Application Note 1076

Determination of Monochloroacetic Acid in Carbocisteine

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Keywords
Haloacetic Acid, Dionex IonPac AS11-HC Column, Ion Chromatography, Pharmaceutical Quality Control/Analysis

Goal
To develop a sensitive and simple ion chromatography (IC) method for the determination of monochloroacetic acid (MCAA) in carbocisteine, thus enabling efficient quality control of the carbocisteine manufacturing process

Introduction
Carbocisteine (S-carboxymethyl-L-cysteine) is a mucolytic drug that can interact with the bronchial epithelium to improve the breathing of patients with chronic bronchitis and bronchial asthma.[1,2] A condensation reaction of L-cysteine with MCAA is the typical method to produce carbocisteine (Figure 1). The substrate MCAA, which is cardio- and hematotoxic, is inevitably present in the carbocisteine product.[3] Therefore, the determination of MCAA is important for the quality control of carbocisteine production. The pharmacopeias of China and Europe recommend a colorimetric method to test the content of chloride in carbocisteine and regulate its content at less than 0.15%; however, the determination of MCAA is not mentioned.[4,5]

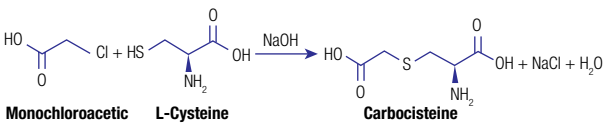

Figure 1. Reaction of L-cysteine with MCAA for the production of carbocisteine.

The commonly used methods to determine MCAA are gas chromatography (GC)[6,7] and IC.[3,8,9] Compared to the GC method that requires precolumn derivatization, the IC method is sensitive and simple, requiring only a direct injection.

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Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ ICS-2100 Integrated Reagent-Free™ IC (RFIC™) system,* including:
  - Isocratic Pump
  - Vacuum Degasser
  - EG Eluent Generator
  - Column Heater
  - High-Pressure 6-Port Injector
  - Conductivity Cell and CD Conductivity Detector
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC III Potassium Hydroxide (KOH) Eluent Generator Cartridge (P/N 074532)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.1
- Thermo Scientific™ Target2™ Nylon Syringe Filter, 0.45 µm, 30 mm (Fisher Scientific P/N F2500-1)

** This application can be run on any Dionex IC system that supports electrolytic eluent generation.*

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Sodium Hydroxide (NaOH) Solution, 50% (w/w/Certified) (Fisher Scientific P/N SS254-500)
- Sodium Chloroacetate, 98% (Fisher Scientific P/N 50-700-3414)

Conditions	
Columns:	Thermo Scientific™ Dionex™ IonPac™ AG11-HC Guard, 4 × 50 mm (P/N 052962) Dionex IonPac AS11-HC Analytical, 4 × 250 mm (P/N 052960)
Eluent:	10 mM KOH
Eluent Source:	Dionex EGC III KOH Eluent Generator Cartridge with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	1.0 mL/min
Inj. Volume:	25 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor,™ 4 mm (P/N 064554) in recycle mode, 31 mA
System Backpressure:	~2000 psi
Background Conductance:	<0.6 µS
Typical Noise:	<1.5 nS
Run Time:	16 min

These conditions apply to Figures 2 and 4.

Preparation of Solutions and Reagents

Working Standard Solutions for Calibration

Prepare a stock standard solution by weighing 125 mg of sodium chloroacetate and diluting to 100 mL in a volumetric flask with DI water. The concentration of the stock standard solution will be 1250 mg/L.

Prepare working standard solutions with concentrations of 0.08, 0.24, 0.48, 0.96, 3.2, and 8.0 mg/L for calibration by adding the proper amounts of stock standard solution and diluting with DI water.

Sample Preparation

The bulk drug, tablets, and oral liquid of carbocisteine were generously donated by a customer.

Preparation of the Drug Samples

Dissolve 0.1 g of carbocisteine bulk drug (i.e., the drug substance) and then dilute to 100 mL with DI water.

Add a ground carbocisteine tablet (labeled amount, 250 mg per tablet) to a 50 mL centrifuge tube, then add 25 mL 0.25% NaOH solution. After 10 min of vortex mixing, 20 min in an ultrasonic bath, and 10 min of centrifugation (setting = 3000 rpm), move the supernatant to a 250 mL volumetric flask and add DI water to the mark.

Add 5 mL carbocisteine oral solution (500 mg per 10 mL) to a 250 mL volumetric flask and add DI water to the mark.

The prepared sample solutions can be diluted with water, if necessary. Prior to injection, filter the solution through a Target2 nylon syringe filter.

Preparation of the Spiked Sample

Prepare a sample with an 800 mg/kg spike by adding 0.4 mL of 1000 mg/L MCAA standard solution to 0.50 g of carbocisteine bulk drug sample. The remainder of the procedure is the same as the preparation described above for the bulk drug sample.

Results and Discussion

Column Selection

The Dionex IonPac AS11-HC column—designed to resolve a large number of inorganic and organic acid anions including MCAA in a single run—has been successfully used for the determination of organic acids and inorganic anions in cranberry and bilberry extracts,[10] fruit juices,[11] and fermentation broths.[12] The –amine and –carboxyl groups contained within the carbocisteine molecule indicate that hydrophilic stationary phases are suitable for the separation of carbocisteine. Therefore, the Dionex IonPac AS11-HC column, which contains a hydrophilic stationary phase that has been used to determine a number of carboxylic acids, was selected for the determination of MCAA in the carbocisteine drug substance and drug products. Figure 2 shows that the Dionex IonPac AS11-HC column resolves the MCAA present in the carbocisteine drug substance from chloride and a peak eluting just before MCAA; the peak resolutions between MCAA and those two peaks both exceed 1.5.

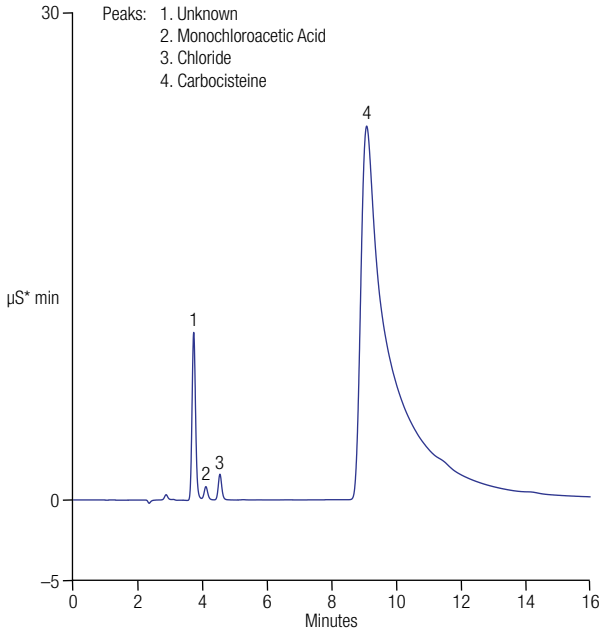

Figure 2. Monochloroacetic acid in 1.0 mg/L of carbocisteine bulk drug sample.

Reproducibility, Calibration, and Detection Limit

Seven consecutive 25 µL injections of 1.0 mg/L of a carbocistein bulk drug sample showed that the RSDs of MCAA retention time and peak area are 0.2 and 1.3, respectively. These results indicate that the method has good short-term reproducibility.

Calibration linearity was investigated by making 25 µL injections of MCAA at six different concentrations: 0.08, 0.24, 0.48, 0.96, 3.2, and 8.0 mg/L (five injections for each concentration). Linearity (Figure 3) was observed from 0.08 to 8.0 mg/L when plotting the concentration (C) versus the peak area (A), and the coefficient of determination was 0.9997. This calibration curve, $A = 0.0855\ C - 0.0048$, was used to quantify MCAA in carbocisteine drug substance and the carbocisteine drug products.

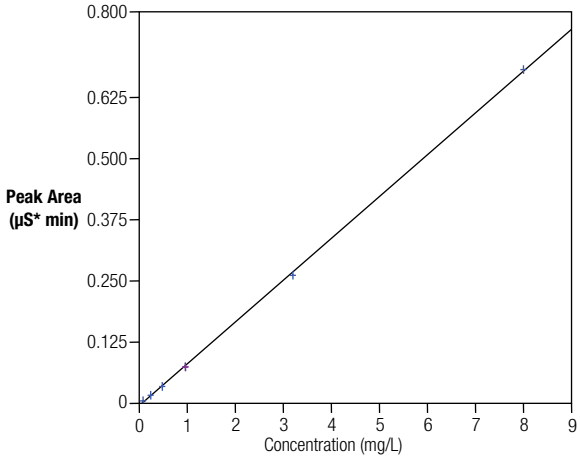

Figure 3. Calibration curve for MCAA.

Five replicate injections of a MCAA standard with a concentration of 0.08 mg/L were used for estimating method detection limit (MDL) using a signal-to-noise ratio (S/N) = 3. The average of calculated S/N was 16.2, and the MDL of MCAA was 0.015 mg/L.

Analysis of Carbocisteine Drug Samples

Figure 4 shows the chromatograms of a carbocisteine bulk drug sample, a 3.2 µg/mL MCAA standard, and the carbocisteine bulk drug sample spiked with MCAA. Monochloroacetic acid was well resolved from the other peaks in the sample. This was also true for the tablet and liquid samples (not shown). Table 1 summarizes the analysis results of carbocisteine as a bulk drug, tablet, and oral liquid. Monochloroacetic acid was detected in all the drug samples. To judge method accuracy, three injections of a carbocisteine bulk drug sample spiked with 0.8 mg/L of a MCAA standard were made; the average recovery was 95%.

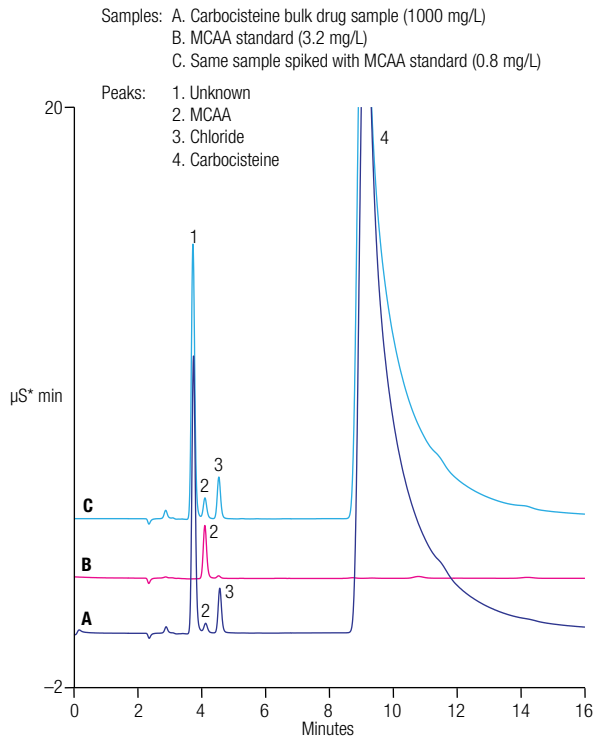

Figure 4. A carbocisteine bulk drug sample (A), MCAA standard (B), and the same sample spiked with MCAA (C).

Conclusion

This work describes a reproducible and accurate IC method for determining MCAA in carbocisteine drug substance and two drug products. This application is easily executed using a Dionex RFIC system controlled by Chromeleon CDS software. The analyst has simply to add water and samples to separate and accurately determine MCAA using the Dionex IonPac AS11-HC column.

References

1. Brockmoller, J.; Staffeldt, B.; Roots, I. Evaluation of Proposed Sulphoxidation Pathways of Carbocysteine in Man by HPLC Quantification. *Eur. J. Clin. Pharmacol.* **1991,** *40,* 387–392.
2. Walash, M.I.; El-Brashy, A.M.; Metwally, M.E.S.; Abdelal, A.A. Fluorimetric Determination of Carbocisteine and Ethionamide in Drug Formulation. *Acta Chim. Slov.* **2004,** *51* (2), 283–291.
3. Lan, X.Y. Determination of Monochloroacetic Acid in Carbocisteine with Ion Chromatography. *Chin. J. Ethnomedicine and Ethnopharmacy* **2011,** *22,* 23–24.
4. *Chinese Pharmacopoeia 2010, Part II: 1086–1088;* Pharmacopoeia Commission of the Ministry of Public Health: Beijing, China.
5. Monograph 0885. *European Pharmacopeia 52;* Strasbourg, France.
6. Sarrion, M.N.; Santos, F.J.; Galceran, M.T. In situ Derivatization/Solid-Phase Microextraction for the Determination of Haloacetic Acids in Water. *Anal. Chem.* **2000,** *72,* 4865–4873.
7. Aikawa, B.; Burk, R.C. Determination of Chlorinated Acetic Acids in Drinking Water by in situ Derivatization and Solid-Phase Microextraction. *International J. Environ. Anal. Chem.* **1997,** *66* (3), 215–224.

Table 1. Sample analysis results.

Carbocisteine Drug Sample	Detected		Added (mg/L)	Found (mg/L)	Recovery (%)
	In Prepared Sample (mg/L)	Equivalence in Original Sample (%)			
Bulk Drug	0.68	0.07	0.80	1.44	95
Tablet	0.45	0.05	—	—	—
Oral Liquid	0.24	0.02	—	—	—

8. Qu, F.; Mou, S.F. Determination of Monochloroacetic Acid and Dichloroacetic Acid for Quality Control of Acetic Acid Chlorination Industry by Ion Chromatography. *Microchem. J.* **1999,** *63,* 317–321.

9. Liu, Y.J.; Mou, S.F. Determination of Bromate and Chlorinated Haloacetic Acids in Bottled Drinking Water with Chromatographic Methods. *Chemosphere* **2004,** *55,* 1253–1258.

10. Dionex (now part of Thermo Scientific) Application Brief 112: Determination of Organic Acids in Cranberry and Bilberry Extracts. Sunnyvale, CA, 2011. [Online] www.dionex.com/en-us/webdocs/87552-AB112-IC-OrganicAcids-31Mar2011-LPN2560-01.pdf (accessed Dec. 17, 2013).

11. Dionex (now part of Thermo Scientific) Application Note 143: Determination of Organic Acids in Fruit Juices. Sunnyvale, CA, 2003. [Online] www.dionex.com/en-us/webdocs/4094-AN143_LPN1415.pdf (accessed Dec. 17, 2013).

12. Dionex (now part of Thermo Scientific) Application Note 123: Determination of Inorganic Anions and Organic Acids in Fermentation Broths. Sunnyvale, CA, 2006. [Online] www.dionex.com/en-us/webdocs/4082-AN123_LPN1030_2.pdf (accessed Dec. 17, 2013).

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