Poster Reprint

ASMS 2024
Poster number WP 810

A Triple Quadrupole GC/MS MRM Database for Forensic and Toxicological Workflows

Celine Gys[1], Anna Klimowska[1,2], Adrian Covaci[1], Remko van Loon[3], Joel Ferrer[4], Anastasia Andrianova[4]

[1]Toxicological Center, University of Antwerp, Wilrijk, Belgium; [2]Department of Toxicology, Medical University of Gdansk, Gdansk, Poland; [3]Agilent Technologies, Middelburg, Netherlands; [4]Agilent Technologies, Wilmington, DE, USA

Introduction

Systematic toxicological analysis in forensic investigation demands continuous adaptability to an ever-evolving toxicant landscape.

The three main challenges are:

1. low concentrations of the toxicants
2. an ever-growing number of analytes to be monitored and quantitated
3. the limitations in obtaining analytical standards for every chemical.

For the volatile compounds, gas chromatography/mass spectrometry (GC/MS) is the method of choice when analyzing forensic drugs and toxicants [1, 2]. GC/MS **forensic toxicological workflow** greatly benefits from **selectivity** and **sensitivity** of multiple reaction monitoring (MRM) approach enabled with triple quadrupole GC/MS (GC/TQ).

The aim of this work was to develop an **MRM database** to help toxicological researchers build screening and quantitation methods simplifying method development.

The database of MRM transitions for relevant toxicants was established and successfully applied to creating GC/TQ methods for analyzing real-world authentic samples with **greater sensitivity and confidence** than the conventional GC/MS approach. The database will be available for download starting July 2024.

Experimental

GC/MS Parameters

Agilent 7000 Series GC/TQ mass spectrometer was used for developing the MRM transitions. Compounds were analyzed underivatised, as well as their trimethylsilylated and acetylated derivatives. Agilent MassHunter Optimizer for GC/TQ was used for developing 1794 MRM transitions.

Parameter	Value
MS	Agilent 7000 Series GC/TQ
Column	Agilent J&W DB-5ms, 30 m, 0.25 mm, 0.25 µm (p/n 122-5532)
Inlet	Multimode inlet, Ultra Inert, splitless, double taper (part number 5190-3983)
Injection volume	2 µL
Injection mode	Pulsed splitless (1.5 min, pulse @25 psi for 1.5 min)
Inlet temperature program	275 °C
Oven temperature program	80 °C for 1 min; 20 °C/min to 290 °C, 8 min hold
Carrier gas	Helium
Column flow	1 mL/min constant flow. Retention Time Locked to cocaine at 12.26 min
Transfer line temperature	300 °C
Quadrupole temperature	150 °C
Source temperature	230 °C
Electron energy	70 eV
TQ mode	dMRM When developing MRM transitions: Scan (m/z 100-450), Product Ion Scan, MRM

Table 1. GC/MS method parameters

Experimental

Database Curation

The starting GC acquisition method was optimized for successful GC analysis of toxicants. The Optimizer software was used exercising *the Start from Scan* workflow, which includes the following steps performed sequentially:

- Acquisition or import of full scan data to identify target compounds
- Precursor ion identification
- Product ion identification
- Collision energy optimization.

MassHunter Unknowns Analysis was used for identifying the target compounds through searching against Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites [3] (Fig. 1).

The resulting 1,794 MRM transitions were sported as a CSV file.
The database created in this work, can be used to simplify creation of dMRM data acquisition methods with the Agilent GC/TQ.

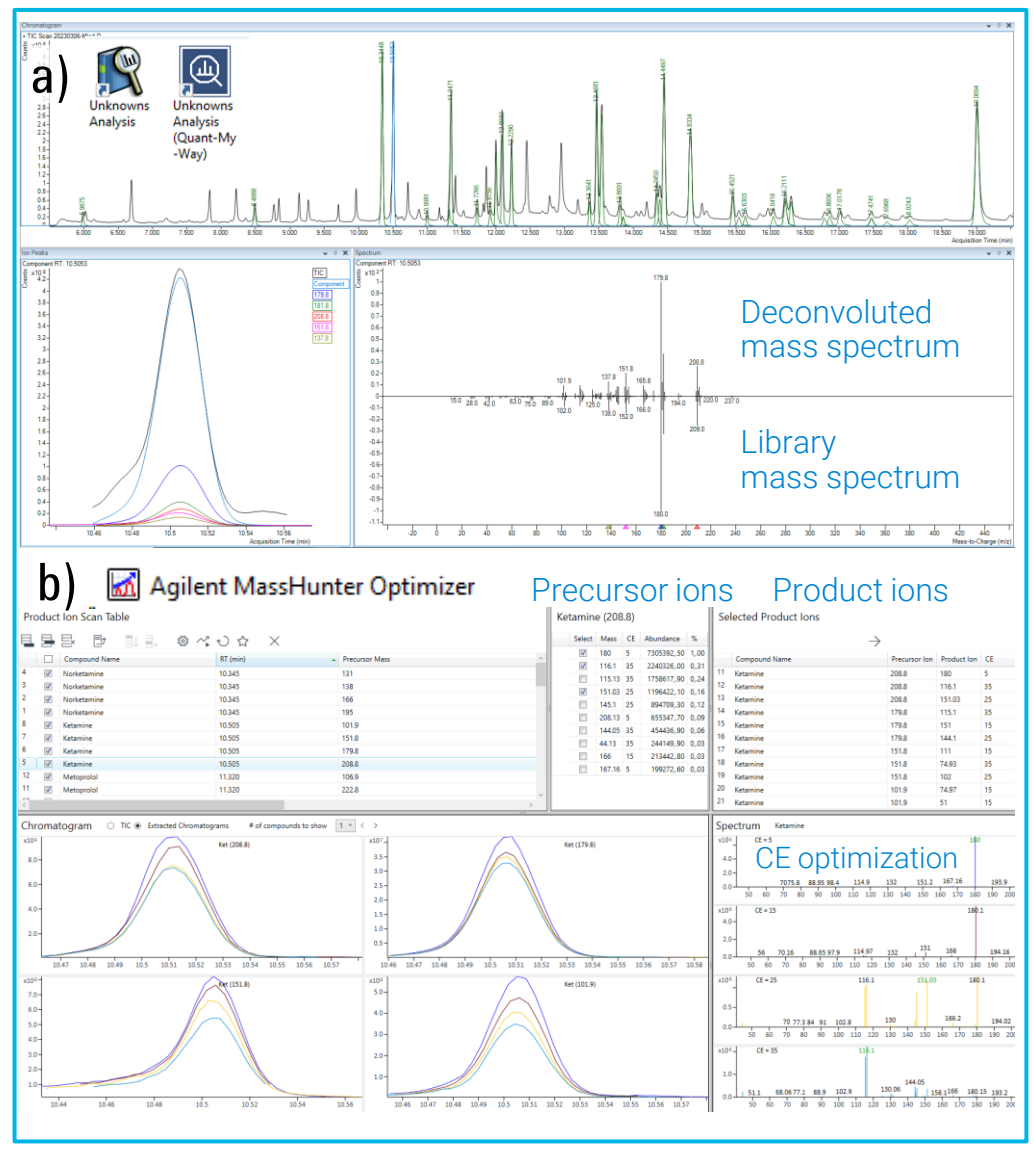


Figure 1. Example: MRM development for ketamine: (A) Compound identification using spectral deconvolution; (B) Optimizer for GC/TQ operating in *Start from Scan* workflow.

Forensic GC/TQ Database

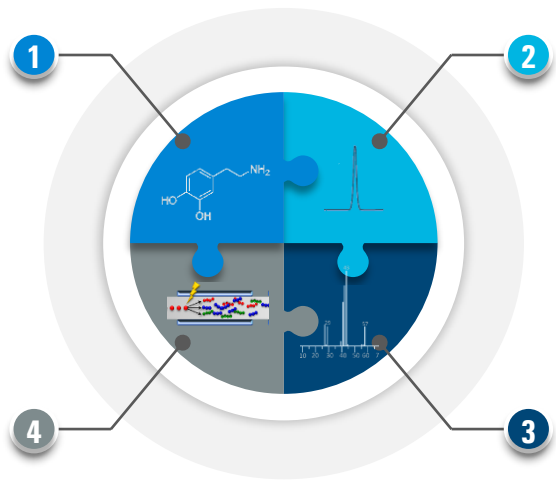
The database created in this work includes 175 entries in total that include 154 unique compounds, out of which 123 are underivatized entries, 32 are trimethylsilylated, and 20 acetylated entries (Fig. 2) The compounds included benzodiazepines, antidepressants, opioids, and drugs of abuse.

175 entries in total

- 154 unique compounds:
  - 123 underivatized entries
  - 32 trimethylsilylated entries
  - 20 acetylated entries

1,794 MRM transitions

- 3-12 transitions per compound
- optimized collision energies



Retention times for all entries

- retention time locked to cocaine
- allows a new column or instrument to have retention times that match the retention times provided in the database

Relative ion abundances

- for additional confirmation and optimal dwell time distribution

Figure 2. The overview of the entries included in the forensic GC/TQ database.

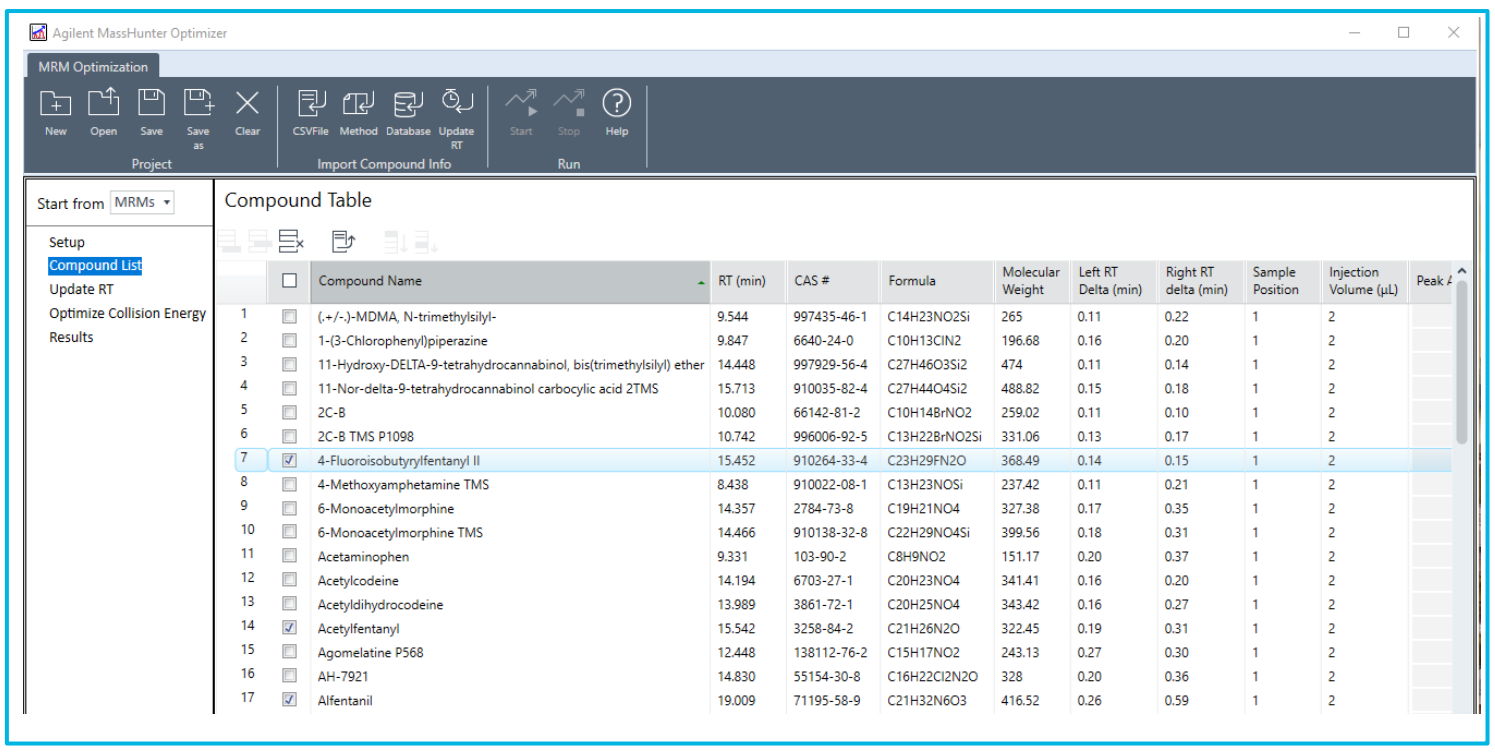
How to Use the Database

The database created in this work, can be used to simplify creation of dMRM data acquisition methods with the Agilent GC/TQ. The Agilent MassHunter Optimizer for GC/TQ can be used to simplify the method creation process as described below. The Optimizer is installed automatically with Agilent MassHunter GC/MS data acquisition 10 and above.

The following simple steps describe how to create a data acquisition method using the database.

1. Set Up: Create and save a GC/MS data acquisition method in MassHunter, using conditions from Table 1. Retention time lock to cocaine at 12.26 min or another compound included in the database.
2. Optimizer Setup: Specify the acquisition method in the Optimizer, retaining the GC parameters.2. Second, in the Optimizer, under Setup, specify the Acquisition method created in step 1, with the GC parameters that will be retained.
3. Import the Database: Under Setup, import the database as a CSV file.
4. Select Compounds: Choose target compounds by unchecking all and then selecting the ones you want.
5. Update Retention Times: If necessary, use Update RT function for adjusting retention times.
6. Review MRM Transitions: Check or uncheck the MRM transitions for the chosen compounds under the Results tab.
7. Save Method: Create and save the acquisition method.
8. Open Method: Open the saved method in MassHunter Acquisition software for review.

Fig. 3 shows the example of the compound table, in which the targets were sorted in alphabetical order and only the compounds from the fentanyls group were checked.



	Compound Name	RT (min)	CAS #	Formula	Molecular Weight	Left RT Delta (min)	Right RT delta (min)	Sample Position	Injection Volume (µL)
1	(.+/-.)-MDMA, N-trimethylsilyl-	9.544	997435-46-1	C14H23NO2Si	265	0.11	0.22	1	2
2	1-(3-Chlorophenyl)piperazine	9.847	6640-24-0	C10H13ClN2	196.68	0.16	0.20	1	2
3	11-Hydroxy-DELTA-9-tetrahydrocannabinol, bis(trimethylsilyl) ether	14.448	997929-56-4	C27H46O3Si2	474	0.11	0.14	1	2
4	11-Nor-delta-9-tetrahydrocannabinol carbocylic acid 2TMS	15.713	910035-82-4	C27H44O4Si2	488.82	0.15	0.18	1	2
5	2C-B	10.080	66142-81-2	C10H14BrNO2	259.02	0.11	0.10	1	2
6	2C-B TMS P1098	10.742	996006-92-5	C13H22BrNO2Si	331.06	0.13	0.17	1	2
7	4-Fluoroisobutyrylfentanyl II	15.452	910264-33-4	C23H29FN2O	368.49	0.14	0.15	1	2
8	4-Methoxyamphetamine TMS	8.438	910022-08-1	C13H23NOSi	237.42	0.11	0.21	1	2
9	6-Monoacetylmorphine	14.357	2784-73-8	C19H21NO4	327.38	0.17	0.35	1	2
10	6-Monoacetylmorphine TMS	14.466	910138-32-8	C22H29NO4Si	399.56	0.18	0.31	1	2
11	Acetaminophen	9.331	103-90-2	C8H9NO2	151.17	0.20	0.37	1	2
12	Acetylcodeine	14.194	6703-27-1	C20H23NO4	341.41	0.16	0.20	1	2
13	Acetyldihydrocodeine	13.989	3861-72-1	C20H25NO4	343.42	0.16	0.27	1	2
14	Acetylfentanyl	15.542	3258-84-2	C21H26N2O	322.45	0.19	0.31	1	2
15	Agomelatine P568	12.448	138112-76-2	C15H17NO2	243.13	0.27	0.30	1	2
16	AH-7921	14.830	55154-30-8	C16H22Cl2N2O	328	0.20	0.36	1	2
17	Alfentanil	19.009	71195-58-9	C21H32N6O3	416.52	0.26	0.59	1	2

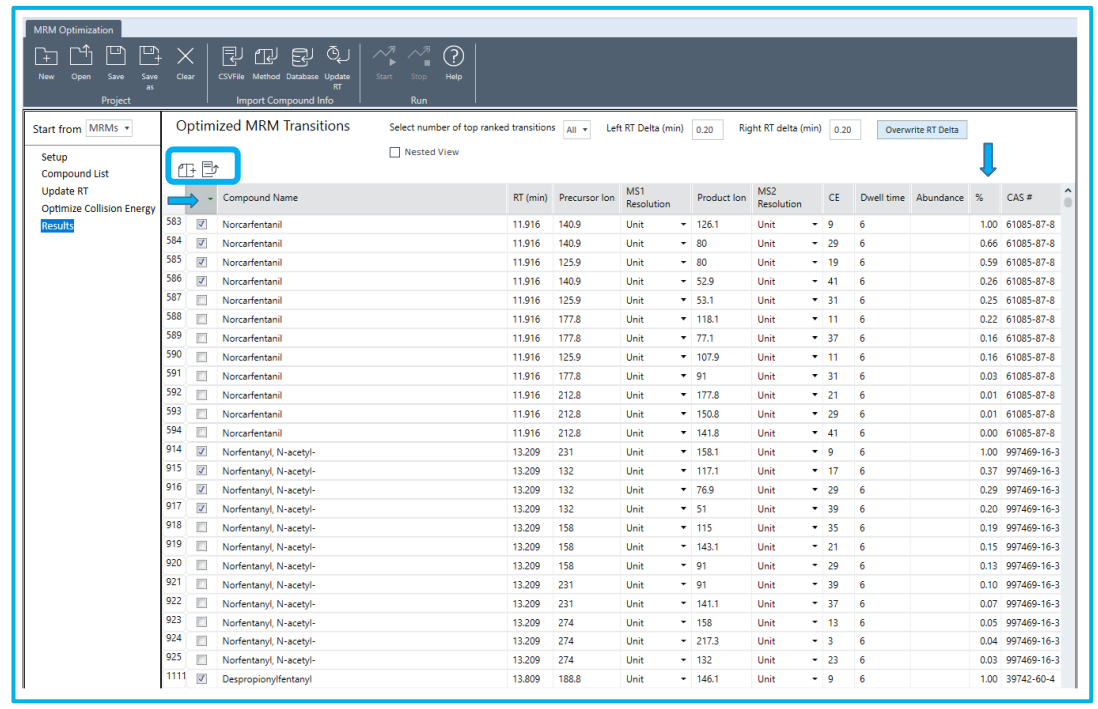
Figure 3. Compound Table in the Optimizer for GC/TQ demonstrating the first 17 entries (alphabetically) from the forensic GC/TQ database, with the selected targets from the fentanyls group checked.

Acquisition Method Creation Using the Database

Figure 4 shows the MRM transitions available for two selected compounds from the fentanyls group. The database includes the information on the compound name, retention time, precursor and product ions, collision energy, ion abundance in % of the most abundant MRM, and the CAS number.

The database includes up to 12 MRM transitions for some targets, hence, the user may prefer to uncheck some of the transitions for the selected targets to limit the number of MRMs per compound in the final method.

The MRM transitions selected in the Results table (Fig. 4) will be included into the final data acquisition method.



		Compound Name	RT (min)	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	CE	Dwell time	Abundance	%	CAS #
583	☑	Norcarfentanil	11.916	140.9	Unit	126.1	Unit	9	6		1.00	61085-87-8
584	☑	Norcarfentanil	11.916	140.9	Unit	80	Unit	29	6		0.66	61085-87-8
585	☑	Norcarfentanil	11.916	125.9	Unit	80	Unit	19	6		0.59	61085-87-8
586	☑	Norcarfentanil	11.916	140.9	Unit	52.9	Unit	41	6		0.26	61085-87-8
587	☐	Norcarfentanil	11.916	125.9	Unit	53.1	Unit	31	6		0.25	61085-87-8
588	☐	Norcarfentanil	11.916	177.8	Unit	118.1	Unit	11	6		0.22	61085-87-8
589	☐	Norcarfentanil	11.916	177.8	Unit	77.1	Unit	37	6		0.16	61085-87-8
590	☐	Norcarfentanil	11.916	125.9	Unit	107.9	Unit	11	6		0.16	61085-87-8
591	☐	Norcarfentanil	11.916	177.8	Unit	91	Unit	31	6		0.03	61085-87-8
592	☐	Norcarfentanil	11.916	212.8	Unit	177.8	Unit	21	6		0.01	61085-87-8
593	☐	Norcarfentanil	11.916	212.8	Unit	150.8	Unit	29	6		0.01	61085-87-8
594	☐	Norcarfentanil	11.916	212.8	Unit	141.8	Unit	41	6		0.00	61085-87-8
914	☑	Norfentanyl, N-acetyl-	13.209	231	Unit	158.1	Unit	9	6		1.00	997469-16-3
915	☑	Norfentanyl, N-acetyl-	13.209	132	Unit	117.1	Unit	17	6		0.37	997469-16-3
916	☑	Norfentanyl, N-acetyl-	13.209	132	Unit	76.9	Unit	29	6		0.29	997469-16-3
917	☑	Norfentanyl, N-acetyl-	13.209	132	Unit	51	Unit	39	6		0.20	997469-16-3
918	☐	Norfentanyl, N-acetyl-	13.209	158	Unit	115	Unit	35	6		0.19	997469-16-3
919	☐	Norfentanyl, N-acetyl-	13.209	158	Unit	143.1	Unit	21	6		0.15	997469-16-3
920	☐	Norfentanyl, N-acetyl-	13.209	158	Unit	91	Unit	29	6		0.13	997469-16-3
921	☐	Norfentanyl, N-acetyl-	13.209	231	Unit	91	Unit	39	6		0.10	997469-16-3
922	☐	Norfentanyl, N-acetyl-	13.209	231	Unit	141.1	Unit	37	6		0.07	997469-16-3
923	☐	Norfentanyl, N-acetyl-	13.209	274	Unit	158	Unit	13	6		0.05	997469-16-3
924	☐	Norfentanyl, N-acetyl-	13.209	274	Unit	217.3	Unit	3	6		0.04	997469-16-3
925	☐	Norfentanyl, N-acetyl-	13.209	274	Unit	132	Unit	23	6		0.03	997469-16-3
1111	☑	Despropionylfentanyl	13.809	188.8	Unit	146.1	Unit	9	6		1.00	39742-60-4

Figure 4. The Results table showing the MRM transitions.

Application of the Database to the Real-World Samples

The proof of concept using the developed database involved the analysis of 25 archived post-mortem blood samples. A comparison was made between full scan data acquisition mode and MRM, with a focus on the identification of compounds. The MRM method was created from the database.

All the compounds were found with the MRM approach, while some of the toxicants present in the sample at a low concentration were missed with the full scan approach.

Fig. 5 shows that fentanyl was detected in the sample with the MRM approach and quantitated at 1.7 ng/mL, while it was not detected in full scan acquisition mode.

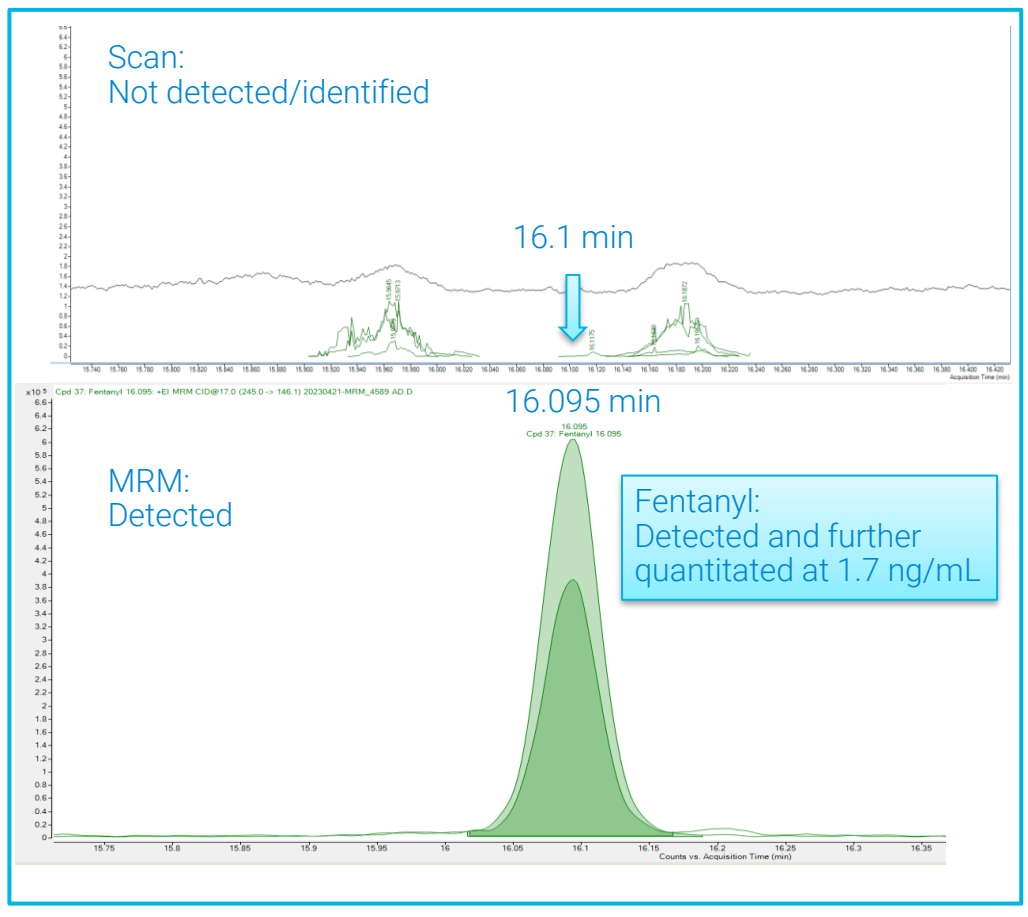


Figure 5. Fentanyl in the archived post-mortem blood sample detected in the MRM GC/TQ data acquisition mode (bottom) and not detected with the spectral deconvolution approach in the full scan data (top).

https://www.agilent.com/en/promotions/asms

Conclusions

- The forensic toxicology database with curated set of 1,794 MRM transitions for 175 toxicologically-relevant compounds, including benzodiazepines, antidepressants, opioids, and drugs of abuse, was successfully developed.
- The application of this MRM method to authentic samples showcased its ability to detect and quantitate toxicants at trace levels due to high sensitivity and selectivity of the MS/MS approach addressing limitations when relying solely on full scan data.
- The developed MRM database can be used for simplified data acquisition method creation, providing a valuable resource for the development of screening and quantitation methods in forensic labs.

References

[1] Lokits, K., Ciotti, R., Diaz, H. QuickProbe Dual Configurations for Forensic Workflows: Providing flexibility and robustness on a single GC/MS system. Agilent Technologies Application Note 5994-6889EN, 2023.

[2] Lokits, K., Willey, A. Evaluation of Hydrogen Carrier Gas and the Agilent HydroInert Source for Forensic Street Drug Analysis. Agilent Technologies Application Note 5994-6982EN, 2023.

[3] Maurer, H.H., Pfleger, K., Weber, A.A. Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites, 2007 (3rd Edition).

University of Antwerp
Toxicological Centre

Agilent
Trusted Answers