



# Determination of Opiates and Other Drugs of Abuse Using the Agilent 500 Ion Trap LC/MS

## Application Note

Drugs of Abuse Testing

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### Abstract

A rapid, sensitive and reproducible method has been developed for LC/MS/MS on the Agilent 500 Ion Trap LC/MS. This method effectively separates, detects, identifies and quantitates seven common drugs of abuse (codeine, oxycodone, hydrocodone, meperidine, cocaine, methadone, and THC).

### Introduction

Drugs of abuse testing requires instrumentation that is robust, sensitive, and reliable. Currently, drug testing labs test for hundreds of illicit drugs in blood, urine and saliva, and are always looking for ways to increase sensitivity and throughput.

For this analysis, a rapid, sensitive, and reproducible method has been developed for LC/MS/MS on the Agilent 500 Ion Trap LC/MS. This method effectively separates, detects, identifies and quantitates seven common drugs of abuse, including codeine, oxycodone, hydrocodone, meperidine, cocaine, methadone, and THC (Figure 1).



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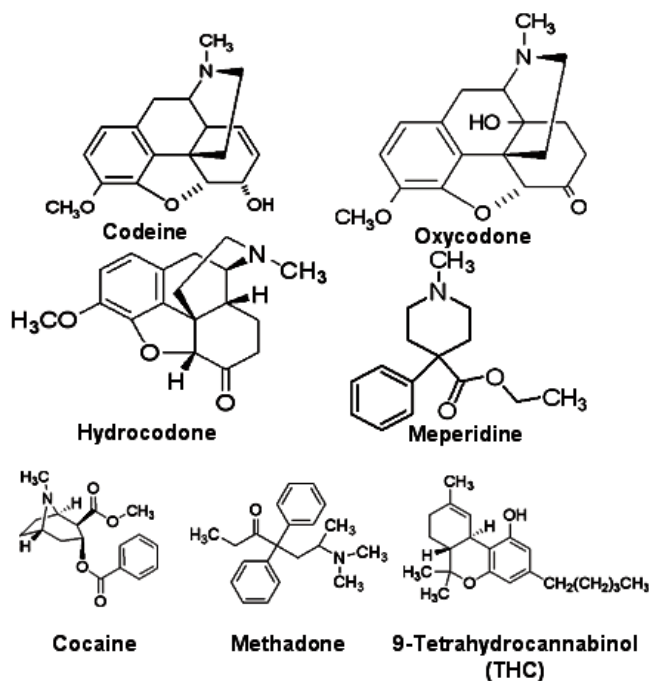


Figure 1. Chemical structures of seven common drugs of abuse.

This analysis uses SelecTemp and SelecFlow to optimize the nitrogen gas temperature and flow rate in the API source. The user can select the optimum conditions for each segment in the analytical run. The ability to change these parameters in the middle of an analytical run is unique to Agilent products, and is especially important for compounds that may be thermally labile.

## Instrumentation

The following instruments were used in this study:

- Agilent 500 Ion Trap LC/MS with ESI source
- Agilent 212-LC Binary Gradient LC/MS pumps
- Agilent Prostar 430 AutoSampler

## Materials and Reagents

All standards, solvents and reagents were purchased from Sigma-Aldrich Co, St. Louis, MO.

## HPLC conditions

Column:	Pursuit C18, 150 × 2 mm, 3 μm (Agilent p/n A3001150X020)			
Solvent A:	Water with 0.1% formic acid			
Solvent B:	Acetonitrile with 0.1% formic acid			
Injection volume:	10 μL			
LC program:	Time (min:sec)	%A	%B	Flow (μL/min)
	00:00	97	3	150
	00:30	97	3	150
	08:00	0	100	150
	15:00	0	100	150
	15:01	97	3	150
	20:00	97	3	150

## MS parameters

Ionization mode:	ESI (positive)
Needle:	5000 V
Shield:	600 V
API nebulizing gas:	Segment 1 – 60 psi Segment 2 – 60 psi Segment 3 – 30 psi Segment 4 – 60 psi
API drying gas:	Segment 1 – 30 psi at 350 °C Segment 2 – 30 psi at 350 °C Segment 3 – 40 psi at 350 °C Segment 4 – 40 psi at 400 °C

Table 1. MS Segment Parameters

Segment	Analyte	Transition	Retention time (min)	Capillary voltage (V)	Excitation amplitude (V)	RF load %
1	Codeine	300 → 215.2+ 225.2+	4.00	110	1.50	88
1	Oxycodone	316 → 298.3	4.14	100	1.10	90
1	Hydrocodone	300 → 199.2	4.23	110	1.50	88
2	Cocaine	307 → 185.0	4.77	58	0.60	87
2	Meperidine	248 → 220.2	4.78	76	0.80	81
3	Methadone	310 → 265.2	5.49	57	1.00	88
4	THC	315 → 259.3+ 233.4	10.50	89	1.00	91

## Results and Discussion

This LC/MS/MS method separates seven common drugs of abuse. The chromatographic separation shown here between the analytes is made possible by the Agilent Pursuit C18 LC column, which provides baseline separation and consistent performance. Figure 2 shows the extracted ion chromatogram (EIC) of the seven analytes at 5 ppb.

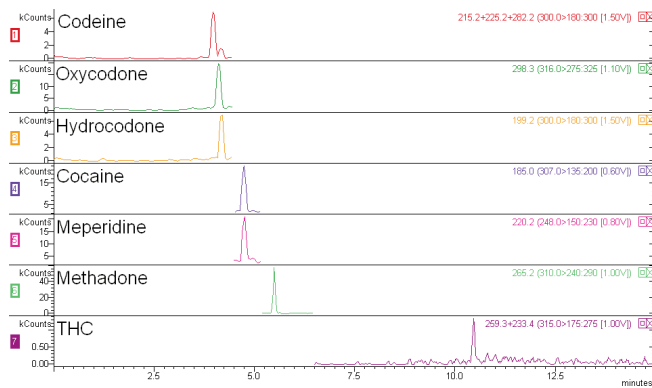


Figure 2. EIC of codeine, oxycodone, hydrocodone, cocaine, meperidine, methadone and THC at 5 ppb.

Calibration curves were found to be linear from 5 to 500 ppb, with %RSD values of 14.75% for codeine, 14.59% for oxycodone, 10.95% for hydrocodone, 10.80% for meperidine, 21.01% for cocaine, 13.64% for methadone and 24.62% for THC. Figure 3 shows the calibration curve for hydrocodone.

### Calibration Curve Report

File: ...of abuse\opiates\_4-(amphet+meth) (ait metasil aq 3 100x2) std.mth  
Detector: 500-MS Mass Spec, Address: 56

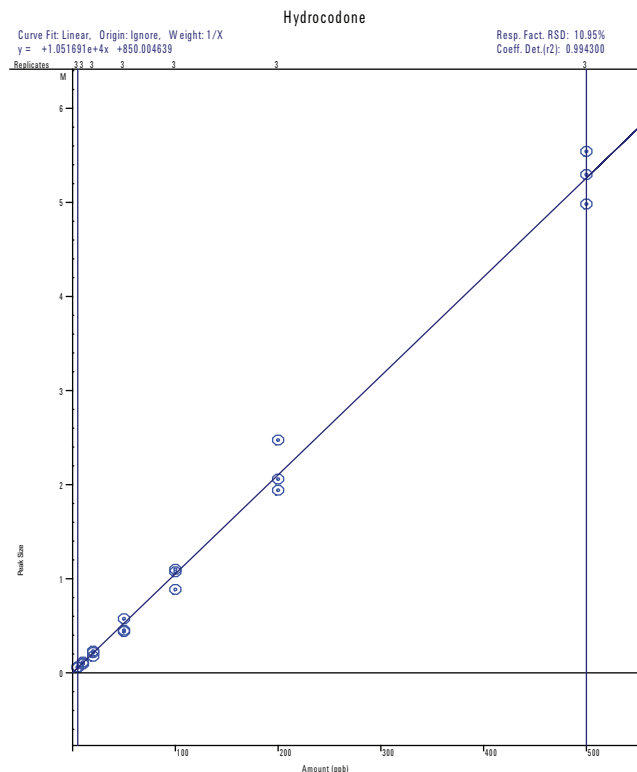


Figure 3. Calibration curve of hydrocodone from 5 to 500 ppb. For this curve, %RSD is 10.95% and  $r^2 = 0.994$ .

## Conclusion

The LC/MS/MS method outlined herein produces excellent chromatographic separation, detection, identification and quantitation of seven common drugs of abuse in a single LC/MS analysis. Quantitative results were obtained from 5 to 500 ppb.

The MS/MS capabilities of the Agilent 500 Ion Trap LC/MS allow for isolation of desired precursor ions followed by collision induced dissociation (CID) and production of characteristic product ions, resulting in the collection of clear, reproducible chromatographic peaks for accurate target compound quantitation.

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