Journal of Chromatography, 640 (1993) 259–270
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2657

Ion-exchange high-performance liquid chromatography in the brewing industry

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ABSTRACT

Isocratic HPLC methods using an ion-exchange column (Aminex HPX) for the determination of carbohydrates, organic acids, alcohols (methanol, ethanol, glycerol), 5-hydroxymethylfurfural and purines in wort, beer, wine and soft drinks are presented. The strategy for selecting the best column geometry, the appropriate counter ion and specific detection systems is discussed.

INTRODUCTION

Beverages such as beer, wine, fruit juice, carbonated soft drinks and their raw materials are complex mixtures of several natural compounds Table I [1]. Because of their different concentrations, chemical behaviour and molecular mass ranges, the isolation, enrichment and determination of distinct compounds may cause serious problems. Isocratic HPLC methods using ion-exchange materials based on polystyrene–divinylbenzene resins in combination with a suitable eluent and specific detection may overcome these problems.

This paper reports a strategy for selecting the best separation system for particular analytical problems in the food and beverage industry. One major advantage of ion-exchange resins is that normally no organic modifier such as methanol or acetonitrile need be used, only pure water or dilute salt solutions being required. Another advantage is that large molecules such as proteins or polysaccharides are excluded from the pores and elute with the void volume. Therefore, a minimum of sample preparation (filtration and dilution) is needed before analysing a sample.

TABLE I

NATURAL COMPOUNDS IN BEVERAGES

Proteins	Carbohydrates (mono-, di-, tri-, oligo- and polysaccharides, sugar alcohols)
Peptides	Glycerol
Amino acids	Polyphenols
Organic acids	Diols
Alcohols	Aldehydes
Phenolic acids	Ketones
Vitamins	Bitter substances
Preservatives	Antioxidants

EXPERIMENTAL AND RESULTS

Reagents

Water for the preparation of the mobile phase must be distilled freshly and degassed with helium. Sulphuric acid, potassium sulphate and ammonium sulphate were of analytical-reagent grade from

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0021-9673/93/$06.00

Merck (Darmstadt, Germany). Calcium oxides have been prepared in the laboratory. All reference standard solutions were prepared from analytical-reagent grade chemicals (Sigma, Deisenhofen, and Aldrich, Steinheim, Germany).

Instrumentation

System A. This system consisted of Model 1350 HPLC pump (Bio-Rad Labs., Richmond, CA, USA), and SPD-2AS UV spectrophotometric detector (Shimadzu, Kyoto, Japan), a CTO-2AS column oven unit (Shimadzu), a Model 1755 refractive index (RI) detector (Bio-Rad Labs., and a Model 7125 sample injection valve (Rheodyne, Cotati, CA, USA).

System B. This system consisted of a Model 64.00 HPLC pump (Knauer, Berlin, Germany), a Model 89.00 column oven unit (Knauer), a Model 87.00 UV spectrophotometric (Knauer), a Chromascope high-speed scanning UV detector (Barspec, Rehovot, Israel); a Model 98.00 RI detector (Knauer) and a Model 7110 sample injection valve (Rheodyne).

Resin-based ion-exchange columns

The following columns were obtained from Bio-Rad Labs.: Aminex HPX 42A (300 × 7.8 mm I.D.) with de-ashing system; Aminex HPX 87H (300 × 7.8 mm I.D.); "Fast-Acid" (100 × 7.9 mm I.D.); Aminex HPX 87C (300 × 7.8 mm I.D.); Aminex HPX 87K (300 × 7.8 mm I.D.); Aminex HPX 72S (300 × 7.8 mm I.D.); Cation H (30 × 4.6 mm I.D.) and Carbo C (30 × 4.6 mm I.D.). All column materials were packed in stainless-steel tubes and refilling by Bio-Rad Labs. was not possible.

The following columns were obtained from Inovex (Vienna, Austria): Inores S259H (250 × 7.5 mm I.D.); Inores S259C (250 × 7.5 mm I.D.) and Ino-Pre (25 × 4.6 mm I.D.). These materials were introduced in 1992. They are comparable to Aminex and gives similar separation patterns. All column materials were packed in PEEK tubes and can be refilled by Inovex.

Strategy for column selection

Resin-based ion-exchange materials offer several possibilities for the selection of suitable separation systems [2]: (a) cation (for neutral or acidic compounds) or anion exchanger (for basic compounds); (b) column geometry (length); short columns for compounds with long retention times (*e.g.*, phenolic acids, aldehydes) or for high-speed separations with specific detection systems (*e.g.*, total sulphite in beer or wine); (c) cross-linkage; separation of oligosaccharides (4%, 6% or 8% divinylbenzene); lower cross-linkage gives greater interstitial pores for separating oligosaccharides up to a degree of polymerization (DP) of 11; (d) counter ion; H^+, Ca^{2+}, K^+, Ag^+, OH^- or SO_4^{2-}; these ions effect the separation of mono- and disaccharides and of sugar alcohols; (e) eluent strength (pH); strong effect on organic acids, but no effect on sugars, diols or alcohols; (f) column temperature; higher temperatures give better separations and resolutions (normally 65–85°C); (g) specific detection; RI for carbohydrates, sugar alcohols, alcohols, diols and glycerol, UV for organic acids, aldehydes and ketones and electrochemical detection for phenols, vitamins and sulphite; (h) sample preparation; for co-eluting compounds with similar detector response; a powerful tool for the extraction of anionic compounds (organic acids) is to use solid-phase extraction (SPE) with quaternary amines.

Determination of carbohydrates in wort, beer, fruit juice and carbonated beverages

The only sample preparation necessary for all of the following examples is degassing, dilution and filtration of the samples.

Matrix wort. Mono-, di-, tri- and oligosaccharides appear along with bitter substances, proteins and phenolic compounds. For controlling the degradation of the polysaccharides during the mash procedure, the best separation is achieved by isocratic HPLC with RI detection using the Aminex HPX 42A column and de-ashing system. The guard column removes both anions and cations. Chloride and sulphate have to be removed before analysis because they give a precipitate with silver counter ions of the column and this may clog the analytical column [3–6]. This system separates carbohydrates up to DP 11 and monosaccharides. No interference from other compounds can be seen. Proteins are eluted with the void volume and the other constituents are not detected by the RI detector.

The HPLC conditions are as follows: column Aminex HPX 42A (300 × 7.8 mm I.D.) with de-ashing system; eluent, doubly distilled water; flow-

rate, 0.5 ml/min; temperature, ambient; detector, RI.

For the determination of fermentable sugars in pitching wort, another separation system is used because only maltotriose, maltose, glucose and fructose can be fermented by brewers yeast. In this system determination up to DP 11 is not necessary. It is possible to decrease the analysing time by using the Aminex HPX 87H or the Inores S259H column. Maltooligosaccharides up to DP 4 and monosaccharides are separated by using dilute sulphuric acid. Chloride or sulphate do not cause problems in this system. Nevertheless, appropriate guard columns should be used to protect analytical columns against contamination.

The HPLC conditions are as follows: column, Aminex HPX 87H (300 × 7.8 mm I.D.) or Inores S259H (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Pre (25 × 4.6 mm I.D.); eluent, 0.005 *M* sulphuric acid; flow-rate, 0.6 ml/min; temperature, 65°C; detector, RI.

This method is accepted by the European Brewery Convention (EBC) for the determination of fermentable sugars in wort [7]. The flow-rate described in the *EBC-Analytica* [7] is set to 0.2 ml/min and the separation therefore takes about 40 min. This very low flow-rate is not necessary because 0.6 ml/min gives a similar resolution and shorter analysis times (< 10 min).

Matrix beer. Beer contains mono-, di- and trisaccharides in addition to bitter substances, organic acids, proteins, fermentation by-products (glycerol, sulphite, alcohols and phenolic compounds). Using the Aminex HPX 87H or the Inores S259H column with RI detection, carbohydrates, glycerol and ethanol can be determined simultaneously [8,9]. Fig. 1 shows an alternative method for the determination of ethanol in alcohol-free beer. Sample preparation includes degassing and dilution (1:10 with the eluent).

The HPLC conditions are as follows: column, Aminex HPX 87H (300 × 7.8 mm I.D.) or Inores S259H (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Pre (25 × 4.6 mm I.D.); eluent, 0.005 *M* sulphuric acid; flow-rate, 0.6 ml/min; temperature, 65°C; detector, RI.

Matrix fruit juice and concentrates. This matrix consists of glucose, fructose and sugar alcohols (*e.g.*, sorbitol) in addition to organic acids. The resin is in the H^+ form and does not separate fructose from sorbitol. Therefore, a column with another counter ion (Ca^{2+}) must be used. Generally the res-

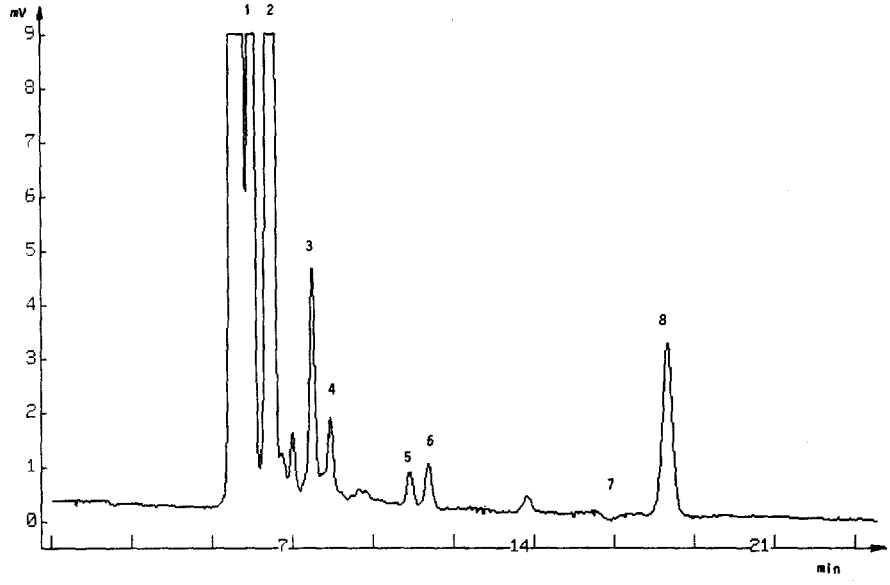


Fig. 1. Determination of ethanol in non-alcoholic beer. Chromatographic conditions as described in text. Peaks: 1 = maltotriose; 2 = maltose + sucrose; 3 = glucose; 4 = fructose; 5 = lactic acid; 6 = glycerol; 7 = carbonate (negative); 8 = ethanol.

in in the Ca^{2+} form separates the sugar alcohols from mono- and disaccharides [9].

The HPLC conditions are as follows: column, Aminex HPX 87C (300 × 7.8 mm I.D.) or Inores S259C (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Pre (25 × 4.6 mm I.D.); eluent, 0.02% Ca azides; flow-rate, 0.6 ml/min; temperature, 85°C; detector, RI.

Sugars in carbonated beverages. Carbonated beverages consist mainly of sucrose (up to 100 g/l), glucose, fructose and organic acids (mainly citric acid). For separating these four compounds, two different columns can be used, either the H^+ or the Ca^{2+} form. Both columns have a similar retention behaviour for sugars. The important difference between this methods is the column temperature. Using the Aminex HPX 87H or the Inores 259H column, the analysis must be done at room temperature because sucrose inverts quickly to glucose and fructose under acidic solutions at higher temperatures. This problem does not occur when using the Ca^{2+} form because the eluent is pure water. High temperatures (up to 85°C) can be applied.

The HPLC conditions are as follows: (I) column, Aminex HPX 87H (300 × 7.8 mm I.D.) or Inores S259H (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Pre (25 × 4.6 mm I.D.); eluent, 0.005 *M* sulphuric acid; flow-rate,0.6 ml/min; temperature, ambient; detector, RI; and (II) column, Aminex HPX 87C (300 × 7.8 mm I.D.) or Inores S259C (250 × 7.5 mm I.D.); guard column, Carbo C; eluent, distilled water or 0.02% Ca azides; flow-rate, 0.6 ml/min; temperature, 85°C; detector, RI.

Separation of sucrose and maltose in dark beer. Sometimes malt is partially substituted by sucrose for brewing dark beers. Neither the H^+ nor the Ca^{2+} form of resin can separate carbohydrates of DP 2 (sucrose, maltose). To solve this problem one must use the K^+ form of the Aminex resin (Fig. 2).

The HPLC conditions are as follows: column, Aminex HPX 87K (300 × 7.8 mm I.D.); eluent, 0.005 *M* K_2SO_4; flow-rate 0.6 ml/min; temperature, 85°C; detector, RI.

Table II gives a compilation of methods for the determination of carbohydrates using ion-exchange chromatography.

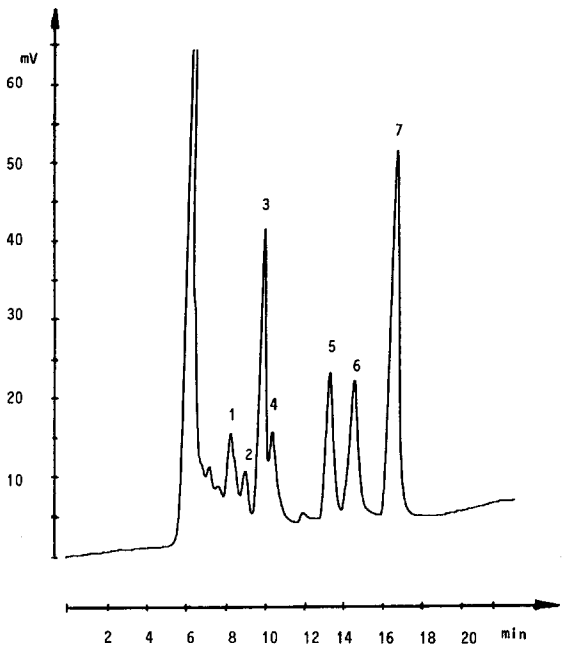


Fig. 2. Separation of maltose and sucrose in dark beer on an Aminex HPX 87K column. Chromatographic conditions as described in text. Peaks: 1 = DP 4; 2 = DP 3; 3 = sucrose; 4 = maltose; 5 = glucose; 6 = fructose + glycerol; 7 = ethanol.

Organic acids

Beer. Organic acids can also be separated by ion-exchange chromatography with UV detection [8,10,11]. It is possible to check the peak purity with a diode-array system. The main problem with UV detection is that not only organic acids are detected, as can be seen in the UV spectra. Phenolic compounds, bitter substances and fructose exhibit a significant response at 210 mm. This problem cannot be solved be changing the columns or chromatographic conditions, and sample clean-up with SPE is necessary (Analytichem SAX; quaternary amine mini-column).

A 10-ml volume of diluted beer sample (1:10 with NaOH, pH 10) was applied to the SPE column. The organic acids were retarded on the strong anion exchanger. The column was washed with 5 ml of NaOH (pH 10), then the organic acids were eluted three times with 1 ml of 1 *M* HCl. The eluates were diluted about 1:20 with eluent (0.005 *M* sulphuric acid). These chromatograms can be seen in Fig. 3a–c. The recovery of the organic acids was 92–97%.

TABLE II

SEPARATION OF CARBOHYDRATES USING DIFFERENT RESIN-BASED CATION-EXCHANGE COLUMNS

Carbohydrates	Aminex HPX column			
	87H	87C	87K	42A
Oligo- and monosaccharides	<DP	<DP5	<DP6	<DP11
Maltose and sucrose	No	No	Yes	No
Fructose and sugar alcohols	No	Yes	No	–
Invert of sucrose at high temperature	Yes	No	No	No
α- and β-glucose	No	Yes	No	No

All co-eluting peaks which are non-organic acids were now present in the primary eluate and in the wash solution, as they passed through the SPE column. Organic acids were in the HCl eluate. The spectra of one specific organic acid peak are shown in Fig. 4.

The retention behaviour of organic acids on this column is sensitive to the eluent concentration and temperature (Tables III and IV). Higher temperatures and lower eluent concentrations give better resolutions of organic acids. For sugar separation, the eluent concentration has no influence on the retention time. One advantage of this type of ion-exchange chromatography for organic acids is the possibility of separating the optical isomers of lactic acid and malic acid (Fig. 5).

The HPLC conditions are as follows: column, Aminex HPX 87H (300 × 7.8 mm I.D.) or Inores S259S (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Res (25 × 4.6 mm I.D.); eluent, 0.005 *M* sulphuric acid; flow-rate, 0.6 ml/min; temperature, 65°C; detector, UV at 210 nm or diode array.

Fruit juice (concentrates). A similar problem occurs in the determination of organic acids in fruit juice. Co-eluting compounds are quinic acid and fructose, which, in contrast to glucose, are also UV-active at 210 nm. Because of the low concentration of quinic acid depending on the other constituents, SPE is not only a cleaning but also a concentration step.

The HPLC conditions are the same as those given above under *Beer*.

Diols and glycerol in wine

Wine contains residual sugars, alcohols, fermentation by-products (glycerol), organic acids, proteins and phenolic compounds. The conditions for the determination of sugars and alcohols are similar to those described for beer [12,13]. The determination of diols by ion-exchange HPLC is not common [11]; they are mostly analysed by GC.

These compounds play an important part in wine adulteration. Well known diols are diethylene glycol, which is added for simulating higher extract, and 1,3-propanediol. The determination of these two substances can be achieved with the H^+ form of the columns. Moreover, acetic acid, methanol and ethanol can be determined in one run (without sample preparation). One example is shown in Fig. 6 (Austrian white wine without diols).

The HPLC conditions are as follows: column, Aminex HPX 87H (300 × 7.8 mm I.D.) or Inores S259H (250 × 7.5 mm I.D.); guard column, Cation H or Ino-Pre (25 × 4.6 mm I.D.); eluent 0.005 *M* sulphuric acid; flow-rate 0.6 ml/min; temperature, 65°C; detector, RI.

With little sample preparation and using ion-exchange SPE, it is possible to determine sugars, diols and alcohols in addition to organic acids [11,14].

Aldehydes in beer: 5-hydroxymethylfurfural (HMF) and furfural

HMF is an indicator for thermal treatment of solutions that contain proteins and carbohydrates. Furfural is an aldehyde which appears during the storage of beer under certain conditions. Therefore, the determination of these two substances is impor-

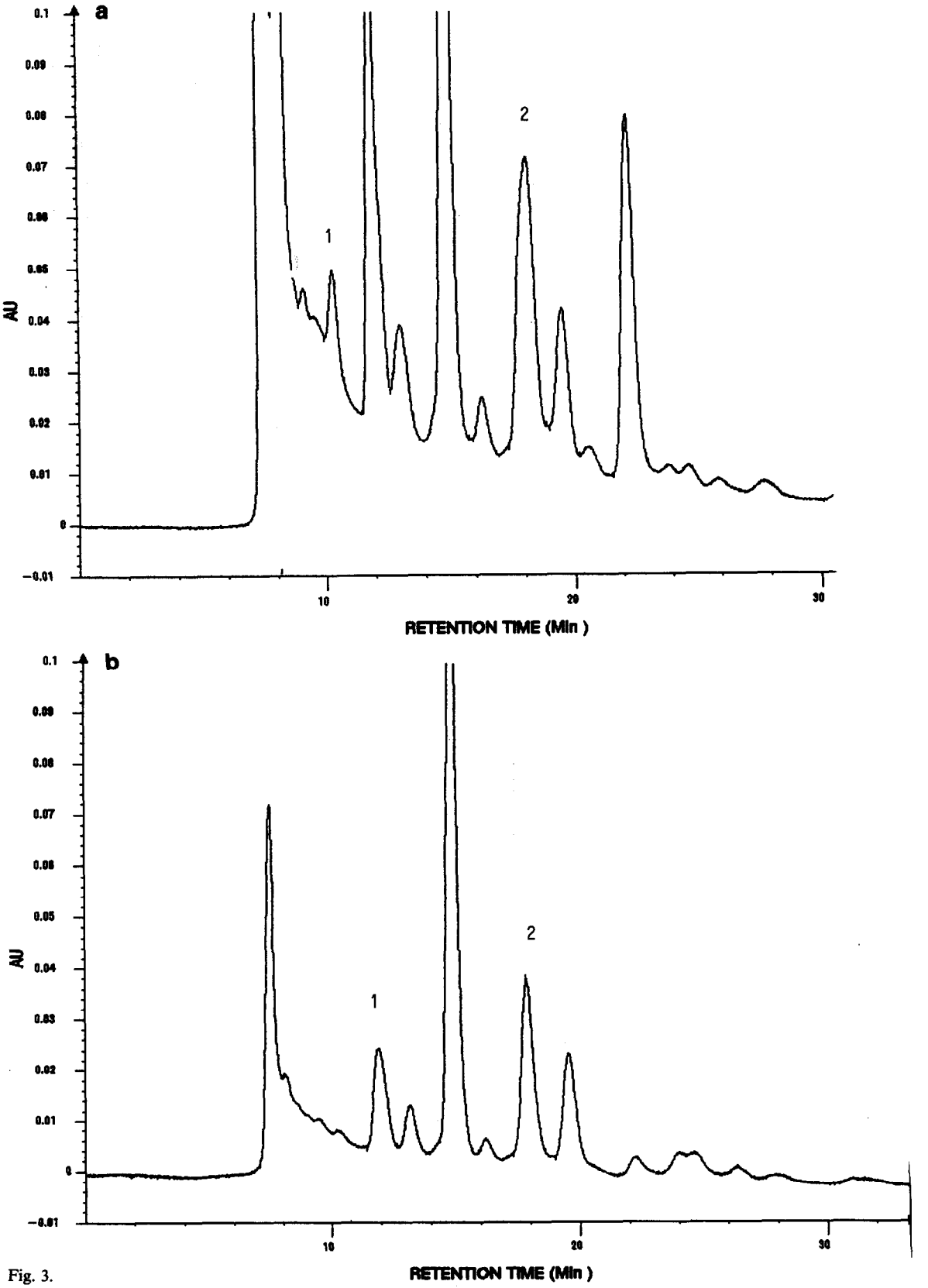


Fig. 3.

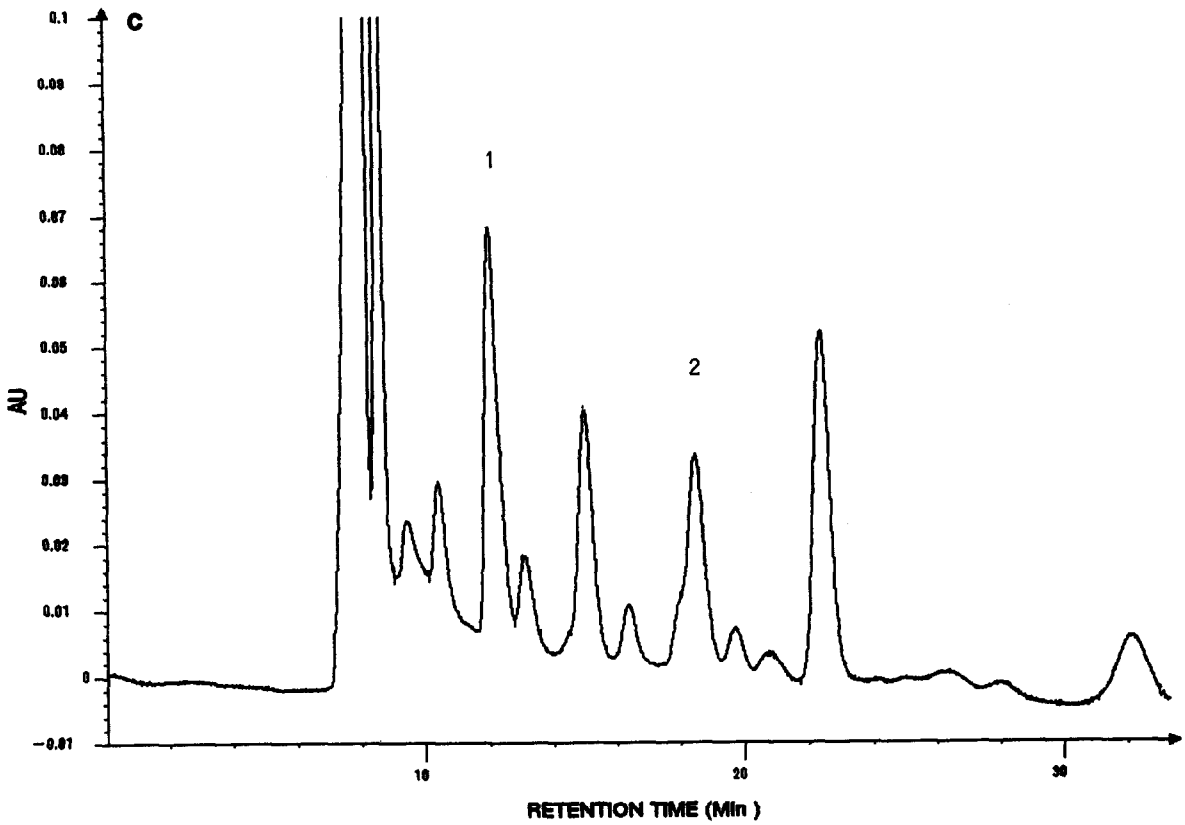


Fig. 3. Chromatograms of (a) beer sample diluted 1:10 with eluent, (b) unretarded compounds and (c) HCl eluate. Chromatographic conditions as described in text.

tant for controlling the storage conditions of bottled beverages [15].

Because of strong interactions between aldehydes and the ion exchangers, it is preferable to use shorter columns for this kind of separation. The analysis takes only about 10 min on the "Fast Acid" column, which is only a third of that with the Aminex HPX 87H column. There are no interferences from other compounds because UV detection is effected at 283 nm. Details of this method can be found elsewhere [16].

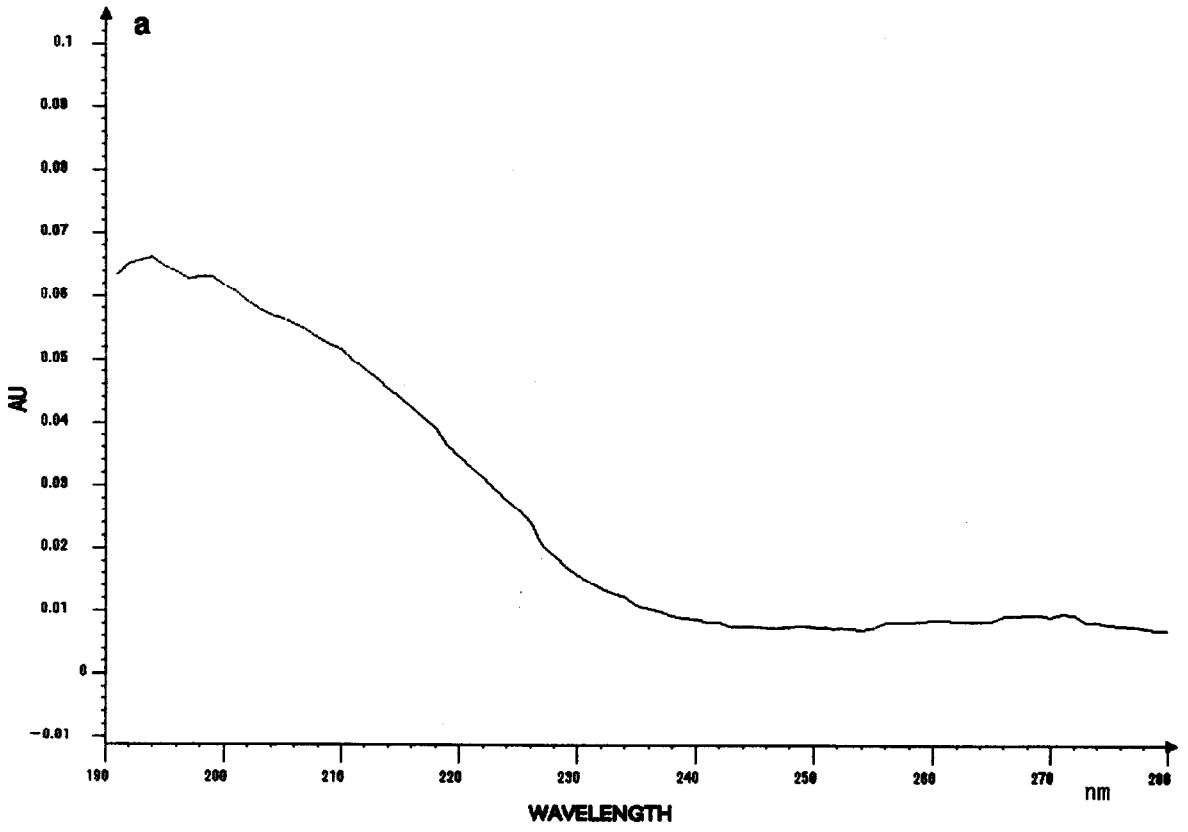
The HPLC conditions are as follows: column, "Fast Acid" (100 × 7.8 mm I.D.), with guard column, Cation H; eluent, 0.005 *M* sulphuric acid; flow-rate, 1.2 ml/min; temperature, 65°C; detector, UV (283 nm).

Preservatives and antioxidants

Substances such as ascorbic acid, sulphite, propionic acid, benzoic acid or sorbic acid are often added to beverages. By using different columns and different detection systems it is possible to optimize the analytical parameters for rapid and accurate analysis.

Determination of ascorbic acid and free and total sulphite in beer, wine and soft drinks. Details of these methods are given in the accompanying paper [17].

Determination of preservatives in soft drinks. Because of the very strong interaction of propionic acid, benzoic acid and sorbic acid with the aromatic backbone of the resin, the guard column (Cation H) is used as an analytical column. Only degassing and dilution of the materials to be analysed are neces-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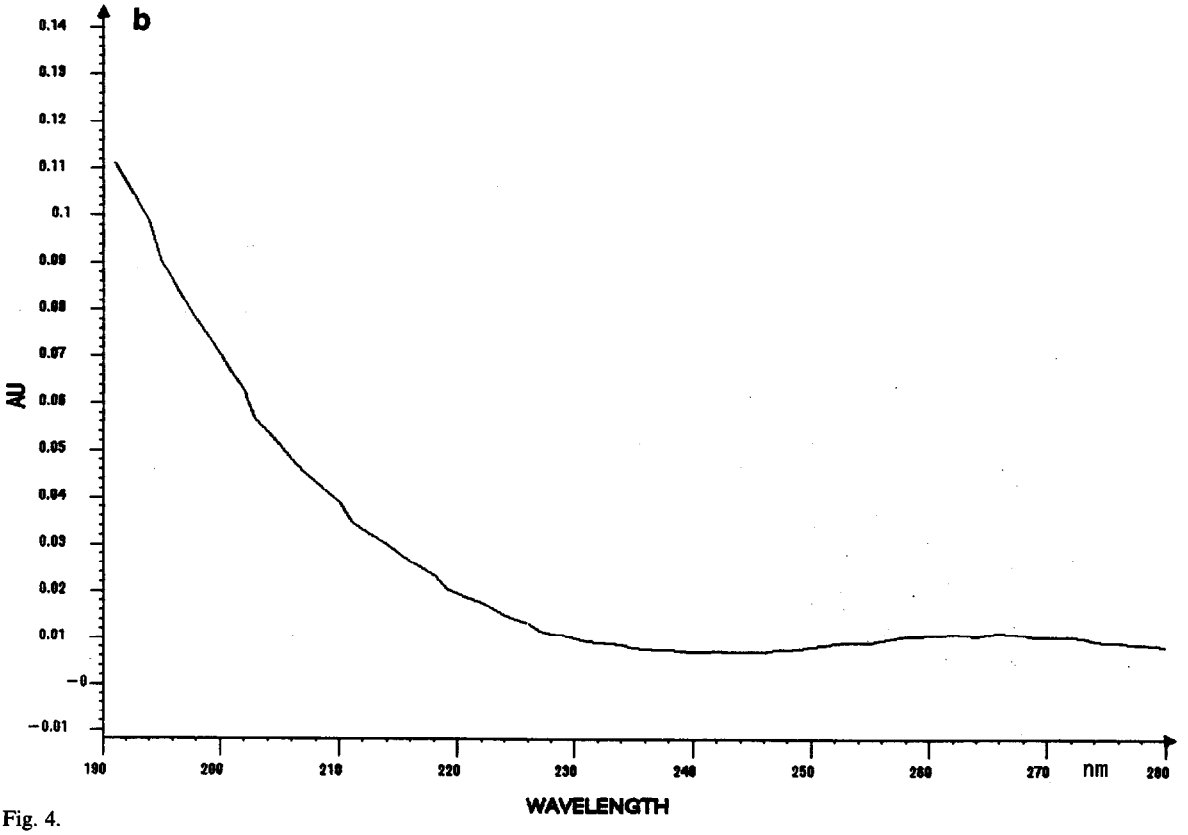
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Fig. 4.

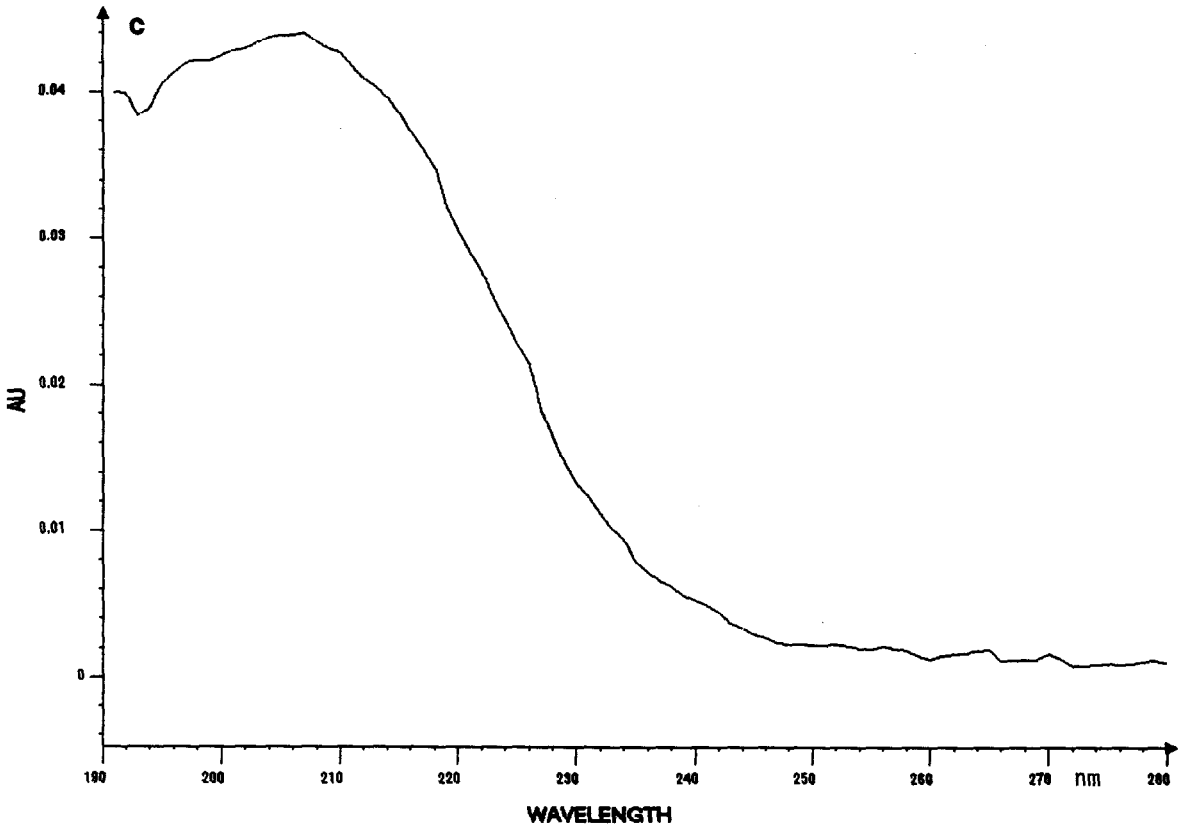

Fig. 4. Spectra from peak 2 in the chromatograms in (a) Fig. 3a, (b) Fig. 3b and (c) Fig. 3c (succinic acid).

sary and the analysis takes about 5 min. The advantage over to reversed-phase HPLC is that no organic modifier (methanol or acetonitrile) need be used.

The HPLC conditions are as follows: column, Cation H (30 × 4.6 mm I.D.) eluent, 0.005 *M* sulphuric acid; flow-rate 0.6 ml/min; temperature, 65°C; detector, UV (240 nm) (benzoic acid, sorbic acid).

For the determination of propionic acid it is better to use the "Fast Acid" column because of the higher retention. With the Cation H column this compound elutes close to the void volume. The best sensitivity is obtained at 210 nm.

Purines in beer

The determination of purines which are fermentation by-products, in beer, causes problems because of their low concentrations and the complex sample matrix. Therefore, clean-up and concentration are necessary [18,19]. The clean-up was done by gel filtration chromatography (Bio-Gel P-2; Bio-Rad Labs.) and three fractions were obtained. These fractions were concentrated by evaporation to dryness under reduced pressure and the residues were dissolved in the eluent. Each fraction was then analysed by ion chromatography.

Purines are basic compounds and can be separated on Aminex-type anion exchangers with UV detection. Table V shows the retention times of these substances.

The HPLC conditions are as follows: Aminex HPX 72S (300 × 7.8 mm I.D.); eluent, 0.13 *M* $(NH_4)_2SO_4$; flow-rate, 1 ml/min; temperature, 65°C; detector, UV (260 nm).

TABLE III

INFLUENCE OF ELUENT CONCENTRATION ON RETENTION BEHAVIOUR OF ORGANIC ACID

Analytical column: Aminex HPX 87H (300 × 7.8 mm I.D.).

Parameter	Conditions		
pH	3.02	2.00	1.24
Temperature (°C)	45	45	45
H_2SO_4 concentration (*M*)	0.0005	0.005	0.05
Acid	Retention time (min)		
Oxalic acid	5.85	6.66	8.44
Citric acid	6.89	7.91	8.21
Isocitric acid	7.24	8.00	8.23
D-Gluconic acid	8.17	9.16	10.88
L-Malic acid	8.50	9.48	9.73
Quinic acid	8.96	9.86	10.12
L-Lactic acid	9.11	11.91	12.77
Glycolic acid	11.26	12.06	12.25
D-Lactic acid	11.71	12.50	12.77
Acetic acid	14.80	14.98	15.15
D-Malic acid	10.76	14.73	15.93
Fumaric acid	10.76	14.73	15.85
Succinic acid	11.40	14.73	15.84
Formic acid	12.51	13.67	13.93

CONCLUSIONS

With consideration of some basic rules, ion chromatography with resin-based materials is a powerful analytical technique in the food and beverage industries: knowledge of the sample matrix; separation behaviour of the different ion-exchange columns (eluent concentration, temperature, flow-rate, counter ion); chemical properties of analyte substances; simultaneously eluted compounds under distinct conditions; chemical reactions during analysis (sucrose); detection systems for specific identification; and sample preparation for special problems. In most instances the sample preparation is reduced to a minimum (degassing, filtration, dilution). Acidic, neutral and basic compounds can be determined in alcoholic or non-alcoholic beverages.

The following applications are possible with resin-based ion-exchange columns: carbohydrates in fermentation broth (wort), beer, wine, fruit juice (appel juice) and soft drinks; diols and glycerol in wine and beer; alcohols in fermentation broth, beer and soft drinks; organic acids in wine, beer and soft drinks; aldehydes (HMF, furfural) in beer, fruit

TABLE IV

INFLUENCE OF TEMPERATURE ON RETENTION BEHAVIOUR OF ORGANIC ACIDS

Analytical column: Aminex HPX 87H (300 × 7.8 mm I.D.).

Parameter	Conditions				
pH	3.02	3.02	3.02	3.02	3.02
Temperature (°C)	25	35	45	55	65
H_2SO_4 concentration (*M*)	0.0005	0.0005	0.0005	0.0005	0.0005
Acid	Retention time (min)				
Oxalic	5.7	5.78	5.85	5.74	5.70
Citric acid	7.01	6.95	6.89	6.82	6.82
Isocitric acid	7.74	7.37	7.24	7.21	7.13
D-Gluconic acid	8.08	8.21	8.17	8.21	8.27
L-Malic acid	8.59	8.64	8.50	8.45	8.36
D-Malic acid	11.55	11.36	10.76	10.52	10.17
Quinic acid	8.99	8.99	8.96	9.12	8.90
L-Lactic acid	8.92	9.20	9.11	9.31	9.34
D-Lactic acid	12.24	11.80	11.71	11.75	11.74
Glycolic acid	11.31	11.40	11.26	11.24	11.19
Acetic acid	14.92	14.99	14.80	14.72	14.61
Fumaric acid	11.56	11.37	10.76	10.53	10.16
Succinic acid	11.70	11.67	11.40	11.21	11.02
Formic acid	12.57	12.70	12.51	12.49	12.42

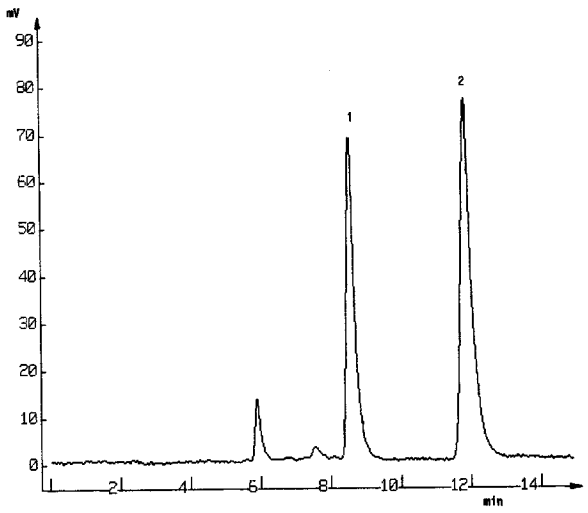

Fig. 5. Separation of optical isomers of malic acid. Chromatographic conditions as described in text. Peaks: 1 = L-malic acid; 2 = D-malic acid.

TABLE V

RETENTION TIMES OF PURINES IN BEER

Fraction	Retention time (min)	Compound
I	11.4	Cytidine
I	13.7	Uridine
I	27.0	Adenosine
I	37.1	Guanosine
II	13.1	Thymidine
II	18.5	Hypoxanthine
II	19.4	Xanthine
III	9.8	Cytosine
III	12.6	Uracil
III	15.9	Thymine
III	35.7	Adenine + guanine

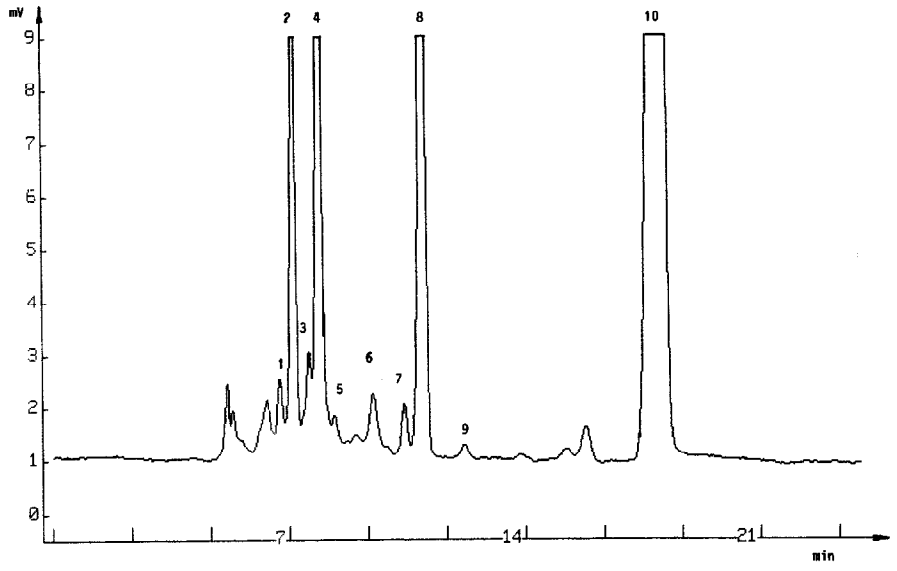

Fig. 6. Separation of Austrian white wine on an Inores S 259H column. Chromatographic conditions as described in text. Peaks: 1 = citric acid; 2 = tartaric acid; 3 = glucose; 4 = malic acid; 5 = fructose; 6 = succinic acid; 7 = lactic acid; 8 = glycerol; 9 = acetic acid; 10 = ethanol.

juice concentrates and brandy; purines in beer; sorbic acid and benzoic acid preservatives in beer and soft drinks; and ascorbic acid, dehydroascorbic acid antioxidants in beer and soft drinks.

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