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Screening of pesticide residues in blood by GC-MS in patients admitted to the department of endocrinology ibn sina chu rabat

Rachid Alami[*1,2], Mohammed Jbilou[1], Yahia Cherrah[2], Khalid Laarej[1], Adil El Yadini[3], Hind Iraqi[4], Abdelmjid Chraibi[4] and My Elabbes Faouzi[5]

[1]*Research Laboratory and Medical Analysis of the Gendarmerie Royale, Rabat, Morocco*
[2]*Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy,Mohammed V University, 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]*Service of Endocrinology, University Hospital Ibn Sina, Rabat, Morocco*
[5]*Pharmacocinetic Team Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco*

ABSTRACT

Repeated exposure or consumption of food contaminated with pesticide residues can cause various disorders for the human body, especially the immune and hormonal disturbances and the onset of certain cancers. The multirésiduel assay method of pesticides in blood by (GC-MS) Clarus® 600/560 DMS PerkinElmer® we developed allowed us to conduct a screening of the majority of pesticide residues used in Moroccan agriculture. The calibration range contains a mixture of the most used pesticides. The extraction was performed in a solid medium by C_{18} SPE cartridges. This method uses a capillary column of 30 meters type Supelco®, the oven temperature was programmed from 90 ° C to 290 ° C with a gradient of 10 ° C / min. Helium was used as carrier gas with a flow rate of 0.8ml / min. Phosalone was used as internal standard (IS). The calibration curves of different pesticides are linear with concentrations ranging between 10 and 70 ppb with satisfactory correlation coefficients (R2> 0.99) and CV <20% for all results. In these analytical conditions we analyzed blood samples of Endocrinology of the University Hospital of Rabat. The results showed that some patients are contaminated with pesticide residue; dimethoate and bifenthrin. The hypothesis of the involvement of these pesticides in the development of hormonal disorders in patients is very likely. This method may be expanded and supplemented to prove this causal link.

Keywords: Pesticides, GC-MS, assay, Patients.

INTRODUCTION

The dangers of pesticides on human health are occurring even with low exposures, these pesticides can have serious consequences for the body such as male infertility, spontaneous abortions, and the occurrence of certain cancers, particularly hormonal disturbances and immunity [1].

Chromatographic techniques of pesticide dosing in human blood are limited and do not allow the determination of all pesticide residues that can contaminate the users (farmers) and consumers of processed food products.

Barr et al [2] have developed a method by GC-HRMS for a multi-residual analysis of 29 pesticides in human blood. The method we suggest is regarding the most traded pesticides residues screening in Morocco for people who may be exposed to pesticides by GC - MS. The application of this method to determine the pesticide contamination of some patients in the Endocrinology department of the Rabat University Hospital is a concrete verification of the method before switching to determining the impregnation of a large population of residues pesticides.

MATERIALS AND METHODS

Chemicals and reagents

Mixed standards and internal standards (EI) are from Scientique Ultra brand (USA, Kingstown) Acetonitrile, Hexane, Ethyl acetate and Iso-octane are ultra-pure HPLC grade solvents from LiChrosolv Merck KGaA 64271 (Darrmstadt, Germany).

The standard solutions are diluted by HPLC grade methanol (VWR, France). The $SPEC_{18}$ extraction cartridges (500 mg, 6 mL) are branded (Agela Cleanert Wilmington, DE 19808, USA).

The sample of human blood free of traces of pesticides that were used as a biological matrix during the development of the method comes from the blood transfusion center of Rabat, Morocco.

Instrumentation

The gas phase chromatographic system coupled with mass spectrometry is a GC - MS Clarus 600 / 560DMS 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 0.25 DF) Elite-5MS phase, the carrier gas is helium at a flow rate of 0.8 mL / min. The vacuum is generated in the system by EDWARDS type external pump.

Chromatographic conditions

We have prepared a calibration range (10, 20, 30, 50 and 70 ppb) by serial dilution of the stock solution of the mixed standard (1 ug / mL) in plasma sample. 1 mL of each sample was mixed with 100 µl of a solution Phosalone (EI) to 100 ppb, the set was mixed by vortex for 20 seconds.

Extraction

The extraction was carried out by adding 1.5 mL of Acetonitril in a conical tube 15 mL containing 1 mL of plasma. The mixture was stirred by vortex and centrifuged 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 µl of isooctane before the injection of 3 µl in the chromatographic system.

Oven Programming

The oven was programmed from 90 ° C to 230 ° C in a gradient of 15 ° C, hold 1 min, ramp 5° C /min to 290 ° C and hold 2 min with a first heating of the transfer line at 325 ° C and 250 ° C source. The automatic injection is splitless manual (50/1 to 250 ° C). Ionization is caused by electron impact (EI).

RESULTS AND DISCUSSION

Chromatographic identification

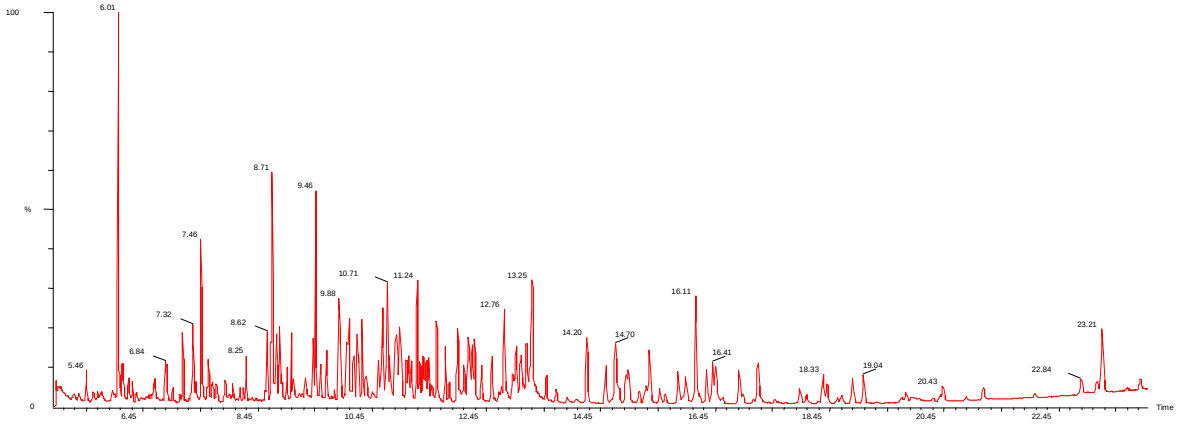

Fig 3: Chromatogram of a point of the calibration range (50ppb)

Figure 3 shows the chromatogram of the injection of the extract of a point of the calibration range, each peak was identified by its mass spectrum. Determining each pesticide retention time was followed by the selective isolation on chromatograms pesticides individually (Figure 4).

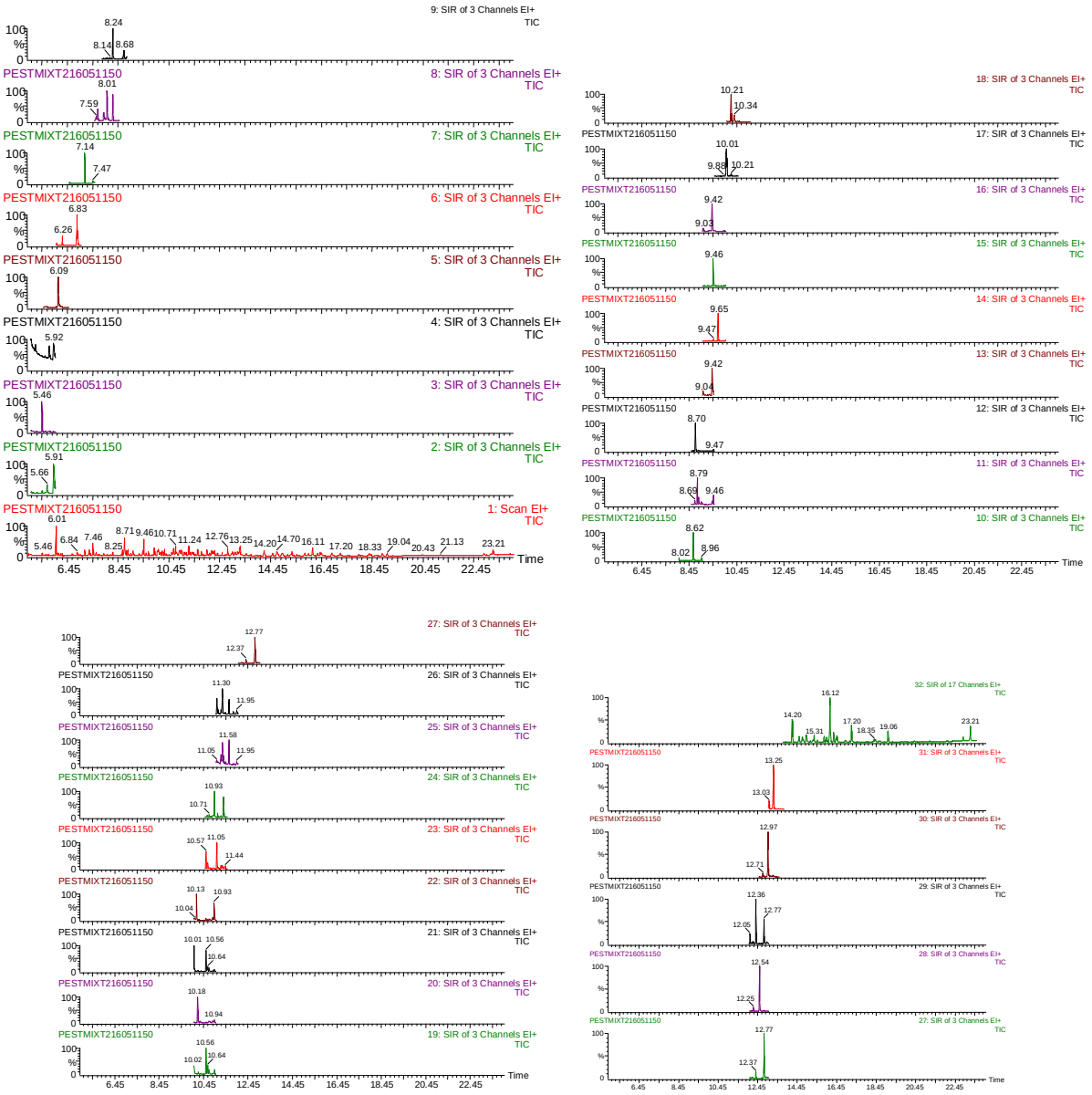

Figure 4: Isolation of pesticides by their retention time and their specific masses

Calibration range

The isolation of peaks allows us to draw straight linear calibrations for each pesticide in the range. The majority of these lines had correlation coefficients greater than 0.99. Figure 5 illustrates the detected calibration curves in patients.

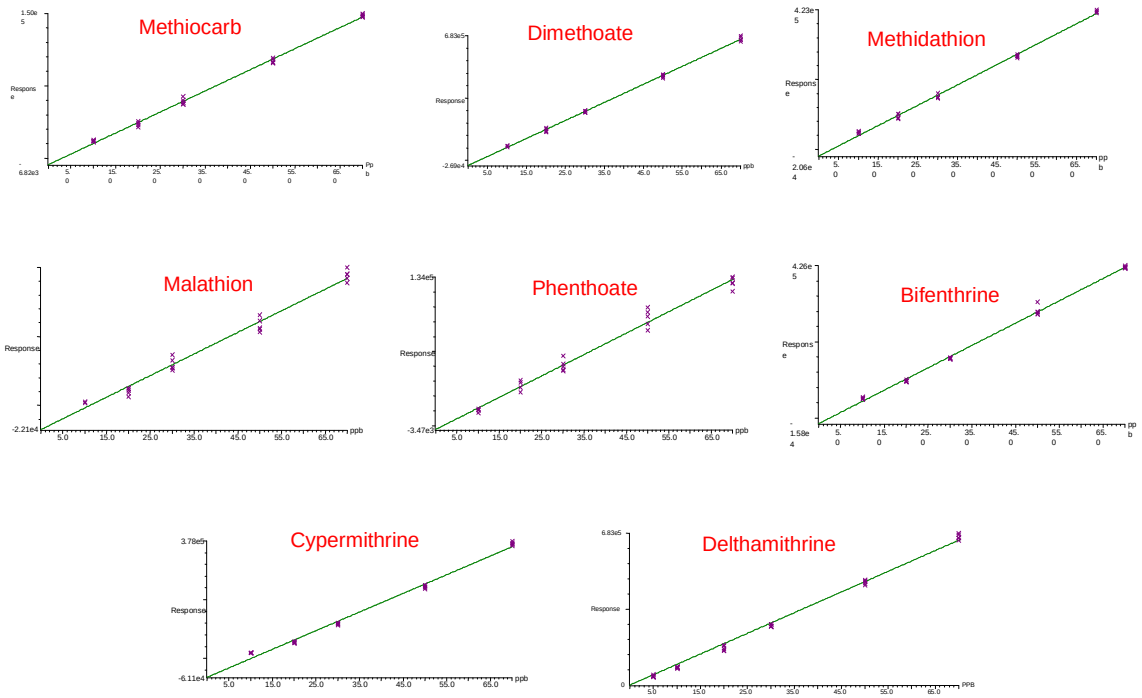

Figure 6: Straight calibration of pesticides detected in patients

Linearity

Linearity [3], is set to check the dosage field and to deduce the characteristics of straight calibration.

Dosage interval consisting of five concentration levels (10ppb, 20ppb, 30ppb, 50ppb and 70ppb), each concentration was repeated 5 times.

The equation of the calibration straight lines (y = ax + b) were calculated by the least squares method on the wholes ranges with satisfactory correlation coefficients for the majority of pesticides and CV <20% (Table 1).

Specificity

The specificity of an analytical method is defined as its ability to selectively show the presence of a compound in a chemical components rich media (degradation products, metabolites, same class of substances as the analyte) [4].

The blank extract obtained from the extraction of a pesticides free blood sample hasn't shown any signals in pesticides specific retention times (Figure 7).

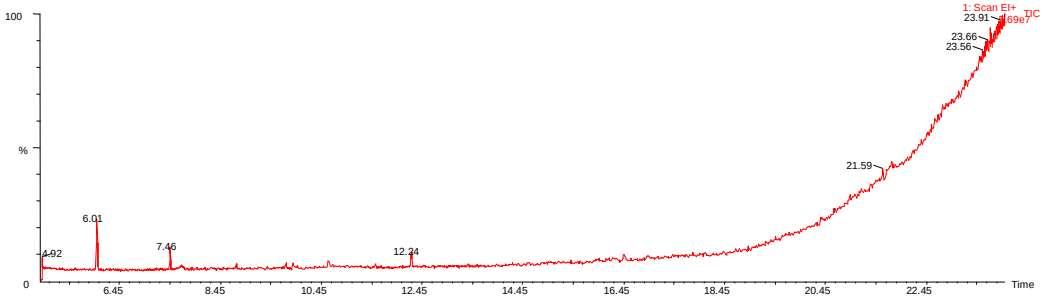

Figure 7: Chromatogram of a blood sample free of pesticides

Limit of detection and quantification

The detection limit is the lowest concentration or amount of substance to be examined in which a sample can be detected but not quantified as an exact value. [5] Residue limit of detection (LOD) is estimated from the background noise of the recording. LOD = 5hmax (h max: maximum amplitude of the signal over a distance equal to 20 times the width at half height of the peak corresponds to the substance to search). The limit of quantification (LOQ) is the smallest amount of a substance to be examined in a sample which can be assayed in the experimental conditions described with defined fidelity and accuracy (LOQ = 10hmax) [6].

The LOD was determined as 5ppb (around five times the background) and the LOQ was 10ppb, hence the validity of this method multirésiduelle assay of pesticide is between 10ppb and 70ppb (table 1).

Table 1: Results of the validation of the assay pesticides in blood

Pesticides	R T (min)	Targest peaks (m/z)	Quant (m/z)	Linearity (ax+b)	R^2	LOQ (ng/ml)	CV%	Accuracy %	
								min	max
Dichlorvos	5.45	109,79,185	109	0.050x-0.0242	0.998	10	05.23	96.87	107.12
Methiocarb	7.99	168,153,109	168	0.067x+0.0146	0.988	10	09.55	96.85	115.03
Chlorpropham	8.78	127,213,129	127	0.041x-0.0625	0.991	10	08.95	100.22	124.35
Sulfotep	9.03	97,322,65	322	0.096x-0.0198	0.994	10	05.19	101.66	123.74
Dimethoate	9.35	87,93,125	87	0.022x-0.0234	0.996	10	03.75	96.63	108.23
Carbofuran	9.44	164,149,122	164	0.051x-0.0467	0.991	10	09.44	90.38	103.67
Aminocarb	9.64	151,150,136	151	0.047x-0.0558	0.986	10	08.70	98.52	108.78
Triazophos	10.03	97,109,161	97	0.018x-0.600	0.999	10	10.58	96.33	107.02
Triazine	10.08	239,178,241	239	0.044x-0.0213	0.999	10	06.08	86.04	102.05
Iprobenphos	10.18	91,204,123	91	0.075x-0.0289	0.997	10	01.06	88.77	101.01
Pirimicarb	10.25	72,166,238	72	0.011x-0.0722	0.975	10	09.91	98.27	115.22
Chlorpyriphos	10.56	125,127,173	125	0.040x-0.0677	0.999	10	10.27	95.06	109.12
Fenpropidin	10.89	98,99,106	98	0.066x-0.0309	0.993	10	05.88	96.98	103.7
Methomyl	10.92	105,58,88	105	0.044x-0.0213	0.994	10	03.77	95.95	115.2
Malathion	11.05	93,127,173	93	0.050x-0.0242	0.991	10	12.56	97.97	120.26
Diethofencarb	11.09	151,124,150	151	0.011x-0.1134	0.990	10	05.85	100.25	108.11
Triadimefon	11.36	57,208,85	208	0.024x-0.0881	0.992	10	07.25	91.15	104.33
Pirimiphos	11.58	276,290,93	290	0.084x-0.0236	0.996	10	08.23	87.57	118.5
Cyprodinil	11.73	224,225,77	224	0.033x-0.0710	0.991	10	06.66	94.75	108.4
Phenthoate	12.05	125,121,93	125	0.014x-0.0361	0.996	10	11.10	93.77	111.18
Procymidone	12.19	283,96,285	283	0.090x-0.0882	0.990	10	13.09	88.15	110.37

Pesticides	R T (min)	Targest peaks (m/z)	Quant (m/z)	Linearity (ax+b)	R^2	LOQ (ng/ml)	CV%	Accuracy %	
								min	max
Methidathion	12.37	85,93,145	85	0.025x-0.0196	0.991	10	10.77	92.59	111.66
Mepanipyrim	12.53	222,223,77	222	0.062x-0.0274	0.994	10	06.28	95.55	110
Flutolanil	12.76	173,145,281	173	0.097x-0.0244	0.999	10	09.10	98,45	121.2
Oxadiazon	13.06	175,177,258	175	0.024x-0.0067	0.995	10	05.35	95.22	102.8
Buprofezin	13.24	106,105,119	105	0.076x=0.0277	0.999	10	10.22	91.31	110
Binapacryl	13.49	83,55,84	83	0.044x-0.0606	0.990	10	13.72	100.22	112.75
Mepronil	14.18	119,91,269	119	0.012x-0.0475	0.999	10	09.75	98.78	121.11
Ofurace	14.55	132,77,160	132	0.015x-0.0805	0.998	10	11.25	94.04	106.67
Trifloxystrobin	14.71	116,131,59	116	0.061x-0.0772	0.990	10	13.95	88.88	110.3
Dicofol	15.11	139,111,251	139	0.044x-0.0632	0.995	10	11.46	96.36	107.77
Oxycarboxin	15.49	175,93,267	175	0.091x-0.117	0.991	10	09.74	97.82	121.01
Bifenthrine	16.03	181,165,166	181	0.050x-0.013	0.995	10	05.03	87.56	102.32
Phosmet	16.07	160,161,77	160	0.020x-0.0250	0.991	10	04.01	98.78	123.06
Fenpropathrin	16.29	97,181,55	97	0.033x-0.0208	0.995	10	15.56	93.25	121.2
Phenamiphos	16.41	303,145.217	303	0.018x-0.0176	0.999	10	03.88	89.08	108.01
Fenarimol	17.94	139,107,219	139	0.040x-0.0508	0.993	10	10.66	91.18	101.3
Fenothrin	18.32	123,183,81	183	0.047x-0.0650	0.993	10	04.22	101.89	120.55
Imiprothrin	18.37	123,81,151	123	0.039x-0.0901	0.990	10	08.75	99.2	105.6
Boscalid	20.44	140,142,112	140	0.031x-0.0780	0.998	10	10.10	90.55	119.8
Cypermethrine	20.51	163,181,165	161	0.015x-0.033	0.995	10	02,19	92.59	111.66
Deltamithrine	23.22	181,253,77	181	0.082x-0.0143	0.995	10	05.67	95.55	110

Pesticide residues assay in patients

The Ethics Committee met on October 6, 2015 has given a favorable opinion on pesticide residues assay in patients of Endocrinology Service of the Rabat University Hospital (N^0 825 of 11 October 2015). Blood samples of patients admitted in the study and the controls have undergone the same extraction protocol than the standard range, check results are consistent, their standard deviations and coefficients of variation are shown in the table 2. The results show that patients are contaminated with pesticide residues; dimethoate (Figure 8), other patients have residues such as perithrinoides and carbamates with peaks exceeding 50 µg/L and especially for bifenthrin in some patients suffering from thyroid dysfunction, while reporting thresholds for the allocation of human health by pesticides are 10 ppb [7].

Table 2 : Résultats of contrôles (30ppb)

Pesticides	Nbr Series (n)			Moy	Bais%	ET	CV%
Dichlorvos	31.76	30.65	33.12	32.49	11.63	01.24	03.80
Methiocarb	35.98	33.60	31.51	33.69	12.33	02.24	06.63
Chlorpropham	32.62	33.00	29.81	31.81	6.03	01.74	05.48
Sulfotep	32.02	32.72	29.33	31.35	4,50	01.79	05.8
Dimethoate	33.64	32.36	30.96	32.32	7.73	01.34	04.15
Carbofuran	36.08	28.13	35.01	33.07	10.23	04.31	01.3
Aminocarb	29.68	28.94	28.84	29.15	2,83	0.46	01.57
Triazophos	32.06	31.65	32.82	32.17	7.23	0.59	01.85
Triazine	30.24	31.10	29.28	30.20	0.66	0.91	03.01
Iprobenphos	30.61	30.68	31.96	31.08	3.60	0.76	02.44
Priimicarb	35.46	33.24	33.18	33.96	13.20	01.30	03.8
Chlorpyriphos	27.77	28.75	27.47	27.99	7.60	0.67	02.40
Fenpropidin	31.68	30.04	31.47	31.06	3.53	0.89	02.87
Methomyl	32.05	29.15	28.65	29.95	0.16	01.84	06.12
Malathion	36.16	33.76	34.05	34.65	15-50	01.30	03.78
Diethofencarb	33.54	31.65	33.28	32.82	9.40	01.02	03.12
Triadimefon	29.93	30.61	29.11	29.88	0.4	0.75	02.51
Perimiphos	30.11	29.14	27.31	28.85	3.83	01.42	04.92
Cyprodinil	28.97	29.06	28.82	28.95	3.50	0.12	04.18
Phenthoate	35.04	30.55	31.28	32.22	7.40	02.41	07.47
Procymidone	29.54	27.70	28.85	28.69	4.36	0.93	03.24

Pesticides	Nbr Series (n)			Moy	Bais%	ET	CV%
Methidathion	30.19	31.90	28.69	30.26	0.86	01.66	05.31
Mepanipyrim	28.35	28.84	28.89	28.69	4.36	0.30	01.04
Flutolanil	30.45	29.29	30.46	29.57	1.43	0.67	02.27
Oxadiazon	31.13	28.51	29.72	29.78	0.73	01.31	04.40
Buprofezin	29.77	30.03	29.30	29.70	1.00	0.37	01.25
Binapacryl	28.49	27.77	28.30	28.52	4.93	0.37	01.31
Mepronil	28.63	29.25	29.39	29.09	2.70	0.40	01.39
Ofurace	28.13	31.55	32.04	30.57	1.90	02.13	06.97
Trifloxystrobin	27.65	30.82	28.40	28.95	3.50	01.66	05.72
Dicofol	26.96	28.82	26.80	28.52	4.93	01.12	03.94
Oxycarboxin	25.57	26.18	28.30	26.68	11.40	01.43	05.37
Bifenthrine	30.31	29.61	29.77	29.89	0.63	0.37	01.23
Phosmet	34.06	34.45	27.36	31.95	6.50	03.99	01.25
Fenpropathrin	31.68	29.53	33.87	31.69	5.63	02.17	06.85
Phenamiphos	27.44	29.39	34.45	30.42	1.40	03.62	01.19
Fenarimol	31.15	31.51	29.96	30.87	2.90	0.81	02.63
Fenothrin	33.03	33.00	30.65	32.22	7.40	01.37	03.66
Imiprothrin	33.77	33.69	31.21	32.89	9.63	01.19	03.61
Boscalid	31.22	29.61	28.33	29.72	0.93	01.18	03.98
Cypermethrine	30.96	29.56	29.88	30.13	0.43	0.73	02.43
Deltamithrine	29.09	31,06	31,11	30.42	1.40	0.74	02.43

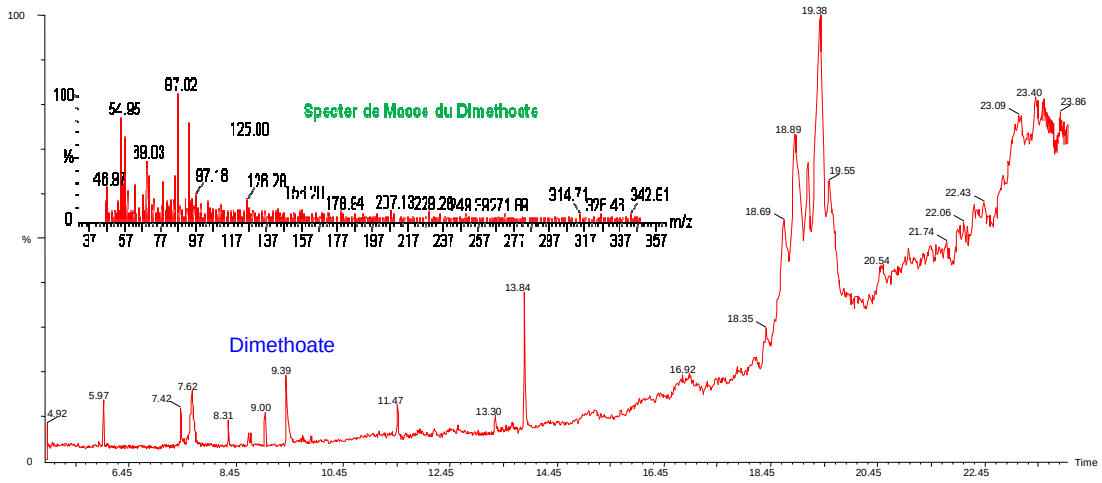

Fig 8: chromatogram of a contaminated patient by dimethoate

CONCLUSION

The multi-residual assay of pesticides in human blood by using GC / MS technic, proves sufficiently sensitive, loyal, specific, accurate, and robust which allows for a screening of the majority of pesticides used in agriculture. It was used for the determination of pesticide residues in blood samples of patients of the Endocrinology department of the University Hospital of Rabat and it can be used for determining the impregnation of a larger population by pesticide residues.

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