Journal of Chromatography B 1092 (2018) 453–458

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of nine anticoagulant rodenticides by ultra-performance liquid chromatography–tandem mass spectrometry with ultrasound-assisted low–density solvent dispersive liquid–liquid microextraction

Zhi Yan[a], Hongge Li[b], Hui Li[c], Guoyin Lai[d], Junhao Chu[e], Hao Guo[f,*], Qingbiao Zhao[e,*]

[a] West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, Chengdu, Sichuan 610000, China
[b] Department of Traditional Chinese Medicine, Weinan Central Hospital, Weinan 71400, China
[c] Anhui Supervision Institute of Veterinary Drug and Feed, Hefei, Anhui 230091, China
[d] Technical Center of Xiamen Entry-Exit Inspection and Quarantine Bureau, Xiamen 361026, China
[e] Key Laboratory of Polar Materials and Devices, Ministry of Education, Department of Electronic Engineering, East China Normal University, Shanghai 200241, China
[f] Chongqing Institute of Forensic Sciences, Chongqing 400021, China

ARTICLE INFO

Keywords:
Anticoagulant rodenticides
Ultra-performance liquid chromatography
tandem mass spectrometry
Low density solvent dispersive liquid-liquid microextraction
Urine

ABSTRACT

A sensitive determination method is developed for nine anticoagulant rodenticides (ARs) in urine samples by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) with ultrasound-assisted low–density solvent dispersive liquid-liquid microextraction (UA–LDS–DLLME) pretreatment. The target analytes are brodifacoum, bromadiolone, warfarin, coumachlor, coumatetralyl, difenacoum, pindone, diphacinone and chlorophacinone. The parameters that influence the extraction recovery in the UA–LDS–DLLME were systematically investigated and optimized. With the optimized extraction parameters, recoveries ranging from 64.6%–124.2% were obtained for the target analytes. The linear range for all analytes was 0.1–100 ng/mL with correlation coefficients higher than 0.99. Very low LODs ranging in 0.003–0.03 ng/mL were obtained. LOQs were in the range of 0.01–0.1 ng/mL for the nine target analytes. The accuracy that was expressed as mean relative error was within ± 5.8% while the precision expressed as relative standard error was less than 5.9%. The combination of UA–LDS–DLLME with UPLC–MS/MS is a feasible, sensitive and rapid analytical approach for the determination of ARs in urine matrix, which is particularly suitable for clinical and forensic purposes.

1. Introduction

Anticoagulant rodenticides (ARs) are poisons that are used for controlling rodents [1–3]. They have wide application in agriculture, households and urban infrastructures. Frequently used ARs include brodifacoum, bromadiolone, warfarin, coumachlor, coumatetralyl, difenacoum, pindone, diphacinone and chlorophacinone. The capability of controlling rodents of ARs derives from the inhibition of vitamin K epoxide reductase, which results in the deficiency of vitamin K and the deactivation of blood clotting factors (II, VII, IX and X) [1, 4].

Many mammals are susceptible to the poisoning effect of ARs. In fact, a large number of poisoning incidents of non-target animals have been reported [3, 5–7]. More importantly, human poisoning and suicide cases involving ingestion of ARs have occurred many times [8–10]. Thus, a sensitive and rapid determination method for ARs is important. Previous studies have focused on animal organs and foods of animal origin, which include determination methods of ARs by heated electrospray ionization tandem mass spectrometry (LC-HESI-MS/MS) for animal liver and blood [11], a UPLC-MS/MS method for animal liver, an LC–MS/MS method for dog plasma and an HPLC–MS method for animal feed, cooked beef and beverages [12].

Six anticoagulant rodenticides were quantitatively determined in faeces from a dog with UHPLC-MS/MS, with liquid-liquid extraction (LLE) pretreatment [13]. ARs were determined in polecat livers with LC-MS/MS coupled with solid phase extraction (SPE), to evaluate the non-target exposure of these compounds [14]. Liver samples of rabbits were determined with UHPLC-MS, and two pretreatment methods, QuEChERS (quick, easy, cheap, effective, rugged and safe) method and a “dilute and shoot” method, were tested [15]. Determination of ARs in liver using UPLC-MS/MS coupled with d-SPE pretreatment was recently reported [16]. ARs in human blood samples was determined with LC-MS/MS in combination with liquid-liquid extraction [17]. ARs were

* Corresponding authors.
E-mail addresses: hao.guo1982@hotmail.com (H. Guo), qbzhao@ee.ecnu.edu.cn (Q. Zhao).

https://doi.org/10.1016/j.jchromb.2018.06.053
Received 3 March 2018; Received in revised form 2 June 2018; Accepted 24 June 2018
Available online 25 June 2018
1570-0232/

determined in tissues samples with UHPLC-MS/MS and sample preparation by online SPE [18].

The sample pretreatment methods of LLE and SPE, etc., typically have drawbacks including consumption of relatively large amount of organic solvents, significant carry-over effect and long operation time. The sample pretreatment by dispersive liquid-liquid microextraction (DLLME) was proposed in 2006 [19]. DLLME method has multiple advantages, such as small consumption of organic solvents, rapidity and convenience. In this work, a highly sensitive and convenient method with UA–LDS–DLLME pretreatment followed by UPLC–MS/MS analysis was developed for extraction and determination of nine ARs in human urine sample. The effect of various experimental parameters on the extraction of the nine ARs was systematically tested and the practicality of the proposed method was investigated for the determination of ARs in real urine samples from patients. The development of a highly sensitive and convenient method for determination of ARs is of significant importance for clinical and forensic applications.

2. Materials and methods

2.1. Reagents and standards

Standards of brodifacoum, bromadiolone, warfarin, coumachlor, coumatetralyl, difenacoum, pindone, diphacinone, chlorophacinone and brodifacoum-d_4 were obtained from Cerilliant Corp (Round Rock, TX, USA). Sodium chloride was supplied by Merck (Darmstadt, Germany). HPLC–grade solvents ethyl acetate, toluene, n–hexane and cyclohexane were purchased from TEDIA (New Delhi, India). LC–MS grade acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany). Deionized water was prepared in-house with a Milli–Q water purification system (Millipore, Billerica, MA, USA) and used throughout the experiments. Buffer solutions of pH 2.0 (citric acid/sodium hydroxide/hydrogen chloride), pH 4.0 (citric acid/sodium hydroxide/hydrogen chloride), pH 6.0 (citric acid/sodium hydroxide), pH 8.0 (boric acid/sodium hydroxide/hydrogen chloride) and pH 10.0 (borax/sodium hydroxide) were used. Stock solutions of the anticoagulant rodenticide standards were prepared with methanol (10.0 mL) at concentration levels of 10 mg/L for all analytes. Working solutions were generated by appropriate dilution of the stock standard solutions. Blank urine samples were provided by healthy volunteer in our lab, who had not taken any drug for at least 3 months before the experiment. Real urine samples were provided by Chongqing Institute of Forensic Science (Chongqing, China) Both the spiked samples and real samples were preserved at −20 °C prior to the analysis.

2.2. Sample pretreatment with UA–LDS–DLLME procedure

Spiked urine sample or real sample (0.5 mL) was injected into a 2.0 mL centrifuge tube, 10 ng internal standard brodifacoum-d_4 was added, and pH value was adjusted to 6 with 0.5 mL buffer solution (citric acid/sodium hydroxide). 0.5 mL NaCl solution (20% W/V) was added. Afterwards, 200 μL of ethyl acetate, serving as the low–density extraction solvent, was added dropwise into the sample solution. Vigorous sonication in an ultrasonic bath (Kunshan) was performed for 3 min, and a cloudy suspension formed. The mixture was then centrifuged for 5 min at 12,000g. Finally, the upper layer of low–density extraction solvent was retrieved with a syringe and 2 μL of the extractant was injected into the UPLC-MS/MS system for analysis.

2.3. UPLC-MS/MS analysis

The analysis was performed by reverse–phase liquid chromatography using a Waters UPLC-Xevo TQS micro instrument. Chromatographic separation was performed with ACQUITY UPLC BEH C18 column, 2.1 × 100 mm with 1.7 μm particle size (Waters, USA). A gradient program for the mobile phase was set as the following: mobile phase A was 5 mM ammonium formate and 0.1% formic acid in water, and mobile phase B was 5 mM ammonium formate and 0.1% formic acid in methanol. A gradient program was used with a total run time of 7 min: 0.00–1.00 min 10% B; 1.01–2.00 min 10–90% B; 2.01–5.00 min 90% B; 5.01–6.00 min 10% B; 6.01–7.00 min 10% B. The flow rate was 0.3 mL/min. The mobile phase was transferred to waste between 0.00 and 1.00 min to prevent the source from contamination; data were collected between 1.00 and 6 min, and after 6.01 min the mobile phase was again transferred to waste. Ionization was performed using an electrospray ionization (ESI) source in negative mode with a Xevo TQS micro instrument. Data were analyzed with Masslynx 4.1 Software (Waters Corporation, Milford MA). MS/MS condition parameters were: capillary voltage −2.5 kV, source temperature 450 °C, desolvation temperature 550 °C, cone gas flow 50 L/h, desolvation gas flow 1000 L/h, dwell time 0.025 s. The MS/MS parameters are summarized in Table 1. MRM chromatograms are shown in Fig. 1.

Table 1
The MS/MS parameters for the analysis of the target analytes.

Compound	Precursor ion (*m/z*)	Quantitative product ion/ qualitative product ion (*m/z*)	Cone voltage (V)	Collision energy (V)
Brodifacoum	521	79[a]/135	6	38/38
Bromadiolone	525	250[a]/181	52	34/44
Coumachlor	341	284[a]/161	42	30/25
Coumatetralyl	291	141[a]/247	55	28/24
Difenacoum	443	135[a]/293	30	32/22
Warfarin	307	250[a]/161	40	24/24
Pindone	229	116[a]/144	24	32/28
Diphacinone	339	145[a]/167	18	22/24
Chlorophacinone	373	201[a]/145	60	22/24
Brodifacoum-D4	527	81[a]/139	18	56/40

[a] Quantitative product ions.

3. Results and discussion

In the whole analysis process, sample pretreatment is an important step. The goal of sample pretreatment is to separate and enrich the target analytes, while in the meantime being compatible with the analytical approach to be utilized [20]. Dispersive liquid–liquid microextraction (DLLME) has multiple advantages, including feasibility, rapidity and high EFs [19, 21]. Ultrasound is often used to emulsify the extraction solvent, and in this case the method is termed ultrasound assisted dispersive liquid–liquid microextraction (UA–DLLME) [22]. Nevertheless, there are certain drawbacks in DLLME method. DLLME method generally involves the utilization of potentially toxic chlorinated solvents as extraction solvent. Moreover, the extraction layer of DLLME cannot be directly injected to LC system for analysis. The extraction solvent needs to be dried and the analytes are then reconstituted with a proper solvent prior to LC procedure. Later on, a modified version of DLLME, ultrasound–assisted low–density solvent dispersive liquid–liquid microextraction (UA–LDS–DLLME) was developed [21, 23]. By using a container with a conical shape with a narrow “neck”, DLLME procedure was allowed to be performed with extraction solvents having a smaller density than water [23]. This method was easy to execute, and steps like injection of chemical demulsifiers were saved.

3.1. Optimization of UA–LDS–DLLME procedure

The extraction efficiency of UA–LDS–DLLME method can be influenced by several factors, including choice of extraction solvents, quantity of the extraction solvents, pH value, amount of NaCl and the extraction time. In this study, these factors were systematically