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Simultaneous determination of amitraz and its metabolite residue in food animal tissues by gas chromatography-electron capture detector and gas chromatography–mass spectrometry with accelerated solvent extraction

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ABSTRACT

A new method has been developed for determination and confirmation of amitraz and its main metabolite, 2,4-dimethylaniline, in food animal tissues using gas chromatography-electron capture detector (GC-ECD) and gas chromatography–mass spectrometry detector (GC–MS). This method is based on a new extraction procedure using accelerated solvent extraction (ASE). It consists of an *n*-hexane/methanol extraction step, a cleaning-up step by BakerBond octadecyl C_{18} silica bonded cartridge, hydrolysis and derivatization to 2,4-dimethyl-7-F-butyramide for GC-ECD analysis. For confirmation using GC–MS, hydrolysis and derivatization were not needed. Parameters for extraction pressure, temperature and cycle of ASE, clean-up, derivatization and analysis procedure have been optimized. Spike recoveries from 50 to 300 μg/kg levels were found to be between 72.4 and 101.3% with relative standard deviation less than 11.5% in GC-ECD, from 5 to 20 μg/kg levels were found to be between 77.4 and 107.1% with relative standard deviation less than 11.6% in GC–MS. The LOD and LOQ are 5 and 10 μg/kg, respectively, for these two analytes using GC-ECD. For GC–MS, LOD and LOQ were 2 and 5 μg/kg, respectively. The rapid and reliable method can be used for characterization and quantification of residues of amitraz and its main metabolite, 2,4-dimethylaniline, in liver and kidney samples of swine, sheep and bovine.

1. Introduction

Amitraz, 1,5-di-(2,4-dimethylphenyl)-3-methyl-1,3,5-tri-aza-penta-1,4-diene, is a member of formamidine pesticide family. It is an acaricide and insecticide indicated for control of ticks, mites, lice and other infestations on sheep, cattle and swine with good therapeutic results. However, amitraz poisoning is often encountered in animals [1,2], and can also find its way into the human body through food chain [3]. Increased concerns in recent years on possible health risk due to amitraz residues have greatly influenced our thinking and impelled us to set up monitoring programs to determine amitraz at low levels. It should be pointed out that amitraz is a very labile pesticide whose degradation products include 2,4-dimethylaniline (2,4-DMA). Thus, analysis of amitratz should also include analysis of 2,4-DMA. In China, amitraz is approved for use in animal husbandry, and the maximum residue limits (MRL) in swine and sheep livers are set at 200 and 100 μg/kg in sheep, and in the kidney of swine, sheep and bovine at 200 μg/kg [4].

Different methods such as high performance liquid chromatography (HPLC) with UV detection [5], gas chromatography [6], mass spectrometry (GC–MS) [7] (most widely used method), cyclic voltammetry [8] and ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry [9], have been published for amitraz in stock solutions and other matrix. However, no attempts have been made to simultaneously analyze amitraz and 2,4-DMA residues in edible tissues such as liver and kidney using GC-ECD and GC–MS.

Sample pretreatment is always a key element in residue analysis, especially when large number of samples is involved where rapid extraction becomes even more essential. However, limited published methods have focused on rapid extraction of amitraz [10–13], and from veterinary residue and food safety points of view, the only matrixes investigated were beeswax or honey. Accelerated solvent extraction (ASE) is a recent advance in sample preparation for trace analyte, and it has been used in environmental and edible animal tissues sample pretreatment [14–17]. Therefore, to develop

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a method using ASE to shorten the extraction time and apply it to different edible tissues is very desirable.

The objective of this work was to develop a rapid and accurate extraction method (ASE) for GC-ECD analysis of residues of amitraz and 2,4-DMA in liver and kidney of swine, sheep and bovine, and to develop a confirmation method using GC–MS. Optimization of ASE was conducted by varying extraction parameters such as extraction solvents, temperature, pressure and cycle times. Clean-up was based on a BakerBond octadecyl C_{18} silica cartridge. This newly developed method was then applied to real tissue sample.

2. Experimental

2.1. Standards and materials

Amitraz and 2,4-DMA were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Stock standard solutions for each compound were prepared in hexane at a concentration of 1 mg/ml and stored at −20 °C in dark. Working mixed standard solution (100 mg/l) was prepared by dilution of stock standards in hexane. Stock standards were stable for 6 months, and the working standard was stable for at least 1 month when stored in amber vials below 4 °C.

Hexane and methanol were chromatographically pure grade. Water used in all experiments was purified on a Milli-Q system from Millipore (Bedford, MA, USA). Sodium dodecyl sulfate (SDS) and dichloromethane (DCM) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Hydrochloric acid (HCl), sodium sulfate (Na_2SO_4) and sodium hydroxide were analytical grade. Sodium hydroxide (NaOH) solution (pH = 9.0) was prepared by dissolving 0.4 mg sodium hydroxide in 1 l purified water.

Solid phase extraction (SPE) cartridges used such as BakerBond octadecyl C_{18} silica bonded phase (6 ml and 300 mg) were from Supelco (Bellefonte, PA, USA), heptafluorobutyric acid (HFBA) was purchased from Sigma–Aldrich (Milwaukee, WI, USA), OASIS SAX SPE cartridges (6 ml and 300 mg) were purchased from Waters (USA), and $–NH_2$ cartridge (6 ml and 300 mg) was from Agela technologies (USA).

2.2. Blank sample

The liver and kidney samples of swine, bovine and sheep were purchased from local market, homogenized in a high-speed food blender, and stored at below −20 °C until the time of analysis.

2.3. Sample preparation

2.3.1. Accelerated solvent extraction (ASE)

ASE was carried out using a Dionex accelerated solvent extractor 200 (Dionex, Sunnyvale, CA, USA) equipped with 22-ml stainless-steel extraction cells. The extraction procedure was applicable to all matrices including liver and kidney. For each cell, 5 g tissue sample was placed in cellulose filters (Dionex) which were in turn placed in the stainless-steel extraction cells. All the cells were heated in a water bath at 40 °C to improve and facilitate the handling of the mixture, using *n*-hexane and methanol as solvents. Optimized extraction conditions were obtained by sequentially varying one experimental parameter while all other parameters remained fixed. Final conditions used in the extraction for amitraz and 2,4-DMA were as follows: time heating cell 2 min, time of solvent in contact with the sample 2 min (static time), pressure 120 bar, temperature 60 °C, time purging with nitrogen to expulse rest of solvent in the cell 60 s, water volume flushing in respect to the cell size in percentage 50%, and three times cycled. At the end of each extraction a total extract volume of 15 ml was obtained, 3 ml of which was mixed with 27 ml of NaOH solution (pH = 9.0) for SPE clean-up.

2.3.2. Clean-up by solid phase extraction

SPE column was activated with 4 ml of methanol and washed with 4 ml of methanol:NaOH solution (pH = 9.0) (1:9, v/v). Next, 30 ml of solution obtained in the sample extraction section was passed through the column. The cartridge was washed twice with 10 ml of methanol:NaOH solution (pH = 9.0, 10:90, v/v) and then dried by applying vacuum for 1 min. The compounds of interest were eluted with 2 ml of methanol. The final volume was adjusted to 2 ml and sodium sulfate (Na_2SO_4, about 0.1–0.2 g) was added to remove residual water. After filtration, this solution was injected into GC–MS for analysis. For GC-ECD, further hydrolysis and derivatization were needed.

2.3.3. Hydrolysis and derivatization for GC-ECD analysis

The solution obtained above in 2.3.2 was added NaOH (pH = 9.0) 0.5 ml and hydrolysis was completed by heating at 70 °C for 40 min. Two grams of Na_2SO_4 were added to remove water in the solution, all the solution was then transferred to another tube, and 10 μl of derivatization agent HFBA was added. The solution was incubated at 70 °C for 60 min. After cooled to room temperature, the solution was dried by a stream of nitrogen. The residue was re-dissolved in 1 ml of methanol, vortexed, and transferred into an auto-sampler vial for GC-ECD analysis. For the spiked sample, a standard solution, either amitraz or 2,4-DMA, with the same concentration was processed along with the samples for a calibration curve, results will be calculated as amitraz concentration.

2.4. GC-ECD conditions

The GC-ECD analysis was performed on SHIMADZU-2010 gas chromatograph equipped with a SHIMADZU AOC-20s automatic sampler coupled to a SHIMADZU electron capture detector. Capillary GC analysis was performed on a Rtx-5 (30 m × 0.25 mm i.d., 0.25 μm) capillary column (5% diphenyl and 95% dimethylpolysiloxane) with nitrogen as carrier gas. GC conditions were initially at 50 °C, at a rate of 7 °C/min to 220 °C, kept for 5 min, column flow rate at 1.0 ml/min and carrier gas (N_2) flow rate of 30.0 ml/min. Injection temperature was 250 °C with a split ratio of 50/1 and ECD temperature was at 300 °C.

2.5. GC/MS conditions

Confirmatory analysis was performed on SHIMADZU-QP 2010 gas chromatography with a mass selective ion detector and a fused-silica capillary column (HP-5-5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). GC performed under the following conditions: initial temperature, 60 °C (1 min), increased at a rate of 15 °C/min to 150 °C, kept for 5 min, then increased at 20 °C/min to 280 °C, and final temperature being held for 3 min; injector temperature, 280 °C; carrier gas, He operated in the splitless mode; purge off time, 1 min; injection size, 1 μl; GC–MS transfer line, 280 °C. MS conditions: solvent delay, 5 min; electron impact ionization voltage, 70 eV; scan rate, 1.5 scan/s; scanned-mass fragment *m*/*z* 162, 293, 121, and 132 for amitraz; *m*/*z* 121, 120, 106, and 77 for 2,4-DMA. Due to the little interference and high response to the detector, 162 and 121 were selected for amitraz and 2,4-DMA quantification, respectively.

2.6. Validation procedure

The optimized analytical method has been validated according to the Decision 2002/657/EC under Council Directive 96/23/EC [18]. Specificity, linearity, limits of detection (LOD), limits of quantification (LOQ), recovery and precision for the method were determined.