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Rapid quantitative and qualitative confirmatory method for the determination of monofluoroacetic acid in foods by liquid chromatography–mass spectrometry

G.O. Noonan *, T.H. Begley, G.W. Diachenko

US Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5100 Paint Branch Parkway, College Park, MD 20740, USA

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Abstract

A rapid quantitative method and a qualitative confirmatory method for the determination of monofluoroacetic acid (MFA) in complex food matrices are presented. The quantitative method utilizes a water extraction, solid phase extraction clean-up and liquid chromatography–mass spectrometry (LC–MS) for determination of MFA. This method showed a high degree of specificity, detecting MFA in all of the spiked samples, while none of the unfortified samples tested positive for MFA. Spike recoveries were high in all matrices analyzed, varying from 85 to 110%, and comparable at low (2 mg/L) and high (20 mg/L) spiking levels. Repeatability tests at the low spiking levels yielded RSDs of less than 5% for all matrices analyzed. The qualitative confirmatory method developed is conceptually different from the quantitative method, ensuring that both methods would not be subject to the same interferences. The method uses the formation of the hydrazide of MFA through derivatization with 2-nitrophenylhydrazine. This derivatization is well established for the determination of carboxylic acids, but this is the first application to the determination of MFA. The derivatization yield was matrix dependent, however the limit of detection (LOD) (0.8 μg/L) was sufficient to confirm the presence of MFA in all spiked matrices. Repeatability tests at the low spiking levels yielded RSDs of approximately 7% for all matrices analyzed.

Keywords: Fluoroacetic acid; Food adulteration

1. Introduction

Monofluoroacetic acid (MFA), also referred to as compound 1080, is a rodenticide and a naturally occurring toxic component of poisonous plants found in Australia, South Africa, and India. Although banned for use in South Africa, MFA is still commonly used in the USA, Australia, and New Zealand [1]. The availability and stability [1] of MFA and its proximity to agricultural products could potentially lead to the accidental or intentional contamination of food. In the event of food contamination early identification of the adulterant can limit further exposure and can also improve patient outcomes when treating cases of human exposure [2]. However, currently available test methods for MFA in biological and environmental matrices are either time consuming [3,4] or not applicable to complex food matrices [5,6]. Therefore, we were interested in developing a fast quantitative and qualitative confirmatory method for the analysis of MFA in foods.

There are a number of analytical methods used for the determination of MFA in simple matrices or in situations where rapid identification of MFA is not critical. Several previous researchers have used the derivatization of MFA with *O*-*p*-nitrobenyl-*N*,*N*′-diisopropylisourea [3] or 4-bromomethyl-7-methoxycoumarin [7], followed by HPLC to analyze samples for MFA. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) have been used for analysis after derivatization of MFA with α-bromo-2,3,4,5,6-pentafluorotoluene [4,8–10], ethanol and sulfuric acid [11], 1-(pentafluorophenyl)diazoethane [12], and 2,4-dichloroaniline [13]. These derivatization methods show excellent sensitivity and specificity, however the procedures are often complex and time consuming. Kimball et al. reported a GC method

* Corresponding author. Tel.: +1 301 436 2250; fax: +1 301 436 2634.
E-mail address: Gregory.noonan@fda.hhs.gov (G.O. Noonan).

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which measured the free acid of MFA in aqueous extracts of sheepskin and wool, avoiding the need for derivatization prior to analysis [14]. However, the production and frequent replacement of the guard column were not straightforward. Additionally, injections from more complex matrix extracts could lead to faster degradation of guard column and chromatographic performance. A number of researchers report the use of HPLC–UV (210 nm) [5,15] or ion chromatography with conductivity detectors [16,17] to determine MFA. Guan and co-workers describe an electrophoretic method which utilized UV detection [6]. Although reversed phase and ion chromatography are straightforward analyses, neither UV or conductivity detection offer adequate specificity or limits of detection in complex food matrices. Specificity is especially problematic using UV detection (210 nm) where a variety of interferences make it difficult to develop a uniform clean-up procedure for all foods. Coenen et al. [18] reported difficulties when using UV or conductimetric detection for the determination of aliphatic monocarboxylic acids with complex matrices such as foods.

Given the limitations of the current methods, our goal was to develop a rapid, straightforward, quantitative method for the analysis of MFA in foods. Ideally, the method would use common materials and instrumentation, have high sample throughout and be applicable to a large variety of food matrices. To limit the number of confirmatory samples, thus decreasing overall analysis time and preventing unnecessary action based on false positive results, the method required a high degree of specificity. Finally, the method needed to detect MFA in foods at or below 2 mg/kg. In contrast to these performance requirements we could allow some flexibility in the quantitative accuracy and precision. With the exception of some teas, food should never contain MFA, therefore detecting the presence of MFA with a high degree of confidence was more important than determining MFA concentration with great accuracy.

Given the health and safety concerns associated with detecting toxic contaminants in foods, it is advantageous to have a secondary method to confirm the presence or absence of MFA. The qualitative confirmatory method developed is conceptually different from the rapid quantitative method, ensuring that both analyses would not be subject to the same interferences. The method uses the formation of the hydrazide of MFA through derivatization with 2-nitrophenylhydrazine. Commonly, derivatization reactions for MFA or other low molecular weight carboxylic acids are performed in organic solvents [3,4,7–13]. Miwa et al. reported the derivatization of fatty acids in aqueous solvent using 2-nitrophenylhydrazine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and pyridine buffer [19,20]. Subsequent work by Miwa and co-workers applied the aqueous phase derivatization to the determination of carboxylic acids in beverages and oils [21]. Other researchers, with slightly modified conditions, used the hydrazide derivatization to determine carboxylic acids in marine sediments and water [22,23]. Although the method is well established for the determination of carboxylic acids in foods and water, this is the first reported application to the determination of MFA.

In this paper we present the development of a rapid, quantitative method and a qualitative confirmatory method for the determination of MFA in foods. The quantitative method uses room temperature water extraction followed by filtration and C18 solid-phase extraction (SPE) cartridge clean-up. Separation by a C18 column, using an ion pair mobile phase and detection by LC–MS, using atmospheric pressure chemical ionization (APCI) and selected ion monitoring, is sensitive and highly selective. The qualitative confirmatory method uses the extract from the quantitative method to reduce sample processing time. The hydrazide of MFA is isolated with a two step SPE clean-up prior to analysis by LC–MS. Combining characteristics of previously reported hydrazine derivatization methods [20,23,24] we successfully developed a method that confirmed the presence of MFA in all of the spiked sample matrices.

2. Experimental

2.1. Reagents

Sodium monofluoroacetate (97%) was purchased from Chem Service (West Chester, PA, USA), isotopically labeled [$^{13}C_2$] chloroacetic acid ([$^{13}C_2$]CAA) (99%), formic acid (96%), and tributylamine (>98.5%, TBA) were obtained from Aldrich (St. Louis, MO, USA) and used as received. EDC and aldehyde–agarose solution were obtained from Sigma (St. Louis, MO, USA). EDC was stored at −20 °C after opening. HPLC–Grade water (J.T. Baker), hydrochloric acid (EM Science), and pyridine were obtained from VWR (West Chester, PA, USA). 2-Nitrophenylhydrazine (2-NPH) was obtained from Alfa Aesar (Pelham, NH, USA) and was purified by recrystallization in water and stored at 4 °C prior to use. Water (18 MΩ) was obtained from an Aqua Solutions (Jasper, GA, USA) water purification system and used for the food extractions.

2.2. Materials and apparatus

SPE cartridges (Discovery DSC18, 3 mL/500 mg) were purchased from Supelco (Bellefonte, PA, USA) and were rinsed with 5 mL of methanol followed by 5 mL of water prior to use. Aldehyde–agarose SPE cartridges were prepared by placing an aliquot (1 mL) of aldehyde–agarose solution (Sigma, St. Louis, MO, USA) into a 3 mL cartridge and rinsing with water (5 mL) to remove residual sodium azide. Nylon syringe filters (0.2 and 0.45 μm) were obtained from Titan (Wilmington, NC, USA) and membrane filters (0.02 μm, Anodisc 47) were purchased from Whatman (Clifton, NJ, USA), all were used without preconditioning. Extractions were performed in 50 mL polypropylene centrifuge tubes purchased from Corning (Acton, MA, USA). Derivatization reactions were performed in amber 1 dram glass vials from Supelco. Food samples were purchased from a local store and kept under normal storage conditions. Opened foods, except for the ice cream, were kept refrigerated (4 °C) for up to 7 days and then discarded. Ice cream was kept frozen (≤−4 °C) and used repeatedly over a 1-month period. Coffee was prepared, using an automatic drip coffee maker, according to manufacturer's instructions and used the day of preparation. A Branson