DETECTION OF CYPERMETHRIN RESIDUES IN TOXICOLOGICAL CONTROL OBJECTS USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY WITH SOLID-PHASE EXTRACTION

IGOR M. FITSEV, OKSANA V. SHLYAMINA, ALSU R. MAKAEVA, GANNA R. NASYBULLINA &
ALEXANDER M. SAIFUTDINOV

*Research Scholar, Federal Center for Toxicological, Radiation and Biological Safety, Scientific Town,
Kazan city, Russia*

ABSTRACT

Method for determination of synthetic pyrethroid (cypermethrin) using gas chromatography-mass spectrometry (GC-MS) in the objects of toxicological control was carried out. The sample preparation via solid-phase extraction (SPE) with the increased capacity / selectivity concentration cartridges «Diapak C18 Plus» was used before GC-MS measurement. Methodological approach was used for quantitative determination of cypermethrin in food material, food end products, and tissues of animal/insect origin. The method is based on combination of GC-MS with electronic ionization (EI, 70 eV) via SPE. It is characterized by cypermethrin limit of detection (LOD) of $3 \mu\text{g}\cdot\text{kg}^{-1}$ and relative standard deviation (RSD) of not higher than 5 %. GC-MS sensitivity of cypermethrin quantitative determination is approached via selective ion monitoring (SIM/SIR-mode) with parameters of $m/z=163$ (principal ion), and $m/z=181, 127$, (confirmation ions) accordingly.

KEYWORDS: Gas Chromatography-Mass Spectrometry; Objects of Toxicological Control; Synthetic Insecticides; Pyrethroids; Cypermethrin; Solid-Phase Extraction

Received: Jun 05, 2020; **Accepted:** Jun 25, 2020; **Published:** Jul 30, 2020; **Paper Id.:** IJMPERDJUN2020529

1. INTRODUCTION.

Cypermethrin-cyano (3-phenoxyphenyl) methyl3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclo-propanecarboxylate-is the second-generation pyrethroid. Despite the fact that cypermethrin first made its appearance on the world market in the beginning of the 1970s, this compound still remains the most utilized insecticide in the world (primarily in the agricultural industry). Cypermethrin as an insecticide / acaricide is manufactured in the form of various commercial products, e.g. “Kinmix”. The latter is a racemic mix of *cis*- and *trans*- isomers (40:60 proportion) of cypermethrin, predominantly *trans*-isomers (beta-cypermethrin). Being a broad-spectrum contact-digestive insecticide against larva and grown insects with chewing and piercing-sucking mouthparts (Tsipi *et al.* 2015) β -cypermethrin and its isomers (just like other synthetic pyrethroids) bind to the lipophilic cell membranes and act on the voltage-gated sodium channel proteins of the nerve cells. The depolarization of the membrane and slowing of sodium channel opening or closing ensues as the result. The speed of sodium ions in such a pyrethroid-modified channel is changing as well as the strong depolarization flow of sodium ions is emerging (due to the CN-group contained in cypermethrin) causing repeating discharges and giving rise to synaptic disruptions (Vijverberg, van den Bercken 1990).

Cypermethrin being a 3rd degree hazard material for humans according to the official classification (Xuanwei *et al.* 2016) is characterized by a widespread application. Even though a variety of methods are

suggested to use for its detection, including gas chromatography (Chen *et al.* 2014; Joia *et al.* 1985; Sassine *et al.* 2004), high performance liquid chromatography (HPLC) with various detectors (Bissacot, Vassilieff 1997; Liu *et al.* 2012; Radford *et al.* 2016), as well as using mass spectrometry of low and high definitions, the task of highly sensitive and selective cypermethrin detection in the objects of toxicological control remains relevant. All-in-all, mass spectrometry method of cypermethrin detection in objects of toxicological control combined with simplicity and affordability of tabletop GC-MS instruments (Fitsev *et al.* 2004; Grimalt, Dehouck 2016; Gurbuz *et al.* 2014; Watson 1976) equipped with the expert database mass-spectra appears to be the optimal solution for its detection in objects of toxicological control.

The goal of this research is the developing a GC-MS cypermethrin detection protocol in the objects of toxicological control using a SPE during the sample preparation.

2. MATERIALS AND METHODS

The following equipment was used: gas chromatograph «Trace 1300» with a mass-spectrometer «DSQ» (Thermo Fisher Scientific, USA), monitoring electronic ionization specters (EI, 70 eV) in the range $m/z=30-550$. Qualitative and quantitative GC-MS analysis was performed on a fused silica column «TraceGold TG-5 MS» (30 m×0.25 mm×0.25µm, Thermo Fisher Scientific, USA). Conditions of GC split: injector temperature – 280 °C, interface temperature – 280 °C, initial thermostat temperature - 70 °C, column's rate of temperature elevation - 10 °C/min, final temperature of column's thermostat – 280 °C, splitless injection, sample size – 1 µl, volume velocity of gas vehicle (He, 99,999%) – 0,9 ml/min – constant flow.

Processing of mass-spectral data was done using software tool «Xcalibur» (Thermo Fisher Scientific, USA), electronic mass-spectrum library «NIST'17», «NIST MS Search Program V. 2.3», «NIST MS Interpreter» (NIST, USA).

Regression analysis of calibration characteristics and mathematical forecasting of peak area response was done using software tool «Origin V. 6.1» (OriginLab Corp, USA), and their processing performed according to the requirements specified in the normative documents (ISO/IEC Guide 98-1 2009; Rabinovich 2017).

In the course of GC-MS research, the following reagents were used for obtaining the calibration curve of “analytical signal - analyte content” as well as for validity and convergence checks: analytical standard «Pestanal®» (98%, CAS №52315-07-8, catalogue №36128, Supelco, USA). Analyte solutions (1000 µg·L⁻¹), as well as working solutions for setting up calibration dependencies were prepared with the absolute acetone of «for HPLC». The following were used as well: hexane, acetonitrile, methanol «for HPLC». Concentration cartridges of increased capacity and selectivity on the basis of sorbent with chemically bonded octadecyl groups «Diapak C18 Plus» were used for SPE (BioChemMac, Russia).

3. RESULTS AND DISCUSSION

GC-MS of analyte standard solution (C=25 µg·L⁻¹) demonstrated the presence of four peaks of total ion current on its chromatogram (Figure 1) which relate accordingly to cis-1 (29.33 min), cis-2 (29.50 min), trans-1 (29.61 min) and trans-2 (29.68 min) respectively diastereomers of cypermethrin. A better split of cis- and trans-isomers in the GC conditions is possible with the use of the greater-length column, however it would not lead to significant results, since the full split of cypermethrin geometrical and optical isomers could be achieved only with the use of Reversed Phase (RP) HPLC (Hu *et al.* 2015; Naobumi *et al.* 1990), which would also allow assessing the biological effectiveness of substances containing cypermethrin based on the information on the content of most active cis-forms. The data presented in figure 1 shows that relative intensity of analytical signals of sum of

cis- and sum of trans- isomers of cypermethrin is characterized by the proportion of 40:60, which is comparable to the data presented in (Kutter, Class 1992; Nosikova, Kochetov 2017; Yu 2000). Mass-spectrum of EI cypermethrin and main directions of dissociative ionization of its molecule are presented in Figure. 2.

Export of mass-spectrum EI peaks with the retention levels 29.33, 29.50, 29.61 and 29.68 min (using software «Xcalibur») into the electronic library of mass-spectrums «NIST'17» and its subsequent identification on the characteristic ions using a *Relative Match Factor (RMF)* generated by software too «NIST MS Search Program» of cypermethrin molecule EI mass-spectrum from the above-mentioned library showed high level of RMF at 930-945 respectively. This circumstance confirms the correct identification of cypermethrin by registering total ion current on its EI mass-spectrum (Figure 2).

The data presented in Figure 2 show that dissociative EI of cypermethrin molecule goes in two competing directions with the forming of ions $m/z=208$ ($C_{14}H_{10}NO^+$, 40 %) and $m/z=207$ ($C_8H_9Cl_2O_2^+$, 39 %). In its turn, the separation of CN-group ($m/z=26$) from $C_{14}H_{10}NO^+$ leads to the formation of ion $m/z=181$ ($C_{13}H_9O^+$, 89 %), and detaching of CO_2 ($m/z=44$) from $C_8H_9Cl_2O_2^+$ comes with the formation of ion $m/z=163$ ($C_7H_9Cl_2^+$, 100 %), which has a maximum intensity in the mass-spectrum of EI cypermethrin molecule. Intensive values $m/z=127$ (45 %) correspond to formation of ion $C_7H_{11}O_2^+$, $m/z=91$ (41 %) – formation of ion $C_7H_7^+$, and $m/z=77$ (33 %) - formation of ion $C_6H_5^+$. Therefore, the ions with $m/z=163$, 181, 127 have the greatest intensity in cypermethrin mass-spectrum.

We subsequently applied the strategy of identifying cypermethrin by its most characteristic and intensive ions of mass-spectrum EI $m/z=163$ (principal ion), $m/z=181$ and $m/z=127$ (confirmation ions) to build the calibration curve «analyte signal – analyte content» for *cis*-1, *cis*-2 and *trans*-1, *trans*-2 isomers of cypermethrin in order to utilize it for the their detection in the objects of toxicological control.

Experimental equations for calibration curves for the sum of cypermethrin *cis*-1, *cis*-2 and *trans*-1, *trans*-2 isomers, received during calibration of MS-detector «DSQ» using analytical standard «Pestanal®» in selective ion monitoring mode (m/z : 163, 181, 127) as well as their metrological characteristics are presented in Table 1.

The data provided in Table 1 show that the LOD of all forms of cypermethrin for GC-MS and calculation with 3·S-criterium was $3 \mu g \cdot kg^{-1}$.

The check of the extraction, accuracy, convergence and results' reproducibility for the determination of cypermethrin *cis*- and *trans*- isomers was done using an «Injected - Detected» method (see Table 2) after the SPE stage according to current approach in analytical chemistry (Goncalves, Alpendurada 2004; Mogadati et al. 1999; Redondo et al. 1996).

SPE using «Diapak C18» cartridges included a standard flushing procedure by 10 ml of acetonitrile, then with an acetonitrile-water mix (1:1) and subsequent elution with 4 portions of 5 ml of the acetonitrile-water mix (4:1) through the cartridge of cypermethrin's acetonitrile-water solution. The fractions collected in the glass receiver were evaporated dry via a rotary evaporator under the temperature of 30 °C, and subsequently dissolved into 1 ml of acetone and analyzed under GC-MS protocol with the registration of selective ions. After receiving the GC-MS analysis results, the analyte content in the sample was calculated as well as the completeness of its extraction from cartridge and the necessary volume of eluent which amounted to 15 ml. The results provided in Table 2 show that completeness of cypermethrin *cis*- and *trans*- isomers' extraction amounts to 94.3 ± 6.1 %.

The developed method of GC-MS cypermethrin detection with SPE sample preparation was applied to analyze the

sample of raw food materials and food products, as well as to the tissues of animal and insects. Determination results are provided in Table 3.

Figure 1: Total Ion Chromatogram (TIC MS) of Analytical Standard Cypermethrin Solution (C=25 Mg,L-1) Cis-1 (29.33 Min), Cis-2 (29.50 Min), Trans-1 (29.61 Min) and Trans-2 (29.68 Min) Respectively Diastereomers of Cypermethrin.

Figure 2: Graphical Representation of EI Mass-Spectrum (70 Ev) and Main Directions of Cypermethrin Molecule's Dissociative Ionization.

Table 1: Characteristics of Calibration Curves for the Cypermethrin Cis-1, Cis-2 and Trans-1, Trans-2 Isomers Received Using Analyte Standard Solutions for MS-Detector «DSQ II»

Determined Compound Form	Ordinary Least Squares Estimation Equation $Y = (A \pm S_a) + (B \pm S_b) \cdot X$, Where Y Is The Relative Ionic Current And X Is The Cypermethrin Concentration, Mg/Kg	Correlation Coefficient (R^2)	LOD (Mg·Kg ⁻¹)	LOQ* (Mg·Kg ⁻¹)
cis-1 isomer	$y = (424.3 \pm 62.3) + (62439,4 \pm 113.2) \cdot x$	0.998 (n=10)	3.0	8.0
cis-2 isomer	$y = (494.0 \pm 52.4) + (51465,2 \pm 125.1) \cdot x$	0.999 (n=10)	3.0	8.0
trans-1 isomer	$y = (489.2 \pm 71.2) + (75116.3 \pm 120.1) \cdot x$	0.997 (n=10)	3.0	8.0
trans-2 isomer	$y = (411.4 \pm 44.2) + (45465.3 \pm 105.1) \cdot x$	0.999 (n=10)	3.0	8.0

Limit of Quantitification

Table 2 Results of Cypermethrin Detection Recovery Rate After Applying SPE «Injected – Detected» Method, Confidence Interval for the Average and Its Standard Deviation Under GC-MS Conditions (by the Sum of Cis- and Trans- Isomers) (N=10, P=0,95)

Injected, $\text{Mg} \cdot \text{L}^{-1}$	Detected, $(\bar{x} \pm \Delta_{\bar{x}}), \text{Mg} \cdot \text{L}^{-1}$	Average Detection Rate, $\bar{x}, \%$	Average Detection Rate Confidence Interval, $\pm \Delta_{\bar{x}}, \%$	Standard Deviation, $S, \%$
$3.00 \cdot 10^{-2}$	$(2.80 \pm 0.19) \cdot 10^{-2}$	94.3	± 6.2	4.2
$5.00 \cdot 10^{-2}$	$(4.72 \pm 0.30) \cdot 10^{-2}$	94.4	± 5.9	4.1
$6.00 \cdot 10^{-2}$	$(5.70 \pm 0.40) \cdot 10^{-2}$	94.2	± 6.1	4.0
$1.00 \cdot 10^{-1}$	$(9.43 \pm 0.62) \cdot 10^{-2}$	94.3	± 6.2	4.0
$1.20 \cdot 10^{-1}$	$(1.13 \pm 0.08) \cdot 10^{-1}$	94.4	± 6.3	4.0

Table 3 Results of Cypermethrin Content Determination (By the Sum of Cis- and Trans-Isomers) in Objects of Toxicological Control (Raw Food Material, Food Products, Animal and Insect Tissues). (N=6, P=0, 95)

Research Object	Found, $(\bar{x} \pm \Delta_{\bar{x}}), \text{Mg} \cdot \text{Kg}^{-1}$	Standard Deviation, $S, \%$	Toxicological Data – Maximum Admissible Concentration, $\text{Mg} \cdot \text{Kg}^{-1}$ According To Criteria82 Cypermethrin (Albert <i>Et Al.</i> 1989)
All parts (except for the root system) of the plant Hordeum (barley) vegetation / seed formation period	$(9.20 \pm 0.60) \cdot 10^{-1}$	4.0	2.0
All parts (except for the root system) of the plant Brassica napus (Colza) vegetation / blossoming period	$(0.90 \pm 0.05) \cdot 10^{-1}$	4.1	0.1
Aboveground portion of Solánium tuberósum (Potato) vegetation / tuberization period	$(1.00 \pm 0.05) \cdot 10^{-1}$	4.0	-
Plant tuber Solánium tuberósum (Potato) egetation / tuberization period	$(0.22 \pm 0.02) \cdot 10^{-1}$	4.0	0.05
Chicken egg	$(0.51 \pm 0.03) \cdot 10^{-1}$	4.0	0.10
Domestic fowl tissue Gallus domesticus (Chicken)	$(0.92 \pm 0.06) \cdot 10^{-1}$	4.0	0.05
Insect tissue Apis mellifera (honey bee)	$(0.23 \pm 0.05) \cdot 10^{-1}$	4.0	$\text{LD}_{50} 3.3 \cdot 10^{-5} \text{ mg} \square / \text{individual}$
Laboratory animal tissue Ratus (rat)	$(2.53 \pm 0.03) \cdot 10^{-1}$	4.0	LD_{50} for rats 250-300 mg

4. CONCLUSIONS

Method for GC-MS quantitative determination of cypermethrin in objects of toxicological control (raw food material, food products, animal and insect tissues) with SPE stage for sample preparation on the basis of sorbent with chemically bonded octadecyl groups in cartridges of «Diapak C18 Plus» was developed. For quantitative determination of cypermethrin by GC-MS (EI, 70 eV) monitoring of selective ions was used with parameters of $m/z=163$ (principal ion), and $m/z=181, 127$, (confirmation ions), which allowed achieving the cypermethrin LOQ of $8 \mu\text{g} \cdot \text{kg}^{-1}$.

5. ACKNOWLEDGMENTS

The team of authors expresses appreciation for the analytical equipment used in the present work of the Test Center of the Federal Center for Toxicological, Radiation and Biological Safety (Kazan).

Conflict of Interest: I.M. Fitsev declares that he has no conflict of interest. O.V. Shlyamina declares that she has no conflict of interest. A.R. Makaeva declares that she has no conflict of interest. G.R. Nasybullina declares that he has no conflict of interest. A.M. Saifutdinov declares that he has no conflict of interest

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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