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DEVELOPMENT AND VALIDATION OF A NEW ASSAY OF PESTICIDES RESIDUES IN HUMAN BLOOD BY GC/MS

Rachid Alami[*1], Mohammed Jbilou[1], Yahia Cherrah[2], Abdelaziz Bouklouze[2], Adil El Yadini[3] and My Elabbes Faouzi[4]

[1]Research Laboratory and medical analysis of the Royal Gendarmerie, (Rabat) Morocco.
[2]Research team Kinetics, Pharmacology and Toxicology Laboratory , Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.
[3]Laboratory of Spectroscopy, Molecular Modelisation, Material and Environment (LS3ME), Faculty of Sciences, University Med V-Agdal, Avenue Ibn Battouta, BP 1014, Agdal, Rabat, Morocco.
[4]Pharmacocinetic Team Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.

Keywords:
Validation, Pesticide residues, Human blood, Analysis, GC / MS.

Abstract

A new method for pesticide residues analysis in human blood by gas chromatography coupled with mass spectroscopy (GC / MS) was developed and validated on human plasma samples loaded by pesticide residues (demethoate, endosulfan, befinthrin, phosmet and deltamethrin). The extraction of these pesticides was conducted in solid phase through C18 SPE cartridges.The method of analysis of pesticide residues by GC / MS proposed, using a capillary column supelco® type oven temperature was programmed from 75 ° C to 320 ° C. Helium was used as carrier gas with a flow rate of 0.8ml / min. Phosalone (100 ppb) was used as internal standard (EI). Calibrations curves pesticides are linear in a concentration range between 10 and 100 ppb with R2 > 0.994 for all the pesticides. The method was validated according to the guidelines of the NF V03-110, in May 2010 in terms of the criteria of accuracy, fidelity, linearity and specificity. The validated method is selective and has a quantification limit of 10 ppb. It can be used routinely for the determination of pesticide residues in samples of human blood.

INTRODUCTION

Pesticides are substances intended to eliminate pests and crops. Insecticides, herbicides, fungicides and rodenticides construction for the main classes of these substances. Thousands of commercial products have been used around the world in various sectors such as agriculture, industry and even public health [1]. These uses have certainly contributed to the increase of agricultural production; however, human contact with these chemical formulas can damage the health of people directly in their applications, whether as a result of consummation of contaminated food by residues pesticides. Scientific studies have shown that exposure to low doses can have long term health effects such as endocrine disruption, a disorder of the immune system, disturbance of the nervous system and the appearance of cancer of certain organs (thyroid, breast, prostate, stomach) [2]. From that made it proves important to assess the pesticide residue concentrations in people in contact with pesticides. The quantitative determination of pesticide residues in biological media can be done by chromatographic methods such as: The method for the simultaneous determination of 12 urinary metabolites of pesticides by GC / MSMS with isotope dilution has been developed by Bravo et al [3]. Oslon et al [4] have developed a multi residue method for the determination of 19 pesticides in human urine while Norrgan et al [5] proposes a multi residual method for the determination of six herbicides urinary metabolites by LC-APCI -MS / MS. In blood, Hernandez et al [6] followed by Pitarch et al [7] have developed a rapid method for simultaneous determination of 16 pesticides in blood by GC-MS / MS. Lacassie et al [8] 47 dose volatile pesticides by GC / MS and 14 polar and thermally labile pesticides by LC / MS in human

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
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serum. Finally for dosing pesticides and their metabolites in maternal blood and umbilical cord Corion et al [9] described a method by GC-EI-MS. The main objective of this work is the precise dosage of pesticide residues in human blood by GC / MS, with high accuracy of people who are in direct contact (workers and farmers) or indirect (patients and consumers) with these residues, in order to prevent against the possible health complications in this population. such as; endosulfan, demithoate, deltamethrin, phosmet, befinthrin because these products and according to the poisons unit in Morocco have to cause many food poisonings especially in rural areas. [10].

MATERIALS AND METHODS

Chemicals and reagents

The standard of pesicides (demethoate, endosulfan, befinthrin, phosmet and deltamethrin) and internal etalon (phosalone) are Scientique Ultra brand (USA, Kingstown).Acetonitrile, hexane, ethyl acetate and isooctane are solvents ultra pure grade HPLC LiChrosolv Merck KGaA 64271 (Darrmstadt, Germany).The standard solutions are diluted by HPLC grade methanol (VWR Prolabo, France). The $SPEC_{18}$ extraction cartridges are brand (Waters USA).The sample of human blood free of traces of pesticides' has been used as a biological matrix during the development of the method was obtained from the blood transfusion center of Rabat, Morocco.

Instrumentation

The gas phase chromatographic system coupled to mass spectrometry is of type GC / MS Clarus® 600 / 600C DMS PerkinElmer® (Bridgeport, USA), equipped with an automatic injector. The system is controlled by a mass Turbo Software (Windows XP SP2).The stationary phase is a column supelco® (L 30m x 0.25 ID x DF 0.25) Elite-5MS phase, the carrier gas is helium at a flow rate of 0.8ml / min.

Chromatographic conditions

Preparation of the calibration range (10 ppb, 20 ppb, 30 ppb, 50 ppb, 60 ppb, 70 ppb and 100 ppb) was performed from the stock solution (1ug/mL) by successive dilutions in plasma samples. 1 ml of each sample was mixed with 100 uL of a solution phosalone (EI) 100 ppb, the whole was mixed by vortexing for 20 seconds.

Extraction

The extraction was performed by adding 2 mL of acetonitrile in a 15 mL conical tube containing 1 mL of plasma sample load. The mixture was stirred by vortex and centrifuge at 4000g for 10 min, the organic phase recovered is passed through a $SPEC_{18}$ cartridge. Elution is made with an organic mixture (hexane / ethyl acetate 6/1 v / v). This organic phase is evaporated under a stream of nitrogen at a temperature of 50°C. The dry extract obtained is taken up in 100 uL of isooctane and injected into the chromatographic system.

Oven Programming

The oven was programmed from 75°C to 32°C at a gradient of 20°C per minute with a pre-heating the transfer line at 325°C and 250°C at source. The automatic injector is in splitless guide (50/1 to 250°C). Ionization is caused by electron impact (EI).

RESULTS & DISCUSSION

After analyzing, the chromatogram has showed in (Fig 1) and the quantitative results have been presented, Rations amplitudes (RA), concentration recovered (CR), and yield (%) in **Table-1**.

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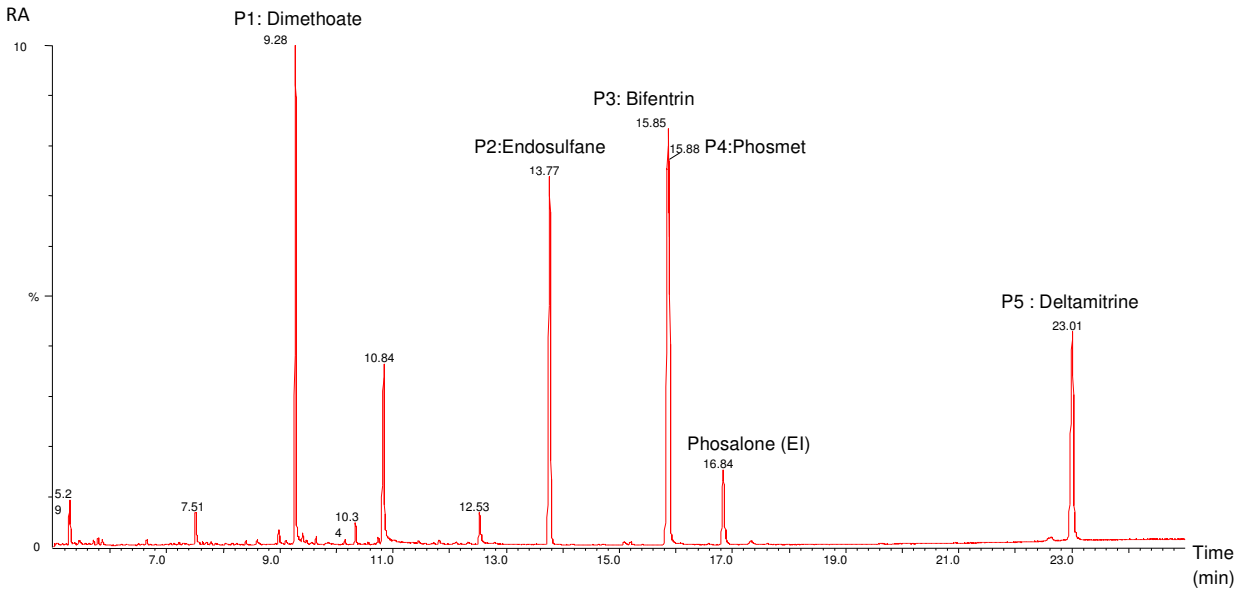


Figure 1: Chromatogram of pesticide calibration

Pesticides names	Concentrations (ppb)	R T (min)	RA	CR (ppb)	Yield (%)
Dimethoate	50	09.29	2.866	52.826	105.650
Endosulfane	50	13.77	0.350	46.128	92,256
Bifentfhrin	50	15.85	2.444	45.155	90.031
Phosmet	50	15.89	2.552	51.575	103.150
Deltamithrine	50	23.02	2.513	48.987	97.974

Table1: Extraction of pesticides doped in human blood

The Dimethoate (Fig 2) was detected in 9.29 min and confirmed by molecular fragmentation (Fig 3).

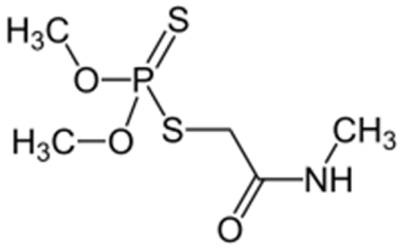


Figure 2: Chemical structure of Dimethoate

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65

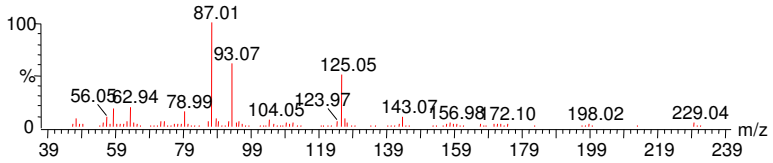


Figure 3: Mass spectrum of dimethoate

The Endosulfan (Fig 4) was detected in 13.77 min and confirmed by molecular fragmentation (Fig 5)

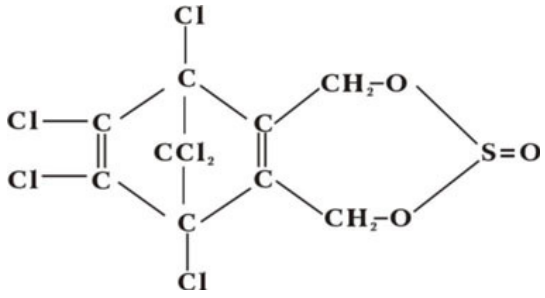


Figure 4: Chemical structure of Endosulfane

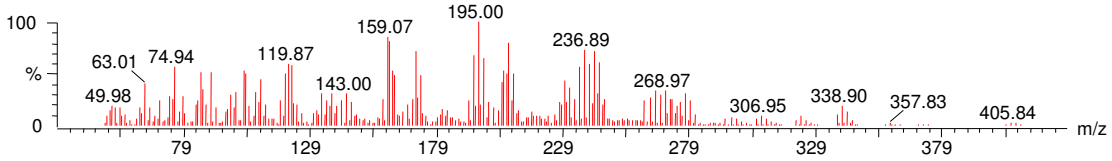


Figure 5: Mass spectrum of Endosulfane

The bifenthrin (Fig 6) was detected in 13.77 min and confirming molecular fragmentation by (Fig 7)

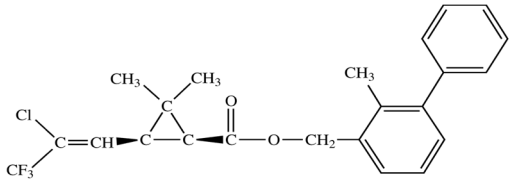


Figure 6: Chemical structure of Bifenthrin

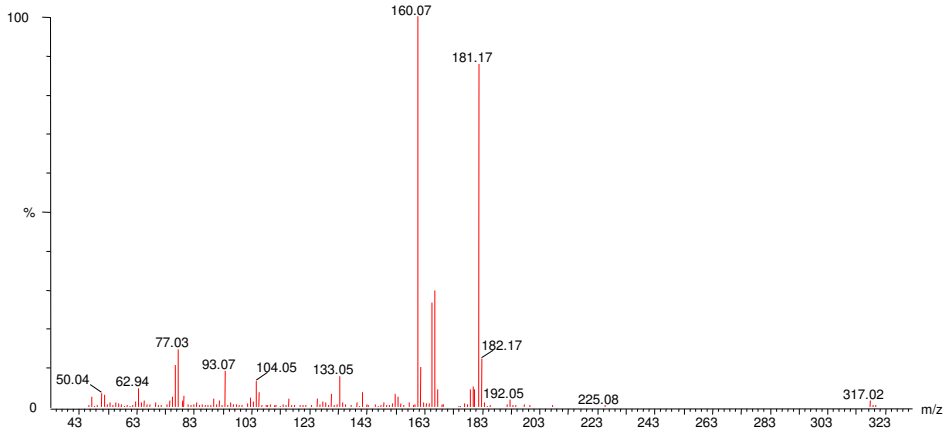


Figure 7: Mass spectrum of Bifenthrin

The Phosmet (Fig 8) was detected in 13.77 min and confirmed by molecular fragmentation (Fig 9)

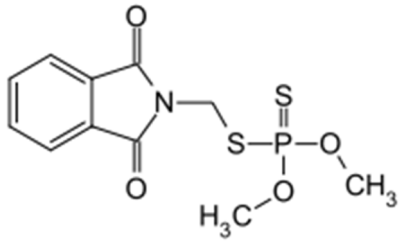


Figure 8: Chemical structure of phosmet

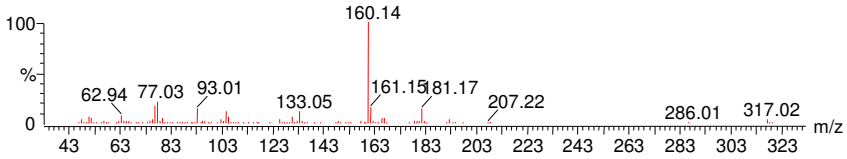


Figure 9: Mass spectrum of phosmet

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65

The Phosalone (Fig 10) was detected in 13.77 min and confirmed by molecular fragmentation (Fig 11)

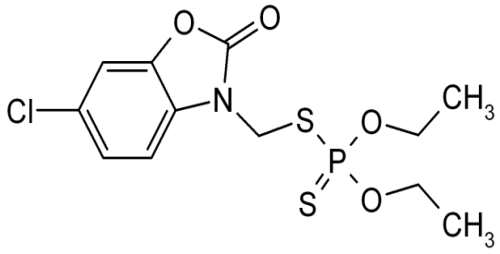


Figure 10: Chemical structure of phosalone (EI)

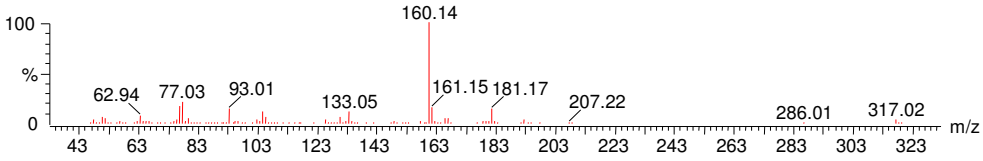


Figure 11: Mass spectrum of phosalone (EI)

The Deltamitrine (Fig 12) was detected in 13.77 min and confirmed by molecular fragmentation (Fig 13)

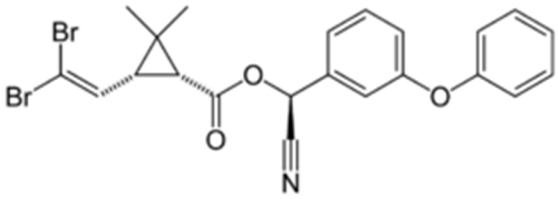


Figure 12: Chemical structure of Deltamitrine

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65

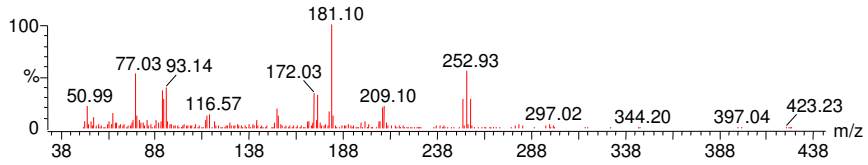


Figure 13: Mass spectrum of Deltamitrine

VALIDATION OF THE METHOD

The validation of this method has been studied on the requirements of the NF V03-110, May 2010 [11], based on the profile accuracy and fidelity.

Selectivity

Selectivity was studied according to the validation standard [12]. White to extract from the extraction of a blood sample free of pesticides showed no signal at their retention time (Fig 14)

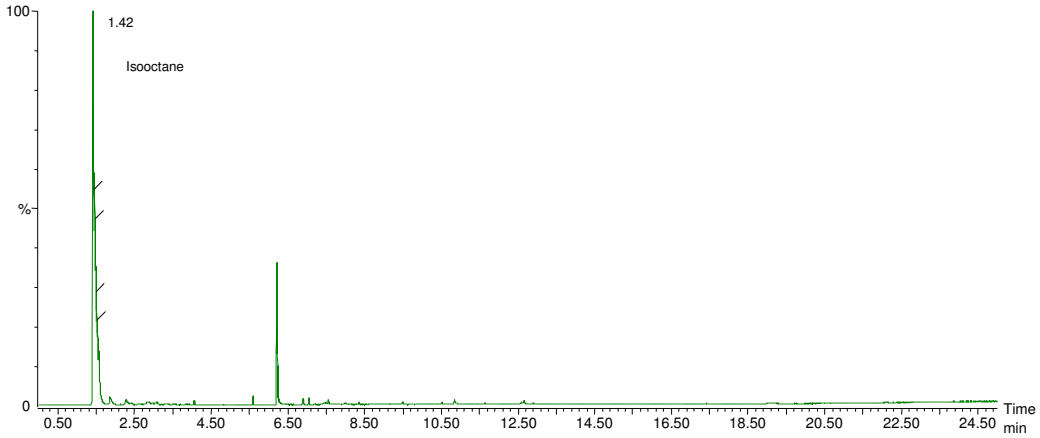
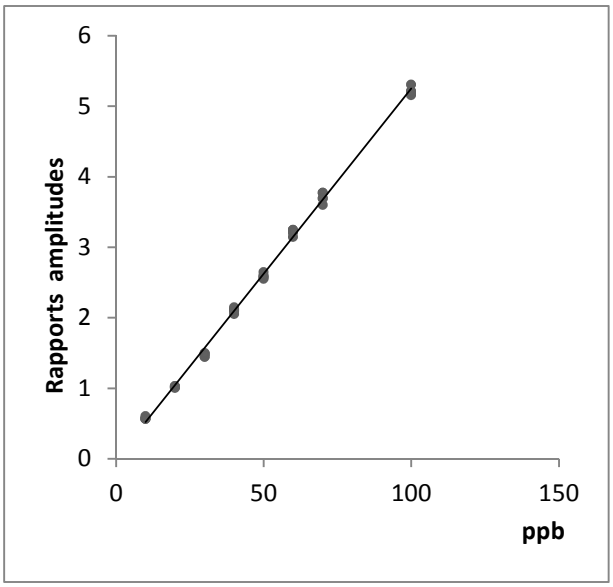


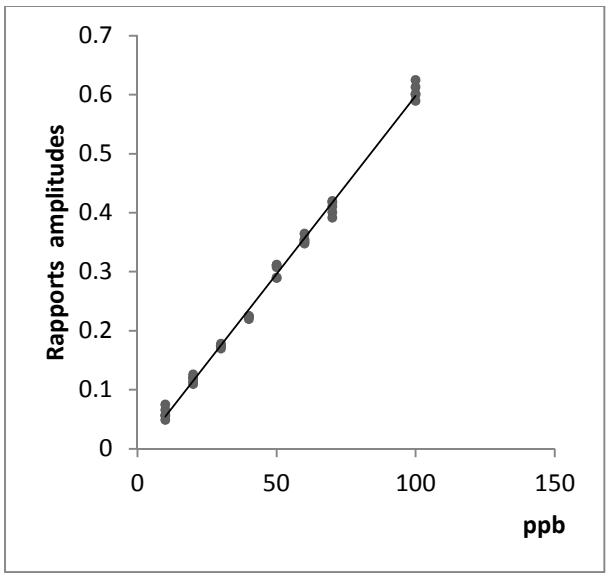
Figure 14:Chromatogram selectivity**Linearity**

The linearity is the ability of an analytical method, within a certain interval to provide an instrumental response [13], it is therefore necessary to check the field strength and to deduce the characteristics of straight calibration pesticide calibration solution on a dosing interval consists of 7 levels of concentrations (10 ppb, 20 ppb, 30 ppb, 50 ppb, 60 ppb, 70 ppb and 100 ppb), each concentration was repeated 5 times (Fig 15). The equations of the calibration straight lines (Y = ax + b) were calculated by the least squares method on the entire range, with their correlation coefficients (R2) **Table-2**.

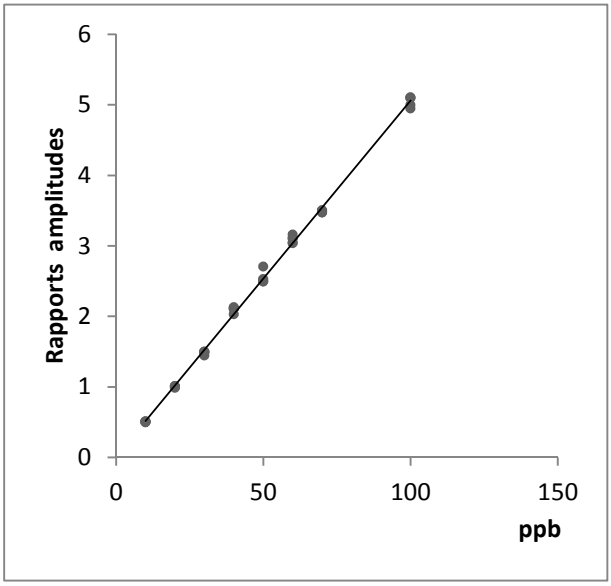
April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65



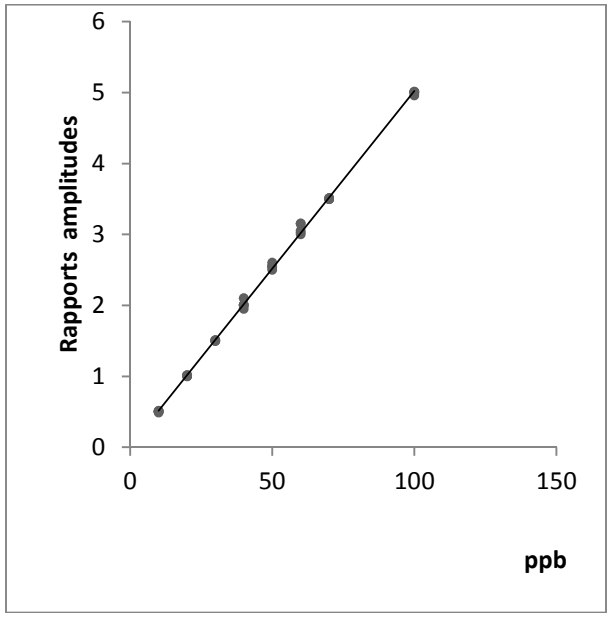
(a)



(b)

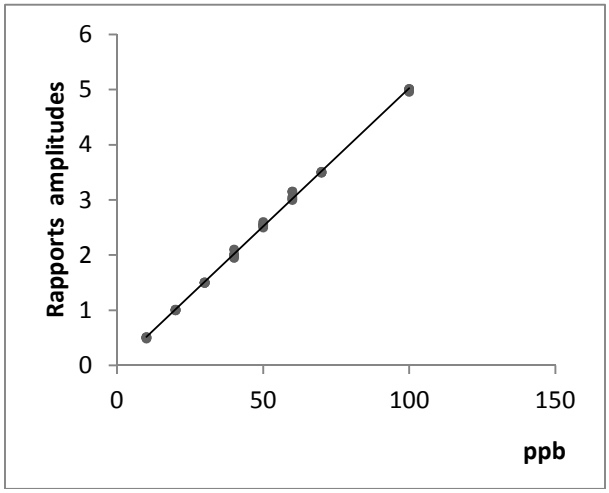


(c)



(d)

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65



(e)

Figure15: Straight calibration of pesticides (a: Demithoate, b: Endosulfane,c: Befintrin, d: Phosmet, e: Deltametrine)

Pesticides	Y = ax + b	R^2
Dimethoate	0.052x - 0.006	0.998
Endosulfane	0.006x - 0.005	0.995
Bifentrin	0.005x + 0.013	0.995
Phosmet	0.005x + 0.013	0.999
Deltamitrine	0.005x + 0.012	0.994

Table 2: Results of Linearity

Fidélité

The loyalty is expressed at two levels; repeatability and intermediate precision. [14]

a-Repeatability:

Repeatability expresses the precision under identical conditions; same analysis, same equipment, same reagent, a short time interval and the same operator. It is evaluated by the coefficient of variation (CVr or RSD) [14].

RSD% or CVr = standard deviation / mean x 100

The repeatability of the method was studied on three levels of concentrations (20 ppb, 30 ppb and 50 ppb), these concentrations were repeated 5 times **Table-3**.

Pesticides	% recouvrement (n=5)	CVr%
Dimethoate	102.91	5.51
Endosulfane	90.01	7.12
Befinthrin	94.94	4.92
Phosmet	95.22	6.88
Deltamethrine	89.55	5.01
CVr		**5.89%**

Table 3: Results of repeatability

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65

b -Intermediate Fidelity

The study of reproducibility eliminates random effects from inaccurate results and errors in judgment or manipulation by scientists.

This study was performed on three independent series of concentration levels of 10 ppb, 20 ppb and 30 ppb. The coefficient of variation (CVR %) 6.27% **Table-4** is less than 15% recommended by the FDA in biological environment confirms the precision of the method.

Pesticides	Serie1	Serie2	Serie3
Dimethoate	8.98	9,90	6.85
Endosulfane	5.50	6.45	4.93
Befinthrin	6.63	10.02	7.70
Phosmet	4.13	6.16	4.28
Deltamethrine	5.01	6.33	6.66
CVR			**6.64%**

Table4: Results of intermediate Fidelity

Accuracy

The accuracy of the method has been studied in plasma samples supplemented with fixed quantities of pesticides

a- accuracy at the limit of quantification (LOQ = 10 ppb)

Five plasma samples were 10 ppb pesticide analyzes in intermediate precision conditions. Each analysis was repeated twice under conditions of repeatability **Table-5.**

Pesticides	Series (n=5)					Moy	Biais %	ET	CV%	LQ+60%xLQ	LQ-60% xLQ
Dimethoate	10.615	11.133	09.005	07.900	7.697	09.270	7.30	0.15	5.330	3.5	1.2
Endosulfane	12.00	10.000	10.823	9.916	14.723	11.500	15.0	0.44	5.816	4.1	5.1
Bifentrin	9.915	13.645	8.566	12.00	9.999	10.825	8.25	0.61	3.022	2.4	3.9
Phosmet	13.16	8.005	8.660	10.100	7.865	09.333	6.67	0.70	6.000	0.8	0.3
Deltamitrine	7.777	10.050	7.572	8.911	11.00	09.066	9.34	0.18	5.190	3.3	1.0

Table 5: Results of accuracy at 10 ppb

Presupposed the limits of quantification are accurate for all pesticides, they are checked by the following two inequalities:

MLQ SLQ + 2 x < LQ +60% x LQ and MLQ - 2 x SLQ> LQ - 60% x LQ [11].

b -study of the accuracy has 3 levels (30 ppb, 50 ppb and 100 ppb)

The verification of accuracy is 30ppb, 50ppb and 100ppb was made under the same conditions as for the study of the limit of quantification **Table-6**.

Pesticides	n	Moy			Biais%		
		30	50	100	30	50	100
Dimethoate	5	28.135	49.067	104.225	6.30	1.90	4.22
Endosulfane	5	32.611	49.034	105.766	8.70	1.97	5.77
Bifentrin	5	32.015	54.534	99.187	6.71	9.06	0.90
Phosmet	5	29.300	51.050	95.233	3.10	2.10	4.80
Deltamitrine	5	30.802	52.000	95.516	2.67	4.00	4.82

Table 6: Summary of the study of the accuracy

April 2016; Volume 3 (Issue 4)ISSN: 2394-9414 DOI: 10.5281/zenodo.50335
Impact Factor- 2.65

The accuracy to show the different concentration levels and tolerance intervals which do not exceed 20%.

CONCLUSION

The Pesticides Residue assay developed from blood samples loaded using GC / MS as technique, proves sufficiently linear, specific and accurate. It may be used with sucked for the quantification of pesticides in human blood samples from persons deemed infected. The developed method is simple, sensitive and for the determination of small amounts in the blood, allowing to detect traces of pesticides in the blood.

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