Toxicology Screening of Whole Blood Extracts Using GC / Triple Quadrupole / MS

Application Note

Forensic Toxicology

Authors

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Abstract

The Agilent 7000 GC / QQQ system can provide both high selectivity and high sensitivity for the analysis of drugs. Low-level detection and confirmation of large numbers of target drugs in blood extracts is possible in a single run. Combined with information from a single quadrupole screening instrument like the Agilent GC / NPD / MSD / DRS system, a much more complete picture of each sample is now possible.

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Introduction

Toxicology screening is challenging due to the need to look for large numbers of target compounds in samples that contain complex matrix interferences. GC/MS methods are widely used and accepted for this analysis. Full-scan EI methods offer many advantages for broad-range screening, such as unlimited numbers of targets, full spectrum identity confirmation, and library searching for identification of nontargets. Several recent advances in Agilent's GC/MS technology, including retention time locking (RTL), deconvolution reporting software (DRS), and capillary flow technology (CFT), have greatly improved the screening process. Samples can now be screened much more rapidly with fewer false positives and negatives [1].

Screening is usually aimed at drugs in concentrations high enough to cause intoxication or death, and GC/MS in full-scan mode usually provides sufficient sensitivity for this task. Labs routinely monitor drugs down to approximately 100 pg in matrix. For those cases where drugs need to be determined at low or trace levels, single ion monitoring (SIM) mode can be used to improve the sensitivity of the analysis. With the introduction of Agilent's SIM/scan, SIM data can be collected simultaneously with scan data, saving significant analysis time [1]. As an example, the method described in reference 1 screens for 725 compounds in SIM/scan mode with a cycle time of 9.6 minutes injection to injection. This time includes the simultaneous acquisition of scan, SIM (for 27 compounds), and NPD data.

For some drugs, however, there are limitations with SIM. Compounds present in the matrix can result in interferences that prevent detection or confirmation of trace levels of certain target analytes. For these situations, there are two main approachs to solving the problem. The first is to increase the chromatographic selectivity using Agilent's heartcutting 2D-GC technology [2]. This approach uses two columns and a Deans switch to chromatographically isolate the analyte(s) from matrix interferences. With the extremely high separation power of this technique, SIM mode can be used to detect analytes at very low levels due to the reduction in interference.

This approach is relatively simple and cost effective, but in practice, only a few analytes can be determined in one run. A second approach is to increase the mass spectral selectivity using a triple quadrupole mass spectrometer (GC/QQQ). The extremely high selectivity and sensitivity with this approach allows detection of drugs down to sub-picogram levels with minimal matrix interferences. A significant advantage is that it can be used to routinely monitor for large numbers of compounds (up to a few hundred) in a single run.

This note describes using GC/QQQ to detect low and trace levels of drugs in extracts of whole blood. The samples were previously analyzed on a system using GC/MS with SIM/scan, DRS, and simultaneous detection with a nitrogen phosphorus detector. The GC/QQQ is shown to be a powerful complement to the GC/NPD/MSD/DRS system for those cases where trace level detection and confirmation is required.

Experimental

Chemicals and Standards

Analytical reference standard solutions of the drugs in Table 1 were purchased from Cerilliant (Round Rock, TX). Calibration solutions were prepared by appropriate dilution of the reference standards in toluene. For method setup using Q1-scan mode and for product ion scans, a test solution of 1 ng/µL of the drugs was used. For calibration in MRM mode, standard solutions at 10 and 50 pg/µL were used.

Samples

Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single-step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume.

Instrumentation

Analyses were performed on an Agilent 7890 GC combined with a 7000A Triple Quadrupole MS system. The system was configured with a capillary flow technology 2-way splitter with makeup (option 889) as described in [3] to allow backflushing the column after every run. This prevents heavy matrix components from the blood extracts from fouling the column by removing them at the end of each analysis [1]. The instrumental conditions are listed in Table 2.

Several MRMs were evaluated for each analyte using the 1 ng/µL standard solution. When possible, four were identified for analysis and are listed in Table 2. Although only two are typically used for GC/QQQ analysis, four were identified in case added certainty in identification of trace analytes was desired.

The whole blood extracts were analyzed on both GC/QQQ and the GC/NPD/MSD/DRS system described in reference 1. The retention times on the GC/QQQ were precisely locked to twice those in reference 1 using Agilent's method translation and RTL software.

Table 1. MRM Parameters and MDLs

	Retention time (min)	Precursor ion	Product ion	Collision energy (EV)	Relative response	*MDL (pg)
Meperidine	5.651					
		246	172.1	10	100	0.2
		247	71	10	80	
		218	172.2	10	36	
		174	70.2	10	32	
PCP (phencyclidine)	6.497					
		200	117.2	15	100	0.1
		200	84.1	15	46	
		242	171.2	25	17	
		243	200.3	10	14	
Methadone	7.728					
		72	42	25	100	0.2
		72	44	25	4	
		223	104.9	10	3	
		178	152	25	3	
Cocaine	8.078					
		82	67	20	100	
		82	41	25	60	
		182	82	10	50	0.2
		303	82	25	20	
Codeine	8.980					
		229	214.1	10	100	2.2
		299	229	15	38	
		162	146.8	20	38	
		162	146	30	25	
Hydrocodone	9.252					
		299	242.8	10	100	1.0
		242	152.8	30	71	
		242	180.9	20	71	
		299	270.1	15	71	
THC	9.321					
		231	173.9	25	100	0.4
		299	81	20	11	
		314	81.3	30	6	
6-Acetylmorphine	9.533					
		215	42.1	30	100	50
		268	252	25	77	
Oxycodone	9.589					
		315	230.1	15	100	0.5
		315	258	10	57	
		230	215.3	10	43	
		201	186.1	25	43	
Heroin	9.970					
		327	215	15	100	0.5
		327	268	10	67	
		369	268	30	33	
		369	204	10	25	
Fentanyl	10.354					
		245	146	20	100	0.2
			189	44	20	
			202	146	10	
			189	146	5	

* Signal-to-noise ratio = 3, noise measured peak to peak

Table 2. Instrument Conditions

GC	
Agilent Technologies 7890A with autoinjector and tray	
Inlet	EPC split/splitless
Mode	Constant pressure
Injection type	Splitless
Injection volume (µL)	1.0
Inlet temperature (ºC)	280
Inlet pressure (psig)	17.8
Purge flow (mL/min)	50
Purge time (min)	0.75
Gas type	Helium
Oven	
Initial oven temperature (ºC)	100
Initial oven hold (min)	0.5
Ramp rate (ºC/min)	20
Final temperature (ºC)	325
Final hold (min)	2.5
Total run time (min)	14.25
Equilibration time (min)	0.5
Column	
Type	DB-5MS UI
Agilent part number	122-5512UI
Length (m)	15
Diameter (mm)	0.25
Film thickness (um)	0.25
Nominal initial flow (mL/min)	2.2
Outlet pressure (psig)	3.8
Column Backflushing	
2-way splitter with makeup (one port plugged)	
Restrictor length (m)	0.8
Restrictor id (mm)	0.15
Backflushing pressure (psig)	75
Backflushing temperature (ºC)	325
Backflushing time (min)	2
Triple Quadrupole MS	
Agilent Technologies 7000A	
Inert EI source, Ionization energy (EV)	70
Mode	MRM
MS1 and MS2 resolution (amu)	1.2
Collision cell nitrogen pressure (psig)	2.6
Helium quench gas pressure	6.25
Solvent delay (min)	1.4
EM voltage	Atune voltage
Quad1 and 2 temperature (ºC)	150
Source temperature (ºC)	300
Transfer line temperature (ºC)	300

Results and Discussion

Figure 1 shows the GC/QQQ TIC in MRM mode for the evaluated compounds. The compounds are not derivatized because the sample preparation for the comparison screening method from reference 1 does not use derivatization. While the amines (amphetamine, phentermine, methamphetamine, MDA, MDMA, and MDEA) all show a sizable response at 1 ng/µL, analysis at lower levels was not possible because of their loss in the chromatographic system before reaching the MS, as is well known. Trace detection of the amines would require derivatization.

With the exception of 6-acetylmorphine, the remainder of the compounds all exhibited detection limits in the low picogram range. The detection limits listed in Table 1 are calculated for a signal-to-noise ratio of three with the noise measured as peak to peak. All MDLs were measured by injecting 1 µL of a 10 pg/µL solution of the compound except for 6-acetylmorphine, for which 1 µL of 50 pg/µL was used. Figure 2 shows the response for 10 pg of heroin at the 4 MRMs listed in Table 1. This example illustrates the high sensitvity provided by the GC/QQQ.

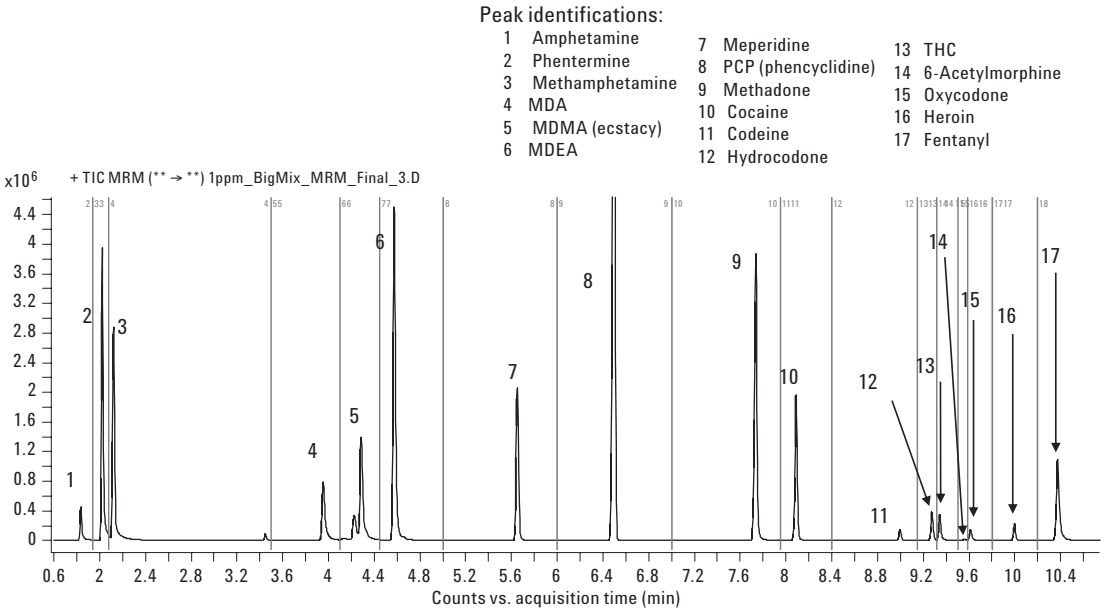

Figure 1. TIC of the Agilent 7000A Triple Quad GC/MS system in MRM mode. Standard solution of 1 ng/uL.

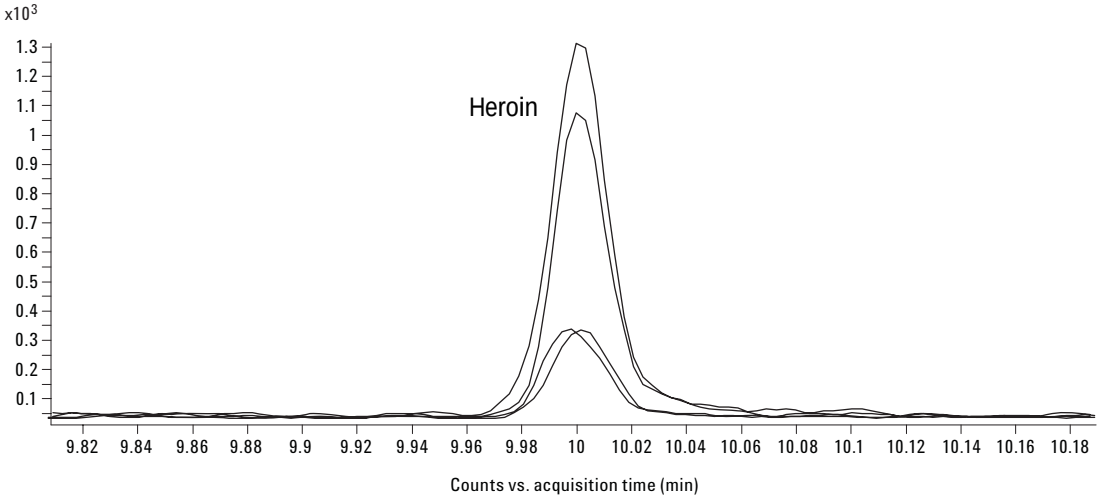

Figure 2. MRM transitions for heroin standard at 10 pg/µL. The transitions are listed in Table 1.

Figure 3 shows the extracted ion chromatograms (EICs) for codeine from the GC/NPD/MSD/DRS system scan data for whole blood extract A. The response at the codeine target ion and a corresponding peak on the NPD chromatogram at the correct retention time for codeine suggests it is present. However, confirmation with qualifier ion ratios is complicated by the low signal-to-noise ratio due to interference and the small quantity of codeine present. The deconvoluted spectrum from the DRS report only had a spectral match quality of 59 (out of 100), which is not high enough to confirm the presence of codeine.

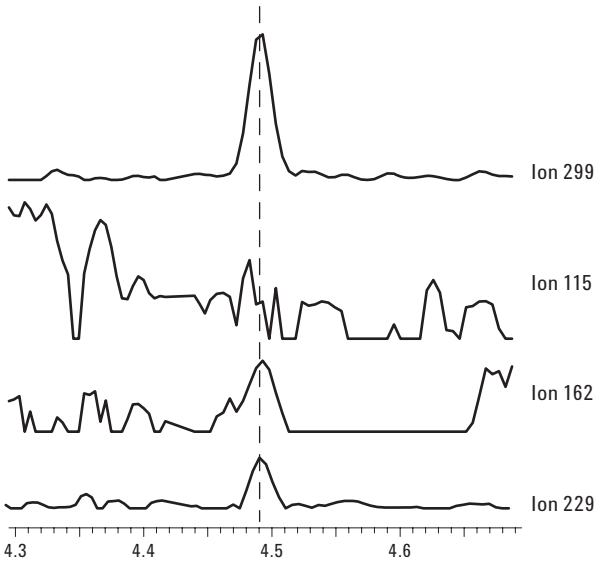

Figure 3. Codeine EICs from GC/NPD/MSD/DRS system scan data for whole blood extract A.

Figure 4 shows the corresponding GC/QQQ results for codeine in the same sample. The much higher selectivity and sensitivity afforded by GC/QQQ clearly confirm the presence of codeine in sample A. The amount detected corresponds to about 150 pg.

Detection of the powerful drug fentanyl in blood extracts is often a challenge because of the relatively small quantities of the drug administered. Confirmation is limited because there are only three ions of significant abundance. Figure 5 shows scan and SIM EICs for fentanyl from the GC/NPD/MSD/DRS system. There are only three ions and ion 189 is marginal at best due to low signal size and some interference. SIM data from SIM/scan had a much better signal-to-noise ratio, but still exhibited the same interferences on ion 189. The NPD response confirms that a nitrogen-containing compound with the same RT as fentanyl is present.

The DRS report for the sample found a marginal spectral match for fentanyl (66) at the correct RT. Based on all the information taken together, it appears that fentanyl is present in the sample.

Figure 6 shows the GC/QQQ MRMs for fentanyl in the same sample. The selectivity of MSMS detection clearly confirms its presence. The amount detected corresponds to about 150 pg.

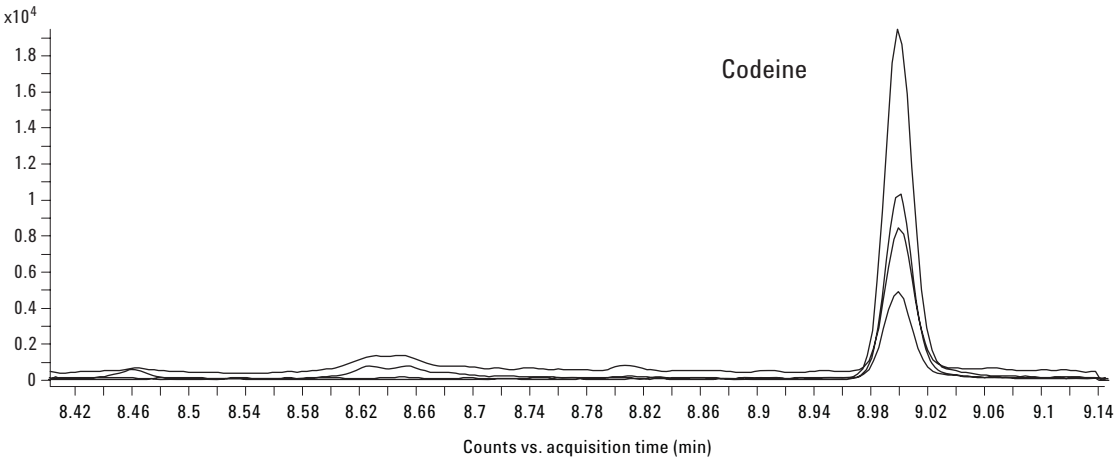

Figure 4. Codeine MRMs from GC/QQQ of whole blood extract A in Figure 3.

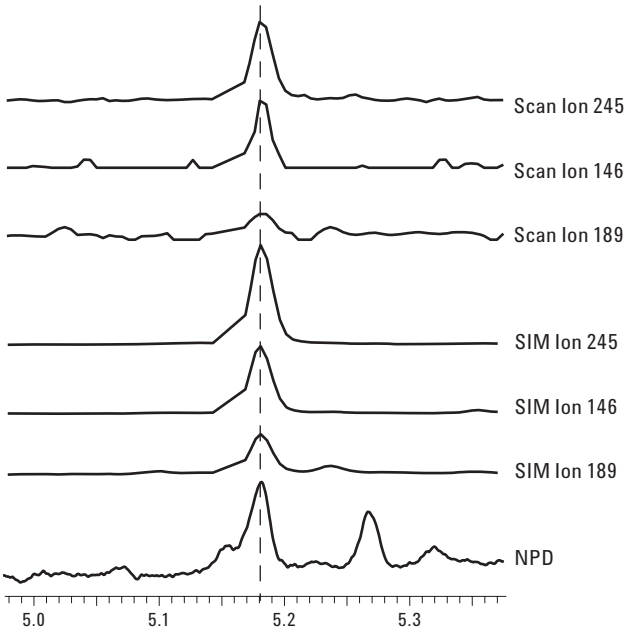

Figure 5. Fentanyl EICs and NPD response from whole blood extract B on GC/NPD/MSD/DRS system.

Figure 7. shows the scan, SIM, and NPD chromatograms for methadone in whole blood extract C from the GC/NPD/MSD/DRS system. Confirmation of methadone is complicated by the fact that its spectrum contains one large ion at a low, relatively common mass (72). The remaining ions are all small, being less than 6% relative abundance. As seen in Figure 7, the qualifier ions, especially 57, exhibit interferences. The deconvoluted spectrum had a match of 74. Note that the match quality value is dominated by the single 72 ion, so the number is artificially skewed a bit higher than normal. The data all point to methadone being present in the sample.

Figure 8 shows the GC/QQQ MRMs for methadone in sample C. The presence of methadone is clearly confirmed. The amount detected corresponds to about 170 pg.

Figure 9 shows the scan, SIM, and NPD chromatograms for oxycodone in whole blood extract B from the GC/NPD/MSD/DRS system. In this case, the amount present is relatively low at about 60 pg. Oxycodone was not reported in the DRS report because the spectral match was only 46, which is typically below the minimum match. The poor match resulted from high interferences and the small quantity of oxycodone present. In the scan EICs, the target ion and the NPD

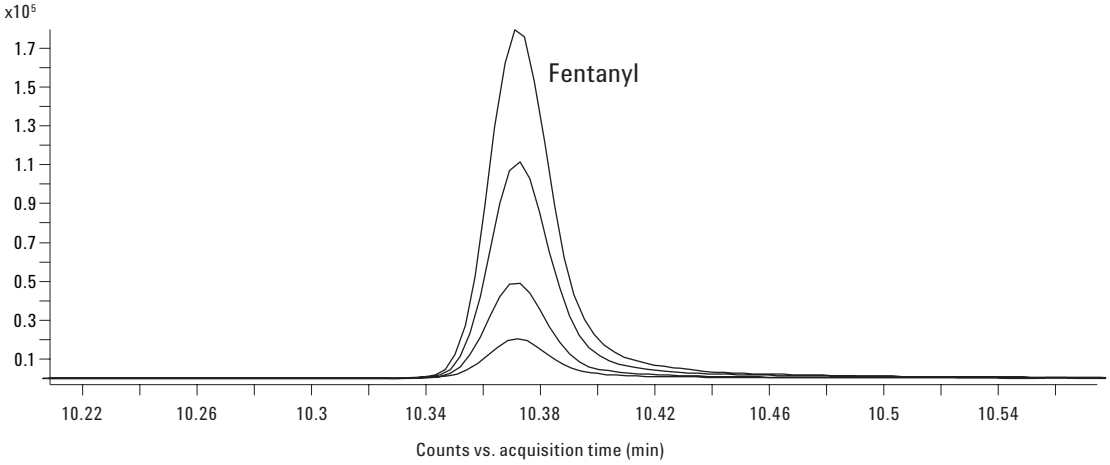

Figure 6. Fentanyl MRMs from GC/QQQ of whole blood extract B in Figure 5.

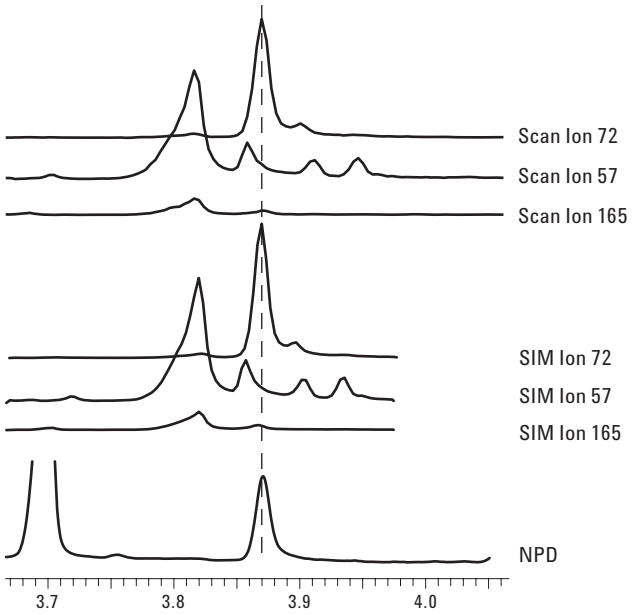

Figure 7. Methadone EICs and NPD response from whole blood extract C on GC/NPD/MSD/DRS system.

response are discernible peaks, but the two qualifiers are unusable. Note that the much higher signal-to-noise ratio provided by SIM allows a choice of ions that are too small to be used in scan mode and which have significantly higher selectivity. This is seen in the SIM chromatograms in Figure 9. The substitution of ion 316 for ion 70 now provides two clean qualifier ions with which to confirm the presence of oxycodone.

Figure 10 shows the GC/QQQ MRMs for oxycodone in the sample B. As with the previous examples, the high selectivity and sensitivity of GC/QQQ makes detection and confirmation of oxycodone straightforward.

The last example is shown in Figures 11 and 12. Figure 11 shows the scan, SIM, and NPD chromatograms for cocaine in whole blood extract A from the GC/NPD/MSD/DRS system. Note there is no indication of cocaine on either the scan or SIM chromatograms. There is what may be a very small response on the NPD, but it is too small to be significant. The GC/QQQ clearly shows the presence of cocaine in the sample at a very low level. The peak represents about 0.7 pg of cocaine, highlighting the low limits of detection available with GC/QQQ.

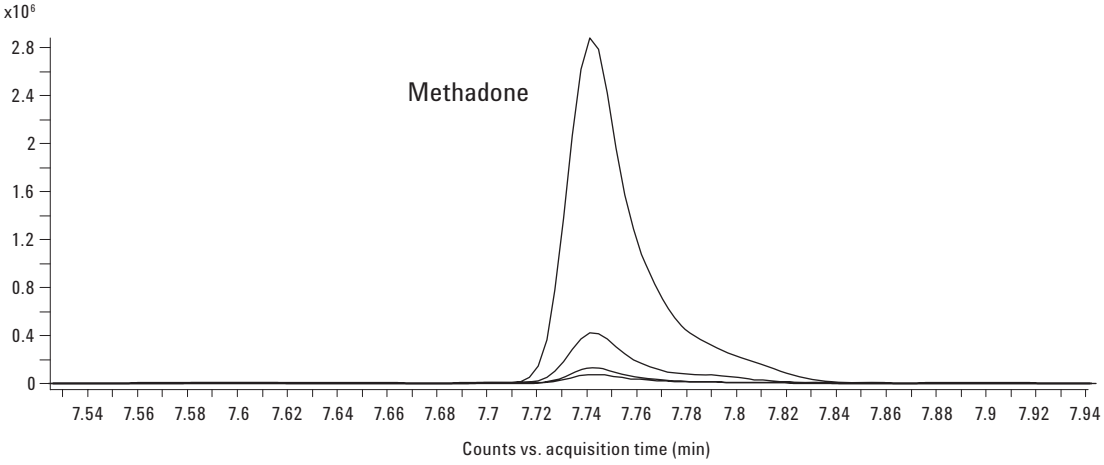

Figure 8. Methadone MRMs from GC/QQQ of whole blood extract C in Figure 7.

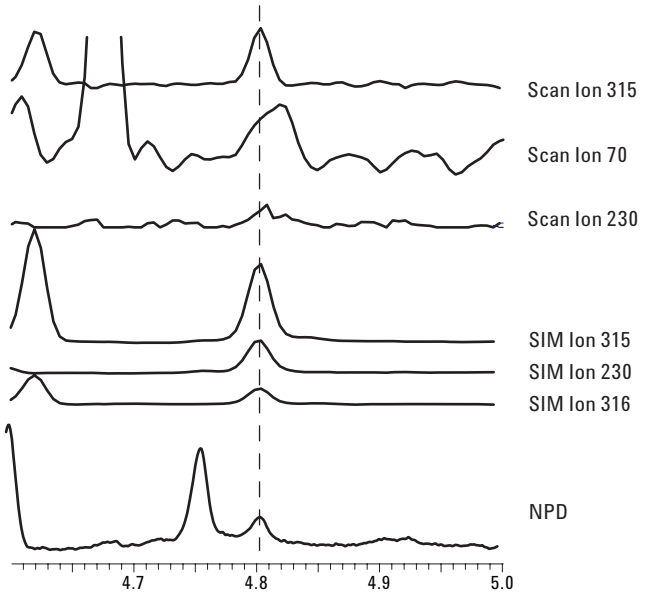

Figure 9. Oxycodone EICs and NPD response from whole blood extract B on GC/NPD/MSD/DRS system.

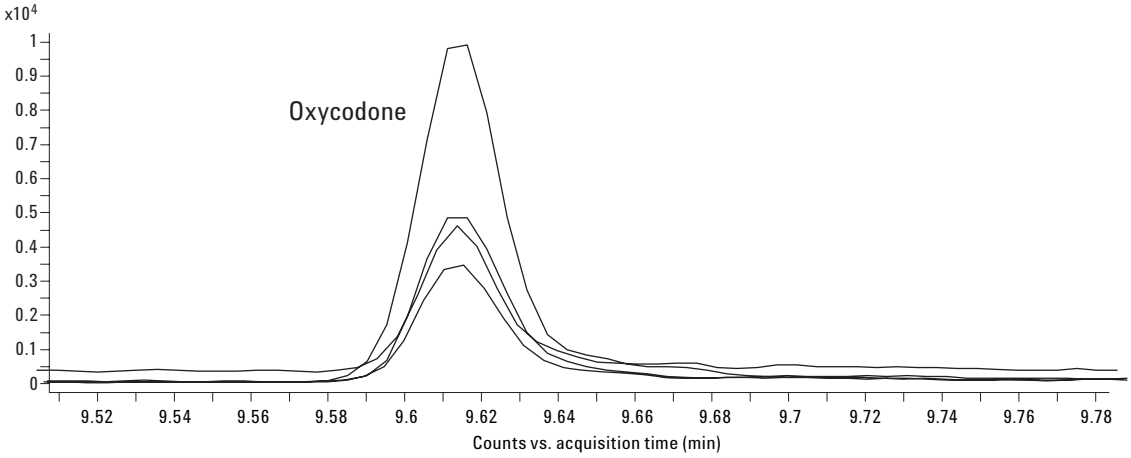

Figure 10. Oxycodone MRMs from GC/QQQ of whole blood extract B in Figure 9.

Conclusions

The Agilent 7000 GC/QQQ system provides both high sensitivity and high selectivity for the analysis of drugs. The system allows the low level detection and confirmation of large numbers of target drugs in blood extracts in a single run. When used in combination with a single quadrupole screening instrument like the Agilent GC/NPD/MSD/DRS system, a much more complete picture of each sample is now possible. The GC/NPD/MSD/DRS system provides the broadest range screen (725 compounds), full spectra and nitrogen selective detection for identifying nontarget compounds, and SIM data for lower level targets. The GC/QQQ provides routine detection and confirmation of up to a few hundred target compounds at low pg levels, even in difficult matrices.

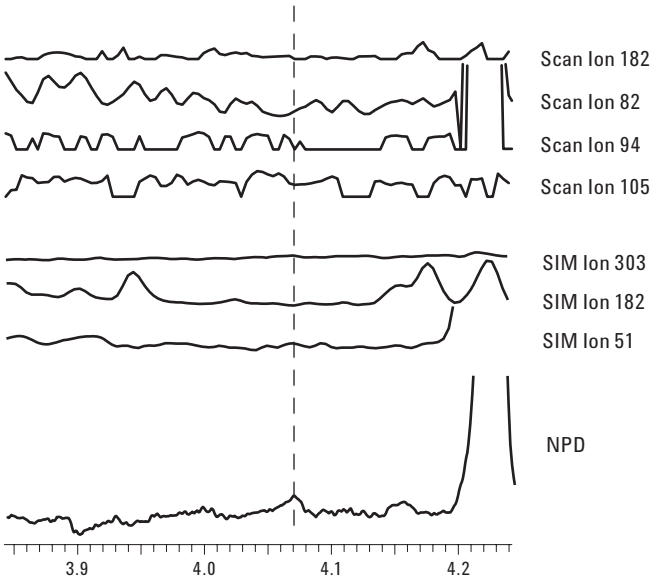

Figure 11. Cocaine EICs and NPD response from whole blood extract A on GC/NPD/MSD/DRS system.

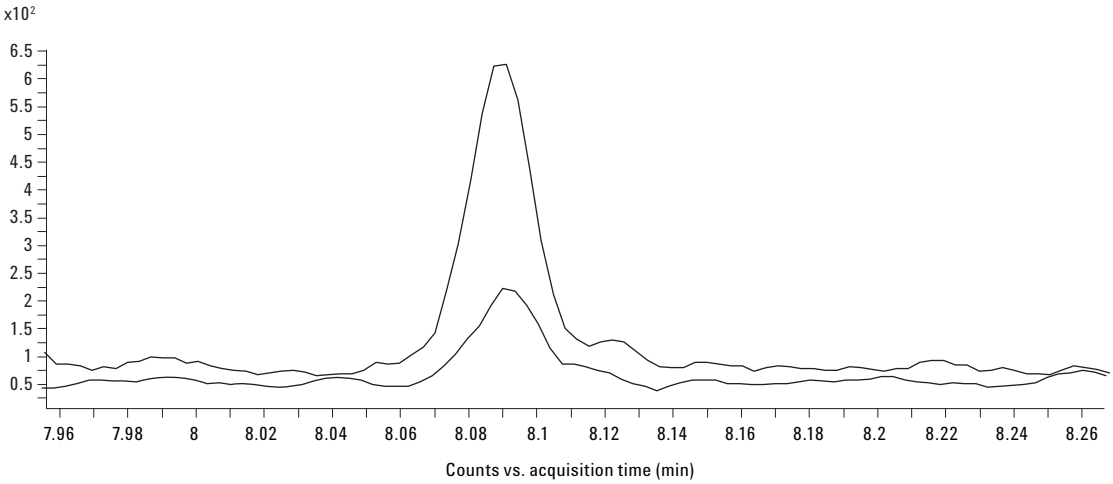

Figure 12. Cocaine MRMs from GC/QQQ of whole blood extract A in Figure 11. Top trace is MRM 182-82, bottom trace is 303-82.

References

1. Bruce Quimby, "Improved Forensic Toxicology Screening Using A GC/MS/NPD System with a 725-Compound DRS Database," Agilent Technologies publication 5989-8582EN.
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3. Chris Sandy, "Analysis of Complex Samples by GC/MS/MS – Pesticides in Marine Biota," Agilent Technologies publication 5989-9727EN.

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