PO-CON1448E

SHIMADZU
Excellence in Science

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

ASMS 2014 TP 583

Klaus Bollig[1]; Sven Vedder[2], Anja Grüning[2]
[1] Shimadzu Deutschland GmbH, Duisburg, Germany;
[2] Shimadzu Europe GmbH, Duisburg, Germany

Fast and highly sensitive analysis
of multiple drugs in ground-, surface- and wastewater

Introduction

Many pharmaceuticals from medical treatments are metabolized or partially degraded in the body. An even larger amount of these compounds is excreted intact and pollutes the aquatic environment. Relevant classes of drugs are human or veterinary antibiotics, antiepileptics, analgetics and lipid lowering drugs or radio-opaque substances. The extent of damage caused to the environment and the resulting health risk for humans or animals should not be underestimated, even though it is not understood in detail so far. The requirement for universal, reliable and fast methods for drug determination in water is steadily increasing. Highly sensitive triple-quad-MS systems are suitable tools for the analysis of residues in ground-, surface- and wastewater, but development of a simple, rapid and robust method for simultaneous measurement of trace levels of various different classes of analytes in complex matrices is a challenge.

Figure 1. LCMS-8050 triple quadrupole mass spectrometer

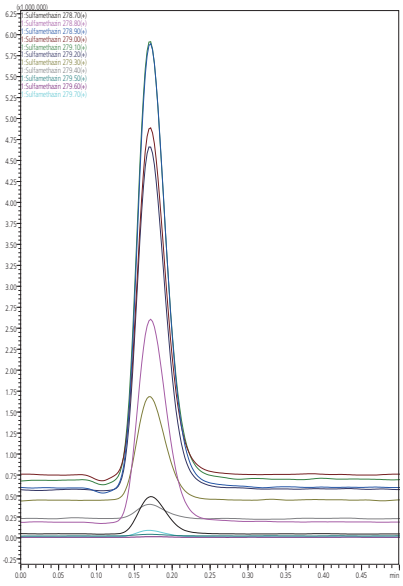
Method

This study describes a fast LC-MS/MS method for the determination of trace levels of different classes of drugs in water. With online SPE no further sample pretreatment is necessary. The quaternary system with low pressure gradient eluent (LPGE) and solvent blending functionality renders addition of a third LC-Pump unnecessary. The solvent blending function was further used for method development. High speed values for MRM recording and the fastest polarity switching time of 5 ms are essential physical parameters for a maximum of data points on various classes of analytes in different polarities during one single analysis.

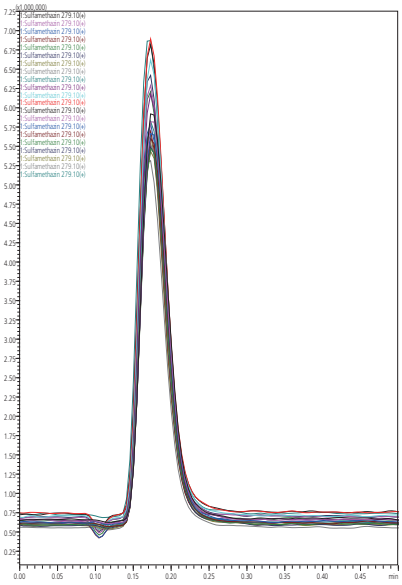
LC-MS/MS Method Optimization

One of the first steps during this automated process is the precursor ion selection, followed by m/z adjustment of the precursor. The collision energy is optimized for the most abundant fragments and finally the fragment m/z is adjusted. Six optimization steps were performed via flow injection analysis, each taking 30 seconds (Figure 2). The result of these automated steps was the automatic generation of a final MRM method (Table 1).

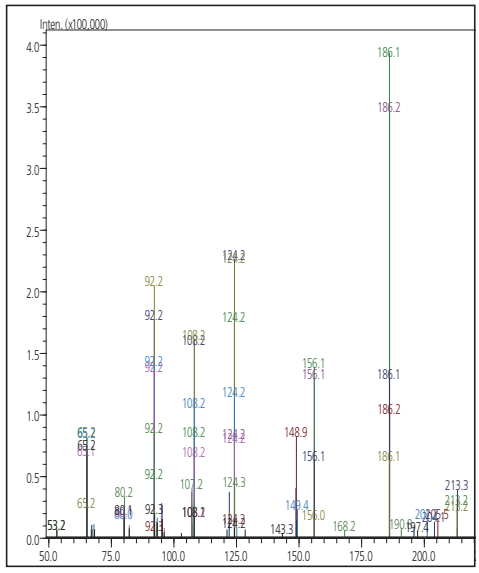
Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater



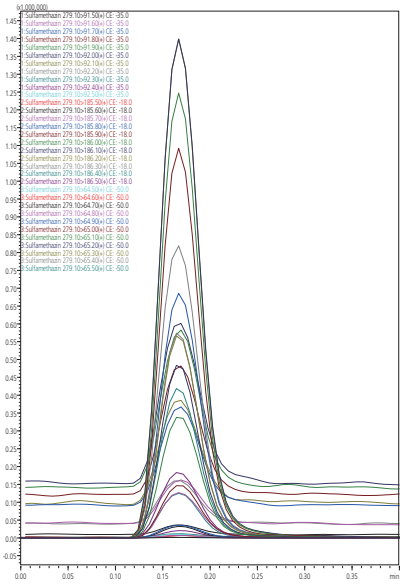
1st Step: m/z Precursor adjustment



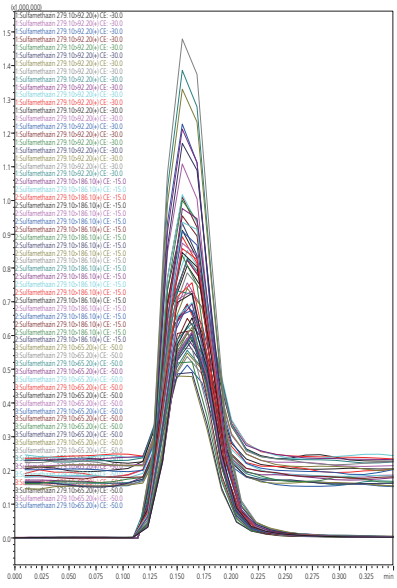
2nd Step: Setting Q1 Prerod Bias



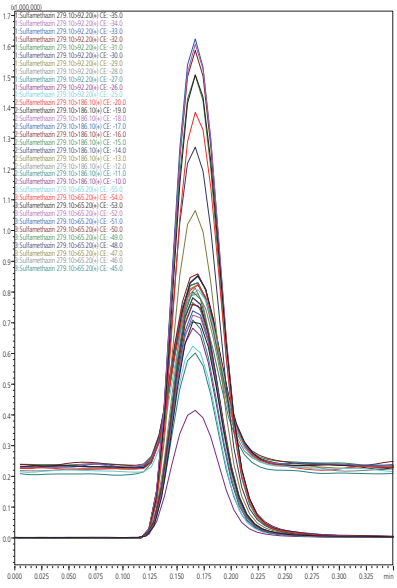
3rd Step: Product Ion / CE selection



4th Step: m/z Product Ion adjustment



5th Step: Setting Q3 Prerod Bias



6th Step: CE fine tuning

Figure 2. Automated MRM Optimization of the drug Sulfamethazin

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

Table 1. Optimized MRM transitions of 9 drugs

Compound	Mode	MRM transitions	Collision energy (kV)
Sulfamethazin	ESI positive	279,10>186,10 / 279,10>92,20	-17 / -31
Sulfamethoxazol	ESI positive	253,90>92,20 / 253,90>156,15	-26 / -15
Bezafibrat	ESI positive	362,10>139,15 / 362,10>316,25	-25 / -15
Carbamazepine	ESI positive	237,10>194,20 / 237,10>179,20	-19 / -34
Diclofenac	ESI positive	296,00>214,15 / 296,00>215,15	-34 / -19
Clofibric acid	ESI negative	213,00>127,00 / 213,00>85,00	15 / 15
Ibuprofen	ESI negative	205,10>161,30	7
Iopamidol	ESI negative	775,80>126,95	22
Iopromid	ESI negative	790,00>127,00	26

Solvent Blending

The solvent blending functionality entails automated mobile phase preparation on a LPGE (low pressure gradient) unit which is integrated in the binary LC pumps. The blending function eliminates the need of mobile phase pre-mixing, as necessary with ordinary binary pumps. Mobile phase composition can simply be changed in the method without physically changing the solvents. Therefore solvent blending is a powerful tool for easy and efficient elucidation of the SPE, the gradient and the starting conditions. During this study the solvent blending function was used for optimization of the SPE conditions. A second LPGE unit was used for the analytical gradient.

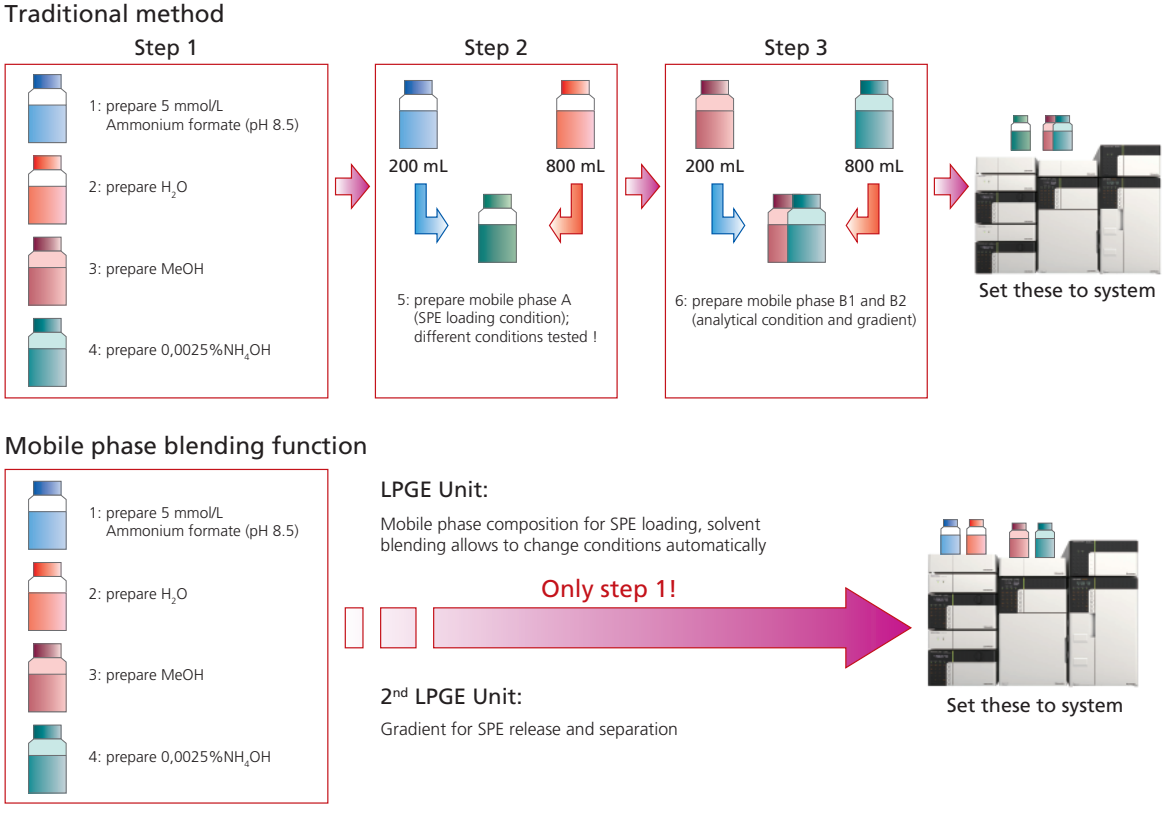


Figure 3. Solvent blending functionality

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

HPLC/MS Workflow

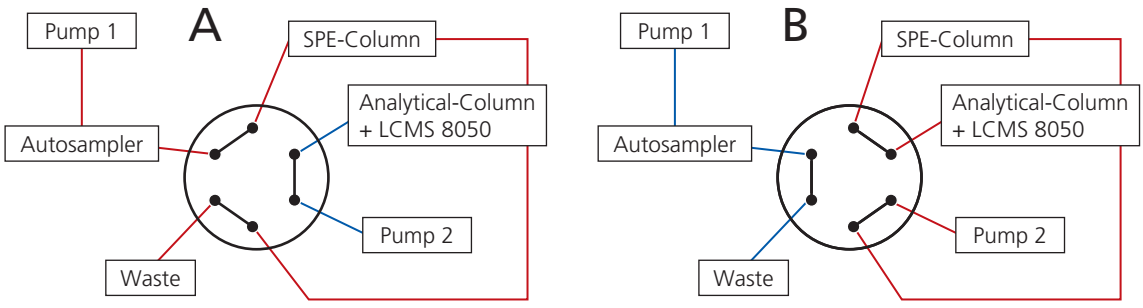


Figure 4. Scheme of online-SPE extraction (A) and analytical separation (B)

Final method

SPE Conditions

Injection volume	: 250 µl
SPE-column	: Strata-X , 25 µm , 20*2 mm
SPE-flow rate	: 1 ml/min
SPE-loading buffer	: 1 mmol/L ammonium formate (LPGE Pump B)

Analytical Conditions (LPGE Pump A)

Column		: Kinetex C8, 2.6 µm, 100*2.1 mm
Flow rate		: 0.5 ml/min
Solvent A		: 0.0025% NH_4OH
Solvent B		: MeOH 1 min – 2.5 min analytical separation
Gradient	: 0 min	: 30% B
	: 1 min	: 30% B
	: 1.5 min	: 95% B
	: 4.5 min	: 95% B
	: 4.51 min	: 30% B
	: 6 min	: 30% B (Stop)

LCMS Conditions

Interface	: ESI
Nebulizing Gas Flow	: 2.2 L/min
Heating Gas Flow	: 12 L/min
Interface Temperature	: 400 °C
Desolvation Line Temperature	: 150 °C
Heat Block Temperature	: 400 °C
Drying Gas Flow	: 6 L/min
Polaritiy Switching Time	: 5 ms

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

Results

In this study we developed a fast method for direct online SPE LC-MS/MS analysis of 9 different drugs in water with a minimal LC configuration of two binary pumps equipped with LPGE units. The solvent blending function was used for method development of the SPE extraction. The second LPGE unit was used for SPE release and analytical gradient separation. Each compound was quantified in a concentration range from 0.05 ng/ml up to 2 ng/ml. Measurements were performed on Shimadzu's LCMS-8050 Triple Quad MS System. The calibration curves and lowest calibration point is shown in figure 5.

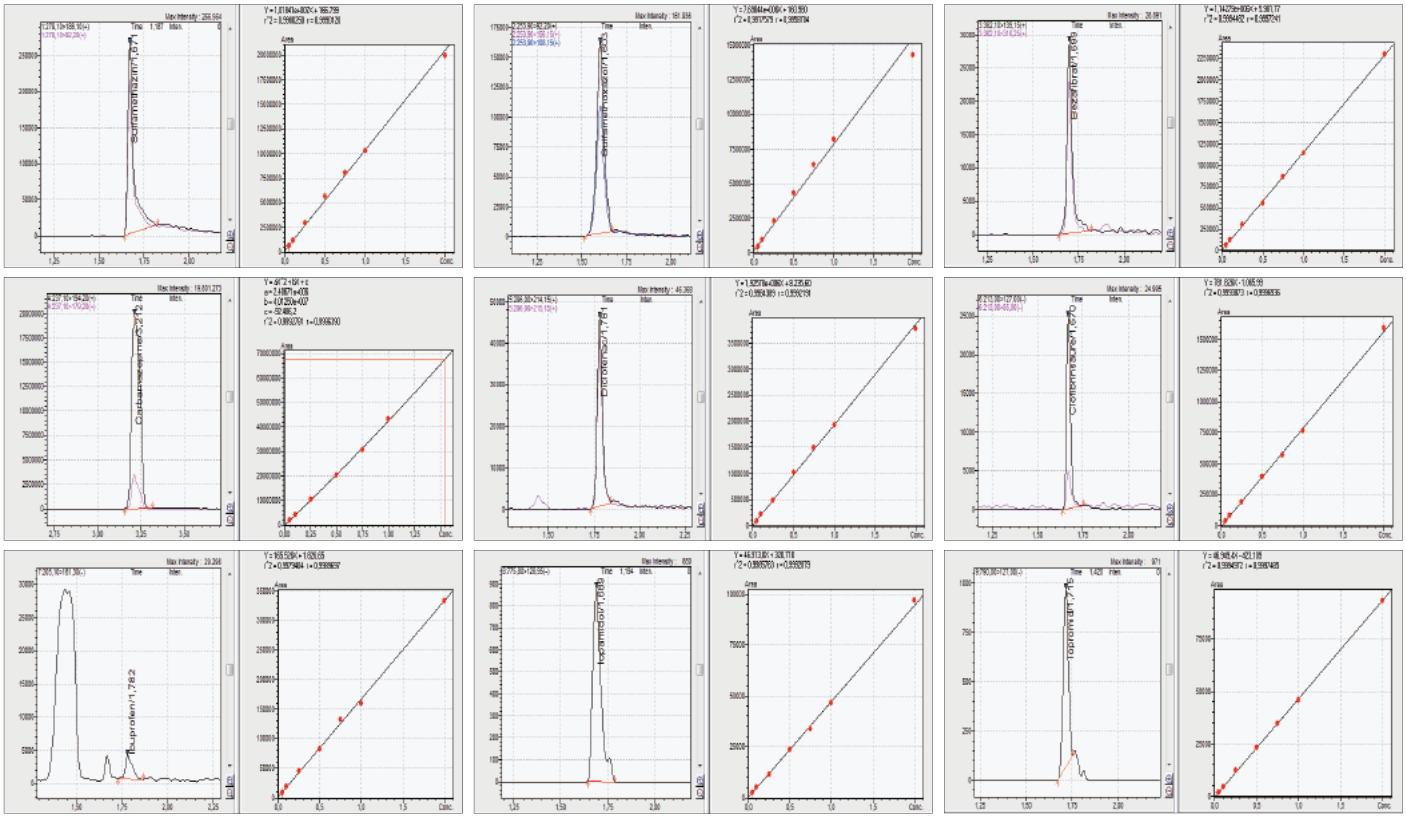


Figure 5. Calibration curve and lowest calibration point at 0.05 ng/ml of each compound

First Edition: June, 2014

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/