Application Note: 390

A Quantitative Test for Multiple Classes of Illicit Drugs and Their Primary Metabolites in Human Biological Fluids by LC-MS/MS for Forensic Use

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Key Words

- TSQ Quantum Discovery MAX
- Surveyor HPLC
- Forensic drugs of abuse testing
- SRM

Introduction

Currently, GC/MS is the method of choice for quantifying drugs of abuse. In recent years, however, many forensic labs have been switching to LC-MS/MS methods, which do not require time-consuming derivatization or extensive sample cleanup necessary in GC/MS analyses. Yet, many of the LC-MS/MS methods described in the literature either assay a limited number of illicit drug classes or do not include their primary metabolites of these illicit drugs (see table 1).[1-5] Herein is described a method to assay multiple drugs of abuse including opiates, stimulants, depressants, and the primary metabolites of these illicit drugs.

Goal

To apply a single LC-MS/MS method to screen for 32 illicit drugs of abuse and their metabolites in biological fluids.

Experimental Conditions

Sample Preparation

Whole blood or urine samples (0.1–0.4 mL) were spiked with 20 ng of isotopically labeled internal standards and purified by solid phase extraction (SPE). Extracted samples were reconstituted to yield solutions with the internal standards at 25 ng/mL.

HPLC

HPLC analysis was performed using the Thermo Scientific Surveyor HPLC System. Each 10 µL sample was injected directly onto a Thermo Scientific Hypersil GOLD PFP 50×2.1 mm, 3 µm analytical column. A gradient LC method used mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.3 mL/min.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Discovery MAX triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) probe. The MS conditions were as follows:

Ion source polarity: Positive ion mode
Ion transfer tube temperature: 370°C
Scan Type: SRM
SRM scan time: 10 ms per transition
Q1, Q3 resolution: unit (0.7 Da FWHM)

Two SRM transitions were monitored for each component to provide ion ratio confirmations (IRC). Table 1 summarizes these SRM transitions.

	Drug of Abuse	Parent *m/z*	Quantifier Product *m/z*	Qualifier Product *m/z*	Ion Ratio
A	*Morphine*	286	201	165	87
B	7-amino-nitrazepam	252	121	94	14.5
C	*Ephedrine*	166	115	133	95
D	Hydromorphone	286	185	157	56
E	Amphetamine	136	119	91	86
F	*Codeine*	300	165	215	97
G	7-amino-clonazepam	286	222	250	85
H	Noroxycodone	302	187	227	97
I	*Methamphetamine*	150	91	119	67
J	Oxycodone	316	241	256	65
K	MDA	180	135	105	92
L	6-MAM	328	165	211	68
M	Norketamine	224	125	179	43
N	Hydrocodone	300	199	171	28
O	*Benzoylecgonine*	290	168	105	24
P	7-amino-flunitrazepam	284	135	227	52
Q	MDMA	194	163	135	30
R	*Ketamine*	238	125	179	40
S	*MDEA*	208	163	135	32
T	Meperidine	248	220	174	55
U	Oxazepam	287	241	269	54
V	*Nordiazepam*	271	140	208	82
W	Cocaine	304	182	82	11.1
X	Lorazepam	321	275	229	25
Y	Nitrazepam	282	236	180	38
Z	Alprazolam	309	281	205	85
AA	Temazepam	301	255	177	11.8
BB	*Clonazepam*	316	270	214	28
CC	Diazepam	285	193	154	70
DD	*Cocaethylene*	318	196	82	15
EE	Flunitrazepam	314	268	239	34
FF	*Methadone*	310	265	105	18

Table 1: Summary of SRM transitions for 32 illicit drugs.

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Results and Discussion

Figures 1 and 2 demonstrate the separation of 32 illicit drugs in less than 10 minutes. Using an SRM dwell time of 10 ms per transition yielded a minimum of 15 data points across an LC peak. The limits of quantitation (LOQs) were determined as either 0.5 ng/mL (lowest calibrator concentration used) or as the concentration where the percent relative errors and %CVs were less than 20% for five replicate injections.

As shown in Figure 3, most calibration curves were fit using linear regression. Some standards (for example, cocaine) yielded better statistical calibration curves using quadratic regression. In these select cases, the target compound used a structurally different isotopically labeled internal standard (for example, cocaine used D5-nor-diazepam as internal standard).

The assay of biological sample extracts identified multiple drugs of abuse and related metabolites. Figures 4A and B demonstrate examples of urine and whole blood extracts assayed for the presence of illicit drugs with the developed LC-MS/MS method. Note that cocaine and benzoylecgonine were detected and qualified below the assay LOQs in a whole blood extract (Figure 4B), indicating that lower LOQs are possible for these compounds.

Conclusion

An LC-MS/MS method for assaying illicit drugs and their metabolites at an LOQ of 0.5–2.5 ng/mL in biological fluids for forensic use has been demonstrated. Confirmation of the drugs of abuse was achieved by monitoring two SRM transitions per compound and measuring their area ratios to within ±20%. Utilizing a low SRM dwell time of 10 ms per transition to achieve sufficient data points across a chromatographic peak had no adverse effects, such as SRM cross-talk, on the quantitation and confirmation of these illicit drugs. To authenticate this assay, extracts from biological fluids were analyzed, showing the presence of several drugs of abuse and their metabolites.

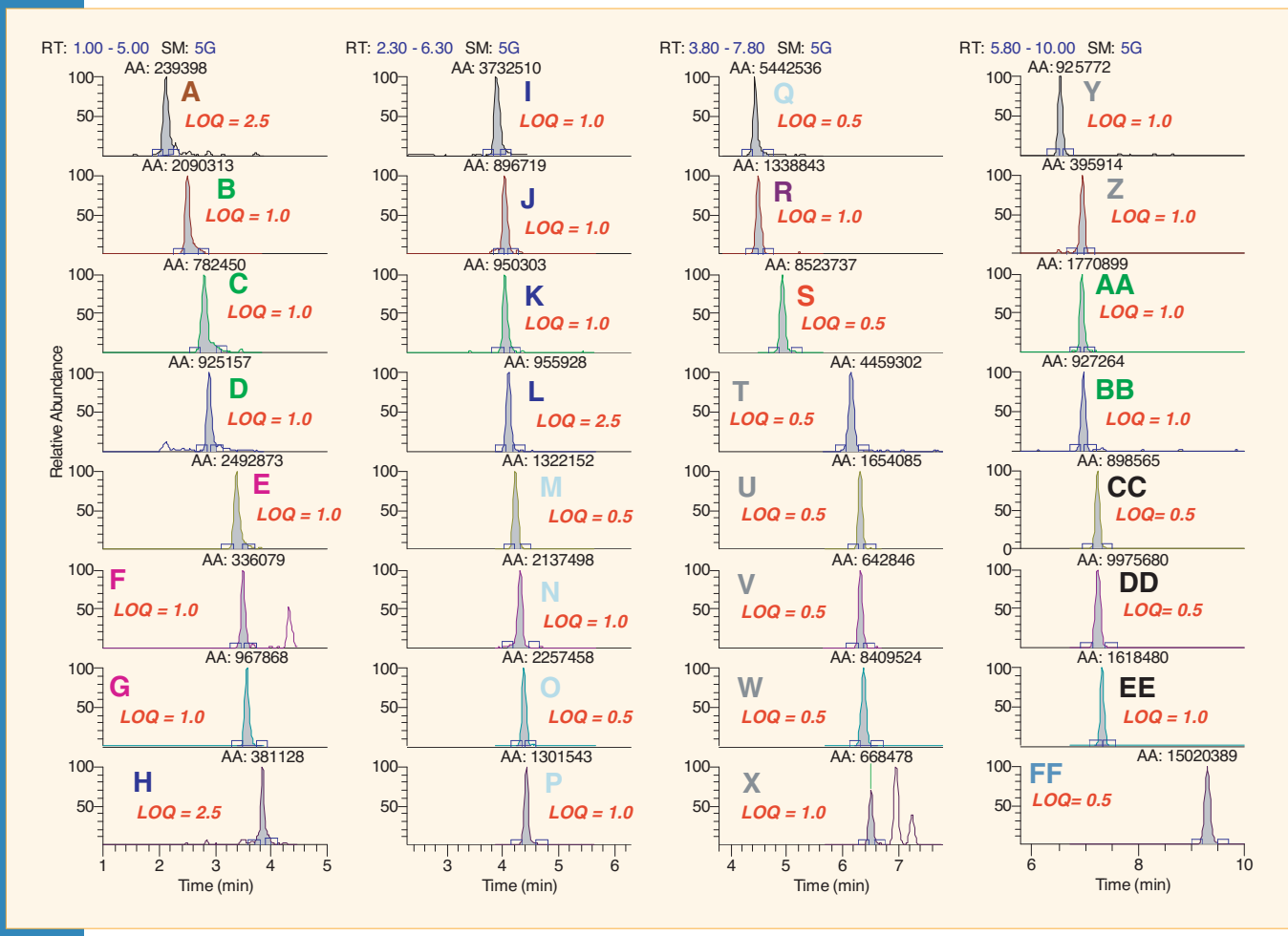

Figure 1: Quantifier SRM transitions for the 2.5 ng/mL standard. For the compound designators, refer to the legend in Table 1.

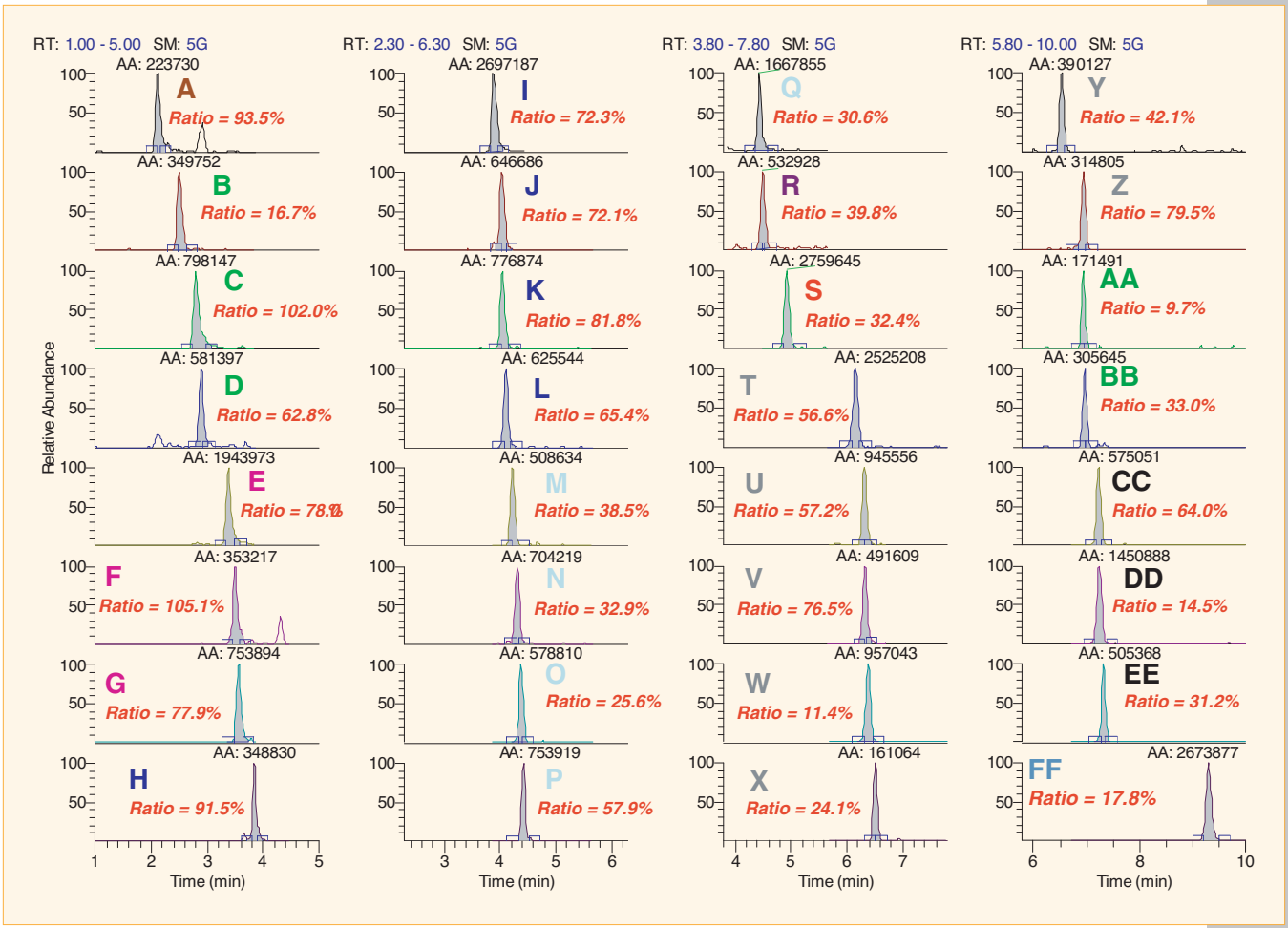

Figure 2: Qualifier SRM transitions for the 2.5 ng/mL standard. For the compound designators and the target ion ratio %, see Table 1.

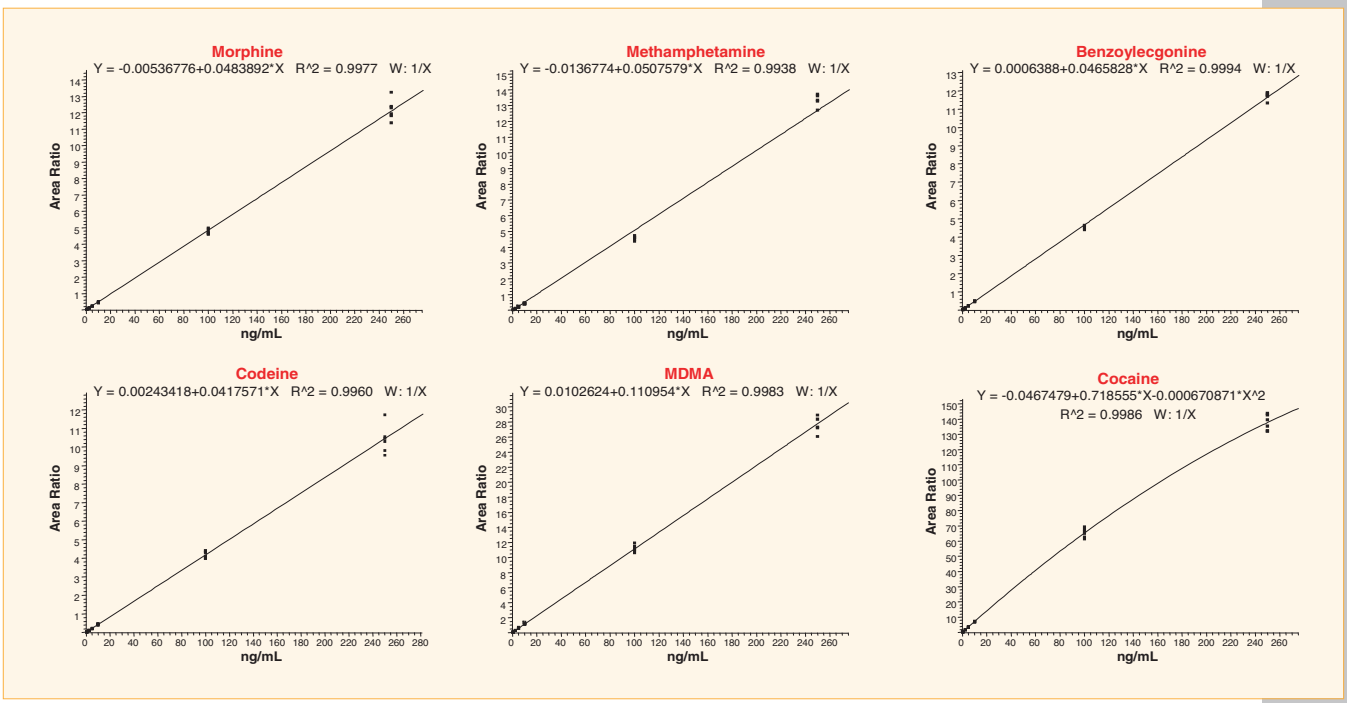

Figure 3: Calibration curves for select drugs of abuse. Regression curve fitting used 1/x weighting from five replicate injections, where $R^2 > 0.993$ for all standards.

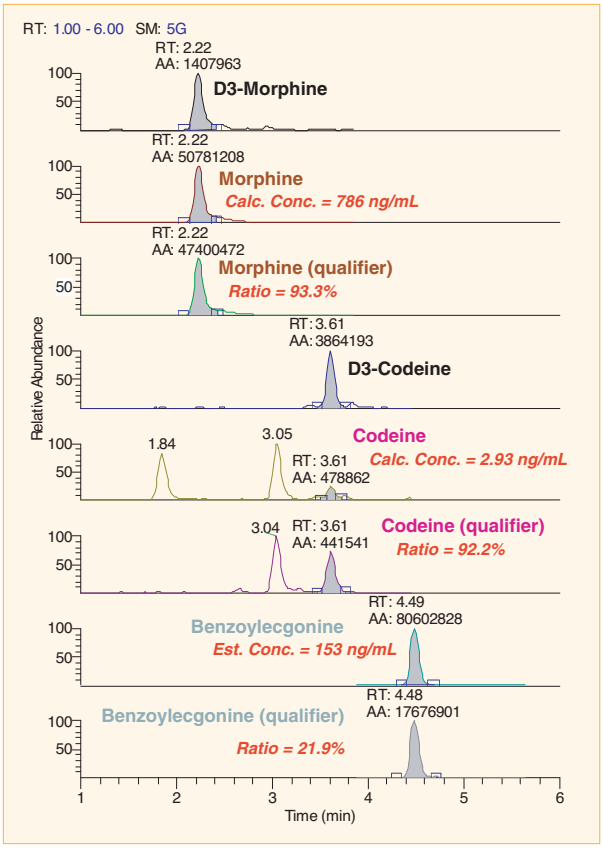

Figure 4A: Assay of urine extract (#423) targeting morphine and its metabolites. The concentration of benzoylecgonine is estimated because a labeled internal standard was not added to the sample extract.

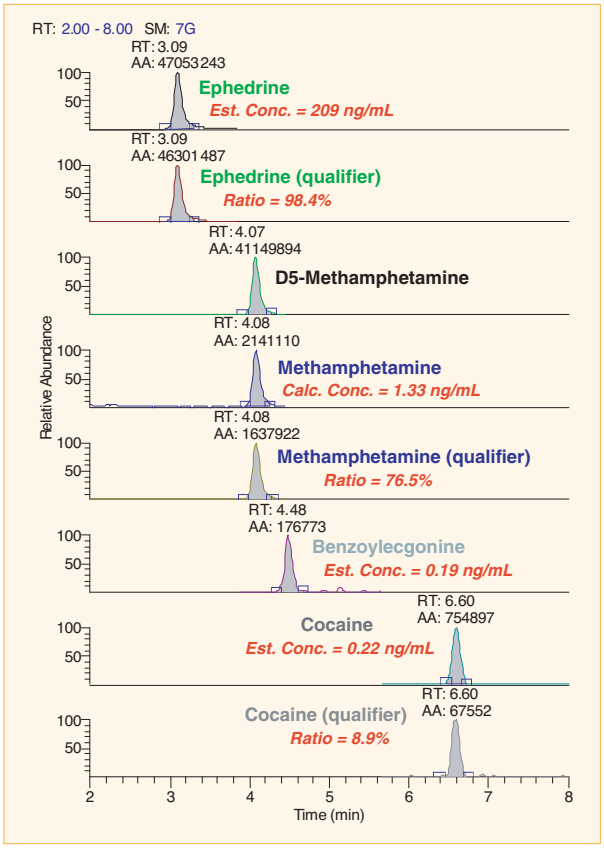

Figure 4B: Assay of whole blood extract (#473) targeting amphetamine and its metabolites. The concentrations of ephedrine, benzoylecgonine and cocaine are estimated because labeled internal standards were not added to sample extract.

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