Analysis of phenolic compounds in industrial wastewater with high-performance liquid chromatography and post-column reaction detection

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Abstract

A new method is presented for the characterization of phenols after reversed-phase high-performance liquid chromatographic (RP-HPLC) separation, even in highly complex matrices. Phenols can be detected at a wavelength of 500 nm, immediately after the addition of N-methylbenzothiazole-2-hydrazone and $Ce(NH_4)_2(SO_4)_3$ in a strongly acidic medium, without heating or the use of a reaction coil. Visible spectra and intensities were independent of the water content using typical HPLC eluents. Thirty common hydroxyaromatic compounds were investigated, covering a wide range of substituents. Nearly all phenols showed their maximum absorbance around 500 nm. Limits of detection were between 1 and 20 ng injected onto the column, except for nitrophenols. Aldehydes do not react under these conditions. Under neutral to basic conditions, interferences of thiophenols can be completely abolished. Aromatic amines show strong hypsochromic shifts and decreases in absorption intensity. Using this method, more than 100 different hydroxyaromatic compounds could be detected in heavily loaded tannery wastewater.

Keywords: Water analysis; Derivatization, LC; Detection, LC; Environmental analysis; Phenolic compounds

1. Introduction

Phenols are regularly found in industrial wastewater. Besides their well-known influence on the taste and odor of fish and water, some of them reveal toxic effects [1,2] to aquatic biota. Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods have been reviewed for analyses of phenols in water [3] and, more recently, capillary electrophoresis has also been applied for some separations [4,5]. In general, HPLC methods [6,7] offer milder conditions for sample preparation

than GC [8,9] methods and, therefore, volatile phenols are prevented from being lost. After HPLC separations, target phenols can be identified by means of a diode-array detector [10,11]. Quite regularly, however, chromatograms exhibit a lot of additional unidentified peaks, which might include a high number of unknown phenolic compounds. Thus, it was an aim of this study to develop a method for characterizing unknown phenols in highly complex matrices that was both sensitive and selective.

There are few reports on unknown compound identification using other coupling techniques such as HPLC–particle beam mass spectrometry (MS) [12,13]. HPLC–Fourier transform infrared spectros-

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copy coupling has not found its way into environmental analysis yet, though it might be valuable. Most recently, characterization of phenols using electrochemical detection for both HPLC and flow injection analysis (FIA) has been suggested [14]. However, passivation of electrode surfaces is still an unsolved problem for heavily loaded samples.

In several reviews, HPLC post-column reactions are shown to be quite useful for the detection of certain functional groups [15,16]. However, only a few attempts have been made to characterize aromatic hydroxy compounds by means of post-column reaction detection. Detection of phenols was greatly enhanced by a post-column photochemical reaction [17], but an additional pre-column dansylating derivatization step was required. 4-Aminoantipyrine has been used as an oxidative coupling reagent for the determination of phenols in wastewater [18] and the reaction has also been applied for a HPLC post-column method [19]. However, this reagent does not permit detection of most 4-substituted phenols [20], and aromatic amines are known to interfere in water analysis [21]. Some further reagents for the determination of total phenols in water, such as *p*-aminophenol [22] or *o*-tolidine [23], are even more restricted to certain classes of phenols and, in general, are less sensitive.

The most promising alternative reagent was N-methylbenzothiazolone-2-hydrazone (MBTH). A general reaction mechanism was proposed (Fig. 1) [24] and *p*-substituted phenols were shown to react with MBTH as well as phenols with substituents in the *o*- and *m*-position [25].

This study demonstrates that the reaction of MBTH with phenols is readily applicable to HPLC post-column characterization of phenolic moieties, in general. Reaction conditions were adopted to HPLC solvents, and visible spectra and intensities of the reaction products of phenolic compounds are compared to interferences with pseudo-phenolics. Criteria for the identification of unknown hydroxy-aromatic compounds are proposed and a method for differentiating between phenolic peaks from aromatic amines and thiophenols is shown. Finally, HPLC analyses of different wastewater samples using post-column reaction detection are presented.

2. Experimental

2.1. Chemicals

Solid-phase extraction cartridges from the following companies were used: 500 mg cartridges Supelclean EnviChrom from Supelco (Bellefonte, PA, USA), 500 mg LiChrolut RP-18e and 250 mg LiChrolut EN from Merck (Darmstadt, Germany). All solvents were from Merck and were of gradient grade quality. Double-distilled water was used for standard solutions. Test compounds, as given in Table 1, were purchased from the following companies: Nos. 1–3, 6–7, 9–10, 13–15, 19–20, 28, 33–34 were from SAF (Deisenhausen, Germany), No. 17 and the hydroxytriazenes, of Pestanal quality, were from Riedel-de Haen (Seelze, Germany) and all others were from Merck. Cellulose acetate filters were obtained from Sartorius (Göttingen, Germany).
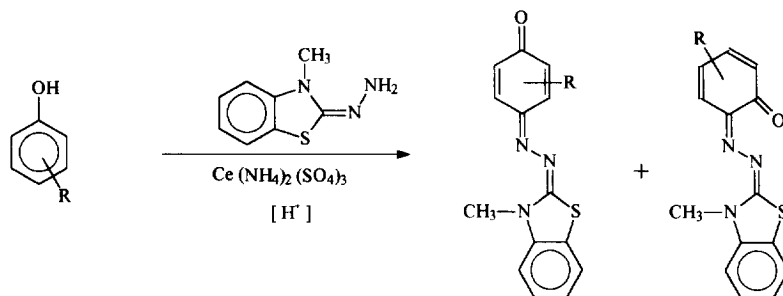

Fig. 1. Reaction of phenolic compounds with MBTH–Ce^{IV}.

2.2. Analytical apparatus and procedure

UV absorbance for manual assays was determined on a lambda-12-spectrophotometer (Perkin-Elmer, Überlingen, Germany) using 1.4-ml sample volumes and the addition of 500 µl of reagent solution to each. HPLC analysis was carried out on a 250×3 mm Eurosphere 100 C_8 column, 5 µm (Knauer, Berlin, Germany). The HPLC system was equipped with an L-6200A gradient pump, an AS-2000 A autosampler and a T-6300 column thermostat (all from Merck-Hitachi, Darmstadt, Germany). UV detection was performed with a Gynkotek 540 diode array detector (Munich, Germany) with a wavelength range from 195–595 nm. Comparison of UV spectra was carried out using spectral libraries provided by Gynkotek (500 entries) and our own library (100 entries). Post-column reagents were added using a 655A-13 reaction pump that was obtained from Merck. A Knauer 64 HPLC pump was used for a stepwise pH shift of the reaction mixture using buffer solutions.

LC separations were performed with acetonitrile–water mixtures. Pure water containing 1 mmol/l NaH_2PO_4 was adjusted to pH 3.05 with H_3PO_4 and served as solvent A. Solvent B was an acetonitrile–water or a methanol–water mixture (95:5, v/v). Standards were analyzed with isocratic elutions of various ratios of A:B at a flow-rate of 0.7 ml/min. Injection volumes were between 2 and 50 µl, depending on the content of analytes in standard samples. Wastewater analyses were begun with 80% A, followed by a 5-min isocratic elution, and by a linear gradient to 30% A at 10 min. The flow-rate was held constant at a flow-rate of 0.7 ml/min. A 0.15% aqueous MBTH–HCl (SAF) solution and a 0.4% solution of $Ce(NH_3)_2(SO_4)_3$ (Merck) in 5% (v/v) H_2SO_4 were employed as post-column reagents. Reagents were sequentially added, each at a flow-rate of 0.2 ml/min, over a T-piece without additional mixing chambers or reaction coils for wastewater samples. Heat dependencies of the reaction were investigated in the HPLC post-column mode using a 15-m self-knitted PTFE capillary (0.5 mm I.D., Latek, Heidelberg, Germany) as an additional reaction coil and a Waters TCM temperature control module (Milford, MA, USA). A 7% NaOH solution, buffered with 2.5% boric acid containing 1% Na–EDTA, shifted the eluent stream into alkaline pH values to abolish interferences of aldehydes, thiophenols and aromatic amines.

3. Samples

The tannery wastewater treatment pilot plant is described in detail elsewhere [26]. Extractions followed a sequential solid-phase extraction scheme that we proposed recently [27] for non-target analyses of both polar and non-polar analytes. Untreated, anaerobically and aerobically cleaned tannery wastewater samples were filtered over 0.45 µm cellulose acetate filters. Each sample was divided into two 250 ml portions, and each portion was passed sequentially through four extraction cartridges. At first, the aqueous samples were passed over a $C_{18}e$ cartridge at pH 7 for the extraction of highly hydrophobic compounds. The resulting filtrate was extracted using an EnviChrom tube at the same pH value. Recovery tests with target phenols [27] concluded that simple phenols and cresols would be recovered at this extraction step, whereas more hydrophilic phenols, such as phenolic carboxy acids or dihydroxy aromatic compounds, require acidic conditions to be extracted. Therefore, the resulting filtrate from the EnviChrom cartridge was acidified to pH 4.5 and was extracted in a third extraction step using a LiChrolut EN phase that has proven to be more suitable for polar phenols. In the final extraction step, a LiChrolut EN phase was used at pH 2.5. Using this procedure, total recoveries of target phenols of 80–97% could be achieved [27].

All cartridges were conditioned with 7 ml of methanol and 10 ml of water prior to use. Extraction rates were 1–2 ml/min. After extraction, the cartridges were washed with 10 ml of water of the pH value and ionic strength of the sample and were eluted with 7 ml of methanol. They were aspirated for about 30 s, to prevent the loss of volatile phenols. After combining the eluates from the two corresponding portions, they were concentrated in a SpeedVac concentrator (Savant, Farmingdale, ME, USA) to a final volume of about 1 ml, at a chamber temperature of 40°C. Therefore, the samples were preconcentrated to about 500-fold. Aliquots (10 µl) were injected onto the column. Each sample was run

twice; once with and the second time without derivatization.

4. Results and discussion

4.1. Optimization

In a former study, reagent concentrations of MBTH and Ce^{IV} were varied independently using a FIA method with 3 m reaction coils [28] for the determination of total phenols in water. Absorption maxima of phenol standards varied between 340 nm (hydroquinone) and 580 nm (p-aminophenol). Therefore, no analytical wavelength for the determination of unknown compounds could be suggested, and use of the method for the determination of total phenols was rejected. The baseline was found to be most stable at a 2.5–5-fold excess of $Ce(NH_4)_2(SO_4)_3$ compared to the MBTH content in the FIA study. Thus, we chose a constant ratio of 2.7 for HPLC post-column reaction detection.

With both methanol–water and acetonitrile–water mixtures, bathochromic and hyperchromic effects were observed [29], so the reaction might perform even better under HPLC conditions. Under aqueous conditions, however, $Ce(OH)_4$ precipitated at pH values that were higher than 2.5. Its solubility might be reduced even further in organic solvents and, therefore, we investigated the dependence of precipitation on pH using organic solvent–water mixtures in this study.

For acetonitrile- or methanol–water mixtures (80:20, v/v), at least 1.8% H_2SO_4 solutions (v/v) of the Ce^{IV} reagent were needed to keep the reaction mixture clear of fine needles. Contrary to results obtained with 0.4% H_2SO_4 solutions for the FIA method, visible spectra of hydroquinone and p-aminophenol overlapped strongly with the phenol spectrum (Fig. 2) under these acidic conditions. However, the reaction was not completed within 2 min for several substituted phenols (e.g. phenolic carboxy acids and hydroxynaphthalene sulfonic acids). Additional time delay reaction coils would have been needed for sensitive detection in the HPLC post-column mode, causing unwanted peak broadening.

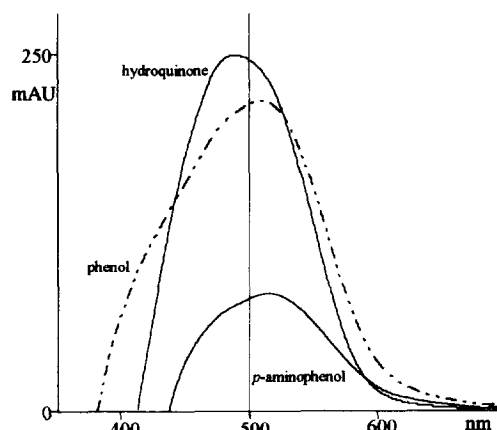The speed of color development was greatly

Fig. 2. Vis spectra of phenols after derivatization with MBTH–Ce^{IV} under highly acidic conditions.

improved by raising the content of H_2SO_4 of the Ce^{IV} reagent up to 5% (v/v). In batch tests, the reaction was complete for nearly all of the tested phenols immediately after addition of the reagents (Fig. 3). Only a few compounds needed more than 5 s for full color development, such as the slowest reacting 5-methoxy-2-hydroxybenzoic acid. Slight hindrances of other molecules (1,2-dihydroxybenzene, 1-hydroxynaphthalene-2,4-disulphonic acid) cannot be explained by molecular structure, since similar compounds reacted immediately (1,4-dihydroxybenzene and 2-hydroxynapthalene-3,6-disulfonic acid). Additional substituents (-alkyl, -chloro, -hydroxy, -amino, -sulfonic acids or -carboxy acid) did not have a significant electronic influence. Steric effects do not severely hamper the reaction, either. Both 4-tert.-butylphenol and 2,4-di-tert.-butylphenol showed immediate and complete reaction, whereas 2,6-di-tert.-butylphenol was just a little slower. According to the reaction mechanism, nucleophilic attack at the m-position is extremely retarded. Using 2,6-di-tert.-butyl-4-methylphenol, there was only a slight reaction after 12 min. Phenol itself showed a slight, but linear decrease in its visible absorption. This effect might be caused by further oxidation of the colored product by excess Ce^{IV} reagent or by protonation of the colored products. However, further investigation of the kinetics and mechanisms of the reaction was beyond the scope of this study.

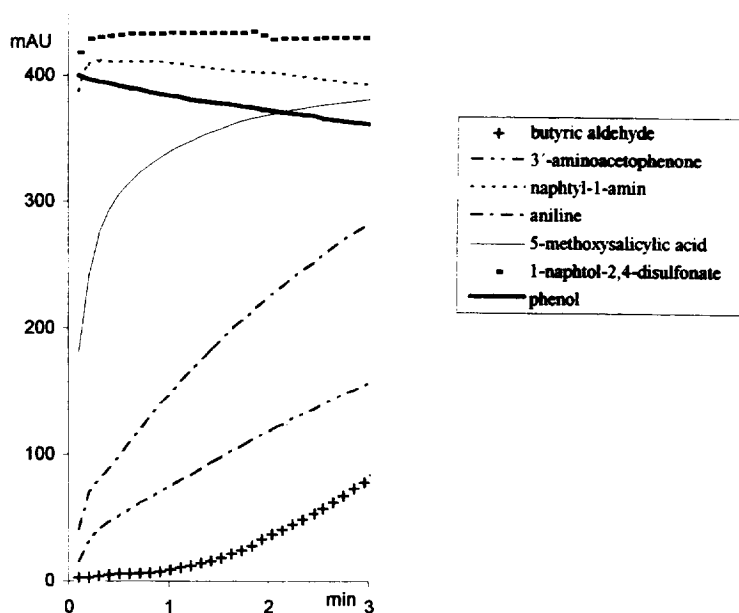In summary, rapid color development was com-

Fig. 3. Reaction development of phenols (20–200 μmol/l), aromatic amines (70–240 μmol/l) and butyric aldehyde (1000 μmol/l) at 500 nm.

plete within 20 s for all of the tested phenols. For each compound, at least 50% intensity was achieved within 3–8 s, compared to its maximal absorbance, which was determined after a 10-min reaction time.

Thirty hydroxyaromatic compounds were tested in manual assays to determine peak maxima and band widths. The results obtained with a Ce^{IV} solution containing 5% (v/v) H_2SO_4 are given in Table 1. The mean maximum absorption wavelength was 517 ± 20 nm. In fact, at 500 nm, nearly all of the phenolic compounds showed at least 85% or more of the intensity at their absorption maximum (average $94 \pm 6\%$). There was no significant difference between phenols (1–13, 16–19, 22–26, 29–30) and naphtols (14, 15, 27). For three compounds, however, Vis spectra were obtained that had less than 80% of the maximum intensity at 500 nm. Two of them (20, 28) were N-containing heterocyclic hydroxyaromatic compounds. For the third (H-acid, 21), azo dye formation might have driven maximum absorption to longer wavelengths, since H-acid is a common dye precursor. Interference of the amino group is rather unlikely since there was no hypsochromic shift and no decrease of intensity between pH 8 and pH 10 (see below). In conclusion, N-atoms attached to the aromatic ring may cause a batho-

chromic shift of about 50 nm.

4.2. Selectivity

Selectivity was investigated using aldehydes, primary aromatic amines, thiophenols, hydroxytriazenes, benzothiazole and methoxybenzene.

Hydroxytriazenes (desethylhydroxyatrazine, desisopropylhydroxytriazine, ammeline, cyanuric acid and ammelide), benzothiazole and methoxybenzene did not react at all. This illustrates that the reaction seems to be limited to true aromatic systems containing XH groups.

Aldehydes are known to react with MBTH and oxidizing Fe^{III} salts [30]. Using $Ce(NH_4)_2(SO_4)_3$ as the oxidizing agent, glyoxylic acid, butyric aldehyde and benzaldehyde showed absorption maxima between 620 and 665 nm and absorption minima between 450–500 nm (10% of their maximum intensities). Additionally, a reaction time of at least 1 min was needed to detect 1 mM solutions of short-chain aliphatic aldehydes in batch tests at 500 nm, and the reaction was not complete within 10 min. Benzaldehyde was even less reactive. So, aldehydes, in general, should not interfere with the HPLC on-line detection of phenols at 500 nm.

Table 1
Vis spectra data of standard compounds after derivatization with MBTH–Ce^{IV} and detection limits for HPLC post-column applications

No.	Compound	λ_{max} (nm) at pH 0	85% band width (nm)	Detection limit at 500 nm (ng)	λ_{max} (nm) at pH 9.5	85% band width (nm)
1	2-Methylphenol	505	473–534	5	503	474–532
2	2,4-Dihydroxybenzoic acid	519	484–557	2	492	460–517
3	4-Hydroxyphenylacetic acid	527	486–578	4	553	522–584
4	4-Hydroxycinnamic acid	534	486–578	7	567	524–607
5	4-Hydroxybenzoic acid	494	455–530	3	522	496–557
6	4-Aminophenol	512	477–540	–	410	350–471
7	1,4-Dihydroxybenzene	485	458–524	4	493	350–534
8	1,2-Dihydroxybenzene	500	472–526	5	527	477–566
9	4-Chloro-2-methylphenol	512	478–540	2	509	484–534
10	2-Methoxyphenol	503	480–525	2	503	481–525
11	2,6-Dimethylphenol	494	473–513	9	494	474–512
12	2-Hydroxy-5-methoxybenzoic acid	523	470–552	18	473	428–508
13	3-Methylphenol	513	470–543	6	510	486–535
14	1-Hydroxynaphthalene-4-sulfonic acid	520	495–544	5	520	486–535
15	2-Hydroxynaphthalene-1,4-disulonfic acid	473	426–502	26	519	493–544
16	4-Amino-2-hydroxybenzoic acid	536	488–576	3	504	495–545
17	Pentachlorophenol	538	442–565	190	524	430–565
18	Phenol	505	462–536	2	516	492–541
19	4-Methylphenol	541	506–570	8	548	519–578
20	8-Hydroxyquinoline	558	532–583	–	512	488–536
21	4-Amino-5-hydroxynaphthalene-2,7-disulfonic acid	585	558–604	19	584	548–619
22	1,3,5-Trihydroxybenzene	474	403–506	5	408	381–442
23	4-tert.-butylphenol	532	484–562	–	547	517–582
24	2,4-Di-tert.-butylphenol	546	400–578	–	529	499–554
25	2,6-Di-tert.-butylphenol	525	493–547	–	–	–
26	2,6-Di-tert.-butyl-4-methylphenol	548^a	350–592	n.d.	492	476–505
27	1-Naphthol	505	484–532	–	510	485–536
28	5-Hydroxbenzothiazole	547	525–572	–	551	530–572
29	2,4-Dinitrophenol	510	455–548	96	n.d.	n.d.
30	4-Nitrophenol	506	459–550	72	n.d.	n.d.
29	3'-Aminoacetophenone	571	549–588	–	472	489–449
30	4-Aminobenzoic acid	564	538–583	–	420	350–436
31	1-Naphthylamine	581	557–602	–	461	442–478
32	Aniline	565	544–581	63	459	440–477
33	1,2-Diaminobenzene	585^b	350–607	–	410	384–439
34	4-Chloroaniline	569	557–579	–	364	350–379
35	Thiophenol	420	400–457	18	396	383–410
36	2-Naphthylthiol	420	396–468	–	392	372–404
37	2-Mercaptobenzothiazole	470	430–491	–	396	382–408

n.d.=not detected; – =not determined.
^{a} 12-min reaction time.
^{b} no clear max., shoulder.

Thiophenols show an immediate reaction with MBTH, resulting in absorption maxima between 420–470 nm (see Table 1). Therefore, thiophenols would give false positive results in phenol analysis at 500 nm with 40–80% of their maximum intensities. As in the case of heteroaromatic hydroxy compounds, nitrogen atoms attached to the aromatic system caused a bathochromic shift of about 50 nm (No. 37). However, these interferences could be abolished completely by adding a 1% solution of Na–EDTA in 7% NaOH and 2.5% H$_3$BO$_3$ at a flow-rate of 0.4 ml/min, to reach a final pH of 8.5.
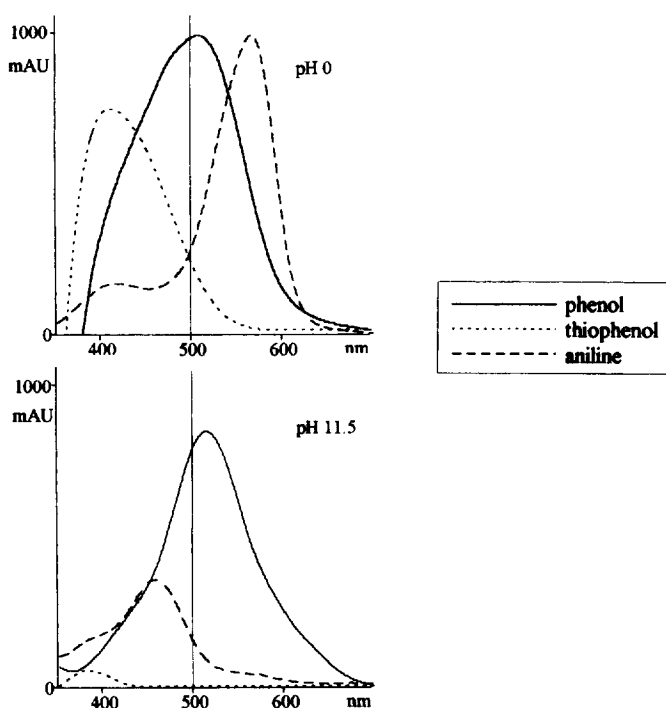
Fig. 4. Interferences of thiophenols and aromatic amines at pH 0 and pH 11.5.

In batch tests, decolorization occurred immediately after addition of buffer solutions (Fig. 4).

Aromatic amines reacted at maximum wavelengths between 565–585 nm with 20–50% of their maximum intensities at 500 nm. Reaction in batch tests was mostly slower and less sensitive than for phenols. Naphthyl-1-amine, however, showed an immediate and sensitive reaction. So, interferences in HPLC post-column characterization of phenolics might occur. In alkaline media, aromatic amines undergo a mean hypsochromic shift of >100 nm (see Fig. 4 and Table 1). Additionally, the intensities at optimal wavelengths were greatly reduced between pH 0 and pH 11 for all of the tested aromatic amines (Fig. 5). However, at 500 nm, the absorption intensities remained essentially the same before and after the addition of alkali.

In contrast to the results obtained with thiophenols and amines, the intensities of both phenols and heteroaromatic hydroxy compounds were not severely affected by pH changes (Fig. 5), and decreases were only due to the added buffer volume. In fact, some phenols even showed hyperchromic effects at

pH 8.5 (Nos. 6, 7, 15 and 20). Some phenolic compounds underwent slight bathochromic shifts at pH 8–10 (see Table 1). Others showed no effect or even slight hypsochromic shifts, therefore, no single analytical wavelength with more than 75% intensity band widths (517 nm) can be suggested for the detection of unknown hydroxy aromatic compounds at alkaline pH values.

4.3. Application as HPLC post-column reaction

As for the batch tests, the reaction of MBTH and phenolic compounds was tested as a HPLC post-column method for Ce^{IV} solutions containing 1.8 and 5% H_2SO_4.

Using Ce^{IV} solutions containing 1.8% H_2SO_4, the baseline at 500 nm was noisy, with peak intensities of up to 2 mAU. Due to the slower reactivity compared to 5% H_2SO_4 solutions, an additional 15 m coil was employed for some further reaction time delay. Heat dependencies were studied between 20 and 80°C, and some analytes showed an increase of intensity up to 60% at reaction temperatures of 80°C.
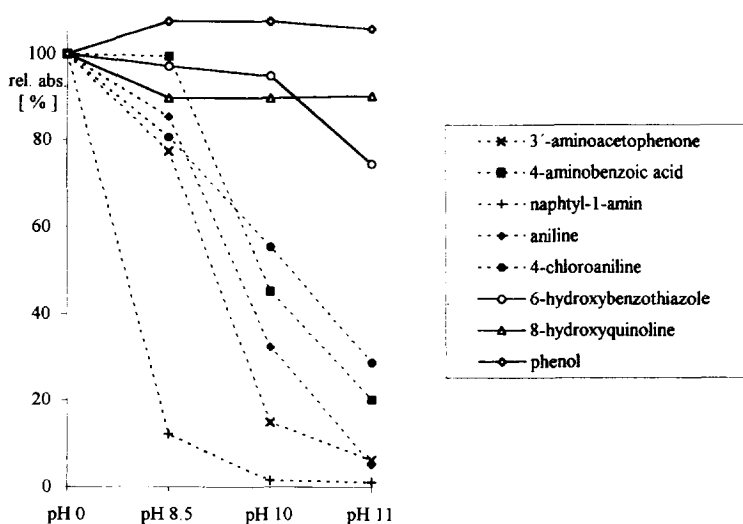
Fig. 5. Influence of pH on absorption intensities at maximum wavelengths for aromatic amines and phenols.

The use of 5% H_2SO_4 solutions reduced the baseline noise consistently to 0.3 mAU and, therefore, this content of sulphuric acid was chosen for all further tests. No heat dependencies were observed and no additional reaction coils were needed, since the reaction time was approximately 6 s, which was due to the distance between the T-piece and the detector cell. According to the batch tests, this time delay was sufficient for most phenolics and possible interferences of aromatic amines were, thus, minimized. The reagent dosage for the HPLC post-column reaction was found to be optimal at 0.2 ml/min per pump for an eluent flow-rate of 0.7 ml/min (Fig. 6). For most compounds, detection limits ($S/N > 3$) were between 2 and 20 ng injected onto the column (Table 1). Absorption maxima and 85% band widths agreed with the data obtained in the batch tests. Using a 2.5-m reaction coil resulted in an additional 25-s time delay, and the detection limits of less reactive compounds such as 2-hydroxy-5-methoxy-benzoic acid could be minimized according to the kinetic studies. Sensitivity was further studied using 10–80% acetonitrile in water. Both Vis spectra and intensities of four test compounds (1, 7, 12 and 30) remained essentially the same. Therefore, the reaction is readily applicable to HPLC gradient separations. In eluents containing 50% methanol, absorption intensities were reduced to 55–75% compared to acetonitrile–water (50:50, v/v) mixtures.

Data on the Vis spectra of nitrophenols (given in Table 1) could only be obtained using the post-column method, i.e., not by batch tests. In fact, absorption maximum was shifted to 378 nm and Vis absorption at 500 nm was greatly reduced using additional reaction coils. So, colored reaction products initially formed could have been rapidly oxidized by excess Ce^{IV} reagent or by protonation due
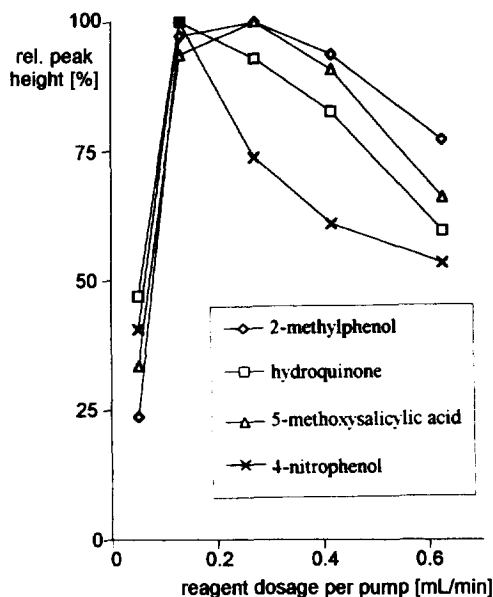

Fig. 6. Optimization of reagent flow of MBTH and Ce^{IV} solutions.

to the acidic medium, in a manner similar to the slow decrease of intensity observed for phenol.

Pentachlorophenol should not have reacted at all since it does not contain a free position in its molecular structure. Less chlorinated impurities probably reacted, although no minor peaks could be detected by HPLC–UV analysis. However, further elucidation of the reaction mechanism was beyond the scope of this study. As expected from its slow reaction, 2,6-di-*tert.*-butyl-4-methylphenol could not be detected in HPLC post-column analysis.

A 40-cm×0.5 mm I.D. capillary was employed for the post-column addition of alkali to exclude interferences of thiophenols and aromatic amines. Stepwise addition of the alkali solution was performed using a second HPLC pump, so the pH value of the resulting reaction mixture could be gradually increased from pH 0 to 11. In fact, decolorization of the reaction products of thiophenols could be achieved at pH>5.5. At pH 9, baseline noise reached about 1 mAU (Fig. 7). Between pH values of 9 and 10, the absorbance maximum of aniline changed from 565 to 459 nm. However, the pH value cannot

be shifted above pH 9.5 due to the increasing baseline noise, however, changes in Vis spectra were sufficiently clear at this pH value for all of the tested amines. The use of gluconic acid as an alternative Ce^{IV} chelating agent [31] resulted in even higher noise levels.

In conclusion, changes in the Vis spectra at neutral-to-basic pH values can be used to differentiate aromatic amines and thiophenols from aromatic hydroxy compounds. However, a diode array detector is needed in doubtful cases. Measurements in a single wavelength mode should only be used for target analysis or in cases where possibly interfering S,N-phenol analogs are known to be absent. Alternatively, fluorescence reaction with *o*-phthaldialdehyde [32] might exclude the presence of primary amines and selective thiol detection could also be achieved using 6,6'-dithionicotinic acid [33]

In summary, two criteria for characterizing an unknown HPLC peak as an hydroxyaromatic compound can be derived from these results: (I) at 500 nm, at least 85% of its maximum intensity should be observed after the reaction; (II) when the first criterion is not fulfilled, a second HPLC post-column reaction is proposed with the subsequent addition of an alkaline EDTA solution to shift the eluent up to pH 9.5. In doing this, the maximum wavelength should not undergo a hypsochromic shift of more than 50 nm, and the peak height at the absorbance maximum should not be reduced by more than 20%.

4.4. Application to wastewater samples

In fact, thiophenols and aromatic amines are normally absent in tannery wastewaters [34]. Additionally, there is no reliable method for preconcentration of thiophenols, and aromatic amines are known to be best extracted under alkaline conditions. Therefore, interferences of these compounds were rather unlikely for the wastewater eluates given below.

Untreated, anaerobically and subsequently aerobically treated tannery wastewaters were extracted using a four-step sequential solid-phase extraction procedure, resulting in an overall preconcentration of 500-fold. Using this procedure, more than 60% of the originally included dissolved organic content (1143 ppm) could be recovered. A comparison of the
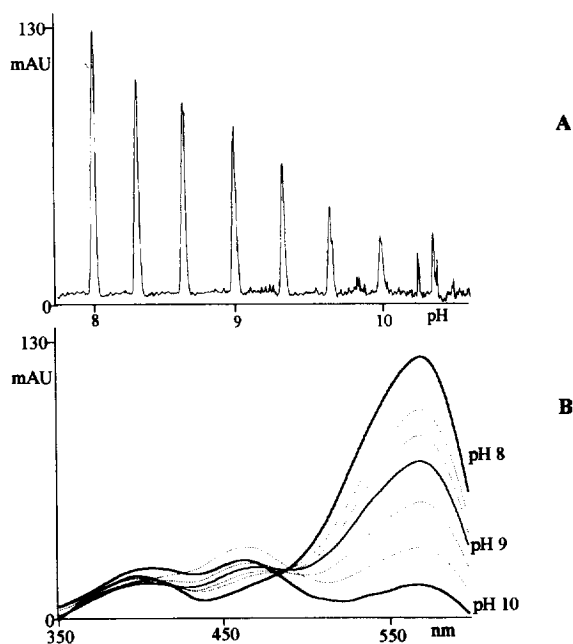

Fig. 7. Repeated injection of aniline (λ=565 nm, A) and changes in its Vis spectra during a stepwise pH shift, after HPLC post-column reaction (B).

four corresponding eluates of an anaerobically treated wastewater sample demonstrates the applicability of the proposed detection method for aromatic hydroxy compounds, even in heavily loaded samples (Fig. 8). HPLC analyses with UV

detection at 220 nm of these eluates resulted in very complex chromatograms. However, single peaks observed in these chromatograms can easily be characterized as phenolic compounds after second injections and post-column reaction detection. No
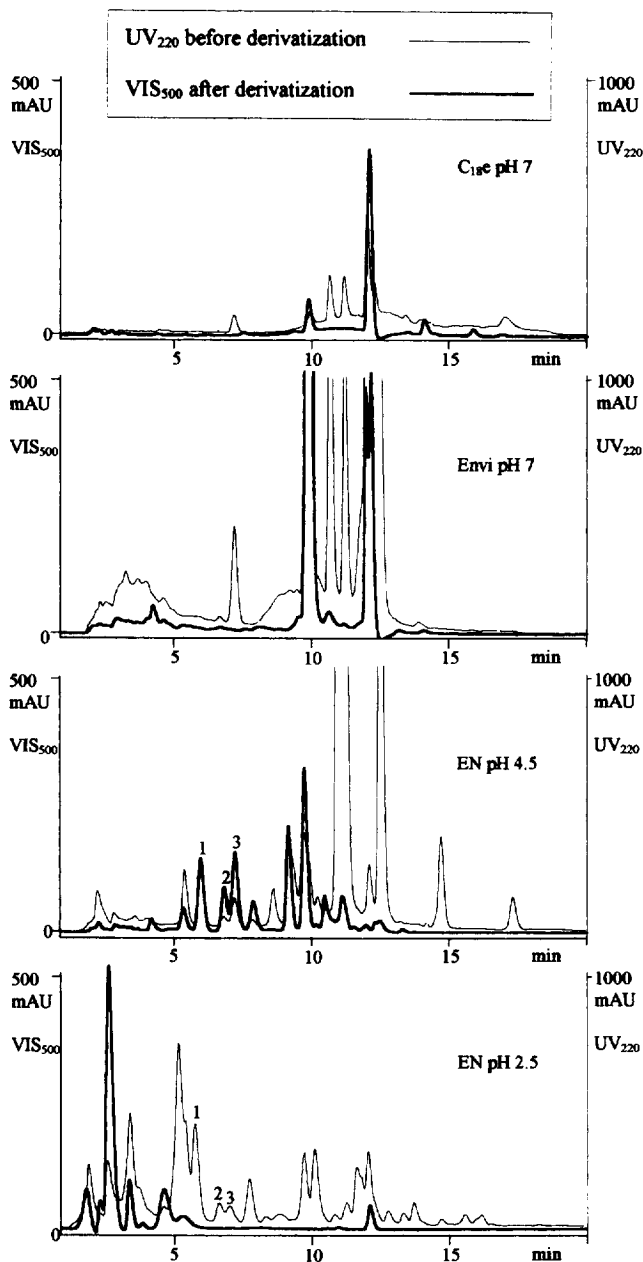

Fig. 8. UV at 220 nm (UV_{220}) and Vis at 500 nm (Vis_{500}) HPLC chromatograms of anaerobically treated wastewater eluates obtained by a sequential solid-phase extraction procedure before and after post-column derivatization. For numbers and further details, see text.

peak broadening occurred, due to the extremely short reaction times. At least seventeen different major (>40 mAU) and 33 minor (<40 mAU) phenolic constituents could be identified by comparison of spectra and retention times. Few hydroxyaromatic compounds apparently occurred in two fractions, and among them, only one had similar amounts in both eluates. Major peaks detected at 9.9 min in the EnviChrom pH 7 and the EN pH 4.5 eluates proved to belong to different compounds (λ_{max} 485 and 505 nm, respectively). Therefore, a fair pre-separation was achieved during the sequential extraction. Following the elution order in HPLC-chromatograms, most hydrophobic phenols were recovered using the first solid-phase cartridge (C_{18}e) and the following fraction (EnviChrom, pH 7). More polar hydroxyaromatic compounds were obtained using the following two EN-phases. These phenols are supposed to include additional acidic functionalities or at least more than one hydroxy group, since samples had to be acidified for quantitative extraction [27].

Only three peaks could be tentatively identified by comparing the UV spectra of our spectra library: 4-methylphenol (C_{18}e eluate, 11.7 min), 4-hydroxyphenylacetic acid (EN pH 4.5 eluate, 9.1 min) and 4-hydroxybenzoic acid (EN pH 4.5 eluate, 7.2 min). UV spectra and retention times of peaks 1–3 in the EN pH 4.5 and the EN pH 2.5 fractions were almost identical before the post-column reaction took place. Normally, these data prove the compound identification obtained using HPLC–diode array detection analysis. In fact, peaks 1–3 were clearly non-phenolic compounds in the EN pH 2.5 eluate, whereas major phenolic peaks were obtained at these retention times in the EN pH 4.5 fraction.

Results of a similar analysis for all wastewater treatment steps after post-column reaction are given in Table 2. Nearly all of the detected peaks could clearly be identified as phenolic compounds by their Vis spectra. Maximum wavelengths were around 500 nm, with spectral shapes and a standard deviation similar to the tested standard compounds. Therefore, the analytical wavelength of 500 nm has been proven to be suitable for the analysis of unknown hydroxyaromatic compounds in complex matrices. Phenolic characterization was doubtful only in a few cases, so the reaction did not have to be repeated at pH 9.5 (to abolish interferences of aromatic amines or thiophenols).

5. Conclusion

Aromatic compounds containing hydroxy groups can be selectively detected after HPLC separation and post-column addition of aqueous solutions of $Ce(NH_4)_2(SO_4)_3$ in 5% (v/v) H_2SO_4 and MBTH. The reaction appeared to be quite universal, since nearly all of the tested phenols reacted spontaneously, with absorption maxima around 500 nm. Therefore, this wavelength is suggested as the analytical wavelength of choice for the characterization of phenols of unknown HPLC peaks. The reaction appeared to be very sensitive and, therefore, it might also be used for target analysis of phenols in complex matrices. It could also be extended to the determination of total phenols in water. Aromatic amines showed absorption maxima above 560 nm and aromatic thiols below 475 nm. These interferences can be differentiated from phenolic compounds and heteroaromatic hydroxy compounds (absorption maxima above 540 nm) by the addition of alkaline EDTA solutions and the use of a diode-array detector.

The HPLC post-column method appears to be easily applicable to most HPLC laboratories, since no reaction coil or temperature control was needed. Therefore, no problems of peak broadening occur.

Table 2
Results of HPLC post-column derivatization of twelve tannery wastewater eluates

Wastewater treatment step	No. of phenolic peaks	Mean maximum wavelength (nm)	No. of possible interfering non-phenolics
Untreated	62	506 ± 16	1 (573 nm max.)
Anaerobic	50	503 ± 18	1 (455 nm max.)
Aerobic	13	487 ± 15	2 (419 nm max.)

Phenolic compounds have been successfully characterized in heavily loaded wastewaters and the reaction should be comfortably extended to other kinds of samples. Using a UV-spectra library before derivatization, only three phenols could be identified in a solid phase eluate of an anaerobically treated tannery wastewater, whereas 50 aromatic hydroxy compounds were detected after post-column reaction detection.

Acknowledgments

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