Service of Forensic Chemistry and Toxicology
National Institute of Legal Medicine and Forensic Sciences, I.P. - Portugal

Development and validation of a multi-substance method for routine analysis of pesticides in *post-mortem* samples by UPLC-MS/MS

Francisco Matos*[1,2], Mário Barroso[1], Mónica Antunes[1,3], João Franco[1], Suzana Fonseca[1]

(1) National Institute of Legal Medicine and Forensic Sciences, I.P. South Branch, Portugal; (2) Faculty of Sciences and Technology, University of Coimbra, Portugal; CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Portugal (3)

*Corresponding author: ciscomatos@gmail.com

INTRODUCTION

Pesticides play an important role in forensic toxicology and are usually classified as a single class of chemicals, but in fact there are several different types of compounds, including organophosphates, carbamates, pyrethroids or organochlorines, among others, with different toxicities. Pesticide analysis in *post-mortem* samples can be difficult due to the complexity of the samples and to the high toxicity of these compounds. The aim of this study was to develop and validate an easy to use, sensitive, and robust method, using ultra-performance liquid chromatography-tandem mass spectrometry to be incorporated in the routine flow for more than fifty pesticide analysis in *post-mortem* blood.

METHODS

Sample Preparation

- 100 µL MeOH/H_2O (1:1, v/v)
- 10 µL Internal Standard
- Standard Mixture
- 100 µL Whole Blood

Protein Precipitation and Post-PPT

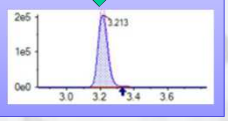
- 900 µL refrigerated Acetonitrile
- Centrifuge: 10 min, 14000 rpm
- Vial: 200 µL Mobile Phase 50 µL Supernatant

Intrumental Analysis by UPLC-QTRAP-MS® 6500+ Sciex

10 µL

Flow: 0,4 mL/min

Time	A	B
0	90	10
0,5	90	10
8	5	95
11	5	95
11,1	90	10
14	90	10



RESULTS

The developed method was linear from 10 to 200 ng/mL (fig. 1) with a weighting factor of $1/x^2$ for the 15 compounds listed in the table. The limits of quantitation were 10 ng/mL. It was successfully evaluated a dilution ratio of 1 to 2, 5 and 10. No interference was observed analysing spiked samples containing more than 250 therapeutic drugs and drugs of abuse. No carryover was observed after sequential injection with all analytes. All substances showed a stability of one month when stored in the freezer.

	Q1	Q3	RT (min)	DP (V)	CE (V)	CXP (V)	Precision (CV %)	BIAS (%)	Ionization Enhancement (%)	LOD (ng/mL)
Acetamiprid	223	126	4,5	61	42	8	4,4	-4,7	11,6	1
Azinphos-ethyl	346	160	7,5	36	11	12	7,1	-5,9	18,3	1
Bendiocarb	224	167	5,8	51	13	12	7,1	-5,0	7,2	1
Carbofuran	222	165	5,9	16	17	10	6,6	-4,3	8,5	1
Chlorfenvinphos	359	155	7,9	74	17	10	5,9	-2,9	17,4	1
Dimethoate	230	125	4,4	51	29	8	5,5	-5,7	10,9	1
Imidacloprid	256	209	4,1	46	23	20	5,7	-5,1	12,4	1
Malathion	331	127	7,2	64	10	10	6,9	-5,4	11,7	1
Methiocarb	226	169	7,0	64	13	10	5,9	-4,6	11,8	1
Methomyl	163	88	3,3	26	13	10	5,2	-6,0	9,6	10
Parathion	292	264	7,7	41	15	16	7,0	0,8	21,9	10
Pirimicarb	239	182	5,4	51	23	10	6,1	-5,4	9,2	1
Strychnine	335	184	3,6	140	50	10	5,5	-5,3	-	10
Tetrachlorvinphos	367	127	7,8	46	21	4	6,1	-5,0	14,5	1
Thiacloprid	253	126	4,7	61	25	20	5,5	-5,4	12,0	1

Fig. 1 – Calibration curve and residues for Carbofuran

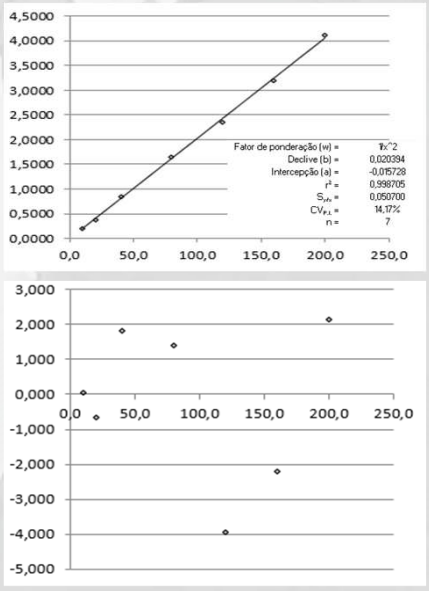
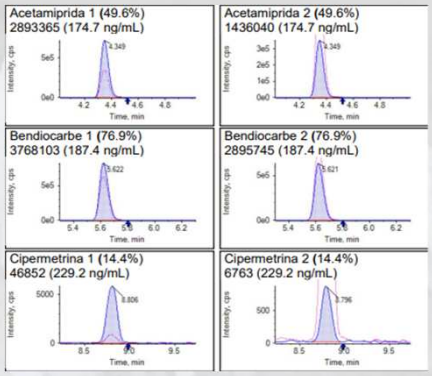


Fig. 2 – Partial report of a 180 ng/mL control



CONCLUSION

The developed analytical method was fully validated according to the guiding principles of the ANSI/ASB Standard 036 and applied to routine post-mortem samples, being already detected more than 15 positive cases. This UPLC-MS/MS method is useful and a powerful tool in a toxicology lab because it is fast, simple, effective, and trustworthy. This method is able to detect and analyse pesticides in *post-mortem* samples. It is set to use for routine analysis and successfully applied to intoxication cases.

The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification, WHO, 2019.
Hrynko et al, Chemosphere vol 263 , p. 128-143, Jan 2021 doi 10 1016 /j.chemosphere. 2020.128143
He et al., J Chromatogr A vol 1633 Dec 2020 doi 10 1016 /j.chroma.2020.461637
Cuypers et al., Forensic Sci Int vol 277 pp 59 65 Aug 2017 doi 10 1016 /j.forsciint.2017.05.016