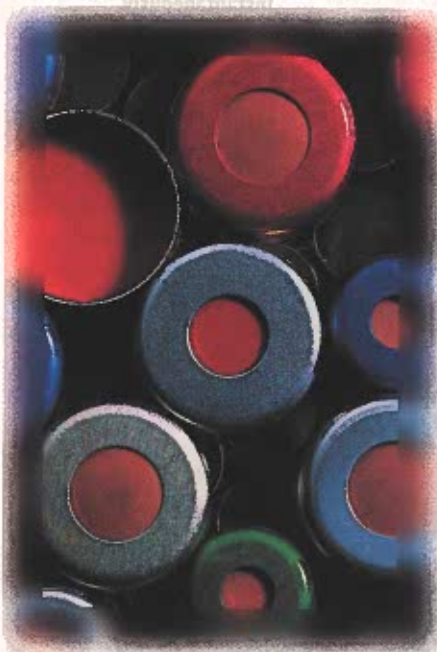


Indirect Suppressed-Conductivity Ion Chromatography



The authors demonstrate a new detection method, indirect suppressed conductivity, for various anions of weak acids separated by ion chromatography. They analyzed three groups of anions, including amino acids such as leucine, taurine, phenylpropionic acid, and phenol; malic and tartaric acids; and lactic, formic, and benzoic acids. The authors describe the mobile phases, detection limits, and correlation coefficient of rectilinear relationships for each anion type.

The first reported example of indirect detection involved ion chromatography in which the eluted ion was the chromophore being monitored by photometry, and the technique was called *indirect photometric chromatography* (1). Indirect detection has been widely used in UV (2–4), fluorescence (5,6), and amperometric techniques (7). The technique converts the above detectors from sample-specific types that monitor a characteristic of the analyte, which normally is absent from the eluent, into something closer to a bulk-property detector, similar to a conductivity detector (8,9), which monitors the change caused by the analyte in a property of the eluent.

Single-column ion chromatography using nonsuppressed-conductivity detection to analyze anions typically uses eluents comprising aromatic acid buffers; for example, phthalate and *p*-hydroxybenzoate (10,11). The increase in conductivity caused by the replacement of these low-conductance species by high-conductance analytes such as chloride, nitrate, or sulfate allows analytes to be measured as positive peaks.

Single-column ion chromatography of cations typically uses eluents comprising strong acids such as nitric acid (11). The decrease in conductivity caused by replacement of the high-conductance hydronium by lower-conductance analytes such as sodi-

um and calcium allows the analytes to be measured as negative peaks.

Suppressed-conductivity detection typically converts all eluent ions to low-conductance species before detection (12). The increase in conductance caused by replacement of these ions with high-conductance analyte ions allows analytes to be measured as positive peaks, regardless of whether they are anions or cations. A major difficulty with suppressed-conductivity detection arises from the fact that weakly ionized analytes are themselves converted to low-conductance species in the course of the suppression process, so they are not well detected (13,14).

To overcome this disadvantage, we developed the following methodology, which uses chemical separation to convert eluent ions to high-conductance species before conductivity detection. The low-conductance analytes then can be readily detected as negative peaks. By analogy with indirect-UV detection, we have chosen to call this technique *indirect suppressed-conductivity* detection. It can be used to detect weak ionic species, and it isn't disturbed by pH.

Experimental

Reagents: All reagents were of analytical grade. All solutions, including the eluents, standard solution, and sample solution, were prepared with distilled water.

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Apparatus: The experimental apparatus we used in these experiments was a model 100T ion chromatograph equipped with a conductivity detector, an Ionpac AG4SC guard column, an AS4SC separating column, and a self-regeneration suppressor (all from Dionex Corp., Sunnyvale, California). Data were obtained using Yinpu chromatographic data station software (Hangzhou, China) installed on a personal computer.

Conditions: For the determination of amino acids, we used 0.75 mM potassium chloride and sodium hydroxide (pH 11.5) as the eluent. For the determination of malic and tartaric acids, we used 0.75 mM sodium sulfate and sodium hydroxide (pH 11.5) as the eluent. For the determination of benzoic and lactic acids, we used 2.0 mM potassium nitrate as the eluent. The flow rate and injection volume for all determinations were 2.0 mL/min and 50 μ L, respectively. The detector time for all determinations was 100 μ s.

Procedure: We filtered samples of a soft drink through a 0.45- μ m filter and diluted them by factor of 10.

Results and Discussion

Determination of lactic, formic, and benzoic acids: *Chromatography condition optimization:* Because formic, lactic, and benzoic acids are weak monoacids with weak retention, we examined potassium chloride and potassium nitrate eluents. We used a 2.0-mL/min flow rate for 2.0 mM potassium nitrate and obtained good resolution and short retention times. Figure 1 shows an example of a chromatogram obtained for the standard stock solution.

Repeatability: The retention times for lactic, formic, and benzoic acids were 3.12, 3.60, and 9.60 min, respectively.

Lactic acid (50 μ g/mL), formic acid (20 μ g/mL), and benzoic acid (50 μ g/mL) were analyzed for repeatability. The peak area relative standard deviations (RSD) of the analytical data for seven injections were 2.2, 0.96, and 2.8%, respectively for lactic, formic, and benzoic acids. The peak height RSDs of the analytical data for seven injections were 1.2, 0.82, and 3.1%, respectively for lactic, formic, and benzoic acids.

The resolution of lactic acid and formic acid was 1.40, and the resolution of formic acid and benzoic acid was 13.44.

Linear ranges and detection limits: The injection solution concentrations of lactic, formic, and benzoic acids were 0–200, 0–80, and 0–200 μ g/mL, respectively. The linear regression equation for lactic acid was

$$H = 6.75 \times 10^4 x + 1.51 \times 10^4 \quad [1]$$

The linear regression equation for formic acid was

$$H = 6.89 \times 10^4 x + 9.65 \times 10^4 \quad [2]$$

The linear regression equation for benzoic acid was

$$H = 5.93 \times 10^4 x + 9.72 \times 10^4 \quad [3]$$

where H is peak height and x is the concentration in micrograms per milliliter. The correlation coefficients (r) for lactic, formic, and benzoic acids were 0.9999, 1.0000, and 0.9991, respectively.

The detection limits for lactic, formic, and benzoic acids were 0.4, 0.16, and 0.4 μ g/mL, respectively.

Interference: Anions such as SO_4^{2-} , Cl^- , and PO_4^{3-} didn't interfere with the detec-

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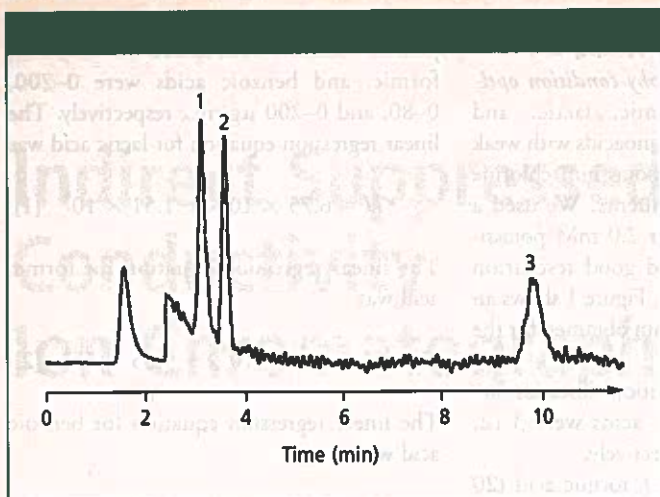


Figure 1: Typical chromatogram from an ion chromatography analysis of a standard stock solution. Peaks: 1 = lactic acid (50 $\mu\text{g/mL}$), 2 = formic acid (20 $\mu\text{g/mL}$), 3 = benzoic acid (50 $\mu\text{g/mL}$).

tion because of the high-conductive-value background. The results showed that the detection of 50- $\mu\text{g/mL}$ lactic acid, 20- $\mu\text{g/mL}$ formic acid, and 50- $\mu\text{g/mL}$ benzoic acid was undisturbed by the addition of 400 mg/L of SO_4^{2-} , F^- , Cl^- , Br^- , NO_2^- , NO_3^- , or PO_4^{3-} .

Sample determination: Samples of soft drinks were filtered through a 0.45- μm filter and diluted by a factor of 10. Table I and Figure 2 show results of the determination.

Determination of amino acids: *Chromatography condition optimization:* Because amino acids such as leucine, taurine, phenylpropionic acid, and phenol are weak

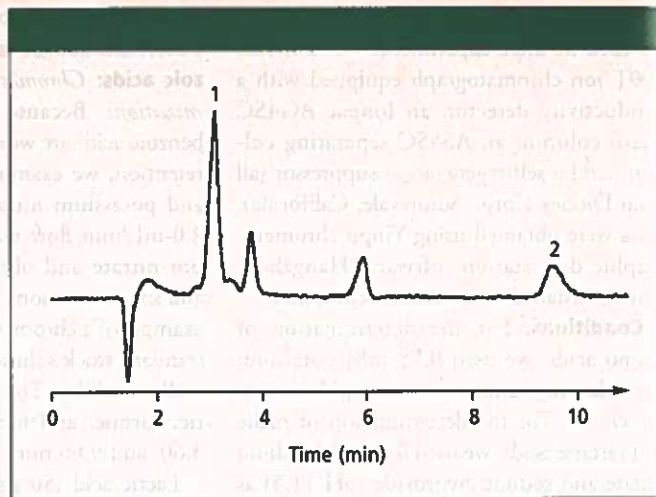
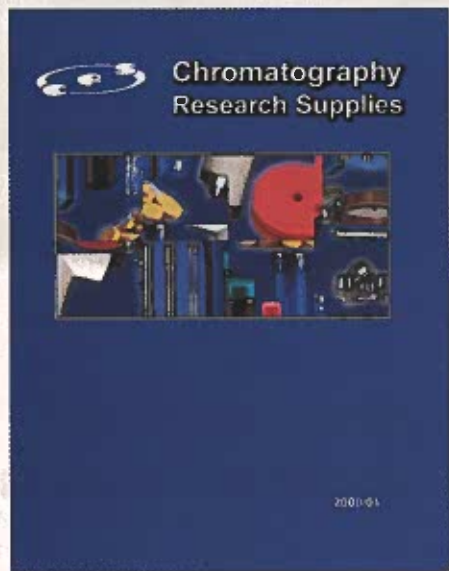


Figure 2: Ion chromatography analysis of lactic and benzoic acids in a soft drink. Peaks: 1 = lactic acid, 2 = benzoic acid.

monoacids and are retained weakly, we examined the use of potassium chloride and potassium nitrate eluents and found that we needed high pH values for ionizing amino acids. We used 0.75 mM potassium chloride and sodium hydroxide (pH 11.5) at a flow rate of 2.0 mL/min and obtained good resolution and a short retention time.

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Table 1: Monoacid results

Species	Concentration (mg/L)	RSD (%)*	Recovery (%)
Lactic acid	67.3	3.5	101.4
Benzoic acid	20.9	3.3	97.1

* n = 7.

Figure 3 shows a chromatogram from an analysis of the standard stock solution.

Repeatability: Retention times for leucine, taurine, phenylpropionic acid, and phenol were 1.52, 1.82, 2.12, and 3.87 min, respectively.

Leucine (50 µg/mL), taurine (50 µg/mL), phenylpropionic acid (50 µg/mL), and phenol (50 µg/mL) were analyzed for repeatability. The peak area RSDs of the analytical data for seven injections were 2.0, 2.8, 2.1, and 2.6%, respectively for leucine, taurine, phenylpropionic acid, and phenol. The peak height RSDs of the analytical data for seven injections were 1.2, 1.3, 1.1, and 2.4%, respectively for leucine, taurine, phenylpropionic acid, and phenol.

The resolutions of leucine and taurine, taurine and phenylpropionic acid, phenyl-

propionic acid and phenol were 1.41, 1.62, and 5.30, respectively.

Linear ranges and detection limits: The injection solution concentrations for leucine, taurine, phenylpropionic acid, and phenol were 0–200, 0–200, 0–100, and 0–100 µg/mL. The linear regression equation for leucine was

$$H = 2.31 \times 10^4 x + 6.82 \times 10^4 \quad [4]$$

The linear regression equation for taurine was

$$H = 2.91 \times 10^4 x - 3.20 \times 10^4 \quad [5]$$

The linear regression equation for phenylpropionic acid was

$$H = 2.75 \times 10^4 x + 1.95 \times 10^4 \quad [6]$$

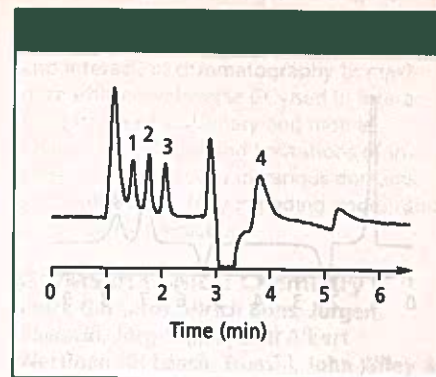


Figure 3: Typical chromatogram from a standard amino acid separation. Peaks: 1 = leucine (50 µg/mL), 2 = taurine (50 µg/mL), 3 = phenylpropionic acid (150 µg/mL), 4 = phenol (50 µg/mL).

The linear regression equation for phenol was

$$H = 4.96 \times 10^4 x - 5.18 \times 10^4 \quad [7]$$

The correlation coefficients for leucine, taurine, phenylpropionic acid, and phenol were 0.9997, 1.0000, 1.000, and 0.9993, respectively.

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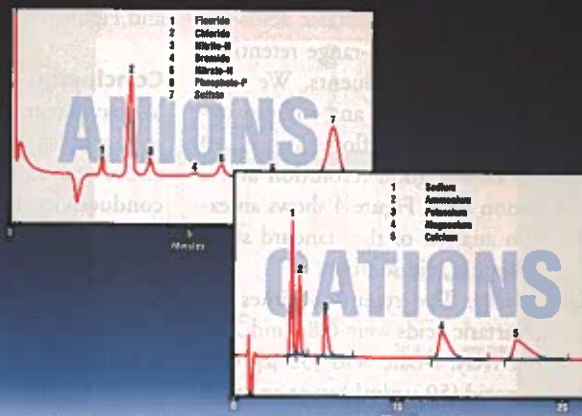
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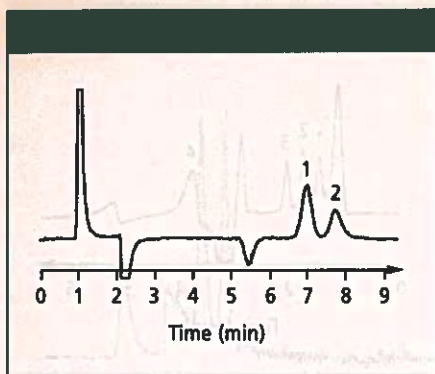


Figure 4: Ion chromatography analysis of a standard stock malic and tartaric acid solution. Peaks: 1 = malic acid (50 $\mu\text{g/mL}$), 2 = tartaric acid (50 $\mu\text{g/mL}$).

The detection limits for leucine, taurine, phenylpropionic acid, and phenol were 0.38, 0.53, 8.3, and 0.76 $\mu\text{g/mL}$, respectively.

Interference: Anions such as SO_4^{2-} , Cl^- , and PO_4^{3-} didn't interfere with the detection because of the high-conductive-value background. As a result, the detection of 50 $\mu\text{g/mL}$ each of leucine, taurine, phenylpropionic acid, and phenol was undisturbed by the addition of 400 mg/L of SO_4^{2-} , F^- , Cl^- , Br^- , NO_2^- , NO_3^- , or PO_4^{3-} .

Determination of malic and tartaric acids: *Chromatography condition optimization:* Because malic and tartaric acids are weak diacids with middle-range retention, we examined different eluents. We used 0.75 mM sodium sulfate and sodium hydroxide (pH 11.5) at a flow rate of 2.0 mL/min to obtain good resolution and a short retention time. Figure 4 shows an example of an analysis of the standard stock malic and tartaric acid solution.

Repeatability: The retention times for malic and tartaric acids were 6.89 and 7.62 min, respectively. Malic acid (50 $\mu\text{g/mL}$) and tartaric acid (50 $\mu\text{g/mL}$) were analyzed for repeatability. The peak area RSDs of the analytical data for seven injections of malic and tartaric acids were 1.46 and 2.99%, respectively. The peak height RSDs of the analytical data for seven injections were 0.57 and 1.60% for malic and tartaric acids, respectively. The resolution of malic acid and tartaric acid was 1.41.

Linear ranges and detection limits: The injection solution concentration for malic acid and tartaric acid was 0–200 $\mu\text{g/mL}$. The linear regression equation for malic acid was

Table II: Diacid results

Species	Concentration (mg/L)	RSD (%) ^a	Recovery (%)
Lactic acid	921.5	0.62	99.8

* n = 7.

$$H = 4.97 \times 10^4 x + 8.56 \times 10^3 \quad [8]$$

The linear regression equation for tartaric acid was

$$H = 2.74 \times 10^3 x + 1.07 \times 10^3 \quad [9]$$

The correlation coefficients for malic and tartaric acids were 0.9998 and 0.9999, respectively.

The detection limits for malic and tartaric acids were 1.31 and 3.04 $\mu\text{g/mL}$, respectively.

Interference: As discussed above for the monoacids, anions such as SO_4^{2-} , Cl^- , and PO_4^{3-} didn't interfere with the detection because of the high-conductive-value background. The results showed that the detection of 50- $\mu\text{g/mL}$ malic acid and 50- $\mu\text{g/mL}$ tartaric acid was undisturbed by the addition of 400 mg/L of SO_4^{2-} , F^- , Cl^- , Br^- , NO_2^- , NO_3^- , or PO_4^{3-} .

Sample determination: Samples of soft drinks were filtered through a 0.45- μm filter and diluted by a factor of 10. Table II and Figure 5 show results from the analysis.

Conclusion

We have demonstrated the determination of weak anions, including mono- and dianions and amino acids, using indirect suppressed-conductivity ion chromatography. Compared with other indirect conductivity ion chromatography methods, indirect suppressed-conductivity detection has some advantages:

- Indirect suppressed-conductivity detection provides a larger conductive difference between eluent ions and analyte anions, so it is more sensitive for determinations.
- The detection technique was undisturbed by pH changes, so it can be used more widely.
- Ionizing substances such as SO_4^{2-} , Cl^- , and PO_4^{3-} didn't interfere with the detection because of the high-conductive-value background.

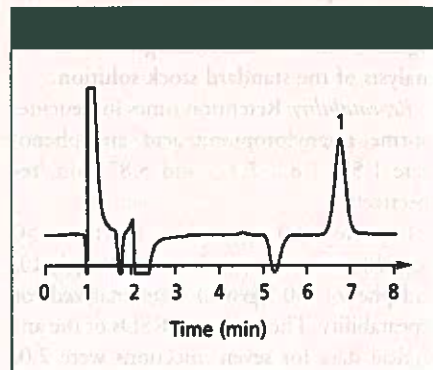


Figure 5: Ion chromatography analysis of a soft drink. Peak: 1 = malic acid.

Acknowledgment

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