Simultaneous Characterization of Highly Polar, Polar and Nonpolar Compounds in River Water using Serial Coupled RPLC and HILIC with a QTRAP® 5500 LC-MS/MS

Identification using MRM Ratios and Enhanced Product Ion Scanning (EPI)

Andrea Boltner[1], Wolfgang Schröder[1], Sylvia Grosse[1], André Schreiber[2], and Thomas Letzel[1]

[1]Technical University of Munich, Chair of Urban Water Systems and Engineering, Garching (Germany); [2]SCIEX Concord, Ontario (Canada)

Introduction

Liquid Chromatography using reversed phase columns (RPLC) coupled to tandem Mass Spectrometry has become a preferred tool for the identification and quantitation of hydrophobic compounds in environmental samples, such as wastewater and surface water.

However, water research lacks information about highly polar and hydrophilic compounds present in water.

In this application note we describe an easy and efficient method based on the serial coupling of RPLC and zwitterionic Hydrophilic Interaction Liquid Chromatography (HILIC) to simultaneously separate polar and nonpolar compounds occurring in wastewater.[1, 2]

Pharmaceuticals, pesticides and industrial chemicals were detected and identified using a SCIEX QTRAP® 5500 LC-MS/MS system operated in MRM-IDA-EPI scanning mode. Information Dependent Acquisition (IDA) combining MRM and EPI enables compound identification based on MRM ratios but also using full scan MS/MS spectra for library searching. Quantitative results were achieved by processing MRM data acquired using the *Scheduled* MRM™ algorithm.

Experimental

Chemicals

Water (LC/MS-grade) was obtained from Sigma Aldrich and acetonitrile (HiPerSolv) was obtained from VWR.

Chemical standards were purchased from different sources: ammonium acetate, acesulfame K, betaine, carbamazepine, diclofenac sodium, gabapentin, ϒ-aminobutyric acid, glyphosate, ibuprofen, isopentylamine, melamine, and vigabatrin from Sigma Aldrich; cyanuric acid and metformin hydrochloride from Fluka, acetylcholinechloride from Acros Organics, and methylparaben from IjSP Biochema Schwaben GmbH.

Sample Preparation

SPE cartridges Strata C18-E (500 mg / 3mL) were obtained from Phenomenex and SPE cartridges ZIC-HILIC (1g / 6 mL) from DiChrom. Prior to analysis the samples were filtered through a 0.22 µm PVDF filter.

150 mL aliquots of river water samples from a sewage treatment plant were cleaned up and concentrated (300x) using SPE prior to LC-MS/MS analysis.

LC Separation

Two Agilent 1260 Infinity LC systems, consisting of a binary pump, an on-line degasser, and a mixing chamber, were used for pumping independent LC gradients for the HILIC column and the RPLC column (Figure 1).

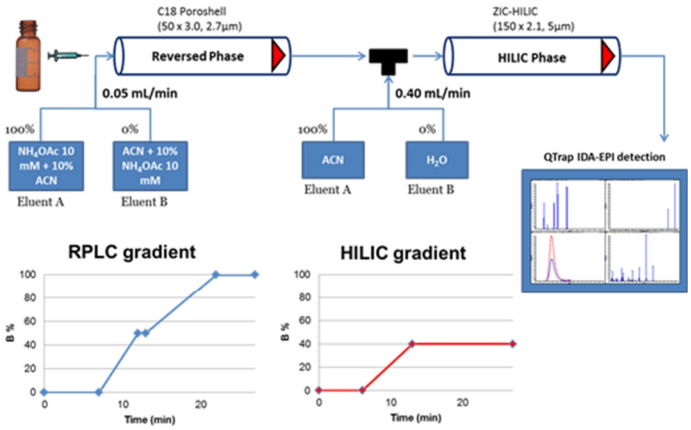

Figure 1. Setup of RPLC and HILIC coupled to MS/MS

For RPLC an Agilent C18 Poroshell (50 x 3 mm, 2.7 µm) column was used with a gradient of water and acetonitrile with 10 mM ammonium acetate.

For HILIC a ZIC-HILIC (150 x 2.1 mm, 5 µm) column was used with a gradient of acetonitrile and water. The LC was kept at ambient temperature and the injection volume was set to 10 µL.

The gradient profile and flow rates are described in detail in Table 1.

Table 1. LC gradient for RPLC and HILIC

	RPLC		HILIC	
Time (min)	Flow (mL/min)	B (%)	Flow (mL/min)	B (%)
0.0	0.05	0	0.4	0
6.0	-	-	0.4	0
7.0	0.05	0	-	-
12.0	0.05	50	-	-
13.0	0.10	50	0.4	40
22.0	0.10	100	-	-
32.0	0.10	100	0.4	40
33.0	0.10	0	0.8	0
53.0	0.10	0	0.8	0
54.0	0.05	0	0.4	0
58.0	0.05	0	0.4	0

MS/MS Detection

A SCIEX QTRAP® 5500 system equipped with Turbo V™ source and an Electrospray Ionization (ESI) probe was used to detect target compounds. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode using the *Scheduled* MRM™ algorithm and positive and negative polarity with a settling time of 50 msec.

Details of the MRM method are described in Table 2. The MRM detection window was 60 sec with a target scan time of 1 sec.

Positive polarity and negative polarity MRM experiments were combined with positive and negative polarity EPI scanning using Information Dependent Acquisition (IDA) to utilize MRM ratios and MS/MS spectra for compound identification. IDA criteria were set to acquire the MS/MS spectrum of the most intense peak of the MRM survey. Dynamic background subtraction was activated and the IDA threshold was set to 1000 CPS. EPI scanning was performed with a scan speed of 10000 Da/sec. The Collision Energy (CE) was set to 40 V with a Collision Energy Spread (CES) of 20 V.

For all four experiments the following ion source settings were used: Curtain Gas (CUR) = 40 psi, IonSpray voltage (IS) = ±1500 V, nebulizer gas (GS1) = 70 psi, heater gas (GS2) = 50 psi, and source temperature (TEM) = 600ºC.

Table 2. Retention time (RT), MRM transitions and compound dependent parameters for all detected compounds

Compound	RT (min)	Q1	Q3	DP (V)	CE (V)
Positive Polarity					
Melamine[c]	7.1	127	68 85	96	37 25
Gabapentin[p]	9.6	172	137 154	56	21 17
Isopentylamine[c]	11.0	88	71 43	61	11 19
Betaine[p]	11.6	118	42 56	51	61 57
Acetylcholine[n]	12.0	146	87 60	61	19 15
Vigabatrin[p]	12.7	130	71 113	46	19 11
g-Aminobutyric acid[n]	13.7	104	69 87	46	21 15
Metformin[p]	16.1	130	71 60	26	29 17
Carbamazepine[p]	26.4	237	179 165	96	47 61
Negative Polarity					
Acesulfam[s]	5.7	162	82 78	-50	-20 -42
Cyanuric acid[c]	6.4	128	85	-55	-12
Glyphosate[h]	12.7	168	63 150	-145	-28 -14
Methylparabene[c]	25.3	151	92 136	-95	-28 -40
Diclofenac[p]	25.6	294	250 214	-50	-16 -26
Ibuprofen[p]	26.0	205	159 161	-30	-10 -10

[c] *industrial chemical,* [p] *pharmaceutical,* [n] *neurotransmitter,* [s] *sweetener,* [h] *herbicide*

Table 3. RT error, LOD, LOQ and linearity for all detected compounds

Compound	Q3	RT error (%)	LOD (µg/L)	LOQ (µg/L)	Linear fit r^2 value
Positive Polarity					
Melamine	68 85	0.74	0.2 0.2	0.8 0.7	0.994 0.978
Gabapentin	137 154	0.00	0.6 2.3	2.2 7.8	0.999 0.999
Isopentylamine	71 43	0.14	1.2 5.0	3.9 16.8	1.000 0.999
Betaine	42 56	0.37	5.8 16.1	19.4 53.7	0.993 0.990
Acetylcholine	87 60	0.89	1.9 5.1	6.3 16.9	0.997 0.996
Vigabatrin	71 113	0.23	1.0 2.9	3.2 9.5	0.984 0.999
g-Aminobutyric acid	69 87	0.33	3.0 3.5	9.9 11.8	0.997 0.998
Metformin	71 60	0.37	0.08 0.002	0.3 0.008	0.990 0.991
Carbamazepine	179 165	0.06	0.2 0.3	0.6 1.1	0.996 0.997
Negative Polarity					
Acesulfam	82 78	1.67	0.008 0.01	0.03 0.04	0.996 0.999
Cyanuric acid	85	0.47	0.5	1.6	0.996
Glyphosate	63 150	0.34	2.1 1.7	6.9 5.6	0.995 0.994
Methylparabene	92 136	0.00	4.1 18.0	13.8 60.1	0.999 0.998
Diclofenac	250 214	0.11	1.4 1.2	4.3 4.1	1.000 1.000
Ibuprofen	159 161	0.00	1.2 7.2	4.0 24.0	0.996 1.000

Results and Discussion

Determination of LOD and LOQ

Serial dilutions of standard mixtures were prepared in water/acetonitrile (50/50) over 2 orders of magnitude to determine instrument detection limits. The limit of detection (LOD) was defined as a signal 3x higher than the background and the limit of quantitation (LOQ) as a signal 10x higher than the background. LOD, LOQ, and r^2 values obtained using a linear fit are reported in Table 3.

Compound Identification

The following criteria were used for compound identification: retention time matching, MRM ratio calculation and MS/MS library searching.

An example of identification of Carbamazepine is shown in Figure 2. MRM ratios were automatically calculated in MultiQuant™ software and MS/MS library searching was performed using MasterView™ software.

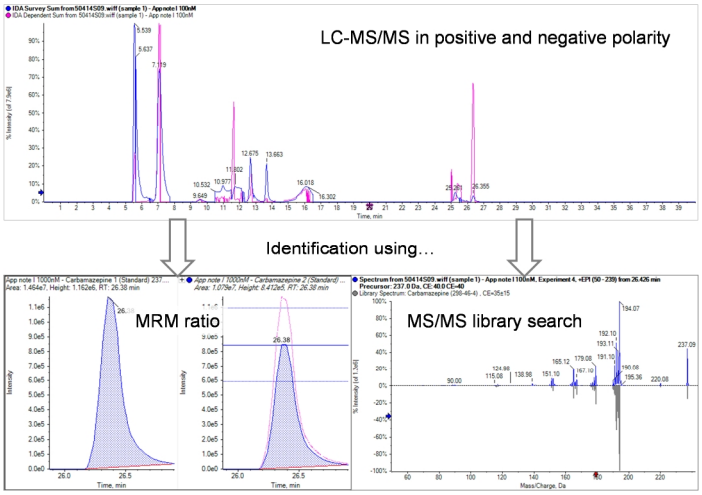

Figure 2. Confident identification of carbamazepine using MRM ratio calculation and MS/MS library searching based on the information saved into the IDA data file

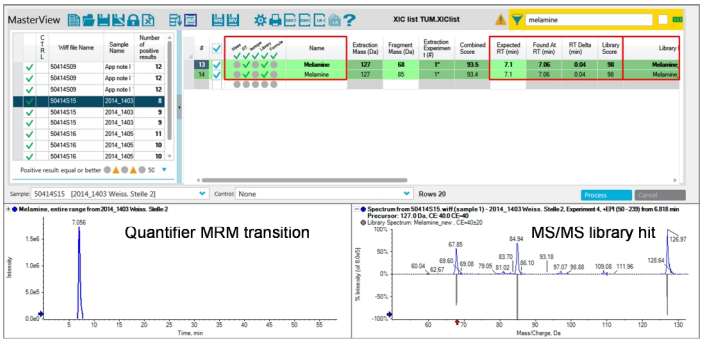

Figure 3a. Identification of Melamine in a river water sample using MasterView™ software

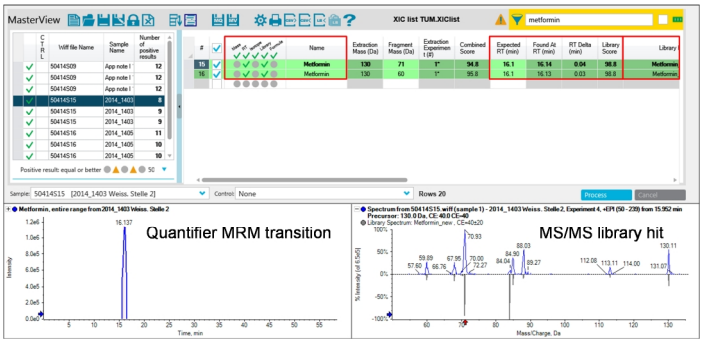

Figure 3b. Identification of Metformin in a river water sample using MasterView™ software

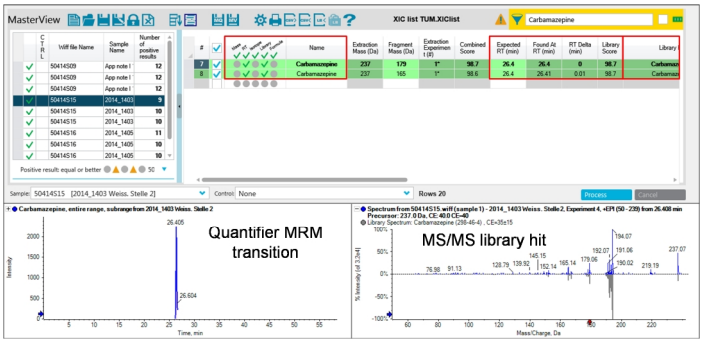

Figure 3c. Identification of Carbamazepine in a river water sample using MasterView™ software

Analysis of River Water Sample

A water sample was extracted and analyzed in triplicate. Four compounds were successfully identified and quantified.

Melamine and Metformin, both separated on the HILIC phase, and Carbamazepine and Methylparabene, both separated on the RPLC phase, were present at sub µg/L concentrations

All 4 compounds were identified with high confidence based on their MRM ratio and by MS/MS library searching (Table 4 and Figures 3a to 3d).

Table 4. Compounds detected in river water (analysis in triplicate, quantitation based on quantifier MRM transition)

Compound	Conc. (µg/L)	RT error (min)	Ion ratio	Ion ratio error	Library FIT (%)
		HILIC			
Melamine	0.05	0.04	0.567	0.58%	98.0
Metformin	0.09	0.04	0.754	2.01%	98.8
		RPLC			
Carbamazepine	0.001	0.00	0.719	1.97%	98.7
Methylparabene	0.04	0.02	0.0009	14.3%	100.0

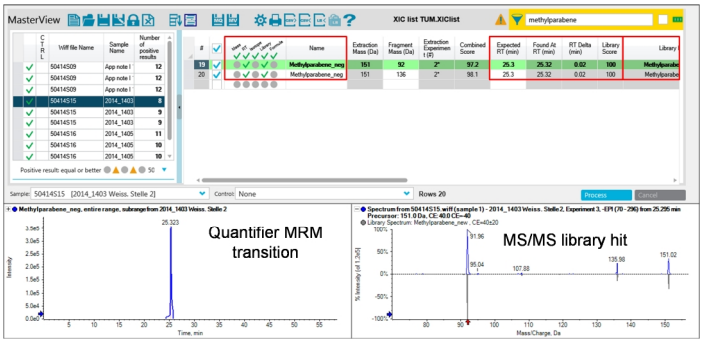

Figure 3d. Identification of Methylparabene in a river water sample using MasterView™ software

FOR-IDENT

Compounds of emerging concern, such as the examples studied in this project, can be observed with non-target screening strategies as currently presented by various laboratories participating in the FOR-IDENT project.

The objective of the FOR-IDENT project is to improve the identification of organic trace substances by standardization of suspect- and non-target screening workflows linking results with open access tools and databases.[3]

For details visit: http://for-ident.hswt.de/

Summary

Here we presented an easy and efficient LC-MS/MS method for the identification and quantitation of hydrophobic and hydrophilic compounds of environmental concern. The LC separation was based on the serial coupling of a RPLC and zwitterionic HILIC column and mobile phase setup. The detection method used a QTRAP® 5500 system operated in IDA mode to combine selective MRM quantitation with identification using MRM ratios and MS/MS library searching.

References

1 G. Greco, S. Grosse, T. Letzel: 'Serial coupling of reversed-phase and zwitterionic hydrophilic interaction LC/MS for the analysis of polar and nonpolar phenols in wine' J Sep Sci. 36 (2013) 1379-1388

2 G. Greco, T. Letzel: 'Main Interactions and Influences of the Chromatographic Parameters in HILIC Separations' J Chromatogr. Sci. 51 (2013) 684-693

3 T. Letzel, A. Bayer, W. Schulz, A. Heermann, T. Lucke, G. Greco, S. Grosse, W. Schüssler, M. Sengl, M. Letzel: 'LC-MS Screening Techniques for Waste Water Analysis and Analytical Data Handling Strategies: Sartans and Their Transformation Products as an Example' Chemosphere 137 (2015) 198-206

Publication number: RUO-MKT-02-3765-A

Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
www.sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.sciex.com/offices