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Short communication

Determination of mono- and dichloroacetic acids in betaine media by liquid chromatography

Alireza Ghassempour [a,*], Soheila Chalavi [b], Assem Abdollahpour [b], Seyed Amir Mirkhani [c]

[a] *Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran*
[b] *Department of Chemistry, Faculty of Science, Shahid Beheshti University, Evin, Tehran, Iran*
[c] *Asalib Company, Semnan Industrial City, Semnan, Iran*

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Abstract

A simple and sensitive method has been developed for the analysis of residue amounts of chloroacetic acids in betaine samples based on derivatization by 1-naphthylamine (NA). The derivatized compounds are analyzed by reverse phase high performance liquid chromatography using methanol and water as mobile phase in the ratio of 32/68 (v/v) and phenyl column and PDA detection at 222 nm. The detection limits (LOD) of monochloroacetic acid (MCA) and dichloroacetic acid (DCA) are 0.1 and 0.15 μg mL^{-1}, respectively. The limits of quantification (LOQ) and the linear dynamic ranges (LDR) of MCA are found to be 1 and 1–400 μg mL^{-1}, respectively, and for DCA are found to be 3 and 3–400 μg mL^{-1}, respectively. The precision at the 5 ppm level for MCA and DCA are about 3% and 2%, ($n = 5$), respectively. The average recovery for MCA and DCA spiked to betaine samples are 98% and 97%, respectively.

Keywords: HPLC; Monochloroacetic acid; Dichloroacetic acid; 1-Naphthylamine; Derivatization; Betaine

1. Introduction

Haloacetic acids (HAAs) are widespread environmental pollutants [1]. They can be found in trace amounts in drinking water as chlorination by-products after disinfection processes [2,3].

Mono- and dichloroacetic acids (MCA, DCA) are supposed to be the products of the hydrolysis of chlorinated acyl chlorides in troposphere chemical reactions [4]. Chloroacetic acids are used as herbicides and defoliants and chemical intermediates in the production of carboxymethyl cellulose, ethyl chloroacetate, glycine, synthetic caffeine, sarcosine, thioglycolic acid, EDTA, some vitamins, dyes and drugs [5]. Also, MCA is used in betaine (this is an amphoteric surfactant in shampoo and body wash formulations) synthesis processes and DCA is usually its impurity. The final betaine includes trace amounts of salts of these acids.

MCA can be absorbed from inhalation, ingestion and (intact) skin exposure. High doses through dermal contact are corrosive and irritating to skin and eyes and DCA has been found to be a carcinogenic agent [6,7]. Because of their environmental impact, there is a growing interest in their determination in aqueous compartments [8–10].

HAAs are usually analyzed after chemical derivatization by gas chromatography with electron-capture (GC-ECD) [9–11] and mass spectrometric (MS) detection [11–13]. Due to their polar character, HAAs are principally well suited for analysis by high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) [14]. Reverse phase ion pair chromatography with indirect UV detection and a mobile phase containing an ultraviolet-absorbing ion has also been performed to determine some of these compounds [1,15]. However, indirect UV detection lacks sufficient specificity in complicated matrices and the detection limits are not

* Corresponding author. Tel.: +98 21 2418679; fax: +98 21 2418679.
E-mail address: a-ghasempour@sbu.ac.ir (A. Ghassempour).

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very good. Also, detection of HAAs after HPLC separations was recently proposed with electrochemical methods [16], ion chromatography followed by conductivity [17,18] and UV detection [19,20].

The objective of this present work is to be establishing a simple and sensitive method for analysis of residual amounts of chloroacetic acids in betaine samples. This work has been performed in a single step, by combining extraction and derivatization in betaine media. Naphthylamine was used as a derivatization reagent, which converts the chloroacetic acids into their UV absorbance derivatives compounds. These derivatives were analyzed by HPLC at 222 nm.

2. Experimental

2.1. Reagents and chemicals

Monochloroacetic acid (MCA), dichloroacetic acid (DCA), 1-naphthylamine (NA), carbodiimide, benzene, hydrochloric acid (HCl), dichloromethane and HPLC-grade methanol (MeOH) were purchased from Merck (Darmstadt, Germany). All solvents were filtered and degassed before be used. Betaine samples were obtained from commercial sources. Water used in the mobile phase was double deionized and filtered through active charcoal and a 0.5 μm filter.

2.2. Derivatization and extraction procedure

To prepare standard derivatives, MCA and DCA were spiked to a prepared betaine sample in which none containing these chloroacetic acids to produce betaine samples with concentrations of MCA and DCA in the range of 0.1–500 $\mu g\,mL^{-1}$.

To perform derivatization, exactly 1 mL of this betaine was dispensed into a round-bottom flask contains 20 mL benzene, 0.1 g naphthylamine and carbodiimide (as dehydrating agent). The resultant solution was refluxed with continues stirring at 90 °C over 30 min and the flask was cooled to room temperature. The resulting precipitate was filtered by sinter glass, washed with benzene and dissolved in methylene chloride and was filtered for separation of salts. The obtained solution was evaporated by blow nitrogen over it and resultant precipitate was dissolved in 1 mL of methanol as solvent to prepare standard solution of MCA and DCA and kept at 4 °C before analysis.

To perform derivatization for real samples, exactly 1 mL of betaine sample, with defined pH, was derivatized like above.

2.3. Investigation of pH effects on derivatization reaction

For investigation of pH effect, standard solution of betaine (10 $\mu g\,mL^{-1}$ respects to MCA and DCA) was selected and different pHs in the range of 2–6 were prepared and used for derivatization. The pH of betaine samples was adjusted with HCl (2N).

2.4. Instrumentation

The HPLC system consisted of a computer-controlled system with chrom gate software and Knauer low pressure HPLC pump K-1001, Knauer solvent organizer K-1500 and PDA detector K-2800 operated at 222 nm for quantitative analysis. The column was Teknokroma tracer extra 120 phenyl (150 mm × 4.6 mm i.d.; 5.0 μm particle size, Spain, Barcelona). The flow rate of mobile phase in all separations was optimized at 0.7 $mL\,min^{-1}$ and the sample injection volume was 20 μL. The experiments were performed at ambient temperature and under isocratic elution conditions using as mobile phase a mixture of MeOH and water in the ratio 32/68 (v/v).

A Shimadzu MS model QP-1100 EX was used. MCA and DCA derivatives were analyzed in the EI mode using an ion source with temperature at 250 °C and pressure of 5×10^{-6} Torr. Ionization was performed at 70 eV.

3. Results and discussion

3.1. Derivatization results

The betaine media is a complex mixture of salts, MCA, DCA and other organic compounds. DCA and MCA have active carboxylic groups as part of their structures which are reacted with naphthylamine during elimination reaction according to the Fig. 1. The end product of this reaction is a chromophor compound which can be isolated from this complex media and monitored by UV detector at 222 nm. The obtained results show that in real samples only chloroacetic acids were derivatized and other compounds do not interfere with MCA and DCA peaks.

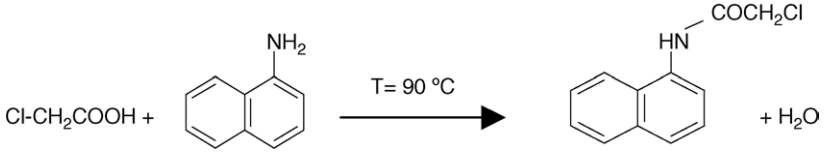

Fig. 1. The derivatization reaction scheme of MCA.

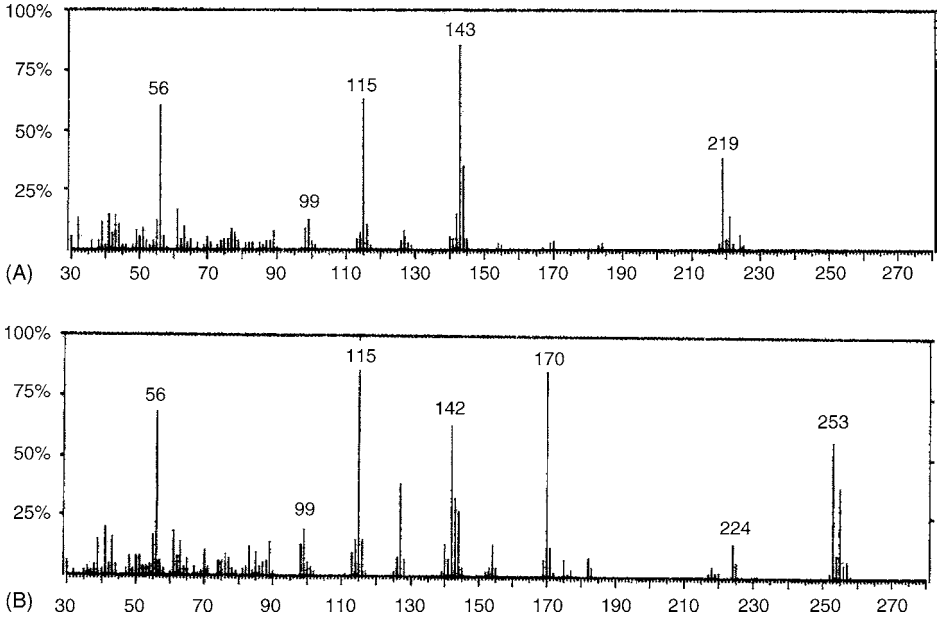

Fig. 2. Full mass spectra of chromophore derivatives (A) MCA; (B) DCA.

3.2. Mass spectrometry results

In order to confirm of derivatization reaction products, the MCA and DCA derivatives were introduced into the ionization source and the fragmentation gives a pattern of peaks at 219 m/z ($M_{MCA} + M_{NA} - M_{Water}$) and 253 m/z ($M_{DCA} + M_{NA} - M_{Water}$) for MCA and DCA derivatives respectively as shown in Fig. 2. These values of m/z confirm these products.

3.3. pH results

The pH control of betaine solution can affect extracted amounts of chloroacetic acids into benzene. Fig. 3 shows the plot of pH of betaine solutions versus MCA and DCA peaks area obtained from HPLC analysis. As shown in this figure, when the pH of betaine solution decreases, the peak areas of MCA and DCA derivatives increase at constant HPLC analysis conditions. These results show that in the low pH, MCA and DCA are in their neutral forms and this has something to do with the success of the extraction. Therefore, the extracted analytes can easily react with the derivatizing reagent in benzene media. Also, the ionic strength of the aqueous phase of betaine samples increase and analytes migrate into the organic phase. The optimum pH has been observed in the range of 2–3 for both of chloroacetic acids.

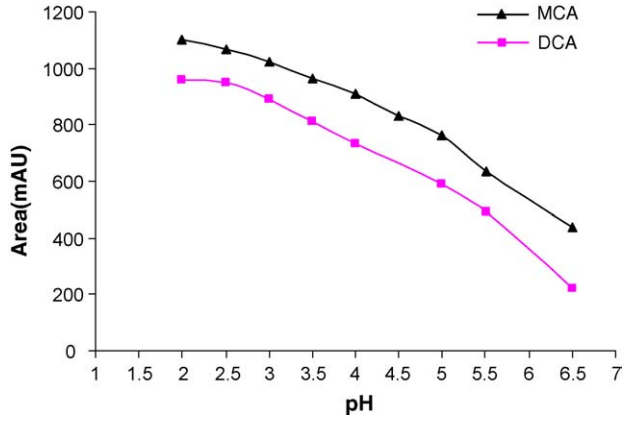

Fig. 3. Effect of pH on extraction of MCA and DCA from betaine media.

3.4. HPLC results

For optimizing the chromatographic conditions, various ratios between methanol and water with phenyl column were evaluated. The obtained results show that increasing of eluting power decreases the analytes retention times. An isocratic mixture as an optimum condition consisting of 32/68 (v/v) MeOH/water and the flow rate of 0.7 mL min^{-1} were selected, because these conditions showed satisfactory peak shapes and area replicates and baseline resolution. These good resolutions are resultant of hydrophobic interactions specially π–π interactions between aromatic groups of stationary phase and chloroacetic acid derivatives. The HPLC chromatogram of standard solution of MCA and DCA was shown in Fig. 4.

The stability of the standard solution of MCA and DCA derivatives were monitored by measuring the area of response of 20 μL injection over a period of 7 days. The RSD (%) values of these solutions were 0.50 for MCA and 0.31 for DCA derivatives within 7 days. The linearity of MCA and DCA in standard solutions was investigated at 10 concentration levels. The calibration curves for these solutions were linear in the range of 1–400 μg mL^{-1} for MCA and 3–400 μg mL^{-1}

Table 1
Calibration results for MCA and DCA

Chloroacetic acid	Equation	RSD (%) ($n = 5$)	R^2	LDR ($\mu g\,mL^{-1}$)	LOD ($\mu g\,mL^{-1}$)	LOQ ($\mu g\,mL^{-1}$)
MCA	$Y = 5.4399X - 7.0502$	<3.1	0.9996	1–400	0.1	1
DCA	$Y = 1.4965X + 1.5729$	<2.4	0.9998	3–400	0.15	3

for DCA. Regression coefficients for five replicates of each concentration were 0.9996 and 0.9998 for MCA and DCA, respectively. Table 1 shows the calibration curve equation, RSD, R^2, LDR, LOD and LOQ of MCA and DCA which have been obtained by this method.

3.5. Recovery test

The procedure was validated by means of recovery experiments. First two solutions of MCA and DCA as standard samples (5 and 20 $\mu g\,mL^{-1}$) were prepared in distilled water. An amount of 1 mL of these solutions was derivatized and obtained compounds were analyzed in optimized LC condition. In the follow, a betaine sample none containing MCA and DCA was prepared and spiked known amounts of MCA and DCA at two levels (5 and 20 μgmL^{-1}) and these samples were derivatized and analyzed like above.

The recoveries were estimated by comparison of results obtained from the spiked betaine samples with those obtained from standard samples under the same experimental conditions. Table 2 shows that the average recovery for MCA and DCA spiked to betaine samples are 98% and 97%, respectively which are indicated of acceptable recovery and reproducibility.

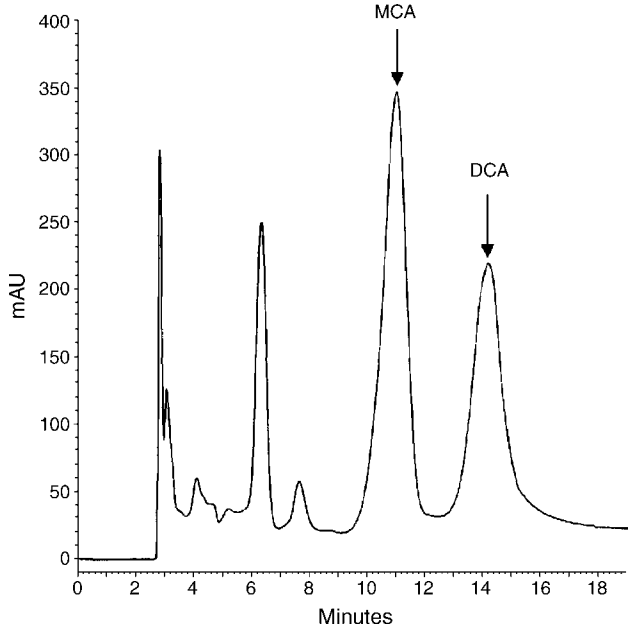

Fig. 4. HPLC chromatogram of standard solution of MCA and DCA derivatives. Eluent: 32/68 (v/v) MeOH/water and detection at 222 nm.

Table 2
Recoveries and coefficients of variation for MCA and DCA in betaine samples spiked at different levels

Spike	MCA		DCA	
	5 $\mu g\,mL^{-1}$	20 $\mu g\,mL^{-1}$	5 $\mu g\,mL^{-1}$	20 $\mu g\,mL^{-1}$
Recovery	95	99	92	96
RSD (%)	2	4	3	3

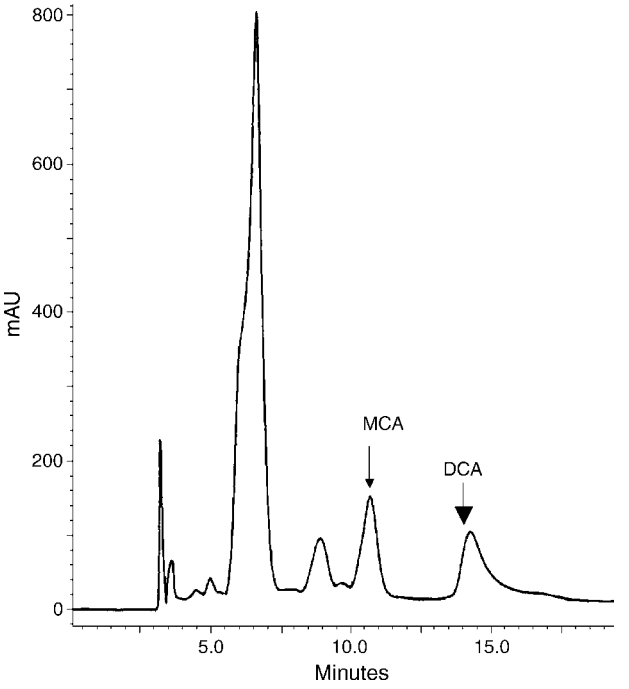

Fig. 5. HPLC chromatogram of MCA and DCA derivatives in real samples. Eluent: 32/68 (v/v) MeOH/water and detection at 222 nm.

The developed method is simple and rapid; because of the small volume (1 mL) of betaine samples are required. Also, the analysis and derivatization reaction consume approximately 1 h for each sample. A typical HPLC chromatogram of real betaine sample is shown in Fig. 5. The analysis of different samples showed that both of these chloroacetic acids are present in the low concentrations.

4. Conclusion

Determination of MCA and DCA has been performed in a single step. This method offers the following advantages: (1) higher selectivity for MCA and DCA; (2) fewer interfering peaks and cleaner baseline; (3) the use of short run time.

Linear range, sensitivity and detection of trace amounts of chloroacetic acids in betaine media with this method are better than other methods, because other methods have been applied for simple media such as drinking water.

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