Rapid Forensic Toxicology Screening Using an Agilent 7890A/NPD/5975C/DRS GC/MSD System

Application Brief

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Laboratories that perform toxicology screens on forensic samples are challenged by the requirement to analyze large numbers of samples containing complex matrix interferences. The system described here addresses these demands by combining fast GC to reduce the run time; simultaneous collection of scan, SIM, and NPD data in one shorter run; backflushing to prevent heavy matrix components from fouling the detectors; and Deconvolution Reporting Software (DRS) to simplify data interpretation. The scan data is deconvoluted and used to identify any of 278 target compounds. SIM data is used to look for select low-level compounds not detectable in scan mode. The nitrogen response of the NPD is used to highlight nontarget compounds, identity confirmation, and can be used for quantitation if needed. Using an extract of a whole blood sample, the system finds all the molecules detected by the conventional method in significantly less time.

Highlights

- DRS simplifies data interpretation, especially in dirty samples.
- Simultaneous collection of SIM, scan, and NPD signals saves time.
- The 7890A GC/MS High Speed Oven Accessory provides high programming rates, even with 120 V service.
- Backflushing reduces ghost peaks in high matrix samples.

Experimental and Results

The Forensic Toxicology GC/MSD RTL Database of 277 compounds was downloaded from Agilent's Web site and converted for use with DRS. The method was scaled to precisely two times faster using Agilent's Method Translation software. 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 1/6th volume. Extracts were analyzed using the conditions in Table 1. The simultaneously acquired chromatograms for scan, SIM, and the NPD for one of the samples is shown in Figure 1. The 245 target ion for fentanyl shown is one of 13 SIM ions monitored. This example is particularly challenging because of the high levels of matrix interferences as seen in the scan TIC. The drug compounds present were identified using a combination of 1) full-spectrum searching of the deconvolved spectra against the target library (AMDIS), 2) target and qualifier ion ratios in the MSD ChemStation, and 3) response on the NPD.

Compounds identified by AMDIS deconvolution but not found by the MSD ChemStation because of out-of-range qualifiers were manually inspected in QEdit. Quantitation was forced if AMDIS indicated an acceptable spectral and retention time match and if there was a corresponding NPD response.

The SIM data was used to screen for several compounds (see Table 1) that are often at levels too low to be detected in scan mode. In this sample, fentanyl was found present at a low level in the scan data and confirmed with the SIM responses. The signal-to-noise ratio of the SIM target ion was 10 times greater than that of the scan.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A		
Inlet	EPC split/splitless		
Mode	Splitless, 2 µL injected		
Inlet temp	280 °C		
Pressure	24.15 psig, retention time locked to oxycodone at 5.505 min		
Purge flow	50 mL/min		
Purge time	1 min		
Gas saver	Off		
Gas type	Helium		
Liner	Agilent splitless inlet liner, single-taper, Part # 5181-3316		
Oven	**240 V**		
Oven ramp	°C/min	Next °C	Hold min
Initial		120	1.00
Ramp 1	40	320	2.50
Total run time	8.5 min		
Equilibration time	0.5 min		
Backflush time	2.0 min		
Backflush temp	320 °C		
Column	Agilent Technologies DB-17 ms, Part # 123-4712		
Length	15.0 m		
Diameter	0.32 mm		
Film thickness	0.25 µm		
Mode	Constant pressure		
Outlet	2-way splitter with solvent vent		
Splitter pressure	3.8 psi during acqusition, 75 psi during backflush with inlet set to 1.0 psi during backflush		
Splitter restrictors	MSD:1.44 m × 0.18 mm id × 0.18 µm film DB-17 ms (Part # 121-4722). NPD:0.75 m of same		
Solvent venting	0 to 1.40 min		
NPD	Capillary NPD with EPC, option 251		
Gas flows	Hydrogen 3.0 mL/min, air 60 mL/min, nitrogen makeup 12 mL/min		
NPD temp	310 °C		
MSD	Agilent Technologies 5975C, Performance Turbo		
Solvent delay	None (solvent vented with splitter)		
EM voltage	Tune voltage		
Mode	SIM/scan		
Scan	42-550 amu, sampling: 2[1]		
SIM ions	Group 1 (PCP) 84, 186, 200, 242; Group 2 at 4.5 min, (norfentanyl butyl derivative, 6-acetylmorphine, heroin, fentanyl) 42, 82, 83, 146, 158,189, 231, 245, 268, 284, 310, 327, 369; Group 3 at 6.5 min (LSD) 221, 323, 181, 207; all dwell times 10 msec		
Quad temp	150 °C		
Source temp	280 °C		
Transfer line temp	280 °C		

Table 3 shows the DRS report for the sample in Figure 1. The report lists the compounds quantitated by the MSD ChemStation and identified by deconvolution. The quantitative results are rough approximations, as the response factors used here were only average responses for screening purposes. Note that there are several nondrug compounds in the target library that are detected as well.

The spectra of peaks found on the NPD that did not correspond to targets were searched against the NIST and Pfleger libraries for identification. The peak on the NPD in Figure 1 labeled with a question mark was not a target compound. Search results of the spectrum indicated it was cyheptamide (later found to be an internal standard added in sample preparation).

For comparison, the sample in Figure 1 was analyzed in the same way but with the 1x method for reference. All drugs found with the original 1x method were found with the 2x method.

The use of the two-way splitter with solvent venting allows the solvent peak (and any other unwanted peaks) to be vented before reaching the detectors. This helps extend the useful life of the NPD bead. The device also allows backflushing at the end of the run. As seen in Figure 1, there are large matrix peaks that elute after the last target compound. Backflushing quickly removes these compounds, saving time and reducing detector and column maintainence.

The significant time savings available with the method described here vs. the original method where three separate runs of scan, SIM, and NPD are needed to access the same information are shown in Table 2.

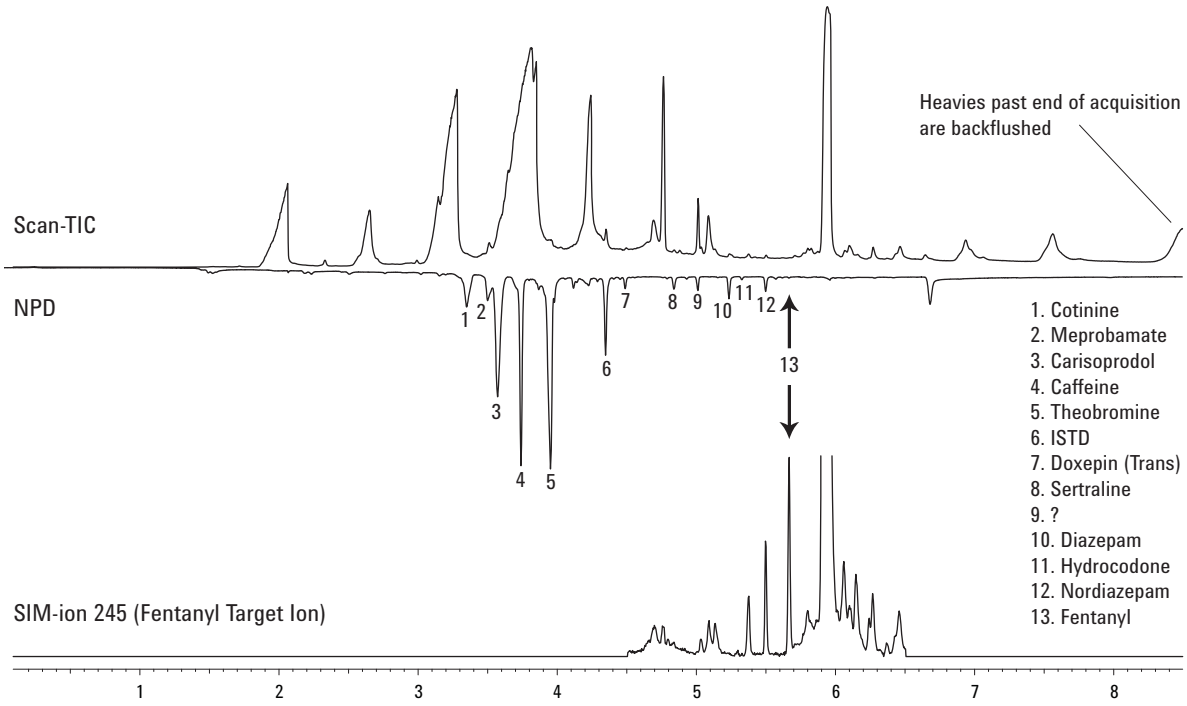

Figure 1. Chromatograms from screen of whole blood sample.

Table 2. Time Savings Using the Agilent 7890A-5975C

	Typical 6890 1X	7890A 2X	Minutes Saved
Run time without matrix bake-out, includes equib	17	8.5	8.5
Run time with matrix bake-out 6890 or Splitter 7890A	24	10.5	13.5
Cool down time from 320 to 120	2.3	1.6	0.7
Autosampler time, 7890A with overlap	1	0.1	0.9
Acquiring scan, SIM, and NPD signals separately vs. simultaneously	81.9	12.2	69.7

Time savings > 85%
Not including time saved using DRS

Table 3. DRS Report from Screen of Whole Blood Sample

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (~ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
2.1124	nc06	DIETHYLPROPRION		61	-3.9		
2.6530	nc013	TETRADECANOIC ACID	44.17	99	-1.1		
3.3558	nc046	COTININE	1.94	78	0.9		
3.5188	nc060	MEPROBAMATE	1.79	74	-2.2		
3.5792	nc063	CARISOPRODOL	1.88	85	-0.1		
3.745	nc079	CAFFEINE	1.43				
3.958	nc089	THEOBROMINE	5.46	75	1.8		
4.3516	nc0123	10,11-DIHYDRO-DIBENZ[B,F] [1,4]OXAZEPIN-	9.25	98	-0.1		
4.494	nc0133	DOXEPIN(TRANS)	1.45				
4.4940	nc0131	DOXEPIN(CIS)		67	3.0		
4.7654	nc0155	BIS[2-ETHYLHEXYL] PHTHALATE	138.58	94	0.4		
4.8420	nc0158	SERTRALINE[2]	0.54	88	-0.1		
5.087	nc0178	DOXYLAMINE METABOLITE	0.21				
5.238	nc0187	DIAZEPAM	0.45	92	0.3		
5.3285	nc0191	HYDROCODONE	0.1	62	0.2		
5.5009	nc0198	NORDIAZEPAM	0.41	62	0.0		
5.6695	nc0210	FENTANYL	0.1	64	0.8		
5.7097	nc0218	GAMMA-TOCOPHEROL	0.94	81	0.5		
5.8750	nc0226	VITAMEN E	0.81	90	2.7		
5.930	nc0233	CHOLESTEROL	65.83	98	0.2		

Conclusions

Significant time savings can be realized in the screening of toxicology samples with the system described. The cycle time required per sample is reduced 85%. Data interpretation time is also reduced with the use of DRS.

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