Journal of Chromatography B, 1027 (2016) 131–138

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Analysis of monofluoroacetic acid in urine by liquid chromatography-triple quadrupole mass spectrometry and preparation of the positive sample by the bioconversion from monofluoroacetamide to monofluoroacetic acid *in vitro*

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ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 13 May 2016
Accepted 16 May 2016
Available online 24 May 2016

Keywords:
Monofluoroacetic acid
Monofluoroacetamide
Bioconversion *in vitro*
Urine
Liquid chromatography–triple quadrupole mass spectrometry

ABSTRACT

Whether as a rodenticide or as a natural product, monofluoroacetic acid (FAcOH) may cause poisoning to humans or animals for its high acute toxicity. Urine is one of the most typical specimens for forensic diagnosis when poisoning case about FAcOH happens. The positive sample containing FAcOH plays a key role for the development of an accurate and reliable analytical method. The bioconversion from monofluoroacetamide ($FAcNH_2$) to FAcOH in urine *in vitro* was studied for the preparation of positive urine sample containing FAcOH without standard spiking or animal experiment. The average bioconversion rates were 0%, 18.6% and 41.3% when incubated the $FAcNH_2$ spiked urine *in vitro* for 21 days at −20 °C, room temperature (RT) and 37 °C, respectively. Afterwards, a fast and sensitive analytical method was developed for determination of FAcOH in urine. Samples were diluted with water containing formic acid and cleaned with polymeric anion exchange (PAX) cartridge. The acid eluate was neutralized with ammonium hydroxide and directly measured by hydrophilic interaction liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) using basic mobile phase condition. The limit of detection and limit of quantification of FAcOH in urine were 2 and 5 ng mL^{-1}, respectively. The linear range was 5–1000 ng mL^{-1} with a correlation coefficient of r = 0.9993 in urine calibrated with internal standard. The recoveries at four spiking levels (5, 10, 50 and 500 ng mL^{-1} in urine) were 87.2%–107% with relative standard deviations ranged between 4.3%–8.8%.

1. Introduction

Monofluoroacetic acid (FAcOH) is a rodenticide with high acute toxicity and is known as sodium monofluoroacetate (1080) in industry. It is permitted to be used as vertebrate pesticide in New Zealand, Australia, Israel and the USA [1,2]. FAcOH (as the format of monofluoroacetate) has been found as well in natural plants such as the Fabaceae (Acacia, Gastrolobium), Rubiaceae (Palicourea), Bignoniaceae (Arrabidaea), Malpighiaceae (Amorimia) and Dichapetalaceae (Dichapetalum, Tapura) in the southern continents of Africa, Australia, South America and India [3,4]. The median lethal dose of mammals (LD_{50}) is less than 1 mg kg^{-1} in animals and estimated to be 2–5 mg kg^{-1} in humans within 6–48 h of eating baits [2,5]. Besides, FAcOH was reported to be the metabolite and biomarker of monofluoroacetamide ($FAcNH_2$, another rodenticide with high acute toxicity) in body [6]. The exposure for both rodenticides to humans or animals includes suicide, ingestion of contaminated food and criminal poisoning [6–11]. FAcOH and $FAcNH_2$ even can be a powerful tool for terrorist threat due to their colorlessness, tastelessness, easy accessibility and extremely toxicity. The typical case was the criminal threat of FAcOH to adulterate infant formula in New Zealand in 2015 [12].

It is important and imperative to develop a fast and sensitive analytical method of FAcOH in biological matrices to quickly respond to poisoning cases. Gas chromatography–mass spectrometry (GC–MS) was reported to measure FAcOH in biological samples while derivatization techniques were required to fit the polarity of the gas chromatography column [10]. For liquid chromatography measurement of the trace levels of FAcOH in complex matrices, chemical modification was also used to fit the reverse phase separation and produce the fluorescent signal [13]. As a polar and water soluble compound, ion pair reagents were used

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http://dx.doi.org/10.1016/j.jchromb.2016.05.026
1570-0232/

to improve the retention ability for direct analysis of FAcOH by liquid chromatography-mass spectrometry (LC–MS) [3,9,14] or liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS) [15]. Solid phase extraction (SPE) cartridges with reverse phase function of C18 [9] or HLB [15] were used for clean-up of FAcOH in foods or biological matrices. FAcOH behaves weak retention ability to reverse phase SPE material and only weak polar matrices were removed. In this study, polymeric anion exchange cartridge (PAX) was used to remove both the weak polar and water soluble matrices in urine. Hydrophilic interaction column Amide was planned to directly separate FAcOH under basic conditions without ion pair reagents.

Urine is one of the most typical specimens for forensic diagnosis when poisoning case about FAcOH happens to humans or animals. The positive sample containing FAcOH plays a key role for the development of an accurate and reliable analytical method. Standard spiking can be used to evaluate the method accuracy while the preferable way to obtain a positive sample is the animal experiment. However, the disadvantage of animal experiment is self-evident considering the cost and the animal ethics. As a hot topic for latest study, it is urgent need to find an alternative way to obtain a positive sample without animal experiment. The bioconversion from $FAcNH_2$ to FAcOH (Fig. 1) was found during the stability study of $FAcNH_2$ in urine *in vitro*. The feasibility for preparation of a positive urine sample containing FAcOH was studied according to this bioconversion. The stopping of the bioconversion, levels of the prepared FAcOH in urine, stability and storage of the positive sample were studied in this study.

2. Experimental

2.1. Materials

All reagents and solvents were of analytical grade unless specified.

FAcOH (Sodium salt, 98%), $FAcNH_2$ (99%) and isotope labeled internal standard FAcOH-$^{13}C_2$ (Sodium salt, 99%) were provided by Dr. Ehrenstorfer (Augsberg, Germany), Chem Service, Inc (West Chester, PA, USA) and BDG synthesis (Wellington, New Zealand), respectively. HPLC-grade methanol and acetonitrile were supported by Merck (Darmstadt, Germany). Formic acid was obtained from ROE Scientific Inc. (New Castle, DE, USA). Ammonium hydroxide (>25%) was provided by Hangzhou Liren Medicine (Hangzhou, China). Sodium chloride and anhydrous sodium sulphate were supported by Huadong Medicine (Hangzhou, China) and baked at 500 °C for 4 h before use. Polymeric anion exchange cartridge PAX (60 mg, 3 mL) was provided by Agela Technologies (Tianjin, China). Ultrapure water was prepared using a Millipore system (Bedford, MA, USA).

2.2. Preparation of the standard solution

Pure FAcOH (Sodium salt), $FAcNH_2$ or FAcOH-$^{13}C_2$ (Sodium salt) was dissolved in water containing 0.1% (v/v) of formic acid to obtain the standard stock solution with a concentration of 10, 10 and 0.1 mg mL^{-1}, respectively. Each stock solution was then repeatedly diluted in acetonitrile to prepare the individual standard spiking solution with the concentrations of 1 μg mL^{-1}. A 7 point calibration curve of FAcOH was prepared with the concentrations of 1, 2, 5, 10, 50, 100 and 200 ng mL^{-1} in 75% (v/v) of acetonitrile/methanol containing 1% (v/v) of formic acid. Each milliliter of the standard solution was spiked with 20 μL of internal standard spiking solution (1 μg mL^{-1}) and 0.1 mL of ammonium hydroxide before injection. A 7 point calibration curve of $FAcNH_2$ was prepared with the concentrations of 2, 10, 25, 50, 100, 250, and 500 ng mL^{-1} in acetonitrile.

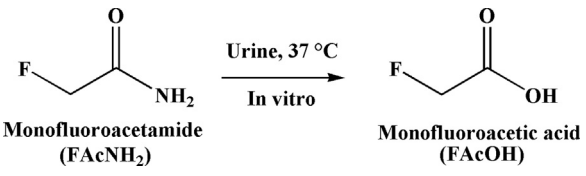

Fig. 1. Bioconversion from $FAcNH_2$ to FAcOH in urine *in vitro*.

2.3. Sample preparation

2.3.1. Homogenized urine blank sample

The FAcOH and $FAcNH_2$ free urine samples were obtained from 5 volunteers. All the samples were mixed together and centrifuged at 4000 rpm for 5 min. The supernatant was used as homogenized urine blank sample.

2.3.2. Incubation of $FAcNH_2$ in urine in vitro

An aliquot of 500 mL of the homogenized urine blank sample was spiked with 100 μL of $FAcNH_2$ standard stock solution (10 mg mL^{-1}), mixed gently for 1 min and stood still for 10 min. After that, 50 mL each of the spiked sample was added to 9 polypropylene plastic test tubes and covered with the screw caps, respectively. Each three test tubes were then incubated at −20 °C (in refrigerator), 37 °C (in an oven) and room temperature (RT, 15–28 °C, in May 2015 in Hangzhou, China), respectively. Two milliliters of the urine was taken out from each test tube after different incubation time (0-21 days) as shown in Table 1 and put into refrigerator at −20 °C at least for 30 min before analysis. The sampled urines were used to analyze FAcOH and $FAcNH_2$ as soon as possible.

2.3.3. Storage of the prepared FAcOH positive sample at −20 °C

The $FAcNH_2$ spiked urine, which was incubated at 37 °C for 21 days as described in Section of 2.3.2., was stored at −20 °C. FAcOH and $FAcNH_2$ were analyzed at the next 7, 14 and 21 days to see the stability of the prepared positive sample at −20 °C.

2.3.4. Incubation of FAcOH in urine in vitro

An aliquot of 500 mL of the homogenized urine blank sample was spiked with 25 μL of FAcOH standard stock solution (10 mg mL^{-1}), mixed gently for 1 min and stood still for 10 min. After that, 50 mL each of the spiked sample was added to 9 polypropylene plastic test tubes and covered with the screw caps, respectively. Each three test tubes were then incubated in −20 °C (in refrigerator), 37 °C (in an oven) and RT, respectively. Two

Table 1
Sampling time for the $FAcNH_2$ spiked urine (2000 ng mL^{-1}) incubated in different temperatures.

Sampling time day[a]	Analytes detected in the sample		
	−20 °C	RT	37 °C
0	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$
1	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$/FAcOH
2	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$/FAcOH
3	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$/FAcOH
4	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$/FAcOH
7	$FAcNH_2$	$FAcNH_2$	$FAcNH_2$/FAcOH
8	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
9	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
10	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
11	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
14	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
18	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH
21	$FAcNH_2$	$FAcNH_2$/FAcOH	$FAcNH_2$/FAcOH

[a] 0 day means tested after spiking but before incubation.