

BIPM Capacity Building & Knowledge Transfer Program

2022 BIPM - TÜBİTAK UME Project Placement

REPORT

Project Name	Improvement of skills and knowledge transfer in the field of organic chemistry with reference to pesticide analysis using gas and liquid chromatography techniques.
Description	Project consisted of the following parts: preparation of primary stock solutions and calibration solutions in previously defined ranges, optimization of instrumental parameters, method development for different groups of pesticides, and training for method validation and measurement uncertainty calculation.
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Motivation & Introduction

The main purpose of this project placement is improvement of technical skills and knowledge transfer in the field of organic chemistry with reference to pesticide analysis using chromatography techniques such as GC-MS/MS (gas chromatography coupled with mass spectrometry) and HPLC-MS (high-performance liquid chromatography coupled with mass spectrometry). We are familiar that all substances that are used against living organisms that harm plants and animals are called pesticides. Pesticides are among the most important contaminants worldwide due to their wide use, persistence, and toxicity. Improper use of pesticides may cause pesticide residues in soil and that is becoming an increasing risk regarding environment but also food safety, since such residues can migrate from soils to food and thus affect the health of consumers.

Considering these facts, developing accurate testing methods for pesticide residue analysis using chromatography techniques and preparing certified reference materials of pesticides in solvent or different matrices can be of great importance when it comes to providing support and metrological traceability for control laboratories. Since the Laboratory for Organic Chemistry within TÜBİTAK UME conducts method development, method validation studies, determines uncertainty values for new measurement methods and has published CMCs in the area of interest for the project, gained knowledge and experience will improve scientific and technical capabilities of IMBIH related to this topic.

It is important to mention that this project placement was part of a wider project already conducting in TÜBİTAK UME which, as a final result, has preparation of certified reference materials (CRMs) of pesticides in the solvent. Our laboratory work regarding stock solutions preparation, instrumental optimization and method development for different groups of pesticides will be the basis for the further process of preparing certified reference materials of pesticides in solvent that will serve mainly to food control laboratories throughout Turkey.

Research

Method development was conducted for two groups of pesticides: pyrethroids (13 analytes) and triazoles (20 analytes). For the third group (neonicotinoids) we managed to optimize some of the measurement conditions. Further research and optimization is needed in order to obtain necessary information about target compounds and linear range of calibration solutions. Methods for pyrethroids and triazoles were developed using Thermo Scientific Trace GC Ultra coupled with TSQ Quantum XLS system for mass spectroscopy (Figure 2).

Preparation of primary stock solutions and calibration solutions

For the purpose of method development individual stock solutions (1000 mg/kg^{-1}) of each pesticide were prepared by dilution in acetonitrile ($\geq 99.9\%$, HPLC grade, HiPerSolv CHROMANORM). Stock solutions were prepared from the initial solid pesticide reference compounds purchased from Dr. Ehrenstorfer (*LGC Standards*), LabStandard (*LabInstruments*) and Sigma Aldrich. These steps were followed by preparation of mixed standard solution for each group of pesticides, and calibration solutions were prepared by diluting mixed standard solution in acetonitrile at five concentration levels for each group in linear range from 0.01 to 0.2 mg/kg^{-1} . All solutions were prepared gravimetrically using Mettler Toledo XP56 balance ($d=0.001 \text{ mg}$) (Figure 1).

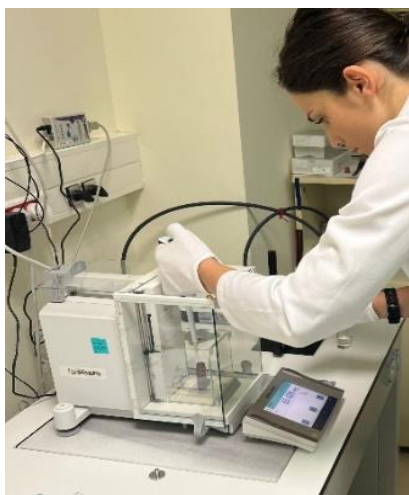


Figure 1. Preparation of stock solutions; **Figure 2.** GC-MS/MS (up) and HPLC-MS system (down);

Optimization of measurement conditions for method development on the GC-MS/MS system

Individual stock solutions, as well as mixed standard solutions for each group of pesticides were tested in full scan mode ($50\text{-}500 \text{ m/z}$ ratio) for the determination of the precursor ions and optimization of the MS/MS measurement conditions for each compound. Selected precursor and product ions as well as adequate collision energies were applied in selective reaction monitoring (SRM) method (Table 1). Selected reaction monitoring (SRM) is generally performed technique on triple quadrupole instruments in which fragmentation is used as a means to increase selectivity. This monitoring offers significant improvements for the screening and confirmation of pesticide residues especially in more complex matrices. Optimized SRM method was used for calibration solutions analysis and generation of linear calibration curve. Since internal standard calibration wasn't used, each concentration was analyzed in five replicates in order to not overlook the error coming from the solvent evaporation.

Chromatographic separation was carried out using Agilent Technologies capillary HP-5MS column (-60 to 325°C, 30m × 250µm and film thickness 0.25µm). Helium gas (99.999% purity) was used as the carrier gas at constant flow rate 1.00 ml/min. Injection volume of 1 µL was employed in splitless injection mode at 280°C. The oven temperature for pyrethroids method was programmed from 60°C (0 min hold) to 150°C (0 min hold) at a rate of 30°C/min, and then to 290°C at a rate of 10 °C/min (6 min hold). For triazoles temperature program was optimized as follows: from 100°C (2 min hold) to 200°C (0 min hold) at a rate of 20°C/min, and then to 280°C at a rate of 10°C/min (5 min hold). Equilibration time was 0.10 minutes, and solvent cut-off time was 10 minutes in order to avoid the peak from solvent. Before and after each injection three washes of needle with solvent (acetonitrile) were employed (solvent volume was 2µL). The MS transfer line temperature was 280°C, and ion source temperature was 230°C. *Xcalibur* software was used for instrument control and data analysis.

Table 1. Summary of SRM method operating parameters on GC-MS/MS (P-pyrethroids, T-triazoles)

Analyte	Pest. group	Retention time (HP-5MS column)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
Tefluthrin	P	8.21	197.03	140.92	10
Tetramethrin	P	13.74, 13.89	163.99	106.96	20
Bifenthrin	P	13.89	181.03	166.09	15
Fenpropathrin	P	14.00	265.07	210.10	15
Cyhalothrin	P	14.85	208.09	181.05	12
Acrinathrin	P	15.05	208.08	181.00	10
Permethrin	P	15.57, 15.69	183.01	167.96	20
Cyfluthrin	P	16.16, 16.25, 16.33, 16.36	226.13	206.06	10
Cypermethrin	P	16.47, 16.57, 16.65, 16.68	208.04	181.01	10
Etofenprox	P	16.77	163.00	134.97	15
Esfenvalerate	P	17.65	419.20	225.10	5
Tau-fluvalinate	P	17.66, 17.72	250.09	54.96	25
Deltamethrin	P	18.33	252.92	92.97	25
Triadimefon	T	9.35	208.05	180.84	10
Tetraconazole	T	9.40	336.13	204.02	50
Penconazole	T	9.79	247.85	191.86	30
Triadimenol	T	9.95, 10.04	167.07	69.86	10
Paclobutrazol	T	10.25	236.09	124.80	10
Flutriafol	T	10.45	219.15	122.92	15
Hexaconazole	T	10.56	231.93	158.73	40
Myclobutanil	T	10.82	178.92	152.06	15
Flusilazole	T	10.87	314.04	233.31	15
Cyproconazole	T	11.07	222.24	124.93	20
Diniconazole	T	11.34	267.93	232.03	20
Propiconazole	T	11.93, 12.04	259.41	172.85	12
Tebuconazole	T	12.22	250.21	124.94	20
Epoxiconazole	T	12.52	192.06	137.93	15
Bromuconazole	T	12.78, 13.20	294.88	172.87	15
Triticonazole	T	13.43	235.01	214.99	15
Fluquinconazole	T	14.47	339.93	107.78	20
Bitertanol	T	14.47, 14.56	169.77	141.08	30
Fenbuconazole	T	15.15	198.15	129.01	5
Difenconazole	T	17.27, 17.38	322.91	265.29	15

Optimization of measurement conditions for method development on the HPLC-MS system

Optimization of measurement parameters was carried out using Zivak Technologies HPLC system coupled with Zivak Tandem Gold Triple quadrupole mass spectrometer (Figure 2). For chromatographic separation selected column was Troyasil C18 (100x4mm diameter and 5 μ m particle size). For the first trial, methanol with 0.1% formic acid (A), and water with 0.1% formic acid and 5% methanol (B) were used as elution solvents. The flow rate was 400 μ L/min and column was thermostated at the temperature of 35°C. Isocratic elution was applied with 90% mobile phase A and 10% mobile phase B for 15 minutes. Since the obtained peaks in the full scan mode (100-700 m/z ratio) for individual compounds were not good, further optimization regarding elution solvents, but also, heating and nebulizer gas pressure are needed. Research and activities regarding this method will continue considering the final purpose of the project which is preparation of certified reference materials of pesticides in solvent (CRMs).

Method validation and measurement uncertainty calculation

Besides method development, project placement included training on method validation and calculation of measurement uncertainty, using methods developed on the GC-MS/MS system as very useful example. For this purpose Eurachem Guides: *The fitness for purpose of analytical methods* (2nd ed., 2014) and *Quantifying Uncertainty in Analytical Measurement* (3rd ed., 2012) were used. All calculations were carried out using Microsoft Excel program. Also, during the project we had the opportunity to attend the e-learning course regarding CIPM MRA framework and all relevant aspects concerning measurement traceability, calibration and measurement capabilities (CMCs), CIPM, RMO and supplementary comparisons.

Conclusions and Future Work

Gained knowledge and experience about pesticides analysis will contribute to the technical capabilities and research potential of IMBIH. Conducting this type of analysis in Laboratory for Chemistry (IMBIH) could lead to significant improvements when it comes to providing support and metrological traceability for control laboratories within our country, participation in Proficiency Testing schemes and publication of the new CMCs in the future.

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