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Organic acid analysis by ion chromatography–particle beam mass spectrometry

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ABSTRACT

Ion chromatography–mass spectrometry (IC–MS) has been investigated, using the particle beam interface, as an analytical technique for the analysis of weak organic acids. Ion-exclusion chromatography is a technique for separating weak acids. Using dilute HCl as the mobile phase, weak acids are separated on a cation-exchange resin. IC–MS was used to detect and identify weak acids in commercial grape juices and red wine. Analyses were performed using both electron (EI) and chemical ionization (CI). Isobutane CI readily produced protonated molecular ions, and combined with structural information obtained from EI analyses, identification of weak acids was possible. A main advantage of this technique is that co-eluting acids can be identified, whereas with conductivity and UV detection this is difficult. To simplify these analyses, solid-phase extraction was used to remove sugars from the juice and wine samples.

INTRODUCTION

Organic acids are found in a variety of complex matrices. They can be found in biological samples, such as urine, blood and bile; in food products, such as wine, juice drinks and milk [1]. Small carboxylic acids are extremely important in wines and juice drinks because they contribute to taste and product stability [2,3].

Monocarboxylic acids can be analyzed by gas chromatography (GC), but dicarboxylic acids are difficult to analyze because of their high polarity and boiling points. A number of investigators have analyzed these compounds by GC using derivatization. Because sample preparation can take as long as 24 h in some cases, acids are often analyzed by liquid chromatography [4–9].

Although there are a variety of liquid chromatographic techniques for the analysis of weak acids, ion-exclusion chromatography [10–15], ion-exchange chromatography [3,11,17–19] and reversed-phase HPLC [2,20–22] have received the most attention. These separation techniques usually employ conductivity, UV or refractive index detection. Ion-exclusion chromatography with conductivity or UV detection has proven to be a sensitive technique for the analysis of weak acids. However, these analyses give limited information about the compounds of interest and provide no information on possible co-elution of compounds.

The focus of this study was to interface ion-exclusion chromatography with mass spectrometry using the particle beam interface. Others have used ion chromatography (IC) coupled to mass spectrometry (MS) [15,16]. Pacholec *et al.* [15] investigated IC–MS using the thermospray interface and was able to produce only the molecular ions for each acid. However, the particle beam interface provides the chromatographer with the ability to distinguish and identify unknown compounds from mass spectral data that contains both molecular mass (CI) and structural information (EI). The mass spectrometer also provides the analyst with a tool to deconvolute the co-elution of acids. Therefore, IC–MS makes identification of weak acids an

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easier process by providing the analyst with spectral information in addition to the retention time of compounds.

EXPERIMENTAL

Reagents

Deionized water was obtained from a Milli-Q reagent water system (Millipore, Bedford, MA, USA). The HPLC-grade methanol, sodium hydroxide (Baker Analyzed), sulfuric acid (Baker Instra-Analyzed Reagent), hydrochloric acid (ULTREX II Ultrapure Reagent) were used as received from J.T. Baker (Phillipsburg, NJ, USA). The organic acids were purchased from Aldrich (Milwaukee, WI, USA). The grape juices were purchased from a local supermarket, and the red wine was from a local winery.

Standard and sample solutions

The acid standards (citric, fumaric, malic, tartaric and succinic acids) were prepared at the 75 ppm (w/w) level in deionized water. The grape juice and red wine samples were diluted 1:5 in deionized water.

The neutral compounds were separated from the organic acids by passing the diluted samples through a 2.8 ml Bond Elut strong anion-exchange (SAX) solid-phase extraction cartridge (Analytichem International, Harbor City, CA, USA). First, the tube was conditioned with 5 ml of methanol followed by 5 ml of deionized water. The pH of the diluted sample was adjusted to pH 7–8 with 10 *M* sodium hydroxide, and then 1 ml of sample was passed through a SAX cartridge at 1 ml/min. The neutral compounds (sugars) were eluted from the SAX cartridge with 2 ml of water at 1 ml/min. The acid compounds were eluted with two 0.5-ml aliquots of 0.5 *M* sulfuric acid at 1 ml/min [17].

Chromatography

The liquid chromatograph consisted of a Hewlett-Packard (HP) 1090 and a Rheodyne 7125 injector fitted with a 100-μl sample loop. A Kratos Spectroflow 783 UV detector (210 nm) was placed in series with the particle beam interface. A flow-rate of 0.5 ml/min and a Waters Fast Fruit Juice (Milford, MA, USA) 15 cm × 7.8 mm column (7 μm particle size) was used for all separations. The mobile phase was 1.0 m*M* HCl and was thoroughly degassed before use.

Mass spectrometer

The experiments were performed on a HP 5988 quadrupole mass spectrometer with a mass range of 2000 u. The HP particle beam LC–MS interface Model 5998A was used to couple the mass spectrometer to the liquid chromatograph and UV detector. The mass spectrometer was operated in the positive ion full scan mode for all experiments. The mass range scanned was 40–400 u and 100–500 u for EI and CI analyses, respectively. The source temperature was 200°C for both EI and CI analyses. The source pressure for isobutane CI was approximately 0.5 Torr (1 Torr = 133.322 Pa). Methane CI was approximately 0.9 Torr. The particle beam interface desolvation chamber was 70°C, the nebulizer helium pressure was 55 p.s.i. (1 p.s.i. = 6894.76 Pa), and the capillary position was extended approximately 0.6 mm from the flush position of the nebulizer.

RESULTS AND DISCUSSION

Chromatography

Ion-exclusion chromatography is an ion chromatography technique that is used to separate weak organic acids from strong acids. The stationary phases are cation-exchange resins in the H^+ form that can vary in the degree of resin cross-linking. The mobile phases are usually prepared from strong acids, such as hydrochloric and sulfuric acids. At an operating pH of 2–3 the cation-exchange resin and organic acids (*i.e.* carboxylic acids) are protonated. The acids are separated by mechanisms which include: Donnan exclusion, steric exclusion and adsorption [23].

Sulfuric acid (1 m*M*) was initially used for the separation, but, because of the interference of the sulfuric acid the mass spectrometer could not be scanned below m/z 98 (M_r of H_2SO_4) during EI analyses. This would have increased the background signal and interfered with the sensitivity of the analysis. Therefore, HCl was chosen as the mobile phase which allowed us to

start scanning the mass spectrometer from m/z 40, just above the HCl background. The use of HCl as a mobile phase with a stainless-steel HPLC system is not ideal, and thorough washing of the HPLC and the particle beam interface with water was necessary to reduce corrosion problems.

Initially, both mono- and dicarboxylic acids were analyzed. Sensitivity for the monocarboxylic acids was poor (500 ppm), while the dicarboxylic acids gave excellent responses at 75 ppm. We believe this behavior is due to the design of the particle beam interface [24]. To eliminate the solvent from entering the mass spectrometer, the interface employs a two-stage momentum separator. Because of the low molecular mass and volatility of the monocarboxylic acids, these compounds are skimmed away with the solvent, and therefore only high concentrations of the monocarboxylic acids will produce a signal in the mass spectrometer. For this reason we concentrated on the analysis of the dicarboxylic acids: fumaric, malic, succinic, tartaric and citric (Fig. 1). The CI total ion chromatograms of succinic, citric, malic, tartaric and fumaric acid standards are shown in Fig. 2.

Mass spectrometry

EI, negative CI (NCI), and positive CI (PCI) techniques were investigated. EI provided the highest counts while the negative and positive CI analyses were essentially identical using flow

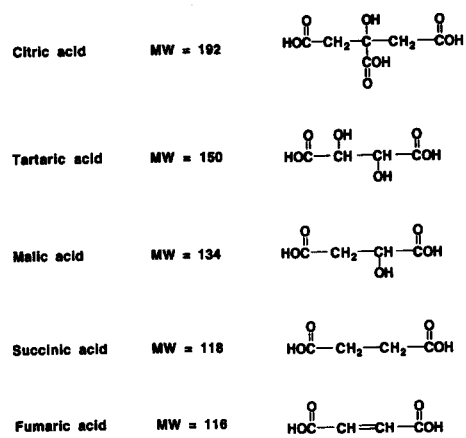

Fig. 1. Chemical structures of weak acid standards. MW = Molecular mass.

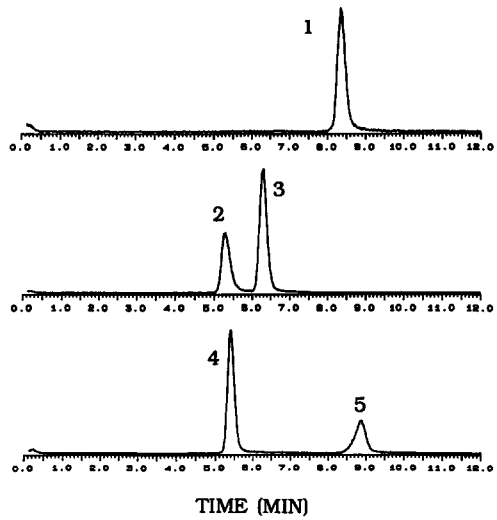

Fig. 2. CI total ion chromatograms for 75 ppm weak acid standards. Peaks: 1 = succinic acid; 2 = citric acid; 3 = malic acid; 4 = tartaric acid; 5 = fumaric acid.

injection analyses of organic acids at a concentration of 500 ppm.

NCI analyses produced molecular ions of the standards with no other significant amount of fragmentation. PCI analyses produced protonated molecular ions of the standards with little fragmentation. The base peak ion in the CI isobutane mass spectra of all the acid standards, except for citric acid, was the protonated molecular ion $(M+H)^+$. The loss of H_2O from the protonated molecular ion produces the ion with the next greatest intensity in the mass spectra. Fig. 3a is an example of succinic acid analyzed by CI. Ion m/z 119 is the protonated molecular ion and ion m/z 101 is the loss of H_2O. Fig. 3b and c are the CI mass spectra of malic and tartaric acids, respectively. The base peak in the CI mass spectra of citric acid is ion m/z 147 which corresponds to the loss of 46 (CH_2O_2) from the protonated molecular ion m/z 193 (Fig. 3d).

The molecular ion peak in the EI analyses of the dicarboxylic acid standards was either weak or absent. This is common for polycarboxylic acids. The loss of CO_2 is often found in the EI mass spectra of dicarboxylic acids [25]. This loss is observed in the EI mass spectra for malic, tartaric and succinic acids which are shown in Fig. 4a, b and c, respectively.

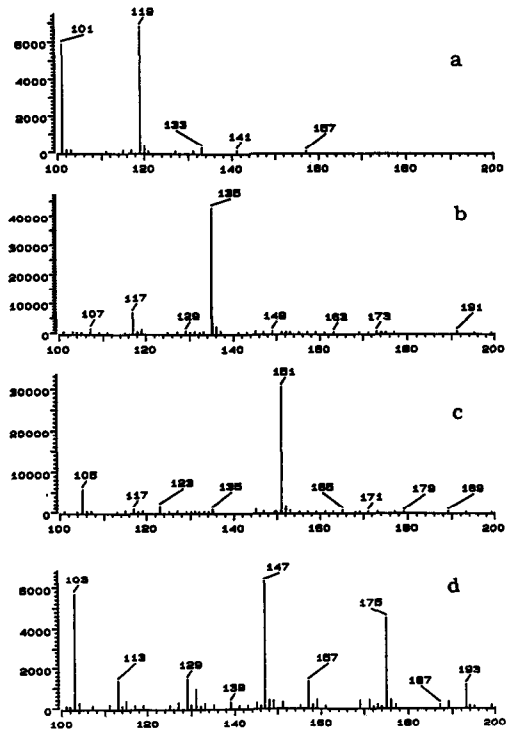

Fig. 3. CI mass spectra for 75 ppm weak acid standards: (a) succinic acid; (b) malic acid; (c) tartaric acid; (d) citric acid.

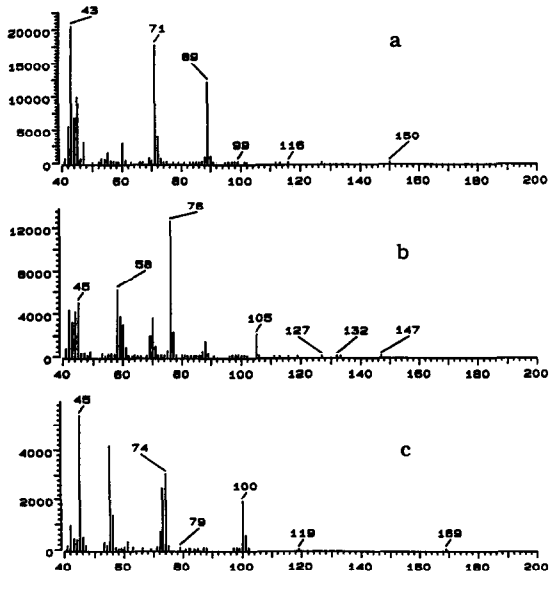

Fig. 4. EI mass spectra for 75 ppm weak acid standards: (a) malic acid; (b) tartaric acid; (c) succinic acid.

Applications

When analyzing commercial grape juice samples that contained sugar, it was necessary to separate the sugars from the sample to simplify the analysis. The sugars do not have a chromophore and are not detected by UV detection. Fig. 5a shows the CI total ion chromatogram for grape juice A before the SAX extraction. This chromatogram is very complex compared to the UV chromatogram of the same sample, Fig. 5b. Although the mass spectrometer has the ability to extract the characteristic ions of the acids from the chromatogram and identify their presence, it was decided for simplicity that solid-phase extraction would be used. Fig. 5c shows the CI total ion chromatogram of acid extract of commercial grape juice A. The major peaks in this chromatogram correlate well with the chromatographic profile obtained for the same sample (using UV detection) before extraction (Fig. 5b). The retention times differ because the analyses were performed on two different days.

The identification of the acid components of a mixture can be readily determined from the extracted ion profiles. The CI total ion chromatogram of the acid extract of commercial grape juice A is shown in Fig. 6a. The extracted ion

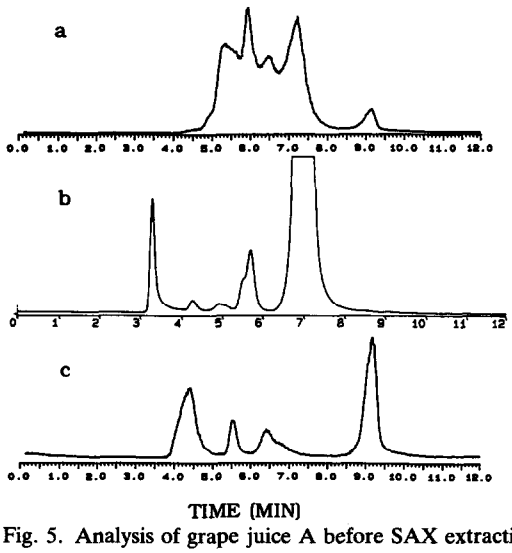

Fig. 5. Analysis of grape juice A before SAX extraction: (a) CI total ion chromatogram; (b) UV chromatogram. Analysis of grape juice A after SAX extraction: (c) CI total ion chromatogram.

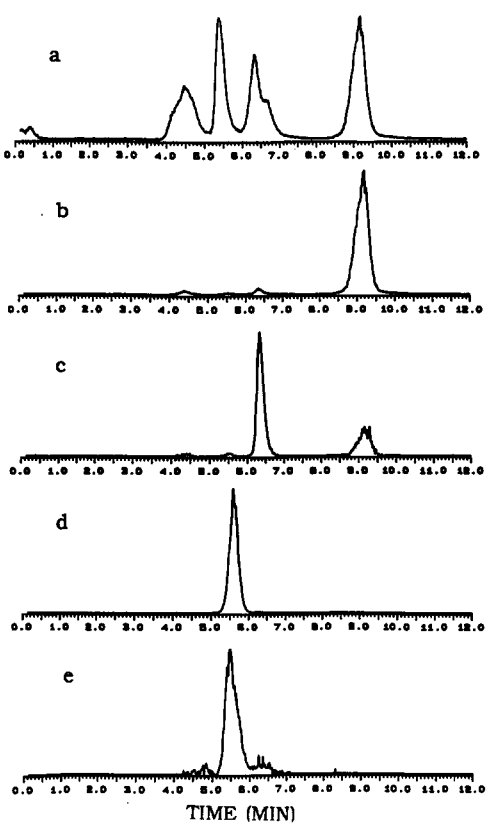

Fig. 6. Analysis of the grape juice A acid extract. (a) CI total ion chromatogram. Extracted ion profiles for (b) fumaric acid (m/z 117), (c) malic acid (m/z 135), (d) tartaric acid (m/z 151) and (e) citric acid (m/z 193).

profiles for ions m/z 117, m/z 135, m/z 151 and m/z 193 correspond to the protonated molecular ions for fumaric, malic, tartaric and citric acids, respectively. The retention times and mass spectra of the peaks confirm the presence of these acids in grape juice A. The extracted ions m/z 151 and m/z 193 (Fig. 6d and e) show the strength of the mass spectrometer to identify the co-elution of tartaric (m/z 151) and citric (m/z 193) acids. The peak at retention time 4.5 min is still unidentified but has characteristic ions of a carboxylic acid. Another acid extract from a different commercial grape juice (B) is shown in Fig. 7. The extracted ion profiles for the protonated molecular ions m/z 135 and m/z 151 correspond to malic and tartaric acids, respectively. The retention times and mass spectra of the peaks confirm the presence of these acids in

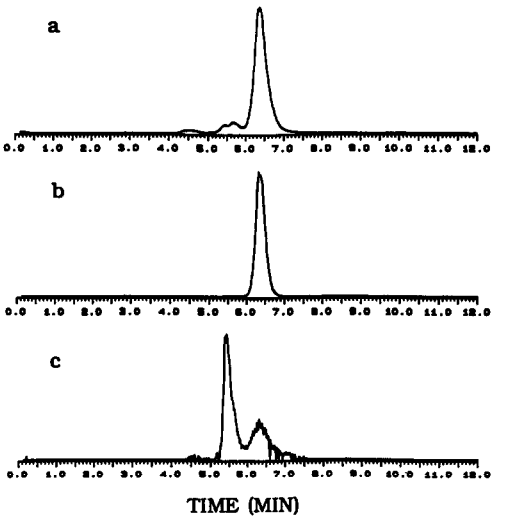

Fig. 7. Analysis of the grape juice B acid extract. (a) CI total ion chromatogram. Extracted ion profiles for (b) malic acid (m/z 135) and (c) tartaric acid (m/z 151).

grape juice B. Malic and tartaric acids were identified in both grape juices, while fumaric and citric acids were identified only in grape juice A.

The CI total ion chromatogram of the acid extract of a red wine is shown in Fig. 8. The extracted ion profiles for the protonated molecular ions m/z 119, m/z 135, m/z 151, and m/z 193 correspond to succinic, malic, tartaric and citric acids, respectively. The retention times and mass spectra of the peaks confirm the presence of these acids in this sample of red wine. Again the deconvolution of ions m/z 151 and m/z 193 (Fig. 8d and e) show the strength of the mass spectrometer to identify the co-elution of tartaric (m/z 151) and citric (m/z 193) acids.

CONCLUSIONS

Ion chromatography coupled to particle beam mass spectrometry has been shown to generate characteristic mass spectra for the identification of weak acids. Due to co-elution of sugars with the weak acids, solid phase extraction was used to eliminate the interference of the sugars present in the samples. This extraction simplified the total ion chromatograms and mass spectra obtained for each analysis. Positive CI analysis produced protonated molecular ions, and EI

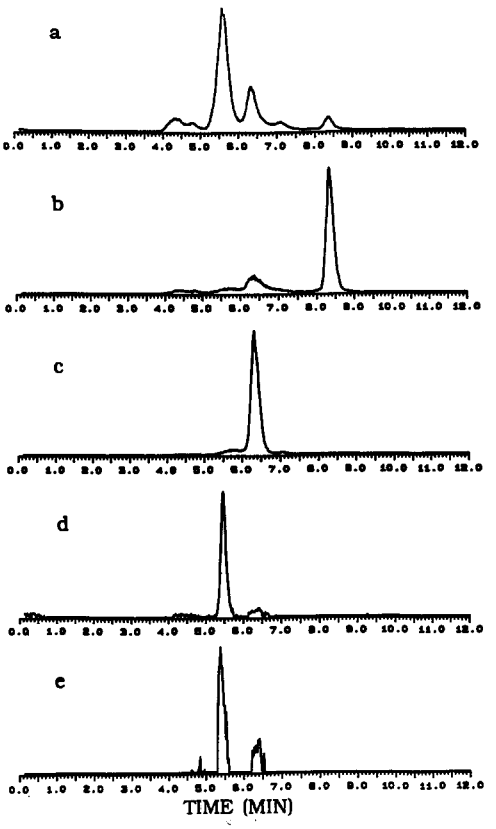

Fig. 8. Analysis of the red wine acid extract. (a) CI total ion chromatogram. Extracted ion profiles for (b) succinic acid (m/z 119), (c) malic acid (m/z 135), (d) tartaric acid (m/z 151) and (e) citric acid (m/z 193).

analysis provided structural information of the acid standards. With the combination of the two analyses, dicarboxylic acids were detected and identified in commercial grape juices and red wine.

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