

**Quantification of 35 pharmaceuticals in surface water by direct injection according to DIN 38407-47 [LCMS-8060]**

**User Benefits**

- ◆ Sensitive and robust quantification for routine analysis
- ◆ Method fulfils the criteria of DIN 38407-47
- ◆ Full solution provided by Shimadzu

▪ **Introduction**

The common use of a wide variety of chemicals like pharmaceuticals led to their introduction into the environment. The World Health Organisation (WHO) and national bodies like German Environmental Agency (Umweltbundesamt, UBA) are not able to conduct toxicological studies on all of these compounds. Therefore, UBA established the concept of a health orientation value (Gesundheitlicher Orientierungswert, GOW). GOW is set by UBA for certain compounds based on own toxicological studies. If a GOW is not set for a compound, a general preventive value ( $GOW_1$ ) of 0.1 µg/l is used. The  $GOW_1$  was calculated from limit and recommended values of 50 compounds that are regulated in drinking water in different countries [1, 2]. The resulting  $GOW_1$  is a general guidance value, lower than 140 limit and recommended values for these 50 exemplary compounds. Only for a few potential highly genotoxic compounds UBA sets a value of 0.01 µg/l ( $GOW_0$ ).

▪ **Materials and Methods**

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in water laboratories.



Figure 1: In this study a Shimadzu LCMS-8060 LC-MS/MS detector coupled to a Nexera X3 UHPLC system was used.

To show the performance of the method for complex samples, surface water sample spikes were injected directly after centrifugation at 3000 rpm for 5 min. Early eluting salts were transferred to waste via a divert valve. The resulting robustness allows to use this method in routine analysis.

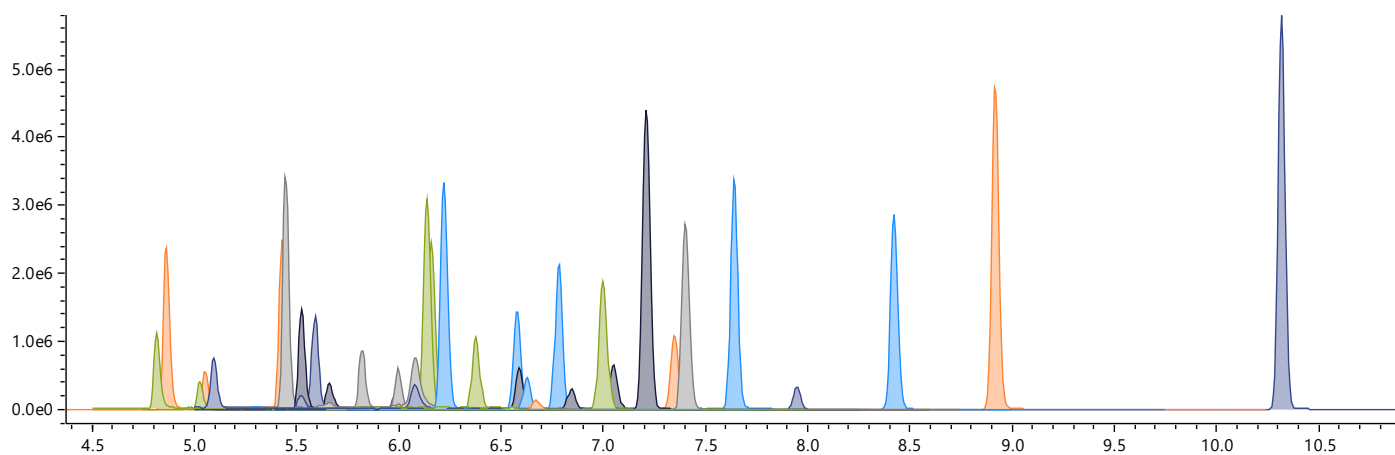


Figure 2: MRM chromatogram, 100 ng/l, surface water, size factor x0.5 for Fenofibrate, Carbamazepine, Bisoprolol

Table 1: Matrix surface water. Results calculated with the calibration method, DIN 32645. Calibration range 0-1000 ng/l, confidence interval 95 %, n = 9-11, depending on LOD. %RSD calculated by peak area. S/N calculated without smoothing. <sup>a</sup> 100 ng/l, <sup>b</sup> GOW<sub>1</sub>, <sup>c</sup> due to high sensitivity. GOW values: [3].

	t <sub>dr</sub> [min] (n = 6)	S <sub>x0</sub> [ng/l]	LOD [ng/l]	Calibration range [ng/l]	r <sup>2</sup>	%RSD (10 ng/l, n = 6)	S/N (10 ng/l)	GOW [ng/l]
Acetaminophen (Paracetamol)	5.094 ± 0.002	0.4	0.8	1-1000	0.9987	5.8	13	100 <sup>b</sup>
Atenolol	4.814 ± 0.003	0.5	1	1-1000	0.9996	4.7	15	100 <sup>b</sup>
Betaxolol	6.475 ± 0.003	2.6	5.6	5-1000	0.9995	6.1	14	100 <sup>b</sup>
Bisoprolol	6.222 ± 0.003	0.3	0.7	1-200 <sup>c</sup>	0.9966	1.5	1224	100 <sup>b</sup>
Candesartan	7.407 ± 0.004	contained in sample (5 ng/l)						300
Carbamazepine	7.214 ± 0.003	contained in sample (4 ng/l)						300
Chloramphenicol	6.675 ± 0.003	0.4	0.8	1-1000	0.9980	8.5	259	100 <sup>b</sup>
Clarithromycin	7.002 ± 0.004	0.4	0.8	1-1000	0.9974	3.4	456	100 <sup>b</sup>
Diazepam	8.426 ± 0.003	0.4	0.8	1-1000	0.9935	1.7	265	100 <sup>b</sup>
Erythromycin A enol ether	6.848 ± 0.004	0.5	1	1-1000	0.9993	7.8	53	100 <sup>b</sup>
Etofibrate	8.920 ± 0.003	0.1	0.3	1-100 <sup>c</sup>	0.9998	0.8	528	100 <sup>b</sup>
Fenofibrate	10.319 ± 0.003	0.3	0.5	1-200 <sup>c</sup>	0.9988	2.4	5568	100 <sup>b</sup>
Gabapentin	5.027 ± 0.003	contained in sample (10 ng/l)						1000
Gabapentin-lactam	6.631 ± 0.003	0.4	0.8	1-1000	0.9993	3.1	25	1000
Metoprolol	5.823 ± 0.003	0.5	1.1	1-1000	0.9996	5	185	100 <sup>b</sup>
N4-Acetylsulfamethoxazol	6.592 ± 0.003	0.8	1.8	2-1000	0.9990	6.8	28	100 <sup>b</sup>
Oxazepam	7.351 ± 0.003	0.5	1	1-400 <sup>c</sup>	0.9989	5.1	159	100 <sup>b</sup>
Oxytetracyclin	5.525 ± 0.004	4.2	8.7	10-100	0.9984	3.5 <sup>a</sup>	35 <sup>a</sup>	100 <sup>b</sup>
Pentoxifylline	6.139 ± 0.003	0.3	0.7	1-500 <sup>c</sup>	0.9990	3.4	172	100 <sup>b</sup>
Phenacetin	6.786 ± 0.003	0.3	0.7	1-400 <sup>c</sup>	0.9993	2.5	162	100 <sup>b</sup>
Phenazon	6.001 ± 0.003	0.4	0.8	1-1000	0.9995	3.1	180	300
Pindolol	5.526 ± 0.003	0.4	0.7	1-1000	0.9992	3.7	277	100 <sup>b</sup>
Pregabalin	5.052 ± 0.003	0.5	0.9	1-1000	0.9955	4.5	36	100 <sup>b</sup>
Primidone	5.986 ± 0.003	0.3	0.8	1-1000	0.9989	9.6	25	3000
Propranolol	6.380 ± 0.004	0.1	0.2	1-1000	0.9993	1.9	44	100 <sup>b</sup>
Propyphenazone	7.644 ± 0.004	contained in sample (1 ng/l)						300
Ritalinic acid	5.449 ± 0.003	0.4	0.6	1-400 <sup>c</sup>	0.9950	2.1	470	100 <sup>b</sup>
Roxythromycin	7.055 ± 0.004	0.6	1.2	1-1000	0.9975	5.7	163	100 <sup>b</sup>
Sotalol	4.861 ± 0.003	0.3	0.6	1-1000	0.9982	2.4	65	100 <sup>b</sup>
Sulfadiazine	5.591 ± 0.003	0.2	0.4	1-1000	0.9995	5.8	111	100 <sup>b</sup>
Sulfamethazine (Sulfamidine)	6.160 ± 0.003	0.5	1	1-1000	0.9988	1.8	414	100 <sup>b</sup>
Sulfamethoxazole	6.581 ± 0.003	0.2	0.5	1-1000	0.9993	3.9	210	100 <sup>b</sup>
Tetracycline	5.523 ± 0.003	0.3	0.7	1-1000	0.9953	3.3	35	100 <sup>b</sup>
Trimethoprim	5.431 ± 0.004	0.4	0.8	1-600 <sup>c</sup>	0.9993	2.9	68	100 <sup>b</sup>
Valsartan	7.951 ± 0.004	0.5	1	1-1000	0.9976	11.9	42	300

Table 2: Liquid chromatography (Nexera X3)

Pump A:	Water ASTM I quality + buffer
Pump B:	Acetonitrile LCMS quality + buffer
Column Oven:	40 °C
Injection Volume:	100 µl (500 µl loop ext. P/N 228-45405-45)
Analytical column:	Shim-pack GIST-HP, C18-AQ, 150x2.1 mm, 3 µm (P/N 227-30765-04)
Flow:	0.4 ml/min (pressure < 230 bar)
Time program:	0-7 min 5>95 %B / 7-10.5 min 95 %B / 10.5-10.8 95>2 %B / 10.8-17 min 2 %B
Divert valve:	0.01 min to waste / 3 min to MS

Table 3: Mass spectrometry (LCMS-8060)

Interface Voltage:	+1.5 / -1.5 kV
Q1/Q3 Resolution:	0.7 Da FWHM (unit)
Desolvation Line:	250 °C
Heating Gas:	15 l/min
Interface Temp.:	400 °C
Nebulizing Gas:	3 l/min
Drying Gas:	5 l/min
Source Distance:	+ 1 mm
Heat Block:	400 °C
Dwell-/Pause-time	2-54 ms / 1 ms
CID	230 kPa
Other Parameters:	Default (tuning file)

The injection volume was 100 µl, an analytical column with moderately polar stationary phase (Shimadzu Shim-pack, GIST-HP, C18-AQ, 150 x 2.1 mm, 3 µm, P/N 227-30765-04) was used. All analytes were detected in MRM mode using 2-3 mass transitions. Method details are shown in tables 2 and 3.

## Results

Good peak shape and sensitivity were achieved. Figure 2 shows an MRM-chromatogram of spiked surface water, 12-90 datapoints per peak. All results are shown in table 1. For each compound, a calibration range from 1 to 1000 ng/l was evaluated. For most pharmaceuticals, calibration curves could be calculated even in the lower range from 1-100 ng/l with correlation coefficients (r<sup>2</sup>) > 0.99 (table 1 and figure 3).

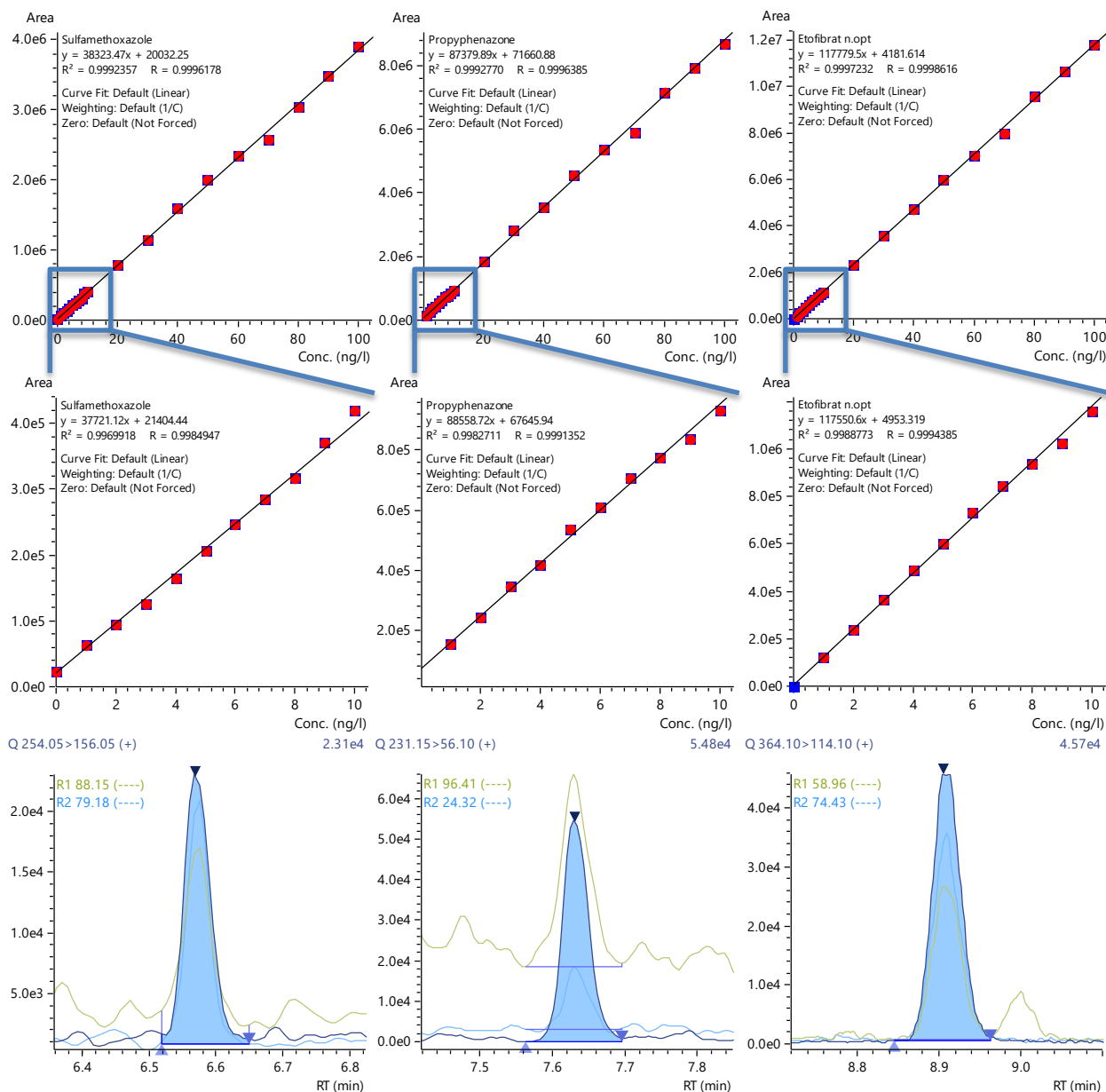


Figure 3: Example for the quantification in the sub-10 ng/l range in surface water. Chromatograms: 1 ng/l. Calibration curves in accordance with DIN 32645.

### Conclusion

The simple method allows the reliable quantification of pharmaceuticals in the lower ng/l-range in surface water. In addition, the divert valve allows an outstanding robustness.

### Literature

- [1] Dieter, H.H. (2014), International Journal of Hygiene and Environmental Health 217(2-3): 117-132
- [2] <https://www.umweltbundesamt.de/themen/wasser/trinkwasser/trinkwasserqualitaet/toxikologie-destrinkwassers/gesundheitslicher-orientierungswert-gow> (downloaded December 22<sup>th</sup> 2020)
- [3] <https://www.umweltbundesamt.de/dokument/liste-nach-gow-bewerteten-stoffe>, version June 2020 (downloaded December 22<sup>th</sup> 2020)



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