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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.

2.6.1. Specificity

Specificity was established by analyzing10 blanks of each tissue type from different sources to evaluate possible endogenous interferences. The sample preparation and chromatographic conditions were optimized to guarantee that no interferences occurred at the retention times of the tested compounds.

2.6.2. Linearity

For GC-ECD calibration purposes, linearity was established through a calibration curve obtained by triplicate analysis of amitraz and 2,4-DMA both at five concentration levels (5, 50, 100, 200 and 400 μg/kg) in the matrix. After extraction and clean-up, each solution was hydrolyzed and derivatized according to Section 2.3.3. For GC–MS calibration purposes, there were five concentration levels at 1, 10, 50, 100, and 200 μg/kg in matrix.

2.6.3. Accuracy and precision

Recoveries were measured in blank tissue samples that were spiked at the levels of 0.5, 1 and 1.5 times of MRL for GC-ECD and 1, 2 and 4 times of LOQ for GC–MS. The recoveries were calculated by comparing peak areas of measured and spiked concentrations. Samples were spiked with amitraz and 2,4-DMA at three concentration levels as described above, with 6 replicates at each level. All were analyzed on the same day with the same instrument and operator. At each concentration, the mean and the relative standard deviation (RSD) were calculated as repeatability. Samples were analyzed on 3 separate days with the same instrument and operator. At each level, the overall mean and RSD were calculated as reproducibility.

2.6.4. LOD and LOQ

Limits of detection (LOD) were established by analyzing 6 blank tissue samples, which were spiked with low concentrations of amitraz and 2,4-DMA, using a criterion of signal-to-noise ratio (S/N) = 3/1. Limits of quantification (LOQ) were determined using the signal-to-noise (S/N) = 10/1 criterion of the above mentioned 6 samples. As discussed above, these levels could be quantified fulfilling the criteria for accuracy and precision [18].

3. Results and discussion

3.1. Optimization of ASE condition

The selection of operating conditions in ASE is a difficult task and was thoroughly investigated in this study. Wide range of conditions was tested for the ratio of *n*-hexane and methanol, temperature, pressure, extraction time and number of extraction cycles. Parameters were optimized one by one, and the results of earlier tests were always used to evaluate the next extraction conditions for optimization.

In the previous study, Korta et al. [13] used hexane–propanol (1/3, v/v) to extract acaricides including amitraz from honey, the total recovery of amitraz was from 53 to 108%. Since 2,4-DMA is much more polar than amitraz, more hexane in the extraction solvent will result in a very satisfactory recovery for amitraz but not for 2,4-DMA and vice versa when more methanol is used. Thus, in order to obtain a compromise optimum solvent ratio for extraction, experiments were carried out with the ratio of *n*-hexane and methanol continuously varied from 5% hexane and 95% methanol to 20% hexane and 80% methanol at 5% steps. The results showed that the best extraction for both amitraz and 2,4-DMA was obtained when *n*-hexane/methanol is at a ratio of 1:9 (v/v).

In some cases, solvent modifiers, such as a surfactant, it has been used to extract PAH from fish tissues [19]. However, our research showed no significant change in the recoveries of the compounds with the addition of modifier such as sodium dodecyl sulfate (SDS) and dichloromethane (DCM).

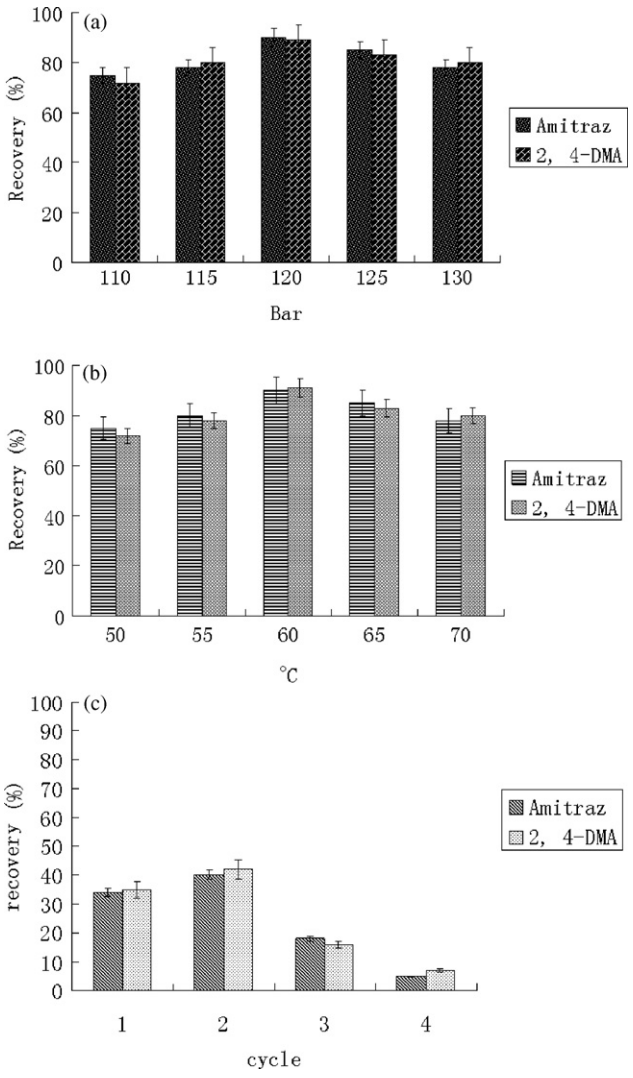

Fig. 1. Influence of pressure (a), temperature (b) and extraction cycle (c) on extraction efficiency.

In ASE, pressure is another important parameter which may influence compound recovery. The pressure was investigated ranging from 110 to 130 bar in this study. It was found that better recovery could be obtained at 120 bar (see Fig. 1a). This is considerably higher than the minimum pressure to keep the solvent liquid. With increased density, the solvating power of the extraction solvent is increased. But at higher densities, the diffusion coefficients decrease, which can lead to low recoveries due to the kinetics of the extraction process [20].

The extraction solvent was tested at different temperatures ranging from 50 to 70 °C. As temperature increases, interactions

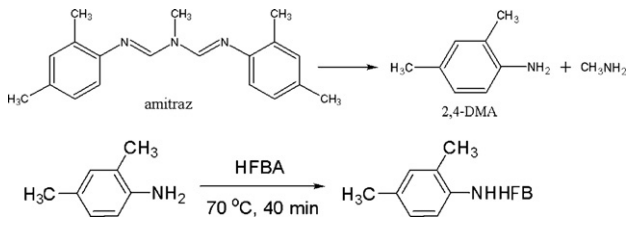

Fig. 2. Derivatization reaction of 2,4-DMA with HFBA.

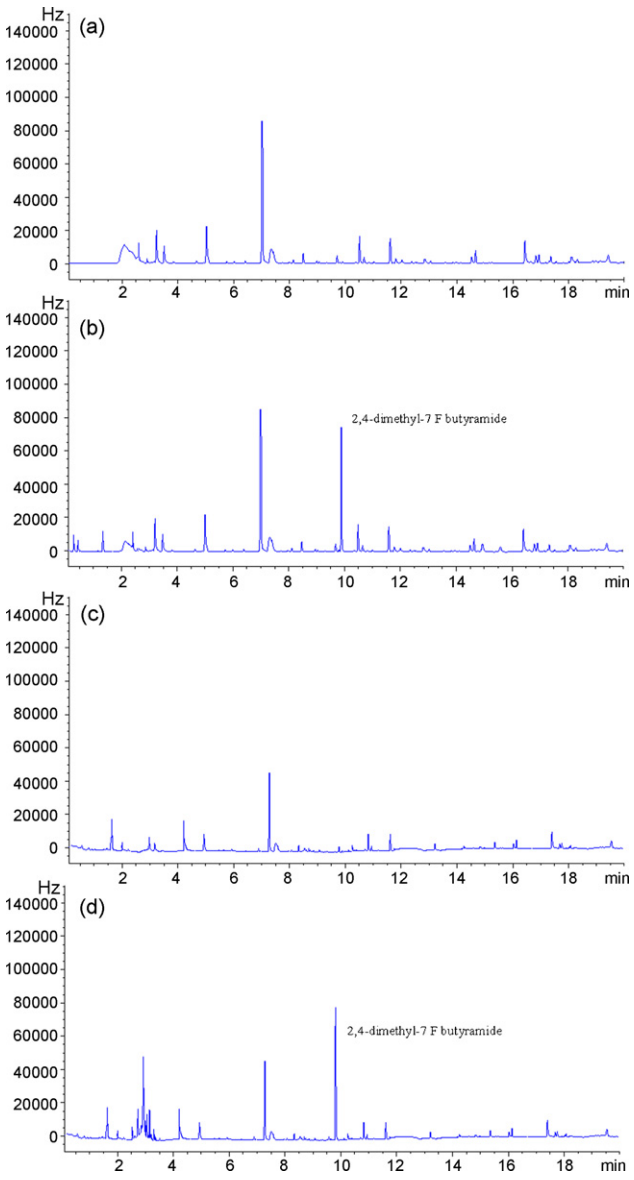

Fig. 3. Typical chromatograms of GC-ECD for blank liver (a) and kidney (c) of swine, spiked liver (b) and kidney (d) of swine with 10 μg/kg of amitraz.

between analytes and matrix components are weakened, and viscosity and surface tension decreased. Extraction efficiency was optimized at 60 °C (Fig. 1b), with recoveries between 80 and 100%. At higher temperatures, the recoveries of amitraz decreased, which is probably due to a destruction or increased formation of 2,4-DMA. Low recoveries below 60 °C for the two compounds are most likely due to decreased desorption and dissolution of the drugs within 4 min of an extraction cycle.

To optimize the number of cycles, four consecutive extractions of analytes from the same sample were made. Significant amounts of the analytes were found in the second extract but the recoveries for both compounds were considered negligible in the fourth cycle, as shown in Fig. 1c. To save solvent and time, three extraction cycles were used, and it allowed us to introduce fresh solvent.

3.2. Optimization of SPE clean-up procedure

Biological sample matrices, especially edible animal tissues, are complex and often contain elements that can interfere with the compounds of interest, therefore direct analysis may not always be possible. Moreover, analyte concentrations are generally low in these samples, and it is usually necessary to purify and concentrate the analytes prior to analysis. The utilization of solid phase cartridges greatly simplifies a pre-workup process. Previously, this technique has been used to the clean-up of amitraz extracts from honey [12]. In the present work, in order to eliminate interferences from the matrix, several cartridges were tested, and finally BakerBond octadecyl C_{18} silica bonded phase was selected because of its high recovery and clean-up efficiency. The result was consistent with a prior study [12]. However, the tris (hydroxymethyl)–aminomethane buffer (0.05 M, pH = 9.0 adjusted with HCl) was replaced with NaOH aqueous solution (pH = 9.0). Different washing and eluting agents were used for the evaluation of clean-up efficiency, 10 ml of methanol:NaOH solution (pH = 9.0, 10:90, v/v) for washing and 2 ml of methanol elution permitted good clean-up and separation.

3.3. Optimization of derivatization procedure

2,4-DMA can be derivatized to amide-based volatile products by reaction with heptafluorobutyric acid (HFBA). The derivation reaction is shown in Fig. 2. In order to select effective derivatization conditions, reaction time and temperature of derivatization were optimized. Standard of 2,4-DMA was derivatized at 70 °C for 20–60 min, and GC-ECD was used to evaluate the efficiency of derivatization. The results showed that HFBA began to derivatize 2,4-DMA into 2,4-dimethyl-7-F-butyramide within 20 min, how-

Table 1
Sensitivity, accuracy and precision of GC-ECD for amitraz and 2,4-DMA in spiked liver and kidney of swine, sheep and bovine (n = 6 at each concentration, on 3 separation days).

Sample	Compound	LODs (μg/kg)	LOQs (μg/kg)	Spiked level (μg/kg)	Overall recovery (%)	Within-day RSD (%)	Between-day RSD (%)
Swine liver	Amitraz	5	10	100–300	81.4–94.6	6.5–8.5	8.2–9.9
	2,4-DMA	5	10	100–300	76.4–92.0	5.2–9.6	7.8–9.6
Sheep liver	Amitraz	5	10	50–150	77.7–82.2	6.7–9. 3	8.1–10.2
	2,4-DMA	5	10	50–150	83.6–85.5	3.7–4.9	7.5–8.9
Bovine liver	Amitraz	5	10	100–300	87.1–90.1	5.3–7.3	9.2–11.0
	2,4-DMA	5	10	100–300	83.6–98.2	4.3–6.9	8.5–10.4
Swine kidney	Amitraz	5	10	100–300	81.7–87.1	4.6–9.0	5.9–9.1
	2,4-DMA	5	10	100–300	72.4–98.2	7.8–9.9	8.9–9.1
Sheep kidney	Amitraz	5	10	100–300	81.4–86.4	5.6–9.6	7.8–11.5
	2,4-DMA	5	10	100–300	77.5–85.0	4.8–9.4	9.9–10.9
Bovine kidney	Amitraz	5	10	100–300	93.1–101.3	6.2–6.6	8.6–10.0
	2,4-DMA	5	10	100–300	84.0–91.4	7.9–9.1	9.4–10.5

Table 2
Sensitivity, accuracy and precision of GC–MS for amitraz and 2,4-DMA in spiked liver and kidney of swine, sheep and bovine (n = 6 at each concentration, on 3 separation days).

Sample	Compound	LODs (μg/kg)	LOQs (μg/kg)	Spiked level (μg/kg)	Overall recovery (%)	Within-day RSD (%)	Between-day RSD (%)
Swine liver	Amitraz	2	5	5–20	84.2–91.2	7.1–10.5	8.6–10.7
	2,4-DMA	2	5	5–20	79.8–92.1	5.2–9.6	9.9–11.6
Sheep liver	Amitraz	2	5	5–20	87.1–90.1	6.7–9. 3	8.4–10.3
	2,4-DMA	2	5	5–20	80.1–85.5	4.3–8.9	6.8–8.6
Bovine liver	Amitraz	2	5	5–20	83.4–82.2	5.3–6.6	6.2–7.8
	2,4-DMA	2	5	5–20	93.1–107.1	3.7–6.9	5.9–8.3
Swine kidney	Amitraz	2	5	5–20	81.7–87.1	4.6–9.0	6.1–11.1
	2,4-DMA	2	5	5–20	77.4–98.2	7.8–9.9	8.9–9.4
Sheep kidney	Amitraz	2	5	5–20	81.7–86.4	5.6–9.6	7.5–10.5
	2,4-DMA	2	5	5–20	79.5–85.0	4.8–9.4	8.0–9.8
Bovine kidney	Amitraz	2	5	5–20	89.6–98.2	6.2–7.3	7.7–9.4
	2,4-DMA	2	5	5–20	86.0–99.0	7.9–9.1	8.9–11.2

Table 3
Confirmation data of amitraz and 2,4-DMA including retention time and relative abundance ion ratios of standards compared to blank and spiked sample.

Analytes	Sample[a]	Retention time (min)	Relative abundance (%)			
			m/z	m/z	m/z	m/z
Amitraz	50 μg/kg standard	16.18	162 (100)	293 (80)	121 (95)	132 (90)
	Blank swine liver	ND[b]	–	–	–	–
	50 μg/kg spiked	16.20	162 (80)	293 (77)	121 (65)	132 (30)
2,4-DMA	50 μg/kg standard	7.20	121 (100)	120 (85)	106 (80)	77 (25)
	Blank swine kidney	ND	–	–	–	–
	50 μg/kg spiked	7.18	121 (70)	120 (78)	106 (67)	77 (15)
Amitraz	50 μg/kg standard	16.18	162 (100)	293 (80)	121 (95)	132 (90)
	Blank sheep liver	ND[b]				
	50 μg/kg spiked	16.19	162 (60)	293 (67)	121 (55)	132 (40)
2,4-DMA	50 μg/kg standard	7.20	121 (100)	120 (85)	106 (80)	77 (25)
	Blank sheep kidney	ND				
	50 μg/kg spiked	7.18	121 (72)	120 (76)	106 (63)	77 (18)

[a] Average of five samples.
[b] None detected.

ever, the peak was not very high. There were no improvements by increasing temperature as there was no change in the peak areas detected by GC-ECD. The sensitivity of HFBA derivatized products increased with increasing reaction time. Therefore, all the derivatization reactions were carried out at 70 °C for 40 min using HFBA as derivatization agent.

3.4. Method validation

Once the sample preparation conditions were optimized, quality parameters of the GC-ECD and GC–MS methods such as linearity, LODs and LOQs, accuracy and precision were determined.

The linearity and regression study was performed separately for each analyte by spiking into each matrix. Calibration graphs were created using external standard technique by plotting concentration against peak area. The matrix spiked curves showed good linearity (r = 0.9987–0.9989) within the tested range (GC-ECD from 5 to 400 μg/kg and GC–MS from 1 to 200 μg/kg), and it indicates that the method can be used to determine levels of the two compounds over a wide concentration range.

For GC-ECD, the LOD for kidneys and livers of swine, sheep and bovine samples was 5 μg/kg, and the LOQ was 10 μg/kg. For GC–MS, the LOD was 2 μg/kg and the LOQ was 5 μg/kg (see Tables 1 and 2).

Representative GC-ECD chromatograms of blank and blank samples spiked with amitraz at 10 μg/kg in swine liver and kidney are shown in Fig. 3. Representative GC–MS chromatograms of a blank and a spiked blank sample at the 5 μg/kg fortification level in swine liver and kidney are shown in Fig. 4. Since the chromatograms of swine, bovine and sheep edible tissues have no significant differences, we only provided the typical chromatograms of swine.

The optimized method was further validated by applying the extraction procedure to the analysis of spiked samples. Table 1 shows the results for recovery and repeatability of the GC-ECD method over the concentration range (50–300 μg/kg) on 3 separate days, and Table 2 shows the results for the recovery and repeatability of the GC–MS method over the concentration range of 5–20 μg/kg on 3 separate days. Recoveries from edible tissue samples were more than 70%, and between-day RSDs were lower than 15%. According to the specific legislation [21] the method provided a wide concentration range.

3.5. Comparison of GC-ECD and GC–MS for the analysis of amitraz and 2,4-DMA

Spiked sheep liver was used to compare the capability of GC-ECD and GC–MS methods. The acquisition results of 50 μg/kg spiked liver of GC-ECD and GC–MS were investigated, and the result was shown in Table 3. For GC-ECD, the effect of matrices was quite high although it had little effect on the detection of 2,4-DMA at the spiked level. For GC–MS, before the analysis, the standard solutions of two compounds were injected to the GC–MS for full-scan to determine the product ions, and the eluate was injected for ions of quantification (m/z) selection. Finally, m/z 162 (100%), m/z 293 (80%), m/z 121 (95%) and m/z 132 (90%) were selected as mass fragments for amitraz and m/z 121 (100%), m/z 120 (85%), m/z 106 (80%) and m/z 77 (25%) for 2,4-DMA, the spectra are quite clean. According

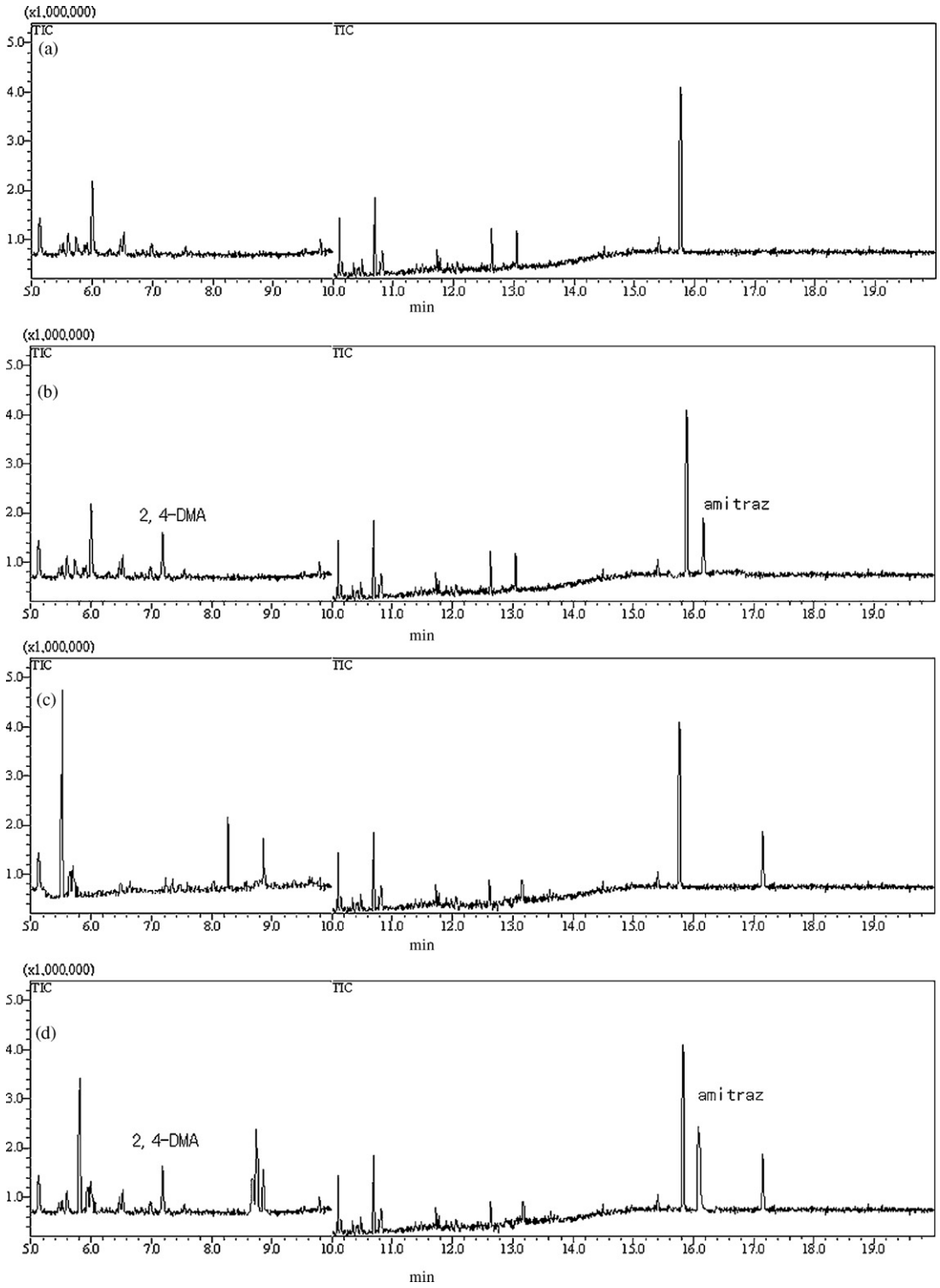

Fig. 4. Typical chromatograms of GC–MS for blank liver (a) and kidney (c) of swine, spiked liver (b) and kidney (d) of swine with amitraz and 2,4-DMA each at 5 μg/kg.

to our calculation, we found that the results of GC-ECD and GC–MS are consisted with each other.

3.6. Stability study

Stability of the analytes was studied in solvent (stock solution), matrix (spiked samples at 50 μg/kg) and eluate. Variations observed in the absorption spectra suggest that amitraz hydrolyzes in basic media as reported by other authors [22,23]. According to our study, the individual stock solutions prepared in *n*-hexane and stored at −20 °C were stable for 2 months. Matrix and eluate solutions were stable for 1 week at 4 °C.

3.7. Analysis of real tissue samples

Using the developed methods, 30 liver samples of bovine and swine were collected and levels of amitraz and 2,4-DMA residues were analyzed by GC-ECD with ASE and calculated. The results are

Table 4
Amitraz residue results in different tissue samples.

Sample	Amitraz (μg/kg)
Swine liver	
MRL	200
Minimum	ND[a]
Maximum	38.5
Average	19.0
Positive (%)[b]	0
Bovine liver	
MRL	200
Minimum	ND
Maximum	209.8
Average	99.7
Positive (%)	1.5

[a] Not detected.
[b] The % of samples above the MRL.

given in Table 4. It shows that amitraz and its metabolite are present at a moderate low level in all these samples, and such moderate content of drugs is not expected to greatly influence the health of edible animals and human beings who consume them.

4. Conclusions

The present study demonstrates that ASE is a simple and reliable extraction technique. Because extraction, clean-up and analysis processes are all carried out in an easy step, the method is efficient for determination of amitraz residues and its metabolite in edible tissues. 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 for GC-ECD were found to be between 72.4 and 101.3%, from 5 to 20 μg/kg levels GC–MS were found to be between 77.4 and 107.1%. The LOD and LOQ are 5 and 10 μg/kg for these two analytes using GC-ECD. For GC–MS, LOD and LOQ were 2 and 5 μg/kg, respectively. The use of GC-ECD combined with GC–MS is recommended for the analysis of large numbers of tissue samples requiring method ruggedness. The rapid and reliable method can be used for characterization and quantification of amitraz and its main metabolite residue, 2,4-dimethylaniline, in liver and kidney samples of swine, sheep and bovine.

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