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Determination of phosphonates in natural waters by ion-pair high-performance liquid chromatography

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Abstract

The paper describes a new method for the determination of phosphonates by ion-pair high-performance liquid chromatography. The phosphonates are complexed with Fe(III) and separated on a reversed-phase polymer column. The eluent consists of a bicarbonate solution at pH 8.3, tetrabutylammoniumbromide as counter-ion and 14% acetonitrile. The complexes are detected at 260 nm. The four phosphonates HEDP (1-hydroxyethylene-1,1-diphosphonic acid), ATMP (aminotris [methylenephosphonic acid]), EDTMP [ethylenediaminetetra(methylenephosphonic acid)], and DTPMP [diethylenetriaminepenta(methylenephosphonic acid)] are well separated within 20 min. The limits of detection are $5 \cdot 10^{-8}$ *M* for ATMP and EDTMP, $1 \cdot 10^{-7}$ *M* for DTPMP and $5 \cdot 10^{-7}$ *M* for HEDP. The method is suitable for the determination of phosphonates in the influent and effluent of wastewater treatment plants. A preconcentration factor of 10 is easily achieved by adsorption of the phosphonates onto freshly precipitated calcium carbonate and subsequent dissolution of the solid-phase by HCl.

Keywords: Environmental analysis; Water analysis; Complexation; Phosphonates

1. Introduction

Phosphonates are chelating agents that contain one or more phosphonic acid groups [C-PO(OH)$_2$]. They are widely applied in water treatment, industrial cleaning and in laundry detergents [1].

Very little is known about the fate of phosphonates in the environment due to the lack of a sensitive, specific method for the determination in natural systems. The standard method for determining the presence of phosphonates is ion chromatography with post-column reaction with Fe(III) and detection of the Fe(III) complexes at 300–330 nm [2–4]. This method has a detection limit of about 2–10 μ*M*. Other methods have been described based on post-column oxidation of the phosphonate to phosphate and detection of phosphate with the molybdenum blue method [5]. Phosphonates are also measured by capillary electrophoresis with detection limits of 1–11 μ*M* [6]. By fractionation of the phosphorous – orthophosphate, polyphosphates and total phosphorous – the sum of organic phosphonates can also be determined in natural samples [7]. All these methods suffer, however, from a high detection limit of several μ*M*. They are therefore not suitable for the specific identification of phosphonates in wastewater treatment plants or natural waters.

This paper is the first description of a method that

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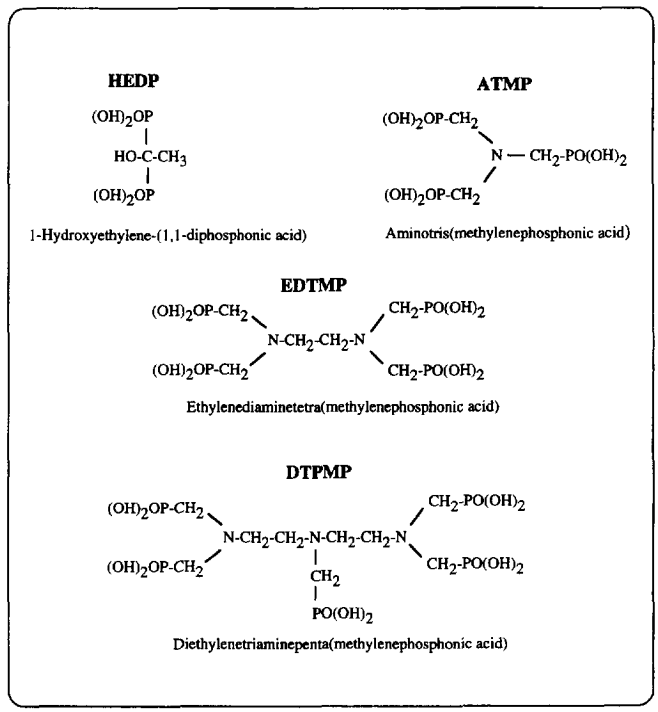

Fig. 1. Names, abbreviations and structures of the phosphonates used in this study.

is able to specifically detect phosphonates in water samples such as the influent of a wastewater treatment plant. The procedure is based on the the HPLC method for EDTA (ethylenediaminetetra-acetic acid), where the Fe(III)–EDTA complex is formed and then separated by ion-pair chromatography on a reversed-phase column [8,9]. This method was adapted for detecting HEDP (1-hydroxyethylene-1,1-diphosphonic acid), ATMP [aminotris-(methylenephosphonic acid)], EDTMP [ethylenediaminetetra(methylenephosphonic acid)], and DTPMP [diethylenetriaminepenta(methylenephosphonic acid)], which are the 4 phosphonates in major use. For structures of the phosphonates see Fig. 1. This paper also presents a new method for the preconcentration of phosphonates from natural waters which is based on the work of Frigge [10].

2. Experimental section

2.1. Apparatus

The liquid chromatography was performed with a Spectra-Physics P4000 pump. The autosampler was equipped with a loop of 200 μl. A polymer reversed-phase column was used (PLRP-S; Polymer Labs.; a polystyrene–divinylbenzene copolymer, particle size 5 μm, 15 cm×4 mm). The eluent was degassed online with a vacuum degasser (Thermo Separation Products). The flow-rate was 1 ml min^{-1}. Detection was made with a UV detector (Spectra Focus) at 260 nm.

2.2. Reagents and chemicals

All water was obtained from a Milli-Q system (Millipore). If not otherwise specified, all chemicals were obtained from Fluka (Switzerland). The mobile phase was prepared by dissolving 0.02 mol $NaHCO_3$ (puriss) and 0.001 mol tetrabutylammonium bromide (TBA-Br) in 1 l water (pH 8.3). A Fe(III) solution was prepared by dissolving 0.001 mol $Fe(NO_3)_3 \cdot 9H_2O$ and 0.01 mol HNO_3 in 1 l water. A buffer solution was prepared with 0.6 mol $NaHCO_3$ and 0.01 mol TBA–Br in 1 l water. An NTA (nitrilotriacetic acid) solution was prepared by dissolving

0.001 mol $Na_3NTA \cdot 2H_2O$ in 1 l water. The eluent was mixed with 13–15% acetonitrile.

ATMP and HEDP were obtained from Fluka (Switzerland). The other phosphonates were obtained from Monsanto as technical products (Dequest 2041, EDTMP; Dequest 2060S, DTPMP). Dequest 2060S was purified as follows: 0.1 moles of DTPMP in 500 ml water were neutralized by NaOH to a pH of 5. Subsequently, 0.6 mol of $CaCl_2$ were added. A white precipitate with the stoichiometry Ca_3H_4DTPMP is formed [11]. The precipitate was washed by repeated centrifugation and resuspension in water. The precipitate can be redissolved at pH 2. The content of phosphonate was verified by measuring total phosphate after digestion with peroxodisulfate. Fig. 1 shows the names, the abbreviations and the structures of the phosphonates used in this study.

2.3. Procedure

The filtrated water sample is passed through a cation-exchange column in the H^+-form (Dionex OnGuard H) to remove Ca and Mg and to bring the pH to about 3. Subsequently, 3 ml of the sample and 100 μl of the Fe(III) solution (final concentration $3 \cdot 10^{-5}$ *M* iron) are mixed and left for 2 h at room temperature. Then 100 μl of the NTA solution is added (final concentration $3 \cdot 10^{-5}$ *M* NTA). After about 30 min, 300 μl of the TBA/HCO_3 buffer is added. The sample is pipetted into autosampler vials and measured within one day.

After several 100 injections, the column is regenerated as follows: 100% acetonitrile for 30 min, 0.1 *M* HNO_3 for 5 h, 100% acetonitrile for 30 min, and finally bicarbonate eluent for 5 h.

2.4. Preconcentration

Samples with low concentrations of phosphonates are preconcentrated by using calcium carbonate as adsorbent. Co-precipitation of phosphonates by calcium carbonate with recoveries of 80% has been reported [10]. This method has been further improved. Calcium carbonate is precipitated by rapid addition of 0.1 moles $CaCl_2 \cdot 6H_2O$ in 500 ml of water to 0.1 mol of Na_2CO_3 in 500 ml of water. After 10 min the suspension is centrifuged for 3 min at 2000 rpm. The supernatant is discarded and the calcium carbonate is resuspended in 100 ml water. The suspension has to be used within few hours. A 0.1-ml volume of this suspension is added to 10–100 ml of the water sample, depending on the expected concentration of phosphonates. After 60 min, the sample is filtered through 0.45-μm filter and the calcium carbonate on the filter dissolved with 6 *M* HCl and diluted with water to exactly 10 ml. This solution is passed through a cation-exchange column in the H^+-form (Dionex Onguard H). Exactly 5 ml of this solution are brought to pH 3 by addition of 10 *M* KOH. The preconcentration factor is calculated as follows: (ml initial sample/10 ml)·[5 ml/(5 ml+ml KOH)]. Afterwards, the complexation is made as described above. Reference solutions are made as follows: 0.1 ml calcite suspension is added to 10 ml of drinking water. After filtration and dissolution of the calcite, the phosphonates are added prior to passing the solution through the cation-exchange column.

3. Results and discussion

3.1. Procedure

Phosphonates that are not complexed exhibit only very low UV-adsorption. The 1:1 Fe(III) complexes, however, absorb strongly in the range from 330 to 220 nm with a maximum around 260 nm. We have always used 260 nm as detection wavelength for all complexes.

If iron(III)-phosphonates are analyzed under the same conditions as EDTA in a formate buffer at pH 3.3 [8,9], no peaks are detected, not even with high concentrations of acetonitrile. Therefore, a mobile phase with a stronger eluting capacity has to be used. We have established that a bicarbonate eluent at pH 8.3 gives the best results. With this eluent, the Fe(III)-phosphonates can be eluted with TBA as counter-ion and the four phosphonates are well separated. Carbonate has been shown to have a pronounced influence on the elution of higher charged ions [12]. 12–15% acetonitrile are necessary to elute the complexes. The concentration of acetonitrile has a strong effect on the retention time and the efficiency of the separation. The retention time of DTPMP can rise from 16 to 25 min if the concentration of acetonitrile is changed from 14 to 13%.

For the other phosphonates this effect is much less pronounced (change of retention time of 1–2 min).

Separation of the four compounds on C_{18}-coated silica was not as good as on the polymer column. Therefore we always used the PLRP-S polymer column for all measurements. The column efficiency decreases after some 100 injections, but the column can be regenerated by purging with 0.1 *M* HNO_3 as described in the methods section. Fig. 2a shows a chromatogram of the three phosphonates HEDP, ATMP, and EDTMP, Fig. 2b, a chromatogram of DTPMP. With a run time of 20 min, the four phosphonates can be very well separated. Some impurities in the DTPMP solution (Dequest 2060S), even if redissolved from the purified calcium salt, interfere with the HEDP peak. If HEDP and DTPMP are determined simultaneously, each phosphonate has to be calibrated in a separate run.

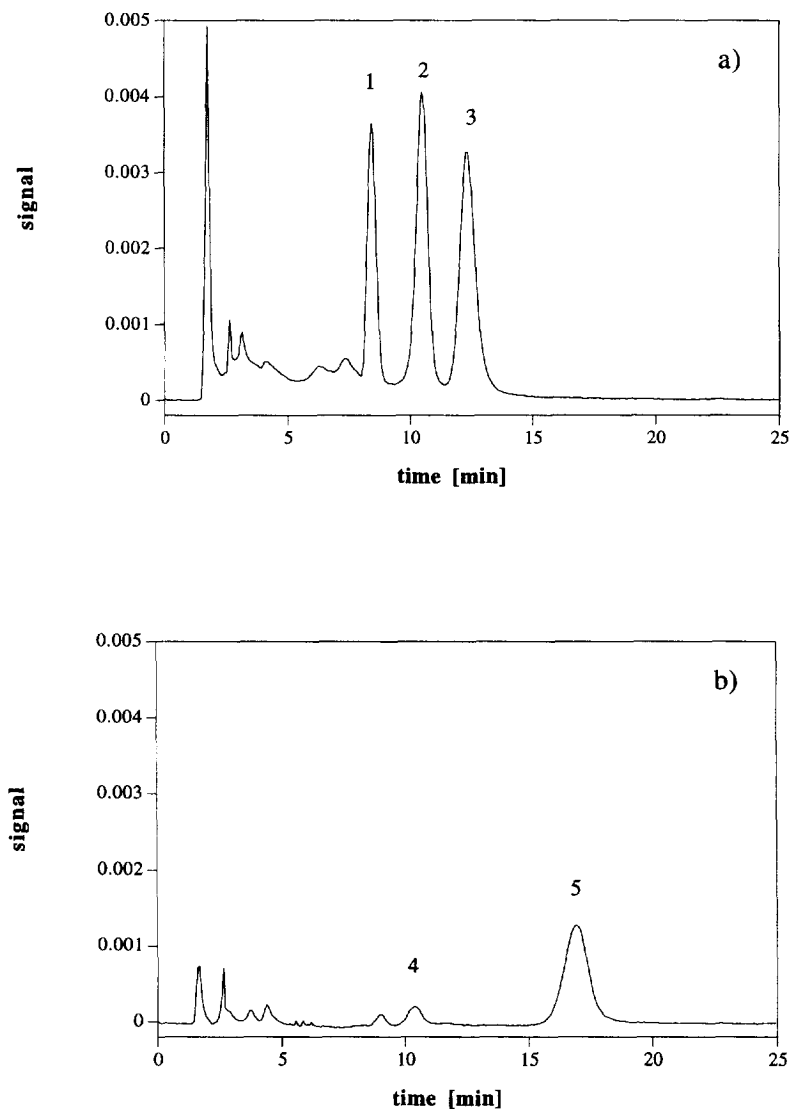

Fig. 2. (a) Chromatogram of 10 μ*M* HEDP (1), 5 μ*M* ATMP (2), and 5 μ*M* EDTMP (3). (b) Chromatogram of 5 μ*M* DTPMP (5) with impurity from Dequest 2060 (4). Conditions as in the Section 3.

Table 1
Influence of different metal–phosphonate complexes on the peak area of the Fe(III)–phosphonate complex

Phosphonate	Fe	Zn	Cu	Ni	Cr(III)
HEDP	100	94	83	94	29
ATMP	100	96	98	100	42
EDTMP	100	97	101	98	41
DTPMP	100	100	97	100	28

The area of the Fe(III) complex was set to 100%.
Conditions: $2.7 \cdot 10^{-6}$ *M* metal–phosphonate, $2.7 \cdot 10^{-5}$ *M* Fe(III) and NTA.

3.2. Natural samples

The high pH (8.3) of the eluent causes some problems, if the phosphonates are initially not present as iron(III) complexes. All metal-phosphonates have to be converted to the iron(III) complexes with iron(III) in excess at low pH. Afterwards, the pH has to be brought to 8.3 before analysis. Iron, that is not complexed with phosphonates or other ligands, precipitates at this pH. The phosphonates coprecipitate or adsorb onto the oxides. An excess of 10 μ*M* iron can totally prevent 10 μ*M* HEDP from being detected. It is therefore necessary to mask the excess iron. This is made possible by adding NTA prior to the bicarbonate. Fe(III)–NTA is a weaker complex than the Fe(III)–phosphonates and therefore does not interfere with the detection. It was found that for solutions with 10 μ*M* or less phosphonates, 30 μ*M* iron and 30 μ*M* NTA give the best results. HEDP with the weakest Fe(III) complexes is best measured with 10 μ*M* iron and NTA.

Phosphonates are present in natural waters complexed with different metals. Using this method it is possible to dissociate most of the complexes and to form the Fe(III)–phosphonates. The results of the measurements for Zn-, Cu-, Ni- and Cr(III)-phosphonates are shown in Table 1. It can be seen that for ATMP, EDTMP, and DTPMP, the metals, with exception of Cr(III), do not significantly influence the complexation with Fe(III). Only Cr(III), which forms very strong and kinetically inert complexes, hinders the complexation reaction. If Cr(III)-phosphonates play a relevant role in natural waters, then the concentration of the phosphonates is underestimated using this method. For HEDP, Cu also decreases the concentration of the iron complex. Contrary to Ni-EDTA, which dissociates only very slowly at room temperature [9], the Ni-phosphonates react very fast. Heating of the solution after addition of Fe(III) is therefore not necessary.

A natural water sample with high calcium concentrations is passed through a cation-exchange column before iron is added. The column (Dionex OnGuard H, H^+-form) removes calcium and magnesium, which interfere with the complexation of Fe(III) by the phosphonates. After the column, the solution has a pH around 3 which is optimal for the complexation. It was tested to assure that the column does not adsorb metal–phosphonate complexes. The application of the column allows the detection of 0.05 μ*M* phosphonates in natural waters without a preconcentration step. The calibration function is linear from 0.05 μ*M* to 10 μ*M*. The calibration function for HEDP is not linear at concentrations below 0.5 μ*M*. The limit of detection based on three times the background noise is $5 \cdot 10^{-8}$ *M* for ATMP

Table 2
Recovery rates of phosphonates in percent from different water samples (drinking water, influents and effluents from wastewater treatment plants)

Sample	*n*	Addition (*M*)	ATMP (%)	EDTMP (%)	DTPMP (%)
Drinking water	6	$2 \cdot 10^{-6}$	97±3	101±2	87±5
Drinking water	4	$1 \cdot 10^{-6}$	98±2	113±2	85±3
Effluent WWTP[a]	6	$2 \cdot 10^{-6}$	82±4	89±3	96±4
Influent WWTP	4	$5 \cdot 10^{-7}$	95±6		
Influent WWTP	5	$5 \cdot 10^{-7}$		102±16	
Influent WWTP	7	$1 \cdot 10^{-6}$			102±9

Conditions as described in the Section 2.
[a] WWTP: wastewater treatment plant.

and EDTMP, $1 \cdot 10^{-7}$ *M* for DTPMP, and $5 \cdot 10^{-7}$ *M* for HEDP.

The recovery rate of the phosphonates in different samples was found to be between 85 and 110% (Table 2). Good recovery rates were also achieved in drinking water and in the influent and effluent from wastewater treatment plants. The standard deviation for 4–7 samples was always between 2 and 5% for drinking water and between 3 and 15% for wastewater treatment plants.

3.3. Analyses

The method was applied to several influents and effluents of wastewater treatment plants. Fig. 3a shows a chromatogram of an influent and the addi-

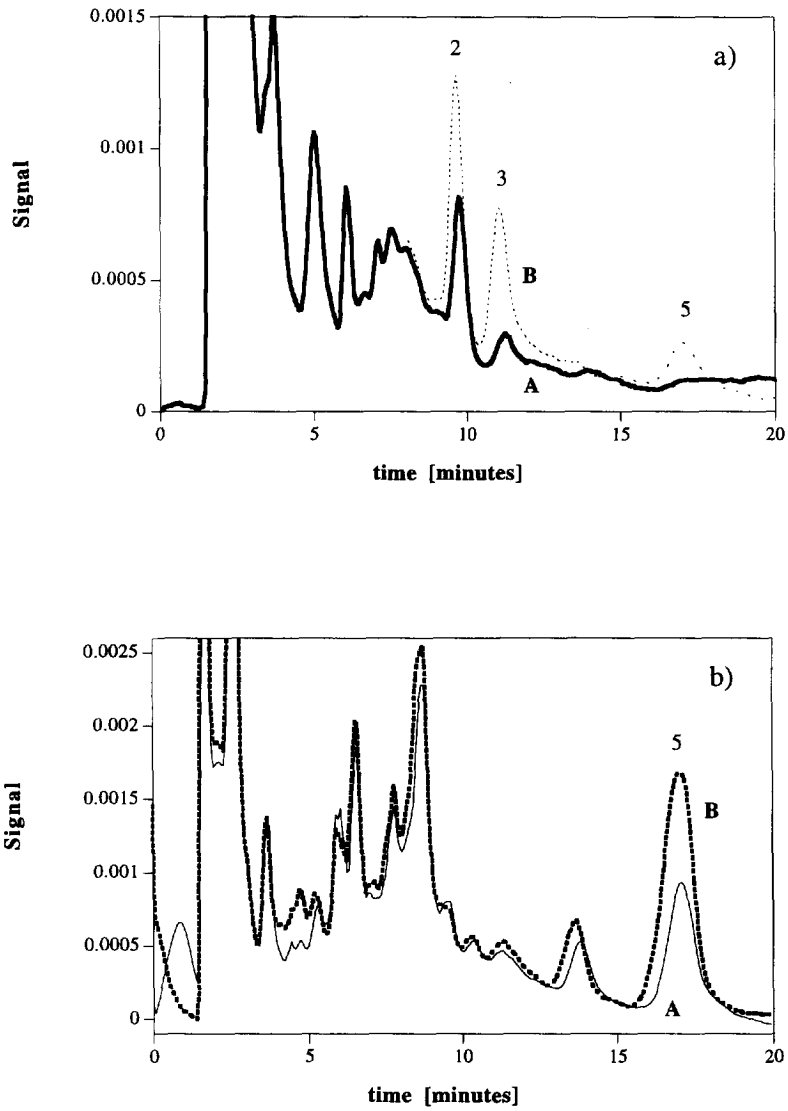

Fig. 3. (a) Chromatogram of the influent of a wastewater treatment plant (A) and addition of $5 \cdot 10^{-7}$ *M* ATMP, EDTMP, and DTPMP to the sample (B). Peaks numbered as in Section 2. (b) Chromatogram of the influent of a wastewater treatment plant (A) and addition of 1 μ*M* DTPMP (B). Peaks numbered as in Fig. 2.

Table 3
Recovery rates of phosphonates in percent from different samples with preconcentration onto freshly precipitated calcium carbonate

Sample	Addition (M)	ATMP (%)	EDTMP (%)	DTPMP (%)
Drinking water	$2 \cdot 10^{-6}$	101	106	98
Effluent WWTP[a]	$2 \cdot 10^{-7}$	97	100	79
Effluent WWTP	$1 \cdot 10^{-7}$	96	90	88

Conditions as described in Section 2.
[a] WWTP: wastewater treatment plant.

tion of $5 \cdot 10^{-7}$ *M* ATMP, EDTMP and DTPMP. The two phosphonates ATMP and EDTMP were detected in concentrations of $7.5 \cdot 10^{-7}$ and $1.5 \cdot 10^{-7}$ *M*, respectively. The identity of the peaks was verified by standard addition. Fig. 3b shows a chromatogram of an influent of another wastewater treatment plant with a high concentration of about 2 μ*M* DTPMP. The identity of the peak was verified by addition of DTPMP. HEDP elutes in a region with high background signals and cannot be quantified.

3.4. Preconcentration

The phosphonates adsorb strongly onto calcium carbonate. It was found that 0.1 ml of a 1 *M* calcium carbonate suspension is able to adsorb 2 μ*M* of each ATMP, EDTMP, and DTPMP in 100 ml drinking water. Freshly precipitated calcium carbonate has the strongest adsorption capacity. After several hours, its effectiveness to adsorb phosphonates decreases, which may be due to a change in the surface area during aging. The calcium carbonate suspension should therefore be used within few h. Table 3 shows the recovery rates of 3 phosphonates from drinking water and the effluent of a wastewater treatment plant. The recovery rates are between 80 and 105%. As shown in Fig. 4, the adsorption onto calcite includes also a clean-up of the sample. Separation of the phosphonate peaks from the background is much better. With a tenfold preconcentration, a detection limit of about $1 \cdot 10^{-8}$ *M* can be achieved.

4. Conclusions

A simple and rapid method for the analysis of phosphonates has been developed. Compared with previous methods, the detection limit is 10–20 times better. By applying the new preconcentration on calcium carbonate, it can be improved further. This method will provide a useful tool for environmental monitoring of phosphonates in wastewater treatment plants and rivers.

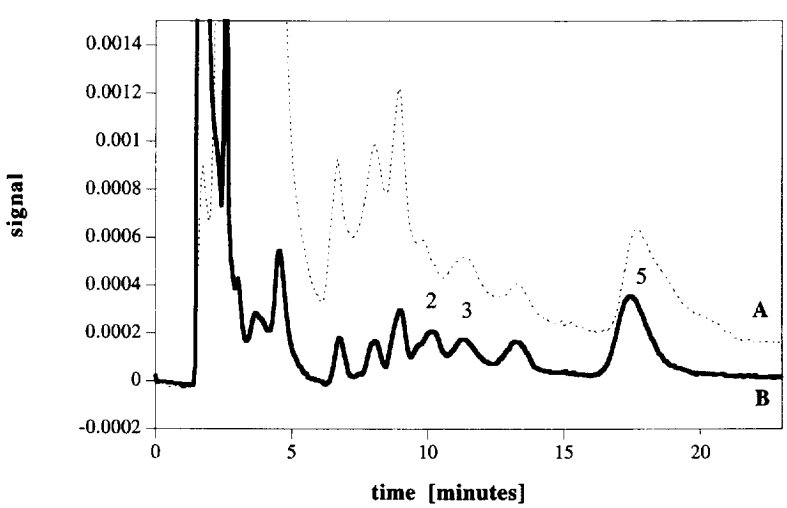

Fig. 4. Chromatograms of the influent of a wastewater treatment plant. A. Direct measurement with conditions as Section 3. B. Adsorption onto calcite and subsequent dissolution of the calcite as described in the text. Peaks numbered as in Fig. 2.

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