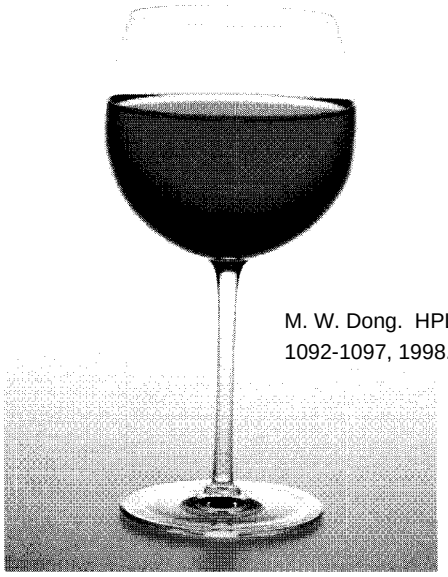
HPLC Analysis of Organic Acids in Juice and Wine Using Resin and Reversed-Phase Columns



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High performance liquid chromatography is the preferred technique for the analysis of organic acids in foods. Although both resin and reversed-phase columns are used routinely, many analysts are unaware of the selection criteria and the practical precautions for effective column use. This article delineates the pros and cons for using both column types and illustrates their practical use in beverage applications. The author concluded that reversed-phase columns were suited for fruit juice analyses and that resin columns were useful for the simultaneous determination of organic acids, sugars, and ethanol in wines and fermentation samples.

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Organic acids occur naturally in foods as a result of biochemical metabolic processes, hydrolysis, and bacterial growth. Organic acids are found in various foodstuffs, including fruit juices, vegetables, meat, wines, vinegars, and dairy products, and also in fermentation broths and biological fluids. The determination of organic acids is important for the quality control of natural and processed foods because some organic acids are organoleptic and others are indicators for ripeness, bacterial spoilage, and adulteration.

Several organic acids are used as food additives such as preservatives, acidulants, antioxidants, and stabilizers (1,2). High performance liquid chromatography (HPLC) is the preferred analytical technique, and separation is accomplished by using either resin or reversed-phase columns (2,3). UV detection at 210 nm provides sensitive and specific detection, and refractive index detection yields additional data about other non–UV-absorbing components in the sample. Organic acids also can be derivatized to form molecules with better chromatographic or chromophoric properties; the derivatized organic acids subsequently can be analyzed with gradient HPLC. The derivatization approach is particularly useful for research samples that contain minor organic acids (4).

Although both resin-based and reversed-phase columns can be used for organic acid analysis, the selection criteria are not widely publicized and can be dependent on the sample. This article delineates the advantages and limitations of both column types and illustrates their usefulness in juice and wine applications. I have developed two quantitative assays: one for organic acids in fruit juice using reversed-phase columns and another for organic acids, sugars, and ethanol in wines using resin columns. This article documents practical guidelines for improving assay robustness.

EXPERIMENTAL

Apparatus and reagents: I used a Turbo LC Plus HPLC system, which comprised a binary liquid chromatography (LC) pump, an autosampler, a column oven, a refractive index detector, and a UV–vis detector. I also used a Turbochrom Professional workstation for data handling and HPLC system control. All HPLC and data-handling equipment were from Perkin-Elmer Corp. (Norwalk, Connecticut).

The reversed-phase column was a 22 cm × 4.6 mm, 5-μm d_p PE Brownlee Spheri-5 RP-18 column with an integral 1.5 cm × 3.2 mm, 7-μm d_p NewGuard C18 column (Norwalk, Connecticut). This column's bonded phase has an 11% carbon loading, a 180-m^2/g surface area, and an 80-Å pore diameter. The resin column was a 22 cm × 4.6 mm, 10-μm d_p PE Brownlee Polypore H column directly coupled with a 3.0 cm × 4.6 mm, 10-μm d_p Polypore H guard column.

I obtained organic acids, sugars, and chemical reagents from Supelco (Bellefonte, Pennsylvania), Sigma Chemicals (St. Louis, Missouri), and Aldrich (Milwaukee, Wisconsin). The HPLC-grade organic solvents and water were from J.T. Baker (Phillipsburg, New Jersey). I purchased juice and wine products at a market in Connecticut.

HPLC operating conditions: For analyzing juice samples I used the reversed-phase column and performed UV detection at 210 nm. I started with a mobile phase of 25 mM monobasic potassium phosphate (pH 2.4) for 8 min, then purged the HPLC system with 30% acetonitrile for 2 min, and equilibrated the system for 10 min. The flow rate was 1.5 mL/min at room temperature and 2400-psi pressure. The sample injection volume was 5 μL of diluted fruit juice.

For wine samples I used the resin column and performed UV detection at 210 nm as well as refractive index detection. The mobile phase was 0.01 N sulfuric acid, and the flow rate was 0.2 mL/min at 60 °C and 300 psi. The sample injection volume was 10 μL of diluted wine or grape must juice.

Sample preparation procedures: I diluted 100 μL of the clear (or clarified) juice and wine samples with 400 μL of mobile phase before HPLC analysis. I filtered and clarified

some juice samples — orange juice and juice from grape must — through 0.5-μm membrane filters before dilution.

RESULTS AND DISCUSSION

Resin columns for organic acid analysis: Organic acids most commonly are analyzed with columns that are packed with 10-μm d_p sulfonated 8% cross-linked polystyrene cation-exchange resins in the hydrogen form (2). Columns similar to the Polypore H resin columns used are available from various manufacturers, including Bio-Rad Laboratories (Hercules, California); Dionex Corp. (Sunnyvale, California); Hamilton Co. (Reno, Nevada); Interaction Chemicals (San Jose, California); Shimadzu

TABLE I: Retention Times of Common Organic Acids on Resin and Reversed-Phase Columns

Organic Acid	Resin Column* (min)	Reversed-Phase Column† (min)
Oxalic acid	4.3	1.45
Citric acid	5.2	4.8
Tartaric acid	5.7	1.75
Ascorbic acid	6.0	2.67
Malic acid	6.2	2.3
Quinic acid	6.2	1.81
Shikimic acid	7.2	2.53
Succinic acid	7.5	5.4
Lactic acid	7.5	2.75
Formic acid	8.3	1.87
Acetic acid	8.8	3.0
Propionic acid	10.2	7.8
Fumaric acid	10.4	5.0

* Columns: PE Brownlee 22 cm × 4.6 mm, 10-μm d_p Polypore H and 3.0 cm × 4.6 mm, 10-μm d_p Polypore H guard column; temperature: ambient; flow rate: 0.3 mL/min.

† Column: PE Brownlee 22 cm × 4.6 mm, 5-μm d_p Spheri-5 RP-18; temperature: ambient; flow rate: 1.5 mL/min.

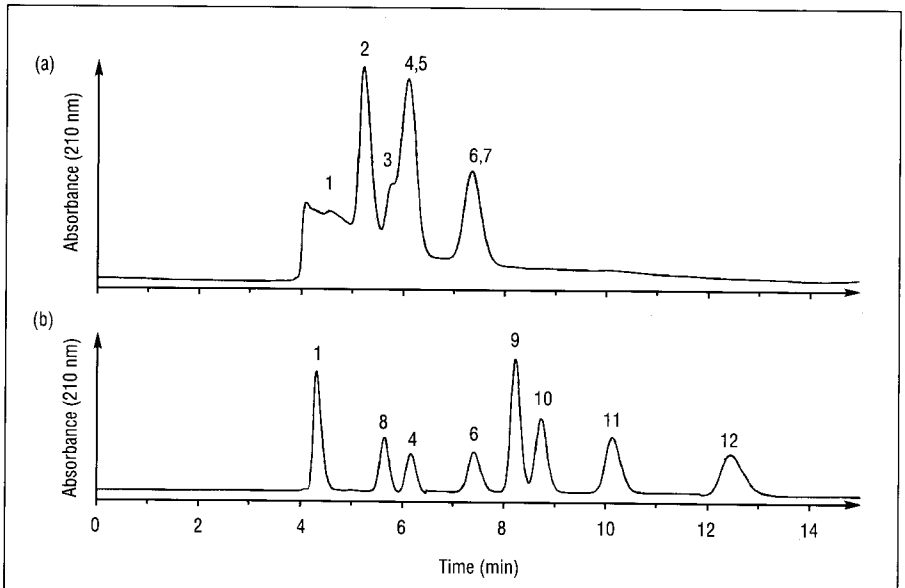

FIGURE 1: Separation of (a) a cranberry juice cocktail and (b) a standard mixture. Column: 22 cm × 4.6 mm Polypore H with a 3-cm guard column; mobile phase: 0.01 N sulfuric acid; flow rate: 0.3 mL/min; temperature: ambient. Peaks: 1 = oxalic acid, 2 = citric acid, 3 = ascorbic acid, 4 = malic acid, 5 = quinic acid, 6 = succinic acid, 7 = shikimic acid, 8 = tartaric acid, 9 = formic acid, 10 = acetic acid, 11 = proprionic acid, 12 = butyric acid.

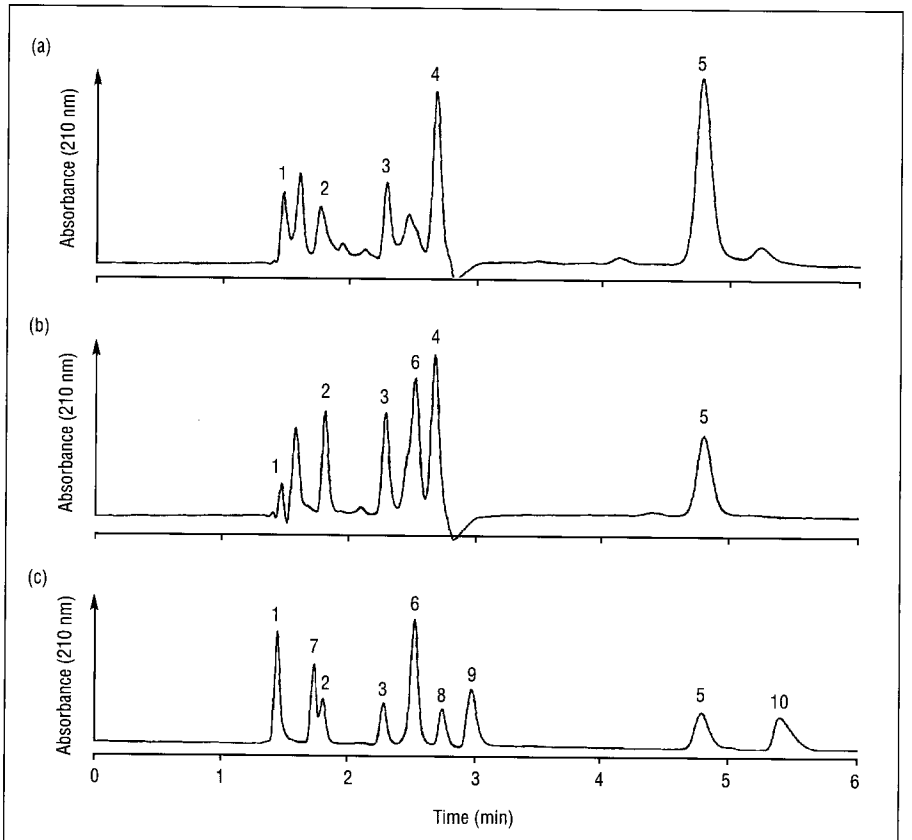

FIGURE 2: Reversed-phase HPLC analysis of (a) an orange juice sample, (b) a cranberry juice cocktail, and (c) a calibration test mix. Conditions are described in the experimental section. Peaks: 1 = oxalic acid, 2 = quinic acid, 3 = malic acid, 4 = ascorbic acid, 5 = citric acid, 6 = shikimic acid, 7 = tartaric acid, 8 = lactic acid, 9 = acetic acid, 10 = succinic acid.

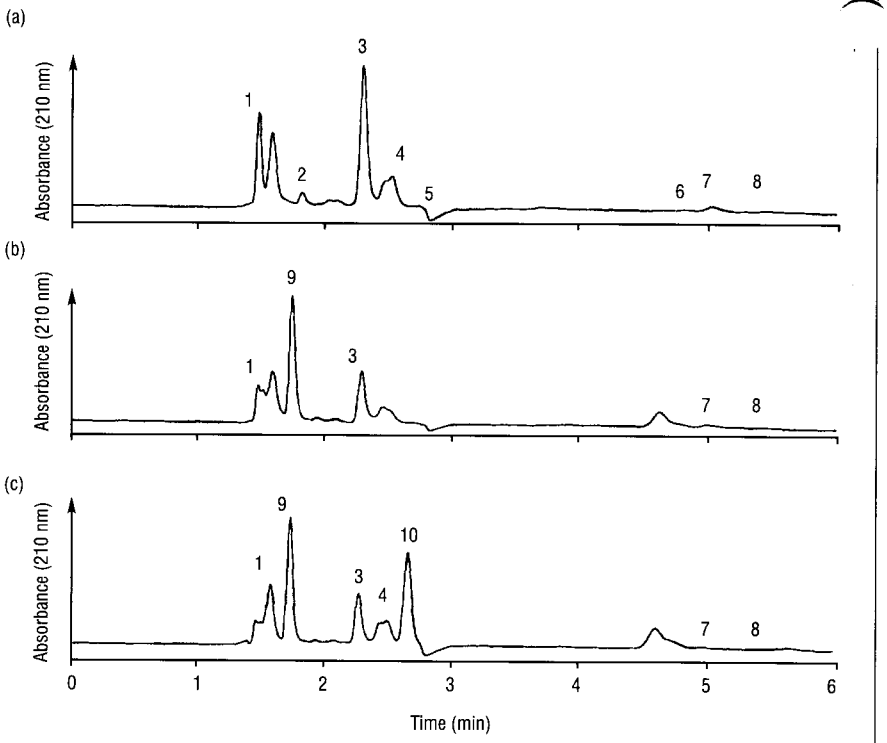

FIGURE 3: Reversed-phase HPLC analysis of (a) apple, (b) purple grape, and (c) white grape juice samples. Conditions are described in the experimental section. Peaks: 1 = oxalic acid, 2 = quinic acid, 3 = malic acid, 4 = shikimic acid, 5 = lactic acid, 6 = citric acid, 7 = fumaric acid, 8 succinic acid, 9 = tartaric acid, 10 = ascorbic acid.

Scientific Instruments (Columbia, Maryland); and Supelco (Bellefonte, Pennsylvania).

Typical operating conditions include 0.2–0.7 mL/min flow rates, pressures less than 1200 psi, 20–80 °C temperatures, and a mobile phase of 0.005–0.5 N sulfuric acid. Column efficiency is 4000–6000 plates for the 22-cm-long column with 3-cm-long guard column. In addition to organic acid analysis, these resin columns are useful for the analysis of aromatic acids, alcohols, aldehydes, ketones, and sugars. The separation mechanism appears to be a combination of ion exclusion, ligand exchange, and hydrophobic interaction with the polymeric backbone (2,3).

Organic acid analysis using resin columns is popular for dairy, industrial, and fermentation samples. The resin column is easy to use because it can be operated at low pressures and requires simple mobile phases, and it yields reproducible retention times. Using this column type for juice analysis is less satisfactory, however, because of the close elution of several key analytes; namely, ascorbic, malic, and quinic acids and succinic, shikimic, and lactic acids as shown in Figure 1 and Table I. Figure 1 shows the separation of a standard mixture and a cranberry juice cocktail sample using a resin column at room temperature with a mobile phase of 0.01 N sulfuric acid. Although I could improve the separation somewhat by increasing the column length and optimizing other operating parameters, I can achieve better results more readily with reversed-phase columns.

Analysis of organic acids in fruit juices using reversed-phase columns: Several researchers have reported the reversed-phase HPLC analysis of organic acids in juice samples using ion suppression with acidic mobile phases (5–7), and the technique was adopted in an Association of Official Analytical Chemists International (AOAC International) method for cran-berry and apple juice (8).

My experimental results for the reversed-phase column confirmed the usefulness of reversed-phase separations. Figures 2 and 3 show excellent resolution that resulted from

TABLE II: Determination of Organic Acids in Fruit Juice Using Reversed-Phase Columns and UV Detection*

Organic Acid	Apple Juice (mg/mL)	Orange Juice (mg/mL)	White Grape Juice (mg/mL)	Purple Grape Juice (mg/mL)	Cranberry Cocktail (mg/mL)
Oxalic acid	0.1	0.1	—	—	0.1
Tartaric acid	—	—	2	2.2	—
Quinic acid	0.5	—	—	—	2.5
Malic acid	3.0	1.0	1.3	2.1	1.3
Shikimic acid	0.04	—	0.01	—	0.1
Ascorbic acid	—	0.3	0.3	—	0.2
Citric acid	0.04	6.0	—	—	2.0
Fumaric acid	0.02	—	0.002	0.01	—
Succinic acid	0.05	—	—	0.1	—

* — = not present or nondetectable.

the high efficiency (more than 8000 plates) and solute selectivity of the 5-μm d_p column. Major organic acids are nearly baseline resolved. The quantitative data of several juice samples are shown in Table II. I used a totally aqueous mobile phase, which is unusual for reversed-phase separations because 5–10% of organic solvent typically is recommended. I also reproduced similar separations in my laboratory using the same mobile phase and several different C8 and C18 columns. An 8.3 cm × 4.6 mm, 3-μm d_p C8 column accomplished the separation in 3 min; however, the resolution between tartaric and quinic acid was better on the longer reversed-phase column.

The rapid (less than 10 min) reversed-phase assay requires little sample preparation, uses a lower-cost column at room temperature, and is useful for applications such as quality control and adulteration screenings of most juice products. The method provided a relative standard deviation (RSD) of less than 1% for retention time and peak area; 10–30 ng detection limits for most organic acids (1–5 ng for oxalic, tar-ic, and shikimic acids); and a 0.1–100 μg ırity range for all organic acids.

For samples such as cranberry juice that contain late-eluted components, the column was purged with 30% acetonitrile for 2 min and then equilibrated for 10 min to prevent interferences in subsequent samples. I replaced the guard column after analyzing 200 samples. I observed occasional resolution loss if air bubbles entered the HPLC system (presumably from the collapse of the hydrophobic bonded-phase under the nonwetting aqueous mobile-phase conditions). In all cases, the column performance was restored by purging the column with 30% acetonitrile for 5 min.

Simultaneous analysis of organic acids, sugars, and ethanol using resin columns: The unique ability of the resin columns to retain and separate neutral molecules such as sugars and alcohols can be exploited for fermentation monitoring during wine making (2,3). Several researchers have demonstrated the simultaneous assay of organic acids such as tartaric, malic, lactic, acetic, citric, and succinic acids; glucose; fructose; glycerol; and ethanol in wine samples (9–12). In my study, I optimized the separation conditions for these 10 key analytes as well as oxalic and shikimic acids using two concatenated resin columns.

Figure 4 shows chromatograms produced under the optimized conditions. I found some ctivity differences — minor elution-order nges — between the resin columns I used and the Bio-Rad Aminex HPX-87H column used by Lopez and Gomez (11). I used a 60 °C column temperature to resolve lactic acid from succinic acid, and I decreased the flow rate to 0.2 mL/min to increase overall column efficiency. Dual refractive index–UV detection enabled the quantitation of several partially resolved or low-concentration components. Refractive index detection provided the primary data channel, which was supplemented by data from the UV channel on tartaric and malic acids. Those two compounds often are masked by higher concentrations of sugars, which have no or low UV absorbance, in the refractive index channel.

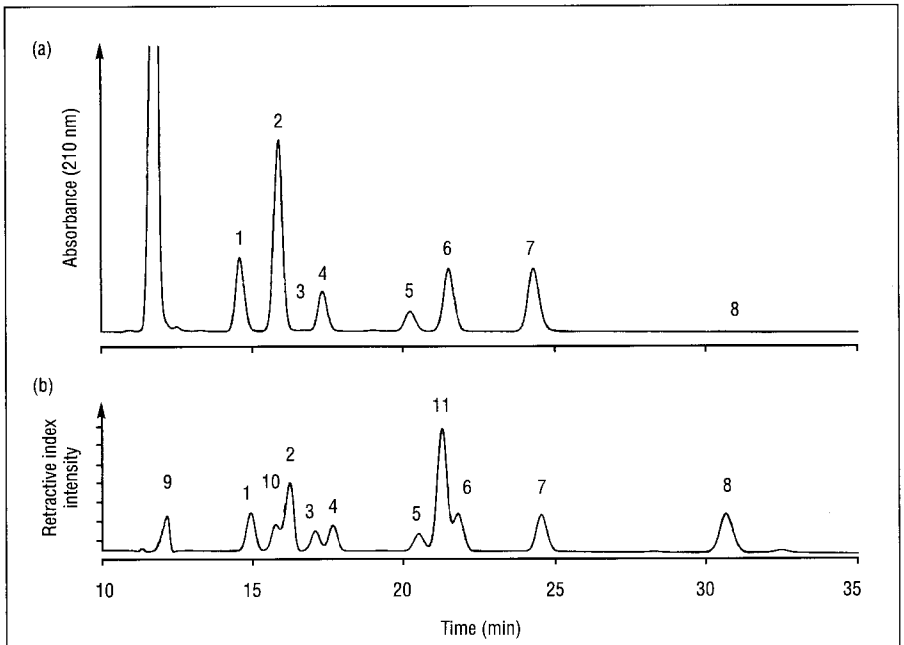

FIGURE 4: Separation of a mixture of 10 key wine analytes and oxalic and shikimic acids using two Polypore H resin columns with dual (a) UV-absorbance and (b) refractive index detection. Other conditions are described in the experimental section. Peaks: 1 = citric acid, 2 = tartaric acid, 3 = fructose, 4 = malic acid, 5 = succinic acid, 6 = lactic acid, 7 = acetic acid, 8 = ethanol, 9 = oxalic acid, 10 = glucose, 11 = glycerol.

Figure 5 shows chromatograms from the analysis of a grape must and a white wine sample (refractive index detection) with insets of the UV channel that reveal the presence of tartaric, malic, shikimic, and lactic acids. This HPLC methodology requires 40 min per sample, and is useful for quality control analyses of wines and grape must samples. Table III summarizes the quantitative data of major organic acids, sugars, glycerol, and ethanol in must, wine, and vinegar samples. The method provided less than 1% RSD precision for retention time and peak area, 10–50 ng detection limits for most analytes when using refractive index detection, and a 0.1–100 μg linearity range for all organic acids. Although a more elaborate LC system with a column oven, dual detectors, and two resin columns is necessary, this method determined all key analytes in wine samples in one assay with little sample preparation.

CONCLUSIONS

The family of very water-soluble organic acids provides some unique separation challenges for chromatographers. The resin columns for organic acid analysis are easy to use and yield exceptionally reproducible retention data in both run-to-run and column-to-column terms. Compared with silica-based columns, resin columns are more expensive and have lower efficiencies and pressure ratings. Although they are perfectly adequate for many sample types, their application in juice analysis is unsatisfactory because of the coelution of several key analytes. However, the unique ability of resin columns to separate sugars and alcohols makes them the ideal columns for monitoring fermentation products during wine making.

In contrast, reversed-phase columns provide higher efficiencies and selectivities, so they clearly are preferable for juice analysis. Because most organic acids are eluted with low capacity factors in both column types (even with 100% aqueous mobile phase), the options for increasing resolution are limited to increasing column length and varying column temperatures or the acid concentrations of mobile phases (10).

This article has documented the use of resin and reversed-phase columns in organic acid analysis. It also described two HPLC methods for quality control analysis of juice and wine products.

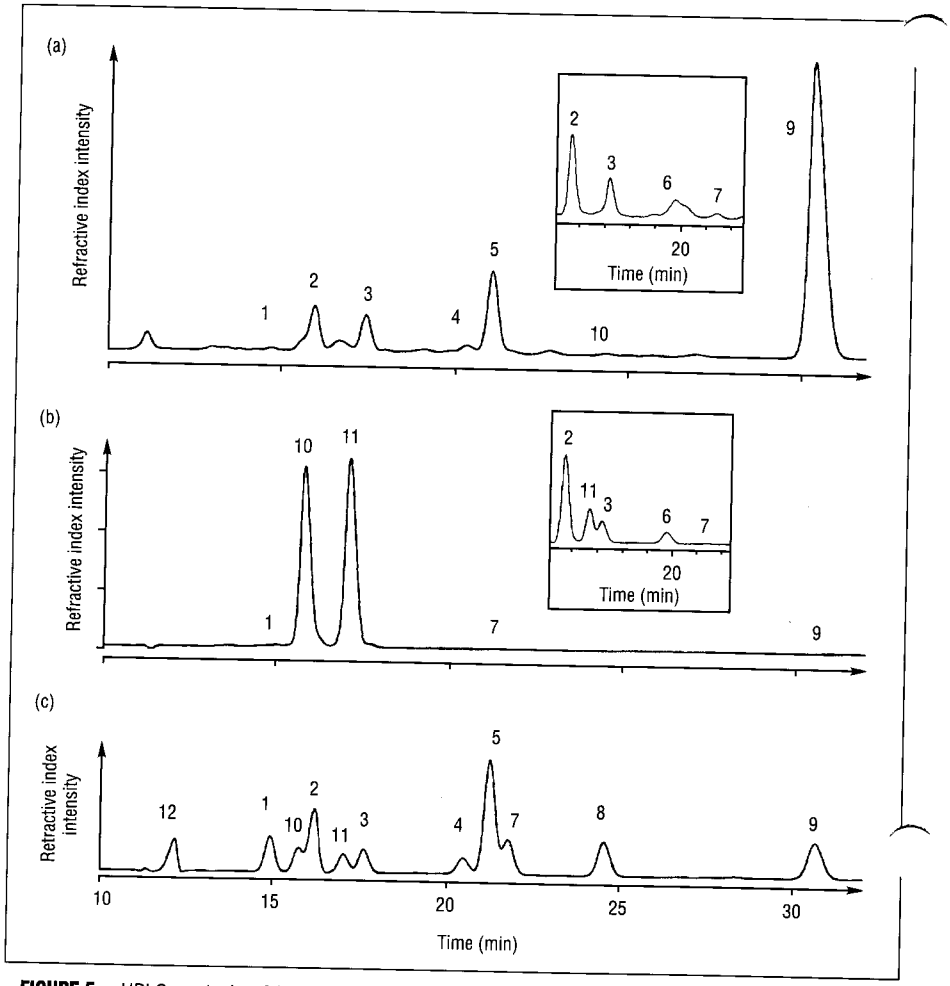

FIGURE 5: HPLC analysis of (a) white wine, (b) grape must, and (c) a standard mixture with refractive index detection. The inset chromatogram segments were generated with UV-absorbance detection. Other conditions are described in the text. Peaks: 1 = citric acid, 2 = tartaric acid, 3 = malic acid, 4 = succinic acid, 5 = glycerol, 6 = shikimic acid, 7 = lactic acid, 8 = acetic acid, 9 = ethanol, 10 = glucose, 11 = fructose, 12 = oxalic acid.

TABLE III: Quantitative Data of Analytes in Grape Must, Wine, and Vinegar Samples Using Two Resin Columns and Refractive Index–UV Detection*

Analyte	Grape Must (mg/mL)	White Wine (mg/mL)	Red Wine (mg/mL)	Dry Sherry (mg/mL)	Red Wine Vinegar (mg/mL)
Tartaric acid†	3.1	1.8	1.6	0.5	0.2
Malic acid†	1.3	1.5	0.15	1.0	—
Shikimic acid†	0.05	0.05	0.1	0.1	—
Lactic acid	0.2	0.8	2.0	0.4	28
Acetic acid	0.8	0.08	0.3	0.1	30
Citric acid	0.2	0.2	0.1	0.1	1.0
Succinic acid	—	0.1	0.4	0.2	—
Glucose	64	0.3	0.6	6.0	13
Fructose	78	—	1.5	8.4	—
Glycerol	—	6.9	9.1	6.4	3.5
Ethanol	—	70	103	137	0.7

* — = not present or nondetectable.
† Quantitated using data from the UV channel.

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