

## Ion chromatography

# Citric acid determination in mixed beer beverages

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**Goal**

To develop a fast isocratic method to determine citric acid in carbonated beverages with a carbonate-based eluent and electrolytically regenerated suppression

**Introduction**

Citric acid, a trivalent carboxylic acid, and its respective sodium, potassium, and calcium salts are among the most commonly used food preservatives, especially in beverages. Typically they are used for inhibiting yeast and mold growth and are effective against a wide range of bacteria.

The CODEX Alimentarius lists citric acid as an acidity regulator, antioxidant, color retention agent, and sequestrant. The maximum advised concentrations are given below. These values are subject to the national legislation of the importing country:

- 3,000 mg/kg for fruit juice, concentrates for fruit juice
- 5,000 mg/kg for complementary foods for infants and young children, fruit nectar, and concentrates for fruit nectar.

Citric acid may be used in beer, malt beverages, and aromatized alcoholic beverages (e.g., beer, wine, spirituous cooler-type beverages, low alcoholic refreshers) under the conditions of good manufacturing practice (GMP) as outlined in the preamble of the Codex General Standard for Food Additives (GSFA).<sup>1</sup>

Mixed beer and lemonade beverages are complex mixtures containing various substances such as coloring compounds, flavoring agents, acidulants (e.g., citric acid), sweeteners, and preservatives. Acidulants are important in modern fruit drink processing, used for flavoring and preservation. Citric acid's ability to chelate trace metals improves the effectiveness of antioxidants in preserving the beverage. Longer-term storage can be achieved by combining citric acid with sodium citrate to precisely control the pH, stabilizing the product.<sup>2</sup>

The following exemplary food regulations describe citric acid as an additive for beer and related products:

- European Union: Citric acid (E330) is a permitted additive for beer and malt beverages under Regulation (EC) 1333/08 and its amendment.<sup>3,4</sup> The specific maximum level of the additive is being set to "quantum satis", i.e., no maximum quantity is given. However, under GMP, the substances shall be used only in the amount necessary to achieve the desired effect.
- USA: The US Code of Federal Regulations Chapter 21, Part 184, Subpart B, affirms the Generally Recognized as Safe (GRAS) status of citric acid and approves its use in all foods on a GMP basis.<sup>5</sup> There is no limitation on using such products specified in the beer standard (27 CFR Part 25).<sup>6</sup>
- Australia and New Zealand: In 2015, Food Standards Australia New Zealand (FSANZ) permitted citric acid as a food additive in beer, lower strength, and flavored beer styles. FSANZ's risk assessment concluded that there is no risk to health and safety from its use under GMP and maximum levels for citric acid are not necessary for these products.<sup>7</sup>

Even if the addition of citric acid is considered harmless, legislation in some countries recommends mentioning it in the ingredients list.<sup>8</sup> Therefore, in conjunction with the necessity to control product quality and the production process, there is a need for an analytical tool to quantify citric acid in complex beverages.

Different analytical approaches are available to determine high concentrations of citric acid in beverages: high-performance liquid chromatography (HPLC) with UV-detection, gas chromatography (GC) after derivatization, ion-exclusion chromatography (ICE) with refractive index, UV- and conductivity detection, and anion-exchange chromatography with suppressed conductivity detection. In Thermo Scientific Application Note 169 and Application Note 72501, the authors describe a rapid anion-exchange determination of citrate and phosphate in carbonated soft drinks utilizing a potassium hydroxide eluent.<sup>9,10</sup> They used a Thermo Scientific™ Dionex™ Reagent-Free™ IC (RFIC™) system, with eluent generation and electrolytically continuously regenerated suppressors for conductivity detection and a dedicated anion-exchange column. The analytical run times were 5 and 8 min, respectively, matching the analytical requirements for high sample throughput in a manufacturing environment.

These applications are considered a gold standard in the beverage industry as the RFIC-technology ensures maximum reliability and the highest level of automation.

However, an IC method using a carbonate-based eluent would be desirable for some laboratories to ensure compatibility with existing applications. A high sample throughput was not the focus; less than 15 min run times were acceptable, and the instrument would need to operate for a longer time with a given eluent supply, thus reducing the cost of operation.

This application describes the use of the Thermo Scientific™ Dionex™ IonPac™ AS29-Fast-4µm column in 2 mm format under isocratic conditions with a sodium carbonate eluent. Due to the smaller column inner diameter, the flow rate was four times lower than with a comparable 4 mm column, resulting in a four-fold increase in instrument availability for a given eluent supply. Citric acid was detected with suppressed conductivity detection using a Thermo Scientific™ Dionex™ AERS™ 500 Carbonate suppressor in the electrolytic AutoSuppression Recycle Mode. Instrument control, digital data acquisition, and evaluation were performed with the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS).

## Experimental

### Equipment

- Thermo Scientific™ Dionex™ Aquion™ Ion Chromatography System with degas ([P/N 22176-60004](#))
- Thermo Scientific™ Dionex™ Aquion™ Column Heater ([P/N 070063](#))
- Thermo Scientific™ Dionex™ AS-DV for 5.0 mL vials ([P/N 068907](#))

\* This application can be run on any Thermo Scientific™ Dionex™ IC system equipped with suppressed conductivity detection. The carbonate eluent (see Method) can be generated electrolytically using Thermo Scientific™ Dionex™ RFIC™ technology.

### Consumables

- Dionex IonPac AS29-Fast-4µm (2 × 150 mm) ([P/N 302835](#))
- Thermo Scientific™ Dionex™ IonPac™ AG29-Fast-4µm (2 × 30 mm) ([P/N 302836](#))
- Dionex AERS 500 Carbonate Suppressor (2 mm) ([P/N 085028](#))
- Thermo Scientific™ Dionex™ PolyVials and Filter Caps (20 µm), 250 each ([P/N 038141](#))

### Software

- Chromeleon CDS, version 7.2.10 or above ([P/N CHROMELEON7](#))

## Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 M $\Omega$ -cm resistivity or better
- Citric acid, 99%, Thermo Scientific™ (P/N A10395.30)
- Sodium carbonate, 99.5%, extra pure, anhydrous, Thermo Scientific™ (P/N 123670025)

A stock solution for citric acid (1 g/L) was prepared by dissolving the starting reagent with DI water. The working standards were made by dilution of the stock solution. The stock solutions can be kept frozen for several weeks, while the working standards should preferably be prepared every working day.

A hundred-fold concentrated eluent stock solution (1.25 M) was prepared by dissolving 13.25  $\pm$  0.05 g Na<sub>2</sub>CO<sub>3</sub> in DI water and diluting it to a final volume of 100 mL. Dilute 10 mL of this stock solution to a final volume of 1 L with degassed DI water (use either vacuum or ultrasonic degassing). The final solution has a concentration of 12.5 mM.

When handling the starting chemicals and preparing the working standards and eluent, wear personal protective gear, gloves, and safety goggles. The work should be carried out under a fume hood.

## Samples

The three samples investigated were purchased in a local grocery store.

## Sample preparation

The carbonated beverages were degassed for a minimum of 10 min using ultrasonication, then diluted with DI water (1 mL sample to a final volume of 100 mL).

## Method

Parameter	Value
Column	Dionex IonPac AG29-Fast-4 $\mu$ m (2 $\times$ 30 mm) (P/N 302836) and Dionex IonPac AS29-Fast-4 $\mu$ m (2 $\times$ 150 mm) (P/N 302835)
Eluent	12.5 mM Na <sub>2</sub> CO <sub>3</sub>
Flow rate	0.25 mL/min
Injection volume	25 $\mu$ L
Temperature	30 $^{\circ}$ C (column heater), 35 $^{\circ}$ C (detector cell)
System backpressure	~1,800 psi (100 psi = 0.6894 MPa)
Detection	Suppressed conductivity, Dionex AERS 500 Carbonate (2 mm), recycle mode (P/N 085028), regeneration current: 21 mA
Background conductivity	<31 $\mu$ S/cm
Run time	15 min
Sampler delivery speed	4 mL/min; sample volume delivered 750 $\mu$ L

## Results and discussion

### Determination of limit of detection (LOD) and limit of quantification (LOQ)

The determination of LOD and LOQ was based on the signal-to-noise ratio (S/N). It is usually obtained by comparing the measured signal from a standard with a low analyte concentration with a blank and establishing the minimum concentration at which the analyte can be reliably detected.<sup>11,12</sup> A S/N=3 is used to estimate LOD and a S/N=10 for the quantification limit (LOQ).<sup>13</sup> A similar approach determines the noise in a segment of a reference chromatogram's baseline close to the peak of interest where no peaks elute over a distance equal to at least five times the width at half-height of the reference peak. This approach is automated and objectified in Chromeleon CDS, and considering the isocratic elution in this work, the time segment (6–11 min) was used for the evaluations. The calculations in Chromeleon CDS follow the European and US Pharmacopeia definition of the signal-to-noise ratio, where half of the noise amplitude is taken into account:<sup>11,12</sup>

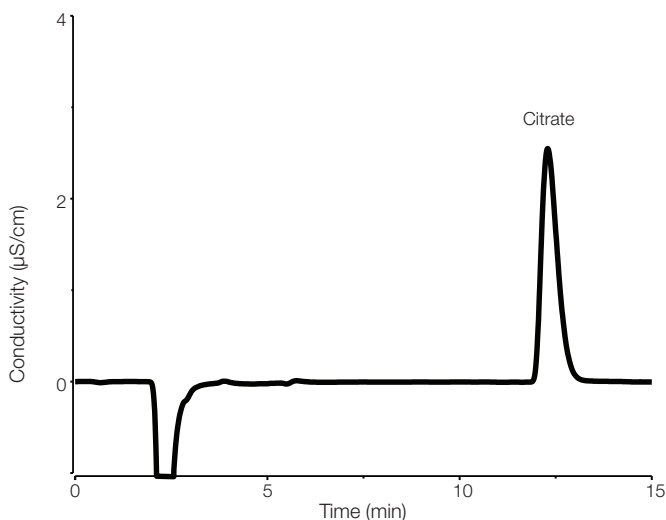
$$\frac{S}{N} = 2 \times \frac{\text{Peak height}}{\text{Noise}}$$

#### Equation 1. Calculation of the signal-to-noise ratio in Chromeleon CDS

In Chromeleon CDS, we added two report columns to the “Peak Results” tab of the “Interactive Reports”, one for the LOD calculation and one for the LOQ calculation to avoid data export to external spreadsheet programs and transcription errors. LOD and LOQ for citric acid were estimated using a 2 mg/L reference solution. The LOD was 0.03 mg/L, and LOQ resulted in 0.09 mg/L, based on a baseline noise of 0.0045  $\mu$ S/cm and a peak height of 0.5054  $\mu$ S/cm.

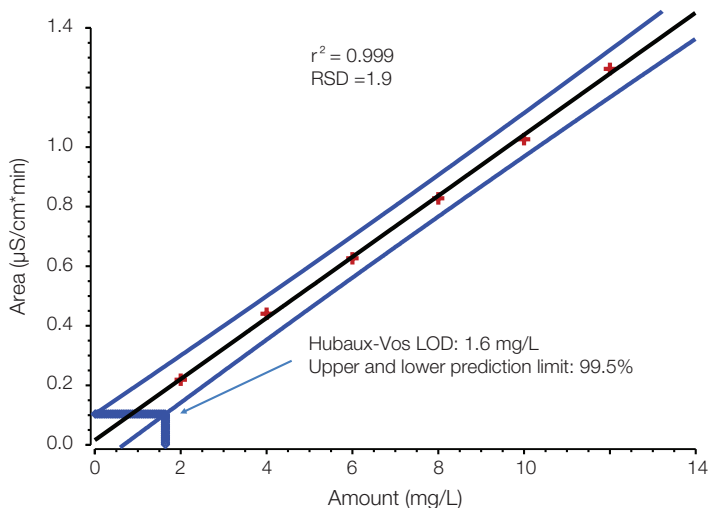
### Calibration and evaluation

Figure 1 shows the isocratic separation of citric acid, a trivalent carboxylic acid, using a carbonate eluent in less than 15 min. The short retention time was achieved using an eluent of 12.5 mM Na<sub>2</sub>CO<sub>3</sub> at a 0.25 mL/min flow rate. The high dynamic exchange capacity of the continuously electrolytically regenerated membrane-based suppressor (Dionex AERS 500 Carbonate) allows the uninterrupted operation of the instrument without external regeneration or external valves or switching operations. The suppressor is used indiscriminately for standards and samples, ensuring complete traceability of the analytical results, which is in high demand in the analytical industry.<sup>14</sup>



**Figure 1. Isocratic separation of citric acid using anion-exchange chromatography.** Concentration: 12 mg/L. Column: Dionex IonPac AS29-Fast-4µm (2 mm), eluent: 12.5 mmol/L Na<sub>2</sub>CO<sub>3</sub>, flow rate: 0.25 mL/min, inject. vol.: 25 µL, detection: suppressed conductivity (Dionex AERS 500 Carbonate, 2 mm).

Figure 2 shows the calibration used to quantify citric acid in the mixed beer beverages. In contrast to conventional calibration plots, where the upper and lower confidence intervals are shown, we decided to show the upper and lower prediction intervals, to access the so-called Hubaux-Vos LOD,<sup>15</sup> another Chromeleon CDS option. This approach allows the quantitative estimation of the standards' influence on the sensitivity. The LODs determined via the S/N-ratio (0.03 mg/L) and Hubaux-Vos approach (1.6 mg/L) are different, and the decision of which type of evaluation to use depends on the individual laboratories' requirements.

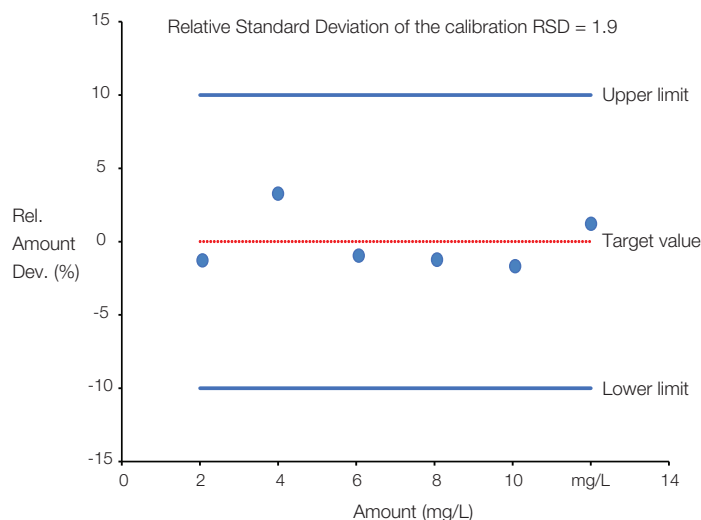


**Figure 2. Calibration plot and data for citric acid.** Calibrated range: 2 to 12 mg/L, evaluation: linear with offset, Hubaux-Vos calculation using an integrated Chromeleon CDS option

The calibration data are summarized in Table 1. The relative amount deviations for the calibration points were plotted against the calibrated amount (Figure 3). No trend was observed, and the resulting RSD of the calibration was 1.9.

**Table 1. Calibration data for citric acid**

Peak name	Ret. time min	Cal. type	Eval. type	Number of points	Rel. std. dev.	Coeff. of determination
Citric acid	12.3	Lin, WithOffset	Area	6	1.9	0.999

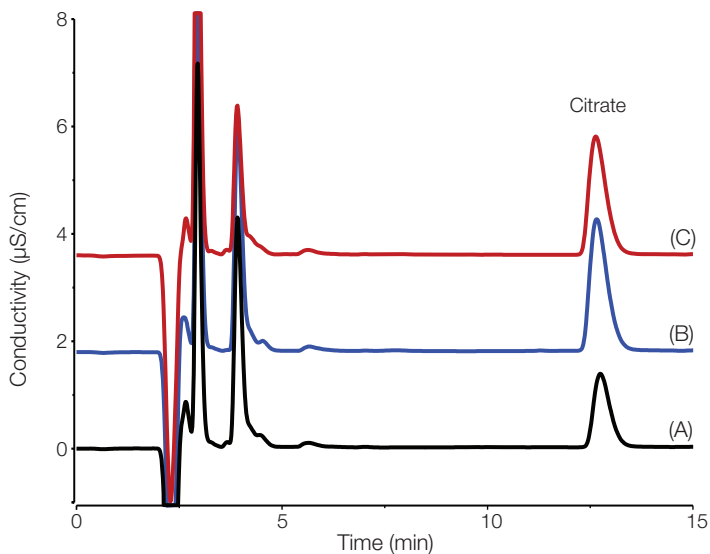


**Figure 3. Relative amount deviation versus the calibrated amount**

### Sample measurement

Three representative chromatograms of local beer with citrus lemonade mixed beverages are shown in Figure 4. The citric acid concentrations were between 0.61 g/L to 1.18 g/L, with RSDs of around 0.3 (n = 6).

Typical lemonade addition to beer ranges from 40% to 60%. Some mixed beer beverages listed citric acid as an acidulant, so the effective citric acid concentration depends on the mixing ratio of beer to lemonade and the citric acid addition.



**Figure 4. Citric acid determination in mixed beer beverages.** Conditions: see Method and Sample preparation; citric acid concentration in sample (A): 0.61 g/L, sample (B): 1.18 g/L, and sample (C): 1.05 g/L

The target concentrations are relatively high, and the degassed samples were diluted one-hundred-fold. The initial requirements for this application demanded the use of a 25 µL injection loop. If the offline sample dilution should be avoided, a different autosampler (Thermo Scientific™ Dionex™ AS-AP) allows automated dilution and partial loop injection.<sup>16,17</sup> Alternatively, a second smaller sample injection loop, automatically selected by Chromeleon CDS, could be used.<sup>18</sup>

The chromatogram run time is 15 min and could be reduced by increasing the analytical flow rate. In this case, it might become necessary to replace the Dionex AERS 500 Carbonate suppressor with a Thermo Scientific™ Dionex™ ADRS 600 suppressor to accommodate the higher regeneration current requirements.

## Conclusions

Citric acid can be determined in mixed beer beverages under isocratic conditions with a 12.5 mM sodium carbonate eluent in less than 15 min. It was separated on a modern polymeric anion exchanger, a Dionex IonPac AS29-Fast-4µm column, in the 2 mm format. Detection was carried out with suppressed conductivity by applying a continuously electrolytically regenerated Dionex AERS 500 Carbonate suppressor (2 mm). The suppressor's high dynamic exchange capacity enables the ion chromatograph's continuous operation. No external regenerants are required for the regeneration, and using a single suppressor ensures traceability of calibration and sample results, adding to the presented solution's ease of use. The instrument was externally calibrated linearly with a coefficient of determination of  $r^2 = 0.999$  and an RSD of 1.9. The degassed samples were diluted and directly injected. Citric acid concentrations were between 0.61 g/L to 1.18 g/L in the investigated samples.

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