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# ION CHROMATOGRAPHY OF INORGANIC AND ORGANIC IONIC SPECIES USING REFRACTIVE INDEX DETECTION

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## SUMMARY

Ion chromatography of inorganic and non-UV-absorbing organic species can be monitored with a differential refractive index detector if aromatic counter ions are used. Practical applications show that in this way refractive index (RI) detection can compete with systems for ion chromatography (IC) using electric conductivity detection. IC with RI detection can be carried out with any liquid chromatograph. Hence high efficiency ion-exchange columns can be used.

## INTRODUCTION

The analysis of ionic species in aqueous solutions is becoming increasingly important. It is well known that the specificity of ion exchange can be utilized for liquid chromatography of ionic compounds. In high-performance liquid chromatography (HPLC), however, the application of ion-exchange chromatography is limited because of poor detection sensitivity. This is not a problem if the ionic solutes absorb in the UV or visible range, but ion-exchange chromatography of inorganic ions is limited by the lack of sensitivity inherent in universal detectors such as the refractive index and electrical conductivity detectors. Thus, the development of ion-exchange chromatography has not been as rapid as that of other liquid chromatographic techniques such as straight phase adsorption, reversed-phase and size exclusion chromatography.

In 1975 Small *et al.*[1] reported a new chromatographic method using electrical conductometric detection and mentioned ion chromatography (IC). In addition to the separation or analytical column they used a stripper or suppressor column. The stripper column removes the buffer used for the elution of the ionic species from the separation column, which results in a low background activity for the conductivity cell. This principle has been adopted in the ion chromatographs of the Dionex Corporation.

Gjerde and co-workers[2,3] demonstrated that a suppressor column is not absolutely necessary for ion chromatography with electric conductivity detection. They used an anion exchanger having a low exchange capacity and an eluent having a very low conductivity. In this way the background conductivity is low enough to allow the

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separated anions to be detected with a simple conductance detector. Molnár *et al.*[4] applied a specially designed conductivity detector both with and without a suppressor column, for the separation of anions and cations on $C_{18}$ columns using secondary equilibria in the mobile phase. For the separation of cations a silicious cation exchanger was used.

Besides ion exchange, some researchers have used ion exclusion for the separation of weak acids. Turkelson and Richards[5] employed this technique for separation of the acids of the citric acid cycle, and Tanaka *et al.*[6] described the ion-exclusion behaviour of a large number of strong and weak acids. Bio-Rad Labs. (Richmond, CA, U.S.A.) recently introduced a so-called organic acid analysis column, based on ion exclusion. The use of diluted sulphuric acid as the mobile phase allows UV detection at 210 nm and thus high sensitivity for a variety of weak organic acids. However, ion-exclusion chromatography is not capable of selectivity for the separation of strong acids.

A large number of applications of the Dionex equipment in the fields of process control and environmental research and in the power industry have been reported[7]. From the chromatographic point of view, there are some drawbacks:

(1) the number of injections is restricted by the capacity of the suppressor column

(2) the suppressor column introduces extra band broadening, which results in lower resolution

(3) special equipment is needed for IC

(4) only those buffers can be applied which, after passage through the suppressor column, result in a low electrical background conductivity.

In this paper, a promising mode of IC monitored by a refractive index detector is described. The method allows IC to be carried out with commercially available HPLC equipment. It differs in certain aspects from the IC technique employed by Dionex, and attains a sensitivity similar to that obtained by conductivity detection.

## THEORETICAL

In an ion-exchange process, counter ions are removed from the ion exchanger on injection of a sample solution. The excess of the counter ions in the mobile phase results in a "solvent peak" at the unretained place (the elution "volume" of compounds not interacting with the ion exchanger, such as glucose) in the chromatogram. In addition, since there is a dynamic exchange process between the solute and the counter ions, the migration of the solute ions through the column is accompanied by a local deficiency of the buffer ions. If a differential refractive index detector is employed the signal obtained will be the result of these two concentration changes, which are always opposite. The sensitivity is determined by the difference in refractive index between the buffer ions and the solute ions.

If, as usual, inorganic buffers are used for the separation of inorganic ionic species, the sensitivity is very low. Therefore, to increase the sensitivity, buffers with a very high (or very low) refractive index relative to that of the solute have to be used, *e.g.*, an aromatic anion for an anion-exchange process and an aromatic cation for a cation-exchange process.

## EXPERIMENTAL

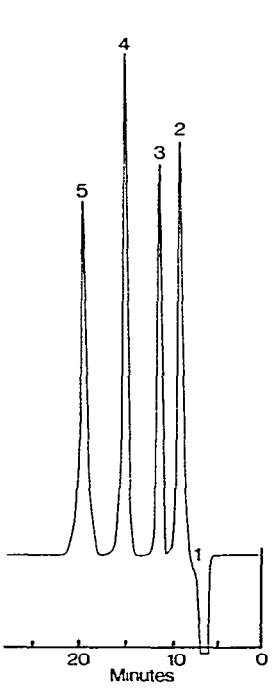
### *Apparatus*

The high-performance liquid chromatograph consisted of a high-pressure pump (Model 6000 A, Waters Assoc.) equipped with a differential refractometer (Waters R 401), a high-pressure sampling valve (Rheodyne RH 7010), a linear potentiometric recorder (Kipp and Zonen BD 8) and a GCA precision thermostat. The latter was used for thermostating in series both the column (via a water-jacket) and the detector.

Three different anion-exchange packings were used in stainless-steel columns Nucleosil SB, 5- and 10-$\mu$m particles, 150 and 250 × 4.6 mm ((Chrompack, Middelburg, The Netherlands); Partisil 10 SAX, 250 × 4.6 mm (Whatman), and Zorbax AX, 250 × 4.6 mm (DuPont). For cation-exchange chromatography use was made of a 250 × 4.6 mm stainless-steel column packed with Nucleosil 10 SA (Chrompack).

### *Chemicals*

The eluents used were prepared from distilled water and reagent grade chemicals (Baker, Deventer, The Netherlands), and degassed carefully.



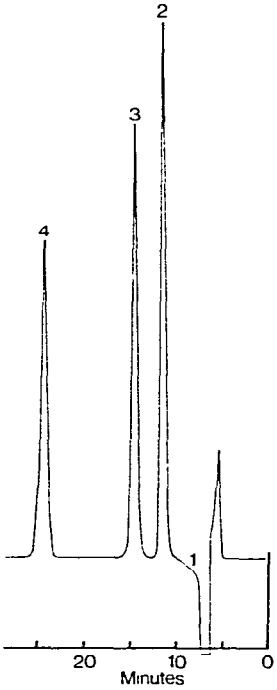




Fig. 1. IC chromatogram of a synthetic mixture of inorganic anions. Column: 250 × 4.6 mm Nucleosil 10 SB. Mobile phase: 0.03 *M* sodium salicylate, pH 4.0; flow-rate 0.5 ml/min. Sample size: 20 $\mu$l. Detection: RI, attenuation × 8. Peaks: 1 = solvent effect; 2 = phosphate (20 $\mu$g); 3 = chloride (10 $\mu$g); 4 = nitrate (20 $\mu$g); 5 = sulphate (20 $\mu$g).

Fig. 2. IC chromatogram of halogens. For conditions see Fig. 1. Peaks: 1 = solvent effect; 2 = chloride (14 $\mu$g); 3 = bromide (25 $\mu$g); 4 = iodide (50 $\mu$g).

## RESULTS AND DISCUSSION

In Figs. 1 and 2 the separations of some mixtures of inorganic anions are given. A good resolution was obtained by using 0.03 mol/l sodium salicylate, pH 4, as the eluent. Since the decrease in the buffer ion concentration is measured, the corresponding decrease of the refractive index has been plotted along the vertical axis.

The selectivity can be altered by changing the counter ion, *e.g.*, benzoate, *p*-hydroxybenzoate, sulphobenzoate or phthalate, and the pH (see Figs. 3 and 4). Moreover, the selectivity depends on the type of column. The Zorbax AX packing (DuPont) has a much higher capacity than the Partisil SAX and the Nucleosil SB material and hence requires stronger eluents.

Organic anions can also be separated, as shown in Fig. 5 for chloroacetates.

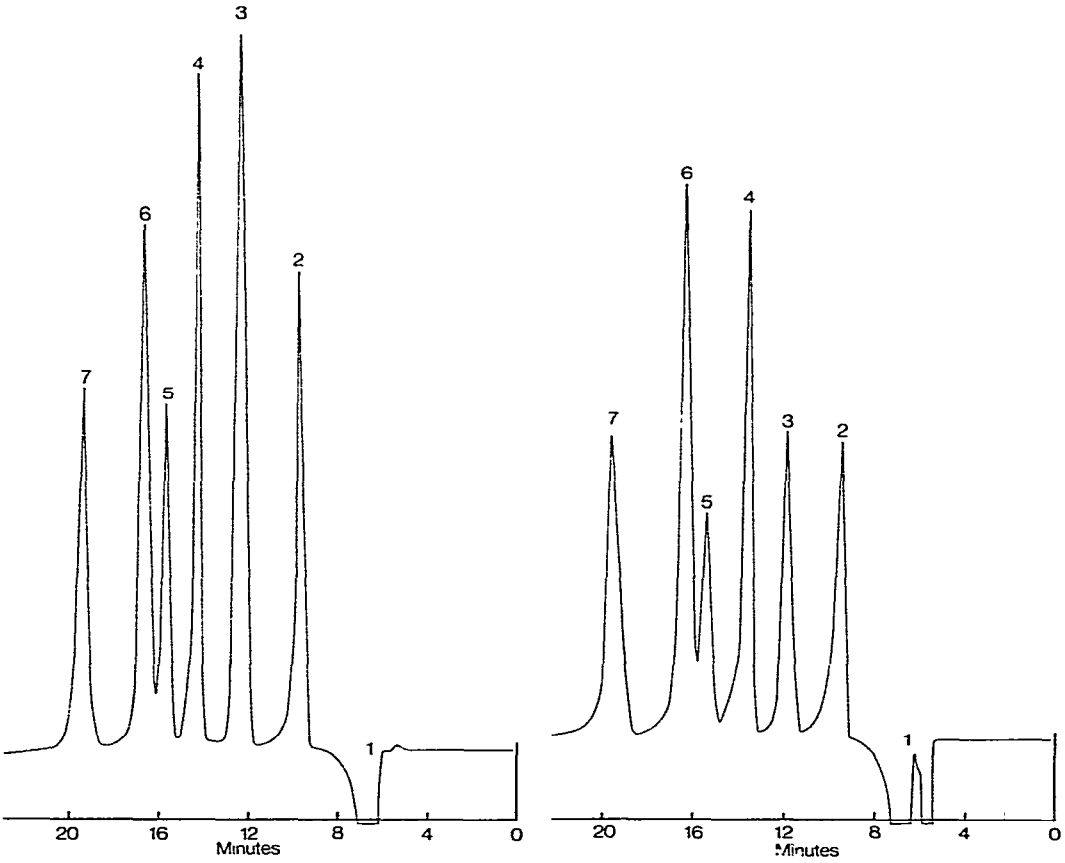



Fig. 3. IC chromatogram of a synthetic mixture. Mobile phase: 0.075 *M* sodium *p*-hydroxybenzoate, pH 5.6. Other conditions as in Fig. 1. Peaks: 1 = solvent effect; 2 = glycolate (20 μg); 3 = chloride (20 μg); 4 = nitrite (20 μg); 5 = chlorate (20 μg); 6 = nitrate (20 μg); 7 = sulphate (20 μg).

Fig. 4. IC chromatogram of a synthetic mixture. Mobile phase: 0.05 *M* potassium biphthalate, pH 3.9. Other conditions as in Fig. 1. Peaks: 1 = solvent effect; 2 = dihydrogen phosphate (25 μg); 3 = chloride (10 μg); 4 = nitrite (25 μg); 5 = bromide (25 μg); 6 = nitrate (25 μg); 7 = sulphate (25 μg).

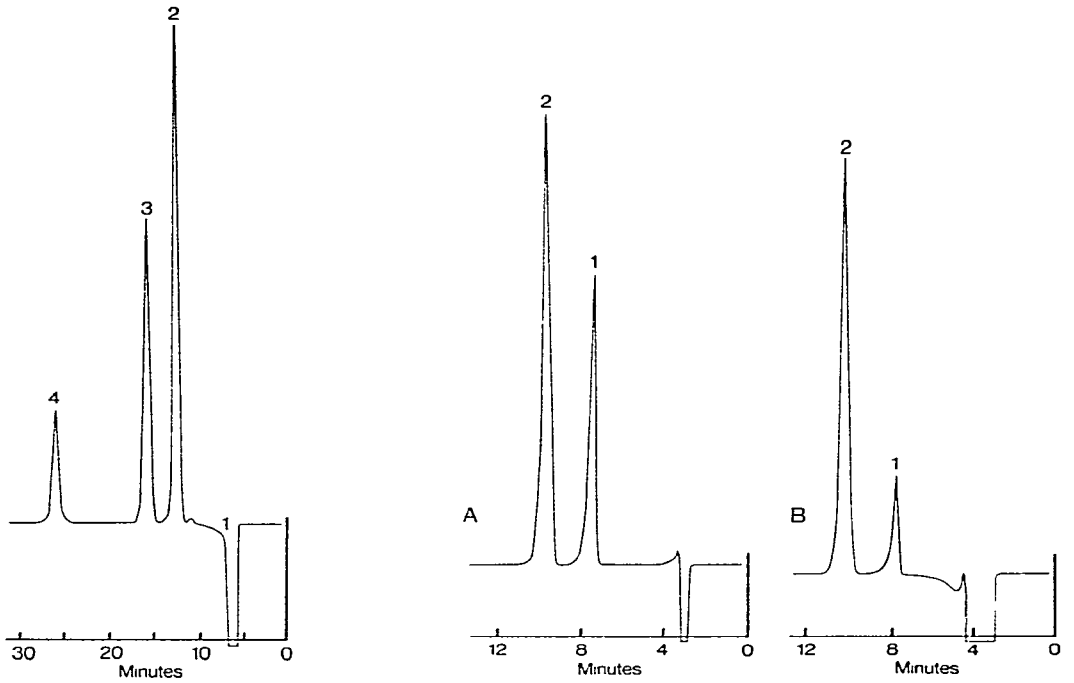



Fig. 5. IC chromatogram of chloroacetates. For conditions see Fig. 1. Peaks: 1 = solvent effect; 2 = monochloroacetate (20 $\mu$g); 3 = dichloroacetate (20 $\mu$g); 4 = trichloroacetate (20 $\mu$g).

Fig. 6. A, IC chromatogram of a synthetic mixture of sodium glycolate (1) and sodium chloride (2). B, IC chromatogram of a water–methanol extract of carboxymethyl-cellulose. Column: 250 × 4.6 mm Partisil 10 SAX. Mobile phase: 0.035 *M* sodium salicylate, pH 5.0; flow-rate 1 ml/min. Sample size: 20 $\mu$l. Detection: RI, attenuation × 8.

The efficiencies of the separations are in principle better than those obtained with the Dionex system[7]. The absence of a suppressor column and the use of silica based ion exchangers with a mean particle size of 5 or 10 $\mu$m result in less peak dispersion.

In Fig. 6A the IC chromatogram of a standard mixture of sodium glycolate and sodium chloride is shown. These two compounds are present in water–methanol extracts of sodium carboxymethyl-cellulose, as is seen in Fig. 6B. Since the efficiency of the carboxymethylation and the degree of substitution can be calculated from the amounts of sodium glycolate and sodium chloride, quantification is important. For these ions a linear relationship between peak area and concentration was established up to an injected amount of 100 $\mu$g chloride and 30 $\mu$g glycolate; the correlation coefficient was better than 0.9999. The relative standard deviation for the two ions, based on data from six independent measurements, was less than 1 % for chloride (at the 20 % level) and about 2 % for glycolate (at the 5 % level).

The sensitivity is reflected in Fig. 7, where the chromatogram of 180 ng nitrate is presented at attenuation position 4 of the Waters R 401 refractive index detector. For this detector the detection limits for some ions are: $Cl^-$, 20 ng; $NO_3^-$, 30 ng; $SO_4^{2-}$, 30 ng; $H_2PO_4^-$, 50 ng and tetramethylammonium ($TMA^+$), 50 ng.

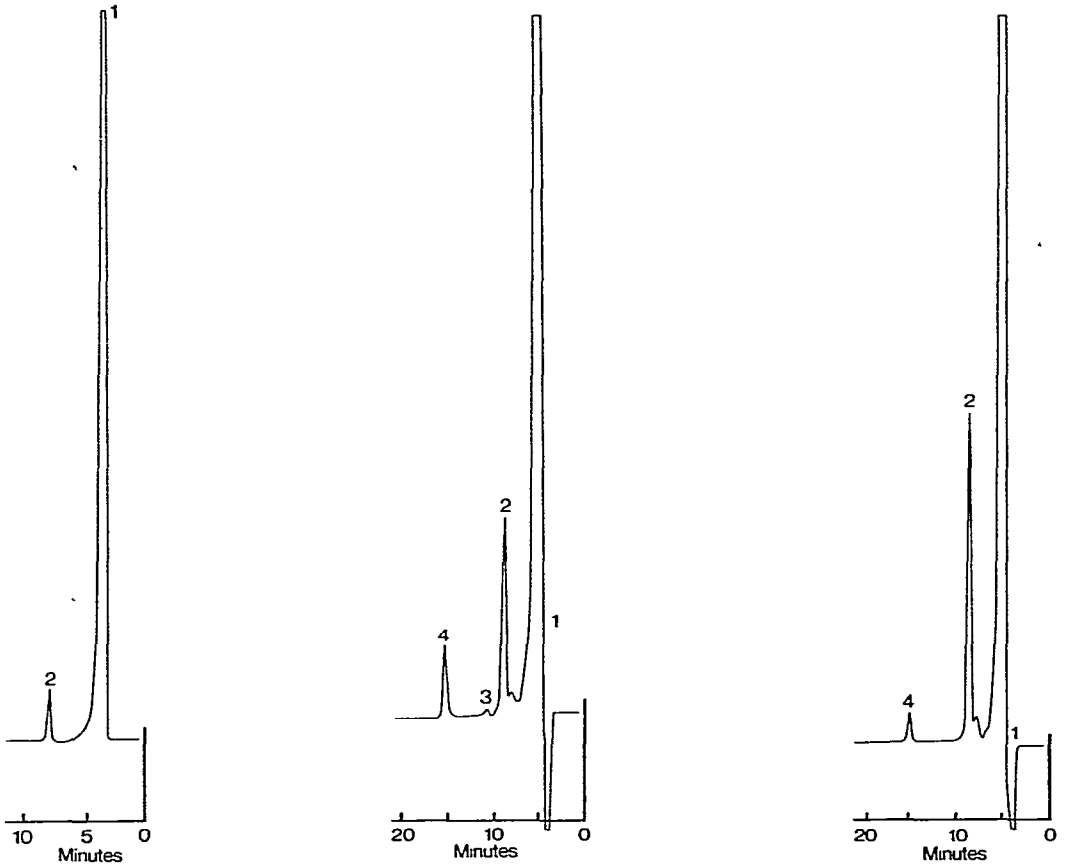



Fig. 7. IC chromatogram of 180 ng nitrate. Column: 150 × 4.6 Nucleosil 5 SB. Mobile phase: 0.03 *M* sodium salicylate, pH 4.0; flow-rate 0.5 ml/min. Sample size: 20 μl. Detection: RI, attenuation ×4.

Fig. 8. IC chromatogram of tap-water from Arnhem. Sample size: 100 μl. Other conditions as in Fig. 7. Peaks: 1 = solvent effect; 2 = chloride; 3 = nitrate; 4 = sulphate.

Fig. 9. IC chromatogram of spring-water from Westervoort. for conditions and peak assignments see Fig. 8.

Figs. 8, 9 and 10 show the chromatograms of 100 μl tap-water (from Arnhem, The Netherlands), spring-water (Westervoort, The Netherlands) and channel-water (Hengelo, The Netherlands), respectively. Calculations with external standards show that the tap-water contains about 12 mg/l chloride, 10 mg/l sulphate and 300 μg/l nitrate. It is evident that the sensitivity, which depends to a small extent on the kind of counter ion, can be improved by increasing the concentration of these ions. In this way, less peak dispersion occurs and, accordingly, the peak height increases. On the other hand, it should be borne in mind that the sensitivity decreases with increasing molecular weight (see Figs. 2 and 5).

The sensitivity can also be improved indirectly by concentration methods. The detection limit of sulphate in brine decreased appreciably when the brine solution was first passed through a small amount of a cation exchanger ($H^+$). The liberated hy-

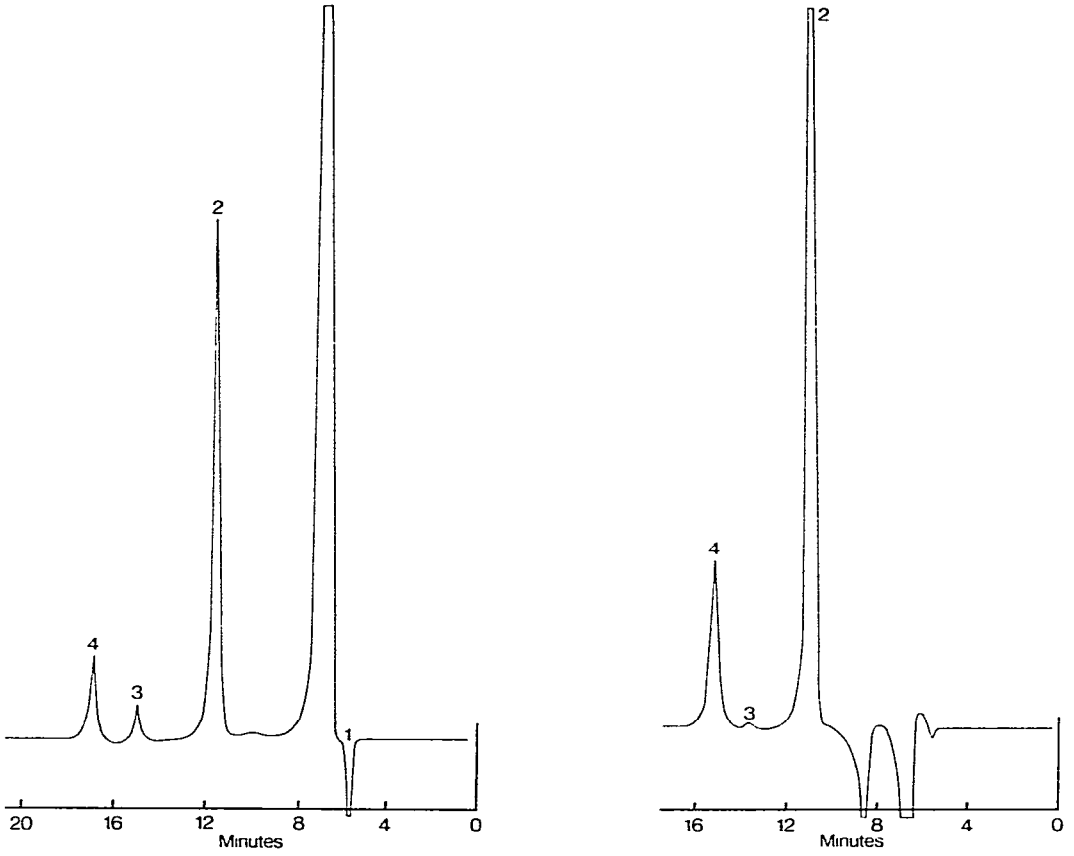



Fig. 10. IC chromatogram of channel-water from Hengelo. Column: Nucleosil 10 SB. Mobile phase: 0.07 *M* sodium *p*-hydroxybenzoate, pH 5.0; flow-rate 0.5 ml/min. Sample size: 20 $\mu$l. Detection: RI, attenuation $\times 8$. Peaks as in Fig. 8.

Fig. 11. IC chromatogram of sulphate in brine after cation-exchange treatment. For conditions and peak assignments see Fig. 10.

drochloric acid was evaporated during the concentration of the sulphuric acid, any loss of the latter being prevented by adding one drop of a 0.01 *M* sodium hydroxide solution. A chromatogram representing 200 mg sulphate per kg brine is given in Fig. 11.

For the separation of cations, aromatic cations have to be used as counter ions. With a Nucleosil 10 SA column and trimethylphenylammonium as the counter ion, a baseline separation has been obtained for mono-, di- and triethylamine (Fig. 12).

The advantages of IC with RI detection can be summarized as follows:

no suppressor column is required so that peak dispersion is less, resulting in a better resolution; moreover, no regeneration step has to be included

any ion-exchange material, silica or polystyrene based, can be applied, not only specially prepared low capacity separation columns

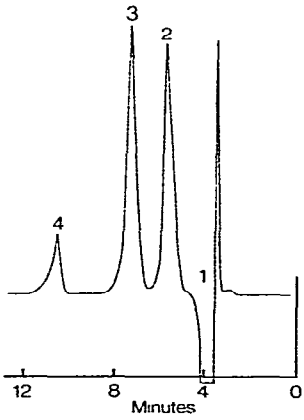



Fig. 12. IC chromatogram of ethylamines. Column: 250 × 4.6 mm Nucleosil 10 SA. Mobile phase: 0.03 *M* trimethylbenzylammonium formate, pH 3.5; flow-rate 1.0 ml/min. Sample size: 20 μl. Detection: RI, attenuation ×8. Peaks: 1 = solvent effect; 2 = monoethylamine (40 μg); 3 = diethylamine (60 μg); 4 = triethylamine (60 μg).

no special equipment is needed; the separations can be achieved on any liquid chromatograph equipped with a differential refractometer

the sensitivity is within the nanogram range.

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