection for Cocaine

Using an Allure™ PFP Propyl Column and HPLC/TOF-MS

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- Monitor cocaine and benzoylecgonine at 0.5pg on-column, ecgonine methylester at 5pg.
- Analysis completed in less than 3 minutes.
- Fast, simple sample preparation—no need for derivatization.

When cocaine is introduced into the body, several main metabolites are produced: benzoylecgonine, ecgonine, and ecgonine methylester. To determine the presence of cocaine and/or these metabolites, urine samples are screened using enzyme immunoassay, and positive results are confirmed using GC/MS. Although GC/MS methods are well established, and provide excellent confirmation data, they can be time-consuming, due to multiple sample preparation steps, including derivatization — and long analysis times.[1] HPLC coupled with electrospray (ESI) time-of-flight mass spectrometry provides an alternate chromatographic confirmation method for cocaine and its metabolites. Using an Allure™ PFP Propyl column in combination with a high-organic mobile phase provides short analysis times and allows detection limits at low picogram levels, without derivatization.

Cocaine, benzoylecgonine, and ecgonine methylester are hydrophilic, basic drugs with pK_a values greater than 8. 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, but the highly aqueous mobile phase causes poor MS response due to inefficient desolvation, and the salts cause ion suppression during ESI.[2] 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).[3]

In contrast, the combination of an Allure™ PFP Propyl column and a high-organic mobile phase provides not only adequate retention and short analysis times, but also excellent sensitivity. All target compounds are eluted from the 30mm column within 3 minutes (Figure 1), with reliable reproducibility of responses (Table 1). 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).

Because the Allure™ PFP Propyl column and the high-organic mobile phase, coupled with HPLC/TOF-MS, produce highly reproducible signal intensities for each mass down to 5pg on-column, we recommend this column and these analysis parameters as an alternative chromatographic approach for confirmation of cocaine and its metabolites.

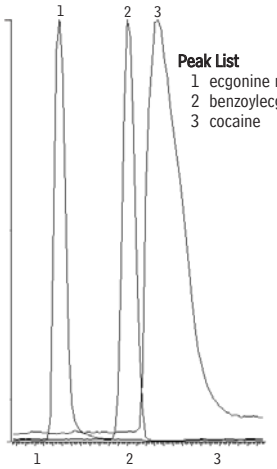
References

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

Allure™ PFP Propyl Column

5µm Column, 2.1mm	cat. #
30mm	9169532

Figure 1 Rapid, sensitive analysis for cocaine and its metabolites, using an Allure™ PFP Propyl column



Peak List	Mass	Ret. Time (min.)
1 ecgonine methylester	200	1.27
2 benzoylecgonine	290	2.00
3 cocaine	304	2.33

Sample:
Inj.: 10µL, 5pg each compound on column
Solvent: mobile phase

Column: Allure™ PFP Propyl
Cat.#: 9169532
Dimensions: 30mm x 2.1mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: acetonitrile:5mM ammonium formate in water, pH=3.0, 80:20
Flow: 0.6mL/min.
Temp.: 25°C

Detection: MS, Micromass LCT Premier
ESI: positive
Capillary: 3000V
Sample cone: 20V
Desolvation temp.: 250°C
Source temp.: 120°C

Table 1 Reproducible responses for cocaine and its metabolites across a wide range of concentrations.

On-column Amount (ng)	Mean % RSD (n = 7) COC	BZE	EME	Cd3
250	1.9	3.2	1.5	3.2
125	0.4	4.7	1.5	4.0
25	0.5	4.8	0.6	4.4
5	0.8	2.9	1.5	2.1
2.5	1.7	4.2	3.2	2.4
0.5	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

COC - cocaine; BZE - benzoylecgonine; EME - ecgonine methylester; Cd3 - cocaine-d3