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# Pharmaceuticals in groundwaters Analytical methods and results of a monitoring program in Baden-Württemberg, Germany

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**Abstract**

In this paper, analytical methods for the trace-level determination of 60 pharmaceuticals in aqueous samples are presented. The list of compounds amenable to the methods comprises analgesics, antiphlogistics, antirheumatics, β-blockers, broncholytics, lipid-lowering agents (or their metabolites), antiepileptics, vasodilators, tranquillizers, antineoplastic drugs, iodinated X-ray contrast media, and antibiotics of different kind, mainly sulfonamides, macrolides, and penicillins. All methods are based on automated solid-phase extraction followed by GC–MS (after derivatization of the acid compounds) or HPLC–electrospray ionization MS–MS. After an intense validation, which included the determination of performance data according to the German standard method DIN 32645 (limit of detection, limit of identification, limit of determination), the determination of linearity, recovery, and repeatability and the study of matrix effects, the analytical methods were applied within a monitoring program on the occurrence of pharmaceuticals in groundwaters of Baden-Württemberg. During this monitoring program, it was found that several of the compounds under investigation could be detected in groundwaters and their occurrence could be traced back to an impact of municipal or industrial waste water. 

*Keywords:* Water analysis; Validation; Environmental analysis; Beta-blockers; Antibiotics; Sulfonamides; Macrolide antibiotics; Penicillins; Drugs

## 1. Introduction

Due to an incomplete elimination in waste water treatment plants, residues of pharmaceutical products are found both in waste and in surface waters (see e.g. Refs. [1–3] and references cited therein). Initiated and financially supported by the Ministry for Environment and Transport in Baden-Württemberg in 2000 a research project was started in order to obtain systematically extensive and sound analytical data on the occurrence and environmental behaviour of pharmaceutical products and estrogenically active compounds in environmental samples of Baden-Württemberg, a county in the south-west of Germany. Within this research program groundwater samples from 105 monitoring wells in Baden-Württemberg were taken and analysed for a large number of pharmaceuticals and endocrine disrupting chemicals. The selection of the wells was based on information available from former monitoring programs in order to have a representative cross-section of the groundwater in Baden-Württemberg. Part of

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the groundwater wells was influenced by waste water, part by agricultural, industrial or urban activities and part of them was more or less not influenced by any human activities.

A list of 74 target compounds was set up for the monitoring program, including 60 pharmaceuticals and metabolites. In Table 1, the 60 pharmaceutical target compounds are given whereby a classification of the substances under investigation was done according to the analytical method used for their determination. The group I pharmaceuticals diclofenac, ibuprofen, ketoprofen, indomethacine, naproxen, and fenoprofen are used as analgesics, antipyretics, antiphlogistics, or antirheumatics. Clofibric acid, bezafibrate, gemfibrozil, etofibrate, fenofibrate, and fenofibric acid are lipid-lowering agents (or metabolites of them), carbamazepine is an antiepileptic, which is also used as antidepressant. Pentoxifylline is used as a vasodilator and diazepam is a tranquillizer. Group II comprises analgesics (phenazone, dimethylaminophenazone, and propyphenazone), β-blockers (metoprolol, propranolol, atenolol, bisoprolol, sotalol, pindolol, and betaxolol), broncholytics and secretolytics (salbutamol, clenbuterol, and terbutaline), two antineoplastic drugs (ifosfamide and cyclophosphamide), and a lipid-lowering agent (simvastatin). Group III comprises four iodinated X-ray contrast media and groups IV to VI comprise antibiotics of different kind, mainly sulfonamides (group IV), macrolides (group V), and penicillins (group VI). Further information on the selected compounds is given e.g. in Ref. [2]. In addition to the compounds listed in Table 1, the monitoring program covered several endocrine disrupting chemicals like natural and synthetic steroidal hormones, bisphenol A or alkyl phenols which will not be discussed in this paper.

For the determination of pharmaceuticals, different analytical methods are reported in literature, which are mainly valid for biological matrices like blood or urine. For environmental samples only a few methods have been published in the last decade. Most of them deal with the determination of acid or neutral pharmaceuticals like lipid-lowering agents, β-block-

Table 1
Pharmaceuticals under investigation (classification according to the analytical methods used)

| | | | |
|---|---|---|---|
| Group I | Diclofenac | Ibuprofen | Ketoprofen |
| | Indomethacine | Naproxen | Fenoprofen |
| | Clofibric acid | Bezafibrate | Gemfibrozil |
| | Etofibrate | Fenofibrate | Fenofibric acid |
| | Carbamazepine | Pentoxifylline | Diazepam |
| Group II | Phenazone | Dimethylaminophenazone | Propyphenazone |
| | Metoprolol | Propranolol | Atenolol |
| | Bisoprolol | Sotalol | Pindolol |
| | Betaxolol | Salbutamol | Clenbuterol |
| | Terbutaline | Ifosfamide | Cyclophosphamide |
| | Simvastatin | | |
| Group III | Iopamidol | Iopromide | Iomeprol |
| | Amidotrizoic acid | | |
| Group IV | Sulfamethoxazole | Sulfadiazine | Sulfadimidine |
| | Sulfamerazine | Ronidazole | Metronidazole |
| | Furazolidone | Trimethoprim | Dapsone |
| Group V | Chloramphenicol | Virginiamycin | Oleandomycin |
| | Erythromycin | Anhydro-erythromycin | Roxithromycin |
| | Clarithromycin | Spiramycin | Tylosin |
| Group VI | Amoxicillin | Oxacillin | Cloxacillin |
| | Dicloxacillin | Nafcillin | Penicillin G |
| | Penicillin V | | |

ers and broncholytics or antiphlogistics in sewage, surface or drinking water [4–11], some with the determination of antineoplastics in sewage water [12] and some with the determination of antibiotics in different environmental samples [13,14]. As one aim of the research project was to set up a data base on the occurrence of pharmaceuticals in groundwaters in Baden-Württemberg by analysing a maximum number of compounds in a maximum number of samples, we attempted to establish a minimum set of different analytical methods without restrictions to the analytical quality. Hence, different classes of pharmaceuticals were analysed together in one multi-method and, in the end, only six different methods were used for the analysis of the 60 compounds. For each group indicated in Table 1 a different analytical method was applied, whereby all methods were based on automated solid-phase extraction and subsequent determination of the analytes by HPLC–electrospray ionization (ESI) MS–MS or GC–MS, respectively. In all cases MS (or even MS–MS) detection was used in order to ensure a reliable detection and identification of the respective target compounds.

## 2. Experimental

### 2.1. Chemicals

All pharmaceutical compounds under investigation were of analytical grade (>90%) and purchased from Sigma–Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), Promochem (Wesel, Germany), Merz and Co. (Frankfurt, Germany), Abbott Labs (Wiesbaden, Germany), ICN Biomedicals (Meckenheim, Germany), Byk Gulden Lomberg (Konstanz, Germany), or Schering (Berlin, Germany). Fenofibric acid was synthesised by saponification of fenofibrate as described in Ref. [15]. Purification was done by recrystallisation in acetone. The synthesised fenofibric acid was free of fenofibrate impurities as was checked by GC–MS. For anhydro-erythromycin, a decomposition product of the macrolide antibiotic erythromycin [16], no reference material is available. Hence, calibration of this compound was done by spiking tap water with erythromycin. After acidification, transformation of erythromycin into anhydro-erythromycin takes place and quantification of the anhydro-erythromycin can be done via the spiked concentration levels of erythromycin. Pentafluorobenzyl bromide used for derivatization was purchased from Fluka (purity >99%). Solvents used for sample preparation and as mobile HPLC phase were of analytical grade and were obtained from Merck Eurolab (Darmstadt, Germany).

### 2.2. Equipment

For automated sample preparation an Autotrace SPE workstation from Zymark (Idstein, Germany) was used. GC–MS analysis was carried out with a GCQ from ThermoQuest (Egelsbach, Germany) equipped with a split/splitless injector and an ion-trap mass spectrometer. HPLC–ESI-MS–MS measurements were performed on a HPLC system 1090, Series II from Agilent Technologies (Waldbronn, Germany) equipped with an API 2000 triple quadrupole mass spectrometer from PE Sciex (Langen, Germany) using ESI under atmospheric pressure.

### 2.3. Analysis of group I pharmaceuticals

Analysis of the pharmaceutical compounds diclofenac, ibuprofen, ketoprofen, indomethacine, naproxen, fenoprofen, clofibric acid, bezafibrate, gemfibrozil, etofibrate, fenofibrate, fenofibric acid, carbamazepine, pentoxifylline, and diazepam was done by GC–MS after solid-phase extraction on to RP-$C_{18}$ material and derivatization of the acid compounds. In order to have one common method for the neutral and acid compounds sample enrichment was done at pH 3. The derivatization procedure has already been described in literature for the analysis of clofibric acid [8].

Water samples (1000 ml) were adjusted to pH 3 by addition of 16 *M* formic acid. Then, 1 μg of 2,3-dichlorophenoxyacetic acid (2,3-D, 100 ng/l solution in acetone) was added, which was used as internal standard for the overall procedure. Automated solid-phase extraction was done on plastic cartridges filled with 1 g of RP-$C_{18}$ material (IST, Mid Glamorgan, UK). After the enrichment step the solid-phase material was dried in a gentle stream of nitrogen. Elution was done with 4 ml of acetone. The acetone was evaporated to 100 μl in a stream of

nitrogen and to dryness in a drying oven at 50°C. The residue was taken up with 200 μl of a 2% solution of pentafluorobenzyl bromide in cyclohexane and 2 μl of triethylamine. Within 120 min reaction time in a drying oven at 100°C the acid compounds are transferred into their pentafluorobenzyl derivatives whereas the neutral compounds (etofibrate, fenofibrate, carbamazepine, pentoxifylline, and diazepam) remain unchanged.

Determination of the group I pharmaceuticals and the respective derivatives was done by GC–MS. Injection temperature was 275°C and 2 μl were injected splitless for 1 min. A fused-silica capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) of DB 35 type (J&W, Folson, USA) was used. Helium with a purity of 99.9990% was used as carrier gas. For GC separation, the temperature program started at 65°C (held for 2 min), set at 30°C/min to 180°C, set at 5°C/min to 300°C and was held isothermally for 12 min. Detector temperature was 200°C. The ion-trap mass spectrometer was run in the full-scan mode from $m/z$ 80 to $m/z$ 400. Mass fragments used for identification and quantification of group I pharmaceuticals are given in Table 2. Calibration was done between 5 and 200 ng/l using tap water which was spiked with different amounts of the compounds under investigation. If higher concentrations were found the samples were diluted and analysed a second time.

Table 2
Main electron impact ionization fragment masses of the group I pharmaceuticals and their pentafluorobenzyl derivatives, respectively

| Compound | Fragment masses ($m/z$) |
|---|---|
| Bezafibrate (d) | 107, 120, 139, 181 |
| Carbamazepine | 165, 191, 192, 193, 236 |
| Clofibric acid (d) | 128, 130, 169, 171, 181 |
| Diazepam | 110, 165, 177, 221, 256, 283 |
| Diclofenac (d) | 179, 181, 214, 216, 242, 244 |
| Etofibrate | 150, 169, 236, 363 |
| Fenofibrate | 121, 139, 197, 232, 273 |
| Fenofibric acid (d) | 121, 139, 181, 197, 232, 234 |
| Fenoprofen (d) | 91, 103, 181, 197, 225 |
| Gemfibrozil (d) | 83, 122, 161, 181, 309 |
| Ibuprofen (d) | 91, 117, 118, 161, 181 |
| Indomethacine (d) | 111, 113, 139, 141, 181 |
| Ketoprofen (d) | 105, 181, 194, 209, 210 |
| Naproxen (d) | 115, 141, 153, 170, 185 |
| Pentoxifylline | 180, 193, 221, 222, 278 |
| 2,3-D (IS, d) | 111, 113, 147, 149, 175, 177, 181 |

Derivatives are marked by (d); underlined fragment masses were used for quantification.

### 2.4. Analysis of group II pharmaceuticals

For the analysis of phenazone, dimethylaminophenazone, propyphenazone, metoprolol, propranolol, atenolol, bisoprolol, sotalol, pindolol, betaxolol, salbutamol, clenbuterol, terbutaline, ifosfamide, cyclophosphamide, and simvastatin the HPLC–ESI-MS–MS technique was used after solid-phase extraction of the analytes on to PPL Bond-Elut material. Water samples (1000 ml) were adjusted to pH 7 (if necessary) and automatically extracted on plastic cartridges filled with 0.2 g of PPL Bond-Elut material (Varian, Darmstadt, Germany). After enrichment of the water sample the material was dried in a gentle stream of nitrogen. Elution was done with 5 ml of methanol. After evaporation of the organic solvent in a stream of nitrogen, the dry residue was taken up with 100 μl of a mixture of 5% acetonitrile and 95% of a 20 m$M$ aqueous ammonium acetate solution. Determination of the analytes was done by HPLC–ESI-MS–MS. For HPLC separation, a Nucleosil 120-3-$C_{18}$ column (250 mm×2 mm, 3 μm) from Bischoff (Leonberg, Germany) was used. Details on the chromatographic conditions are given in Table 3. An electrospray interface was used and MS detection was done in the positive ionisation mode with an ionisation voltage of +5500 V, except for chloramphenicol which was detected in the negative ionisation mode with an ionisation voltage of −4500 V. Orifice voltage and focusing ring voltage were optimised for each compound by direct injection experiments. Optimum values for both parameters are given in Table 4. Nitrogen (purity 5.0) was used as curtain gas (20 p.s.i.), nebulizer gas (35 p.s.i.), and as turbo gas (45 p.s.i.) (1 p.s.i.=6894.76 Pa). Heater temperature was 200°C. MS–MS detection (MRM, multiple reaction monitoring) was used in all cases. Details on the selection of precursor and product ions are also summarised in Table 4. Again, calibration was done for the overall procedure from tap water samples spiked in a concentration range between 5 and 200 ng/l.

Table 3
Chromatographic conditions for the HPLC separation of group II and group IV pharmaceuticals

| | |
|---|---|
| Separation column | Nucleosil 120-3-$C_{18}$ (250 mm×2 mm, 3 μm) |
| Injection volume | 12.5 μl |
| Flow-rate | 0.2 ml/min |
| Eluents | A: 20 m*M* ammonium acetate in MilliQ water (pH 6.8) |
| | B: 20 m*M* ammonium acetate in acetonitrile–methanol (2:1, v/v) |
| *Timetable* | |
| 0 min | 98% A, 2% B |
| 1 min | 98% A, 2% B |
| 6 min | 90% A, 10% B |
| 20 min | 100% B |
| 29 min | 100% B |
| 29.5 min | 100% A |

### *2.5. Analysis of group III pharmaceuticals*

The analysis of the four iodinated X-ray contrast media iopamidol, iopromide, iomeprol, and amidotrizoic acid was done by HPLC–ESI-MS–MS after solid-phase extraction on to LiChrolut EN material. Water samples (1000 ml) were adjusted to pH 3 by addition of hydrochloric acid and automatically extracted on plastic cartridges filled with 0.2 g of LiChrolut EN material (Merck Eurolab). The further analytical procedure was the same as described for group II pharmaceuticals in Section 2.4, whereby elution was done with 5 ml of methanol and subsequently by 5 ml of acetonitrile. Again, determination of the analytes was done by HPLC–ESI-MS–MS. Details on the chromatographic conditions are given in Table 5. General interface and MS–MS conditions were the same as for group II pharmaceuticals. Details on the optimum orifice and ring voltages as well as precursor and product ions for MS–MS detection are summarised in Table 6. Calibration was done from tap water samples spiked

Table 4
Optimum orifice and ring voltage, precursor and product ions for the MS–MS determination of group II pharmaceuticals

| Compound | Orifice voltage (V) | Ring voltage (V) | Precursor ion (*m*/*z*) | Product ion I (*m*/*z*) | Product ion II (*m*/*z*) |
|---|---|---|---|---|---|
| Atenolol | 41 | 380 | 267.3 | 190.2 | 145.2 |
| Betaxolol | 71 | 60 | 308.3 | 55.2 | 56.2 |
| Bisoprolol | 66 | 240 | 326.6 | 116.3 | 56.1 |
| Clenbuterol | 26 | 360 | 277.1 | 203.0 | 168.2 |
| Cyclophosphamide | 41 | 380 | 261.1 | 140.0 | 106.0 |
| Dimethylaminophenazone | 26 | 290 | 232.1 | 111.0 | 56.2 |
| Ifosfamide | 41 | 380 | 261.1 | 92.0 | 63.2 |
| Metoprolol | 56 | 90 | 268.4 | 116.1 | 74.1 |
| Phenazone | 31 | 50 | 189.0 | 104.3 | 77.1 |
| Pindolol | 21 | 370 | 250.1 | 56.2 | 72.0 |
| Propranolol | 56 | 180 | 260.2 | 183.3 | 116.1 |
| Propyphenazone | 36 | 370 | 231.3 | 189.2 | 56.2 |
| Salbutamol | 21 | 370 | 240.3 | 166.2 | 148.2 |
| Simvastatin | 111 | 70 | 419.0 | 285.3 | 199.3 |
| Sotalol | 26 | 350 | 273.4 | 213.1 | 133.1 |
| Terbutalin | 36 | 320 | 226.1 | 152.2 | 107.0 |

Table 5
Chromatographic conditions for the HPLC separation of group III and group VI pharmaceuticals

| | |
|---|---|
| Separation column | Nucleosil 120-3-$C_{18}$ (250 mm×2 mm, 3 μm) |
| Injection volume | 12.5 μl |
| Flow-rate | 0.2 ml/min |
| Eluents | A: 2 m*M* ammonium formiate in MilliQ water (pH 7.0) |
| | B: 2 m*M* ammonium formiate in acetonitrile–methanol (2:1, v/v) |
| *Timetable* | |
| 0 min | 95% A, 5% B |
| 1 min | 95% A, 5% B |
| 22 min | 50% A, 50% B |
| 23 min | 100% B |
| 29 min | 100% B |
| 29.5 min | 100% A |

in a concentration range between 5 and 500 ng/l due to the elevated levels of iodinated contrast media quite often found in the environment.

### *2.6. Analysis of group IV, group V, and group VI pharmaceuticals*

The analysis of the antibiotics comprised in groups IV–VI was done by HPLC–ESI-MS–MS using different separation and detection conditions but after a common solid-phase extraction on to Isolut ENV+ material. First, 500-ml water samples were adjusted to pH 5 by addition of hydrochloric acid. Then, 1.3 g of ethylenedinitrilotetraacetate (EDTA, disodium salt) were added and the water sample was automatically extracted on plastic cartridges filled with 0.1 g of Isolut ENV+ material (Separtis, Grenzach-Wyhlen, Germany). After sample enrichment of the analytes and drying of the solid-phase material in a stream of nitrogen, elution was done using 5 ml of acetonitrile and subsequently 5 ml of a mixture of acetonitrile–water–triethylamine (90:9.5:0.5, v/v/v). After complete evaporation of the solvent mixture in a stream of nitrogen the residue was taken up again with 100 μl of a mixture of 5% acetonitrile and 95% of a 20 m*M* aqueous ammonium acetate solution. Determination of the three groups of antibiotics was done by HPLC–ESI-MS–MS with three subsequent injections under different HPLC–ESI-MS–MS conditions. Group IV antibiotics could be separated by the same chromatographic conditions as group II pharmaceuticals (Table 3). Details on the optimum orifice and ring voltages and the selection of precursor and product ions for group IV pharmaceuticals are summarised in Table 7. For group V antibiotics slightly different chromatographic conditions were used (Table 8). Details on the optimum set of parameters for MS–MS detection are given in Table 9. Finally, for the HPLC separation of group VI antibiotics the same conditions could be used as for group III pharmaceuticals (Table 5). Optimum pa-

Table 6
Optimum orifice and ring voltage, precursor and product ions for the MS–MS determination of group III pharmaceuticals

| Compound | Orifice voltage (V) | Ring voltage (V) | Precursor ion (*m*/*z*) | Product ion I (*m*/*z*) | Product ion II (*m*/*z*) |
|---|---|---|---|---|---|
| Amidotrizoic acid | 76 | 300 | 614.6 | 361.0 | 233.2 |
| Iomeprol | 81 | 340 | 778.1 | 687.0 | 405.2 |
| Iopamidol | 86 | 350 | 778.1 | 558.8 | 387.0 |
| Iopromide | 96 | 270 | 791.8 | 573.0 | 300.1 |

Table 7
Optimum orifice and ring voltage, precursor and product ions for the MS–MS determination of group IV pharmaceuticals

| Compound | Orifice voltage (V) | Ring voltage (V) | Precursor ion ($m/z$) | Product ion I ($m/z$) | Product ion II ($m/z$) |
|---|---|---|---|---|---|
| Dapsone | 41 | 380 | 249.0 | 155.9 | 107.9 |
| Furazolidone | 41 | 370 | 226.1 | 138.8 | 121.8 |
| Metronidazole | 36 | 190 | 172.1 | 127.8 | 82.0 |
| Ronidazole | 16 | 380 | 201.0 | 140.1 | 109.9 |
| Sulfadiazine | 26 | 380 | 251.0 | 155.9 | 107.7 |
| Sulfadimidine | 26 | 380 | 278.9 | 186.2 | 92.0 |
| Sulfamerazine | 26 | 350 | 265.1 | 172.0 | 156.0 |
| Sulfamethoxazole | 31 | 380 | 254.1 | 155.8 | 107.8 |
| Trimethoprim | 61 | 300 | 291.1 | 261.2 | 230.2 |

Table 8
Chromatographic conditions for the HPLC separation of group V pharmaceuticals

| | |
|---|---|
| Separation column | Nucleosil 120-3-$C_{18}$ (250 mm×2 mm, 3 μm) |
| Injection volume | 12.5 μl |
| Flow-rate | 0.2 ml/min |
| Eluents | A: 20 m*M* ammonium acetate in MilliQ water (pH 6.8) |
| | B: 20 m*M* ammonium acetate in acetonitrile–methanol (2:1, v/v) |
| *Timetable* | |
| 0 min | 80% A, 20% B |
| 3 min | 80% A, 20% B |
| 13 min | 20% A, 80% B |
| 18 min | 100% B |
| 29 min | 100% B |
| 29.5 min | 80% A, 20% B |

Table 9
Optimum orifice and ring voltage, precursor and product ions for the MS–MS determination of group V pharmaceuticals

| Compound | Orifice voltage (V) | Ring voltage (V) | Precursor ion ($m/z$) | Product ion I ($m/z$) | Product ion II ($m/z$) |
|---|---|---|---|---|---|
| Chloramphenicol | −41 | −350 | 321.1 | 152.0 | – |
| Clarithromycin | 61 | 360 | 748.3 | 590.2 | 158.2 |
| Erythromycin | 51 | 380 | 734.5 | 576.4 | 158.2 |
| Anhydro-erythromycin | 56 | 330 | 716.6 | 558.4 | 158.2 |
| Oleandomycin | 46 | 380 | 688.5 | 544.4 | 158.2 |
| Roxithromycin | 61 | 360 | 837.4 | 679.3 | 158.1 |
| Spiramycin | 91 | 290 | 843.4 | 174.1 | 100.9 |
| Tylosin | 96 | 380 | 916.4 | 772.3 | 174.1 |
| Virginiamycin | 61 | 340 | 526.5 | 355.2 | 109.2 |

Table 10
Optimum orifice and ring voltage, precursor and product ions for the MS–MS determination of group VI pharmaceuticals

| Compound | Orifice voltage (V) | Ring voltage (V) | Precursor ion ($m/z$) | Product ion I ($m/z$) | Product ion II ($m/z$) |
|---|---|---|---|---|---|
| Amoxicillin | 11 | 360 | 365.8 | 349.0 | 208.2 |
| Cloxacillin | 51 | 380 | 435.9 | 277.0 | 160.2 |
| Diclocaxillin | 56 | 380 | 469.8 | 311.1 | 160.1 |
| Nafcillin | 46 | 320 | 414.8 | 199.2 | 171.0 |
| Oxacillin | 46 | 360 | 401.8 | 243.2 | 160.2 |
| Penicillin G | 21 | 380 | 335.0 | 176.1 | 160.1 |
| Penicillin V | 46 | 360 | 350.8 | 192.2 | 160.2 |

rameters for MS–MS detection of group VI pharmaceuticals are summarised in Table 10.

## 3. Results

### *3.1. Validation of the analytical methods*

The validation procedure of the different analytical methods comprised the determination of performance data according to the German standard method DIN 32645 [17] (limit of detection, limit of identification, limit of determination), the determination of linearity, recovery, and repeatability and the study of matrix effects. For the calculation of the performance data, a calibration was carried out in spiked tap water with 10 concentration levels in the range of 5–50 ng/l. From the resulting calibration curve the regression coefficient was calculated, characterising the linearity of the calibration function. Furthermore the limits of detection, identification and determination were calculated for each pharmaceutical using the equations given in DIN 32645. For the determination of the repeatability five water samples spiked at a concentration level of 25 ng/l were analysed in parallel. From the results the standard deviation could be calculated. These experiments were carried out both in tap water and in surface water (River Rhine at Karlsruhe). A comparison of the resulting peak areas to the peak areas of a direct injection at the same concentration level yields the recoveries for the solid-phase extraction procedure. Concentrations of the pharmaceuticals under investigation in the original surface water have been taken into account. For the derivatization step no reaction yields could be calculated as the respective derivatives are not available as reference materials. In addition to that, a comparison of the data for the tap water samples and the surface water samples gives information on the impact of the matrix on the analytical procedure. The ''matrix effect'' determined this way comprises extraction effects, derivatization effects (for some of the group I compounds), and ion suppression or enhancement effects for the compounds determined by the HPLC–ESI-MS–MS technique.

In Tables 11–16, some of the resulting validation data are presented. It can be seen that the performance data for most of the pharmaceutical compounds under investigation are excellent. Regression coefficients are $>0.99$ in most cases, indicating a good linearity of the calibration function in the concentration range from 5 to 50 ng/l. Additional measurements proved that this linearity holds true up to a concentration level of 500 ng/l. Even in those cases where the $r$-value was near or below 0.99, no deviation from linearity could be recognised but just a stronger scattering of the data points. For many compounds recoveries are between 75 and 125% in both, tap water and surface water. For a few compounds, recoveries in surface water are higher than 125% (e.g. for bezafibrate and pentoxifylline). This can be explained by very low concentration levels of these compounds in the original surface water which could not be detected before spiking. For the iodinated X-ray contrast media (group III pharmaceuticals) the recoveries are low ($<50\%$, in some cases even $<10\%$). This can be attributed to the extremely high polarity and water solubility of these compounds [18]. But as the other performance data, especially repeatability (standard deviation of a

Table 11
Regression coefficient $r$, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group I pharmaceuticals

| Compound | $r$-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Bezafibrate | 0.985 | 93 | 151 | 7.5 | 24 |
| Carbamazepine | 0.976 | 80 | 74 | 9.6 | 32 |
| Clofibric acid | 0.991 | 77 | 103 | 5.3 | 18 |
| Diazepam | 0.997 | 73 | 99 | 6.9 | 22 |
| Diclofenac | 0.979 | 70 | 70 | 8.7 | 29 |
| Etofibrate | 0.986 | 95 | 101 | 6.7 | 22 |
| Fenofibrate | 0.995 | 86 | 116 | 3.7 | 13 |
| Fenofibric acid | 0.989 | 82 | 113 | 6.4 | 21 |
| Fenoprofen | 0.997 | 71 | 99 | 3.3 | 12 |
| Gemfibrozil | 0.993 | 49 | 89 | 5.2 | 17 |
| Ibuprofen | 0.997 | 67 | 110 | 3.5 | 12 |
| Indomethacine | 0.990 | 86 | 114 | 5.4 | 18 |
| Ketoprofen | 0.991 | 80 | 104 | 4.8 | 16 |
| Naproxen | 0.996 | 68 | 105 | 3.8 | 13 |
| Pentoxifylline | 0.989 | 90 | 134 | 6.5 | 22 |

For details see text.

fivefold analysis <10%) and sensitivity (limit of detection, lod <5 ng/l), are excellent, the low recovery is no drawback for a reliable determination of the contrast media. The same is true for some antibiotics which also have relatively low recoveries (especially the sulfonamides) but excellent data for repeatability (data not presented here) and sensitivity. Limits of detection are below 10 ng/l for all compounds under investigation, underlining the good performance data discussed before.

In conclusion, the validation data proved that the methods established for the groundwater monitoring program are well suitable for the reliable determination of the 60 pharmaceutical compounds listed in

Table 12
Regression coefficient $r$, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group II pharmaceuticals

| Compound | $r$-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Atenolol | 0.998 | 86 | 67 | 2.4 | 8.2 |
| Betaxolol | 0.996 | 70 | 45 | 3.7 | 13 |
| Bisoprolol | 0.996 | 67 | 44 | 3.3 | 11 |
| Clenbuterol | 0.994 | 68 | 37 | 3.8 | 12 |
| Cyclophosphamide | 0.970 | 102 | 71 | 10 | 32 |
| Dimethylaminophenazone | 0.993 | 72 | 66 | 4.3 | 14 |
| Ifosfamide | 0.994 | 87 | 73 | 4.2 | 14 |
| Metoprolol | 0.998 | 96 | 54 | 2.2 | 7.9 |
| Phenazone | 0.996 | 81 | 59 | 3.4 | 12 |
| Pindolol | 0.991 | 83 | 75 | 5.0 | 17 |
| Propranolol | 0.993 | 84 | 48 | 4.6 | 15 |
| Propyphenazone | 0.995 | 89 | 48 | 3.7 | 13 |
| Salbutamol | 0.998 | 80 | 66 | 2.6 | 9.1 |
| Simvastatin | 0.939 | 70 | 53 | 13 | 44 |
| Sotalol | 0.998 | 76 | 81 | 2.3 | 8.0 |
| Terbutalin | 0.993 | 44 | 39 | 4.5 | 15 |

For details see text.

Table 13
Regression coefficient *r*, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group III pharmaceuticals

| Compound | *r*-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Amidotrizoic acid | 0.996 | 9.0 | 7.2 | 3.6 | 12 |
| Iomeprol | 0.993 | 15 | 7.4 | 4.8 | 16 |
| Iopamidol | 0.992 | 19 | 28 | 4.5 | 14 |
| Iopromide | 0.998 | 46 | 29 | 2.3 | 8.0 |

For details see text.

Table 14
Regression coefficient *r*, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group IV pharmaceuticals

| Compound | *r*-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Dapsone | 0.999 | 19 | 6.1 | 2.1 | 7.3 |
| Furazolidone | 1.000 | 29 | 36 | 1.0 | 3.7 |
| Metronidazole | 0.998 | 37 | 33 | 2.7 | 9.3 |
| Ronidazole | 0.996 | 59 | 52 | 3.2 | 11 |
| Sulfadiazine | 0.998 | 25 | 14 | 2.6 | 9.1 |
| Sulfadimidine | 0.997 | 25 | 11 | 2.7 | 9.2 |
| Sulfamerazine | 1.000 | 23 | 11 | 1.0 | 3.5 |
| Sulfamethoxazole | 0.999 | 23 | 21 | 1.8 | 6.2 |
| Trimethoprim | 0.999 | 55 | 50 | 1.3 | 4.8 |

For details see text.

Table 1. Additionally, the successful participation in a round robin test where 35 pharmaceutical compounds (including the four X-ray contrast media) were analysed in both waste water and surface water samples gave a further confirmation for the good performance of the analytical methods [19].

### *3.2. Occurrence of pharmaceuticals in groundwaters in Baden-Württemberg*

In September and October 2000 an extensive monitoring program was carried out in Baden-Württemberg including samples from 105 groundwater wells which were analysed for the 60 pharmaceutical compounds given in Table 1. A statistical evaluation of the overall analytical data yielded that, based on a limit of detection of 10 ng/l, in 66 out of 105 samples no pharmaceutical compound could be found. However, in 39 samples, i.e. in more than one-third of all groundwater samples under investigation pharmaceuticals could be detected. In 24 of these 39 samples only one pharmaceutical compound was found, but there were also samples with four and more positive results. In one sample, nine and in another one even 10 pharmaceuticals occurred.

Among the pharmaceuticals which were detected in at least one sample in a concentration above 10 ng/l, were β-blockers (metoprolol, bisoprolol, sotalol), analgesics (phenazone, propyphenazone),

Table 15
Regression coefficient *r*, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group V pharmaceuticals

| Compound | *r*-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Chloramphenicol | 0.999 | 84 | 84 | 1.8 | 6.4 |
| Clarithromycin | 0.996 | 103 | 108 | 3.6 | 13 |
| Erythromycin | 0.995 | – | – | 3.6 | 12 |
| Anhydro-erythromycin | 0.993 | – | – | 4.2 | 14 |
| Oleandomycin | 0.998 | 79 | 76 | 2.2 | 7.5 |
| Roxithromycin | 0.992 | 82 | 99 | 4.5 | 15 |
| Spiramycin | 0.995 | 68 | 43 | 3.8 | 13 |
| Tylosin | 0.999 | 57 | 59 | 1.9 | 6.7 |
| Virginiamycin | 0.998 | 82 | 75 | 2.2 | 7.8 |

For details see text.

Table 16
Regression coefficient $r$, recovery in tap ($R_{tap}$) and surface water ($R_{surface}$), limit of detection (lod) and limit of determination (LOD) according to DIN 32645 for group VI pharmaceuticals

| Compound | $r$-value | $R_{tap}$ (%) | $R_{surface}$ (%) | lod (ng/l) | LOD (ng/l) |
|---|---|---|---|---|---|
| Amoxicillin | 0.994 | 36 | 36 | 4.6 | 15 |
| Cloxacillin | 0.995 | 98 | 101 | 3.9 | 13 |
| Dicloxacillin | 0.992 | 112 | 119 | 4.6 | 15 |
| Nafcillin | 0.994 | 69 | 82 | 4.4 | 15 |
| Oxacillin | 0.993 | 77 | 76 | 4.6 | 15 |
| Penicillin G | 0.991 | 33 | 44 | 5.0 | 16 |
| Penicillin V | 0.984 | 58 | 63 | 6.5 | 21 |

For details see text.

the antiepileptic carbamazepine, the antirheumatic diclofenac, some antibiotics like the sulfonamides sulfadiazine, sulfadimidine, and sulfamethoxazol, ronidazol, dapson, roxithromycin, and anhydro-erythromycin, the degradation product of erythromycin, and the iodinated X-ray contrast media amidotrizoic acid and iopamidole. As a rule, concentrations of these compounds were between 10 and 100 ng/l but in a few cases, especially for sotalol, diclofenac, carbamazepine, sulfamethoxazol, amidotrizoic acid and iopamidole, concentrations of several hundred ng/l could be found. Table 17 gives a summary of those compounds that could be detected in at least three groundwater samples.

In addition to the analysis of pharmaceuticals the concentration of boron was determined in all groundwater samples. Boron is a good tracer for waste water, especially municipal waste water. A more detailed evaluation of the analytical results showed that in most samples where pharmaceuticals were found, boron concentrations were elevated. Furthermore, other tracer compounds for waste water like the nonylphenols or bisphenol A were also often found in these samples, indicating that the occurrence of the pharmaceutical compounds found in the groundwater is (at least) mainly due to the direct or indirect impact of waste water. This conclusion is supported by the fact that the pattern of compounds found in the groundwater is the same as that found in many surface waters where the impact of waste water of course is more evident.

Table 17
Number of positive results and maximum concentration for all pharmaceuticals which were detected at least three times in 105 groundwater samples

| | No. of positive results (105 samples) | Maximum concentration (ng/l) |
|---|---|---|
| Sotalol | 3 | 560 |
| Phenazone | 5 | 25 |
| Diclofenac | 4 | 590 |
| Iopamidol | 5 | 300 |
| Amidotrizoic acid | 21 | 1100 |
| Carbamazepine | 13 | 900 |
| Anhydro-erythromycin | 10 | 49 |
| Sulfamethoxazole | 11 | 410 |

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