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Simultaneous determination of inorganic anions and cations by ion chromatography with indirect photometric detection

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ABSTRACT

Inorganic cations and anions were separated simultaneously on a silica-based Shim-pack WAX-1 column. The method is shown to provide efficient separation of Ca^{2+}, Mg^{2+}, Cl^- and NO_3^-. Using 1 mM phthalate–6 mM EDTA at pH 6.57 as the eluent, a mixture containing these ions was separated in less than 6 min with detection limits of 1.2–2 ng. The effects of the eluent pH, concentration and composition on the retention behaviour of the analytes were studied. Two practical applications are described.

INTRODUCTION

Ion chromatography coupled with indirect photometric detection (IPC), described by Small and Miller [1], is a simple but powerful technique for the separation and detection of many non-UV-absorbing inorganic and organic ions. A feature of this photometric approach is the use of UV-absorbing eluents that can displace the sample ions from the chromatographic column with detection in the effluent. Detection involves measuring the absorbance decrease (negative peak) as the analyte displaces a UV-active component in the mobile phase. This single-column method is at least as sensitive as the more conventional conductimetric detection method [2], and allows greater control over instrumental sensitivity. The method has been used for the separation of inorganic ions in a number of applications [3–7].

In general, anions are separated on an anion-exchange resin and cations on a cation-exchange resin. However, Fritz et al. [8] and Mou and Liu [9] reported that some transition metal ions could be separated with chloride or oxalic acid as eluent using a single anion-exchange column and a post-column derivatization detection method with 4-(2-pyridylazo)resorcinol. Matsushita [10] effected the chromatographic separation of inorganic anions and alkaline earth metal ions with EDTA as eluent, which converted metal cations into metal chelate anions using a single anion-exchange column and direct UV detection in series with a conductivity detector. The use of IPC in conjunction with anion- and cation-exchange columns in series for the simultaneous determination of anions and cations but independently of each other using a single chromatograph has been described [11]. Recently, IPC for the simultaneous determination of inorganic cations and anions on a mixed bed of anion and cation exchangers was reported by Pietrzyk et al. [12].

Most of the commonly used columns in IPC are low capacity ion-exchange resins or silica-based packing exchangers. In this work, a Shim-pack WAX-1 column (50 × 4 mm I.D.; 3 μm)

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was selected. Surface silanol groups of the pack-
ings are chemically modified with a hydrophobic
polymer to produce a neutral silica surface. A
weakly basic stationary phase is chemically
bonded to this silica support, producing a stable
packing material for high-performance anion-
exchange chromatography. The ion-exchange
groups introduced on to the silica surface are
tertiary amino groups. However, the column
conditions for the simultaneous separation of
cations and anions in a single anion-exchange
column and using an indirect photometric detec-
tor must be modified. This paper describes a
procedure by using 1 mM phthalate–6 mM
EDTA as the eluent. The effects of the eluent
composition, concentration, and pH value on the
retention behaviour and detection sensitivity are
discussed.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph used consisted of a
Rheodyne Model 7125 valve injector (20-μl
loop) and a Shimadzu (Kyoto, Japan) system
including a solvent-delivery pump, an SPD-6AV
UV–Vis spectrophotometric detector, a C-R3A
Chromatopac and a Shim-pack WAX-1 column.
UV spectral scans were produced with a U-3400
spectrophotometer (Hitachi).

All reagents were of analytical-reagent grade
and aqueous solutions were prepared using
doubly distilled water.

Chromatographic procedures

Mobile phases were prepared using analytical-
reagent grade phthalic anhydride dissolved in
water. The pH of each mobile phase was ad-
justed by dropwise addition of 1.0 M sodium
hydroxide solution. All mobile phases were
filtered through a 0.45-μm filter and degassed in
an ultrasonic bath prior to use. Further chro-
matographic conditions are given in the figure
captions.

RESULTS AND DISCUSSION

The UV spectrum from 250 to 330 nm of a
phthalate solution (pH 3.72) shows an absorp-

tion maximum at 280 nm with a molar absorp-
tivity of 1279 l/mol·cm. No other peaks are
present in the spectrum. The UV spectrum from
200 to 330 nm of an EDTA solution (pH 5.56)
shows no absorption. We chose 280 nm as the
detection wavelength throughout this work.

The dissociation constants, pK_a, for phthalic
acid (H$_2$P) are 2.95 and 5.41 [13]; the pK_a values
for EDTA(H$_4$Y) are 0.9 (pK_{a_1}), 1.6 (pK_{a_2}), 2.0
(pK_{a_3}), 2.67 (pK_{a_4}), 6.16 (pK_{a_5}) and 10.26
(pK_{a_6}) [13]. It can be calculated that the solu-
tions of phthalate and EDTA at pH 7.21 contain
98.44% P^{2-}, 91.74% HY^{3-} and 8.18% H$_2$Y^{2-}.
It can be concluded from the above that P^{2-} and
HY^{3-} are the predominant anions in the eluent
if pH $\geqslant$ 6.2. Under these conditions, the reaction
between an alkaline earth metal ion M^{2+} to form
a chelate anion with HY^{3-} can be written as

$$M^{2+} + HEDTA^{3-} \rightleftharpoons MEDTA^{2-} + H^+$$

The separation of Ca^{2+}, Mg^{2+}, Cl$^-$ and NO$_3^-$
using a solution containing 1 mM H$_2$P and 6 mM
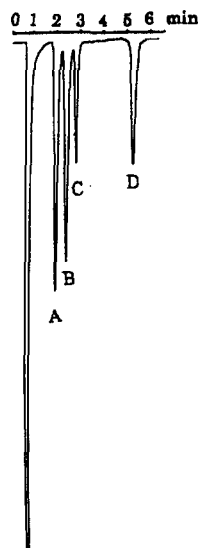EDTA at pH 6.57 as the eluent is shown in
Fig. 1.

Fig. 1. Separation of a mixture of cations and anions by IPC.
Eluent, 1 mM H$_2$P–6 mM EDTA (pH 6.57); flow-rate, 0.6
ml/min; column temperature, 25°C; detection wavelength,
280 nm; absorbance units full-scale, 0.04. Peaks; A = 0.9 μg
Ca^{2+}; B = 0.5 μg Mg^{2+}; C = 0.8 μg Cl$^-$; D = 1.4 μg NO$_3^-$.

Effect of basicity on eluent strength

In this work, the acidity of the eluent over a wide range did not affect the capacity of the strong ion-exchange column, but mainly affected the dissociation of H_2P and EDTA in the eluent. Some details are as follows.

In the anion-exchange process A^{x-} as a counter ion undergoes an ion exchange with the chelate anion and inorganic anion. The exchange process is

$$yA^{x-}_{(S)} + xB^{y-}_{(M)} \rightleftharpoons yA^{x-}_{(M)} + xB^{y-}_{(S)}$$
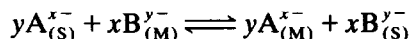
where S and M represent the stationary and mobile phase, respectively, and A^{x-} and B^{y-} represent the counter ion and sample ion, respectively. Assuming activity coefficients to be equal to unity, we express the selectivity coefficient K^B_A as

$$K^B_A = \frac{[A^{x-}]^y_{(M)}[B^{y-}]^x_{(S)}}{[A^{x-}]^y_{(S)}[B^{y-}]^x_{(M)}} \quad (1)$$

The distribution coefficient for B^{y-} is

$$D_M = \frac{[B^{y-}]_{(S)}}{[B^{y-}]_{(M)}} = \left\{ \frac{[A^{x-}]_{(S)}K^{B\,1/y}_A}{[A^{x-}]_{(M)}} \right\}^{y/x} \quad (2)$$

On the other hand, an ion-exchange equilibrium between OH^- and A^{x-} also exists:

$$A^{x-}_{(S)} + xOH^-_{(M)} \rightleftharpoons A^{x-}_{(M)} + xOH^-_{(S)}$$
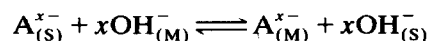
Similarly, the selectivity coefficient K^{OH}_A is

$$K^{OH}_A = \frac{[A^{x-}]_{(M)}[OH^-]^x_{(S)}}{[A^{x-}]_{(S)}[OH^-]^x_{(M)}} \quad (3)$$

By combination of eqns. 2 and 3:

$$D_M = \frac{[B^{y-}]_{(S)}}{[B^{y-}]_{(M)}} = \frac{K^{B\,1/x}_A[OH^-]^y_{(S)}}{K^{OH\,y/x}_A[OH^-]^y_{(M)}} \quad (4)$$

From eqn. 4, we concluded that the higher the basicity of the eluent, the weaker is the retention of the sample ions. This is consistent with the experimental results shown in Fig. 2.

In Fig. 2, the peaks for the first three anions are completely overlapped at pH 5.30, the peaks for Mg^{2+} and Cl^- are completely overlapped in the pH range 5.30–5.94 and the peaks for Ca^{2+} and Cl^- are completely overlapped below pH
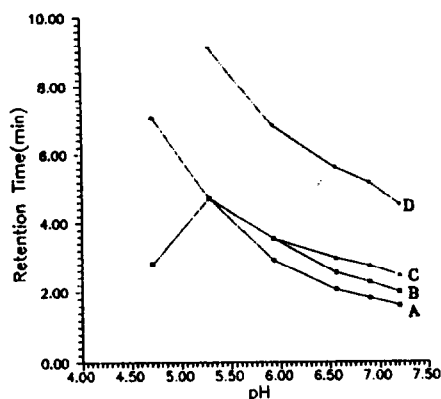

Fig. 2. Retention time *versus* the pH of the eluent. Eluent, 1 mM H_2P–6 mM EDTA. Other conditions as in Fig. 1.

5.30. However, NO_3^- was well resolved, but the retention time for NO_3^- was extremely long at pH <5.30. It was noticeable that the retention time for Mg^{2+} increased with increasing basicity of the eluent at pH <5.30. We presumed that MgY^{2-} was dissociated below pH 5.3. According to the conditional equilibrium constants [14], 4.1 for CaY^{2-} and 2.1 for MgY^{2-} at pH 5.0, the stability of CaY^{2-} is 100 times higher than that of MgY^{2-}, and the chelate MgY^{2-} cannot exist stably in this pH range.

The effects of pH on the retention behaviour of Cl^- and NO_3^- were expected to be dependent on the acid dissociation constants of H_2P and EDTA. However, the effects of pH on the retention behaviour of Ca^{2+} and Mg^{2+} were expected to be dependent on both the conditional stability constants of metal EDTA chelates and the acid dissociation constants of H_2P and EDTA.

Effect of basicity of eluent on detection sensitivity

Hydroxyl ions in a weakly acidic eluent can act as counter ions when the eluent pH is much greater than its pK_a value. Both A^{x-} and OH^-, which are retained by the anion exchanger, can be displaced by the sample ions. This makes the sensitivity of detection lower than the molar absorptivity of phthalate. Hence a pH of the eluent generally maintained in the acid range will produce a powerful separation and a more sensi-
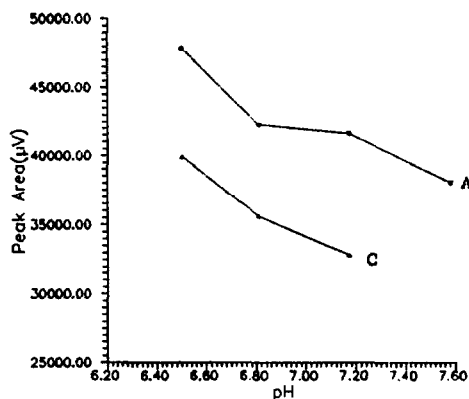
Fig. 3. Plot of sample peak area *versus* eluent pH. Conditions as in Fig. 1.

tive detection. The plot in Fig. 3 verified the point that the detection sensitivity decreases with increasing hydroxyl ion concentration using a weakly acidic mobile phase with $pH > pK_a$ with the IPC method described in this paper.

The result indicates that the ideal eluent pH for complete and optimum separation of the four analytes should be controlled in the range 6.2–7.0; this would eliminate the deleterious effect on detection caused by hydroxyl ions and could avoid damage to the chromatographic column.

Effect of eluent concentration on signal-to-noise ratio

The peak area or peak height for analytes depends on the concentration of the IPC eluent (Fig. 4). Low eluent concentrations are recommended for achieving low background absorb-
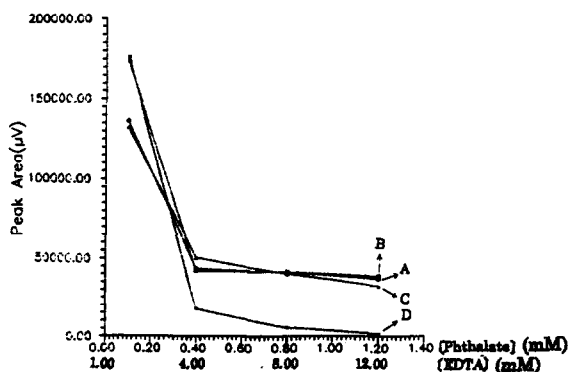

Fig. 4. Plot of sample peak area *versus* eluent concentration. Conditions as in Fig. 1.

ance and good detection limits [1]. This was also predicted by Yeung [15] using the equation

$$C_{\text{lim}} = C_m/(D_R T_R) \qquad (5)$$

where C_{lim} is the concentration limit of detection, C_m is the concentration of the eluent, D_R is the dynamic reserve (ratio of background signal to background noise) and T_R is the transfer ratio, which is expressed as the number of eluent molecules displaced by one analyte molecule. Eqn. 5 shows a direct proportionality between C_{lim} and C_m. These trends are typical and consistent with all indirect detection techniques, because the relative change in the baseline absorbance is more pronounced at low than at high background absorbance levels. A low eluent concentration provides high sensitivity and powerful separation compared with more concentrated eluents. However, it is important to note that a too dilute eluent will result in overlong run times and a decrease in sensitivity due to band spreading.

Effect of eluent composition on retention behaviour

The elution mechanism of IPC using a mixed eluent as in this work is complicated. A series of mixed eluents containing various concentrations of H_2P and EDTA at pH 7.4 were studied, and also two single eluents, H_2P (1 mM, pH 6.95) and EDTA (6 mM, pH 7.02). Fig. 5 illustrates the chromatogram of Ca^{2+}, Mg^{2+}, Cl^- and NO_3^- using EDTA as eluent with detection at 230 nm. All the analytes were well resolved, but baseline noise cannot be deleted. In addition, no peaks appeared when the wavelength was set at 280 nm using the same eluent, because EDTA and the EDTA chelate do not absorb at this wavelength. Fig. 6 shows a chromatogram of the samples obtained using H_2P as eluent with detection at 280 nm. Only two negative peaks for Cl^- and NO_3^- were observed, and peaks for Ca^{2+} and Mg^{2+} were not found because Ca^{2+} and Mg^{2+} cations could not be retained by the anion-exchange column. The experiment was carried out by injecting MY^{2-} into the chromatographic column and two negative peaks were obtained.
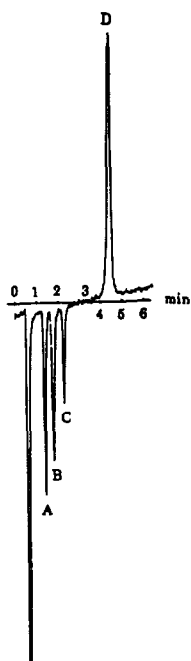
From the above experiments, two theoretical

Fig. 5. Separation of a mixture of cations and anions. Eluent, 6 mM EDTA (pH 7.02); detection wavelength, 230 nm.

models were presumed. The separation of Ca^{2+} and Mg^{2+} was probably based on two factors: the competitive formation balance between metal cations and EDTA and the anion-exchange balance between EDTA anion chelate
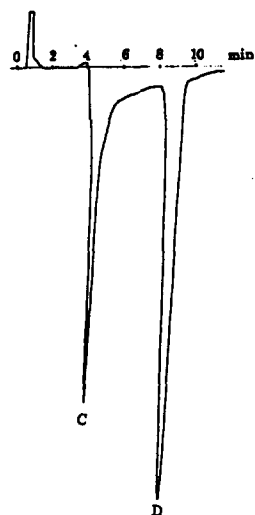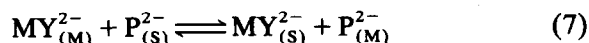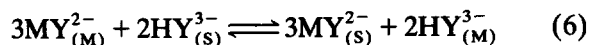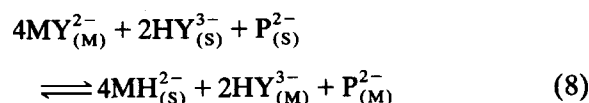

Fig. 6. Separation of chloride and nitrate. Eluent, 1 mM H_2P (pH 6.95); detection wavelength, 280 nm.

and counter ions. When HY^{3-} and P^{2-} displaced MY^{2-} from the column into the eluent, the H_2P concentration was changed, which resulted in a negative absorption at 280 nm, corresponding to an indirection detection mode. The mechanism can be explained as follows:

$$3MY^{2-}_{(M)} + 2HY^{3-}_{(S)} \rightleftharpoons 3MY^{2-}_{(S)} + 2HY^{3-}_{(M)} \quad (6)$$

$$MY^{2-}_{(M)} + P^{2-}_{(S)} \rightleftharpoons MY^{2-}_{(S)} + P^{2-}_{(M)} \quad (7)$$

By combining eqns. 6 and 7 we obtain

$$4MY^{2-}_{(M)} + 2HY^{3-}_{(S)} + P^{2-}_{(S)}$$
$$\rightleftharpoons 4MH^{2-}_{(S)} + 2HY^{3-}_{(M)} + P^{2-}_{(M)} \quad (8)$$

The selectivity coefficient $K^{MY}_{Y,P}$ is

$$K^{MY}_{Y,P} = \frac{[MY^{2-}]^4_{(S)}[HY^{3-}]^2_{(M)}[P^{2-}]_{(M)}}{[MY^{2-}]^4_{(M)}[HY^{3-}]^2_{(S)}[P^{2-}]_{(S)}} \quad (9)$$

The molar fraction of MY^{2-} in solution is

$$\Phi = \frac{[MY^{2-}]}{[M^{2+}] + [MY^{2-}]} \quad (10)$$

The distribution coefficient for M^{2+} can be deduced by combining eqns. 9 and 10:

$$D_M = \frac{[MY^{2-}]_{(S)}}{[M^{2+}]_{(M)}}$$

$$= K^{MY\ 1/4}_{Y,P} \frac{\Phi}{1-\Phi} [HY^{3-}]^{1/2}_{(S)}[P^{2-}]^{1/4}_{(S)}$$

$$\times ([HY^{3-}]^2_{(M)}[P^{2-}]_{(M)})^{-1/4} \quad (11)$$

The logarithmic form of eqn. 11 is

$$\log D_M = \frac{1}{4} \log K^{MY}_{Y,P} + \log \left(\frac{\Phi}{1-\Phi}\right)$$

$$+ \frac{1}{2} \log [HY^{3-}]_{(S)} + \frac{1}{4} \log [P^{2-}]_{(S)}$$

$$- \frac{1}{4} \log ([HY^{3-}]^2_{(M)}[P^{2-}]_{(M)}) \quad (12)$$

where $K^{MY}_{Y,P}$, $[HY^{2-}]_{(S)}$ and $[P^{2-}]_{(S)}$ are constants. However, Φ can also be a constant if the concentration of EDTA in each of the eluents is much higher than that of metal cations. In this event, a straight line should be obtained on

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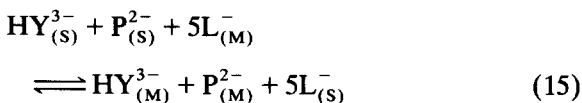plotting log K' vs. $\log\{[HY^{3-}]^2_{(M)}[P^{2-}]_{(M)}\}$, and the theoretical slope value should be -0.25.

Experiments showed that the correlation coefficients for Ca^{2+} and Mg^{2+} are -0.9994 and -0.9999, respectively, and the actual slope values for both are exactly the correct value, -0.25. Apparently the actual counter ions in the eluent for Ca^{2+} and Mg^{2+} are HY^{3-} and P^{2-}, which are compatible with the hypotheses described above.

The separation model for monovalent anions can be described as follows. It was assumed that Cl^- and NO_3^- were displaced from the column by the counter ions HY^{3-} and P^{2-}, which also resulted in a negative absorption at 280 nm, corresponding to an indirect detection mode:

$$HY^{3-}_{(S)} + 3L^-_{(M)} \rightleftharpoons HY^{3-}_{(M)} + 3L^-_{(S)} \qquad (13)$$

$$P^{2-}_{(S)} + 2L^-_{(M)} \rightleftharpoons P^{2-}_{(M)} + 2L^-_{(S)} \qquad (14)$$
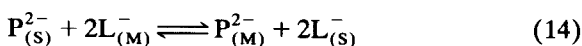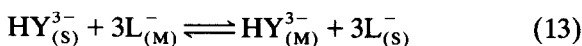
By combining eqns. 13 and 14, we obtain

$$HY^{3-}_{(S)} + P^{2-}_{(S)} + 5L^-_{(M)}$$

$$\rightleftharpoons HY^{3-}_{(M)} + P^{2-}_{(M)} + 5L^-_{(S)} \qquad (15)$$

The logarithmic form of D_M for L^- can be deduced similarly:

$$\log D_M = \frac{1}{5}\log K^L_{Y,P} + \frac{1}{5}\log[P^{2-}]_{(S)}$$

$$+ \frac{1}{5}\log[HY^{3-}]_{(S)}$$

$$- \frac{1}{5}\log([HY^{3-}]_{(M)}[P^{2-}]_{(M)}) \qquad (16)$$

where $K^L_{Y,P}$, $[P^{2-}]_{(S)}$ and $[HY^{3-}]_{(S)}$ are constants. A linear relationship should exist between $\log D_M$ and $\log\{[HY^{3-}]_{(M)}[P^{2-}]_{(M)}\}$, and the theoretical slope value should be -0.20.

Experiments indicated that the correlation coefficients for Cl^- and NO_3^- are -0.9952 and -0.9963, respectively, and the actual slope values are -0.22 and -0.20, respectively.

Six monovalent anions were separated under the same conditions and their slope values and correlation coefficients are given in Table I.

The binary eluent used is effective in separating some other common cations and anions, and the absolute retention times for these ions are given in Table II.

TABLE I

SLOPE VALUES AND CORRELATION COEFFICIENTS FOR ANIONS

Eluent, 1 mM H_2P–6 mM EDTA (pH 7.03); injection volume, 10 μl (1 mM). Other conditions as in Fig. 1.

Anion	Slope	Correlation coefficient
BrO_3^-	-0.20	-0.9960
Cl^-	-0.20	-0.9968
NO_2^-	-0.19	-0.9961
ClO_3^-	-0.19	-0.9951
Br^-	-0.19	-0.9937
NO_3^-	-0.24	-0.9793

Applications

The correlation coefficients of the calibration graphs for Ca^{2+}, Mg^{2+}, and Cl^- are excellent, varying from 0.9992 to 0.9995. The detection limits for Ca^{2+}, Mg^{2+} and Cl^- are 1.2, 1.4, and 2 ppm, respectively (signal-to-noise ratio = 2) when using the optimized mobile phase.

The IPC method described was used to determine alkaline earth metal ions and chloride in tap water and Yellow River water. The concentrations of these three ions determined by this technique are given in Table III.

TABLE II

ABSOLUTE RETENTION TIMES FOR CATIONS AND ANIONS

Ion	Absolute retention time (min)	Ion	Absolute retention time (min)
Ba^{2+}	3.08[a]	BrO_3^-	2.37[b]
Pb^{2+}	3.56[a]	Cl^-	3.02[b]
Ni^{2+}	7.05[a]	NO_2^-	3.67[b]
Mn^{2+}	4.81[a]	ClO_3^-	4.00[b]
Co^{2+}	6.48[a]	Br^-	5.12[b]
Zn^{2+}	6.65[a]	NO_3^-	5.65[b]
Cu^{2+}	14.86[a]		
Bi^{3+}	3.33[a]		
Rh^{3+}	3.34[a]		

[a] Eluent, 1.2 mM H_2P–1.2 mM EDTA (pH 5.54); injection volume, 10 μl (1 mM). Other conditions as in Fig. 1.
[b] Eluent, 1.2 mM H_2P–1.2 mM EDTA (pH 7.02); injection volume, 10 μl (1 mM). Other conditions as in Fig. 1.

TABLE III

ANALYTICAL RESULTS FOR TWO WATER SAMPLES

Eluent, 1 mM H$_2$P–6 mM EDTA (pH 7.25); injection volume, 20 μl.

Sample	Ca^{2+} (ppm)^a	Mg^{2+} (ppm)^a	Cl$^-$ (ppm)^a
Tap water	72.0	22.6	43.2
Yellow River water	50.5	16.7	35.0

^{a} Values are (w/w).

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