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Divalent pairing ion for ion-interaction chromatography of sulphonates and carboxylates

Maria Concetta Bruzzoniti, Edoardo Mentasti, Corrado Sarzanini*

Department of Analytical Chemistry, University of Turin, Via P. Giuria 5, 10125 Turin, Italy

Abstract

An ion-interaction method for the simultaneous separation and UV detection of compounds with weak or strong ionic groups, using a divalent cationic ion pair reagent, namely hexamethonium bromide, has been studied and developed. The analytes considered were tartaric, fumaric, pyruvic, maleic, phthalic, benzoic, sorbic, 4-hydroxybenzoic, benzene and toluene-4-sulphonic acids, and they were chosen for their practical importance. The chromatographic optimization for their separation has been achieved by varying eluent composition (methanol, NaCl and hexamethonium concentrations) and studying the performance of different stationary phases (octyl- and octadecyl-silica based columns). The method developed has been successfully applied to benzoate and sorbate determination in orange juices.

Keywords: Ion-pairing reagents; Fruit juices; Food analysis; Sulfonates; Carboxylates; Organic acids

1. Introduction

Ion-interaction chromatography (IIC) is widely used as an analytical method for the separation of ionic species, including weakly and fully ionized compounds like carboxylic [1–7] and sulphonic acids [8–14], respectively. Ion-pair reagents usually employed for anion determinations are mainly lipophilic quaternary ammonium ions, such as tetrabutylammonium and cetyltrimethylammonium. To the best of our knowledge, only a few examples of the use of multicharged pairing ions in IIC have been reported [10,15,16]. Moreover, no experimental studies dealing with the simultaneous separation of carboxylic and sulphonic acids are available.

The dionium-type lipophilic ions show some peculiar properties and good suitability in the extraction of divalent analytes with opposite charge in the presence of an organic phase. The mechanism involved is supposed to be an interaction throughout a two-point ion-pair formation between the divalent pairing ion and the divalent analyte [10]. For these properties, divalent cations, such as diammonium- or dipyridinium-type, have been employed in the preparation of ion-selective electrodes for the determination of divalent anions [17,18].

The advantages in the use of ion-pair reagents with many-sided interaction points for chromatography lie in the enhanced selectivity for the separation of isomeric ions. The chromatographic systems already investigated, deal with a comparison of the retention ability of dionium reagents at different lipophilicity, but in no case has a method been developed and optimised for both separation and determination of analytes.

The aim of this work is to study a chromatographic method, based on the ion-interaction mechanism, in the presence of a divalent pairing ion, hexamethonium. The method was developed for the simultaneous separation of weakly and strongly

*Corresponding author.

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ionised compounds (carboxylates and sulphonates), having different charges. The analytes were chosen for their practical importance as natural compounds or additives in foods and beverages. The system was optimised through a detailed study on the effect of different stationary phases and eluent composition (NaCl, CH_3OH, hexamethonim) on the capacity factors. The procedure shows good suitability for the determination of the analytes in real samples, such as orange juices.

2. Experimental

2.1. Instrumentation

The chromatographic system used was a Varian (Walnut Creek, CA, USA) Model 9010 pump equipped with a Rheodyne injector with a 100 μl sample loop and a Model 332 UV–Vis variable-wavelength detector (Kontron Instruments, Milan, Italy). The chromatograms were recorded by an Axxiom Chromatography (Calabasas, CA, USA) Model 727 data station. The columns used, 10 μm (250×4 mm I.D.) all by Merck, were LiChrosorb RP-18, LiChrospher 100 RP-18 and LiChrospher 100 RP-8. LiChrocart RP-18 and LiChrocart RP-8 were used as guard columns, 5 μm (4×4 mm I.D.). Orange juice samples were pretreated with Dionex (Sunnyvale, CA, USA) cartridges OnGuard-RP. The chromatographic optimisation was carried out on a LiChrosorb RP-18 column, unless otherwise stated.

The eluent flow-rate was 1.0 ml/min and UV absorbance detection was performed at 210 nm. Experiments were performed at room temperature. Retention times were the means from triplicate injections. The dead volume of the columns were measured by injections of water.

2.2. Reagents and solutions

Eluents were prepared by dissolving analytical-reagent grade chemicals in high purity water obtained with a Milli-Q system (Millipore, Bedford, MA, USA) and filtering through a 0.22-μm filter.

Hexamethonium bromide [hexamethylenebis-(trimethylammonium) bromide] was from Aldrich. Benzenesulphonic acids and toluene-4-sulphonic acid sodium salt were Merck products, while benzoic acid, methanol and ammonia were from Carlo Erba (Milan, Italy). Fumaric acid disodium salt anhydrous, 4-hydroxybenzoic acid sodium salt, maleic acid disodium salt anhydrous, pyruvic acid sodium salt, sorbic acid potassium salt, disodium tartrate dihydrate and potassium hydrogen phthalate were from Fluka (Buchs, Switzerland).

Concentrated stock solutions of the analytes were prepared by dissolving the salts in a water–methanol (40:60, v/v) mixture. Standard solutions of proper concentrations were prepared diluting the concentrated solutions in the eluent. During all experiments, aqueous eluent pH was kept constant at 7.0 with ammonia. According to the pK_a values of the carboxylic acids considered, at this pH value a complete deprotonation takes place for all the acids.

3. Results and discussion

The separation of the analytes was optimised by evaluating the effect of the components of the mobile phase. After preliminary experiments, a mobile phase containing 36 m*M* NaCl was selected in order to study the effect of hexamethonium concentration on the k' values of the analytes, Fig. 1. This study pointed out that 1 m*M* hexamethonium gives a poor stability of the baseline, probably due to a difficult achievement of partition equilibrium between the mobile and the stationary phase for the molecules of hexamethonium; higher concentrations of hexamethonium did not give any baseline stability problem.

The effect of increased hexamethonium concentration in the eluent is to increase the retention of each analyte, as usually observed with monovalent pairing ions. The different hydrophobicities of the analytes are reflected in the different k' values shown at every hexamethonium concentration. In fact, analytes of higher lipophilicity as aromatic acids (toluene-4-sulphonic, benzoic and benzenesulphonic acids) or aliphatic acids with a longer chain (sorbic acid) are more retained and more influenced by hexamethonium concentration. The opposite has been noted for analytes of lower lipophilicity (tartaric, pyruvic, fumaric and maleic). Phthalic and 4-hydroxybenzoic acids represent an intermediate behaviour. In detail, the comparison of k' values for

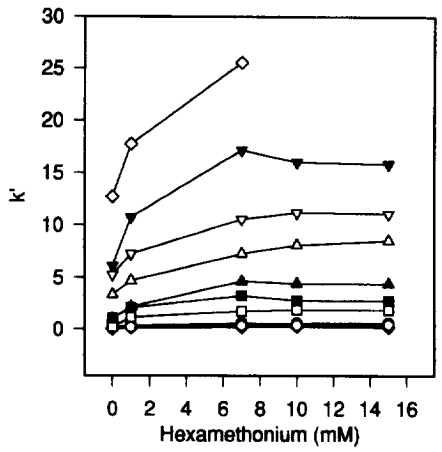

Fig. 1. Effect of hexamethonium concentration in the eluent on the capacity factors (k') of ○ fumaric acid, □ maleic acid, △ benzenesulphonic acid, ▽ benzoic acid, ◇ toluene-4-sulphonic acid, ● pyruvic acid, ■ 4-hydroxybenzoic acid, ▲ phtalic acid, ▼ sorbic acid, ♦ tartaric acid. Stationary phase: LiChrosorb RP-18, 10 μm (250×4 mm I.D.); eluent composition: 36 m*M* NaCl, NH_3 up to pH 7.0. Hexamethonium concentration as shown.

benzoic and 4-hydroxybenzoic acids evidenced that –OH group makes the analyte more hydrophilic and then less retained. The hydroxyl substituent induces preferential interactions with the mobile phase (e.g., hydrogen bonding) whose extent is greater in the presence of hexamethonium than in an only reversed phase mechanism. The presence of a hydrophobic group gives rise to an opposite effect, as could be inferred by the different retention of toluene-4-sulphonic and benzenesulphonic acids (electrostatic interactions with the sulphonic group being equal for the two analytes). In fact, the hydrophobic interactions with the stationary phase between the pairing ion and $-CH_3$ group of toluene-4-sulphonic acid are important, both in the presence or in the absence of hexamethonium in the mobile phase. This effect is magnified at hexamethonium concentrations higher than 7 m*M*, where toluene-4-sulphonic acid is irreversibly retained, while benzenesulphonic acid can still be eluted.

The retention order of the two geometrically isomeric solutes, namely maleate (*cis* form) and fumarate (*trans* form) is opposite to the one observed by Ohki et al. [10] using octamethylenebis-(tributylphosphonium) as divalent pairing ion. The inverse retention order can be ascribed to the compatibility of ionic center distances, as already verified with divalent pairing ions having the same ionic centers but with different length of hydrocarbon chains [10].

In a comparison of the retention behaviour of (−1) and (−2) charged analytes with similar lipophilic structure, it can be seen that benzoic is more retained than phthalic acid. Even if the retention of doubly charged analytes, as generally shown in literature, is higher than that of singly charged analytes, this reverse behaviour is not peculiar for divalent pairing ions. In fact, according to Ståhlberg et al. [19], when tetraethylammonium or tetrabutylammonium are present in the mobile phase, naphthalene-1,5-disulphonate elutes before naphthalene-2-sulphonate, while we observed the opposite retention order when cetyltrimethylammonium is used [14]. The elution order seems then to be related to the different affinity of the pairing ion and analyte for the stationary phase in a chromatographic system, as we reported in a theoretical discussion [20].

The effect of hexamethonium on k' values has also been studied in an octyl column in a wider range (0–25 m*M*). Owing to the reduced lipophilicity of the stationary phase, the retention times at the same mobile phase compositions were lower when compared with those obtained by the octadecylic phase. Other effects of the shorter lipophilic chains are a less pronounced rise of the curves and a delay in the achievement of the plateau, while no changes in the elution order has been observed.

As the mobile phase containing 7 m*M* hexamethonium provided good separation between most of the analytes considered, this value was chosen as optimal for the next studies.

The experimental results obtained showed that mobile phases without methanol gave high retention times and tailing peaks, especially for the analytes stongly retained. The study of retention behaviour as a function of percentage of methanol in the eluent, Fig. 2, showed the exponential dependence typical for IIC separations performed with monovalent pairing ions. The presence of methanol improves the shape of the peaks and the reproducibility of the method. In fact, the values of standard deviations on retention times are reduced from ±10%, without methanol, to ±2% with methanol in the eluent. The presence of 6% (v/v) methanol in the eluent has

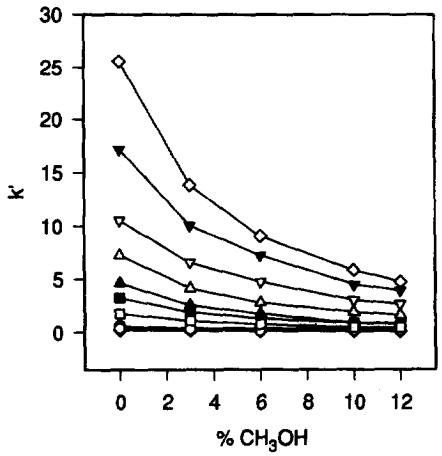

Fig. 2. Effect of methanol percentage in the eluent on capacity factors (k'). Stationary phase: LiChrosorb RP-18, 10 μm (250×4 mm I.D.); eluent composition: 7 m*M* hexamethonium, 36 m*M* NaCl, NH_3 up to pH 7.0. Methanol percentage as shown. Symbols as Fig. 1.

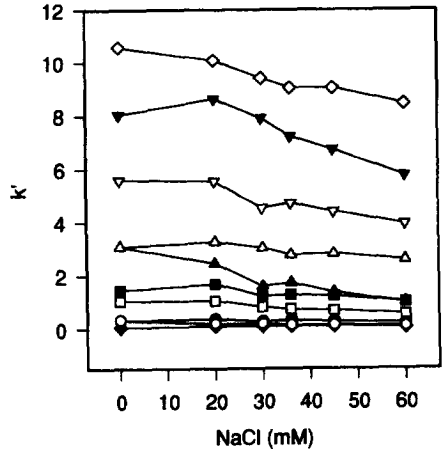

Fig. 3. Effect of NaCl concentration in the eluent on capacity factors (k'). Stationary phase: LiChrosorb RP-18, 10 μm (250×4 mm I.D.); eluent composition methanol–water (6:94, v/v), 7 m*M* hexamethonium, NH_3 up to pH 7.0. NaCl concentration as shown. Symbols as Fig. 1.

been chosen as the optimal concentration. It should be noted that, owing to the increased hydrophilicity of the dianionic pairing ion, in respect to a singly charged one, the methanol content in the eluent is lower than percentages commonly used in ion-interaction chromatography for the same classes of analytes. The unquestionable advantages in the reduced use of an organic solvent are the decreased costs for the analysis and for the subsequent selling off of the eluate.

The last parameter of the mobile phase investigated was the ionic strength, NaCl content. The results obtained at increasing salt concentration are shown in Fig. 3. The dependence of k' values on NaCl concentration is not the one usually reported in IIC literature with monovalent pairing ions, where a decrease in retention is commonly observed for increasing salt concentrations. The profile of k' is the result of two opposite effects of NaCl in the chromatographic system. In fact, the competing effect of the Cl^- ions with the anionic analytes for the cationic sites of hexamethonium in the stationary phase decreases k' values, while the salting-out effect, given by increasing NaCl concentrations, increases the interactions with the stationary phase [21], leading to higher retention times. If NaCl concentration does not affect significantly the retention of analytes, it improves the symmetry for the peaks of phthalic, benzoic and sorbic acids, as shown by comparing Fig. 4a and Fig. 4b obtained with the same eluent, 0 m*M* and 60 m*M* NaCl, respectively. The 60 m*M* NaCl concentration was found to be the optimal mobile phase composition for the separation of the considered analytes using an octadecylic stationary phase (LiChrosorb RP-18). In these conditions, the standard deviations of retention times were ±2%.

The effect of stationary phases on the separation was also studied. Phthalic, benzenesulphonic, benzoic, sorbic and toluene-4-sulphonic acids have been chosen as test analytes to study the performance of different stationary phases at the same mobile phase composition (7 m*M* hexamethonium, 36 m*M* NaCl, 6% CH_3OH). For the sake of clarity, the physicochemical data of the columns used (LiChrosorb RP-18, LiChrospher 100 RP-18, LiChrospher 100 RP-8), according the producer informations [22], were collected in Table 1.

The main feature of a LiChrosorb RP-18 column is the irregularity of the silica particles of the packing material, while LiChrospher 100 RP-8 and LiChrospher 100 RP-18 columns have spherical silica particles in their packing support. The chromatograms obtained showed an increased retention

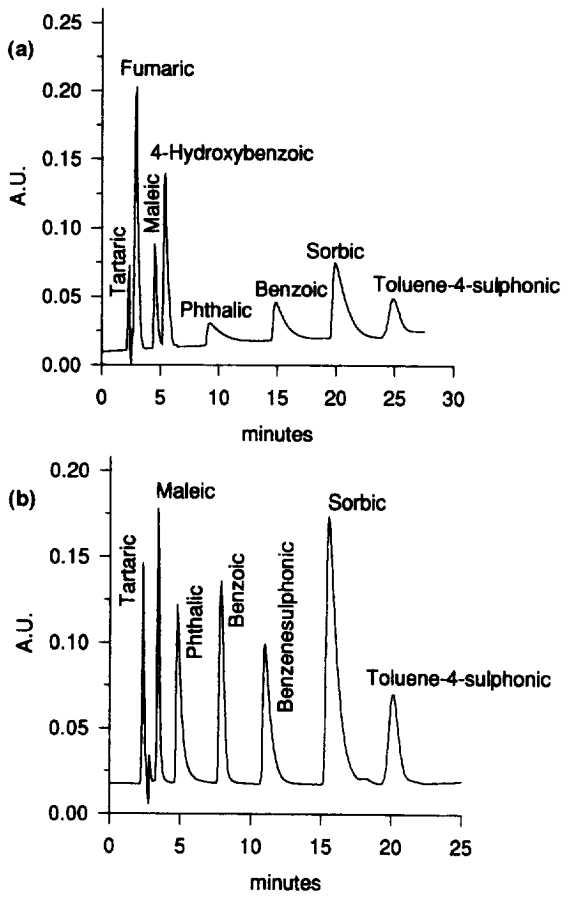

Fig. 4. Effect of NaCl on the symmetry of the peaks. Stationary phase: LiChrosorb RP-18, 10 μm (250×4 mm I.D.). Analyte concentrations: $1\cdot10^{-2}$ *M* tartaric acid, $1\cdot10^{-3}$ *M* benzenesulphonic acid and $1\cdot10^{-4}$ *M* for the other analytes. Mobile phase composition: methanol–water (6:94, v/v), 7 m*M* hexamethonium, NH_3 up to pH 7.0. (a) 0 m*M* NaCl, (b) 60 m*M* NaCl.

when a LiChrospher 100 RP-18 column is used and an improvement of peak symmetry on LiChrospher columns. The irregularity of the silica particles, rather than the length of the alkyl chains, is univocally responsible for peaks tailing. The coverage of the silica material in this case is less homogeneous than in the presence of spherical paricles, inducing secondary, non-specific interaction between the stationary phase and the analytes. The increased retention times obtained with the LiChrospher 100 RP-18 are attributed to the higher percentage of carbon, which functionalised the silica material (see Table 1).

3.1. Detection limits and dynamic linear range

Detection limits (calculated as three times the background signal) and the dynamic linear ranges of detector response were evaluated for all the species (Table 2). The standard deviations for each analyte are included in a ±10% range.

3.2. Determination of sorbate and benzenesulphonate in orange juices

The method developed has been applied to the determination of sorbic and benzoic acids in three different orange juice samples, which we will arbitrarily label as juice A, B and C. These analytes are commonly employed as preservers of foods or beverages.

3.3. Sample pretreatment

The orange juices samples were degassed and were adjusted to pH 7.0 with concentrated ammonia. The samples were centifugated at 5000 rpm for 30 min and filtered through 0.22 μm filters. The samples obtained were then diluted 1:10 and passed through an OnGuard RP-18 cartridge in order to retain lipophilic compounds such as dyes which could interfere in the chromatographic analysis. Before sample (12 ml) treatment, the RP-18 cartridges were washed and preconditioned with 12 ml of methanol and 12 ml water, respectively. The

Table 1
Physico-chemical data of the column used

Designation	V_p (ml/g)	d_p (μm)	%C	Surface coverage (μmol/m^2)	Theoretical plates (*N*/m)
LiChrosorb RP-18	1.0	10	16.2	3.0	15 000
LiChrospher 100 RP-18	1.25	10	21	3.61	20 000
LiChrospher 100 RP-8	1.25	10	12.5	4.04	20 000

Table 2
Detection limits (mol/l and ppm) and dynamic linear range for the analytes studied

Analyte	DL		Dynamic linear range	r
	mol/l	ppm		
Fumaric acid	$1\cdot10^{-5}$	2	$1\cdot10^{-5}$–$5\cdot10^{-4}$	0.991
Maleic acid	$1\cdot10^{-6}$	0.2	$5\cdot10^{-6}$–$5\cdot10^{-4}$	0.999
Pyruvic acid	$5\cdot10^{-6}$	0.6	$5\cdot10^{-5}$–$5\cdot10^{-2}$	0.978
Tartaric acid	$5\cdot10^{-4}$	115	$1\cdot10^{-3}$–$8\cdot10^{-2}$	0.958
Sorbic acid	$1\cdot10^{-6}$	0.2	$5\cdot10^{-6}$–$1\cdot10^{-4}$	0.997
Benzoic acid	$2\cdot10^{-6}$	0.2	$5\cdot10^{-6}$–$5\cdot10^{-3}$	0.998
4-Hydroxybenzoic acid	$1\cdot10^{-6}$	0.2	$5\cdot10^{-6}$–$5\cdot10^{-3}$	0.982
Phthalic acid	$3\cdot10^{-6}$	0.6	$5\cdot10^{-6}$–$5\cdot10^{-3}$	0.999
Benzenesulphonic acid	$3\cdot10^{-5}$	5	$1\cdot10^{-4}$–$1\cdot10^{-2}$	0.981
Toluene-4-sulphonic acid	$2\cdot10^{-6}$	0.4	$5\cdot10^{-6}$–$1\cdot10^{-3}$	0.999

samples after the pretreatment were clear and colorless. This procedure was optimised in respect to analyte recoveries by evaluating standard solutions ($1\cdot10^{-2}$ *M* tartaric acid, $1\cdot10^{-3}$ *M* pyruvic and benzenesulphonic acids and $1\cdot10^{-4}$ *M* for the other analytes) diluted both in water and in eluent (Table 3). Analyte recoveries were calculated by comparing the peak areas obtained by injection of standards before and after cartridge treatment. As the recoveries for benzoic and sorbic acids were higher and more reproducible when the sample was diluted in eluent, this procedure was used for the orange juices dilution.

3.4. Standard additions

The samples after pretreatment were injected into the chromatographic system and the concentrations of sorbic and benzoic acid were evaluated by the standard additions method. The optimised mobile phase composition was used with two different columns (LiChrosorb RP-18 and LiChrospher 100 RP-8). Fig. 5 shows the chromatograms of orange juice A, after dilution as such, and spiked with $2\cdot10^{-4}$ *M* benzoic and $1\cdot10^{-4}$ *M* sorbic acids. The concentration of sorbic and benzoic acids found in the sample were $3.3\cdot10^{-4}$ and $1.1\cdot10^{-3}$ *M* using the octadecyl column and $3.8\cdot10^{-4}$ and $1.2\cdot10^{-3}$ *M* using the octyl column, with standard deviation included in ±3% for both stationary phases. The analysis on juices B and C did not give evidence of the presence of sorbic and benzoic acid.

Table 3
Recoveries % on OnGuard RP-18 cartridges of the analytes studied after 1:10 dilution in water or in eluent

Analyte	Recovery (%)	
	In water	In eluent
Fumaric acid	105±3	99±1
Maleic acid	95±3	99±1
Pyruvic acid	94±11	98±1
Tartaric acid	84±11	98±5
Sorbic acid	60±5	63±2
Benzoic acid	73±1	81±2
4-Hydroxybenzoic acid	97±1	60±1
Phthalic acid	95±1	101±3
Benzenesulphonic acid	98±9	88±1
Toluene-4-sulphonic acid	33±3	28±4

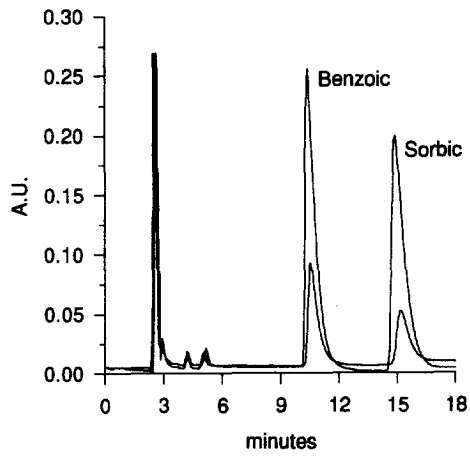

Fig. 5. Analysis of 1:10 diluted orange juice A by the method developed. Chromatograms of sample as such and spiked with $2\cdot10^{-4}$ *M* benzoic and $1\cdot10^{-4}$ *M* sorbic acids.

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