Application Note: 366

Analysis of Multiple Illicit Drugs, Methadone, and their Metabolites in Oral Fluid using a Linear Ion Trap Mass Spectrometer

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Key Words

- Finnigan™ LXQ™
- Surveyor Plus™
- Forensics Analysis
- MS³ Quantification

Introduction

Traditionally, the analysis of urine samples has been the major approach for the monitoring of drugs of abuse.[1] However, a common risk for this type of analysis is adulteration or manipulation of the sample at the point of collection. As an alternative, the analysis of oral fluid provides an easy method of sample collection and has the advantage of providing a relatively clean matrix. Because of the reduced sample volume this technique requires a high sensitivity and robust analytical method to make saliva/oral fluid-based diagnostics an attractive alternative to conventional methods.

In this report, a rapid and rugged LC-MS/MS method using the Finnigan LXQ is described for analyzing a mixture of twenty drugs and their metabolites using intelligent automated mass spectrometry (INTAMS). The detection limits for the mixture of drugs and dynamic range are superior to results reported previously.[2] In addition, this method provides for the simultaneous identification and quantification of drugs and their metabolites.

Experimental Conditions

Sample Preparation:

Ten milliliters of oral fluid collected from a volunteer were protein precipitated using 30 mL acetonitrile. The sample was vortexed and then centrifuged at 5,000 rpm for 10 minutes. The supernatant was evaporated to dryness under nitrogen and reconstituted in 5 mL water. Table 1 provides a list of 20 drugs along with the parent and product ion masses. For quantification experiments, known amounts of a stock solution of the 20 drug mixture were spiked into the treated oral fluid to prepare the standards in concentrations ranging from 50 fg/µL to 1 ng/µL.

Compound	Parent ion *m/z*	Product ions *m/z*
EEE[a]	214.3	196.2
Normorphine	272.3	201.0
AEM[b]	182.3	150.1, 122.1
Morphine	286.3	229.1, 211.2
Norcodeine	286.3	243.3, 225.3, 215.0
Codeine	300.3	175.0, 225.3
6-Acetylmorphine	328.3	268.3, 193.2
m-Hydroxybenzoylecgonine	306.2	168.2
Benzoylnorecgonine	276.2	154.1
Benzoylecgonine	290.3	168.2
Acetylcodeine	342.3	282.3, 225.2
Heroin	370.3	310.2, 328.2, 268.3
Cocaine	304.3	182.1
Norcocaine	290.2	168.1, 136.2
Cocaethylene	318.3	196.2
Norcocaethylene	304.2	182.1, 136.1
Methadol	312.3	223.1, 249.2, 171.2
EDDP[c]	278.0	249.2
Propoxyphene	340.1	266.1
Methadone	310.9	266.2

Table 1: List of 20 drugs and metabolites with their respective parent and product ion masses. EEE: ecgonine ethyl ester; AEM: anhydroecgonine methyl ester; EDDP: 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium

HPLC:

LC System: Surveyor Plus

Column: Hypersil GOLD™
(20×2.1 mm, 1.9 µm particle size)

Mobile phase:

(A) water with 0.1% formic acid and 10 mM ammonium acetate

(B) acetonitrile with 0.1% formic acid

Flow rate: 400 µL/min

Injection volume: 10 µL

Gradient:

t (min)	A%	B%
0.00	95	5
0.10	95	5
1.00	85	15
4.20	50	50
4.21	95	5
7.00	95	5

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Mass Spectrometer:

The Finnigan LXQ linear ion trap mass spectrometer was operated in positive atmospheric pressure chemical ionization (APCI) mode. The corona discharge needle voltage was 4.5 kV and the vaporizer temperature was 400 °C. The capillary temperature was 220 °C and the sheath gas flow was 25 units. All scan events were acquired with one micro scan. No internal standard was used. The set up of the acquisition method using INTAMS is shown in Figure 1.

Results and Discussions

INTAMS data acquisition software was used for the simultaneous identification of 20 drugs in oral fluid. The extracted ion chromatogram is shown in Figure 2. INTAMS software enables the maximum number of scans to be acquired under a given chromatographic peak by obtaining MS/MS spectra on only the masses identified within a specified time window which helps facilitate a faster duty cycle.

In addition, the excellent ion statistics and the fast cycle time of the Finnigan LXQ linear ion trap mass spectrometer enabled the simultaneous quantification and identification of these analytes. Calibration curves based on MS/MS spectra were generated using the standards for the drug mixture spiked in oral fluid over a concentration range from 50 fg/µL to 1.0 ng/µL. Figure 3 shows calibration curves for 8 of the 20 compounds analyzed simultaneously. The R^2 values of these curves are better than 0.996 and they exhibit linear dynamic range over 3 to 4 orders of magnitude. The detection limits (LOD and LOQ) for each analyte in oral fluid are listed in Table 2 along with the linear dynamic ranges. Compared with data published previously[2], the Finnigan LXQ linear ion trap provided up to 10 times lower detection limits and an increased linear dynamic range.

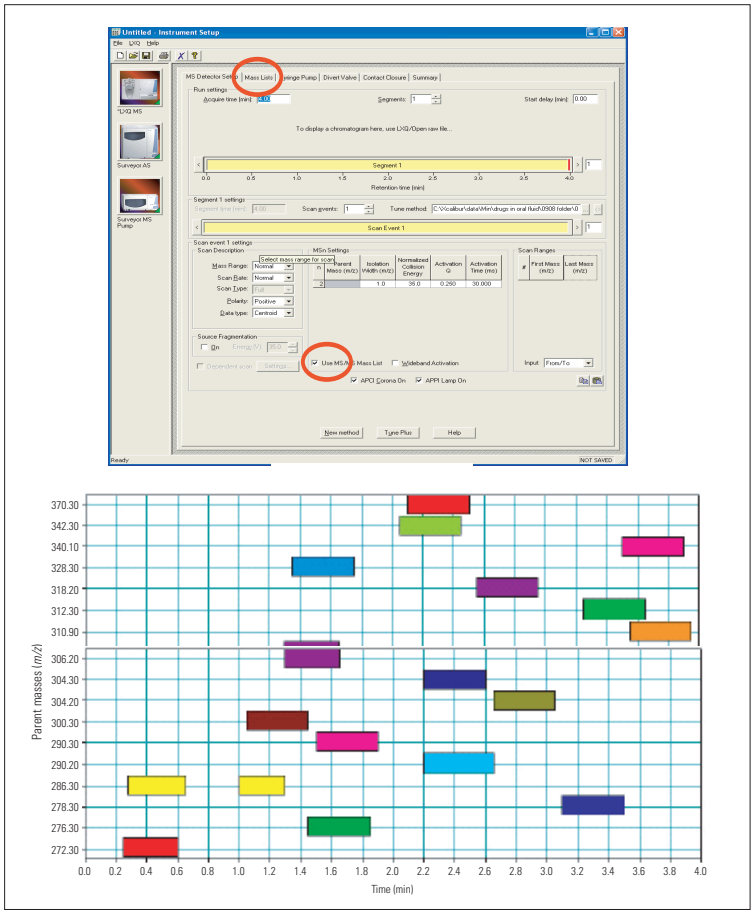


Figure 1: INTAMS (Intelligent Automated Mass Spectrometry) data acquisition software setup for simultaneous analysis of 20 compounds

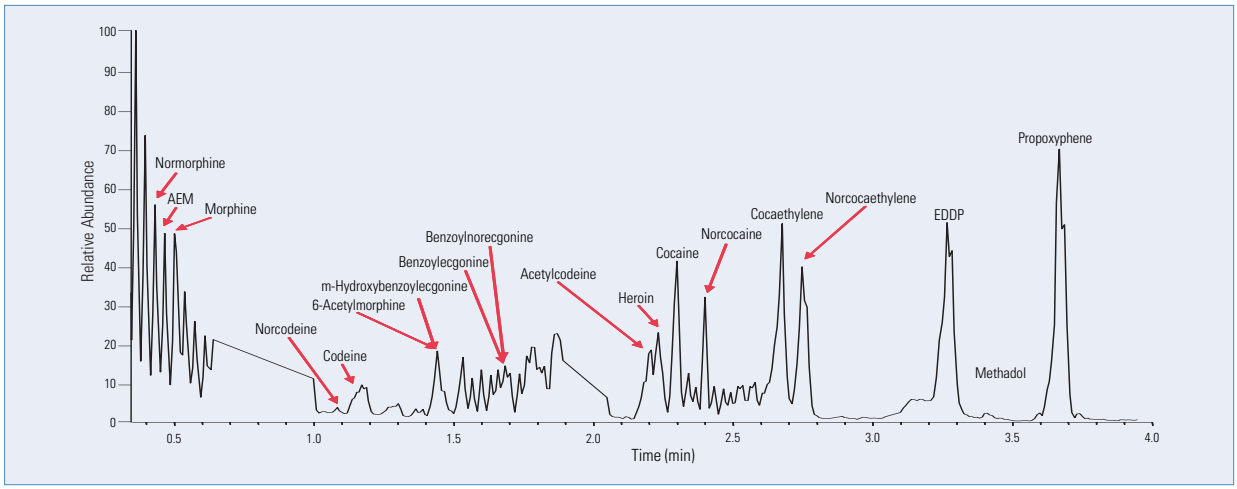


Figure 2: Chromatogram of the drugs and metabolites in oral fluid using LC-MS/MS with INTAMS data acquisition software

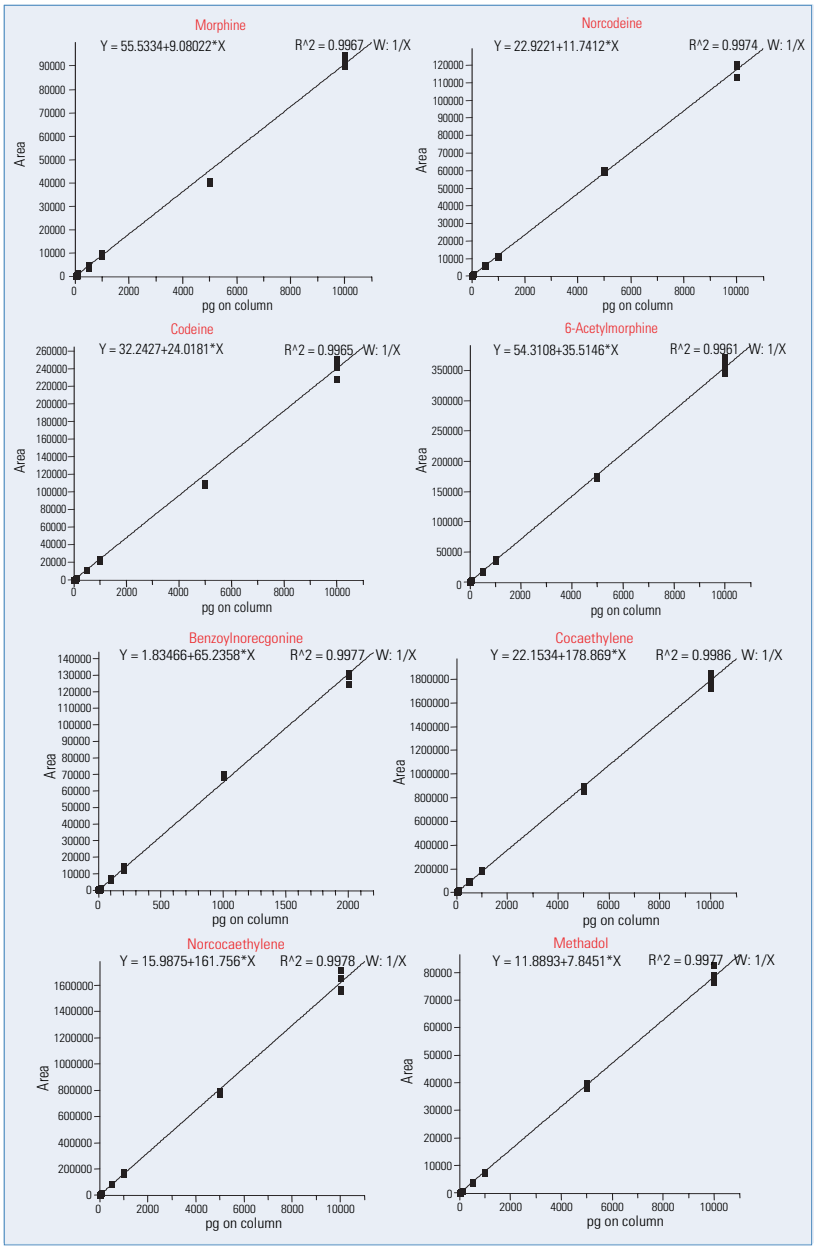


Figure 3: Representative calibration curves for eight drugs in oral fluid

Further confirmatory information and higher specificity results were also easily generated by performing quantification based on MS^3 data. The use of MS^3 quantification is demonstrated for the ecogonine ethyl ester sample (EEE) which undergoes a neutral loss of water molecule upon ion activation. When spiked in oral fluid, interference from the matrix masked the analyte peak. This was overcome as shown in Figure 4. The signal-to-noise ratio (S/N) of the extracted ion chromatogram obtained from MS^3 data (top chromatogram) is dramatically higher than that obtained from the MS/MS data. The high quality of the MS^n spectra obtained using the LXQ also results in greater sensitivity over a wider linear dynamic range (Figure 4b and 4c).

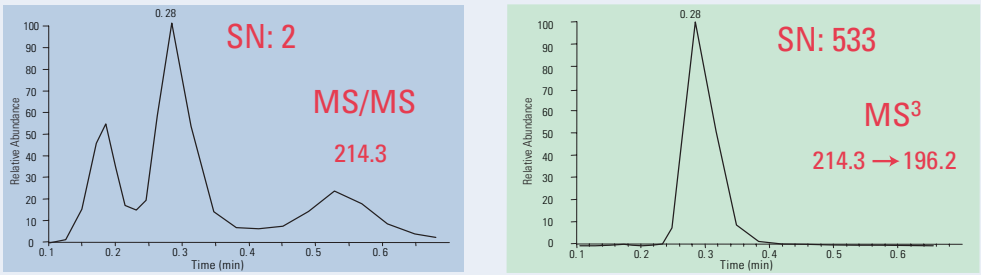
The quantitative study was completed by analyzing two QC oral fluid samples, each containing a mixture of ten drugs. The results shown in Table 3 demonstrate a high level of quantification accuracy, with a deviation of less than 10% for all the analytes. In addition, excellent reproducibility was demonstrated with the %RSD being less than 9% for all the compounds within five injections.

Compound	LOD (pg)	LOQ (pg)	Linear dynamic range (pg)
EEE	1	5	5-5000
Normorphine	5	10	10-10000
AEM	5	10	10-10000
Morphine	5	10	10-10000
Norcodeine	5	10	10-10000
Codeine	1	5	5-10000
6-Acetylmorphine	1	5	5-10000
m-Hydroxybenzoylecgonine	0.2	1	1-2000
Benzoylnorecgonine	0.2	1	1-2000
Benzoylecgonine	0.5	1	1-10000

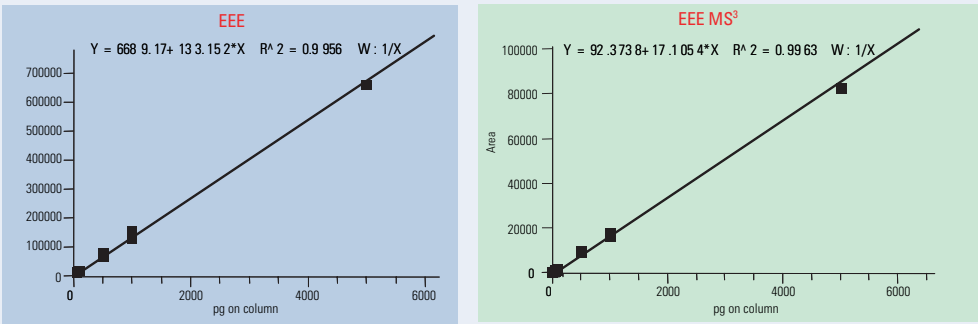
Compound	LOD (pg)	LOQ (pg)	Linear dynamic range (pg)
Acetylcodeine	0.5	1	1-10000
Heroin	0.5	1	1-10000
Cocaine	0.5	1	1-10000
Norcocaine	0.5	1	1-10000
Cocaethylene	0.5	1	1-10000
Norcocaethylene	0.5	1	1-10000
Methadol	1	5	1-10000
EDDP	0.5	1	1-10000
Propoxyphene	1	5	5-10000
Methadone	0.5	1	1-10000

Table 2: LOD (limit of detection), LOQ (limit of quantification) and linear dynamic range for analysis of 20 drugs and metabolites in oral fluid using the Finnigan LXQ linear ion trap mass spectrometer

a) Sensitivity and Specificity



b) Calibration curves using MS/MS and MS^3 product ions



c) %CV for five injections of EEE in oral fluid using MS/MS and MS^3 calibration curves

Amount (pg on column)		5	10	50	100	500	1000	5000
%CV	MS/MS			12.0	8.8	8.3	8.6	3.3
	MS^3	11.2	9.4	6.9	9.2	3.8	3.7	2.4

Figure 4: Analysis of EEE (Ecgonine Ethyl Ester) in oral fluid using MS/MS and MS^3 spectra product ions

	QC Sample I (5 injections)				QC Sample II (5 injections)			
Compound	**Conc (pg)**	**Calc. conc. (pg)**	**% Diff**	**% RSD**	**Conc (pg)**	**Calc. conc. (pg)**	**% Diff**	**% RSD**
EEE[a]	200.0	183.2	-8.4	4.6	40.0	37.7	-5.7	5.6
Morphine	200.0	189.2	-5.4	7.6	40.0	40.4	1.0	8.9
Norcodeine	200.0	190.8	-4.6	5.5	40.0	40.1	0.3	7.8
6-Acetylmorphine	200.0	182.2	-8.9	8.1	40.0	41.0	2.6	8.4
Cocaethylene	133.3	120.1	-9.7	7.4	26.7	26.3	-1.5	1.6
Norcocaethylene	200.0	190.6	-4.7	5.5	40.0	42.0	4.9	7.4
Methadol	200.0	184.6	-7.7	9.6	40.0	37.6	-6.1	3.8
EDDP	133.3	121.4	-8.9	4.9	26.7	24.8	-7.1	4.4
Propoxyphene	200.0	190.4	-4.7	4.0	40.0	42.4	6.3	5.8
Methadone	133.3	122.5	-9.5	7.2	26.7	24.9	-6.8	3.9

Table 3: Quantification results for the analysis of unknown levels of drugs in oral fluid. *[a] based on MS^3 results*

Data Analysis

Mass Frontier™ software includes a number of tools for structure identification. The powerful search features and database management make it valuable for identifying drugs, metabolites and related compounds. A library of target drugs can be easily searched. As an example, the MS/MS spectrum obtained from 6-acetylmorphine in oral fluid was searched against an NIST library using Mass Frontier. In addition to being the top hit (Figure 5), the chromatographic elution time and the mass of the precursor ion provide added degrees of confidence for identification.

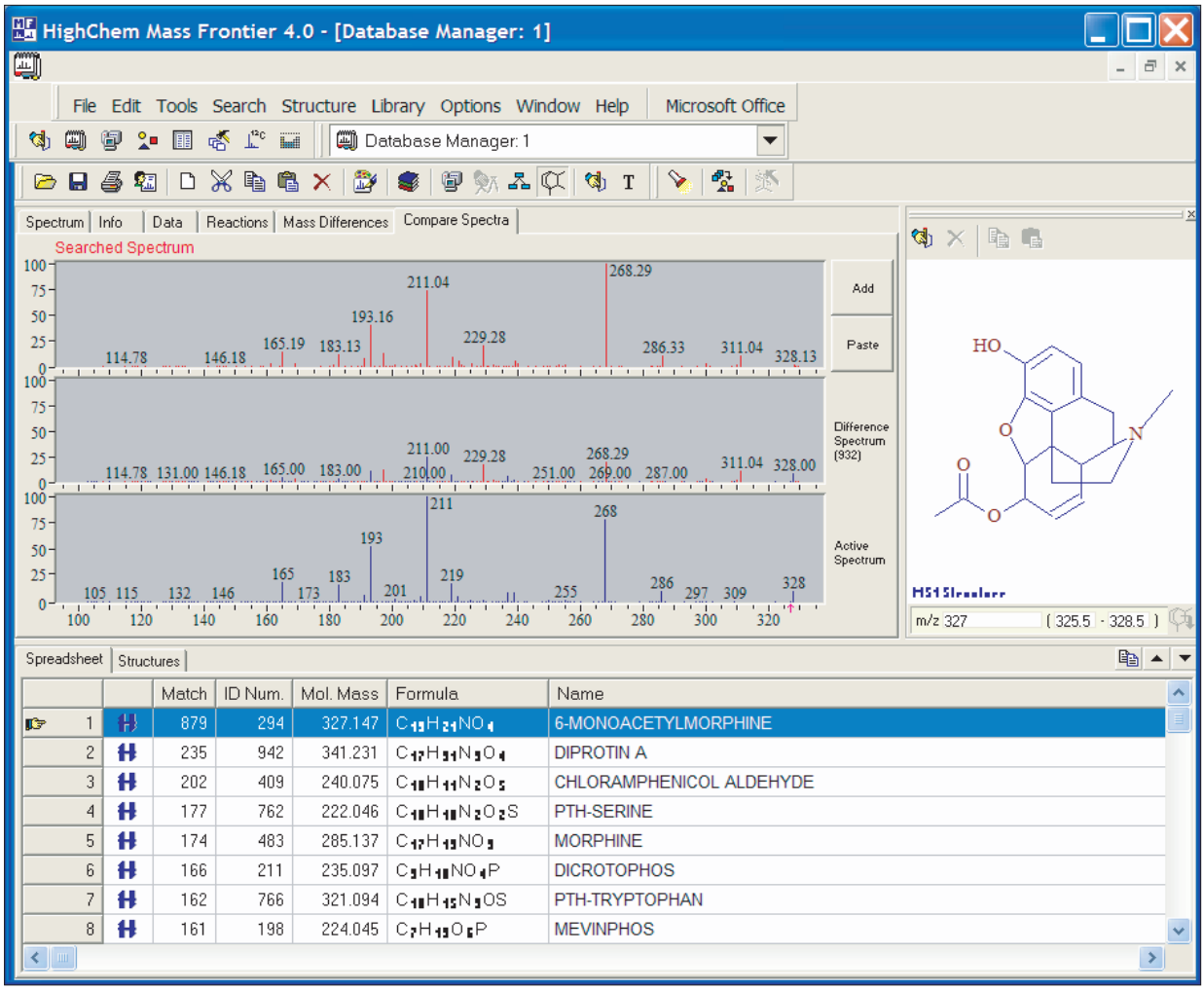


Figure 5: Library search results for 6-acetylmorphine using Mass Frontier. High match score is highlighted

Conclusions

Rigorous simultaneous characterization and quantification of a large number of drugs and their metabolites in a biological matrix can be performed in a fast and robust LC/MS/MS method using a Finnigan LXQ linear ion trap mass spectrometer. The superior sensitivity and faster cycle time of the LXQ makes this possible in a single chromatographic run, resulting in high throughput analyses. High specificity quantification was done using MS^3 data which can reduce overall chemical noise even if there is a co-eluting isobaric interfering ion. Additional compound confirmation was obtained using Mass Frontier, where a high match score to a library search provided enhanced confidence in the compound identification.

Acknowledgements

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References

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[2] Dams, R.; Murphy, C.M.; Choo, R.E.; Lambert, W.E.; De Leenheer, A.P.; Huestis, M.A. *Anal. Chem.* 2003, 75, 798-804

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