REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF METAL CHELATES OF 4-(2-PYRIDYLAZO)RESORCINOL AND 4-(2-THIAZOLYLAZO)RESORCINOL. SIMULTANEOUS DETERMINATION OF LOW CONCENTRATIONS OF Co, Ni AND Fe

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The behaviour of stable and inert metal chelates of 4-(2-pyridylazo)resorcinol and 4-(2-thiazolylazo)resorcinol in RP HPLC and in its ion-pair modification (IP RP HPLC) on Separon SGX RPS silica gel was studied. Good results were obtained by the ion-pair variant in water–methanol solutions at pH 7 in the presence of cetyltrimethylammonium bromide. The technique proved to be convenient for the preconcentration, separation and quantitation of low concentrations of Fe, Co, and Ni in waters.

N-Heterocyclic 2-substituted azo dyes are among the most promising and most frequently used analytical reagents for metal ions. Important is their high analytical selectivity for transition metal ions, ions with the outer electron configuration $d^{10}(s^2)$, rare earth elements (REE), and some platinum metals; the high sensitivity of reactions of some structure variants of the reagents is of importance as well[1].

Owing to the intense colour, high stability, and kinetic inertness of the chelates of some elements, the reagents can be employed with advantage in normal as well as reverse-phase HPLC on hydrophobized sorbents, mostly of the chemically modified silica gel type[2].

In this relation, attention has been paid to the proven and well available 4-(2-pyridylazo)resorcinol (PAR)[3–14] and, more recently, its analogue 4-(2-thiazolylazo)resorcinol (TAR)[15–17], as well as to other azo dyes[18–26] whose complexation equilibria in solutions were studied earlier[1,27–29].

Various modifications of HPLC separation making use of the kinetically inert chelates of Cu(II), Co(II), Ni(II), Fe(II), and also V(V), Cr(III), Mo(VI), Pd(II), Nb(V), Ga(III) and Ta(V) with PAR, and of Cu(II), Co(III), Ni(II), Fe(II) and platinum metals with TAR have been published, although data for their practical analytical application are often lacking or incomplete[23,30].

This work is a detailed study of the separation and quantitative evaluation of PAR and TAR chelates of Cu, Ni, Co and Fe by reverse-phase HPLC (RP HPLC) and by ion-pair reverse-phase HPLC (IP RP HPLC). A procedure was developed for the simultaneous determination of low concentrations of Co, Ni, and Fe on the sorbent Separon SGX-RPS using an aqueous-methanolic mobile phase after preconcentration on a column packed with sorbent of the same kind. The procedure is convenient for the determination of low concentrations of the elements in waters.

EXPERIMENTAL

Apparatus

A Hewlett–Packard HP 1050 liquid chromatograph with a quaternary pump, a VW detector, controlled by a VECTRA QS/16 computer using supplier's HPLC CHEM STATION software.

A home-made liquid chromatograph comprising a Varian 8500 pump, an LCD 2040 photometric detector (Laboratorni pristroje Prague), and an LCI 30 loop injector (ECOM Prague) with a 20 μl loop. Gilson micropipettes 200 and 1 000 μl.

An OP 208/1 pH-meter fitted with an OP 0808P combined electrode (Radelkis, Hungary), calibrated with standard NBS buffers at pH 4.00, 7.00, and 9.18 at 25 °C. The reported pH values for the mobile phases refer to those of the particular buffers in aqueous solution; no corrections for the presence of the organic component in the mobile phase were made.

Columns and Stationary Phases

The following columns, supplied by Tessek, Prague, were used: 250 × 4 mm packed with Separon SGX-RPS 10 μm; 150 × 3 mm packed with Separon SGX-RPS 7 μm; and 20 × 9 mm packed with Separon SGX-RPS 60 μm.

Mobile Phases

Mixtures of methanol and aqueous buffers were degassed on an ultrasonic bath for 10 min. The flow rate through the column was 0.5 and 1 ml min^{-1} for the columns 150 × 3 mm and 250 × 4 mm, respectively. The compositions of the mobile phases are all reported in vol.%, only the surfactant concentrations are in wt.%.

Chemicals

Standard solutions of metal ions were prepared from the pure metals or their nitrates and were standardized by titration with EDTA.

4-(2-Pyridylazo)resorcinol (PAR), supplied by Lachema, Brno, 2 mmol l^{-1} in NH_2OH . HCl solution (0.1 mol l^{-1}, pH 8.7). 4-(2-Thiazolylazo)resorcinol (TAR), obtained from the same supplier, 2 mmol l^{-1} in a solution of 50 vol.% CH_3OH + 50 vol.% NH_2OH . HCl (pH 8.7). The reagents were multiply recrystallized from aqueous-methanolic solutions, and their purity was checked by TLC on silufol.

Tetrabutylammonium hydroxide (TBA), Lachema, Brno. Cetyltrimethylammonium bromide (CTMA), Lachema, Brno, 10 mmol l^{-1} in methanol. TRITON X-100, scintillation purity, Koch Light (U.K.), 1 wt.% solution in warm water.

Buffer solutions contained 0.1 mol l^{-1} of NH_2OH . HCl, tris(hydroxymethyl)aminomethane (TRIS), 2-(*N*-morpholino)ethanesulfonic acid (MES), hexamethylenetetramine (HMT), $NH_4H_2PO_4$, and acetic acid. The pH was controlled by adding NaOH or H_2SO_4 solutions (1 mol l^{-1}).

Doubly distilled water was prepared in a quartz still (Heraeus, Germany).

All chemicals were of reagent grade purity or were recrystallized.

Retention Characteristics and Calibration

The behaviour of the metal chelates on the column was characterized by the capacity factor $k = (t_R - t_M)/t_M$, where t_M is the dead retention time and t_R is the retention time, and by the resolution (for two adjacent peaks i and j), $R_{i,j} = 1.18\ (t_{R,i} - t_{R,j})/(Y_{1/2,i} + Y_{1/2,j})$ where $Y_{1/2}$ is the half peak width. The t_M value was measured as t_R of thiourea injected into the test mobile phase recommended by the manufacturer. For the column 250 mm long, t_M was 2.2 min (mobile phase flow rate 1 ml min^{-1}), whereas for the column 150 mm long, t_M was 1.3 min (mobile phase flow rate 0.5 ml min^{-1}).

Calibration plots were obtained for the peak heights using linear regression methods. The sensitivity of the method was characterized by the calibration straight line slope. The detection limit was expressed for an analyte signal equal to triple baseline noise.

Preparation of Metal Chelate Solutions to Be Injected

Solutions of the reagent (2 mmol l^{-1}) and the metal cations (0.1 mmol l^{-1}) were mixed and diluted with electrolyte whose composition was the same as that of the reagent solution, so that the final concentration of reagent was 1 mmol l^{-1} and that of the metal was 0.005 – 0.04 mmol l^{-1}. The solution was allowed to stand for 5 min prior to injection.

Determination of Low Concentrations Fe, Ni, and Co in Waters

Off-line procedure. A volume of 1 ml of PAR solution (2 mmol l^{-1}) was added to 100 ml of water containing 1.1 – 2.4 μg of the elements determined at pH 6 (alkaline solutions could not be used because the sorbent might decompose). In 5 minutes the solution was fed at a flow rate of 10 ml min^{-1} on a plastic precolumn 20 × 9 mm packed with Separon SGX-RPS (60 μm). After the sorption, the trapped PAR chelates were eluted in the countercurrent mode with 3 ml of methanol at a flow rate of 1 ml min^{-1}. The eluate was diluted to 5 ml with methanol, and a 20 μl aliquot was injected on column packed with Separon SGX-RPS. A mobile phase consisting of 30 vol.% HMT buffer (20 mmol l^{-1}, pH 7) and 70 vol.% methanol containing CTMA (1 mmol l^{-1}) was used. The column was washed with 5 ml of redistilled water both before and after the sorption. The recovery was never lower than 95%. The ultimate time of storability of the columns with the samples was not examined.

On-line procedure. A preconcentration glass column 3 × 30 mm packed with Separon SGX (60 μm) was attached to the adapted LC-30 injection valve in place of the injection loop. Co, Ni and Fe cations (5 – 60 ng) were sorbed at a rate 10 ml min^{-1} from 100 ml of a solution of their PAR chelates at pH 6 in the presence of PAR (0.5 μmol l^{-1}). By turning the injection valve, the whole contents of the column were emptied in the countercurrent mode on the analytical column and chromatographed as in the off-line procedure. When PAR (0.1 μmol l^{-1}) was applied on the preconcentration column in advance and a pure aqueous solution of the metal ions was sorbed, the resulting sensitivity of their determination was 10-fold lower. The column was washed with 5 ml of redistilled water both before and after the sorption. The recovery could not be determined because the volume and concentration of the sample applied after elution from the preconcentration column were unknown.

RESULTS AND DISCUSSION

Complex Equilibria with PAR and TAR

Cations of the majority of transition metals give with PAR and TAR in aqueous or aqueous-alcoholic solutions stepwise several chelates of various stability and protonated to various degrees, with component ratios M : L = 1 : 1 and 1 : 2, in dependence on the pH, reagent concentration and composition of the solution, which affect the resulting chelate charge[1]. The most stable chelate $M^{II}L_2^{2-}$ or $M^{III}L_2^-$ predominates at pH ≥ 8 in the presence of excess reagent. The kinetically inert chelates CoL_2, FeL_2 and NiL_2 are mostly formed in the presence of even a low excess of reagent[1,27]. Chelates of Pd^{2+} and Cu^{2+} with M : L = 1 : 2 do not form or form reluctantly even if the reagent is present in a high excess[31]. The reaction of Co^{2+} with H_2L is accompanied by the oxidation of Co giving $Co^{III}L_2^-$. Once formed, the $Fe^{III}L_2^-$ chelate reduces spontaneously in alkaline solution to $Fe^{II}L_2^{2-}$. In comparison with PAR, the stability of the TAR chelates, as well as the solubilities of the reagent and chelates, are lower[1,27,28].

Effect of Preparation and Composition of Chelate Solutions on Their Chromatographic Properties in RP HPLC and IP RP HPLC

The effect of pH and reagent concentration on the chelate formation was examined for Fe(II,III), Ni(II), Co(III), and Cu(II). For the highest sensitivity (peak height), pH 8.7 is optimum for the chelate formation. If the chelates are formed at pH 6.5, which is the pH of the mobile phase, the sensitivity of determination is 5 – 10% lower. Assuming the formation of the ML_2^{2-} or ML_2^- species in the chelate solution, the molar concentration of the reagent must be in practice at least tenfold with respect to the total concentration of the Cu, Fe, Co and Ni cations whose chelates are separated chromatographically. This corresponds to 1 mmol l^{-1} for a total concentration of the metals of 0.08 mmol l^{-1}.

RP HPLC Variant of the Separation and Determination of PAR and TAR Chelates

Absorption Characteristics of the Chelates Chromatographed

Owing to the chromatographic separation of reagent, the pure spectra of the chelates, unaffected by the reagent, can be obtained. After separation in RP HPLC the mobile phase flow was stopped for the peak maximum, and the spectrum was measured with the VW HP 1050 detector (Fig. 1) at mobile phase pH 6.5. The wavelengths in the absorption peak maxima are given in Table I.

The wavelength of 500 nm was chosen for the detection and evaluation of peaks of the components in mixture with PAR or TAR; alternatively, 530 nm was used to suppress the intense peak of TAR.

Effect of Mobile Phase Composition on the Retention of the Chelates

The capacity factors, or retention times, for the metal chelates of PAR and TAR decrease with increasing pH. This decrease is due to the increased dissociation of the free *p*-OH group in PAR and TAR (pK_a = 5.6 and 6.23 for PAR and TAR, respectively[1]),

TABLE I
Wavelengths (nm) of absorption maxima[a]

Metal	PAR	TAR
Co	509	510
Ni	496	502
Fe	497	483
Cu	511	–
Reagent	413	473

[a] Mobile phase: 70 vol.% HMT solution (20 mmol l^{-1}, pH 6.5) + 30 vol.% methanol.

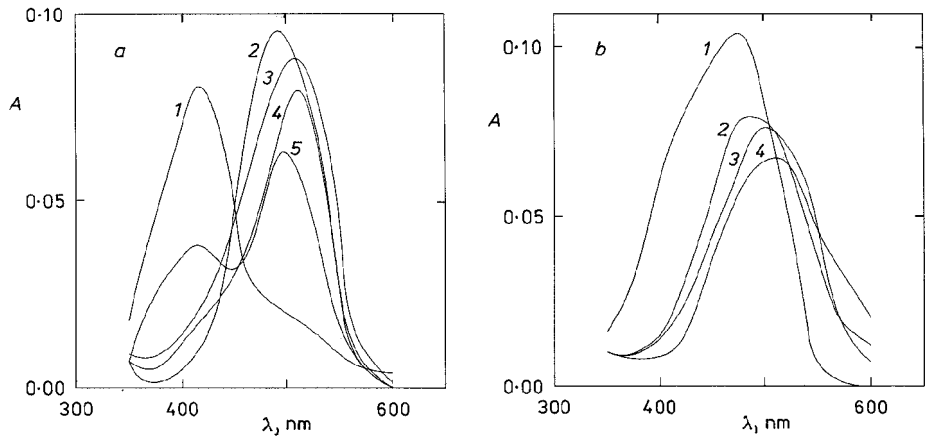

FIG. 1
Absorption spectra of PAR and TAR complexes measured after their separation on a chromatographic column 250 × 4 mm packed with Separon SGX-RPS (10 μm); mobile phase: 70 vol.% HMT (20 mmol l^{-1}; pH 6.5) + 30 vol.% methanol; *a* (PAR): *1* PAR, *2* Ni, *3* Co, *4* Cu, *5* Fe; *b* (TAR): *1* TAR, *2* Fe, *3* Ni, *4* Co

which, in addition, is proportional to the increasing stability of the chelates. The retention time decrease is most marked for the Fe chelate. The optimum response, with a fast and sufficiently efficient separation, occurs at mobile phase pH 6.5 – 7.0 (HMT buffer), which corresponds to the upper limit of usability of the Separon SGX-RPS column (Fig. 2).

The rate of elution of the chelates and reagents from the column also increases if the methanol content of the mobile phase is increased (Fig. 3). The fraction of 30 vol.% methanol is the optimum, at which the separation of the chelates is sufficiently fast; for the last chelate peak, k is approximately 10, in agreement with published data[32].

The buffer has a considerable effect on the retention of the chelates and their resolution (Table II), affecting the shifts of t_R of the chelates and the reagent itself.

The shifts of the retention times of the chelates and reagent are presumably due to interactions of the chelates with the buffer anions and cations in the mobile phase. The HMT buffer, causing a sufficient separation of the chelates, was chosen. Only the peak of the Cu chelate interferes with that of the reagent, and this can be eliminated by adding phosphate buffer.

The Cu chelate with TAR gives no signal in the conditions applied. This is due to the fact that its stability constant is about 3 orders of magnitude lower than that of the chelate with PAR (for M : L = 1 : 1), (ref.[28]).

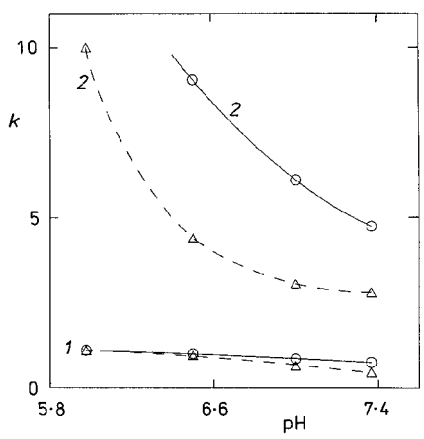

FIG. 2

Dependence of k on mobile phase pH in RP HPLC. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm), mobile phase: 70 vol.% HMT (20 mmol l^{-1}) + 30 vol.% methanol; c_M = 0.02 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}. Full lines: PAR, broken lines: TAR; *1* Co, *2* Fe

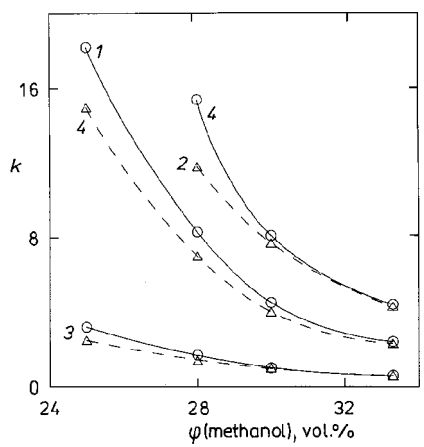

FIG. 3

Dependence of k on the methanol content of the mobile phase in RP HPLC. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm), mobile phase: HMT (20 mmol l^{-1}, pH 6.5) + methanol; c_M = 0.02 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}. Full lines: PAR, broken lines: TAR; *1* PAR, *2* TAR, *3* Co, *4* Fe

Calibration Dependences

Solutions with metal concentrations c_M = 0.005 – 0.025 mmol l^{-1} and a concentration of PAR or TAR of 1 mmol l^{-1}, in the presence of NH_2OH . HCl (0.1 mol l^{-1}, pH 8.7), were used. After 5 min standing, volumes of 20 μl of such solutions were injected into the mobile phase consisting of 35 vol.% methanol and 65 vol.% [HMT (20 mmol l^{-1}) + phosphate buffer (2 mmol l^{-1})] at pH 6.5. The calibration curves so obtained were linear for Co, Ni, Fe and Cu chelates with PAR and for Co, Ni, and Fe chelates with TAR. The peak absorbances are evaluated in Table III. The relative standard deviation

TABLE II
Values of t_R and $R_{i,j}$ of chelates with PAR using various buffers[a]

Ion	Phosphate		Ion	HMT		Ion acetate	
	t_R, min	$R_{i,j}$		t_R, min	$R_{i,j}$	t_R, min	$R_{i,j}$
Cu	4.9	5.3	Co	3.5	8.1	5.6	0.1
Co	9.2	11.1	Cu	10.5	0.7	5.7	6.3
PAR	21.5	2.9	PAR	11.0	7.8	14.5	4.3
Ni	26.2	2.7	Ni	22.8	4.4	19.0	0.7
Fe	31.3	–	Fe	31.3	–	20.5	–

[a] Mobile phase: 70 vol.% buffer (pH 6.5) + 30 vol.% methanol; column: 250 × 4 mm packed with Separon SGX-RPS (10 μm).

TABLE III
Evaluation of calibration curves[a,b]

Ion	PAR chelate		Ion	TAR chelate	
	a, l mol^{-1}	m_{lim}, ng		a, l mol^{-1}	m_{lim}, ng
Co	2 120 ± 150	1	Ni	970 ± 150	2
Cu	142 ± 60	13	Co	1 950 ± 30	1
Ni	1 100 ± 90	2	Fe	166 ± 20	10
Fe	310 ± 60	5			

[a] Mobile phase: 35 vol.% methanol + 65 vol.% HMT (20 mmol l^{-1}) and acetate (2 mmol l^{-1}) buffer, pH 6.5, stationary phase: Separon SGX-RPS; λ = 500 nm; [b] calibration plot slope $a = \delta A/\delta c(\pm t\, s_a)$, detection limit for injection of 20 μl $m_{lim} = 3s_0\, M_M\, 2\, .\, 10^4/a$ where the baseline standard deviation s_0 is 0.0005.

of a determination for 5 replicate measurements, with the metal concentration 0.01 mmol l^{-1}, was 2 – 4%.

Typical chromatograms of the chelates with PAR and TAR are shown in Fig. 4.

Effect of Nonionic Surfactant in the Mobile Phase on the Retention of the Chelates

The retention times of the chelates of Co, Ni, and Fe with PAR and TAR decrease in the presence of Triton X-100. The order of elution of the components alters as well, so that the Ni chelate with PAR is eluted before the reagent itself (Fig. 5). The change in the chromatographic behaviour of the chelates may be due to their solvation by molecules of the surfactant and competition of the solvates with molecules of the surfactant on the stationary phase. A sufficient excess of the surfactant in the mobile phase (1 – 2 wt.%) can replace completely the organic solvent, i.e. methanol. The shape of the calibration curves does not change on the addition of the surfactant.

Mutual Influence of the Chelates During Sorption on Column

The height of the Co(III) chelate with PAR and its retention time are unaffected by a 50-fold excess of Fe(II,III) for 0.5 mmol l^{-1} Fe and 1 mmol l^{-1} PAR in conditions under

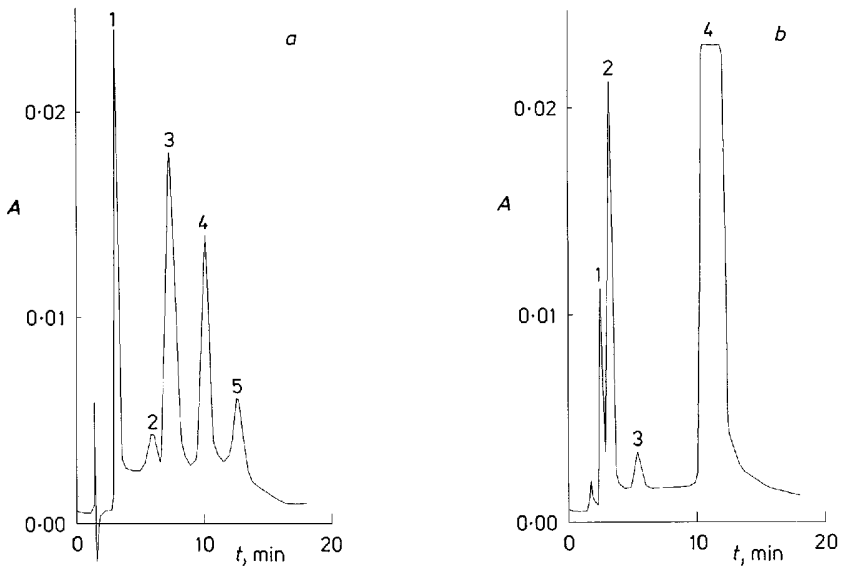

FIG. 4

RP HPLC chromatograms of PAR and TAR chelates. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm), mobile phase: 65 vol.% HMT (20 mmol l^{-1}) and phosphate (2 mmol l^{-1}) buffer at pH 6.5 + 35 vol.% methanol; c_M = 0.01 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}, λ = 500 nm. *a* 3 PAR and its chelates with 1 Co, 2 Cu, 4 Ni, 5 Fe; *b* 4 TAR and its chelates with 1 Ni, 2 Co, 3 Fe

which the calibration curve was measured. For the remaining chelates (Ni and Cu), their mutual influence is negligible unless the concentration of one cation is more than 20-fold with respect to the other, provided that the reagent is present in a sufficient excess. Hydroxylamine reduces Fe(III) in the presence of the reagent at pH 8.7, so that a single chelate, viz. $Fe(II)L_2^{2-}$, is formed, giving a single response for Fe^{3+} and Fe^{2+}. If no reductant (hydroxylamine, ascorbic acid) is present, the Fe(III) chelate gives a separate peak with t_R approaching that of the Co(III) chelate with PAR, using a mobile phase consisting of 50 vol.% methanol and 50 vol.% HMT (20 mmol l^{-1}, pH 5.0) and the column 150 mm long. In this system, the retention times of the Fe(III) chelate with PAR, the reagent itself, and the Fe(II) chelate with PAR are 1.8, 4.1, and 5.5 min, respectively. The response for Fe(III), however, decreases by 60% in 20 min due to spontaneous reduction of the Fe(III) chelate to the Fe(II) chelate.

Effect of Additional Cations in Sample on the Retention of the Co, Fe, Ni, and Cu Chelates

Ca^{2+} and Mg^{2+} at a concentration of 5 mmol l^{-1} do not interfere with the determination of the Ni, Co, Cu, and Fe chelates with PAR and TAR, whereas no chromatographic response of the chelates with PAR is obtained if Zn^{2+}, Al^{3+}, UO_2^{2+}, REE, Hg^{2+}, Mn^{2+}, Pb^{2+}, Zr(IV), Th(IV), Cd^{2+}, Tl^{+}, Sc^{3+}, or In^{3+} is present at a concentration of 250 – 400 mmol l^{-1}. If the chelates are formed in the solution, they are decomposed on the column during sorption on the stationary phase employed, using the mobile phase consisting of 35 vol.% methanol + 65 vol.% HMT (20 mmol l^{-1}) with phosphate buffer (2 mmol l^{-1}) at pH 6.5. With some elements (Mn, Hg, Zr), slight indications of the peaks or a decrease in the height of the dye itself were observed. Only the Ga^{3+} chelate gave a peak with a retention time of 6.1 min.

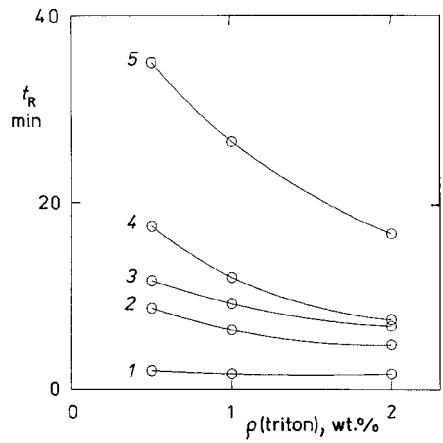

FIG. 5
Dependence of t_R on the concentration of Triton X-100 in the mobile phase. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm), mobile phase: HMT (20 mmol l^{-1}, pH 6.5) with Triton X-100; c_M = 0.02 mmol l^{-1}, c_{PAR} = 1 mmol l^{-1}; *1* Co, *2* Cu, *3* Ni, *4* PAR, *5* Fe

Effect of Some Cations in the Mobile Phase on the Retention of the Ni, Co, and Fe Chelates

When NH_4, Na, K or Li nitrates were used at concentrations up to 0.1 mol l^{-1}, extension of the retention times of the peaks was observed. Using a concentration of 0.1 mol l^{-1}, the increase in t_R was most marked with NH_4^+ (by 160 – 300%), followed by Na^+ (by 60 – 170%), K^+ (by 60 – 120%), and Li^+ (by 0 – 90%). The changes in t_R were most marked for PAR itself, followed by Ni, Fe and Co, using the mobile phase of 40 vol.% methanol + 60 vol.% HMT (pH 7.2) containing the nitrate.

IP RP HPLC of the PAR and TAR Chelates

Effect of Ion-Pair Reagents in the Mobile Phase on the Retention of the Chelates

As the concentrations of tetrabutylammonium hydroxide (TBA) neutralized with sulfuric acid and cetyltrimethyammonium bromide (CTMA) in the mobile phase are increased, the equilibrium shifts in favour of the formation of ion-associates of the PAR and TAR chelates with the ion-pair reagents (IPR), and their retention times increase. If IPR is present in a high excess, the retention times decrease, as can be seen in Fig. 6*a* for the well-soluble TBA at 20 – 50 mmol l^{-1}.

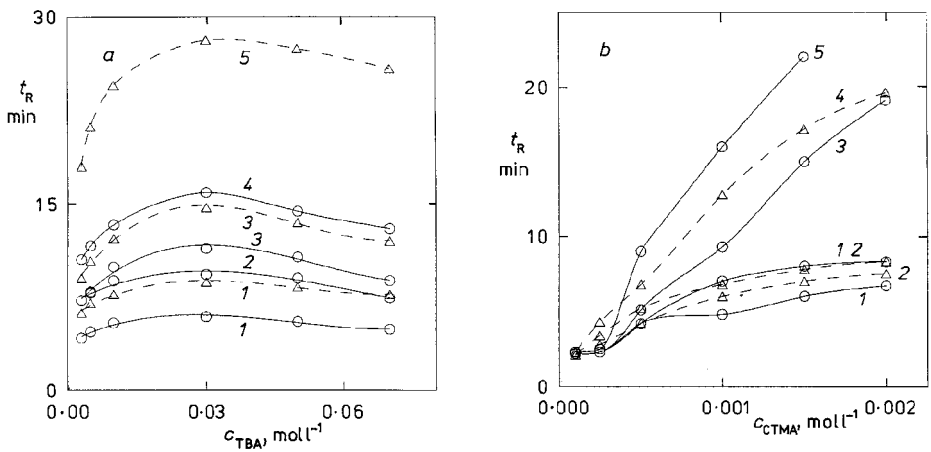

FIG. 6

Dependence of t_R on the concentration of the ion-pair agent in IP RP HPLC. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm), mobile phase: *a* 50 vol.% HMT (20 mmol l^{-1}, pH 7) with TBA + 50 vol.% methanol; *b* 30 vol.% HMT (20 mmol l^{-1}, pH 7) with CTMA + 70 vol.% methanol; c_M = 0.02 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}. Full lines: PAR, broken lines: TAR. Curves: *a 1* Co, *2* Fe, *3* Ni, *4* PAR, *5* TAR; *b 1* Fe, *2* Co, *3* Ni, *4* PAR, *5* TAR

Using the ion-pair reagent with a long carbon chain, the retention time increase is particularly marked for the NiL_2^{2-} chelates with PAR and TAR and for the HL^- anion of the reagent at CTMA concentrations exceeding 2 mmol l^{-1} (Fig. 6*b*).

Effect of pH and Kind of Buffer in the Mobile Phase on the Retention of the Chelates

In the presence of TBA or CTMA, the retention times increase markedly with increasing pH within the range of pH 5.0 – 7.6. The shape of the $t_R = f(\text{pH})$ dependences is opposite to that in RP HPLC of the PAR and TAR chelates (Fig. 7). The longer retention times are due to the dissociation of the polar *p*-OH substituent at the PAR or TAR molecule, a more pronounced formation of the charged ML_2^{2-} and ML_2^- chelates, and consequently an increase in the conditional stability constant of the sorbing ion-associates. The dependence is particularly marked for free PAR and TAR. For CoL_2^-, t_R is constant in the presence of TBA. 20 mmol l^{-1} HMT, $H_2PO_4^-$, 2-(*N*-morpholino)ethanesulfonic acid, and tris(hydroxymethyl)aminomethane at pH 5.0 – 7.6 have a negligible effect on the chromatographic behaviour of the PAR and TAR chelates because the interaction of the chelate with the ion-pair reagent is considerably stronger than with the buffer ions.

Effect of Methanol in the Mobile Phase on the Retention of the Chelates

As the concentration of methanol is increased, the t_R values of the Ni, Fe, and Co chelates, as well as of the reagent itself, decrease significantly both for PAR and TAR in the

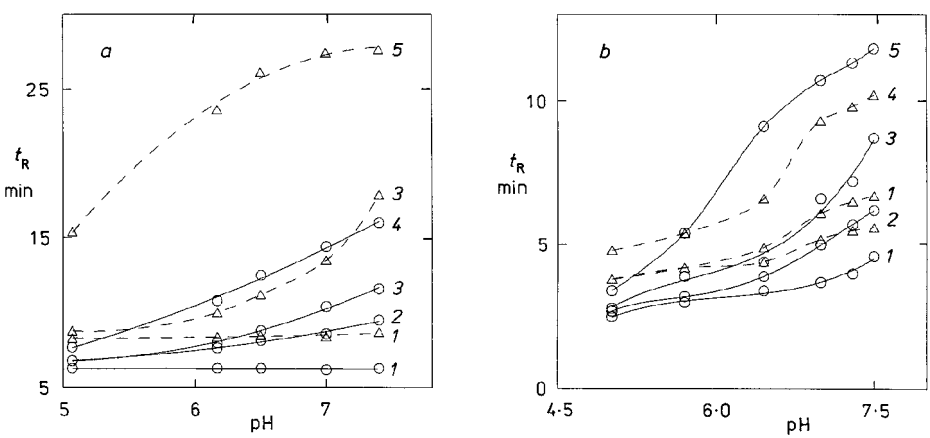

FIG. 7

Dependence of t_R on mobile phase pH in IP RP HPLC. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm); mobile phase: *a* 50 vol.% HMT (20 mmol l^{-1}) with TBA (50 mmol l^{-1}) + 50 vol.% methanol; *b* 28 vol.% HMT (20 mmol l^{-1}) with CTMA (1 mmol l^{-1}) + 72 vol.% methanol; c_M = 20 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}. Full lines: PAR, broken lines: TAR. Curves: *a 1* Co, *2* Fe, *3* Ni, *4* PAR, *5* TAR; *b 1* Fe, *2* Co, *3* Ni, *4* TAR, *5* PAR

presence of TBA or CTMA. Since CTMA forms less polar and more hydrophobic ion-associates with the PAR and TAR chelates as well as with the reagents themselves than TBA does, the methanol content of the mobile phase used was higher, viz. 70 – 75 vol.% (Fig. 8).

Calibration Dependences

The calibration curves of the Co, Ni, and Fe chelates with PAR and TAR in the ion-associates with TBA or CTMA were evaluated based on the peak heights for metal concentrations of 0.005 – 0.020 mmol l^{-1} and a total PAR or TAR concentration in the mother solution of the chelates before injection of 1 mmol l^{-1}. The results are given in Table IV. The relative standard deviation of one determination for the Co, Ni, and Fe chelates with PAR and TAR (0.01 mmol l^{-1}) is 1 – 4% (from 5 replicate determinations).

Interferences – mutual and from accompanying ions – are the same as in the RP HPLC mode.

Examples of chromatograms for the various alternatives are shown in Figs 9 and 10. A comparison of Figs 4*b*, 9*b* and 10*b* demonstrates the effect of the detection wavelength on the suppression of the peak of the free TAR.

Effect of Reagent in the Mobile Phase on the Retention of the Chelates

The behaviour of the chelates was compared for mobile phase which was free from the reagent or which contained it at concentrations of 0.1 or 0.2 mmol l^{-1}; both the RP and IP RP modes were examined.

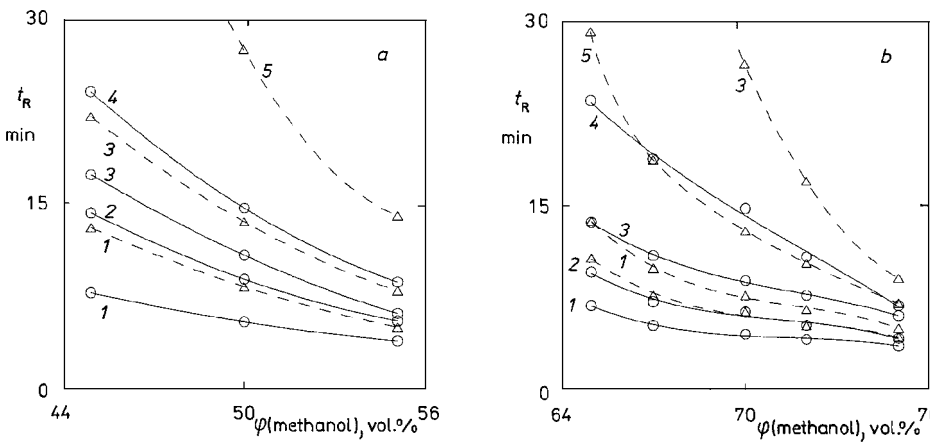

FIG. 8

Dependence of t_R on the methanol content of the mobile phase in IP RP HPLC. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm); mobile phase: HMT (20 mmol l^{-1}, pH 7) + methanol with TBA (50 mmol l^{-1}) (*a*) or CTMA (1 mmol l^{-1}) (*b*); Full lines: PAR, broken lines: TAR. Curves: *a* *1* Co, *2* Fe, *3* Ni, *4* PAR, *5* TAR; *b* *1* Fe, *2* Co, *3* Ni, *4* PAR, *5* TAR

The t_R values decreased slightly (10 – 20%) with increasing reagent concentration in both modes. While the sensitivity remained virtually constant, the reproducibility in both modes was substantially poorer if the reagent was present in the mobile phase.

If the metal cations, without reagent, were injected into the flowing mobile phase containing the reagent, the response was several times lower than if chelates prepared in advance were injected; thus, the chelate formation kinetics plays a role.

Interactions of the Chelates on the Column

In the absence of ion-pair reagents in the mobile phase, the orders of elution are as follows (Fig. 4): Co < Cu < PAR < Ni < Fe, and Ni < Co < Fe < TAR.

The order is affected by the resulting charge of the chelate, which is given by the charge of the cation, the chelate stoichiometry, and the actual dissociation constant of the hydroxy proton in the *para* position with respect to the azo group in the protonated PAR and TAR chelates[2]. The degree of dissociation of the proton is proportional to the stability constants of the forming chelates $M^{II}L_2H^-$, $M^{II}L_2^{2-}$, or $M^{III}L_2^-$ (ref.[28]) and so, besides the simultaneous formation of the neutral chelates $M^{II}(LH)_2$ and $M^{II}L$, is one of the major factors affecting the behaviour of the chelates. The anionic chelates after the detachment of the hydroxy protons, which are more stable, exhibit shorter retention times and capacity factors. The retention is also affected by the kinetic inertness and coordination saturation of the chelate[32,33].

TABLE IV
Calibration curve parameters for PAR and TAR chelates of Co, Fe, and Ni in IP RP HPLC[a,b]

Reagent	Metal	TBA		CTMA	
		a, l mol^{-1}	m_{lim}, ng	a, l mol^{-1}	m_{lim}, ng
PAR	Co	1 260 ± 120	1.4	3 600 ± 330	0.5
	Ni	820 ± 120	2.1	1 490 ± 240	1.2
	Fe	280 ± 60	6.0	1 240 ± 150	1.4
TAR	Co	1 600 ± 180	1.1	4 300 ± 330	0.4
	Ni	1 210 ± 150	1.5	1 550 ± 150	1.1
	Fe	470 ± 120	3.5	570 ± 120	3.0

[a] Mobile phase: for PAR: 50 vol.% HMT (20 mmol l^{-1}, pH 7.0) + 50 vol.% methanol with TBA (50 mmol l^{-1}), or 28 vol.% HMT (20 mmol l^{-1}, pH 6.95) + 72 vol.% methanol with CTMA (1 mmol l^{-1}); for TAR: 45 vol.% HMT (20 mmol l^{-1}, pH 7.0) + 75 vol.% methanol with CTMA (1 mmol l^{-1}), or 25 vol.% HMT (20 mmol l^{-1}, pH 7.2) + 75 vol.% methanol with CTMA (1 mmol l^{-1}); stationary phase: Separon SGX-RPS, λ = 500 nm for PAR and 530 nm for TAR; [b] symbols as in Table III.

Comparison with the results of earlier publications was only possible using the phosphate buffer. The chelate elution order was as reported in ref.[4]. The limits of detection observed by us for Ni, Co, and Fe were mostly better than as published before[3]. In the IP RP mode, the anionic chelates react with the cationic ion-pair reagent, and two mechanisms are assumed to operate in the separation of the ion-associates formed, viz.[30,31]:

a) Sorption of the ion-associates on the stationary phase;

b) ion exchange, due to which the ion-pair reagent binds to the sorbent first and the chelates separate subsequently.

Usually the two mechanisms operate simultaneously.

For neutral chelates or if the ion-pair reagent has a charge of the same sense as the chelate, the chelate and ion-pair reagent molecules act upon each other repulsively and the effect of the ion-pair reagent on the retention of the chelate is opposite – the retention times decrease with increasing concentration of the ion-pair reagent[23].

The orders of elution of the components in mixtures are as follows (Figs 9 and 10): Co < Fe < Ni < PAR and Co < Fe < Ni < TAR in the presence of TBA, and Fe < Co < Ni < PAR and Co < Fe < TAR < Ni in the presence of CTMA.

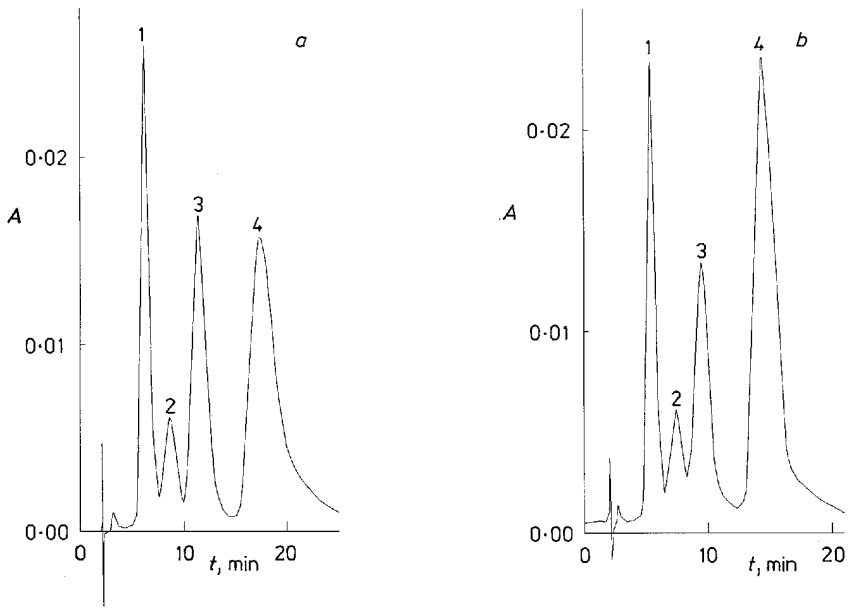

FIG. 9

Chromatograms of chelates of PAR (*a*) and TAR (*b*) in IP RP HPLC with TBA. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm); mobile phase: *a* 50 vol.% TRIS (pH 7) with TBA (30 mmol l^{-1}) + 50 vol.% methanol; *b* 45 vol.% MES (20 mmol l^{-1}, pH 7) with TBA (40 mmol l^{-1}) + 55 vol.% methanol; c_M = 0.02 mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}; λ = 500 nm (*a*), 550 nm (*b*). Peaks: 1 Co, 2 Fe, 3 Ni, 4 PAR or TAR

At pH > 7, the most stable chelates with PAR and TAR, viz. $M(LH)_2$, M(LH)L, and ML_2 predominate in solution[1,27]. Cu^{2+} is an exception, giving the M : L = 1 : 2 chelate only reluctantly with PAR and not at all with TAR. With the latter, only the CuL chelate is formed, whose log β value is 13.6 in 30 vol.% ethanol[34] (β = [CuL]/[Cu][L]). For this reason, no stable ion-associate is formed and no sorption occurs in the IP RP mode.

IP RP HPLC on a column of Separon SGX C18 with a mobile phase containing HMT buffer (20 mmol l^{-1}, pH 6.9 – 7.2) with CTMA (1 mmol l^{-1}) and methanol exhibits the highest sensitivity and the lowest limit of detection. The procedure is also less affected by changes in the aqueous component of the mobile phase than the RP HPLC variant.

The results obtained with the cationic tenside CTMA were better than with the conventional ion-pair reagent TBA (refs[5–7,9,11,12,15,17,20,22,23,25]).

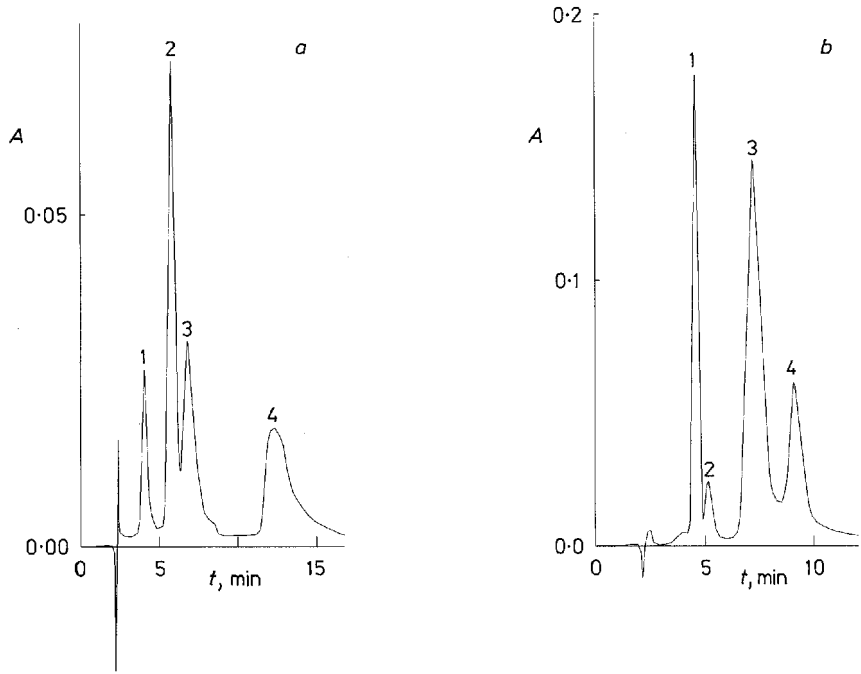

FIG. 10

Chromatograms of chelates of PAR (*a*) and TAR (*b*) in IP RP HPLC with CTMA. Column: 250 × 4 mm packed with Separon SGX-RPS (10 μm); mobile phase: *a* 30 vol.% HMT (20 mmol l^{-1}, pH 6.95) with CTMA (7.5 mmol l^{-1}) + 70 vol.% methanol; *b* 25 vol.% HMT (20 mmol l^{-1}, pH 7) with CTMA (1 mmol l^{-1}) + 75 vol.% methanol; c_M = 0.02 (*a*) or 0.04 (*b*) mmol l^{-1}, $c_{PAR,TAR}$ = 1 mmol l^{-1}; λ = 500 nm (*a*), 530 nm (*b*). Peaks: *a* 1 Fe, 2 Co, 3 Ni, 4 PAR; *b* 1 Co, 2 Fe, 3 TAR, 4 Ni

The results of RP HPLC determination and separation of Fe, Co, Ni, and Cu chelates with PAR at pH 11 – 13 as reported by Timerbaev and Petrukhin[30] are questionable because at such pH the C18 silica gel will decompose and dissolve in the mobile phase.

Determination of Low Concentrations of Fe, Ni, and Co in Waters after Preconcentration as PAR Chelates

The elements in quantities of 0.01 – 2.4 μg were preconcentrated by direct sorption of their PAR chelates from 100 ml volumes on Separon SGX-RPS (60 μm). The procedure was tested on redistilled water with various standard additions of solutions of Fe, Ni, and Co nitrates and on samples of drinking and surface water. The chromatographic peaks were evaluated using 3-point calibration curves, which were measured as in the determination proper.

The results obtained in the off-line and on-line modes are given in Tables V and VI, respectively. The Fe concentrations were corrected for the Fe content of the blank (PAR in redistilled water) arising from the stainless steel capillaries and pump.

The drinking and surface water samples were filtered through a 0.45 μm nylon filter prior to analysis. A volume of 5 ml of the sample was taken to the on-line determination; the PAR concentration was 0.01 mmol l^{-1}. The concentrations of Fe, Co, and Ni were determined by using the standard addition method (additions of 0.5, 1, and 1.5 μg per 100 ml) in order to eliminate any interfering effect of the matrix. An amount of 0.5 μg of Co and Ni per 100 ml of sample was added prior to the determination. The results of three replicate determinations for each sample are given in Table VII. This preconcentration method gave very good results and, with respect to its simplicity and reliability, is superior to procedures recommended in the literature[35].

TABLE V
RP HPLC determination of Co, Ni, and Fe in water after off-line preconcentration on Separon SGX-RPS in the form of PAR chelates[a]

Sample	Metal	Added, μg	Found[b], μg
1	Co	2.36	2.39 ± 0.06
	Ni	2.35	2.29 ± 0.06
	Fe	2.23	2.17 ± 0.06
2	Co	1.18	1.24
	Fe	1.12	1.11
	Ni	1.18	1.22

[a] Mobile phase: 30 vol.% HMT (20 mmol l^{-1}, pH 7) + 70 vol.% methanol with CTMA (1 mmol l^{-1}), sample volume 100 ml; [b] evaluation for 3 replicate determinations.

TABLE VI
RP HPLC determination of Co, Ni, and Fe in water after on-line preconcentration on Separon SGX-RPS in the form of PAR chelates[a]

Sample	Metal	Added, ng	Found[b], ng
1	Co	29.5	29.1 ± 0.9
	Ni	29.4	28.7 ± 1.1
	Fe	27.9	28.3 ± 0.8
2	Co	58.9	60.7
	Fe	55.9	58.1
	Ni	58.7	59.3
3	Fe	5.6	6.1
	Co	5.9	5.2
	Ni	5.9	5.4

[a] Mobile phase: 30 vol.% HMT (20 mmol l^{-1}, pH 7) + 70 vol.% methanol with CTMA (1 mmol l^{-1}), sample volume 100 ml; [b] evaluation for 3 replicate determinations.

TABLE VII
RP HPLC determination of Co, Ni, and Fe in natural waters after preconcentration on Separon SGX-RPS in the form of PAR chelates[a]

Sample			Preconcentration	
			off-line	on-line
	metal	added µg	found[b] µg	found[b] µg
Drinking water I	Fe	0	0.93 ± 0.09	0.95 ± 0.08
	Ni	0.5	0.46 ± 0.06	0.48 ± 0.05
	Co	0.5	0.49 ± 0.05	0.50 ± 0.05
Drinking water II	Fe	0	1.3 ± 0.1	1.3 ± 0.1
	Ni	0.5	0.51 ± 0.06	0.47 ± 0.06
	Co	0.5	0.48 ± 0.05	0.48 ± 0.04
River water	Fe	0	2.3 ± 0.2	2.2 ± 0.2
	Ni	0.5	0.52 ± 0.03	0.53 ± 0.05
	Co	0.5	0.51 ± 0.05	0.52 ± 0.04

[a] Mobile phase: 30 vol.% HMT (20 mmol l^{-1}, pH 7) + 70 vol.% methanol with CTMA (1 mmol l^{-1}), sample volume 100 ml in the off-line mode, 5 ml recalculated to 100 ml in the on-line mode; [b] evaluation for 3 replicate determinations.

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