The Development and Validation of an Analytical Method for Simultaneous Determination of Amitraz and Its Metabolite in Air Samples

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ABSTRACT

Background: To determine the atmospheric contamination by pesticides, conducting laboratory studies is necessary before operating field studies. The aim of this research study was to develop an analytical method to sample and simultaneously determine airborne amitraz and its metabolite.

Methods: A modified fritted impinger with acetonitrile as the liquid sorbent was used in order to study the air concentration of amitraz. Air samples were extracted using a rotary evaporator and then under a soft stream of nitrogen gas. The determination of amitraz and its metabolite in the air samples was made using gas chromatography–mass spectrometer (GC–MS). Quality control of the method was determined at three concentration levels of 50, 500 and 5000 ng/mL for both analyses. The findings revealed that the average values of extraction efficiency were 97.3% and 97.9% for amitraz and its metabolite, respectively, while the detection limits (LOD) for amitraz and 2,4-dimethylaniline were 0.01µg and 0.009µg per one cubic metre of air, respectively.

Results: Furthermore, the percentage values of accuracy were 97.5% for amitraz and 97.9% for its metabolite, whereas the precision values were determined as 1.4 and 1.2 for amitraz and its metabolite, respectively. In addition, the least stability of amitraz and its metabolite was found at room temperature 25°C, while the most stability was determined at -20°C.

Conclusion: The technique developed was a simple, sensitive, specific, and reproducible one that allowed the determination of low-levels of substances of interest in air samples.

Keywords: Air Samples, Amitraz, Amitraz Metabolite, Development, Validation.

INTRODUCTION

Airborne pesticides have been detected and measured by a large variety of sampling, monitoring and analytical devices (1). Laboratory studies are necessary to be conducted before operating field studies. Seemingly, the choice of analytical procedures will depend on the materials being studied, and therefore, is left to the decision of the investigator. The method must be sufficiently sensitive and properly coupled with the chosen trapping and extraction procedures (2). In this regard, many analytical methods have been developed to determine the atmospheric contamination by pesticides(3-8).In the analysis, the air is drawn through an adsorbent and the pesticide is extracted and analysed later (9). Gas chromatography (GC) using a GC detector is one of the most common methods for determination of organic concentration in the atmosphere (10).

Apparently, the problems with personal measurements are that they are costly and time consuming. Besides, proper monitoring devices are not available for all pesticides in the atmosphere (11). Notably, sampling time and air volume sampled for pesticide assessments are dominant factors in determining air sampling method (9). However, a pump which is capable of producing airflow of about

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2 L/min should be used and its batteries should be capable of sustaining maximum airflow for at least four hours without recharging (2). Following this, many researchers have studied different air samplers for pesticide assessment in the atmosphere. These samplers have been reported to involve the use of liquid or solid adsorbents and impingers (6,12).

In laboratory, solvent free samplers generally give higher results than impingers and bubblers. In spite of that, most field comparisons have found impinger and bubbler methods to give higher results than solvent-free methods (12). Although no studies have reported the efficiency of midget impingers for collecting airborne amitraz, using impinger is a method that has been common for collecting pesticides from the atmosphere during the past three decades (1).

Amitraz is a non-systemic formamidine insecticide and acaricide with contact and respiratory action (13). Amitraz under the propriety name Mitac, 20% emulsifable concentrate, has been widely used in Iran for the last 15 years for controlling pistachio pests. Hence, the aim of this study was to develop a method to sample and simultaneously determine airborne amitraz and its environmentally stable metabolite (i.e. 2,4-dimethylaniline).

MATERIALS AND METHODS

Air Sampling

A liquid sorbent and a modified fritted impinger were used in this study in order to investigate the air concentration of amitraz. The SKC impinger with a fritted nozzle was modified. The head of impinger was adapted to a 250 ml round bottom flask. The modified impinger associated with a mini pump air sampler at 2L min^{-1} flow rate was used for air sampling. In addition, the SKC midget impinger had a cylindrical fritted nozzle tip, where it functioned to increase the contact surface between the aerosol and liquid, and subsequently increased the collection efficiency of the impinger (14). The airflow was 2 l/m of air sampling according to Briand *et al*. (2002), while the impinger was filled with 60mL acetonitrile. To protect the pump from splashing impinger liquid, a standard impinger as a trap was installed between the impinger and the pump. The outlet of the modified impinger was connected by a tube to the inlet of the trap. On the other hand, this trap outlet was connected by a short piece of tube to the pump's inlet.

Retention Efficiency Test

To ensure that the collected material was not lost from the medium during sampling, the compounds were tested for breakthrough. This was done by analyzing for any residue that was collected by a trap (the second impinger) placed downstream to the medium being tested (the first impinger). Conversion/collection efficiency studies were carried out by attaching the spiked sampling media to the sampling pumps and pulling air through the sampling media for two hours. For determination of retention efficiency test on amitraz, five impingers containing 60mL of acetonitrile were each spiked with 8.0µg/mL of amitraz, 2,4-dimethylaniline and thymol. Air, 240 L at 2 L/min, was drawn through the solutions. Blank acetonitrile-impingers were used as the backup traps.

Conversely, the backup impingers also contained 60mL acetonitrile. Trapping experiments were run under laboratory conditions of 25°C and approximately 35% humidity. The solutions were then analyzed utilising GC-MS. The retention efficiency was calculated using the following equation (15).

$$\text{Retention efficiency} = \frac{\text{amt trapped}}{\text{Amt fortified} - \text{amt recovered in backup trap}} \times 100$$

where the amount that actually evaporated was the original amount fortified in the impinger minus the amount found in the impinger after the experiment was completed.

Air Samples Extraction Procedure

The round-bottom flask was attached to a rotary evaporator and the sample was

evaporated to around 3.0mL at 50°C. The sample solution for each impinger was transferred to a separate 6.0-mL glass tube with a Teflon cap. Then, each impinger was washed using 1.5 mL of acetonitrile. This process was repeated and combined with the appropriate sample solution. After that, the solvent was removed under a soft stream of nitrogen gas for about five minutes without heating it.

The evaporation process was stopped when 1.0ml of solution remained. A 20-µl volume of internal standard solution thymol (500 ng/mL in acetonitrile) was added to the extract, and later, the caps of glass tubes were kept tight and wrapped with aluminium foil and immediately shipped out to a place for analysis. Finally, quantification and confirmation of results were made using gas chromatography–mass spectrometer (GC–MS). All glassware used for the laboratory analysis was detergent-washed and thoroughly rinsed with tap water and distilled water.

The impingers were also detergent-washed and rinsed with tap water and distilled water before reuse.

GC-MS Analysis

Standard Solution of Chemicals

Concentrated stock solutions of 1µg mL^{-1} were prepared by diluting pure amitraz and 2,4-dimethylaniline in acetonitrile.

GC-MS Apparatus and Conditions

An analysis was carried out on a GC system coupled with quadruple mass spectrometer (GCMS-QP5050, Shimadzu Corporation, Japan). The compounds were separated on ZB-Multiresidue-1 capillary column (Phenomenex, USA, 30m×0.25mm i.d.×0.25µm film thickness). The injection, GC–MS interface, and ion source temperatures were 280, 230 and 230°C, respectively.

The GC oven temperature programme utilized an initial temperature of 100°C and an initial holding time of 5 minutes. Subsequently, the temperature was increased from 20°C/min to 136°C at which it was held for 2 minutes, and then increased from 20°C/minutes to 300°C and held for 5 minutes. Helium was used as the carrier gas with a linear speed of 25cm/s. Amitraz and its metabolite, 2,4-dimethylaniline, were analyzed using a selected full scan mode, where the ionizing energy was 70eV. 1µL aliquot of each extract was injected into gas chromatograph, and notably, the injection was splitless. During the analysis, the mass spectrometer was calibrated weekly.

Injection

One microliter aliquot of the sample solution was injected into the gas chromatograph. The syringe was cleaned with pure acetonitrile and dried thoroughly between injections; hence, it was ready for use to take up the sample for injection.

Measurement of Peak Area

The peak area was measured by the area under the resulting peak, and compared with the areas obtained from the injection of standards to prepare for calibration curve as discussed below.

Calibration Curves

An eight-point standard calibration curve was made by the analysis of amitraz and 2,4-dimethylaniline. Standard solutions of both analytes were prepared by dissolving the above compounds in acetonitrile to yield final concentrations of 50, 250, 500, 1000, 2000, 4000, 8000 and 10000 ng/mL. Thymol (500 ng/mL) was used as the internal analytical standard.

Furthermore, an addition of only acetonitrile (C=0) was used as control. Meanwhile, peak area ratio (PAR) was obtained from the GC-MS analysis of each compound at different concentrations (ng/mL). Calibration curves were constructed by plotting with the PAR of the analyses and IS on the Y-axis and concentration on the X-axis.

Linearity

A series of calibration standards were used to determine the linearity for amitraz and 2,4-dimethylaniline. The linearity of each analysis was calculated by using linear regression equation. After simultaneous analyses of amitraz and its metabolite in the air samples, parameters, such as the intercept, the slope of linear function as a mean, the standard error of the mean (SEM), and the linear correlation coefficient (r), were assessed.

Calculations

The concentrations of analyte for the samples were obtained from the calibration curve in terms of micrograms of amitraz per sample. The air concentrations were calculated using the following formula:

$$\mu g/m^3 = \frac{\text{(micrograms of amitraz per sample) (1000)}}{\text{(litres of air sampled)}}$$

$$ppb = (\mu g/m^3)(24.46)/(293) = (\mu g/m^3)(0.0835)$$

where 24.46 = molar volume (litres) at 25°C and 760 mm Hg
293 = molecular weight of amitraz

Quality Control of Method

Methods should be validated before use to ensure they give results with accuracy appropriate to the measurement task (16). Quality control was determined in this study based on the method that was described by Watson (2005). The initial spike solutions were prepared by dissolving the chemicals, including amitraz and 2,4-dimethylaniline, in acetonitrile to yield three concentration levels of 50, 500, and 5000 ng/mL as the quality control (QC) samples. Meanwhile, blank samples were used as control for each test.

Recovery Efficiency

The recovery of the compound was necessary to eliminate any bias in the analytical method. For this reason, the extraction recovery had to be determined in duplicate and had to cover the concentration ranges of interest. If the recovery were less than 95%, the appropriate correction factor had to be done to determine the true value. The extraction recovery was determined by comparing the peak area ratios of amitraz and 2,4-dimethylaniline with the IS of the extracted samples with the peak-area ratios obtained from direct injection of a standard solution containing the same concentration of amitraz or its metabolite and the IS (500 ng). Hence, to determine recovery, three impingers were spiked with the analyses in order to yield 0.05, 0.5 and 5.0 µg/mL concentrations.

Amitraz and metabolite were diluted in acetonitrile and extracted with the same procedure as previously described. Seven replicates were made at each fortification to calculate the mean and standard deviation of recovery. A parallel blank was also prepared except that no sample was added to it. The recovery efficiency was calculated using the following equation (17):

$$\text{Recovery}\% = \frac{(OC_{extract} / IS_{extract})}{(OC_{spike} / IS_{spike})} \times 100\%$$

Where;
OC extract = peak area for the organic compound (OC) in the extract
IS extract = peak area for the internal analytical standard in the same extract
OC spike = peak area for the OC in the spike solution
IS spike = peak area for the internal analytical standard in the same spike solution

LOD and LOQ

The limit of detection (LOD) is the minimum concentration of a substance that can be measured and reported with 99% confidence. Five blank samples were extracted and prepared in the same manner of the samples; then 1 microliter of each was injected into GC-MS instrument. The peak area of the biggest noise in chromatographic baseline within a time range of 0.5 minutes before and after the peak (signal) was assessed for each blank sample. The instrument detection limit (IDL) was calculated using the following formula:

IDL (ng/mL) = $x_B + 3SD_B$

Where:

x_B _ = the signal from the analytical blank

SD_B = the SD of the reading for the analytical blank

The criterion for a reading reflecting the presence of an analyte in a sample is that the difference between the reading taken and the reading for the blank should be three times the SD of the blank reading. For this experiment, the LOD was determined for a 4-hour sample taken with a flow rate of 2.00 L/min, and extracted with 1.0 mL of solvent.

$$\text{LOD (ng/ m}^3\text{)} = \frac{\text{IDL (ng/ mL)} \times 1.0\text{ (mL)}}{(2.0\text{ L/min}) \times (60\text{ min/h}) \times (4\text{ h}) \times 1/1000\text{ (m}^3\text{/L)}}$$

The limit of quantification (LOQ) is defined as the smallest amount of analyse which can be quantified reliably with an RSD (Relative Standard Deviation) for repeat measurement of < ± 20% and should give a peak > ten times the standard deviation of the chromatographic baseline during chromatographic analysis. In this research, the instrument quantification limit (IQL) was assessed, as follows:

$$\text{IQL (ng/mL)} = x_B + 10SD_B$$

The LOQ was, however, calculated utilising this formula :

$$\text{LOQ (ng/ m}^3\text{)} = \frac{\text{IQL (ng/ mL)} \times 1.0\text{ (mL)}}{(2.0\text{ L/min}) \times (60\text{ min/h}) \times (4\text{ h}) \times 1/1000\text{ (m3/L)}}$$

The analytical peak in the LOQ sample should be identifiable, discrete, and reproducible with a precision of 20% and an accuracy of 80–120% (18).

Accuracy and Precision

The repeatability is given as the relative standard deviation on the results from the analysis of identical samples by the same operator on the same instrument and within a short period of time (19). Therefore, to evaluate the accuracy and precision, three concentration levels of the QC samples (i.e. 0.05, 0.5, and 5µg/mL) were assessed. The intra-day accuracy and precision were determined by running five replicates of three QC samples in one day, while the inter-day accuracy and precision were assessed by five replicates of these three QC samples on three separate days. The extraction procedure was done as already mentioned for the air sample extraction. The results were tabulated for each analyse by blank air samples (C=0) as control (1 in 10). Following this, a comparison was made between the obtained values and the experimental values.

Accuracy was determined as the percentage difference from the actual percentage of DFA (20). The means of the results were calculated and compared to the spiked value to determine the percentage of DFA according to the following formula:

% DFA = (mean/spiked) 100

On the other hand, the precision was expressed as the percentage relative standard deviation (% RSD).

%RSD = (standard deviation/mean)100

The precision determined at each concentration level should not exceed 15% RSD (18).

Reproducibility

Reproducibility is defined as the percentage of relative standard deviation (%RSD) on the results obtained under reproducibility conditions with the same method on the same sample by different operators within a relatively long period of time (19). Six samples were prepared by solving one microgram of amitraz, 2,4-dimethylaniline and timol (IS) onto the acetonitrile impingers. These samples and a draft copy of the extraction procedure were given to a chemist who was not associated with this study.

These six samples were analyzed by the chemist based on the draft copy of the method already described in this work. Reproducibility was calculated according to the following formula:

% RSD = (standard deviation/mean)100

Reproducibility (%) = 100 - %RSD

Stability

If trapping media were to be stored after exposure, a test for the stability of the

compound of interest must be documented (21). The storage samples were generated by spiking impingers with five replicates of three concentration levels of amitraz and 2,4-dimethylaniline. The storage stability samples were extracted and analyzed by the same methods that were employed for field samples. The impinger solutions were then transferred to the glass vials. The stability of amitraz and 2,4-dimethylaniline in air was studied in dark condition at room temperature 25°C for 48h, 4°C for one week and -20°C for four weeks. The stability was considered acceptable if the mean value was within 15% of the theoretical value at each concentration.

Data Analysis

Statistical analyses were performed using Excel statistical software. P-values < 0.05 (two-tailed) were considered statistically significant. Descriptive data of QC of the analytical methods were presented as arithmetic means and standard deviation (mean ± SD), as well as frequencies. The linearity of each analyse in the air samples was calculated by linear regression equation. The equation of a straight line takes the form:

$$y = a + bx$$

Where:

a is the intercept of the straight line with y axis

b is the slope of the line (22).

RESULTS

Calibration Curves

Calibration graphs of the peak area ratio of the analyses and IS on Y-axis, versus concentration (ng/mL) on X-axis obtained from amitraz and 2,4-dimethylaniline are shown in Figures 1 & 2.

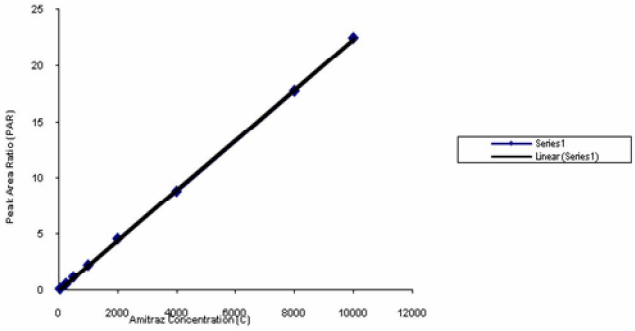

Figure 1. Calibration curve of amitraz in air

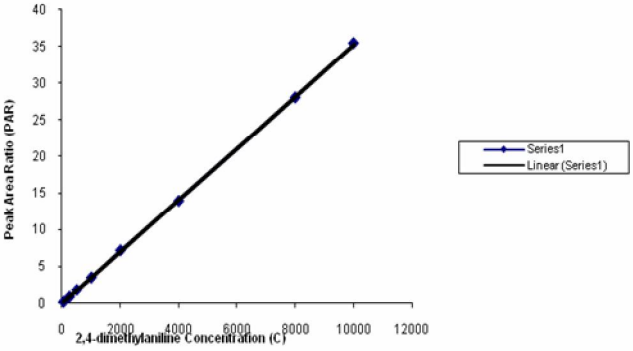

Figure 2. Calibration curve of 2,4-dimethylaniline in air

Linearity

A good linearity was obtained after the simultaneous analysis of amitraz and its metabolite in the air. For amitraz over the concentration range of 50–1000 ng/ml, the intercept was 0.0058 (SEM: 0.0483), the slope was 0.0022 (SEM: 1.0048E-05) and $r = 0.999$. Therefore, the estimated model was as follows: $Y = 0.002x + 0.005$. Similarly, for 2,4-dimethylaniline over the same concentration

range, the intercept was 0.0116 (SEM: 0.0639), the slope was 0.0035 (SEM: 1.3289E-05) and *r* = 0.999. Thus, the estimated model was: Y = 0.003x - 0.011. The r of 0.999 implies that the predictor variable explained about 99% of the variance/variation in the PAR (Y). This was quite a good and respectable result. In addition, the ANOVA data revealed that the F-statistics, i.e. *F* = 49629.23 and *F* = 70583.65 for amitraz and its metabolite, respectively, were very large and the corresponding P-value was highly significant (0.001) or lower than the alpha value of 0.05. This indicates that the slope of the estimated linear regression model line was not equal to zero confirming that the data fitted the proposed simple linear regression model of the study.

Retention Efficiency Test

Seemingly, the collection efficiencies for both compounds were excellent. The top impingers were found to have an average of 99.2 and 98.8% of the spiked amount of amitraz and 2,4-dimethylaniline, respectively. There was no amitraz and its metabolite was not found on any of the backup impingers.

Chromatogram of Standard Solutions

A chromatogram is shown in Figure 3, which reveals the injection of 1000 ng/mL amitraz and 1000 ng/mL 2,4-dimethylaniline standards equivalent to 2.08 µg/m^3 of a 480-L air sample for both analyses. The mass-spectrum of amitraz and 2,4-dimethylaniline is illustrated in Figure 4. The retention time in this chromatogram was 22.64 min for amitraz and this was 7.11 min for its metabolite.

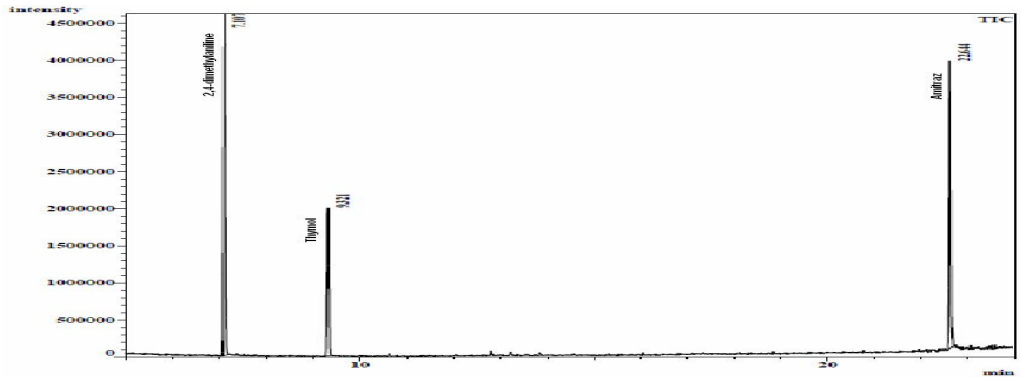

Figure 3. Chromatogram of standard solutions of amitraz and 2,4-dimethylaniline in an air sample

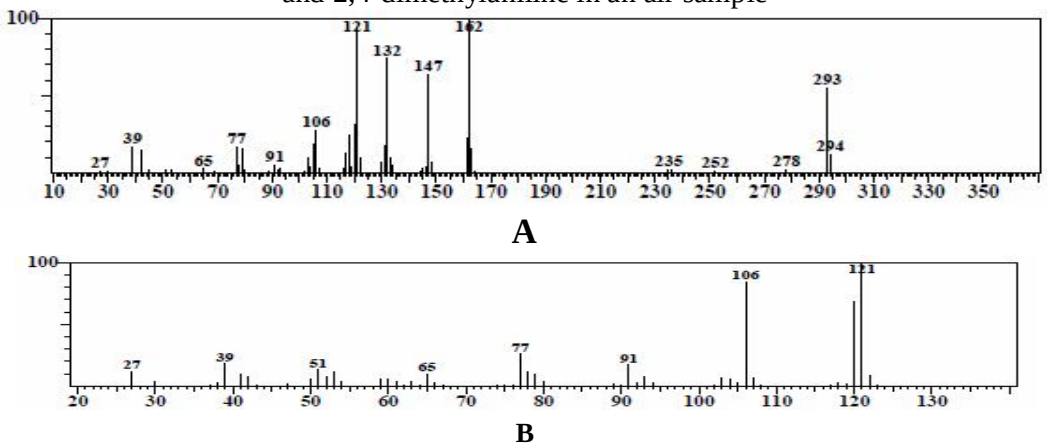

Figure 4. Mass-spectrum of standard solutions of amitraz and 2,4-dimethylaniline in an air sample

Validation Method

Recovery Efficiency

For each concentration (i.e. 0.05, 0.5, and 5.0 µg/mL), seven sample impingers were spiked and analysed with amitraz and 2,4-dimethylaniline in order to determine the extraction efficiency. Acceptable recoveries, ranging from 95.2 to 98.7%, were reported for both spiked analyses. The results are presented

in Table 1. The recovery percentage range was reported from 95.2 to 98.5% for amitraz, whereas this the range for 2,4-dimethylaniline was 96.7 to 98.7%. Meanwhile, the average values of extraction efficiency for the seven impingers spiked at the target concentration were 97.3% and 97.9% for amitraz and its metabolite, respectively. The average recovery values obtained were at least 95.2%, and as such, no recovery correction factor was needed in determining the true values.

Table 1. Recovery of amitraz and 2,4-dimethylaniline in the air samples

Chemicals	µg/mL	Recovery (%)	C.V (%)
Amitraz			
	0.05	95.2 ± 1.1	1.1
	0.5	98.5 ± 1.0	1.0
	5.0	98.3 ± 0.8	0.8
	Mean	97.3 ± 1.0	1.0
2,4-dimethylaniline			
	0.05	96.7 ± 1.0	1.0
	0.5	98.7 ± 0.6	0.6
	5.0	98.2 ± 0.8	0.8
	Mean	97.9 ± 0.8	0.8

LOD and LOQ

The detection limits for amitraz and 2,4-dimethylaniline were 0.01µg and 0.009µg per one cubic metre of air, respectively, whereas, the quantitation limits (LOQ) were 0.014 µg/m^3 for amitraz and 0.011 µg/m^3 for 2,4-dimethylaniline. These amounts were judged to give a measurable response. Apparently, all blanks were less than the LOD. The results of the limit of detection (LOD) and the limit of quantitation (LOQ) are presented in Table 2.

Table 2. Limit of detection (LOD) and quantification (LOQ) of amitraz and its metabolite in the air

Compound	IDL (ng/mL)	LOD (ng/m^3)	IQL (ng/mL)	LOQ (ng/m^3)
Amitraz	4.91	0.01	6.727	0.014
2,4-dimethylaniline	4.171	0.009	5.314	0.011

Accuracy and Precision

Desirable results were obtained for accuracy and precision (Table 3). The percentages of accuracy obtained were 97.5% and 97.9% for amitraz and its metabolite, respectively. Additionally, the precision values were determined as 1.4 for amitraz and 1.2 for its metabolite. Subsequently, the %RSD values were found to be 1.4 for both analytes. Meanwhile, the intra-day accuracy values were 97.5% and 97.7% for amitraz and 2,4-dimethylaniline, respectively. As for inter-day precision, different solutions of amitraz and 2,4-dimethylaniline were injected separately on three different days and the %RSD-values were found to be 0.3 for both chemicals. Evidently, the inter-day accuracy data were 97.8% for amitraz and 98.0% for its metabolite.

Table 3. Accuracy and precision data of amitraz and its metabolite in the air

Type of test	Compound	Concentration (ng/mL) 50 (n=5)	500 (n=5)	5000 (n=5)	Mean
Accuracy (%)	Amitraz	96.0	98.3	98.3	97.5
	Metabolite	96.6	98.6	98.6	97.9
Precision (RSD %)	Amitraz	1.6	1.1	1.4	1.4
	Metabolite	1.2	1.3	1.2	1.2
Intra-day Accuracy (%)	Amitraz	95.8	98.6	98.2	97.5
	Metabolite	96.5	98.6	98.4	97.7
Intra-day Precision (RSD %)	Amitraz	1.6	1.1	1.5	1.4
	Metabolite	1.4	1.5	1.4	1.4
Inter-day Accuracy (%)	Amitraz	96.0	98.7	98.7	97.8
	Metabolite	96.6	98.7	98.6	98.0
Inter-day Precision (RSD %)	Amitraz	0.2	0.3	0.3	0.3
	Metabolite	0.3	0.2	0.3	0.3

Reproducibility

The results of reproducibility test revealed that the precision data were 3.8% and 3.0% for amitraz and 2,4-dimethylaniline, respectively. On the other hand, the reproducibility values were 96.2% for amitraz and 97.0% for its metabolite.

Stability

The results of storage tests for the air samples are summarized in Table 4. It was revealed that the lowest stability data was related to 25°C (room temperature), where the values were 83.5% for amitraz and 91.9% for 2,4-dimethylaniline. Moreover, the recovery of amitraz and its metabolite from the samples used in a 7-day storage test remained above 96%, giving the values 96.6% and 97.9% for amitraz and the metabolite, respectively. In addition, when the samples were stored in -20°C, the recovery was more than 99%.

Table 4. Stability of amitraz and its metabolite in acetonitrile

Chemicals (µg/mL)	25 °C (48 h)	4 °C (1 week)	-20 °C (4 weeks)
	Amitraz		
0.05	82.7 ± 2.9	95.6 ± 2.8	99.5 ± 3.4
0.5	83.6 ± 2.6	96.9 ± 1.5	99.8 ± 1.7
5.0	84.2 ± 3.4	97.2 ± 1.9	99.7 ± 3.0
Mean	83.5	96.6	99.7
	2,4-dimethylaniline		
0.05	92.3 ± 3.7	97.7 ± 1.2	99.6 ± 2.4
0.5	91.7 ± 4.3	97.6 ± 2.2	98.9 ± 1.1
5.0	91.8 ± 5.2	98.5 ± 1.5	99.5 ± 1.7
Mean	91.9	97.9	99.3

DISCUSSION

The methods for the determination of amitraz in air have not been previously described. A general design criterion for a personal sampling device is that it may be small and compact so that the normal daffy functions and jobs can be accomplished with little or no interference from this sampling device (1). Apparently, low-volume samplers

which are generally used for personal monitoring are portable, battery operated, relatively quiet and easy to use. Besides, the flow rates of 0.5–1.5 L/min are typically recommended for pesticides (11). Nevertheless, in our study, the applied airflow was two litres/minute, rather than 1.5 litre/minute so as to ensure the collection of all atmospheric phases of amitraz. The samples should not be considered as valid if the final airflow through the sampling medium is found to be less than 25% of the initial airflow (2).

Different air sampling methods for pesticide determination have been reported, including the use of liquid or solid adsorbents and filters (6). Most field comparisons have found that the impinger and bubbler methods give higher results than the solvent-free methods (12). Midget impingers or bubblers collect many pesticides as aerosols or vapours although they are not well suited to personal sampling since they are cumbersome and breakable and the liquid medium frequently spills during normal work movements. In addition, even though the filters trap aerosols, they do not retain the pesticide vapours. Conversely, solid sorbents retain pesticide vapours but may not efficiently collect or trap aerosol forms (1). Based on these limitations, an air collection method by using an impinger connected to personal samplers was applied for assessing potential inhalation exposure to amitraz.

To design the air sampler, optimising the volatilisation was the first step. The shape of the impinger was important for minimizing sample loss caused by volatilization which can occur during extended sampling periods. For this reason, a round flask of 250ml was chosen for the impinger. In the same way, Durham and Wolfe (1962) stated a method for sampling the air by utilising a modified impinger and using a 500-mL Pyrex glass ball.

The second step was to choose a proper solvent for use in the impinger. The most suitable medium for a particular investigation will depend on the chemicals being studied. In fact, the medium should entrap a high percentage of the chemical passing through it and should allow the elution of a high percentage of the entrapped chemical for analysis. In addition, the chemical should be recovered without any conversion to other reaction products, and the medium should not produce a significant restriction of airflow (2). Notably, since the early 1970s, ethylene glycol has been used as the standard media for collecting the pesticides in the air (23). Cyclohexane has also been used in the impinger for air collection (3). While amitraz is unstable in pure methanol, it is stable in acetonitrile (24). Hence, acetonitrile was chosen as a suitable solvent for air sampling of amitraz.

According to U.S. EPA (1996a), while it would be desirable to know the trapping efficiency of media using aerosols or particulates, no completely satisfactory procedure is currently available for this type of testing. Therefore, when pesticides with very low vapour pressures are investigated for trapping efficiency test, the investigator has to determine the retention efficiency of fortified media rather than the trapping efficiency (2). Studies also showed that not only the type of the collection liquid, but also the volume also affects the collection efficiency. A higher level of liquid means there is more time between bubble formation at the fritted tip and bubble bursting at the surface of the liquid, and thus, more time for particles to diffuse from the air inside the bubbles into the liquid (14). For this reason, the impingers were filled with 60mL acetonitrile in this study. Furthermore, the results of a study by Haraguchi *et al*. (1994) showed that many pesticides exist in a gaseous state rather than in a solid state in air. As mentioned earlier, a retention efficiency test was run for trapping the amitraz by using the impinger.

Amitraz and its metabolite did not identify on backup traps and good efficiency results were obtained on this test.

The methods should be validated before use in order to ensure that they will give accurate results appropriate to the measurement task (16). The results obtained showed that the analyses were not lost in the process. Obtaining recoveries in both analyses were more than 97% and RSD was less than 1%. The extraction efficiency of laboratory fortified controls will be considered acceptable if the lower limit of the 95 percentile interval is greater than 75 percent (2). Following this, the intra-day and inter-day accuracies in this study

were more than 97%. Moreover, the intra-day and inter-day extractions showed consistent recoveries. Evidently, the recoveries for intra-day and inter-day showed that this liquid-liquid extraction method using rotary-evaporator had high precision and consistency. Based on the replicate analysis of the fortified control samples, the methods met the requirements for both intra-day and inter-day accuracies. In addition, the low percentage of RSD values via peak areas confirmed the good precision of the developed method for extracting amitraz and its metabolite in the air samples.

If the extracts from the field samples were to be stored prior to analysis, a documented study of stability had to be made. The fortified media must be stored under the same conditions that would be used for field samples (2). The replicate samples were extracted and analyzed immediately before and at appropriate periods during storage. The samples were found to be stable when stored in the refrigerator at 4°C and freezer at -20°C for seven and thirty days, respectively. The stability percentage of amitraz and its metabolite at room temperature (25°C) was more than 80%. Evidently, this data showed that amitraz was relatively stable in acetonitrile. The analytical methods for air were demonstrated to be valid for the simultaneous determination of amitraz and 2,4-dimethylaniline. This method has been developed to be quick, easy, efficient, and safe.

CONCLUSION

The analytical methods for air were demonstrated to be valid for the simultaneous determination of amitraz and 2,4-dimethylaniline. This study presented a simple, specific, rapid and safe methodology based on gas chromatography analysis and mass spectrometry detection in order to assess the exposure of pesticide applicators to amitraz by inhalation dose measurements. The results obtained showed that the analytes were not lost in the process. The calibration curves were best fitted to a linear curve. The low percentage of RSD values via peak areas confirmed the good precision of the developed method. In addition, the air samples were found to be stable when stored in the refrigerator at 4°C and freezer at -20°C for seven and thirty days, respectively. The device that was used in this study for air collection had some advantages since it was compact and did not interfere with workers' normal daily duties. The findings of this study indicated a very good capability for using a liquid sorbent in direct sampling of amitraz because this solvent can be extracted directly with a liquid-liquid extraction method.

REFERENCES

1. Hill RH, Arnold JE. A personal air sampler for pesticides. Arch Environ Contam Toxicol 1979; 8(5):621-8.
Archives of Environmental Contamination and Toxicology 8 (1979) 621.
2. U.S.EPA, in, United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances (7101) EPA 712-C-96-263, 1996.
3. Briand O, Bertrand F, Seux R, Millet M. Comparison of different sampling techniques for the evaluation of pesticide spray drift in apple orchards. Sci Total Environ 2002 Apr 15;288(3):199-213.
4. Sanusi A, Millet M, Mirabe P, Wortham H. Comparison of atmospheric pesticide concentrations measured at three sampling sites: local, regional and long-range transport. Sci Total Environ 2000 Dec 18; 263(1-3):263-77.
5. Siebers J, Mattusch P. Determination of airborne residues in greenhouses after application of pesticides Chemosphere 1996; 33(8):1597-607.
6. Martínez Vidal JL, Egea Gonzáleza FJ, Glassb CR, Martínez Galeraa M, Castro Cano ML. Analysis of lindane, α- and β-endosulfan and endosulfan sulfate in greenhouse air by gas chromatography. Journal of Chromatography 1997;765(1):99-108.
7. Tsiropoulos NG, Bakeas EB, Raptis V, Batistatou SS. Evaluation of solid sorbents for the determination of fenhexamid, metalaxyl-M, pyrimethanil, malathion and myclobutanil residues in air samples: application to monitoring malathion and fenhexamid dissipation in greenhouse air using C-18 or Supelpak-2 for sampling. Anal Chim Acta 2006; 28:573-4.
8. Turpin BJ, Saxena P, Andrews E. Measuring and simulating particulate organics in the atmosphere: problems and prospects. Atmospheric Environment 2000;34(18):2983-3013.
9. Gil Y, Sinfort C. Emission of pesticides to the air during sprayer application: A bibliographic

review. Atmospheric Environment 2005;39(28):5183-93.
10. Sipin MF, Guazzotti SA, Prather KA. Recent advances and some remaining challenges in analytical chemistry of the atmosphere. Analytical Chemistry 2003;75(12): 2929-40.
11. Hoppin JA, Adgate JL, Eberhart M, Nishioka M, Ryan PB. Environmental exposure assessment of pesticides in farmworker homes. Environ Health Perspect 2006; 114(6):929-35.
12. Streicher RP, Kennedy ER, Lorberau CD. Strategies for the simultaneous collection of vapours and aerosols with emphasis on isocyanate sampling. Analyst 1994; 119(1):89-97.
13. Agin H, ÖCalkavur Ö, Uzu H, y Ba, M. Amitraz Poisoning. Clinical and Laboratory Findings. Indian Paediatrics 2004;41:482 –5.
14. Miljevic B, Modini R, Bottle S, Ristovski ZD. On the efficiency of impingers with fritted nozzle tip for collection of ultrafine particles. Atmospheric Environment 2009; 43(6):1372-6.
15. Hall GL, Mourer CR, Shibamoto T, Fitzell D. Development and validation of an analytical method for Naled and Dichlorvos in air. J. Agric. Food Chem 1997;45:145-8.
16. Harper M.Review Assessing workplace chemical exposures: the role of exposure monitoring J Environ Monit 2004;6(5):404-12
17. Liu S, Pleil JD. Human blood and environmental media screening method for pesticides and polychlorinated biphenyl compounds using liquid extraction and gas chromatography-mass spectrometry analysis. Journal of chromatography 2002;769/1:155-67.
18. Saito T, Yamamoto R, Inoue S, Kishiyama I, Miyazaki S, Nakamoto A, et al. Simultaneous determination of amitraz and its metabolite in human serum by monolithic silica spin column extraction and liquid chromatography-mass spectrometry.J Chromatogr B Analyt Technol Biomed Life Sci 2008;867(1):99-104.
19. Herrmann SS, Poulsen ME. In National Food Institute, The Danish Technical Universi. 2007.
20. Karnes HT, March C.Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. Pharm Res 1993;10(10):1420-6.
21. U.S.EPA in United States Environmental Protection Agency, Washington, D.C., 1996, p.161pp.
22. Watson DG. Pharmaceutical Analysis 2nd ed. Elsevier Churchill Livingstone,2005.
23. Oudbier AJ, Bloomer AW, Price HA, Welch RL. Respiratory route of pesticide exposure as a potential health hazard.Bulletin of Environmental Contamination & Toxicology 1974;12(1):1-9.
24. Pierpoint AC, Hapeman CJ, Torrents. Kinetics and Mechanism of Amitraz Hydrolysis. J Agric Food Chem 1997;45:1937-9.