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Determination of amitraz and its metabolites in whole blood using solid-phase extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A method was developed for determination of amitraz and its metabolites, *N*-[2,4-(dimethylphenyl)-*N*′-methylformamidine (DMPF), 2,4-dimethylformamidine (DMF), 2,4-dimethylaniline (DMA) in whole blood. The analytes were extracted by solid-phase extraction (SPE) using dichloromethane, acetonitrile and methanol (2:1:1) mixture as elute solution. Analysis was performed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) in the positive ion mode using multiple reaction monitoring (MRM) technique. Collision-induced dissociation (CID) of amitraz at the electrospray source in MS/MS was observed in the analytic conditions. The method was validated in human whole blood spiked at three concentration levels. The low limit of detection (LOD) and the low limit of quantification (LOQ) for all the analytes were below 0.5 μg/L and 2 μg/L, respectively. Recoveries were between 90.2% and 104.5%, Bias and relative standard deviation (RSD) were below 15% ($n = 6$). The good linear relationships were obtained in certain concentration ranges of amitraz and its metabolites. The results demonstrated the method is exclusive, sensitive and accurate, and can be applied in forensic toxicology.

1. Introduction

Amitraz (*N*′-2,4-(dimethylphenyl)-*N*-[2,4-(dimethylphenyl) imino]-*N*-methyl-methanimidamide) is a formamidine derivative insecticide and acaricide, which has been widely used in agriculture and horticulture for control of ticks and manage mites in animals [1,2]. Amitraz poisoning of human beings occurs in many countries, especially in China, where it is authorized for numerous applications [3]. In the body, it interacts with the α-2 adrenocepter and causes a series of symptoms, such as central nervous, respiratory systems depression, bradycardia, hypotension and convulsions [4,5]. The amitraz undergoes a rapid degradation or/and metabolism in the body yielding *N*-[2,4-(dimethylphenyl)-*N*′-methylformamidine (DMPF) which is also used as insecticide, 2,4-dimethylformamidine (DMF), and end product 2,4-dimethylaniline (DMA). The chemical structures of amitraz and its metabolites are shown in Fig. 1. Subchronic toxicity studies with metabolites showed that their toxicity on molar base is comparable to that of the parent compound [6,7]. The incidence of amitraz ingestion has occurred every year in China. During the past five years, there were ten more amitraz poisoning cases happened in our territory. Due to the death investigation and forensic application, sensitive and reliable analytic method of amitraz and its metabolites is required.

Several techniques, such as gas chromatographic–mass spectrometry (GC–MS) [8–10], liquid chromatographic–mass spectrometry (LC–MS) [11–13], high performance liquid chromatography (HPLC) [14], have been used to analyze amitraz and its metabolites, but most of publications have described the methods in wines, fruits, and beeswax. Only few reports exist for biological matrices: Chou et al. and Saito et al. reported a GC–MS and LC–MS method for quantitatively measuring amitraz and its metabolites in urine and serum, respectively [10,11]. In forensic science practices, whole blood analysis is essential for hemolyzed blood samples [15]. However, there has been no method reported for determination of amitraz and its metabolites in whole blood to the best of our knowledge, In whole blood, the presence of matrix interference could adversely affect analyte quantification and identification. Therefore, the extract clean-up method is so critical in toxicological analysis that it could avoid the matrix interference and even more, reduce the detection limits. In general, protein precipitation (PP), liquid–liquid extraction (LLE) and solid phase extraction (SPE) are currently used as pretreatment techniques in toxicological analyses [16]. Although PP and LLE are simple methods, inadequate purification of samples may cause contamination to the instrument and problems with reliability of quantitative values. While SPE, which is

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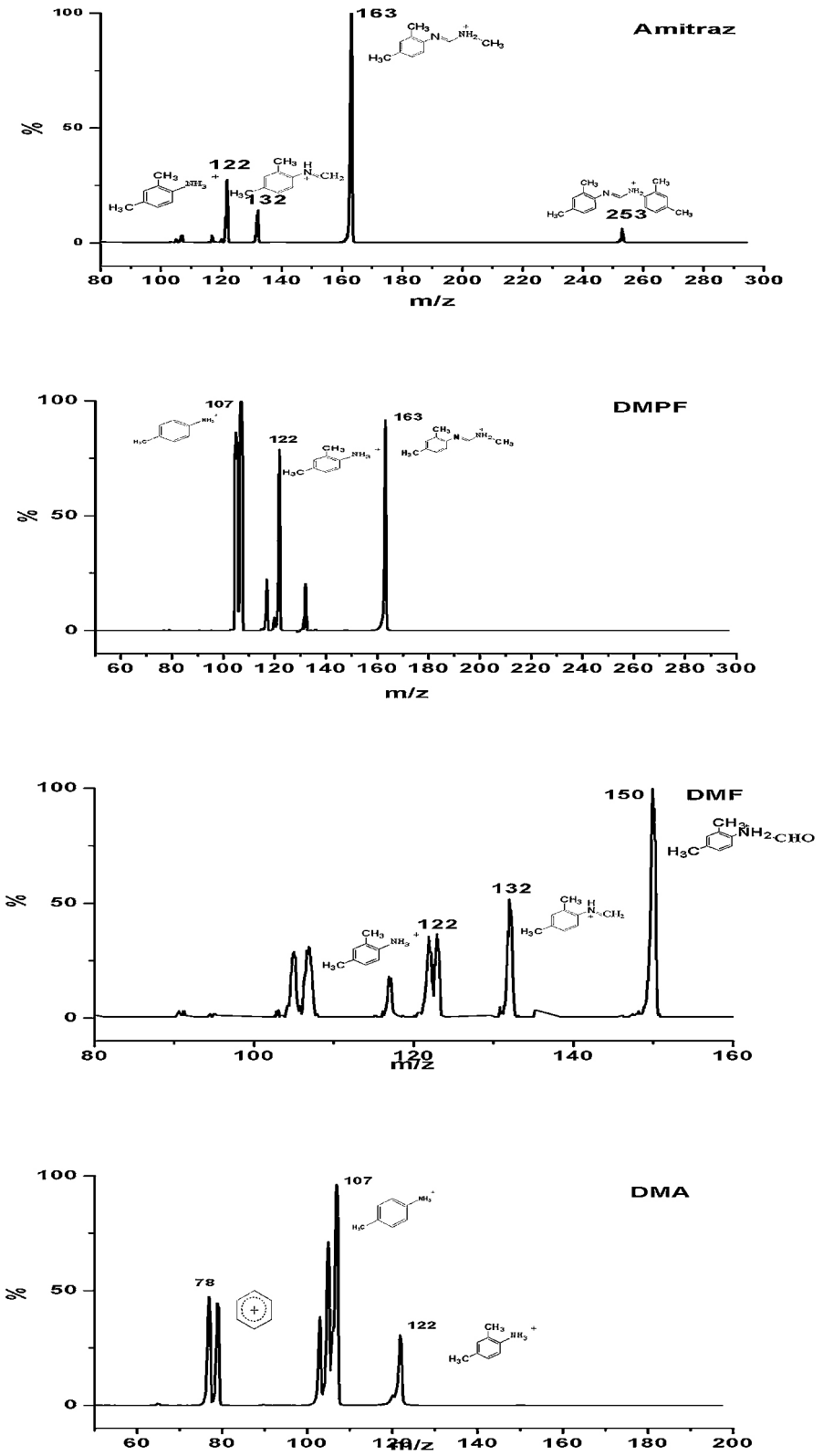

Fig. 1. Product ion spectra of a standard solution and proposed fragments structures of (a) amitraz, (b) DMF, (c) DMPF and (d) DMA.

a combination of clean-up and exaction procedure, has high selectivity and the extraction may contain fewer interfering substances than after PP or LLE, the procedure for extraction is relatively laborious and time-consuming. In recent years, SPE has been widely used in forensic analysis to separate the analyte in whole blood [17].

The present work described and validated a sensitive, reliable and specific method to determine amitraz and its metabolites in human blood samples. The method involves SPE procedure and liquid chromatography–tandem mass spectrometry (LC–MS/MS) determination. The applicability of the proposed method was