Waters™

Nota de aplicación

Identification of Potential Metabolites of Pharmaceutical Residues Detected in an Environmental Water Sample

Gareth E. Cleland, Mark Wrona, Lauren Mullin, Jennifer A. Burgess

Waters Corporation

Abstract

Previous work presented described the use of the Waters Screening Platform Solution in combination with Waters' toxicology library to initially screen a local well water sample for the presence of a large number (>1000) of PPCPs, pesticides and drugs of abuse.[2] In this application note, we have processed the same dataset with the metabolite identification aspect of the integrated software system to isolate known and potential metabolites of the confident screening matches in the dataset. Using the metabolite identification functionality of UNIFI, three metabolites of carbamazepine were identified with confidence in an enriched local well water sample.

Benefits

- HRMS Screening of a large target list, with adducts
- Fast UPLC analysis with the ACQUITY UPLC HSS C_{18} Column
- Incurred residue metabolite identification

Introduction

In recent years, there has been increasing concern regarding the presence of pesticides, pharmaceuticals, and personal care products (PPCPs) in water bodies throughout the world.[1] A greater demand is being placed on techniques not only used to screen for these compounds, but to screen for the presence of their metabolites.

Data obtained from a non-targeted acquisition on a high resolution mass spectrometer can be used to target a theoretical unlimited number of compounds. Moreover, information rich datasets collected using UPLC/MS ^{E} can be used to reduce the large number of false detects that arise when targeting a large number of compounds verses accurate mass as a sole point of contaminant identification. MS^{E} provides accurate mass measurements for both precursor and fragment ion information in a single experiment by alternating scans between low and high collision energies. In combination with UNIFI, an integrated scientific information system, it is now possible to screen for the presence of PPCPs, their adducts, and potential metabolites in a routine laboratory environment.

Previous work presented described the use of the Waters Screening Platform Solution in combination with

Waters' toxicology library to initially screen a local well water sample for the presence of a large number (>1000) of PPCPs, pesticides and drugs of abuse.[2] In this application note, we have processed the same dataset with the metabolite identification aspect of the integrated software system to isolate known and potential metabolites of the confident screening matches in the dataset. Once discovered, metabolites were made available for future screening experiments by adding the detection results (retention time and identified fragment ions) into a scientific library.

Experimental

A locally obtained well water sample was enriched one thousand times as previously described.[2,3] A comprehensive dataset, collected using UPLC-MS^E was obtained within UNIFI. The toxicology screening solution within UNIFI contains pre-defined LC-MS conditions and processing parameters. The toxicology library in UNIFI is comprised of over 1000 compounds including many PPCPs, such as drugs of abuse, veterinary medicines, and pharmaceuticals. Library entries also contain retention times and accurate theoretical fragment masses. Experimental conditions, sample preparation protocols, and data processing parameters are available in a previous application note by the same authors.[2]

Results and Discussion

From a previous application note,[2] the screening of a local well water sample against the full toxicology library in UNIFI, with up to three adducts ($H,^+$ $Na,^+$ K^+), indicated the presence of the four compounds in Table 1.

Component Summary — View: *Qualitative View

	Component na...	Formula	m/z	Retention Time Error (min)	Mass error (ppm)	Identified High Energy Fragments	Response	Adducts
1	Carbamazepine	C15H12N2O	237.1021	0.21	-0.62	3	10282	+H
2	Hexamine	C6H12N4	141.1136	0.38	0.92	3	40806	+H
3	Imidacloprid	C9H10ClN5O2	256.0597	0.18	0.66	1	7907	+H
4	Tramadol	C16H25NO2	264.1956	0.42	-0.68	1	16859	+H

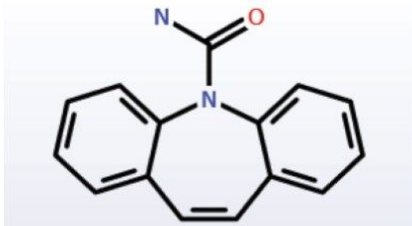
Table 1. Component summary table in UNIFI showing details of confident matches made duringa screening of the extracted well water sample against a library of over 1000 compounds

The inclusion of retention times and accurate mass fragment ions in the toxicology screening library allowed for confident matches to be made since they were based on more information other than accurate mass of the precursor ions alone. As indicated, this is critical for reducing false detection rates, enabling rapid data review for screening experiments.

Further investigation of the comprehensive dataset was possible using the metabolite identification functionality of UNIFI's screening solution software. This functionality requires a target molecule with mol file and a list of possible transformations, that are shown in Figure 1.

Selected transformations

	Name	Delta Mass (Da):	Formula	Classifier
1	Ketone to alcohol	2.0157	+H2	Phase I
2	Oxidation	15.9949	+O	Phase I
3	Glucosylation	162.0528	+C6H10O5	Phase II
4	Methylation of alcohol	14.0157	+CH2	Phase II
5	Glucuronide conjugation of anything	176.0321	+C6H8O6	Phase II
6	Sulfate conjugation	79.9568	+SO3	Phase II



Carbamazepine
+H

Figure 1. Transformations and an example mol file used to identify potential metabolites of compounds found in a screening experiment.

Primarily, using chemical intelligence,[4] the target mol file is systematically cleaved. This essentially increases the target list to include parent compounds and potential breakdown products in the metabolite search.Interrogation of the low energy function of the MS^E comprehensive dataset was performed, which automatically extracted the masses corresponding to the parent as well as the permutations of provided transformations, with and without systematic cleavages of the parent molecule. The list of possible

metabolites for carbamazepine is shown in Table 2 and Figure 2.

No metabolites were observed for the other three compounds found in the screening experiment.

Component Summary — View: *Metabolite Summary

	Component name	Formula	m/z	Observed RT (min)	Mass error (ppm)	Response	Percentage of Parent Response (%)	Identification status
1	Carbamazepine	C15H12N2O	237.1021	7.49	-0.62	10282	100.000	Identified
2	Carbamazepine+O	C15H12N2O2	253.0975	4.34	1.49	5927	57.645	Identified
3	Carbamazepine+O	C15H12N2O2	253.0964	5.82	-2.80	6263	60.907	Identified
4	Carbamazepine+O	C15H12N2O2	253.0984	3.44	4.96	431	4.194	Identified

Table 2. Component summary of potential metabolites found for carbamazepine using the transformations and mol file shown in Figure 1.

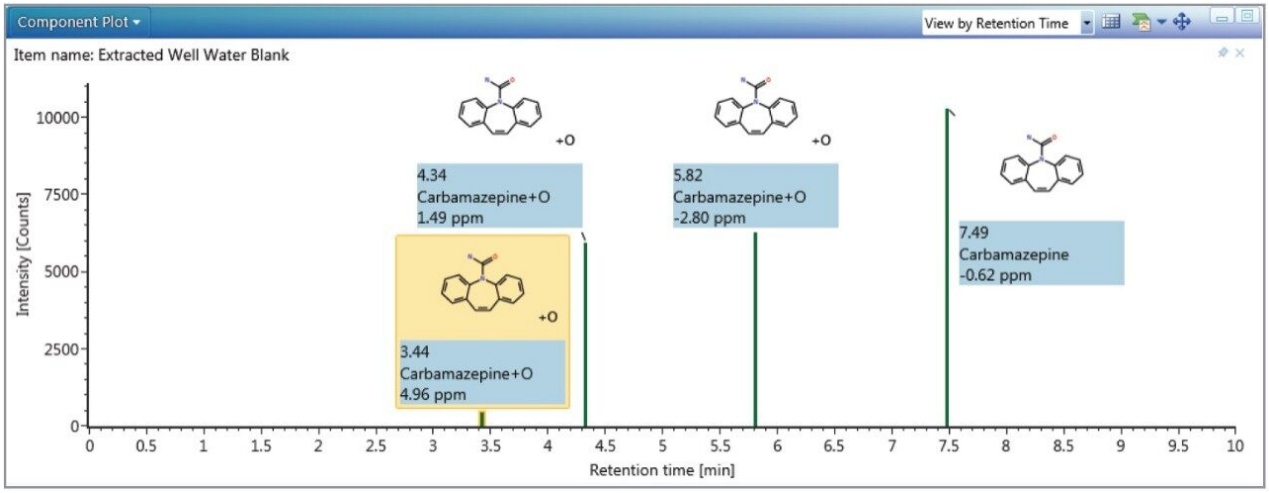


Figure 2. Component plot showing potential metabolites found for carbamazepine using the transformations and mol file shown in Figure 1.

Figures 3 and 4 show the full UI information details for the identification of carbamazapine and a carbamazepine oxidation respectively. Fragment match functionality within UNIFI uses similar intelligence as the cleavage algorithm above. It systematically dissects the mol file of the parent or proposed metabolite and assigns potential accurate mass fragment ions from the high energy function of the MS^E data. Identified fragment ions are annotated, as shown in Figure 3 for the mass 194.06691 Da, and in Figure 4 for the masses 210.09098 Da and 236.07105 Da.

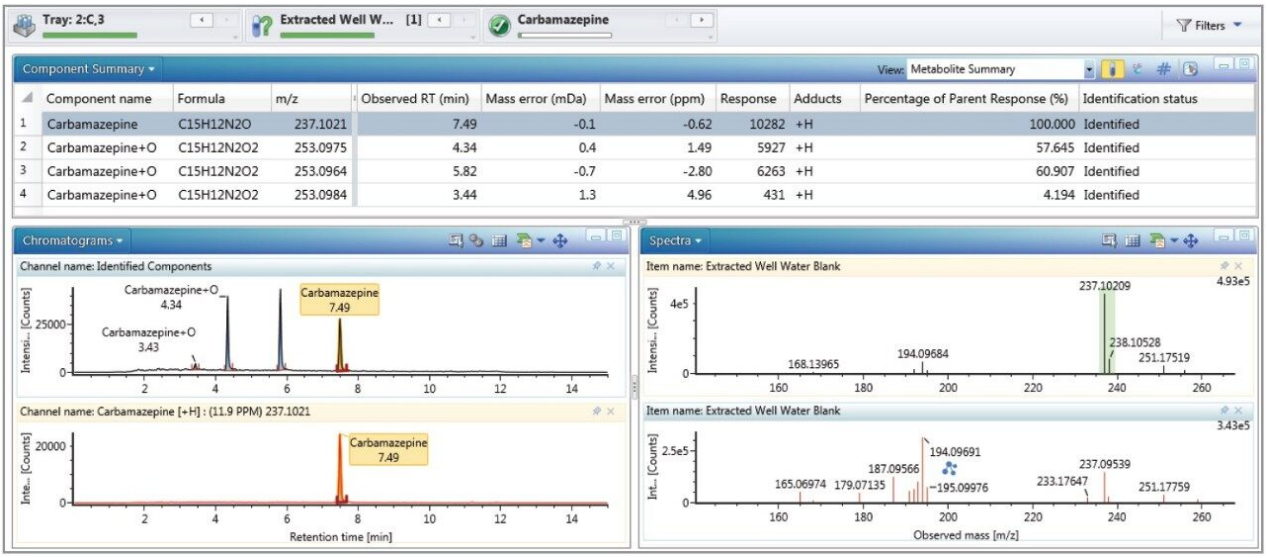


Figure 3. Full user interface (UI) information within UNIFI showing identification details of the carbamazepine parent. Component summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the component summary. The spectra section shows precursor and fragmentation spectra for the highlighted component

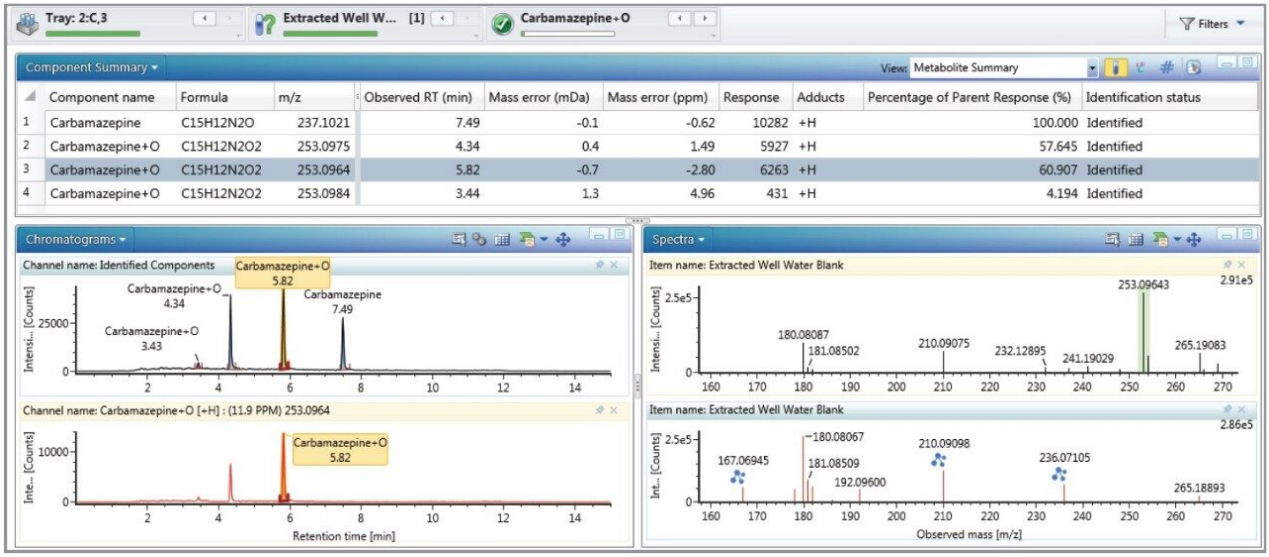


Figure 4. Full user interface (UI) information within UNIFI showing identification details of a proposed carbamazepine metabolite. Component Summary shows identification details while the chromatogram shows extracted ion chromatograms of all identified components with the component highlighted in the component summary. The spectra section shows precursor and fragmentation spectra for the highlighted component

Just as in screening experiments, the high energy fragment ions provided increased confidence that identified metabolites were correct. Common fragment and neutral loss discovery tools, readily available in UNIFI, can also be used to enhance the confidence in metabolite identification. Figure 5 shows the results of running a common fragment search. The two +O metabolites of carbamazepine at 4.3 and 5.8 minutes are shown to be related to each other by the fragment 210.0910 Da, which is the loss of 43.005 Da from the parent 253.0964 Da. This is the same neutral loss from the carbamazepine parent (237.1021 Da) to the primary fragment (194.0969 Da) thus giving further confidence in the metabolites identified.

Figure 5. Results from a common fragment search of 210.09098 Da, performed within the elucidation toolset in UNIFI.

Once the presence of a metabolite has been confirmed, the entry can be easily exported to an existing or new scientific library within UNIFI with the right click of the mouse, as shown in Figure 6. Details such as formula, retention time, theoretical accurate mass fragment ions, and spectra are made available for future users and analyses.

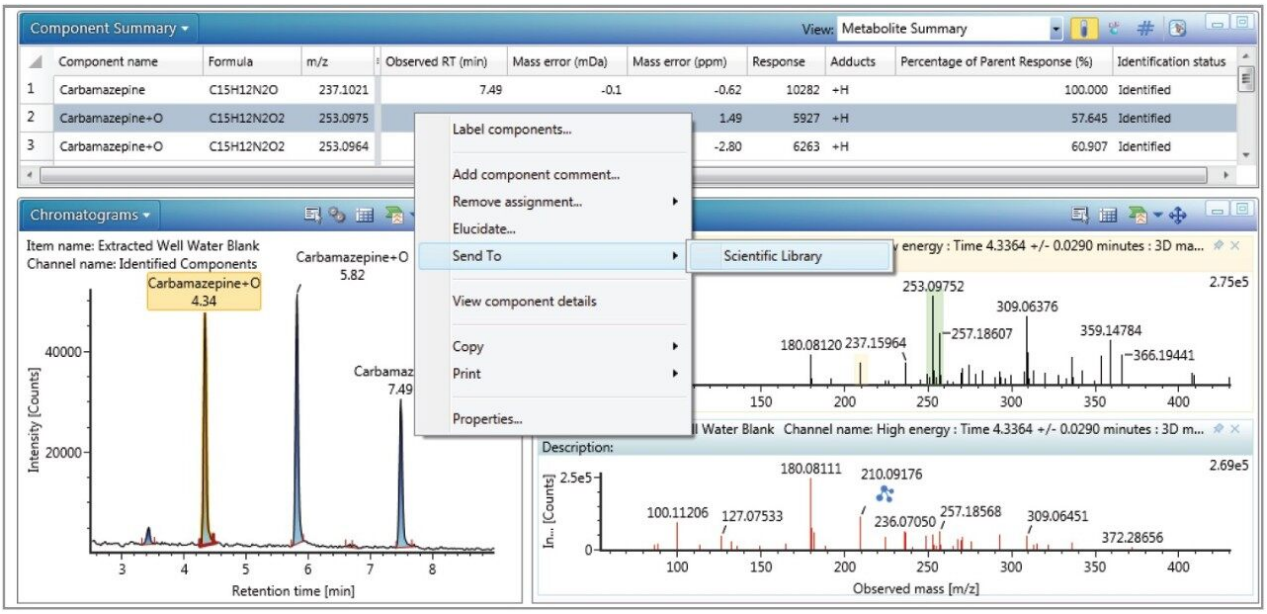


Figure 6. Sending reviewed metabolites to UNIFI's scientific library.

Conclusion

- Information rich MS^E acquisition and an integrated scientific information system make it possible to screen for the presence of compounds of interest, their adducts, and potential metabolites in a routine laboratory environment.
- The presence of retention times and accurate mass fragment ions in scientific libraries within UNIFI allowed identifications to be made on more information than accurate mass of the precursor ions alone. This proves critical for reducing false detection rates and enabling rapid data review for screening experiments.
- Using the metabolite identification functionality of UNIFI, three metabolites of carbamazepine were identified with confidence in an enriched local well water sample.
- Identified metabolites can easily be added to UNIFI's scientific library to expand the list of compounds targeted in future screening analyses.

References

1. S Richardson. Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. *Anal Chem*. (2012) 84: 747–778.
2. C Mallet, G Cleland, J Burgess. Screening Environmental Samples For a Diverse Range of Compounds With Accurate Mass LC-MS and an Integrated Scientific Information System. (2013) Waters Application Note no. 720004810en.
3. C Mallet, G Cleland, J Burgess. Multi-residue analysis of pharmaceuticals and personal care products (PPCPs) in water using the ACQUITY UPLC H-Class and the Xevo TQD tandem mass spectrometer. (2013) Waters Application Note no. 720004813en.
4. R J Mortishire-Smith, J M Castro-Perez, K Yu, J P Shockcor, J Goshawk, M J Hartshorn, A Hill. Generic dealkylation: a tool for increasing the hit-rate of metabolite rationalization, and automatic customization of mass defect filters. *Rapid Commun Mass Spectrom*. (2009) Apr 23, (7): 939–48. doi: 10.1002/rcm. 3951.

Featured Products

Screening Platform Solution with UNIFI <https://www.waters.com/134682903>

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

720004812, July 2017

©2019 Waters Corporation. All Rights Reserved.