Application Note: 494

Analysis of Basic and Acidic Pharmaceutical Products in Drinking Water Using Online Sample Preparation and LC-MS/MS

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Key Words

- EQuan System
- TSQ Quantum Access
- Water Safety

Introduction

In Germany over 8,700 pharmaceutical preparations with around 3,000 different active ingredients are approved for use in human medicine. The amount of pharmaceuticals prescribed for human use is 31,000 metric tons[1] (2006). In addition, there are approximately 800 metric tons of pharmaceuticals sold for veterinary medicine[2]. As an example, beta blockers are one of the most prevalent groups of drugs used in the western world. The list of the top twenty most frequently prescribed drugs includes three preparations of the beta blocker metoprolol alone. A total of more than 170 metric tons of the active ingredients of these beta blockers – atenolol, sotalol, and metoprolol – were prescribed in Germany in 2006.[1]

Through various means, remnants of pharmaceuticals find their way into ground and surface water. At present, over one hundred active ingredients are found in these waters. In the method described here, beta blockers, anticonvulsants, and lipid-lowering agents, as well as selected pharmaceutical metabolites, were analyzed in water.

With most drugs, only a small portion of the active ingredient leaves the body in an unaltered form. The rest is excreted in the form of metabolites. However, in the case of metoprolol, for example, approximately 30% is unaltered when excreted from the body and approximately 70% is metabolized, mainly in the form of α-hydroxy metoprolol. These intermediate catabolic products are generally significantly more polar than the original substances, and, as a result, can become problematic when abstracting drinking water as they are more soluble in water. For this reason, analyzing pharmaceutical products and their metabolites in bodies of water is becoming increasingly important. As the number of test samples increases, so too does the need for faster analysis time and minimized sample preparation.

Goal

To develop a robust analytical method for the analysis of 29 acidic and basic pharmaceutical residues in drinking water using online sample preparation. At the same time, we hope to reduce time spent on sample preparation and reduce opportunities for human error.

Experiment

In the method described here, 10 mL of the sample to be analyzed was filtered and internal standards were added to determine the pharmaceuticals. The enrichment of the analytes and the spiking of the buffer solutions were fully automated with the aid of the Thermo Scientific EQuan environmental quantitation system. In this way, sample preparation was reduced to a minimum by simultaneously purifying and increasing the concentration of the samples.

Sample Preparation

A volume of 10 mL was required to test a water sample for the substances listed in Table 1. This sample volume was placed in a vial using a syringe filter (pore size 0.2 µm).

Table 1: Pharmaceuticals and metabolites analyzed

Basic / Neutral Pharmaceuticals	Atenolol
	Betaxolol
	Bisoprolol
	Carbamazepine
	Fenoterol
	Furosemide
	Metoprolol
	Oxcarbazepine
	Phenazone
	Pindolol
	Primidone
	Propranolol
	Salbutamol
	Sotalol
	Timolol
	Tramadol
Metabolites of Basic / Neutral Pharmaceuticals	4-Acetylaminoantipyrine
	4-Formylaminoantipyrine
	α-Hydroxymetoprolol
	Carbamazepine-10,11-epoxide
	Phenylethylmalonamide
Acidic Pharmaceuticals	Bezafibrate
	Diclofenac
	Fenoprofen
	Gemfibrozil
	Indomethacin
	Ketoprofen
	Naproxen

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Sotalol-d6, carbamazepine-d10 and diclofenac-d4 were then added as internal standards. Additional sample preparation steps were not necessary; the analytes were enriched online using the EQuan™ environmental quantitation system illustrated in Figure 1.

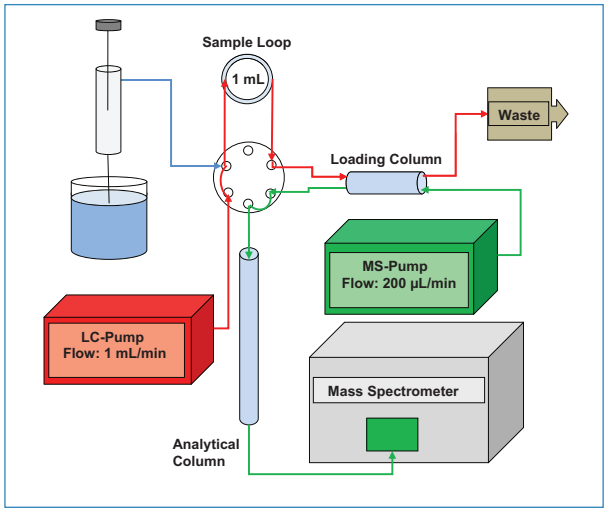

Figure 1: Schematic diagram of the EQuan system

The analysis took place in two automated steps: basic and neutral pharmaceuticals were measured in the first run, while acidic samples were analyzed in a second run. The autosampler placed 1 mL of the sample into a sample loop then it was transferred to a Thermo Scientific Hypersil GOLD aQ 20 mm x 2.1 mm, 12 µm particle size loading column together with the eluent A (water with 1% methanol and 0.1% formic acid). When the clean-up and enrichment steps were complete, a 6-way valve on the autosampler switched over and the liquid chromatography (LC) gradient flowed through the enrichment column to the analytical column. The LC system was equipped with two pumps to facilitate this form of enrichment.

After the basic pharmaceuticals were measured for all samples, the autosampler spiked ammonium acetate buffer to all samples and standards to analyze the acidic pharmaceuticals in the same way.

Liquid Chromatography-Mass Spectrometry

MS analysis was carried out using the EQuan system including the Thermo Scientific TSQ Quantum Access triple stage quadrupole mass spectrometer.

A gradient method using eluents C (water with 1% methanol and 0.1% formic acid) and D (methanol) was used to measure the basic and neutral pharmaceuticals. Eluents D and E (water with ammonium acetate, 2 mmol/L) were used for the acidic pharmaceuticals. The analytical column was a Hypersil GOLD™ Phenyl phase with the dimensions 50 mm x 2.1 mm and particle size 3 µm; the flow rate was 200 µL/min.

The mass spectrometry detection utilized electrospray ionization (ESI) in positive mode for the basic and neutral pharmaceuticals and negative mode for the acidic pharmaceuticals. The substances were identified using at least two specific transfers of mass in connection with the retention time.

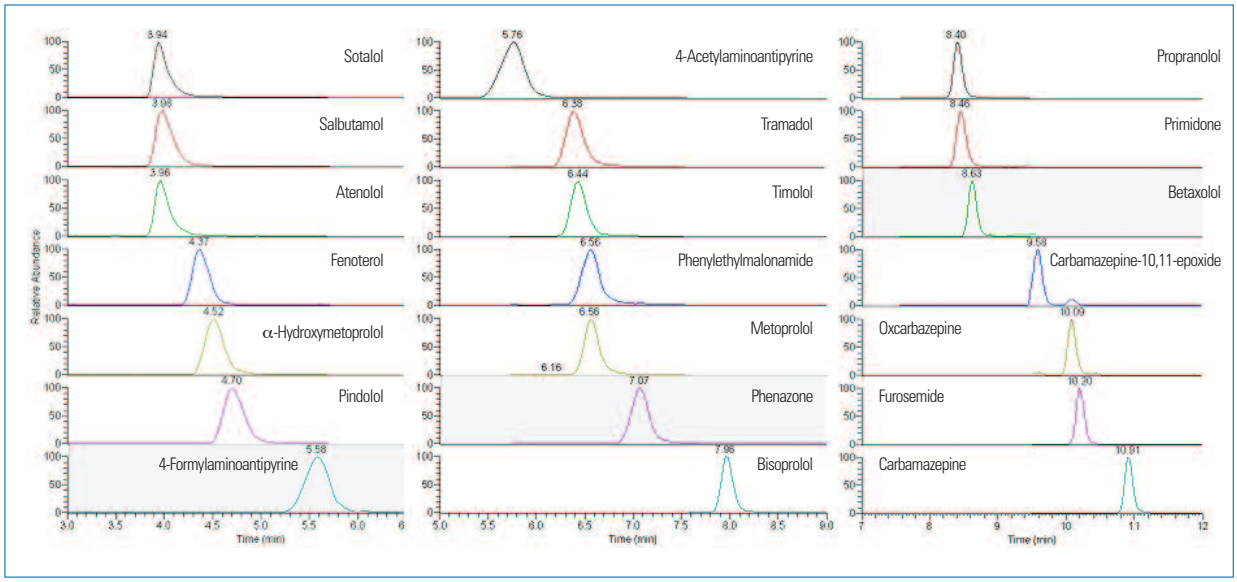

Figure 2: SRM chromatograms of a selection of basic and neutral analytes at a concentration of 50 ng/L monitored in positive ESI.

Results and Discussion

Due to the number of analytes, the LC-MS analytical method was divided into four segments for the basic pharmaceuticals.

The method was calibrated with seven standard solutions over a range of 5 - 150 ng/L, depending on the expected concentrations of the samples. The analytical methods were calibrated prior to each series of measurements and were checked by measuring control samples. Figure 4 shows calibration curves for some of the substances tested. The calibrations were linear in the concentration ranges examined.

The limits of quantitation achieved were between 5 and 10 ng/L depending on the substance and matrix tested.

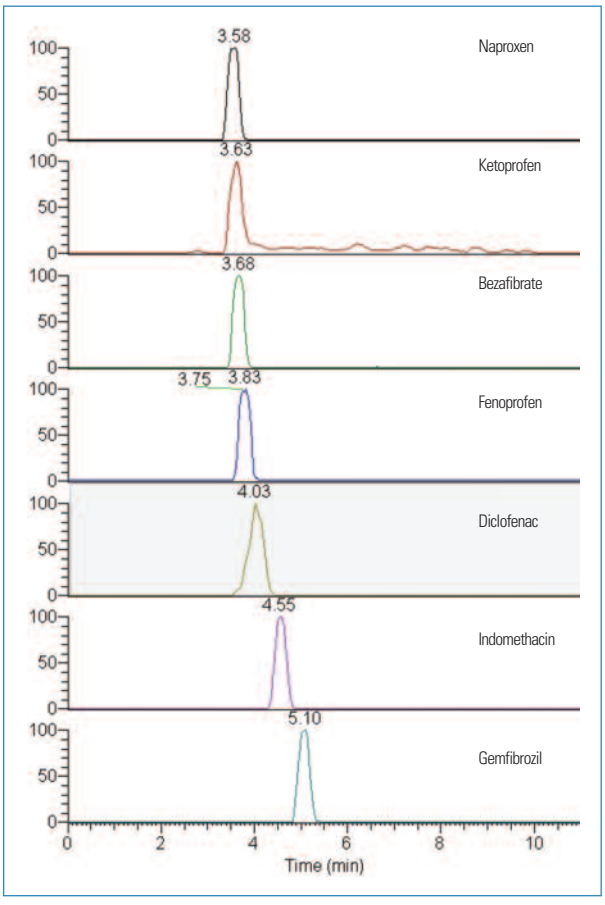

Figure 3: SRM chromatograms of a selection of acidic analytes at a concentration of 50 ng/L monitored in negative ESI

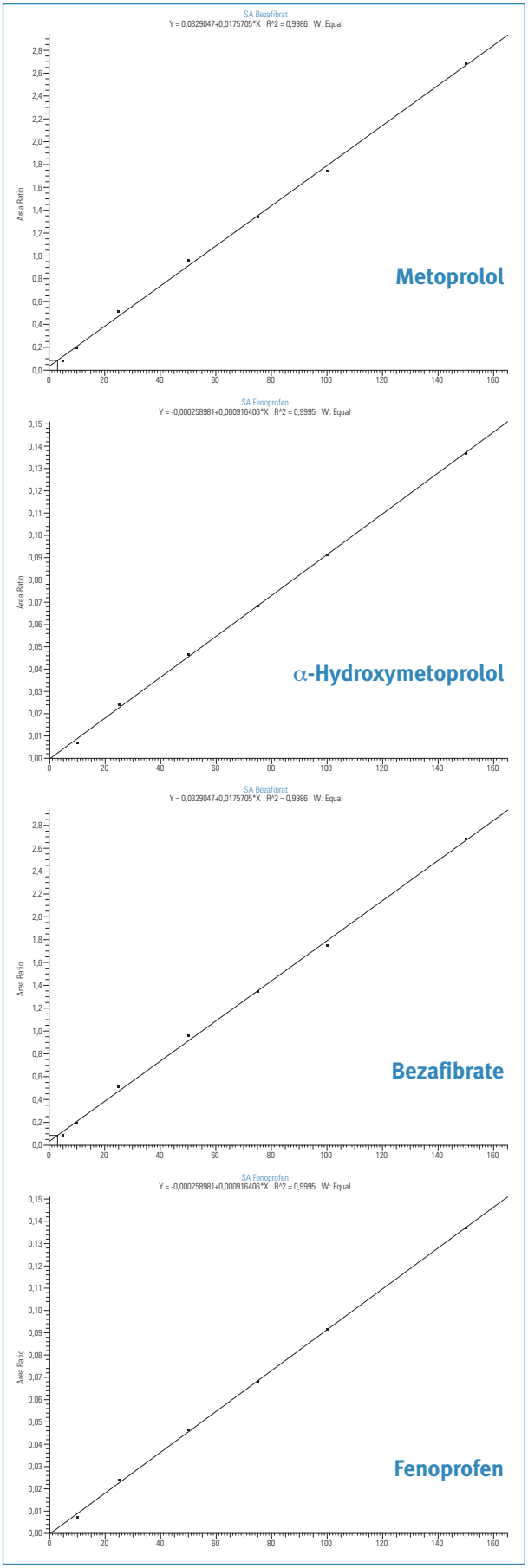

Figure 4: Calibration curves for 4 of the compounds analyzed. The calibration range is from 5-150 ng/L for Metoprolol and Bezafibrate, and 10-150 ng/L for α-Hydroxymetoprolol and Fenoprofen.

Conclusion

An EQuan LC-MS/MS method is described here for the analysis of the most frequently prescribed pharmaceuticals in water with a good level of sensitivity. Comparing the previous offline extraction method to the EQuan online sample preparation method described in this application note, the offline techniques required 20 hours of sample preparation for 12 samples versus 1 hour for the EQuan online technique. Additionally, sample collection requirements were reduced from 1000 mL of water to only 10 mL. This allowed for reductions in shipping costs and made sample collection and transport easier by only requiring a small, 10 mL sample.

References

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