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High-speed on-line LC-MS/MS analysis of pharmaceuticals in the environment using overlapping injections

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Introduction

Pharmaceuticals comprise a group of emerging contaminants which have received considerable attention in recent years. Many common drugs can be found in the environment and sometimes even in drinking water. These drugs and their metabolites get into the waste water through excretion via the urine or feces and may reach surface water, groundwater and also drinking water after the passage in the sewage treatment plants. Conventional sewage treatment plants are failing to eliminate the biodegradable substances completely. Many of these compounds are ubiquitous, persistent and biologically active with recognized endocrine-disruption functions. Paying attention to the hazardous nature of these compounds, it is essential to provide fast and sensitive multi-residue methods that are able to analyze multiple substance classes within one analytical procedure.

LCMS-8060 triple quadrupole mass spectrometer

Materials and Methods

This study describes a novel, fast LC/MS/MS method for the determination of trace levels of different classes of pharmaceuticals in water. Utilizing on-line SPE combined with the benefit of the overlapping injection function of Shimadzu's Nexera X2 featured with the high speed values of Shimadzu's LCMS-8060 for MRM recording and the fastest polarity switching time of 5 msec the duration for analyzing various classes of analytes in different polarities during one single analysis could be decreased tremendously (Figure 1).
The applied method uses a fast and optimized chromatographic gradient which results in various calibration curve levels between 1 - 500 ng/L depending on the compound. Real water samples were tested without any sample pretreatment for 22 different pharmaceuticals.

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Analytical Conditions

SPE system	
LC pump	: Nexera X2 (Shimadzu, Japan)
SPE Column	: Strata-X on-line SPE cartridge (2.0 mm x 20 mm, 25 µm)
Mobile Phase	: 0.0025% NH4OH
Column Temperature	: RT
Injection Volume	: 500 µL
Analytical system	
LC system	: Nexera X2 (Shimadzu, Japan)
Analytical Column	: Raptor Biphenyl (2.1 mm x 100 mm, 2.7 µm)
Mobile Phase A	: 0.0025% NH4OH
Mobile Phase B	: Methanol
Column Temperature	: 40 °C
MS	
MS system	: LCMS-8060 (Shimadzu, Japan)
Ionization	: HESI (positive/negative)
Nebulizing Gas Flow	: 3.00 L/min (N_2)
Drying Gas Flow	: 10.0 L/min (N_2)
Heating Gas Flow	: 10.0 L/min (Air)
DL Temperature	: 250 °C
Block Temperature	: 400 °C
Interface Temperature	: 300 °C

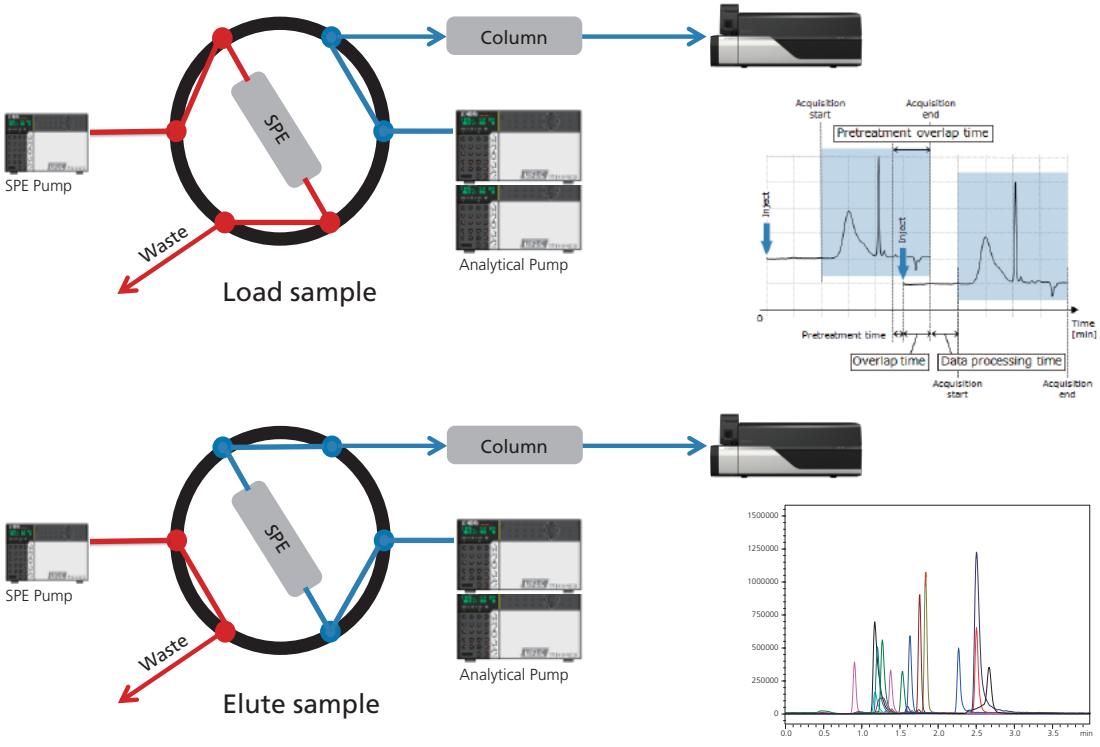

Figure 1. Setup of the on-line SPE analytical system

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Results

Successful establishment of an automated method using the overlapping injection function for the separation, identification and quantification of a mixture of different types of pharmaceuticals out of water samples is demonstrated. The method allows measurements in a concentration range of ng/L within a time range of about 5 miutes. This is possible due to the fact that sample pretreatment and injection of a sample to the on-line SPE column takes place while the preceeding analysis is still ongoing. Furthermore, the time needed for washing of the on-line SPE column is used for re-equilibration of the analytical column. The calibration curves obtained for the 22 compounds are shown in Figure 2. QCs in water were prepared for accuracy evaluation (Table 1). Analysis results of the surface water samples are reflected in Figure 3.

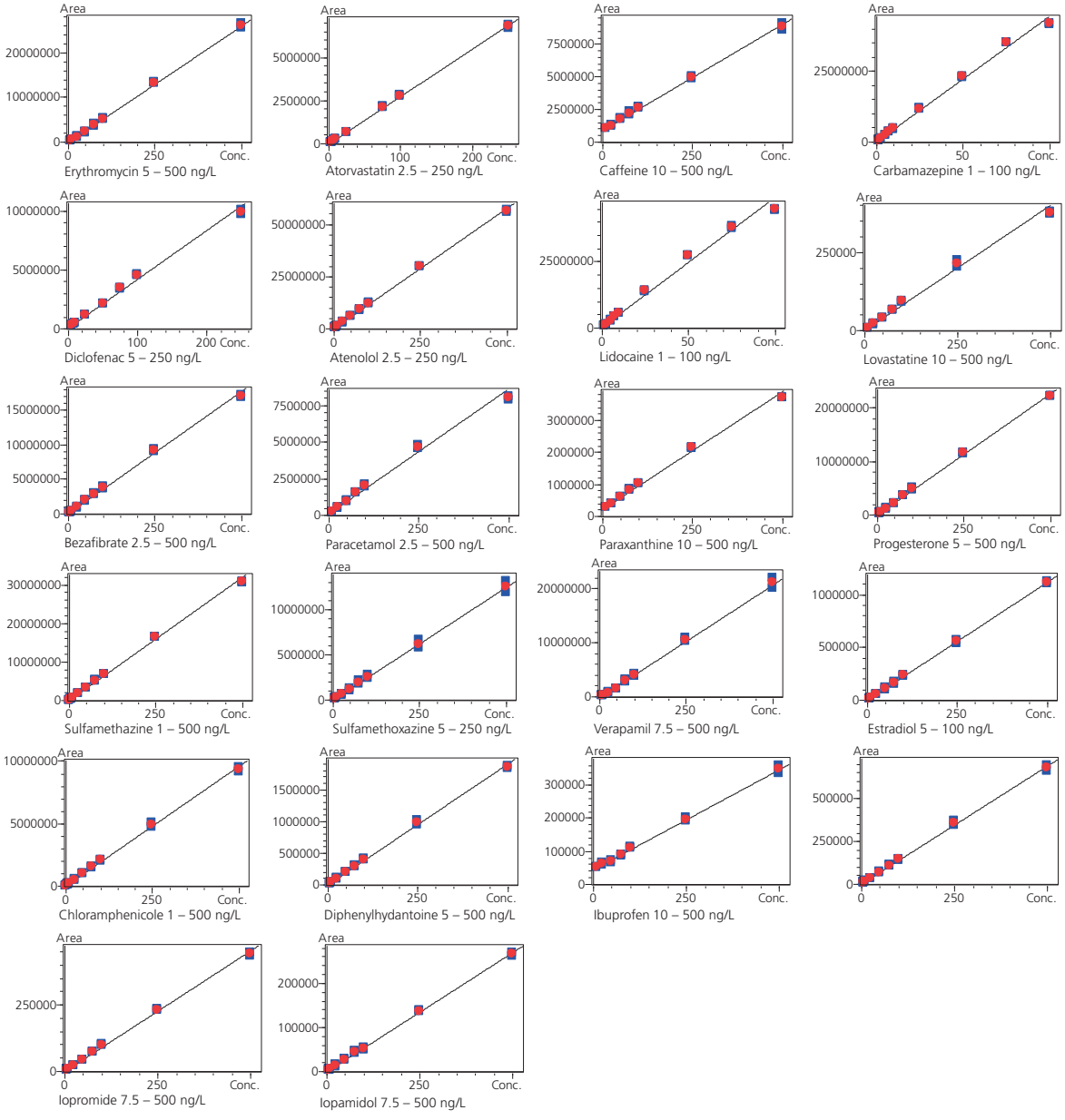

Figure 2. Calibration curves of 22 pharmaceutical compounds determined in duplicate obtained from water samples after on-line SPE treatment. R^2 was better than 0.99 for all calibration curves.

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Table 1. Analysis results for QC samples

QC 75 ng/L (n=6)					
	Accuracy[%]	%RSD		Accuracy[%]	%RSD
Erythromycin	98.7	2.7	Progesterone	107.9	2.7
Atorvastatin	98.5	6.6	Sulfamethazine	108.1	2.1
Caffeine	110.2	3.1	Sulfamethoxazine	107.2	3.0
Carbamazepine	104.4	1.4	Verapamil	97.4	4.2
Diclofenac	103.9	3.2	Estradiol	101.3	4.5
Atenolol	108.7	3.6	Chloramphenicole	105.4	3.3
Lidocaine	103.4	1.2	Diphenylhydantoine	104.6	4.1
Lovastatine	105.9	4.1	Ibuprofen	101.3	2.4
Bezafibrat	106.8	1.7	Indomethazine	109.7	4.8
Paracetamol	107.5	1.9	Iopromide	106.0	7.4
Paraxanthine	106.4	3.6	Iopamidol	96.7	8.7

The measurements of the real water samples illustrate that stream and ship canal which have no additional tributary waters show nearly no contamination wtih pharmaceuticals while different rivers contain detectable pollution. As expected the final effluent of a sewage plant contains the highest contamination since clarification of such compounds is not carried out in the sewage plants at the moment.

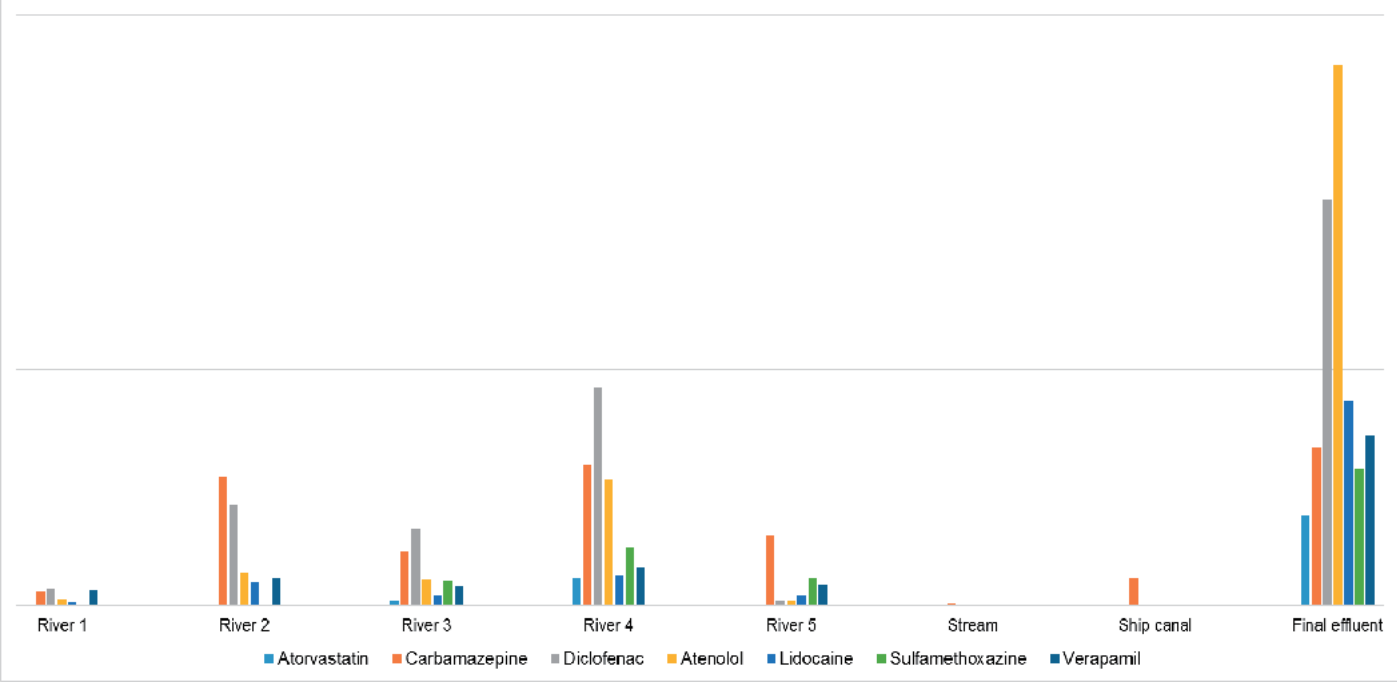

Figure 4. Analysis of surface water samples from different sources

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Conclusions

Using the benefit of the overlapping injection function of Shimadzu's Nexera X2 combined with the high speed values for MRM recording and the fastest polarity switching time of 5 ms on the Shimadzu LCMS-8060 analysis of various classes of pharmaceuticals in water samples can be performed in about 5 minutes including on-line SPE sample pretreatment, analysis and column re-equilibration with sufficient sensitivity.

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