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Journal of Chromatography A, 785 (1997) 251–261

JOURNAL OF CHROMATOGRAPHY A

Gas chromatographic determination of organic acids from fruit juices by combined resin mediated methylation and extraction in supercritical carbon dioxide

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Abstract

A procedure in which anionic analytes, trapped on ion exchange resin, are simultaneously methylated and released using methyl iodide in either supercritical carbon dioxide or acetonitrile has been extended to polyfunctional organic acids. The combined SFE methylation of fruit juice acids trapped onto ion exchange resin proceeds in good yield producing the methyl esters of fumaric, succinic, malic, tartaric, isocitric and citric acids which are readily separated by GC. Using this procedure low concentrations of one acid can be detected and quantitated in the presence of very high concentrations of another. This new method detects tartaric acid at levels of 10 ppm in juices containing 10 000 ppm citric acid. Quantitation was performed either by using GC–FID with triethyl citrate or diethyl tartrate as internal standards or with the element specific calibration capability of the GC–AED. A simple new technique for the determination of citric/isocitric acid ratio is now available. Also, in contrast to HPLC methods, the identity of an analyte is readily confirmed by GC–MS. © 1997 Elsevier Science B.V.

Keywords: Succinic acid; Fumaric acid; Tartaric acid; Malic acid; Citric acid; Isocitric acid; Derivatisation; Methylation

1. Introduction

Quantitation of acids such as citric, malic and tartaric, typically present in fruit juices has been carried out routinely by liquid chromatography using either refractive index or low wavelength UV detection [1–5]. However, HPLC methods are very sensitive to matrix interferences and these interferences often precluded the reliable detection and/or accurate quantitation of some acids at levels below 50 ppm. Despite the limitations of HPLC methodology, GC determination of fruit juice acids has not been widely employed because of the difficulty in the isolation and derivatisation of these acids.

Analysis of organic acids has been important in detecting the adulteration of fruit juices [6,7]. It is frequently necessary to determine low concentrations of one acid in the presence of high concentrations of another. The extension or adulteration of orange juice by the addition of deacidified grape juice can be revealed by the presence of part per million (ppm) levels of tartaric acid in samples which contain 0.5–1% citric acid. Present analytical methods will not allow the unambiguous detection of the tartaric acid arising from such adulteration of orange juice at levels below about 50 ppm.

The derivatisation of polar analytes in supercritical carbon dioxide has been the subject of much recent interest for extending the applicable range of supercritical fluid extraction [8–10]. The preparation of less polar derivatives during extraction not only improves the solubility of an analyte in supercritical carbon dioxide, but also yields products more amenable to GC analysis. We have previously reported

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0021-9673/97/$17.00
PII S0021-9673(97)00411-1

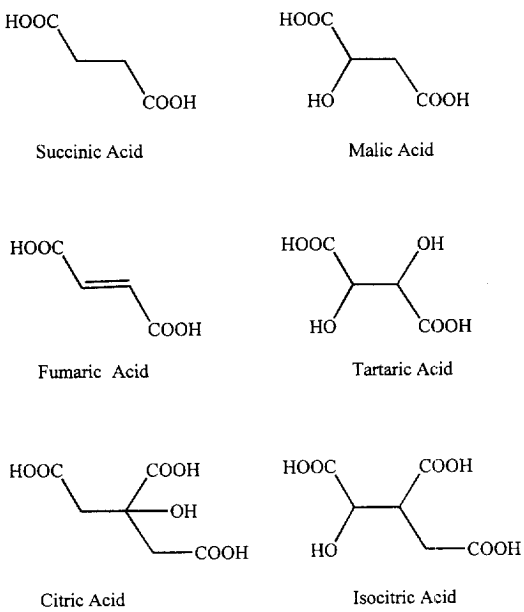


Fig. 1. Structures of six polybasic organic acids.

[11] the trapping of acidic organic analytes onto ion exchange resins followed by their release as methyl ester derivatives using direct derivatisation at 100°C with methyl iodide, either in a solvent or supercritical carbon dioxide. This technique has also been employed and modified by Field and co-workers [12] for the trapping and derivatisation of both acidic herbicides and surfactant metabolites in environmental water samples using Empore™ ion exchange discs.

Our procedure, in which anionic analytes trapped on an ion exchange resin are simultaneously methylated and eluted using methyl iodide in supercritical carbon dioxide, has been extended to polybasic organic acids. The structures of the polybasic organic acids determined are shown in Fig. 1.

2. Experimental

2.1. Reagents and standards

Analytical grade macroporous anion exchange resin of 200–400 mesh (AG MP-1; BioRad, Sydney, Australia) was converted to the fluoride form prior to use as described previously [11]. 3M Empore™ extraction discs (strong anion exchange, Varian, Sydney, Australia) were cut into smaller discs (diameter 13 mm) with a cork borer and used directly.

Citric, isocitric, succinic, fumaric, tartaric and malic acids were obtained from Aldrich Chemical Company (Milwaulkee, WI, USA) or the Sigma Chemical Co. (St Louis, MO, USA). Diethyl and dimethyl tartrate were also obtained from Aldrich. Other reagents used in this work were sourced as follows: methanol (EM Science, Gibbstown, NJ, USA), ethanol (CSR Ltd, Australia), methyl iodide (Sigma or BDH Lab Supplies, Poole, England), Extrelut (Merck, Darmstadt, F.R. Germany), Acetonitrile (Mallinckrodt, Paris, Kentucky, USA), deionised water was prepared using a Milli-Q system (Millipore, Bedford MA, USA). Carbon dioxide (purity >99.5%) was from Linde Gas Co. (Fairfield, NSW, Australia), supplied pressurised to 12 MPa with helium. Reagent gases used with the atomic emission detector were high purity oxygen (99.99%) and hydrogen (Linde).

Trimethyl citrate (TMC) and triethyl citrate (TEC) were prepared by refluxing citric acid (20 g) with the requisite alcohol (100 ml) and concentrated sulfuric acid (15 ml) on a water bath for 8 h. The reaction mixture was cooled and poured onto crushed ice (500 g) and extracted with diethyl ether (3×30 ml). The combined ether extracts were washed with 5% aqueous sodium carbonate (2×10 ml) and dried over anhydrous sodium sulfate. The ether was removed in vacuo to yield the requisite esters. Triethyl citrate was purified by vacuum distillation at 30 mm to yield a colourless oil, whilst trimethyl citrate gave a crystalline solid. Solutions of the prepared material gave a single peak by GC–MSD with the following mass spectra (total ion current mode, 70 eV): Trimethyl citrate: *m/z* 59.10 (17), 69.00 (8), 101.05 (57), 143.00 (100), 144.00 (6), 152.95 (4). Triethyl citrate: *m/z* 69.00 (5), 87.05 (7), 111.00 (8), 114.95 (30), 129.00 (6), 138.95 (4), 157.05 (100), 158.05 (7), 203.00 (11).

2.2. Sample preparation

Fresh fruits or vegetables were squeezed by hand, and the liquid pulp centrifuged in a microcentrifuge (8000*g*, 13 000 rpm). Aliquots of the supernatants were then diluted 1:10 with 0.1 *M* NaOH solution,

and the acids isolated by passage of this diluted sample through the anion exchange resin. A standard mixture containing fumaric, succinic, malic, tartaric, citric and isocitric acids at a concentration of 100 ppm in deionised water was also prepared and loaded directly onto anion exchange resin as above.

To prepare orange juice samples spiked with isocitric or tartaric acids, the orange juice supernatant was diluted 1:10 in 0.1 *M* NaOH, and the acid added to the diluted sample to give a final concentration of 10, 25, 50 and 100 ppm isocitric or 20 ppm tartaric acid in the final solution. The spiked samples were loaded directly onto the anion exchange resin.

2.3. Isolation and derivatisation of acids

2.3.1. Isolation on AG MP-1 resin

AG MP-1 anion exchange resin (0.10 g, fluoride form) was loaded dry into a Pasteur pipette that contained a glass wool plug. The resin was washed with methanol (1 ml) and from this point not allowed to dry out until after sample loading. The resin bed was then washed with deionised water (2 ml) in readiness for sample loading. Samples (1 ml) were allowed to drip through the resin bed. After sample loading the resin was washed with deionised water (1 ml) and methanol (3×1 ml), then dried by air aspiration (30 min).

2.3.2. Derivatisation of acids and extraction with methyl iodide in supercritical carbon dioxide

An ISCO (Lincoln, NE, USA) SFX 220 extraction unit fitted with a 50 μm I.D. fused silica capillary restrictor was used throughout this work. Pressurised liquid carbon dioxide was supplied to the extraction units by an ISCO 260D syringe pump and ISCO SFX 200 pump controller.

The AG MP-1 anion exchange resin that contained the absorbed acids was placed into an SFE cartridge (total sample volume 0.5 ml) half filled with Extrelut (synthetic diatomaceous earth matrix). The remaining volume was then filled with a further portion of Extrelut. Methyl iodide (200 μl) was added onto the top surface of this mixture and the cartridge immediately re-assembled and placed into the SFE chamber. SFE extractions were carried out with neat supercritical CO_2 at 100°C and 250 bar. An initial static extraction period (15 min) was followed by a dynamic extraction (25 ml, pressurised CO_2 flow rate approximately 1 ml/min) with collection through the capillary restrictor into acetonitrile (5 ml). The volume of acetonitrile was then reduced to less than 1 ml under a gentle stream of nitrogen.

2.3.3. Isolation on Empore™ discs

Empore™ anion exchange discs (13 mm diameter) were loaded into stainless steel filter holders and a reservoir (10 ml) attached. The discs were washed under vacuum with acetone (2 ml, 0.5 min) then methanol (2 ml, 0.5 min) and not allowed to dry out before sample loading. Samples (1 ml) were pipetted into the reservoir and allowed to drip through the disc under gravity. After sample loading, the disc was washed with deionised water (2 x 3 ml), dried initially via air aspiration (30 min) and then placed in an oven (100°C, 30 min).

2.3.4. Derivatisation and extraction with methyl iodide in acetonitrile (Empore™ disc only)

The dry Empore™ disc that contained the absorbed acids was folded, placed in a flat-bottomed GC autosampler vial and acetonitrile (1 ml) and methyl iodide (100 μl) added. This vial was capped and then heated (80°C, 60 min). Excess methyl iodide was removed from the sample by reducing the volume to about 200 μl under a gentle stream of nitrogen.

2.4. Gas chromatographic analysis

2.4.1. Quantitation of acids with GC–FID

Samples from either the SFE derivatisation or the GC vial derivatisation were made up to a final volume (~1 ml) in acetonitrile containing 50 μg of internal standard (TEC). Quantitation of methyl ester derivatives was performed using a Hewlett–Packard Model 5890 GC with 7673A autosampler and flame ionisation detector (GC–FID). Separations were carried out using a 2 μl split injection (10:1) on an Alltech (Sydney, Australia) SE-54 column (30 m× 0.25 mm I.D.×0.25 μm film thickness). Helium (1 ml/min) was used as the carrier gas and nitrogen (30 ml/min) as the makeup gas. The injector temperature was held at 260°C and the detector temperature at 285°C. The initial oven temperature of 85°C was

held for 1 min, increased to 110°C at 4°C/min then to 280°C at 23°C/min where it was held for 1 min. Standard curves were constructed for both trimethyl citrate and dimethyl tartrate over the range 0–250 μg/ml and the ratio of the analyte peak height to the internal standard peak height was plotted against the concentration.

2.4.2. Quantitation of acids with GC–AED

Samples from the SFE derivatisation were made up to a final volume of approximately 2 ml in acetonitrile containing 100 μg of internal standard (TEC). Quantitation of methyl ester derivatives was performed using a Hewlett–Packard Model 5890 GC with 7673A autosampler interfaced to a HP5921A atomic emission detector (GC–AED). Separations were carried out using a 2 μl splitless injection on a HP Ultra 2 column (25 m×0.32 mm I.D.×0.25 μm film thickness). Helium (2 ml/min) was used as the carrier gas. The injector temperature was held at 280°C and the detector temperature at 285°C. The initial oven temperature of 70°C was held for 0.5 min, increased to 90°C at 10°C/min and then to 300°C at 20°C/min where it was held for 8 min. The AED transfer line was held at 260°C and the cavity temperature at 260°C. Reagent gases were high purity hydrogen and oxygen. Quantitation was performed using the carbon emission line at 193.0315 nm.

2.4.3. Gas chromatography–mass spectrometry

GC–MS analyses were conducted on a Hewlett–Packard 5890 GC with 7673A autosampler interfaced to a 5971A mass-selective detector (GC–MSD). Separations were carried out using a 2 μl split injection (20:1) on a HP-1 column (12m×0.22 mm I.D.×33 μm film thickness). Helium was used as the carrier gas. The injector temperature was held at 250°C and the detector temperature at 280°C. The solvent delay was set to 3.5 min. The initial oven temperature of 140°C was held for 0.5 min, then increased to 300°C at 20°C/min where it was held for 3 min. The scan range of the MSD was *m/z* 50–450.

3. Results and discussion

The purpose of this work was to extend the potential of our previous work on resin-mediated methylation to the in situ derivatisation and extraction of highly polar polybasic carboxylic acids from fruit juices and other aqueous systems by the heterogeneous derivatisation with methyl iodide in either supercritical carbon dioxide or another solvent, and also compare this method with the recently reported "in-vial" technique [12]. The present work investigated the ability of the resin-mediated procedure to methylate two or more carboxylate moieties in the same molecule to produce di- and trimethyl esters in high yield.

3.1. Sample loading onto anion exchange resin

Anion exchange resins have a much stronger affinity for di- and tribasic carboxylic acids than they do for monobasic aliphatic acids. The capacity of the anion exchange resin in its fluoride form to retain acids in orange juice was assessed by a series of experiments in which increasing quantities of orange juice were loaded onto 100 mg of resin. Results shown in Fig. 2 demonstrate that up to 200 μl of juice can be processed before this quantity of the resin becomes overloaded. Other investigations showed a linear recovery of methyl citrate for volumes of fresh orange juice between 15 and 225 μl. Consequently, volumes of either 100 or 165 μl of juice were used for further investigations.

3.2. In situ derivatisation and extraction of fruit acids using methyl iodide in supercritical carbon dioxide

3.2.1. Extraction–derivatisation conditions

Previous work in this laboratory on derivatisation of organic acids under SFE conditions indicated that, once minimum conditions of temperature, pressure, reaction times and reagent concentrations had been met, large variations in the experimental parameters had relatively little effect on overall recoveries of derivatised products [11]. As was the case with the simple monobasic acids, a large range of reaction conditions gave similar results for the polybasic succinic, fumaric, malic, tartaric, citric and isocitric acids, and once again good derivatisation yields were obtained.

Increasing the SFE temperature from 60 to 90°C resulted in improvements in the methylation yield of

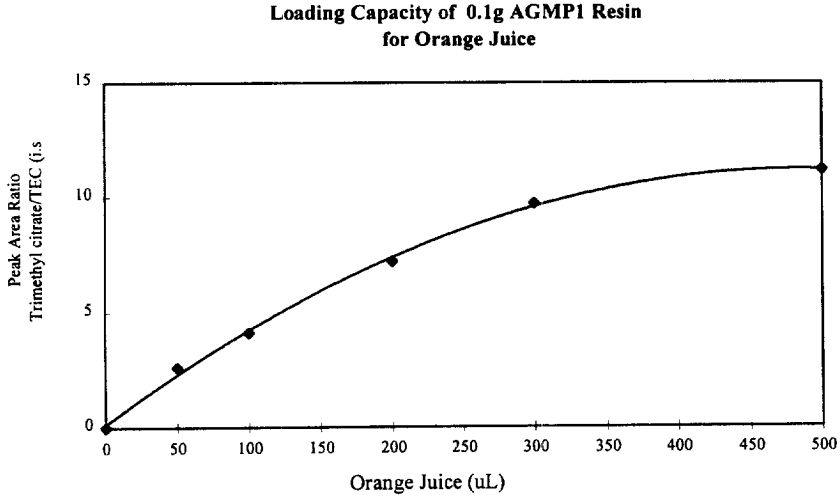


Fig. 2. Capacity of 100 mg AG MP-1 resin to retain the equivalent of 50–500 μl of undiluted orange juice.

all acids, particularly tartaric acid (refer to Fig. 3) for which a maximum recovery of over 85% was attained, but no increase in recoveries was apparent above 90°C. Carbon dioxide pressures of 200 bar or above appeared to give equivalent results, but lower recoveries were obtained below 200 bar. When the volume of methyl iodide added to the extraction cartridge was varied from 10 to 200 μl it was found that, at quantities of 50 μl and above, there was little effect on product yield. Static reaction times of 10 min gave lower recoveries and a static reaction time of 15 min was required to ensure best recoveries were consistently achieved. The volume of supercritical CO_2 used for dynamic extraction which gave best recoveries under these conditions was found to be at least 25 ml of pressurised CO_2.

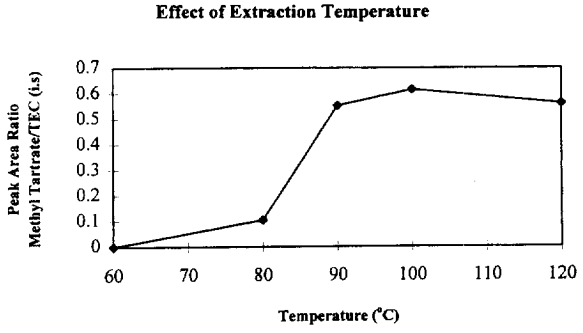


Fig. 3. Effect of temperature on the SFE methylation and extraction of 1 ml of 100 ppm tartaric acid using the procedure described.

3.3. *In situ derivatisation of acids from fruit juices using supercritical carbon dioxide and methyl iodide*

Isolation of acids from fruit juices by ion exchange resins combined with direct methylation of the absorbed acids with methyl iodide gave good yields of methyl esters. The methyl esters of fumaric, succinic, malic, tartaric, isocitric and citric acids were readily separated by GC. In contrast to HPLC methods, there were no matrix interferences apparent and detection and quantitation of acids at concentrations down to 10 mg/l was readily achieved by either GC–FID or GC–AED. GC–MS was suitable for confirmation of analyte identity but suffered from lower sensitivity and less accurate quantitation relative to GC–FID and GC–AED determinations.

3.4. *Recoveries for combined resin-mediated trapping and methylation*

Although the presence, absence or profile of non-volatile organic acids are used as indicators of possible adulteration or extension of fruit juices [6,7], GC methods are not widely used because of problems in derivative preparation. In this work we have shown that a standard mixture of polybasic acids can be trapped onto ion exchange resins and released as their permethyl esters in good yield by methylation with methyl iodide in supercritical car-

Table 1
Reproducibility data obtained by two independent operators using the SFE methylation procedure or the "in-vial" methylation procedure on aqueous standard solutions of 6 organic acids (100 or 10 ppm)

Acid	I Operator A (n=7) 100 ppm soln %C.V. (%Rec.[a])	II Operator B (n=7) 100 ppm soln %C.V. (%Rec.[a])	III Operator B (n=6) 100 ppm soln %C.V. (%Rec. [a])	IV Operator B (n=2) AED %Rec.	V (in-vial) Operator A (n=6) 100 ppm soln %C.V. (%Rec.[a])
Fumaric	5.1	5.8	6.9	93	6.8
Succinic	5.1	4.8	3.8	92	6.3
Malic	20.0	4.6	7.3	93	4.5
Tartaric	4.7 (89)	6.4 (64)	14.8 (64)	69	6.3 (73)
Citric	3.9 (84)	5.3	8.0	84	2.4 (92)
Isocitric	3.7 (58)	5.9	8.7	53	2.2 (60)

[a] Recovery data not available for some analytes, as the replicates were analysed by GC–FID and authentic standards were unavailable.
[b] Recovery of the methylated acids from aqueous solution calculated using the independent calibration capability of the GC–AED (TEC used as the internal standard).

bon dioxide. Table 1 shows the reproducibility of the recoveries for six polybasic organic acids obtained by two operators and, where authentic standards were available, percentage recoveries (citric and tartaric acids) by GC–FID. When determined using the GC–FID, the efficiency of this extraction procedure was found to be linear for standard mixtures in the range 5–500 μg/ml.

Methylated standards of fumaric, succinic, malic and isocitric acid were unavailable. The recovery of these methylated acids from an aqueous solution (100 μg/ml) was therefore calculated using the independent calibration capability of the GC–AED with TEC used as the internal standard. The recoveries of the six polybasic organic acids using this method are also tabulated in Table 1. The results obtained compare well with those obtained by external standard calibration using GC–FID and methylated standards, where available.

The GC separation of the methyl esters of a mixture of acids (each at 100 mg/l) from water using resin-mediated isolation–derivatisation with atomic emission detection (GC–AED) is shown in Fig. 4, along with examples of methyl ester profiles of the acids isolated from orange, grape and apple juices. Fig. 5 shows GC–FID chromatograms for a mixture of the acids (each at 100 mg/l) and a typical orange juice sample.

3.5. Determination of isocitric acid

Isocitric acid is a minor component of fruit juices present at concentrations much lower than other organic acids. Nevertheless, the ratio of citric to isocitric acids is one parameter that has been used to help establish a chemical profile for authentic fruit juices. Because other acids predominate in juices it has not proved practical to determine the concentration of isocitric acid concurrently with the analysis of the major acidic components. Therefore the determination of isocitric acid has involved a separate analytical method which has been conducted almost exclusively by a biochemical enzyme assay procedure [13,14].

In the present work, not only was isocitric acid converted into its trimethyl ester during resin-mediated esterification but trimethyl isocitrate was well separated from trimethyl citrate and all other methyl esters in the gas chromatogram (see Fig. 5C). The procedure described therefore provides a new instrumentally-based method for measuring isocitric acid in fruit juice in the presence of large amounts of citric acid by a single GC procedure.

Fig. 6 demonstrates that isocitric acid can be detected at the low levels present in fruit juices. Addition of a suitable concentration of an internal standard such as triethyl citrate allows the quantitation of trimethyl isocitrate in the presence of a very large excess of other acids. Triethyl citrate was used for the external standard quantitation of both trimethyl isocitrate and trimethyl citrate using GC–FID.

Orange juice was spiked with isocitric acid at 4 levels (10, 25, 50 and 100 ppm) and seven replicate

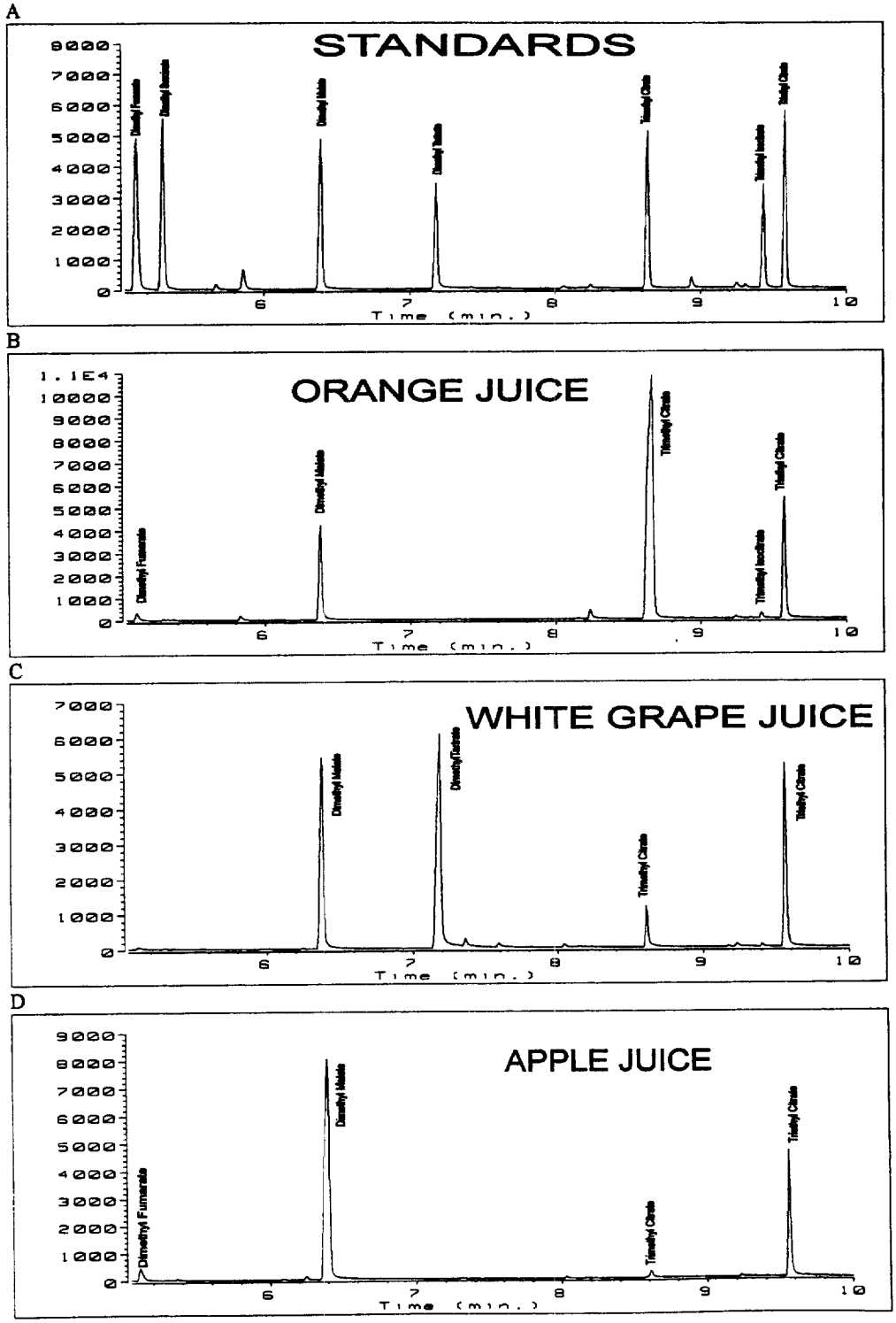
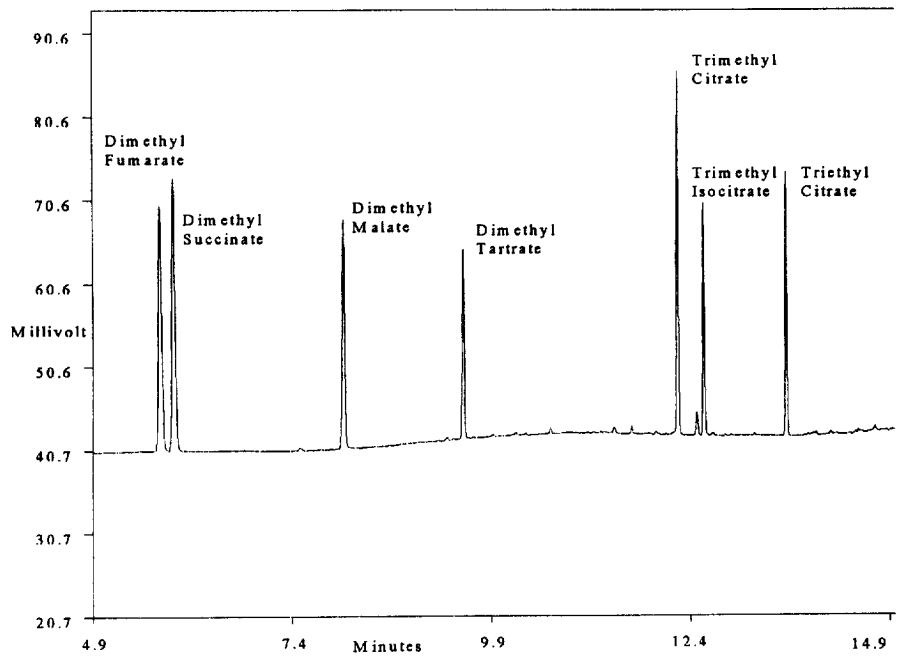


Fig. 4. Gas chromatographic separation (GC–AED) of polybasic fruit acids following SFE methylation. (A) Extract of mixed aqueous standard containing 100 μg/ml of each acid. (B) Extract of 165 μl orange juice. (C) Extract of grape juice. (D) Extract of apple juice.

A


90.6
80.6
70.6
60.6
Millivolt
50.6
40.7
30.7
20.7
Dimethyl Fumarate
Dimethyl Succinate
Dimethyl Malate
Dimethyl Tartrate
Trimethyl Citrate
Trimethyl Isocitrate
Triethyl Citrate
4.9
7.4
Minutes
9.9
12.4
14.9

B

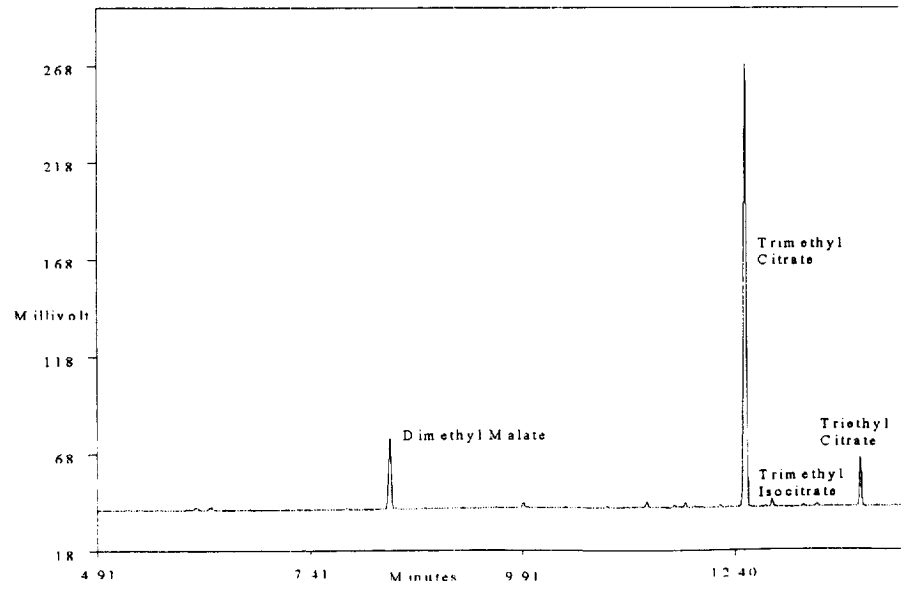

268
218
168
Millivolt
118
68
18
Dimethyl Malate
Trimethyl Citrate
Triethyl Citrate
Trimethyl Isocitrate
4.91
7.41
Minutes
9.91
12.40

Fig. 5.

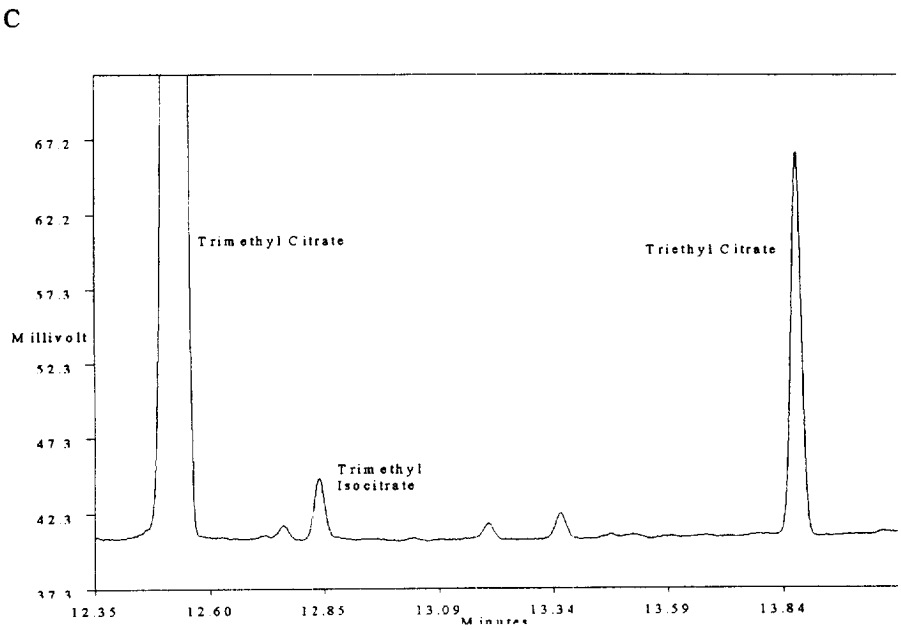


Fig. 5. Gas chromatographic separation (GC–FID) of polybasic fruit acids following SFE methylation. (A) Extract of mixed aqueous standard containing 100 μg/ml of each acid. (B) Extract of 100 μl orange juice. (C) Extract of 100 μl orange juice showing separation of citric and isocitric methyl esters (enlargement).

analyses carried out at each level. The recovery was consistent (60–65%, average 64%) across this concentration range (see Table 2, Fig. 6), and hence this systematic error can be corrected for using the average recovery of the method.

The lower recoveries of isocitric acid (~60–65%) compared with the other acids could indicate that the extraction or derivatisation of the isocitric acid on the resin is somehow less efficient. This may be related to structure, although the reason for the lower recoveries of isocitric acid is unclear from the present data. Recoveries for either spiked orange juice or the standard mixture of acids in water were similar, which suggests that the recovery range of 60–65% was probably not due to any matrix effects.

The orange juice used above was analysed by both

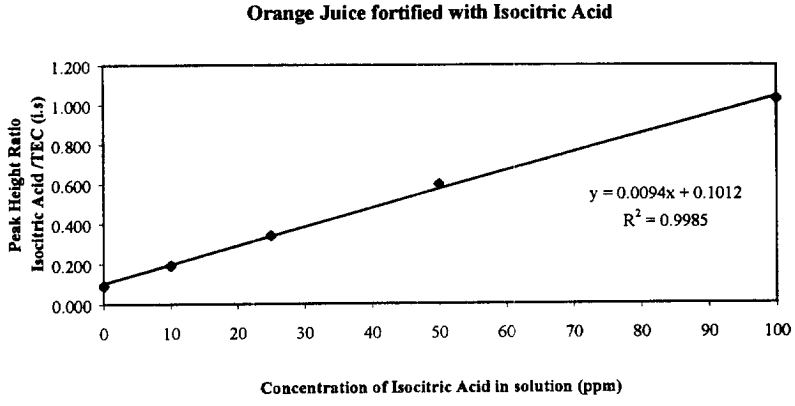


Fig. 6. Linearity of the efficiency of the procedure for the extraction of orange juice fortified with isocitric acid.

Table 2
Reproducibility and recovery data for the SFE methylation analysis of orange juice fortified with isocitric acid

Spiking level (ppm)	%Recovery ($n=7$)	%C.V.
Not spiked	–	7.6
10	65.3	22.1
25	64.2	3.1
50	64.8	5.4
100	60.3	5.2

the SFE method and a commercially available enzymatic kit method (Boehringer Mannheim) and the results compared below. Using the SFE procedure, the concentration of isocitric acid in the orange juice sample was determined to be 35.6±0.8 ppm ($n=7$) not corrected [55.9±1.3 ppm ($n=7$) corrected for average recovery (64%) of the method] and 62 ppm ($n=1$) by the enzymatic technique. An aqueous solution of isocitric acid (50 ppm) was also analysed by the SFE technique and a result of 52.9 ppm ($n=3$, corrected for method recovery) was obtained.

3.6. Determination of tartaric acid

The suitability of this procedure for the determination of tartaric acid in fruit juice was investigated by seven replicate analyses of a diluted orange juice solution fortified at 20 ppm (equivalent to 200 ppm in the juice). A good recovery (88%) and low coefficient of variation (3.6%) was obtained. Increased loading of the resin (150 μl of orange juice per ml loaded onto 100 mg of resin), followed by concentration of the final extract to 200 μl allowed the detection and quantitation of tartaric acid at levels of 10 ppm in orange juice using this method (recovery 93%, %C.V. 5.9 for $n=7$).

Apart from the quantitative capability of the technique, use of GC–MSD allows confirmation of the identity of the fruit acid components of a sample. The method has recently been used qualitatively in this laboratory to confirm the presence of tartaric acid in suspect orange juice and pear juice samples. This acid is not found in authentic juices of this type.

3.7. Derivatisation of acids by the "in vial" technique in acetonitrile

We previously reported that resin mediated methylation was also possible by reaction of the acidic analyte absorbed onto macroporous anion exchange resin with methyl iodide in acetonitrile at 80°C for 1 h or more [11]. This methodology has been elegantly extended by Field [12] who obtained excellent methylation efficiency by trapping the acids onto strong anion exchange Empore™ discs followed by heating in a capped GC autosampler vial with methyl iodide. We found that this procedure could also be applied to the extraction and derivatisation of polybasic organic acids found in fruit juices, and recoveries were equivalent to those observed for the SFE method.

The results of the "in-vial" extraction ($n=6$) of an aqueous solution of 100 ppm of each of the 6 acids are presented in Table 1 (Column V) and compared reasonably well with the SFE technique. The %C.V. values for the "in-vial" technique were slightly better and the experimental technique comparatively simpler than the more demanding SFE method. However, the aim of this work was to demonstrate the applicability of supercritical CO_2 as both a derivatisation and extraction solvent for the quantitation of polybasic organic acids found in fruit juices as an extension of our previous work. The data in Table 1 demonstrate that the SFE method is at least the equivalent of the "in-vial" technique with respect to recoveries of three of the acids under test.

The analysis of fruit juices (for example, apple juice) which contain polyhydroxy acids, such as quinic and shikimic, could be problematic using the "in-vial" technique. The methyl ester derivatives of these acids, which are soluble in acetonitrile, would be injected onto the GC column and could eventually result in column damage. The use of supercritical CO_2 as a derivatisation and extraction "solvent" could overcome this problem; work in our laboratory has found that the esters of these polyhydroxy acids are not extracted into supercritical CO_2.

4. Conclusion

A method has been developed for the determination of six polybasic organic acids found in a variety of fruit juices, by isolation on an anion exchange resin followed by conversion to the methyl ester using methyl iodide in either supercritical CO_2

or acetonitrile ("in-vial"). Good recoveries of both tartaric and citric acids were obtained, whilst recovery of isocitrate was reasonable (~64%) for the SFE method. The "in-vial" technique was found to be experimentally simpler than the SFE method and gave comparable results. The developed method may be used for evaluation of fruit juice acid profiles, determination of citrate/isocitrate ratios in a single instrumental procedure and positive mass spectral confirmation of the identity of markers of adulteration in fruit juice.

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