Rapid and Accurate Forensics Analysis using High Resolution All Ions MS/MS

Application Note

Forensic Toxicology

Authors

Martin Josefsson, and Markus Roman
National Board of Forensic Medicine
Linköping, Sweden

Bernhard Wüst, Stephen Madden, Tiffany Payne, and Lester Taylor
Agilent Technologies, Inc.
Santa Clara, California

Abstract

This application note describes the use of the All Ions MS/MS technique to rapidly screen and identify drugs of forensic toxicological interest.

The analytical method based on a published paper [1] uses a high resolution TOF or Q-TOF mass spectrometer to quickly and easily analyze samples, determine whether target compounds are present, and at what concentration. Blood samples fortified with a 30 compound test mixture at a concentration of 0.1 µg/g in replicates (n=3) were analyzed using an LC-TOF instrument with All Ions MS/MS software processing tools to validate the effectiveness of the technique for rapid generation of accurate results, despite the presence of similar and isomeric compounds at different concentrations. In addition, two post-mortem blood samples were analyzed to test the capability of All Ions MS/MS for the identification of isobars and isomers. By combining MS and MS/MS experiments and using All Ions MS/MS, users experience increased analytical speed and accuracy, significantly improved identification of compounds of interest, and increased productivity in forensic toxicology laboratories. Laboratories that analyze panels of drugs can import All Ions MS/MS results directly into their MassHunter Quantitative Analysis software for more productive compound identification and quantification.

Agilent Technologies

Introduction

The demand for greater analytical productivity combined with legally defensible results for the screening of forensic samples have placed substantial demands on traditional analytical techniques. Scientists analyzing target drugs in forensic toxicology investigations require high quality results quickly. The analytical results must also withstand legal scrutiny. Given this, forensic laboratories must have high confidence in their results.

The Agilent All Ions MS/MS software for high resolution accurate mass data enables screening and identifi cation of compounds in a single analytical run. The technique uses both accurate mass TOF or Q-TOF LC/MS instruments and features easy setup of analytical methods, verifi cation of the compounds using MS/MS spectral libraries and chromatographic coelution of the precursor and product ions. It is also possible to include product ions as qualifi ers. Identifi cation of specifi c isomers is achieved by using retention time information and assessing the quality of product ion chromatograms.

Experimental

Authentic post-mortem blood samples were prepared using liquid/liquid extraction with acetonitrile/ethanol 90/10 (250 mg blood + 500 µL extraction solvent). An 2 µL aliquot of this extract was injected.

In order to compare results from MS and All Ions MS/MS experiments, a 30-compound test standard and a blood sample spiked at 0.1 µg/g were used.

HPLC conditions		
UHPLC column	Acquity UPLC, HSS T3, 2.1 mm × 150 mm, 1.8 µm	
Mobile phase	A: 10 mM ammonium formate + 0.05% formic acid in water B: 0.05% formic acid in acetonitrile	
Gradient program	Min	% B
	0	1%
	0.6	1%
	0.7	5%
	8.0	50%
	10.0	95%
	11.0	95%
	11.1	1%
Stop time	12.0	
Flow rate	0.5 mL/min	

Ion source parameters	
Gas temperature	300 °C
Gas flow	6 L/min
Nebulizer	35 psi
Sheath gas temperature	375 °C
Sheath gas flow	10 L/min
MS parameters (MS-mode)	
Scan range	50–1,000 amu
Scan rate	1.5 spectra/s
All Ions MS/MS mode	
Three scan segments at 0 eV, 20 eV, and 40 eV	
Scan range	50–1,000 amu
Scan rate	3 spectra/s
Internal reference	
Automatic internal reference mass correction was applied using purine (*m/z* 121.0509) and HP 921 (*m/z* 922.0098).	

Results and Discussion

The data were analyzed using the Find by Formula (FbF) algorithm in MassHunter Qualitative Analysis rev. B.06.00. FbF evaluates a chemical formula and determines if a compound with that formula is present in the high resolution mass spec data. This approach uses a modified version of the FbF algorithm which supports the All Ions MS/MS technique. Mass peaks in the low energy channel are first searched against the Forensic Toxicology Personal Compound Database and Library (PCDL) rev. B.04.01 (aka Broecker, Herre & Pragst MS/MS library, version 4.1) for compounds which have the same *m/z* values. Figure 1 shows that FbF was able to identify zopiclone in the sample and automatically extract corresponding chromatograms for the precursor and one of the fragments. The compound spectrum for zopiclone was also extracted and showed excellent isotope matching. The isotopic cluster was displayed with a red overlay, indicating the theoretical abundance and spacing.

Once the search is complete, a set of putative identifications is automatically compiled. For this compiled list of compounds, the fragment ions in the MS/MS spectra from the PCDL are compared to the ions detected in the high energy channel in order to confirm the presence of the correct fragments. Figure 2 shows an extracted spectrum of a high energy scan of zopiclone with its fragments noted.

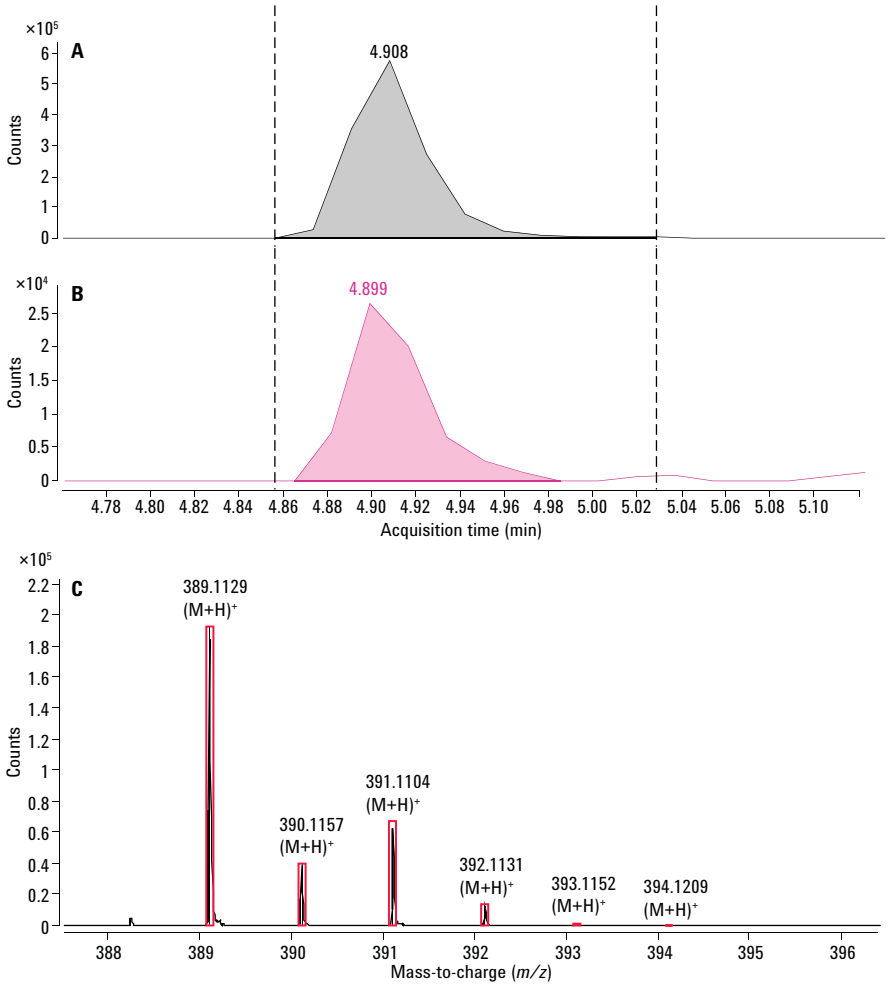

Figure 1. FbF identification of zopiclone. A) Extracted Ion Chromatogram (EIC) of zopiclone molecular ion, B) EIC of one of the fragments of zopiclone, and C) extracted spectrum of zopiclone with isotope matching information.

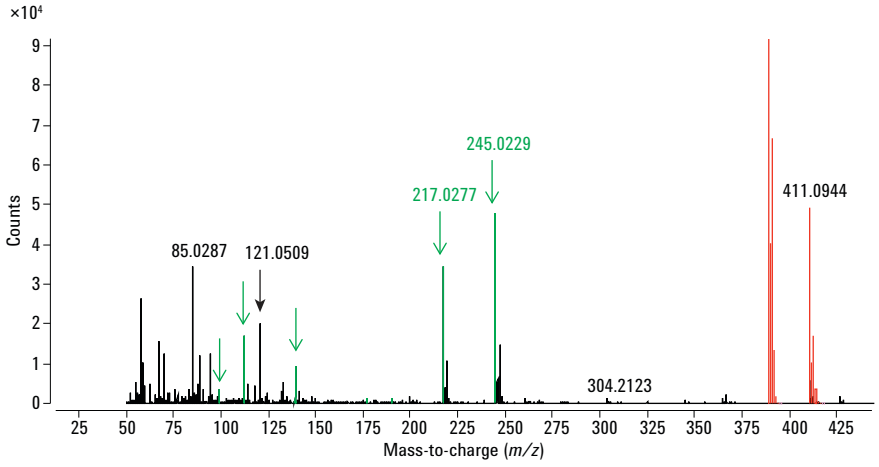

Figure 2. High energy spectrum of zopiclone.

Both the precursors and product ions are extracted as ion chromatograms (Figure 3A) and evaluated using a coelution score. The coelution score is derived from a technique which is similar to the Peak Purity method used in UV chromatography[2]. The software calculates a number that accounts for factors including abundance, peak shape (symmetry), peak width, and retention time. The scores can be plotted and made available to the user for inspection in a Coelution Plot (Figure 3B).

Data analysis of the sample also identified alprazolam and methadone from the sample. It was immediately apparent, from the EIC, that alprazolam and methadone coeluted (Figure 4). The responses also indicated that the concentration of methadone was much greater than alprazolam. This demonstrated that the instrument provided excellent sensitivity to detect these compounds.

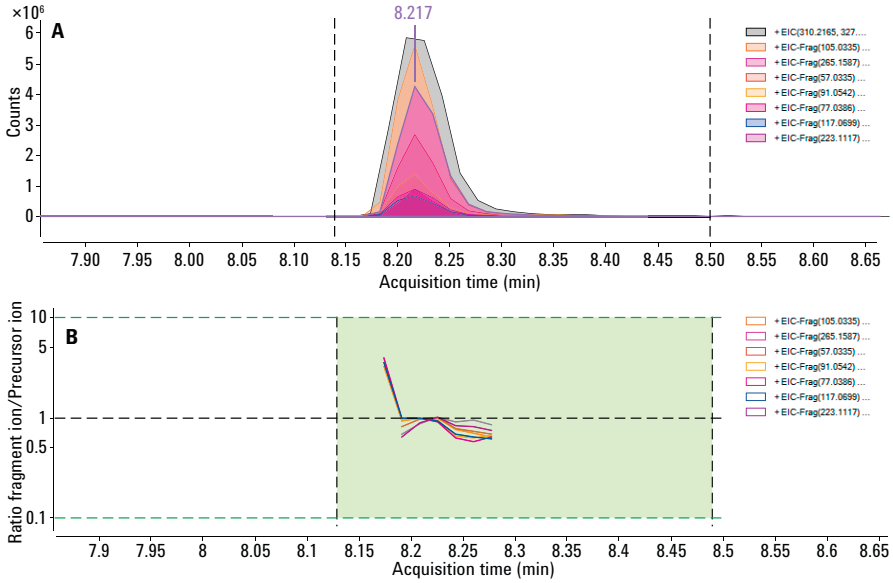

Figure 3. Overlaid ion chromatograms (A), calculated coelution plot (B).

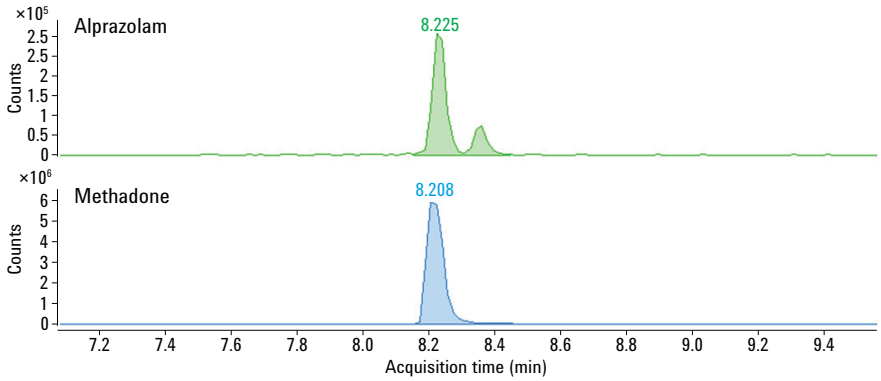

Figure 4. Extracted ion chromatograms of alprazolam and methadone.

Since methadone and alprazolam have similar precursor ion values (*m/z* 309.088 and 310.217), it may appear difficult to distinctly identify either compound (Figure 5). Using another high resolution accurate mass technique, for example, targeted MS/MS or data-dependent MS/MS would have produced a mixed MS/MS spectrum that was difficult to interpret. However, an examination of the coelution scores and plots automatically generated by the All Ions MS/MS software clearly identified the compounds in the sample using both fragment ions from the PCDL and information for their isotopic clusters. Figure 6A shows that the software was able to match seven fragment ions from methadone with coelution scores better than 90. The extracted compound spectrum also showed perfect isotope match for the molecular ion (Figure 6B). Alprazolam was also easily identified based on > 90 coelution scores for two of its fragments and excellent match of the *m/z* value plus the isotopic pattern (Figure 7).

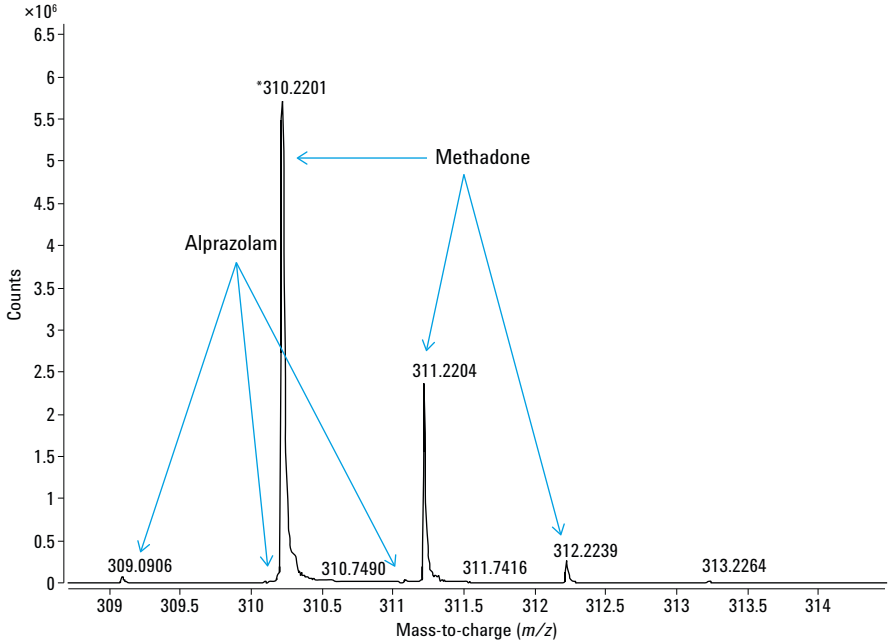

Figure 5. Mass spectrum of alprazolam and methadone.

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff
	methadone	C21 H27 N O	310.2168	309.2096	309.2093	-1.11	99.56	8.208	8.317	-0.109

Coelution Score	CE	Flags(Fls)	FV	Height	m/z	Compound Name	RT	RT Diff	SNR
98.7	40	Qualified		5622078.5	105.0335	methadone	8.217	0.009	2457.9
98.6	20	Qualified		4247226.5	265.1587	methadone	8.217	0.009	4782.3
98.7	20	Qualified		2687710.2	57.0335	methadone	8.217	0.009	593.9
97.3	40	Qualified		1396836.4	91.0542	methadone	8.217	0.009	366.5
96.9	40	Qualified		894049.9	77.0386	methadone	8.217	0.009	179
97.8	40	Qualified		691156.1	117.0699	methadone	8.217	0.009	382.5
98.2	20	Qualified		887147.3	223.1117	methadone	8.217	0.009	1589

Figure 6A. Methadone with seven qualified fragments with coelution scores > 90.

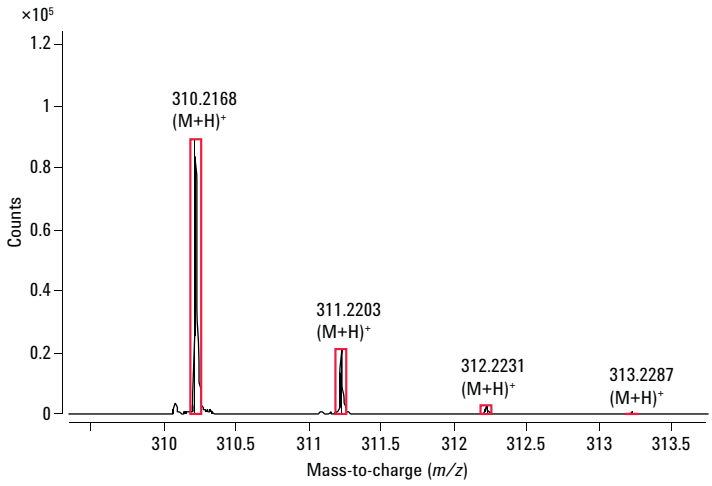

Figure 6B. Isotopic matching of methadone where red box shows theoretical isotope abundance and position.

Each fragment was flagged as either Qualified or annotated with the reason it was not qualified (Figure 7A). The isotopic cluster was displayed with a red overlay indicating the theoretical abundance and spacing. There was excellent agreement for both compounds despite the large difference in their concentrations.

During data analysis, a compound with molecular formula $C_{15}H_{11}ClN_2O_2$ was also identified in the sample. It could be matched to one of the isomers, namely norclobazam and oxazepam. Manual review of the fragment and coelution results gave the correct identification for oxazepam (Figure 8) as five of the fragments matched well to oxazepam while the only two fragments that could match to norclobazam showed very poor coelution scores.

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)
◉	alprazolam	C17 H13 Cl N4	309.0895 331.0723	308.082	308.0829	2.98	97.55	8.225	8.295

Coelution Score	CE	Flags(FIs)	FV	Height	m/z	Compound Name	RT	RT Diff	SNR
93.2	40	Qualified		17295.1	205.0761	alprazolam	8.234	0.009	36.8
93.2	40	Qualified		25488	281.0714	alprazolam	8.234	0.009	112.3
89.4		Low coelution score		5595.5	274.1213	alprazolam	8.234	0.009	15.6
		No peaks for eic			165.0214	alprazolam			
		No peaks for eic			240.0449	alprazolam			
		No peaks for eic			219.0917	alprazolam			
		No peaks for eic			241.0527	alprazolam			

Figure 7A. Alprazolam with two qualified fragments with coelution scores > 90.

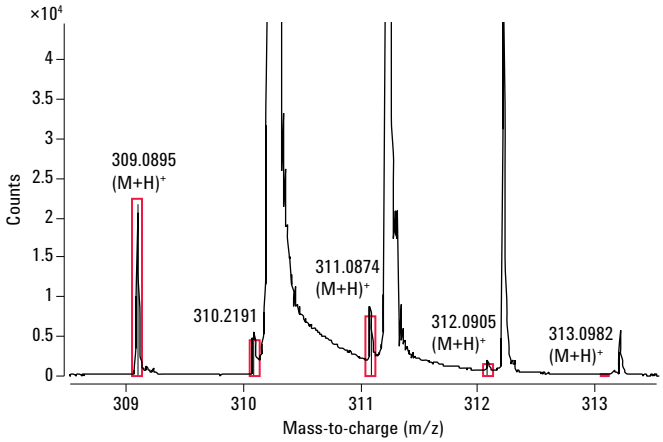

Figure 7B. Isotopic matching of alprazolam.

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT
○	Norclobazam	C15 H11 Cl N2 O2	287.0582 309.0403	286.0509	286.0509	0.07	99.34	7.763
◉	Oxazepam	C15 H11 Cl N2 O2	287.0582 309.0403	286.0509	286.0509	0.07	99.34	7.763

Coelution Score	CE	Flags(FIs)	FV	Height	m/z	Compound Name	RT	RT Diff	SNR
97.7	20	Qualified		30221.8	241.0527	Oxazepam	7.772	0.009	35.2
96.9	40	Qualified		17922.1	104.0495	Oxazepam	7.772	0.009	14.6
96.2	40	Qualified		4666.8	163.0053	Oxazepam	7.772	0.009	7
96.1	20	Qualified		12840.2	269.0476	Oxazepam	7.772	0.009	31.9
94.3	20	Qualified		4794.1	231.0684	Oxazepam	7.772	0.009	7.4
9.3		Low coelutio...		1410.9	210.0788	Norclobazam	7.892	0.129	4.2
6.2		Low coelutio...		3708.5	245.0476	Norclobazam	7.892	0.129	10.8

Figure 8. Fragment and coelution results indicate the correct identification is oxazepam.

Results of the analysis can be reviewed within two modules of the Qualitative Analysis software: Navigator View, which allows the viewing of multiple data files chosen from a Data Navigator window, and Compound Details View (Figure 9), which shows identification results, coelution plots, MS, and fragment spectra for one compound at a time. Users are able to manually review and change compound selections in the Compound Details View by browsing through them in the Compound List.

Conclusions

Drugs of interest in forensic toxicology investigations have been used to demonstrate the analytical utility of the All Ions MS/MS software capability in MassHunter. We rapidly generated a data processing method for high resolution accurate mass data from a Q-TOF mass spectrometer. The All Ions MS/MS method was used to screen for the presence of target compounds. These compounds were identified first using the accurate mass and isotopic pattern of the precursor ion, and then confirmed using the associated fragment ions.

The All Ions MS/MS approach enables easier method setup, more sophisticated data processing, and results in more confidence in target compound identifications. Overall this leads to a more productive analysis. Moreover, the data can be reanalyzed to look for additional compounds without the need to reinject samples. The Compound Details View summarizes important compound information in a single window which makes data review much easier. With these tools, large batches of sample results can be processed and reviewed quickly for even greater productivity.

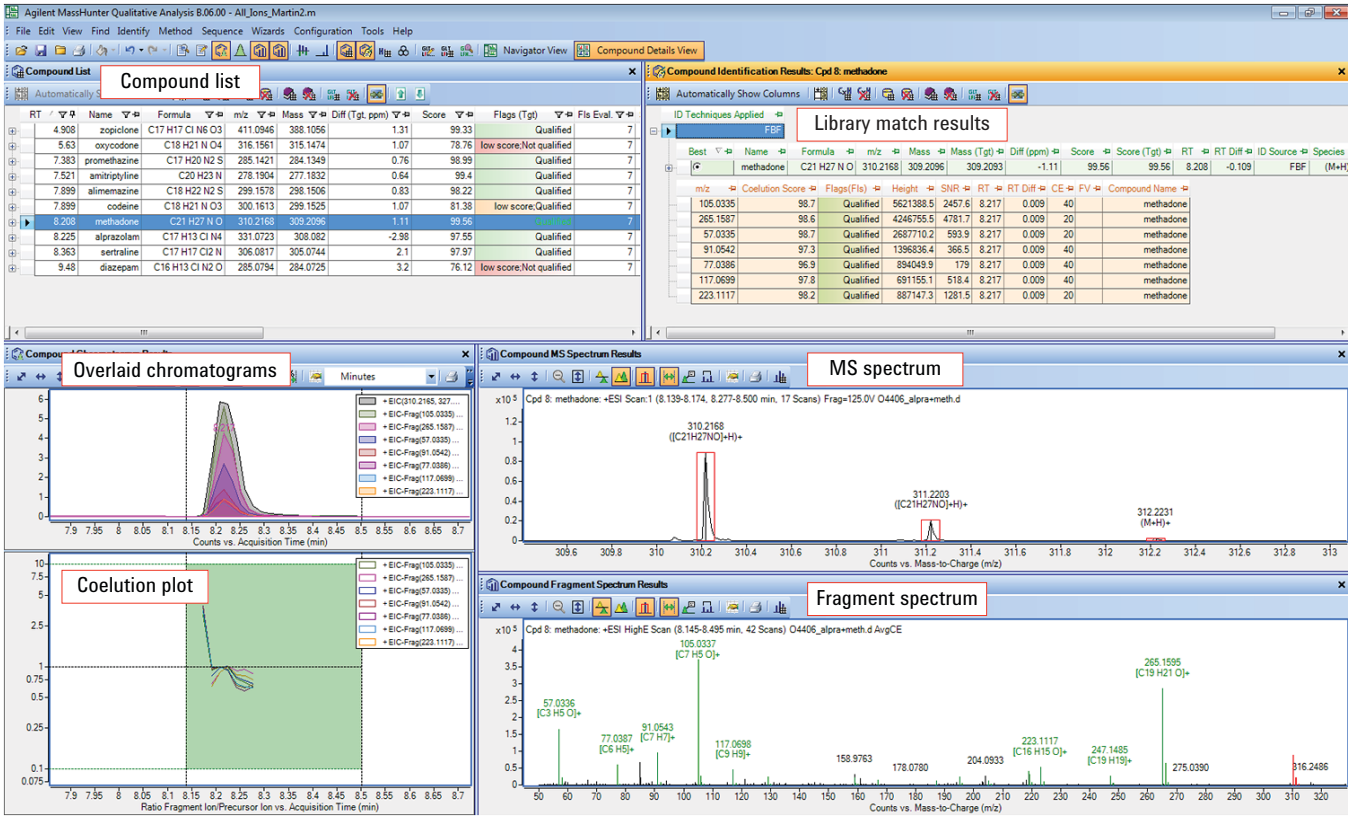

Figure 9. Compound Details View.

References

1. Roman, Markus *et al*. Liquid chromatography/time-of-flight mass spectrometry analysis of postmortem blood samples for targeted toxicological screening. Anal Bioanal Chem 2013, DOI 10.1007/s00216-013-6798-0.

2. Sievert, Hans-Jürgen P.; Drouen, Anton C.J.H.;. Spectral matching and peak purity in Diode Array Detection in High Performance Liquid Chromatography. 51-125, New York: Marcel Dekker. 1993.

www.agilent.com/chem/QQQ

For Forensic Use.

This information is subject to change without notice.

Published in the USA, May 6, 2013
5991-2319EN

Agilent Technologies