Rapid Analysis of Drugs of Abuse by LC/Triple Quadrupole Mass Spectrometry

Application Note

Forensic Toxicology

Authors

Michael C. Zumwalt, PhD
Agilent Technologies, Inc.
Englewood, CO
USA

Christine Moore, PhD
Immunalysis, Corp.
Pomona, CA
USA

Abstract

A fast, sensitive and reproducible technique for confirming the presence of drugs of abuse (DOA) in oral fluids (OF) using the Agilent G6410AA Triple Quadrupole Mass Spectrometer (QQQ) is presented. The sensitivity of the QQQ easily meets the cutoff levels required by the United States Substance Abuse and Mental Health Services Administration (SAMHSA) for workplace testing. The DOA analyzed in this work include THC, cocaine, amphetamine, methamphetamine, and MDMA ("Ecstasy") in OFs, which have been prepared using solid phase extraction (SPE). The sample preparation is then followed by reverse-phase LC/MS/MS using a 1.8-µm, C18 column for high-chromatographic resolution with high-speed separation. As a result, elution times for both analytes and internal standards are less than 4.2 minutes for THC, and less than 1.5 minutes for the remaining drugs. The technique is applied successfully to the quantification of quality controls.

Introduction

In 2004, the United States SAMHSA, proposed a new rule that would allow Federal agencies to use sweat, saliva, and hair in Federal drug testing programs that now only test urine [1]. This initiative effectively confirmed the analysis of oral fluids as a viable test matrix for the determination of drug levels in humans in the workplace, which is logically extended to other areas of testing including police checkpoints for possible driving while under the influence of drugs (DUID) violations.

Confirming the presence of DOA in OF using liquid chromatography/tandem mass spectrometry (LC/MS/MS) provides a faster analysis than gas chromatography/mass spectrometry (GC/MS) because the sample derivatization step, usually required for GC/MS analysis, is bypassed without sacrificing required levels of sensitivity. The use of a C18 column with 1.8-µm particle size for liquid chromatography (LC) results in nicely resolved, symmetric peaks at high flow rates. The multiple reaction monitoring (MRM) capability of the QQQ allows for the highly selective MS/MS analysis of coeluting analyte compounds and their corresponding internal standards, along with monitoring more abundant product ions for quantification and less abundant product ions as qualifier ions for confirmation. The MRM provides for highly specific detection in a complex matrix such as OF.

In this work five DOA are analyzed in two separate runs of less than 4.2 minutes for THC (tetrahydrocannabinol) and less than 1.5 minutes for cocaine, amphetamine, methamphetamine, and MDMA (3,4-methylenedioxymethamphetamine). The sensitivity requirements set forth by SAMHSA for these drugs are easily met. The corresponding cutoff levels are shown in Table 1.

Table 1. SAMHSA Cutoff Levels for Drugs of Abuse

Compound	Cutoff level (ng/mL of OF)
THC	2
Cocaine	8
Amphetamine	50
Methamphetamine	50
MDMA	50

Experimental

Sample Preparation

For each sample, 1 mL of OF is collected using the FDA-approved Quantisal™ collection device, which is then dissolved in 3 mL of a proprietary buffer solution already contained in the sample collection device. One mL of this sample is used for further analysis, which corresponds to 250 µL of OF. For the quality control (QC) samples, reference solutions of each analyte are added to drug-free OF, along with the internal standard (ISTD) at low and medium concentrations of each drug. To the unknown samples only internal standards are added, and for the calibration standards the prescribed levels of analytes and ISTDs are added after the extraction.

The extraction method is the same as used for analysis of these drugs by GC/MS, with any derivatization step omitted and the final residue dissolved in the initial mobile phase rather than in a typical GC solvent.

To the OF/buffer aliquot 2 mL of 0.1 M potassium phosphate buffer is added and then vortexed. The SPE (part number 691-0353T, SPEWare, San Pedro, CA), is conditioned with 0.5 mL of methanol for THC, and 3 mL of methanol for cocaine, etc., followed by 100 µL of 0.1 M acetic acid for THC, and 2 mL of 0.1 M phosphate buffer for cocaine, etc. The SPE is performed by adding the sample to the SPE column followed by successive washes, which include methanol and deionized water, followed by 98:2 hexane:acetic acid for THC, 78:20:2 CH_2Cl_2/IPA/NH_4OH for cocaine, or 2% NH_4OH in ethyl acetate for amphetamine, methamphetamine, and MDMA.

After evaporating the sample to dryness, it is reconstituted in the initial LC mobile phase (0.1% formic acid/water). For the calibration standards, analytes, ISTDs, and mobile phase are added to make 1-mL volumes.

Note that the objective of this work was to test QQQ instrument capability and not the quality of the extraction procedure. Therefore, it was decided that spiking blank OF extracts with both reference and ISTDs after the extraction process would eliminate the variability of sample recovery. However, QCs were spiked with both analytes and ISTDs before the extraction, and the unknown samples were only spiked with ISTDs before the extraction.

Compounds Analyzed

The target compounds and their molecular ion masses are given in Figure 1.

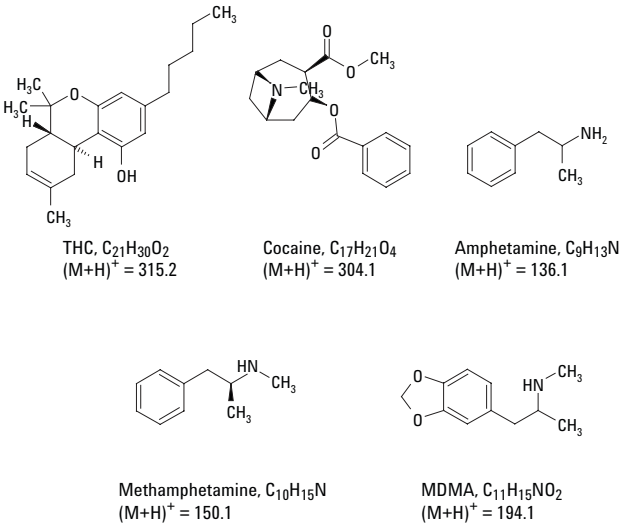

Figure 1. Target compound structures, and their molecular ion masses.

LC/MS/MS Instrumentation

The LC/MS/MS system used in this work consists of an Agilent 1100-series vacuum degasser, binary pump, well-plate autosampler, thermostatted column compartment, the Agilent G6410AA Triple Quadrupole Mass Spectrometer, and an electrospray ionization source (ESI). System control and data analysis is provided by the Agilent QQQ Control (R&D version), Qualitative and Quantitative Data Analysis software programs. Detailed LC and MS conditions are shown below.

The objective of the method development was to obtain a fast and sensitive analysis for quantifying and confirming the presence of drugs of abuse in oral fluids. For speed, while maintaining good chromatographic resolution and peak symmetry, different solvents, flow rates, and column parameters were optimized. It was found that not only would a simple solvent system using water, acetonitrile, and formic acid, work very well, but a very fast 1-minute gradient on a 1.8-µm particle

size C18 column would elute the compounds in times very competitive with most techniques available in GC/MS as well as LC/MS.

LC Conditions

Column:	Agilent ZORBAX SB-C18, RRHT 2.1 × 50 mm, 1.8 µm (p/n 822700-902)
Column temp:	40 °C
Mobile phase:	A = 0.1% Formic acid in water B = 0.1% Formic acid in acetonitrile
Flow rate:	0.5 mL/min
Gradient:	5% B at 0 min 95% B at 1 min 95% B at 6 min Post run time = 2.5 min
Injection vol:	80 µL (THC); 20 µL (for cocaine, etc)

MS Conditions

Mode:	Positive ESI using the Agilent G1948A ionization source
Nebulizer:	40 psig
Drying gas flow:	10 L/min
Drying gas temp:	350 °C
V_{cap}:	4000 V
Q1 Resolution:	0.7 amu (FWHM)
Q2 Resolution:	0.7 amu (FWHM)
Collision energy:	23 V (THC); 5 V (all other analytes)
MRM:	4 transitions for THC; 16 transitions for cocaine, amphetamines, methamphetamines, and MDMA as shown in Table 2

LC/MS Method Details

Determination of the optimal MRM transitions for both quantifier and qualifier ions was carried out by infusing the individual standards at concentration levels around 1 ng/µL. The quantifier ion was chosen as the most abundant product ion and the qualifier ion was chosen as the second-most abundant product ion.

At the time of this writing, the preliminary version of software only allowed one collision energy and one time segment for the entire chromatographic run. Therefore, a single fragmentation energy of 23 V was used for all transitions of for THC and ISTD, and 5 V was used for all of the transitions of the cocaine, etc., compounds and their associated ISTDs, even though these settings were not optimal for each transition. In addition, MRM transitions were monitored continuously throughout the entire run. As a result, while the data shown here satisfies the requirements of SAMHSA, even better sensitivity should be achievable with optimization of collision energy and time programming of MRM events.

Table 2. Data Acquisition Parameters for MRM Transitions

Compound	RT (min)	Pseudo-molecular ion $(M+H)^+$	Quantitation product ion (*m/z*)	Qualifier product ion (*m/z*)
THC	4.2	315.3	193.1	259.1
D3-THC	4.2	318.3	196.1	262.1
Cocaine	1.5	304.1	182.0	82.0
D3-cocaine	1.5	307.1	185.1	85.1
Amphetamine	1.3	136.1	91.0	119.0
D5-amphetamine	1.3	141.1	93.0	124.0
Methamphetamine	1.3	150.1	91.0	119.0
D5-methamphetamine	1.3	155.1	92.0	121.0
MDMA	1.4	194.1	163.0	135.0
D5-MDMA	1.4	199.1	165.0	135.0

Results and Discussion

The chromatograms corresponding to one-half the cutoff value for THC, or 1 ng/mL, are shown in Figure 2. This level is easily seen and the on-column injection amount corresponds to 20 pg. The area reproducibility among three injections is 3.6%. The root-mean-squared (RMS) signal-to-noise (S/N) is estimated conservatively as five times the RMS S/N. This corresponds to a S/N value of 32:1. The limit of quantitation (LOQ) is about half this value, which corresponds to 0.5 ng/mL, and was confirmed by injecting smaller volumes.

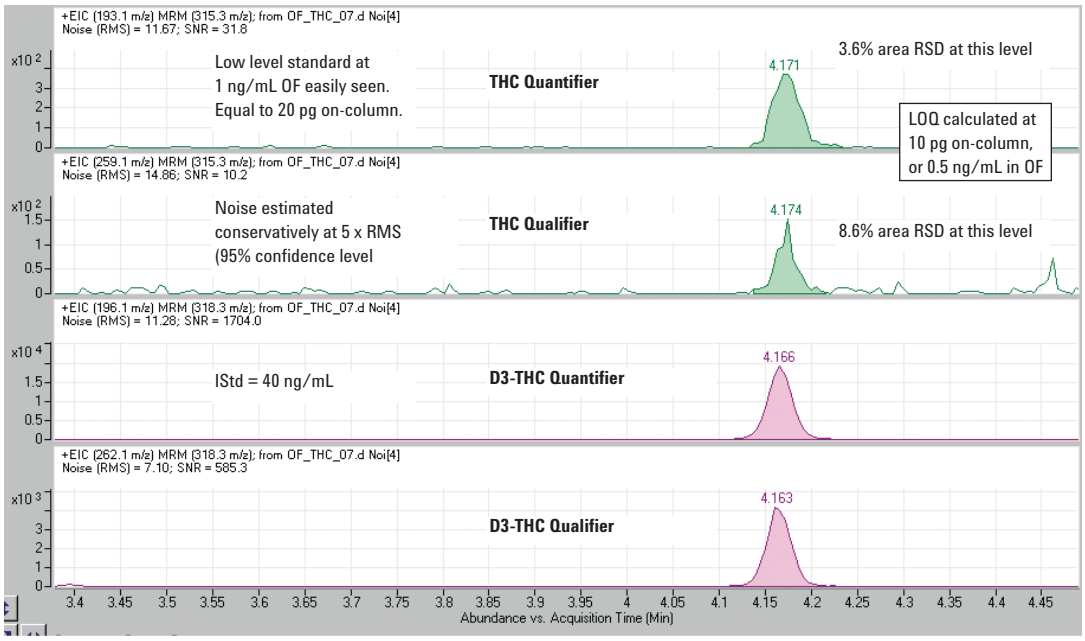

Figure 2. Product ion chromatograms for THC and D3-THC. Generation of chromatograms and integration of peaks is automated with opening of data file by the Agilent Qualitative Analysis software. Peak elution times less than 4.2 minutes. No smoothing applied.

In Figure 3, and using the same reasoning for THC, the LOQs for cocaine (coc), MDMA, methamphetamine (meth), and amphetamine (amp) are estimated to be 0.2, 0.5, 0.6, and 2.5 ng/mL in OF, respectively.

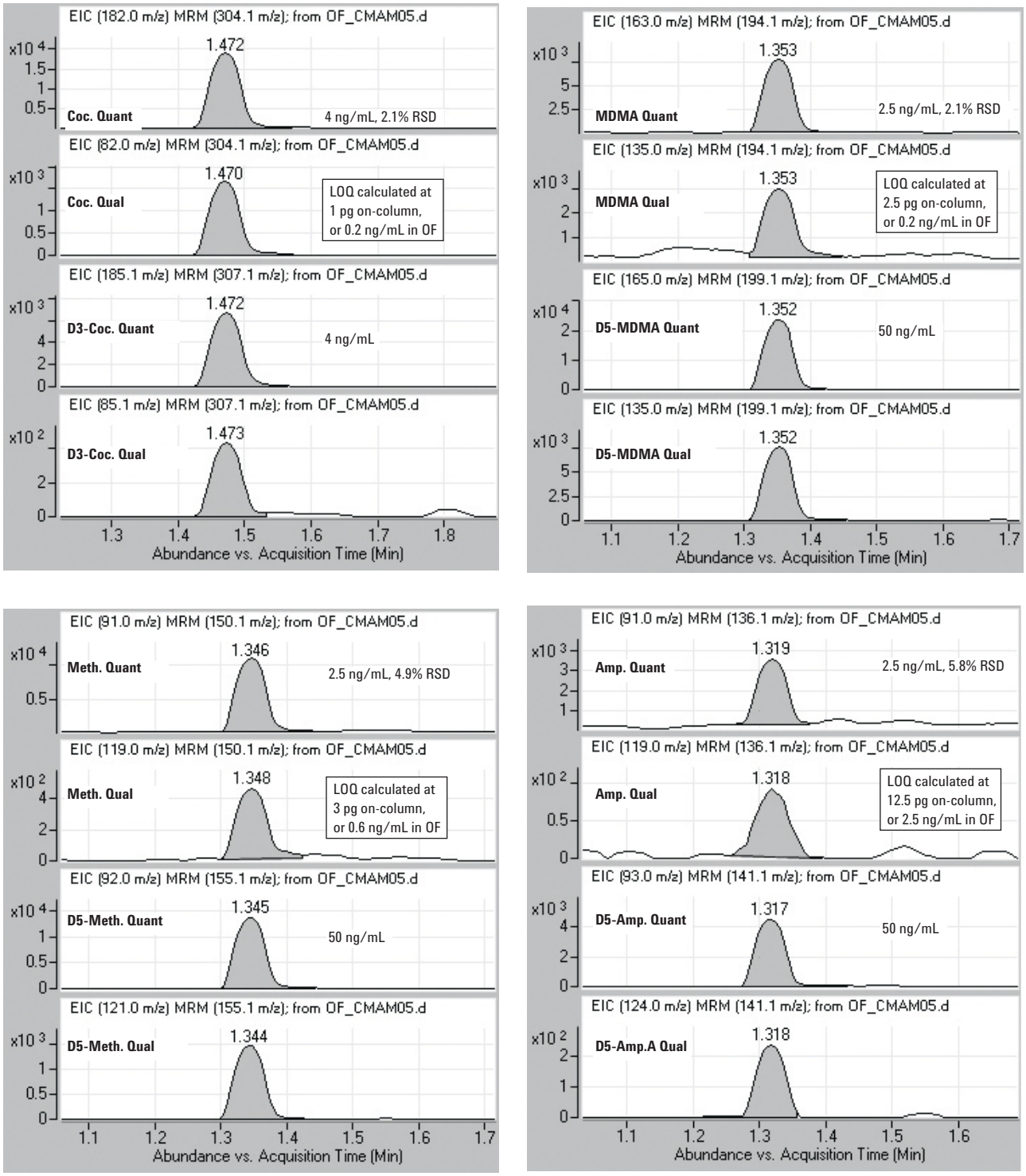

Figure 3. Product ion chromatograms for lowest level standard containing cocaine, D3-cocaine, MDMA, D5-MDMA, amphetamine, D5-amphetamine, methamphetamine, and D5-methamphetamine. Peak elution times less than 1.5 minutes. No smoothing applied.

Along with the quantifier ions for each of the compounds and associated ISTDs, the qualifier ions are also shown in Figure 4. The requirement for each qualifier ion is that its measured area falls within a range of specified ratios with respect to the area of the quantifier ion. For example, with the THC qualifier ion, as determined experimentally by the Agilent G6410AA instrument, the ratio of its measured area to that of the THC quantifier ion should be 22%. Applying a window of acceptance that is ±20% gives an overall range of 17.6% to 26.4%. As long as the ratio of the areas falls within this range, the acceptance criteria for confirmation is met. For all THC compounds, both calibration standards and QCs, this criteria was satisfied. A similar criteria was established for the ISTD.

For the remaining compounds, the qualifier ion area ratio criteria were established as 4% for cocaine, 9% for MDMA, 95% for methamphetamine, and 26% for amphetamine. As was the case for THC, criteria were established for the associated ISTDs as well. All calibration standards and QCs met these criteria.

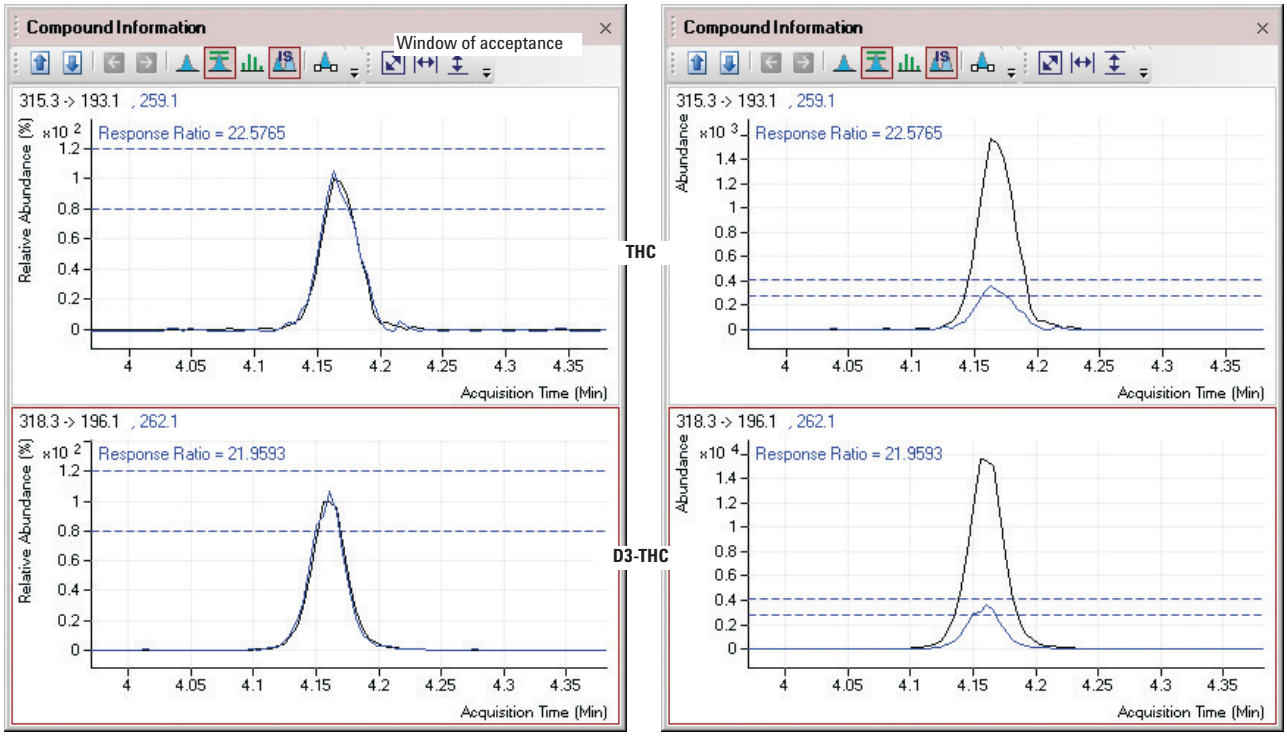

Figure 4. **For confirmation of THC, the qualifier ion area must be 22% that of the quantifier ion area and within a window of ±20% of that value, or from 17.6% to 26.4% overall. The two ways to display this for fast confirmation in the Quantitative Analysis software is normalized by area (left) and un-normalized (right), both of which show the overlap of the qualifier ion on the quantifier ion. If the ion ratio is outside the window of acceptance, the integrated area of qualifier ion will be shaded blue, but transparently to still observe overlap.**

The calibration curves generated for all compounds are shown in Figure 5. The most conservative fitting options are used to generate the line; that is, a linear fit with no weighting and no origin treatment. Each line is based on calibration levels extending across nearly two orders of magnitude.

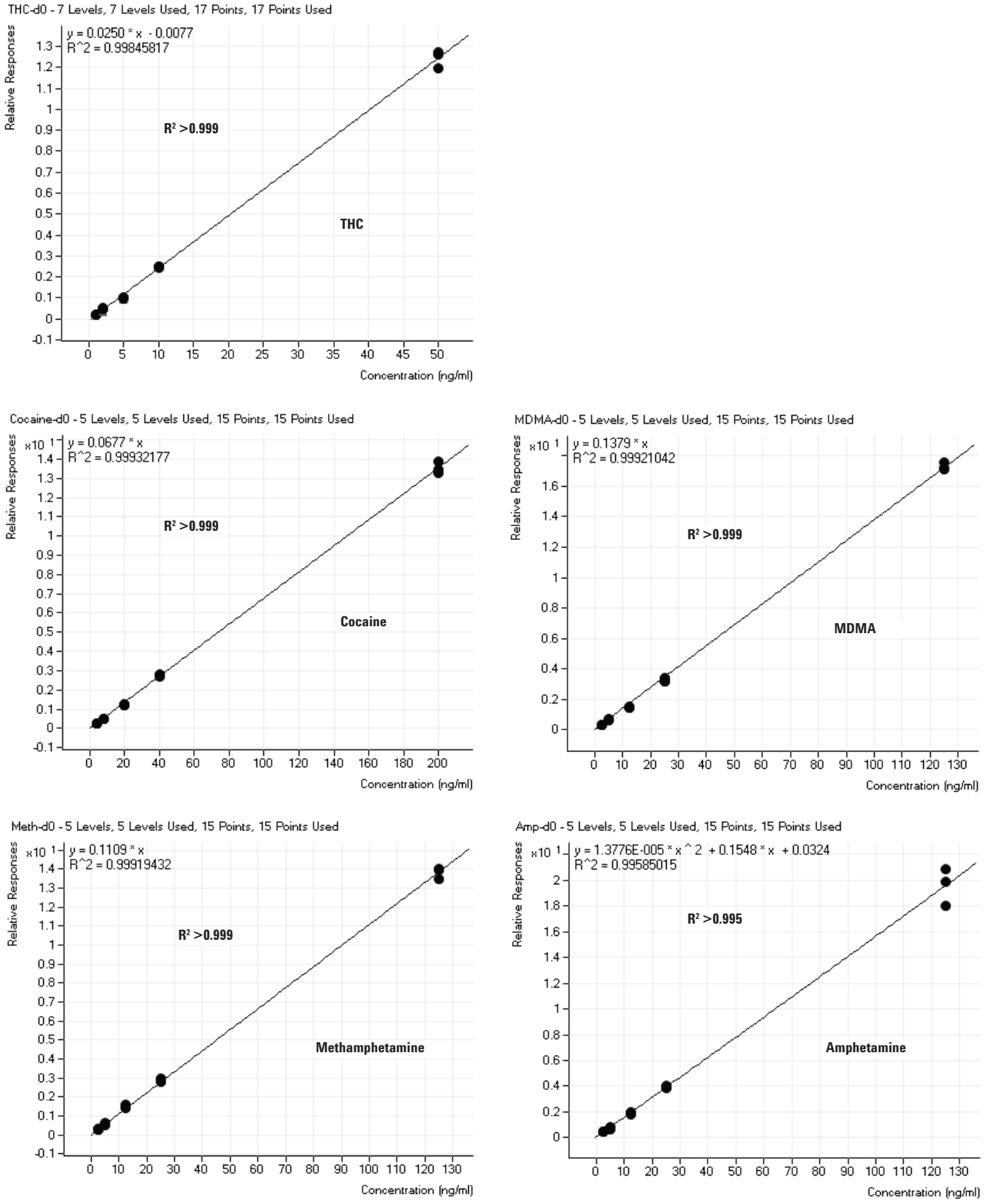

Figure 5. Calibration curves for each DOA using a linear line fit with no weighting and no origin treatment.

The reproducibility for THC is shown in Table 3, and as expected, the %RSD values are lower for higher concentrations. The %RSD is calculated from the area counts for three repeat injections.

Table 3. Reproducibility for THC

Level (ng THC/mL OF)	%RSD
1	3.6
2	2.5
5	2.3
10	1.0
50	1.7

Based on the calibration curves, the QC samples and unknowns are quantified as shown in Table 4. Also shown are the expected amounts of the QCs as prepared by Immunalysis Corporation and the unknown sample THC as measured by GC/MS.

Table 4. Measured Levels of QC and Unknown Samples

Sample	Expected concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)
THC QC1	2	1.81	9.5
THC QC2	5	4.21	16.0
THC Unknown	10*	9.39	6.1
Coc QC1	8	7.51	6.1
Coc QC2	8	7.68	4.0

* Measured by GC/MS

Further Work

Other work has shown that the analysis of THC using atmospheric pressure chemical ionization (APCI), and even atmospheric pressure photoionization (APPI), are more sensitive techniques than ESI [2]. At the time of this writing, the G6410AA Triple Quadrupole Mass Spectrometer instrument was still in its prototype stage and did not support the Agilent G1948A APCI Source, or the Agilent G1978A Multimode Source, which includes simultaneous ESI and APCI capability. Using the APCI Source for the THC could lead to better sensitivity and using the Multimode Source could allow for the analysis of the cocaine, MDMA, methamphetamine, and amphetamine compounds in ESI mode during the first 2 minutes of the run, and the switching to APCI for the remainder of the run when the THC elutes.

As mentioned earlier in this note, the capability to use optimal fragmentation voltages for each MRM transition would lead to an increase in sensitivity. Nevertheless, the G6410A easily meets SAMHSA requirements even without optimization of collision energies or ionization modes.

Conclusions

The LC/MS/MS method described here provides procedures for identification of multiple DOAs in OF with very fast analysis times. Sensitivity levels required by SAMHSA are met for workplace testing, and MRM of several fragmentation transitions are carried out not only for quantitation using designated quantifying ions, but also for confirmation using designated qualifier ions. Using the Agilent C18 column with 1.8-µm particles allows for excellent resolution and peak shape at a relatively high flow rate of 500 µL/min for a 2.1-mm id column and an ESI interface.

References

1. "Rules Proposed for Workplace Drug Testing", *SAMHSA News*, 12 (3) May/June 2004; a publication of the United States Department of Health and Human Services.
2. J. M. Hughes, D. M. Andrenyak, D. J. Crouch, and M. Slawson, "Comparison of LC-MS Ionization Techniques for Cannabinoid Analysis in Blood", Society of Forensic Toxicologists, 2002 Annual Meeting, Dearborn, MI.

For More Information

For more information on Agilent products and services visit our Web site www.agilent.com/chem

For more details concerning this note, please contact Michael Zumwalt at Agilent Technologies, Inc.

Acknowledgments

A special thanks to Agilent colleague John M. Hughes for very valuable review and comments.

Printed in the USA
May 18, 2006
5989-4856EN

Agilent Technologies