Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) in Environmental Samples using Advanced TripleTOF® MS/MS Technology

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Overview

Pharmaceuticals and Personal Care Products (PPCP) are environmental pollutants of growing concern. In order to properly assess the effects of such compounds on our environment, it is necessary to accurately monitor their presence.[1-3]

Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is a preferred tool for the analysis of a wide range of environmental pollutants. Here, a method is presented using the AB SCIEX TripleTOF® 4600 LC/MS/MS system to identify and quantify selected PPCP in water samples. Different scan functions are applied and compared with respect to selectivity, sensitivity, accuracy, reproducibility, and confidence of identification. Data was processed in MultiQuant™ and PeakView® software using a new mass spectral library containing high resolution and accurate mass MS/MS spectra acquired on a TripleTOF® system

Introduction

LC-MS/MS is a powerful analytical tool for the analysis of a wide molecular weight range of polar, semi-volatile and thermally labile compounds, such as PPCP, pesticides, drugs of abuse, perfluoroalkyl substances (PFAS), brominated flame retardants (BFR), etc.

Triple quadrupole-based mass analyzers are popular for targeted quantitation of environmental pollutants because of their high selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode.[3]

Advancements in LC-MS/MS technology, including hybrid systems like quadrupole-quadrupole Time-of-Flight (TripleTOF®), now provide the ability to perform qualitative and quantitative screening on a routine basis. TripleTOF® technology combines the best attributes of triple quadrupole and accurate mass TOF analyzers in a single instrument allowing quantitation with triple quadrupole-like performance (sensitivity, accuracy, linearity) and at the same time high confidence identification based on accurate mass MS and MS/MS information.

The AB SCIEX TripleTOF® 4600 system combines:

- Industry-proven design of the Turbo V™ source and Curtain Gas™ interface delivering unmatched robustness and ruggedness when analyzing dirty samples while minimizing carry-over and contamination.
- A high performance triple quadrupole front-end containing the QJet® ion guide and a high pressure Q0 for best ion focusing and transmission.
- A LINAC® collision cell to accelerate product ions after collision to increase MS/MS speed and data quality.
- The innovative Accelerator TOF™ analyzer, a state of the art accurate mass analyzer, combining speed with high mass accuracy (2 ppm or less) and high resolution (30,000) of an accurate mass instrument, even at low mass for better analysis of small molecules.
- A Calibrant Delivery System for fully automatic calibration of the mass analyzer in MS and MS/MS mode.
- Powerful and intuitive data processing software for quantitative and qualitative analysis.
- A new mass spectral library containing high resolution and accurate mass MS/MS spectra acquired on a TripleTOF® system.

Method Details

UHPLC Separation

A Shimadzu NEXERA UHPLC system was used with a Phenomenex Kinetex 2.6u C18 100x3mm column at 30ºC. Separation was achieved with a gradient of water and methanol with 0.1% formic acid at a flow rate of 600 µL/min. The injection volume was set to 10 µL but larger injection volumes are possible to further decrease detection limits.

MS/MS Detection

The AB SCIEX TripleTOF® 4600 LC/MS/MS system was operated with the DuoSpray™ ion source operated in electrospray ionization (ESI). The APCI probe of the source was used for fully automatic mass calibration using the Calibrant Delivery System. CDS injects a calibration solution matching polarity of ionization and calibrates the mass axis of the TripleTOF® system in all scan functions used (MS or MS/MS).

The TripleTOF® system was operated in TOF-MS, MRM^{HR}, and TOF-MS-IDA-MS/MS mode. These different scan functions result in highly selective and sensitive information allowing quantitation and confident identification of targeted analytes. The combination of TOF-MS and MS/MS with Information Dependent Acquisition (IDA) in a single method, additionally, allows the identification of non-targeted and unexpected compounds through powerful software tools.[4, 5]

The data were processed in MultiQuant™ software version 2.1 and PeakView® software version 1.2 with the XIC Manager.

Results and Discussion

Multiple Reaction Monitoring (MRM) is the most common mode of analysis using a triple quadrupole MS/MS for quantitative analysis. The first quadrupole filters a specific precursor ion of interest. The collision cell is optimized to produce a characteristic product ion by collision induced dissociation (CID). Generated product ions are transferred into the third quadrupole where only a specific m/z is allowed to pass (Figure 1). Thus MRM mode works like a double mass filter which drastically reduces noise and increases selectivity.

The TripleTOF® system can be operated in TOF-MS and TOF-MS/MS mode. In TOF-MS mode, the precursor ion is transmitted directly into the TOF analyzer and measured accurately using its flight time. In TOF-MS/MS product ions of a selected precursor are generated through CID in the collision cell and the m/z of these fragments is measured accurately (Figure 1). Furthermore, IDA allows combining both scan functions in a single method to acquire MS and MS/MS simultaneously.

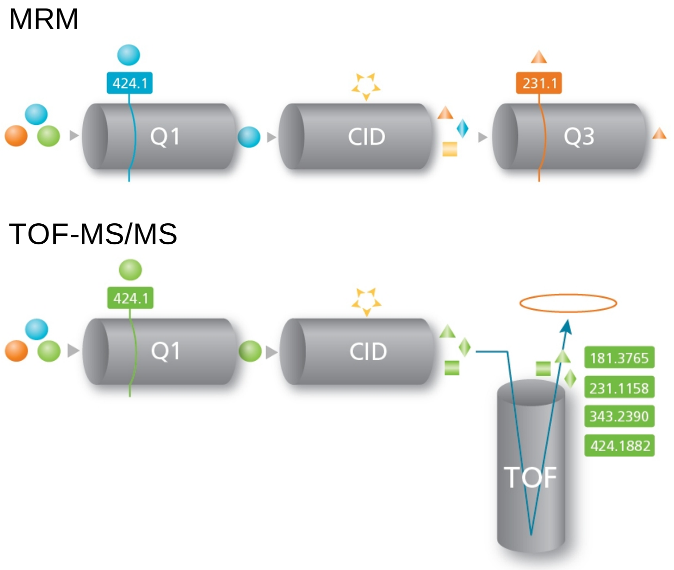


Figure 1. Operation of a triple quadrupole system in Multiple Reaction Monitoring (MRM) and a TripleTOF® system in TOF-MS/MS mode

Data files contain high resolution and accurate mass information of precursor ions and product ions of compounds of interest. The data files can be explored following two strategies, (1) targeted (quantitation and identification) and (2) non-targeted (unknown identification):

1. Narrow Extracted Ion Chromatograms (XIC), typically 5 to 20 mDa wide, can be extracted from MS and MS/MS data to quantify analytes with high selectivity. The accurate mass MS and MS/MS information saved into the spectra allows confident identification of targeted compounds at the same time. XIC generated from high resolution MS/MS (MRM^{HR} workflow using an AB SCIEX TripleTOF® system) provide increased selectivity over XIC from high resolution MS and the MRM mode of a triple quadrupole system (Figure 2).
2. The accurate mass of the molecular ion, its isotopic pattern, and adduct ion information can be used to empirically calculate potential molecular formulas of the detected compound. The accurate mass product ion information can be searched against MS/MS libraries for identification or used to investigate the fragmentation pathway to tentatively identify the structure of the unknown compound.

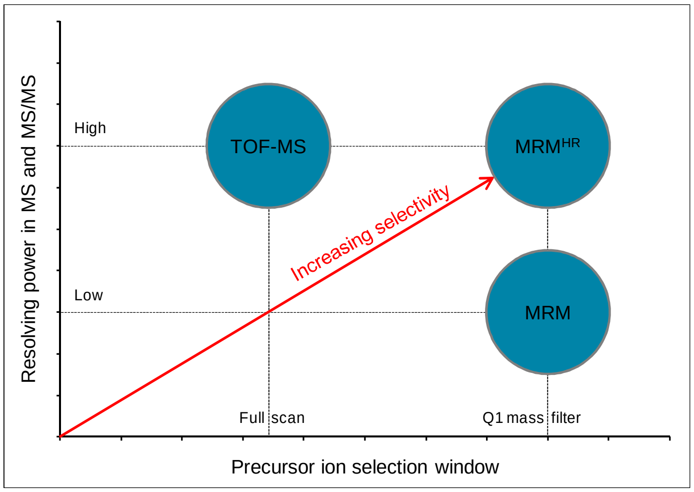


Figure 2. Increasing selectivity for quantitation through MS resolving power or precursor ion selection, highest selectivity is achieved using the MRM^{HR} workflow by combining precursor ion selection at unit resolution with high resolution MS/MS detection

In the following paragraphs, examples of PPCP analysis using different TripleTOF® scan functions and data processing tools are presented.

Quantitation of PPCP using TOF-MS

The high resolution and accurate mass data saved in TOF-MS chromatograms enables the user to use narrow XIC windows for increased selectivity resulting in better Signal-to-Noise (S/N) and lower Limits of Quantitation (LOQ). The chromatograms in Figure 3 show XIC of Carbamazepine spiked into a water sample at a concentration of 100 ng/L. An XIC window of 10 ppm (± 2.37 mDa) was found most effective to reduce background and remove interferences.

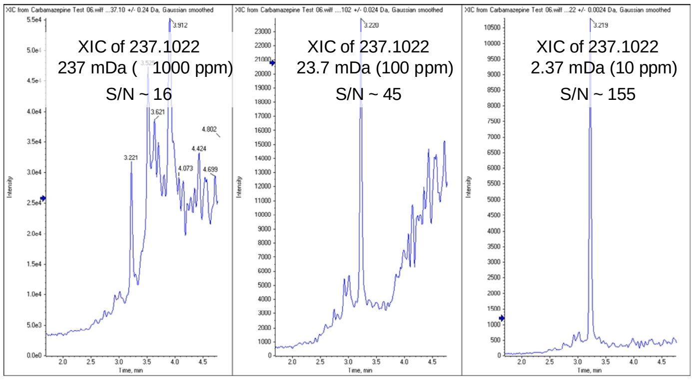


Figure 3. Increasing selectivity and S/N using narrow extracted ion chromatograms (XIC) to measure 100 ng/L Carbamazepine in water

Figure 4 shows typical method performance criteria when using TOF-MS to quantify carbamazepine in water samples. An LOQ of 10 ng/L was achieved using an injection volume of 10 µL. Repeatability was found to be better than 10% with accuracies well between 85 and 115% over the linear range studied (Table 1). The regression coefficient was 0.999 using linear regression with 1/x weighting.

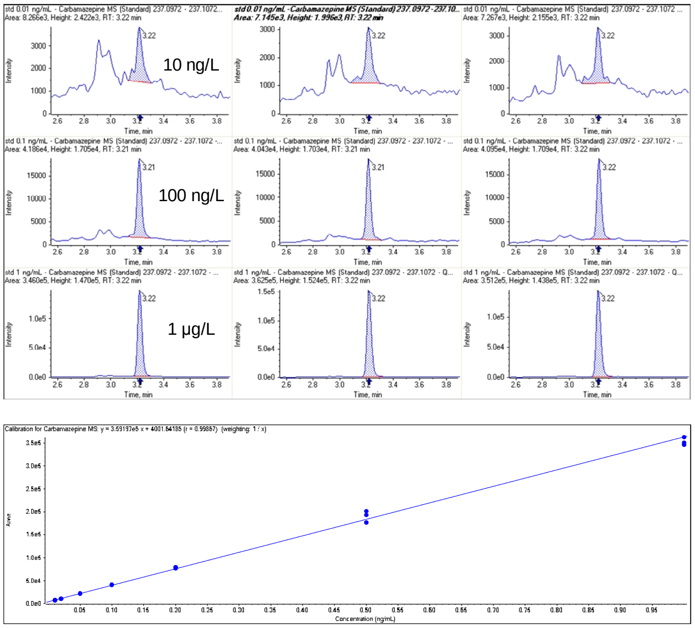


Figure 4. TOF-MS chromatograms of repeat injections of carbamazepine spiked into water at different trace level concentrations and the resulting calibration line (linear with 1/x weighting: r = 0.999 with accuracies between 85 and 115%, and repeatability less than 10%)

Table 1. Comparison of accuracy and repeatability of Carbamazepine at different concentrations detected in TOF-MS and MRM^{HR} mode

	TOF-MS		MRM^{HR}	
Concentration	**Accuracy (%)**	**%CV**	**Accuracy (%)**	**%CV**
10 ng/L	99.03	17.29	97.9	8.3
20 ng/L	94.63	7.60	94.2	2.1
50 ng/L	98.32	1.55	110.5	9.6
100 ng/L	103.22	1.95	109.5	5.9
200 ng/L	103.79	2.22	107.4	4.4
500 ng/L	103.77	6.77	103.0	5.0
1 µg/L	97.23	2.42	95.9	2.7

Quantitation of PPCP using MRM^{HR}

The combination of precursor ion selection with high resolution MS/MS detection results in increased selectivity for quantitation. In the case of Carbamazepine, background interferences were successfully removed and the S/N increased from 6.4 in TOF-MS to 89 in MRM^{HR} mode (Figure 5). The S/N was measured using a Peak-to-Peak algorithm.

Quantitative performance criteria in MRM^{HR} are comparable to TOF-MS. Repeatability was found to be better than 10% with accuracies well between 85 and 115% over the linear range studied. The regression coefficient was 0.998 using linear regression with 1/x weighting.

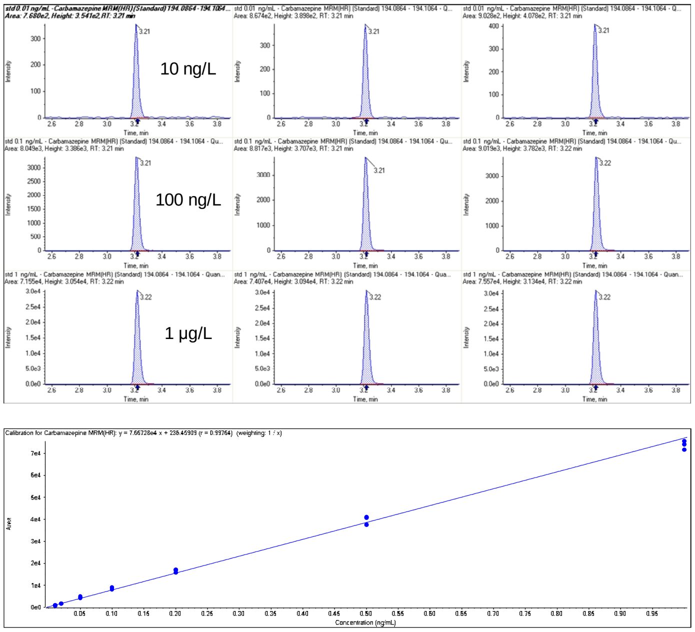
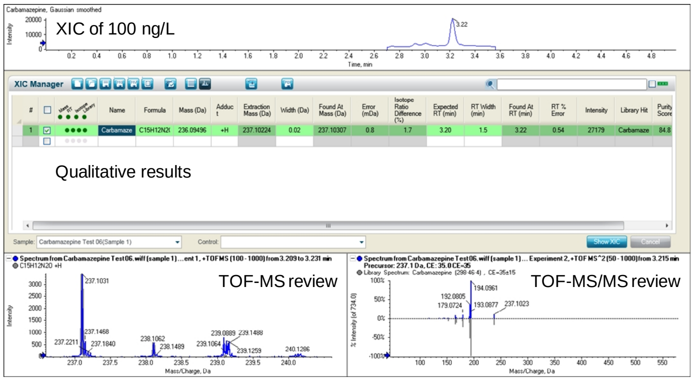


Figure 5: MRM^{HR} chromatograms of repeat injections of carbamazepine spiked into water at different trace level concentrations and the resulting calibration line (linear with 1/x weighting: r = 0.998 with accuracies between 85 and 115%, and repeatability less than 10%)

Quantitation and Identification of PPCP using TOF-MS-IDA-MS/MS

The data acquisition speed of the AB SCIEX TripleTOF® 4600 system (up to 50 Hz) allows combining the acquisition of TOF-MS and TOF-MS/MS. In addition, the build-in IDA logic including dynamic background subtraction makes it possible to collect all relevant MS and MS/MS information in a single analysis. TOF-MS-IDA-MS/MS data files can be explored quantitatively and qualitatively. Qualitative information, including retention time, mass accuracy, isotope pattern and MS/MS fragmentation, is available for identification. Compound identification can be automated using the XIC Manager add-in of PeakView® software.[6]

The example presented in Figure 6 shows a screenshot of the XIC Manager displaying all qualitative information to identify Carbamazepine in water. Confidence scores achieved at different concentrations are summarized in Table 2. Retention time, mass, isotope ratio error, and mass spectral library search score are calculated automatically and visualized using traffic lights in the XIC Manager. A new mass spectral library containing high resolution and accurate mass MS/MS spectra acquired on a TripleTOF® system was used for the MS/MS library search. Standardized collision energy settings of (CE = 35 V with CES = ±15 V) were used when building the mass spectral library.



#	☐	Mass RT Isotope Library	Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (mDa)	Isotope Ratio Difference (%)	Expected RT (min)	RT Width (min)	Found At RT (min)	RT % Error	Intensity	Library Hit	Purity Score
1	☑	●●●●	Carbamaze	C15H12N2(	236.09496	+H	237.10224	0.02	237.10307	0.8	1.7	3.20	1.5	3.22	0.54	27179	Carbamaze	84.8
	☐	○○○○																

Sample: Carbamazepine Test 06(Sample 1)

Control:

Figure 6. Automatic identification of Carbamazepine based on qualitative information saved into the TOF-MS-IDA-MS/MS data

Table 2. Confidence scores achieved at different concentrations of Carbamazepine spiked into water

Concentration	Retention Time Error (%)	Mass Error (mDa)	Isotope ratio Error (%)	Library Search Purity (%)
100 ng/L	0.54	0.8	1.7	84.8
1 µg/L	0.27	0.2	0.7	89.9
10 µg/L	0.36	0.4	1.7	91.9

Quantitation and Identification of PPCP using the *Scheduled* MRM^{HR} Workflow

Scheduled MRM^{HR} is an alternative workflow for multi-target analysis using the TripleTOF® system. Here, TOF-MS/MS experiments are scheduled throughout the chromatographic run to monitor each compound of interest only around the expected retention time. This allows monitoring more target compounds than in regular MRM^{HR} mode. When operating the TripleTOF® system in *Scheduled* MRM^{HR} mode, the TOF-MS data are still recorded to allow retrospective exploration to find unexpected compounds.

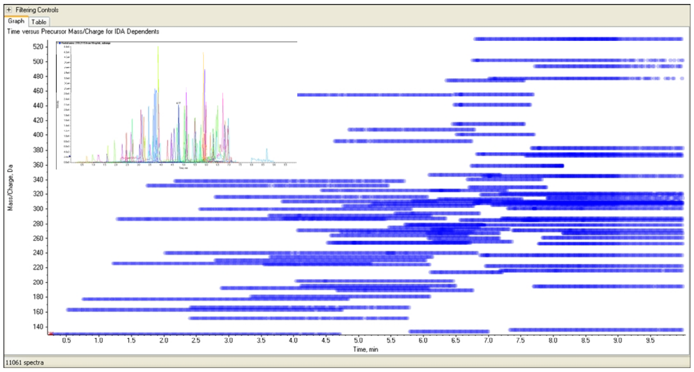
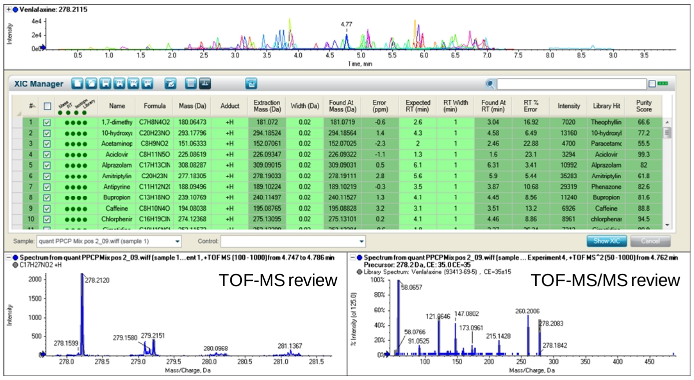


Figure 7. Automatic scheduling of MRM^{HR} experiments to monitor over 80 PPCP in water samples using the Scheduled MRM^{HR} workflow

An example chromatogram of analyzing more than 80 PPCP in a spiked water sample is presented in Figure 7.



#	☐	Mass RT Isotope Library	Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)	RT % Error	Intensity	Library Hit	Purity Score
1	☑	●●●●	1,7-dimethy	C7H8N4O2	180.06473	+H	181.072	0.02	181.0719	-0.6	2.6	1	3.04	16.92	7020	Theophyllin	66.6
2	☑	●●●●	10-hydroxy	C20H23NO	293.17796	+H	294.18524	0.02	294.18564	1.4	4.3	1	4.58	6.49	13160	10-hydroxyl	77.2
3	☑	●●●●	Acetaminop	C8H9NO2	151.06333	+H	152.07061	0.02	152.07025	-2.3	2	1	2.46	22.88	4700	Paracetamc	55.5
4	☑	●●●●	Aciclovir	C8H11N5O	225.08619	+H	226.09347	0.02	226.09322	-1.1	1.3	1	1.6	23.1	3294	Aciclovir	99.3
5	☑	●●●●	Alprazolam	C17H13ClN	308.08287	+H	309.09015	0.02	309.09031	0.5	6.1	1	6.31	3.41	10992	Alprazolam	82
6	☑	●●●●	Amitriptylin	C20H23N	277.18305	+H	278.19033	0.02	278.19111	2.8	5.6	1	5.9	5.44	35283	Amitriptylin	61.8
7	☑	●●●●	Antipyrine	C11H12N2(	188.09496	+H	189.10224	0.02	189.10219	-0.3	3.5	1	3.87	10.68	29319	Phenazone	82.6
8	☑	●●●●	Bupropion	C13H18NO	239.10769	+H	240.11497	0.02	240.11527	1.3	4.1	1	4.45	8.56	11240	Bupropion	81.6
9	☑	●●●●	Caffeine	C8H10N4O	194.08038	+H	195.08765	0.02	195.08828	3.2	3.1	1	3.51	13.2	6926	Caffeine	88.8
10	☑	●●●●	Chlorphenir	C16H19ClN	274.12368	+H	275.13095	0.02	275.13101	0.2	4.1	1	4.46	8.86	8961	chlorphenar	94.5

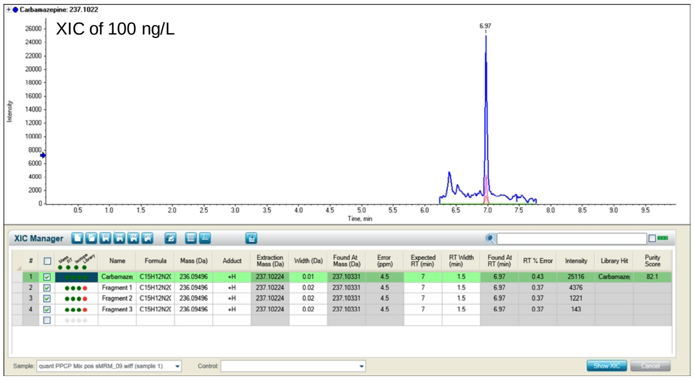
Sample: quant PPCP Mix pos 2_09.wiff (sample 1)
Control:

Spectrum from quant PPCP Mix pos 2_09.wiff (sample 1…ent 1, +TOF MS (100 - 1000) from 4.747 to 4.786 min
C17H27NO2 +H

Spectrum from quant PPCP Mix pos 2_09.wiff (sample … Experiment 4, +TOF MS^2 (50 - 1000) from 4.762 min
Precursor: 278.2 Da, CE: 35.0 CE=35
Library Spectrum: Venlafaxine (93413-69-5) , CE=35±15

Figure 7. Identification of PPCP in a water sample spiked with over 80 PPCP using accurate mass MS and MS/MS information, the confidence score of identification is automatically calculated and visualized using traffic lights in the XIC Manager

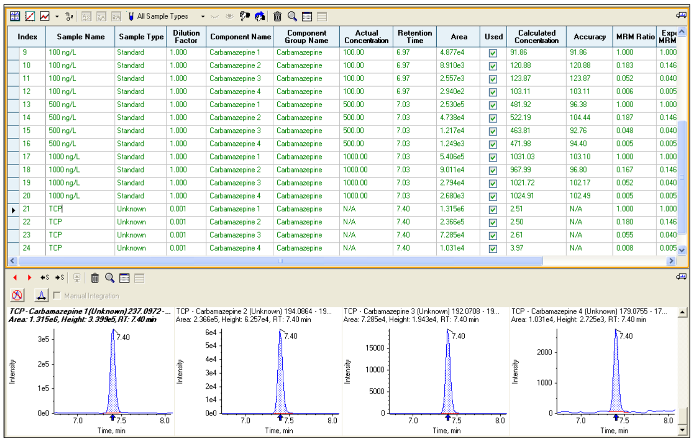
The XIC Manager also allows extracting MS/MS fragment ions of the *Scheduled* MRM^{HR} data file (Figure 8). The resulting XIC allows identification and quantitation of compounds with highest selectivity combing precursor ion selection and high resolution MS/MS detection. Like in TOF-MS-IDA-MS/MS mode, retention time, mass accuracy, isotope pattern and MS/MS fragmentation can be used for identification. In addition, the ratio of different MRM^{HR} transitions can be calculated for further increase in confidence. An example of MRM^{HR} ratio calculation is shown in Figure 9. The sample from a water treatment plant was processed using the ‘Multicomponent’ query in MultiQuant™ software.



#	☐	Mass RT Isotope Library	Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)	RT % Error	Intensity	Library Hit	Purity Score
1	☑	●●●●	Carbamaze	C15H12N2(	236.09496	+H	237.10224	0.01	237.10331	4.5	7	1.5	6.97	0.43	25116	Carbamaze	82.1
2	☑	●●●●	Fragment 1	C15H12N2(	236.09496	+H	237.10224	0.02	237.10331	4.5	7	1.5	6.97	0.37	4376		
3	☑	●●●●	Fragment 2	C15H12N2(	236.09496	+H	237.10224	0.02	237.10331	4.5	7	1.5	6.97	0.37	1221		
4	☑	●●●●	Fragment 3	C15H12N2(	236.09496	+H	237.10224	0.02	237.10331	4.5	7	1.5	6.97	0.37	143		
	☐	○○○○															

Sample: quant PPCP Mix pos sMRM_09.wiff (sample 1)
Control:

Figure 8. Automatic identification of Carbamazepine based on qualitative information saved into the *Scheduled* MRM^{HR} data



Index	Sample Name	Sample Type	Dilution Factor	Component Name	Component Group Name	Actual Concentration	Retention Time	Area	Used	Calculated Concentration	Accuracy	MRM Ratio	Exp MRM
9	100 ng/L	Standard	1.000	Carbamazepine 1	Carbamazepine	100.00	6.97	4.877e4	☑	91.86	91.86	1.000	1.000
10	100 ng/L	Standard	1.000	Carbamazepine 2	Carbamazepine	100.00	6.97	8.910e3	☑	120.88	120.88	0.183	0.146
11	100 ng/L	Standard	1.000	Carbamazepine 3	Carbamazepine	100.00	6.97	2.557e3	☑	123.87	123.87	0.052	0.040
12	100 ng/L	Standard	1.000	Carbamazepine 4	Carbamazepine	100.00	6.97	2.940e2	☑	103.11	103.11	0.006	0.005
13	500 ng/L	Standard	1.000	Carbamazepine 1	Carbamazepine	500.00	7.03	2.530e5	☑	481.92	96.38	1.000	1.000
14	500 ng/L	Standard	1.000	Carbamazepine 2	Carbamazepine	500.00	7.03	4.738e4	☑	522.19	104.44	0.187	0.146
15	500 ng/L	Standard	1.000	Carbamazepine 3	Carbamazepine	500.00	7.03	1.217e4	☑	463.81	92.76	0.048	0.040
16	500 ng/L	Standard	1.000	Carbamazepine 4	Carbamazepine	500.00	7.03	1.249e3	☑	471.98	94.40	0.005	0.005
17	1000 ng/L	Standard	1.000	Carbamazepine 1	Carbamazepine	1000.00	7.03	5.406e5	☑	1031.03	103.10	1.000	1.000
18	1000 ng/L	Standard	1.000	Carbamazepine 2	Carbamazepine	1000.00	7.03	9.011e4	☑	967.99	96.80	0.167	0.146
19	1000 ng/L	Standard	1.000	Carbamazepine 3	Carbamazepine	1000.00	7.03	2.794e4	☑	1021.72	102.17	0.052	0.040
20	1000 ng/L	Standard	1.000	Carbamazepine 4	Carbamazepine	1000.00	7.03	2.680e3	☑	1024.91	102.49	0.005	0.005
21	TCP	Unknown	0.001	Carbamazepine 1	Carbamazepine	N/A	7.40	1.315e6	☑	2.51	N/A	1.000	1.000
22	TCP	Unknown	0.001	Carbamazepine 2	Carbamazepine	N/A	7.40	2.366e5	☑	2.50	N/A	0.180	0.146
23	TCP	Unknown	0.001	Carbamazepine 3	Carbamazepine	N/A	7.40	7.285e4	☑	2.61	N/A	0.055	0.040
24	TCP	Unknown	0.001	Carbamazepine 4	Carbamazepine	N/A	7.40	1.031e4	☑	3.97	N/A	0.008	0.005

TCP - Carbamazepine 1 (Unknown) 237.0972 - …
Area: 1.315e6, Height: 3.399e5, RT: 7.40 min

TCP - Carbamazepine 2 (Unknown) 194.0864 - 19…
Area: 2.366e5, Height: 6.257e4, RT: 7.40 min

TCP - Carbamazepine 3 (Unknown) 192.0708 - 19…
Area: 7.285e4, Height: 1.943e4, RT: 7.40 min

TCP - Carbamazepine 4 (Unknown) 179.0755 - 17…
Area: 1.031e4, Height: 2.725e3, RT: 7.40 min

Figure 9. Quantitation of Carbamazepine in a water sample from a treatment plant using the *Scheduled* MRM^{HR} workflow with calculation of ion ratios using the ‘Multicomponent’ query in MultiQuant™ software

Summary

A fast and reliable method for the quantitation and identification of PPCP in environmental samples was developed. UHPLC with a Phenomenex Kinetex core-shell column was coupled to an AB SCIEX TripleTOF® 4600 system for this study. Different scan functions were applied and compared with respect to selectivity, sensitivity, accuracy, reproducibility, and confidence of identification.

TOF-MS using a narrow XIC window resulted in triple quadrupole-like quantitative performance. The use of MRM^{HR} increased selectivity resulting in reduced background and interference for quantitation and an overall improvement of limits of quantitation.

Additionally, the accurate mass MS and MS/MS information saved into TOF-MS-IDA-MS/MS and Scheduled MRM^{HR} data aided in compound identification with highest confidence. Retention time matching, comparison of theoretical and measured mass accuracy and isotope pattern, and automatic MS/MS library searching was performed using the XIC Manager add-in of PeakView® software.

New mass spectral libraries containing high resolution and accurate mass MS/MS spectra acquired on a TripleTOF® system enable excellent compound identification using high resolution mass spectrometry. The available mass spectral libraries contain accurate mass MS/MS spectra of approximately 500 pesticides, 250 veterinary drugs (including antibiotics, hormones etc.), and 300 relevant pharmaceuticals and drugs of abuse.

References

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