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Ultra-trace analysis of phenols in water using high-performance liquid chromatography with on-line reaction detection

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Abstract

An ultra-sensitive HPLC method for the determination of phenols in water based on flow-through reaction is described. The phenols are concentrated by a two-step liquid–liquid extraction with dichloromethane and after evaporation of the organic solvent the separation is carried out on an octadecyl-silica column by a gradient of water–acetonitrile. Detection is performed by an on-line twin detection system. First dinitrophenols are detected by UV-absorption measurement followed by oxidation of phenols with cerium(IV) in a tubular flow-through reactor and fluorescence measurement of cerium(III). Detection limits in the lower ppt range can be achieved. The influence of reaction time, temperature and reagent concentration on reaction yield was investigated. Identification and quantitation is improved by use of internal standards. The method was applied to samples of surface and drinking water.

1. Introduction

The determination of phenols in very low concentrations in water is a problem frequently encountered in environmental analysis [1–4]. In drinking and surface water they occur in the ppt mass fraction range. It is therefore necessary to carry out an enrichment step prior to separation and detection.

Commonly used methods for sample preconcentration are liquid–liquid and liquid–solid extraction. Liquid–liquid extraction is carried out off-line either discontinuously or continuously [5] or by use of liquid–liquid extraction columns (*e.g.* Chem Elut, Extrelut). Trace enrichment by liquid–solid extraction is achieved by adsorption [2,4,6–10] or by anion exchange [6,11,12] on solid surfaces. For non-polar phenols octadecyl-silicas, for medium polar species copolymer-based adsorbents (XAD, PRP-1, PLRP-S) were used, whereas highly polar phenols are efficiently adsorbed on porous graphitic carbon [13]. Enrichment on solid sorbents can be automated and performed on-line during analysis of the previous sample. If, however, enrichment takes longer than the following analysis steps it is best carried out not in sequence but simultaneously by offering the possibility of enrichment of several samples at the same time. For high-speed operation membrane extraction disks can also be regarded as an alternative [14].

Numerous phase systems have been applied to

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solve the separation problem by HPLC such as reversed-phase columns with isocratic elution [15–24] or gradient elution [4,18,19,25–30], ion-exchange chromatography [1,31,32] and solvent-generated ion-exchange chromatography [33].

Existing solutions for the selective detection in very low concentrations still need improvement. In acidic aqueous solution phenols show an absorption maximum in the 265–280 nm wavelength range, but measuring in this range is hardly selective and the absorption coefficients of most phenols allow mass detection limits in the higher nanogram range [17–19,27–29]. Measuring the native fluorescence of some phenols is more selective and can lead to lower mass detection limits in the higher picogram range [6,28], but most phenols show only low fluorescence or no fluorescence at all. Electrochemical detection is rather sensitive and allows determinations down to the higher picogram range, but the method suffers from a lack of long time stability due to contamination of the electrode surface and a severely drifting baseline at gradient elution [1,3,4,34–37].

Attempts have therefore been made to solve the detection problem by means of reaction detection either by pre-column or post-column reactions leading to reaction products with better detection properties for chemiluminescence [38], electrochemical detection [2,37] or UV absorption [39–44]. Pre-column reactions in which a group is attached to the phenol in order to achieve a more sensitive detection have the disadvantage that phenol derivatives are formed which are more similar than the original phenols and therefore are more difficult to separate. Post-column reactions can be carried out batchwise after collecting fractions of the eluate or in flow-through mode. Carrying out the reaction batchwise is tedious and time consuming, especially in the case of high-performance separations of many analytes, since a large number of fractions has to be analyzed in order to preserve the resolution achieved in the separation. On the other hand post-column reaction detection requires rapid reactions and reaction products with favourable detection properties in high yields. Wolkoff and Larose [45] have described the oxidation of phenols by cerium(IV) sulphate in a flow reactor and the detection of the cerium(III) produced by fluorescence measurement in an attached flow detector. This paper deals with the optimization of this principle and its application for the determination of phenols in surface and drinking water. The combination of a liquid chromatographic separation with the on-line oxidation by cerium(IV) and the selective fluorimetric detection add up to a method selective for phenols with mass detection limits down to the low-nanogram range.

2. Experimental

2.1. Chemicals

For sample preparation the analytical-grade solvents dichloromethane and methanol (E. Merck, Darmstadt, Germany) and the analytical-grade reagents hydrochloric acid, formic acid, acetic acid and sodium hydroxide (E. Merck) were used.

For HPLC analysis acetonitrile (LiChrosolv, E. Merck) and analytical-grade sodium acetate trihydrate (E. Merck) were used. The water was bidistilled in a quartz distillation apparatus.

Analytical-grade cerium(IV) sulphate tetrahydrate, sodium bismuthate (E. Merck) and sulphuric acid (Loba, Fischamend, Austria) were used for post-column reaction.

A priority pollutant phenol standard mixture (Supelco, Bellefonte, PA, USA) was applied for chromatographic calibration. A series of diluted solutions in methanol–water in the ppb to ppt range was prepared.

As internal standards analytical-grade *p*-cresol, 2-chloro-5-methylphenol (E. Merck) and 3,5-dichlorophenol (Aldrich, Steinheim, Germany) were added to the samples. All standard solutions were protected from light and stored at 4°C.

2.2. Apparatus

The chromatographic system used is shown in Fig. 1. It consists of a high-pressure liquid

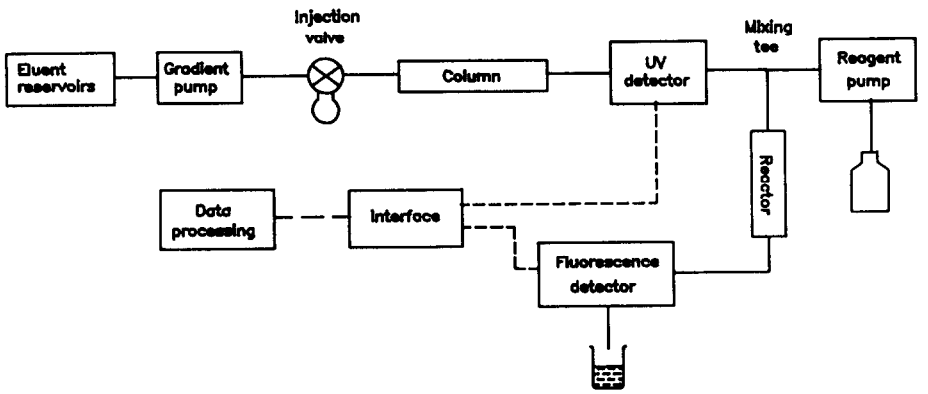

Fig. 1. Scheme of the HPLC apparatus.

chromatograph for gradient elution equipped with an UV–Vis-absorption detector and a flow-through reactor coupled on-line to a fluorimetric detector.

The high-performance liquid chromatograph uses a low-pressure binary gradient former (Model 5000; Varian, Walnut Creek, CA, USA) and an injection valve (Model 7125; Rheodyne, Berkeley, CA, USA) with a 1-ml sample loop.

Separation was performed on a 250 mm × 4 mm octadecyl-silica column (Hibar, LiChrospher RP-18, E. Merck), packed with 5-μm particles.

Absorption detection was carried out by a variable-wavelength spectrophotometer (Model UV 50, Varian) with a 7.9-μl flow cell.

For post-column reaction detection the mobile phase was mixed in a mixing tee with a cerium(IV) solution delivered by a reagent pump (Model 655A-13, E. Merck). A PTFE capillary with the dimensions 1.6 m × 0.5 mm I.D. was used as flow reactor and thermostatted by a column heater (WO Industrial Electronics, Langenzersdorf, Austria). Detection of the reaction product cerium(III) was performed by a flow-through fluorescence spectrophotometer (Model F1000, E. Merck) equipped with a 12-μl flow cell.

Both detector signals were digitized by an intelligent data acquisition module (Model 763; Nelson Analytical, Cupertino, CA, USA). Data storage and data processing were carried out on a personal computer (Model XT; IBM, Boca Raton, FL, USA) by use of chromatographic software (Nelson Analytical).

2.3. Operation

Sample pretreatment

For liquid–liquid extraction of phenols from aqueous solutions solvents offering a high solubility of phenols in the organic phase are used [19]. Dichloromethane was chosen as extraction solvent showing satisfactory large extraction coefficients for phenols in aqueous solution.

The glassware, employed for sample preparation, is cleaned with chromic sulphuric acid, water and methanol. A 200-ml volume of a water sample was filtered through a paper filter (Machery–Nagel, Düren, Germany) to remove solid particles, transferred into a separation funnel and acidified with 2 ml of 4% (v/v) hydrochloric acid, adjusting a pH value between 2 and 2.5. After addition of 200 μl internal standard solution the sample is extracted twice with 20 ml dichloromethane. The combined organic extracts are transferred into a 100-ml round-bottom flask and 0.8 ml of an aqueous solution of 8% sodium hydroxide are added. The organic layer is evaporated at room temperature in a rotating evaporator (Büchi Laboratoriums-Technik, Flawil, Switzerland) and the aqueous residue is transferred to a 25-ml pointed flask, rinsing twice with methanol. The organic solvent is evapo-

rated and the residue (volume *ca.* 0.8 ml) acidified to pH 2.5 to 3.0 with 4 to 5 drops of a solution of formic acid–hydrochloric acid–water (40:10:50, v/v/v). The total volume is used for HPLC analysis.

HPLC separation

Separation is carried out on a hydrophobic adsorbent in acidic aqueous medium suppressing the ionisation of phenols. Elution is performed at constant flow-rate with a binary gradient as given in Table 1. The polar eluent constituent consists of bidistilled water acidified to pH 3.7 with acetic acid which is favourable to achieve peak sharpening and which suppresses asymmetric peak shapes. Acetonitrile is used as organic modifier since it was observed that it causes a lower noise level in fluorescence reaction detection compared to methanol, another organic modifier commonly used for the LC separation of phenols.

Detection

UV absorption detection is performed at 260 nm setting the slit width to 8 nm.

Fluorescence reaction detection is carried out by monitoring the concentration of cerium(III), produced by reaction of cerium(IV) with phenols. The formation of cerium(III) is catalyzed by heterogeneous surfaces like glass walls or precipitants. Special attention must therefore be paid to the selection of the material for the reagent reservoir and the preparation of the reagent solution. Dark glass bottles are used since they were found to be preferable to plastic bottles with respect to the stability of the reagent solution. The reaction is carried out in a 310-μl tubular flow-through reactor. For fluorescence detection the excitation wavelength is set to 260 nm, measuring the emission wavelength at 350 nm.

3. Results and discussion

3.1. Recovery of extraction

For recovery experiments, 200 ml of bidistilled water were spiked by addition of 200 μl of internal standard mixture and variable volumes of the phenol standard mixture (60 ppt–1 ppb). These test solutions were extracted and analyzed by HPLC. Table 2 presents the recoveries found for the phenols included in the study, ranging

Table 1
Gradient programme of one analytical cycle

Time (min)	B (%)	Flow-rate (ml/min)	Comments
0	0	0.8	Analysis
3	27	0.8	
5	32	0.8	
9	35	0.8	
12	37	0.8	
19	37	0.8	
22	40	0.8	
29	47	0.8	
37	100	0.8	
41	100	0.8	End of analysis
43	100	1.5	Cleaning of the column
44	0	1.5	Reequilibration of the column
53	0	1.5	
54	0	0.8	End of the cycle

Eluents: A = water adjusted to pH 3.7 with acetic acid; B = acetonitrile.

Table 2
Recoveries of phenols for a two-fold liquid–liquid extraction from 200 ml acidified water with 20 ml of dichloromethane each time

Analytes	Extraction with dichloromethane and solvent evaporation (%)	Back extraction into alkaline solution (%)
Phenol	61	57
4-Nitrophenol	44	43
2-Chlorophenol	82	75
2-Nitrophenol	93	78
2,4-Dimethylphenol	88	14
4-Chloro-3-methylphenol	94	68
2,4-Dichlorophenol	92	72
2,4,6-Trichlorophenol	95	88
Pentachlorophenol	90	57
2,4-Dinitrophenol	96	93
4,6-Dinitro-*o*-cresol	98	96
Internal standards		
3,5-Dichlorophenol	91	88
p-Cresol	82	55
2-Chloro-5-methylphenol	85	80

Reproducibility: ± (2–4)%, except pentachlorophenol ± 10%.

from 44 up to 98%. Dichloromethane is generally a good solvent for many organic substances. Therefore a reduction of the coextracted compounds was aspired by back extraction of phenols into an alkaline aqueous solution leading to a decrease of the amounts of other substances absorbing in the UV range. Back extraction was achieved with 0.8 ml of an aqueous solution of sodium hydroxide (pH 11), followed by evaporation of the organic solvent. Table 2 shows the recoveries obtained by this procedure. For almost all phenols satisfactory recoveries ranging from 43 to 96% were found except for 2,3-dimethylphenol, for which a recovery of only 14% was observed. Even extraction with a more alkaline solution (pH 12 and above) did not improve the recovery significantly. In view of the fact that the pK_a values of phenols are within a small range, this result may be due to the low solubility of this compound in water [46].

In addition different batches of dichloromethane were tested for interferences in the chromatograms with the phenol peaks at the trace level. Only such batches showing negligible interferences with the analyte peaks were used.

3.2. Separation of priority phenols by HPLC

A chromatogram of a standard mixture of phenols is shown in Fig. 2. In the first part of the chromatogram strongly hydrophilic phenols are eluted, followed by moderate hydrophilic phenols and finally hydrophobic chlorophenols. All phenols are well resolved. Each cycle is completed by a cleaning and reequilibration step.

3.3. Optimization of detection

For selection of an appropriate reservoir vessel several bottles of dark glass were filled with a 10^{-5} *M* solution of cerium(IV) sulphate and allowed to stand for several days. The bottles were then filled with the reagent solution and those showing the lowest cerium(III) level were selected for analysis. Generally the lowest cerium(III) levels are achieved by using fresh double distilled water for the preparation of the reagent. After addition of sulphuric acid and cerium(IV) sulphate, sodium bismuthate was added to reduce the formation of cerium(III)

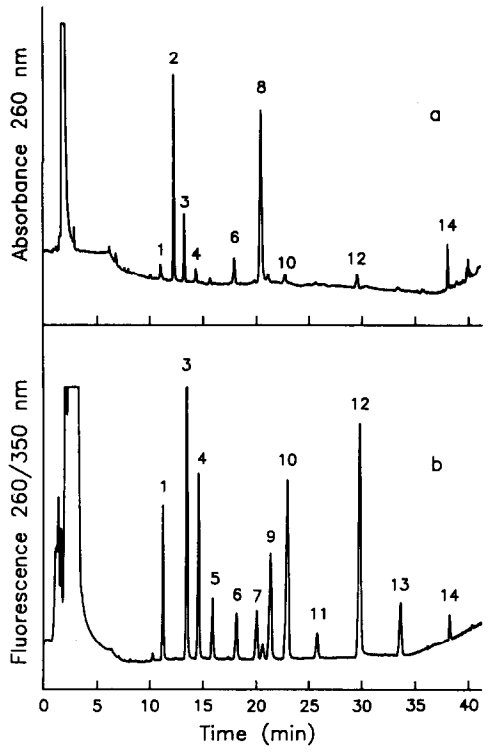

Fig. 2. Chromatograms of standard mixtures of phenols. (a) Absorption detection at 260 nm, (b) fluorescence reaction detection at 260/350 nm. Column: LiChrospher RP-18, gradient see Table 1, eluent flow-rate 0.8 ml/min. Injection volume 200 μl. Reactor: temperature 25°C, reagent 400 mg/l $Ce(SO_4)_2$, reagent flow-rate 0.5 ml/min. Sample: 1 = 50 ng phenol; 2 = 150 ng 2,4-dinitrophenol; 3 = 250 ng 4-nitrophenol; 4 = *p*-cresol (reference); 5 = 50 ng 2-chlorophenol; 6 = 50 ng 2-nitrophenol; 7 = 50 ng 2,4-dimethylphenol; 8 = 250 ng 4,6-dinitro-*o*-cresol; 9 = 2-chloro-5-methylphenol (reference); 10 = 250 ng 4-chloro-3-methylphenol; 11 = 50 ng 2,4-dichlorophenol; 12 = 3,5-dichlorophenol (reference); 13 = 150 ng 2,4,6-trichlorophenol; 14 = 150 ng pentachlorophenol.

due to aging. A solution with a concentration of 400 mg/l $Ce(SO_4)_2$, prepared and stored in the above described manner has a stable cerium(III) concentration for 8 days at a temperature beyond 25°C. Higher temperatures (above 30°C) lead to an untolerable raise of the baseline due to an accelerated formation of cerium(III) within 2 days. The addition of greater amounts of sodium bismuthate (100–200 mg/l) brings only a slight reduction of the cerium(III) concentration, but reduces significantly the yield of the oxidation of phenols.

At the conditions chosen no precipitation of cerium was observed.

The oxidation of phenols by cerium(IV) is strongly influenced by their structure. An overview of the relative reactivity at two different reagent concentrations is given in Table 3. The highest reaction yield is found for phenol itself. Electron-attracting groups like nitro and halogen substituents decrease the reaction rate. If two nitro groups or several halogen atoms are attached to the ring system, the reactivity is drastically decreased.

The reaction yield improves with increasing concentration of the reagent, especially for less reactive phenols. A tenfold increase of the cerium(IV) concentration leads to a 4.4-fold improvement of the yield for 2,4,6-trichlorophenol and to a 5.4-fold improvement for pentachlorophenol and nitrophenols. Therefore analysis was carried out at a high concentration of 400 mg/l cerium(IV) sulphate and operating the detector in the least sensitive mode. In this manner the signal response of accompanying substances with fluorescence properties is reduced.

If two nitro groups are attached to the aromatic ring, the reaction yield is lowered to such an extent that measuring the absorption signal at 260 nm is more favourable than fluorescence. Pentachlorophenol can be measured with comparable intensity by both detection systems, but more selectively with fewer disturbances from accompanying substances by reaction fluorescence detection.

The oxidation reaction is further influenced by temperature. Table 3 shows the influence of temperature on the reactivity of phenols. Increasing the reaction temperature from 30 to 60°C strongly increases the reaction yield of the less reactive compounds. 2,4-Dinitrophenol shows a 19-fold increase, 2,4,6-trichlorophenol and pentachlorophenol a 2.6-fold increase. The baseline shifts however from 10 to 41% full scale, the noise level shows a 2.2-fold increase and the signal intensity of accompanying oxidizeable substances is also increased.

The degree of conversion is further controlled by the reaction time. The reaction yields at 30°C

Table 3
Dependence of the oxidation reaction of phenols with cerium(IV) on the reagent concentration and the temperature

Analytes	(Area units × 10^{-3})/ng analyte			
	Temperature 25°C, concentration $Ce(SO_4)_2$ (mg/l)		Concentration $Ce(SO_4)_2$ 80 mg/l, temperature (°C)	
	40	400	30	60
Phenol	198	199	205	215
4-Nitrophenol	33	90	73	97
2-Chlorophenol	78	105	79	86
2-Nitrophenol	45	66	62	84
2,4-Dimethylphenol	70	102	81	110
4-Chloro-3-methylphenol	78	90	78	90
2,4-Dichlorophenol	44	59	48	69
2,4,6-Trichlorophenol	8	37	16	42
Pentachlorophenol	2	9	7	18
2,4-Dinitrophenol	<1	2	<1	2
4,6-Dinitro-*o*-cresol	<1	2	1	2

Eluent flow-rate 0.8 ml/min; reagent flow-rate 0.4 ml/min, reaction time 0.24 min; reagent concentrations: 40 mg/l $NaBiO_3$, 25 ml/l H_2SO_4; amount of analytes: 50 to 250 ng.

depending on reaction time are shown in Fig. 3. Many phenols have a maximum at 1.5 to 2.0 min. Long reaction times lead to broader peak profiles affecting the chromatographic resolution. Using capillaries with an I.D. of 0.5 mm as reactors and changing the reaction time by using capillaries of varying length from 0.5 to 1.5 min leads at a flow-rate of 0.4 ml/min to a 1.6-fold increase of the peak variance and corresponding decrease of resolution. Simultaneously a shift of the baseline and a magnification of the noise level of the baseline is observed, resulting in reduced dynamic and detection limit.

In conclusion the following conditions for reaction detection were chosen: the optimum reagent solution contains 25 ml/l sulphuric acid, 40 mg/l sodium bismuthate and 400 mg/l cerium(IV) sulphate in bidistilled water.

3.4. Method validation

The analytes are identified chromatographically by measuring their retention times (Table 4). The retention times are highly reproducible. For five injections of a standard mixture of phenols, the relative standard deviation of the retention

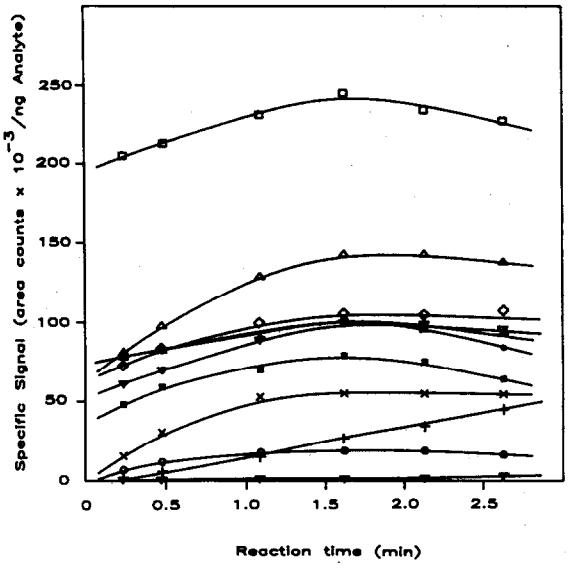

Fig. 3. Effect of reaction time on reaction yield. Column as in Fig. 2. Flow reactor: capillaries of varying length with 0.5 mm I.D., temperature 30°C, reagent flow-rate 0.4 ml/min, reagent concentrations: 80 mg/l $Ce(SO_4)_2$, 40 mg/l $NaBiO_3$, 25 ml/l H_2SO_4. Sample: □ = phenol; △ = 2,4-dimethylphenol; ■ = 2,4-dichlorophenol; ▲ = 4-chloro-3-methylphenol; ● = 2-chlorophenol; × = 2,4,6-trichlorophenol; ○ = pentachlorophenol; ▼ = 2,4-dinitrophenol; ◇ = 4-nitrophenol; + = 4,6-dinitrophenol; ▽ = 2-nitrophenol.

Table 4
Retention time in column and detector

	Analytes	Time (min)	Selectivity
1	Phenol	11.1	
2	2,4-Dinitrophenol	12.1	1.11
3	4-Nitrophenol	13.3	1.12
4	*p*-Cresol (reference)	14.4	1.10
5	2-Chlorophenol	15.7	1.11
6	2-Nitrophenol	18.0	1.17
7	2,4-Dimethylphenol	19.8	1.11
8	4,6-Dinitro-*o*-cresol	20.2	1.02
9	2-Chloro-5-methylphenol (reference)	21.1	1.05
10	4-Chloro-3-methylphenol	23.1	1.11
11	2,4-Dichlorophenol	25.5	1.12
12	3,5-Dichlorophenol (reference)	29.5	1.17
13	2,4,6-Trichlorophenol	33.3	1.14
14	Pentachlorophenol	37.8	1.15

Conditions see Experimental. Hold up time in the reaction detection system is 0.24 min.

times was found to be better than ± 0.9%. Possible retention shifts, due to the influence of accompanying substances, are corrected by the use of internal standards. Identification is performed by calculation of relative retentions with reference to the internal standards *p*-cresol, 2-chloro-5-methylphenol and 3,5-dichlorophenol.

Quantitation of the analytes is achieved by determining the peak area by integration. The chromatographic system is calibrated by repetitive injections of series of dilutions ($n = 4$) made from a standard mixture of phenols. The injection volume was 40 to 500 μl corresponding to 5 ng for the phenol with the lowest concentration and to 1375 ng for the phenol with the highest concentration. The calibration factors, C_i, of the reaction detection system for the phenols were calculated by linear regression of the experimental data according to $x_i = C_i(y_i - y_0)$, where x_i = amount (ng) of analyte i, y_i = peak area (area units), y_0 = intersection of the calibration line and C_i = slope of the calibration line = calibration factor. The values of the calibration factors for the phenols tested are given in Table 5. The calibration factor range describes the selectivity of the reaction detection system. The correlation coefficients of the calibration functions were better than 0.995 with one exception (0.991 for 4-nitrophenol). The precision of the reaction detection system was found to be in the order of 3% for 50 ng except for pentachlorophenol for which a precision of 5% was found. The mass detection limits for the tandem detection system are listed in Table 6. They were found to be in the order of 1 ng except for pentachlorophenol (14 ng). The overall detection limit of the total procedure including sample

Table 5
Calibration: linear regression data

Analytes	Calibration factor (pg/area unit)	Correlation coefficient
Fluorescence detection (260/350 nm)		
Phenol	$1.63 \cdot 10^{-2}$	0.998
4-Nitrophenol	$4.26 \cdot 10^{-2}$	0.991
2-Chlorophenol	$3.10 \cdot 10^{-2}$	0.999
2-Nitrophenol	$3.98 \cdot 10^{-2}$	0.999
4-Chloro-3-methylphenol	$3.55 \cdot 10^{-2}$	0.997
2,4-Dimethylphenol	$2.94 \cdot 10^{-2}$	0.999
2,4-Dichlorophenol	$5.22 \cdot 10^{-2}$	0.999
2,4,6-Trichlorophenol	$7.53 \cdot 10^{-2}$	0.999
Pentachlorophenol	$2.99 \cdot 10^{-1}$	0.996
Absorption detection (260 nm)		
2,4-Dinitrophenol	$6.96 \cdot 10^{-1}$	0.999
4,6-Dinitro-*o*-cresol	$6.56 \cdot 10^{-1}$	0.999

Conditions see Experimental.

Table 6
Detection limits in amounts and mass fractions for a signal-to-noise ratio of 3

Analytes	Detection limit	
	Mass (ng)[a]	Mass fraction (ppt)[b]
Fluorescence detection (260/350 nm)		
Phenol	0.7	5.7
4-Nitrophenol	1.6	16
2-Chlorophenol	1.4	8.7
2-Nitrophenol	1.5	8.5
2,4-Dimethylphenol	1.4	8.5
4-Chloro-3-methylphenol	1.6	8.8
2,4-Dichlorophenol	2.5	11
2,4,6-Trichlorophenol	4.0	17
Pentachlorophenol	14	78
Absorption detection (260 nm)		
2,4-Dinitrophenol	2	8.3
4,6-Dinitro-*o*-cresol	2	8.1

Conditions see Experimental.
[a] Mass detection limits for the detector.
[b] Mass fraction detection limits including sample preparation.

enrichment with respect to the concentration in a water sample of 200 ml can be calculated considering the yield of extraction given in Table 2 and using the data presented in Table 6. The results of this estimation are included in Table 6.

The noise of the reaction detection system was mainly caused by the low frequency of the reciprocating reagent pump. At a reagent concentration of 400 mg/l $Ce(SO_4)_2$ a mean noise level of 0.75% full scale was found. An improvement of the detection limit can be expected by using a pump with a better damping characteristic.

The internal standards were also used to control the sample preparation procedure. They are added to the sample at the beginning of the extraction step and deviations from their standard recoveries (Table 2) allows the calculation of correction factors for the analytes. If a negative deviation greater than 35% from the target value is found for the standards, the result is dismissed and the analysis is repeated. The peak of 3,5-dichlorophenol, which is not disturbed by other test components and was not found in representative surface water samples, was chosen as internal reference for quantitative calculations.

3.5. Application in water analysis

The method was tested by analysing more than 200 samples from rivers and lakes of the Austrian territory and the results were published elsewhere [47]. Samples were collected in regular intervals within one year and treated in the manner described above. Two typical chromatograms of samples of the Danube, taken from the upper and lower course in Austria, are shown in Fig. 4. The legislative stated tolerance limits for priority pollutant phenols (2 ppb) were not exceeded, whereby the relatively highest values were determined in Vienna. Generally the highest content of phenols was found in samples taken in Winter whereas the lowest levels were determined in Summer probably because of the increased biological activity causing degradation of phenols. It was also found that rivers which were known in the past to be polluted, at present have very low concentrations of phenols due to

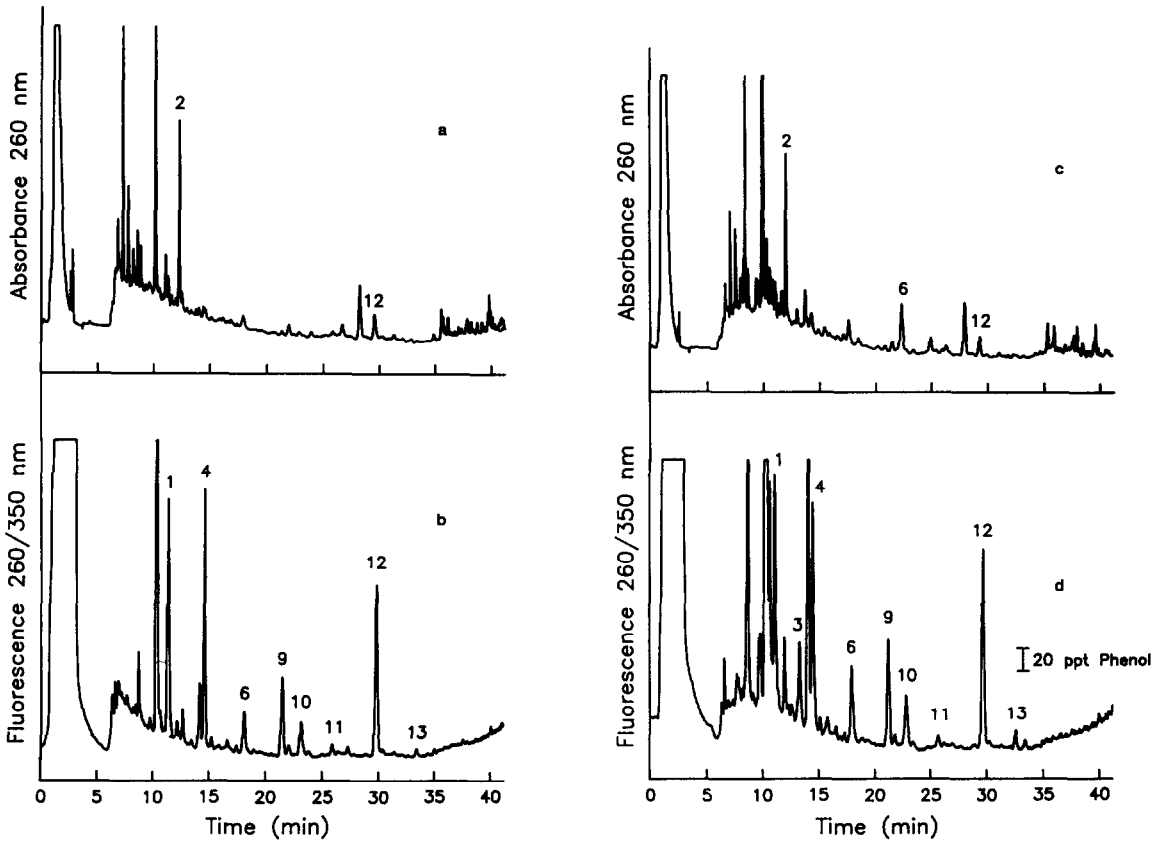

Fig. 4. Chromatograms of samples from the Danube. Working conditions as in Fig. 2 except injection volume 0.8 ml. (a, b) Sampling location at the border to Germany: (a) absorption detection at 260 nm, (b) fluorescence detection at 260/350 nm. (c, d) Sampling location at the border to the Slovak Republic: (c) absorption detection at 260 nm, (d) fluorescence detection at 260/350 nm.

preventions which were taken to improve their water quality.

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