



# IDENTIFICATION AND QUANTIFICATION OF MALATHION USING IMAGE ANALYSIS ALGORITHMS IN INFRARED SPECTROMETRY

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## ABSTRACT

The infrared spectroscopy (IR) is a chemical analysis method cheaper and faster. Reason why it is important to deepen its application in the quantification of pesticides in food, especially for the detection of harmful amounts for human health. Using Matlab® tool, an algorithm capable of analyzing images of IR spectra was developed for transmittance and absorbance, without the need of using a calibration curve, as was commonly used, in order to identify and determine the amount of Malathion in a matrix. The algorithm calculates the area under the curve at intervals of 620-715 cm<sup>-1</sup>, 1018-1047 cm<sup>-1</sup>, 1200-1300 cm<sup>-1</sup> and 1700-1800 cm<sup>-1</sup>, for C-S, P-O, C-O and C = O bonds respectively, from the absorbance spectrum. Once the problems of the matrix effect, the molecular dynamics and the information obtained on the image analysis, have been considered. The direct influence of the number of times that the bond repeats and the IR spectrum were achieved. A mathematical relationship was developed, which was capable to find the amount of analyte in a sample. Finally, the algorithm evaluation was developed, having two IR spectra of Malathion, the first reported in the data base SDDBS and the second from a commercial sample at 57% w/v. It was obtained an error of 4.62% and 11,76% respectively.

**Keywords:** malathion, IR, infrared spectroscopy, IR quantification.

## INTRODUCTION

Nowadays, the gas chromatography technique (GC) is currently used for detecting pesticides in fruits [1], which is a complex and expensive technique to carry out a quality control in foods. This situation disturbing due to the health problems that can produce the consumption of pesticides and their residues. One of the most recent studies reveal how they affect the neurobehavioral in Thai children, who come to suffer cognitive impairment in their development [2].

Considering the above, economic and fast methods for the identification of pesticides in fruits were required. Under these, the infrared spectroscopy (IR) has been studied as one of the most attractive alternative, a cause of its importance in the qualitative and quantitative chemical analysis, for its speed and simplicity [3]. With this technique, the functional groups that exist in a sample are identified, which absorb energy in a range of wavelength, presenting a spectrum for each compound being similar to the fingerprint of a person.

The infrared spectrometric, such as near-infrared (NIR) and with Fourier transform (FTIR) were used in food industry for quality control of milk, fruits, wine, meat, oil, corn and other products of daily consumption. This analysis was performed in order to determinate the moisture, free fatty acids, fiber, proteins, which have been characterized, classified and studied by this method [4] [5] [6].

In order to analyze pesticides in fruits, and other plant, first of all a liquid-liquid extraction process has to be carried out. In this camp there have been several studies to find the best solvent or solvent mixture for this purpose. One of the most recent works, shows the extraction process of seven organophosphorus pesticides, where the solvents were evaluated doping the sample [7]. It was found, that depending on the pesticide there a suitable solvent, however, acetone presents high efficiency in all

the extractions performed, with a recovery percentage between 87% and 100%, same than the acrylonitrile with a recovery percentage between 95% and 100% [8] [9].

In the department of analytical chemistry at the University of Valencia in Spain, different studies have been developed for the analysis of pesticide formulations in commercial samples, where FTIR and Raman spectrometry are used with Fourier Transform for quantifying Folpes, Metalaxyl, fluometuron, imidacloprid and Malathion [10] [11] [12] [13]. Other authors propose the creation and training of a neural network, which is capable of identifying a range of wavelength of the IR spectrum, and depending on the area under the curve determines the amount of analyte [14]. In these reports, the pesticide always was determined using a calibration curve, which relates linearly the signal obtained with the amount of this in the sample.

A cause of the efficiency that shows the chemical analysis by FTIR, it is important to continue research and innovation in this technique. Therefore, in this work was presented an algorithm for the identification and quantification of Malathion without the need of a calibration curve, determining linear functions that relate the signals obtained in wavelengths with the amount of the link that it presents, in this case the links C-S (620-715 cm<sup>-1</sup>), P-O (1018-1047 cm<sup>-1</sup>), C-O (1200-1300 cm<sup>-1</sup>) y C=O (1700-1800 cm<sup>-1</sup>) [13] [14].

## METHODS AND MATERIALS

The development of the algorithm to specify the amount of Malathion in a sample was performed in three phases: first, the digital image processing of IR spectra, then the obtaining of a database to analyze the types of links presented in the molecule of interest. Finally, an algorithm capable of quantifying the pesticide, which was checked by the evaluation of two IR spectra. In Figure-1, an outline of the algorithm.



### Image analysis

A digital image processing was performed in Matlab®, generating a table of values from an IR spectrum in transmittance (T) or absorbance (A), from this, a graph of absorbance was made. Thus, the information was obtained for building the database, in which was the base of the algorithm.

### Database

SDBS Data base (Spectral Database for Organic Compounds) was used [15], in order to acquire the FTIR spectra of the selected pesticides; Malathion, Acephate, Dimethoate, Formothion, Disulfoton, Imidan and Methidathion. These, have a similar molecular structure, for that reason, the spectra will coincide in some vibrations in which the patterns to perform the algorithm can be identified.

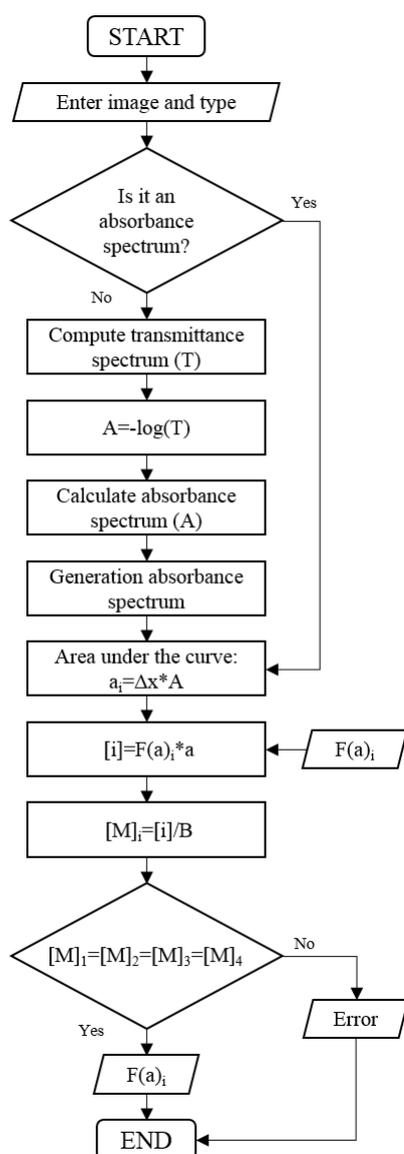


Figure-1. Algorithm for quantify Malathion.

By digital image processing described in Figure-1. It was determine the area under the curve of the graph of absorbance for four wavelength ranges, analyzing the following types of links: C-S (620-715 cm<sup>-1</sup>), P-O (1018-1047 cm<sup>-1</sup>), C-O (1200-1300 cm<sup>-1</sup>) and C=O (1700-1800 cm<sup>-1</sup>) [13], [14], as show in Figure-2.

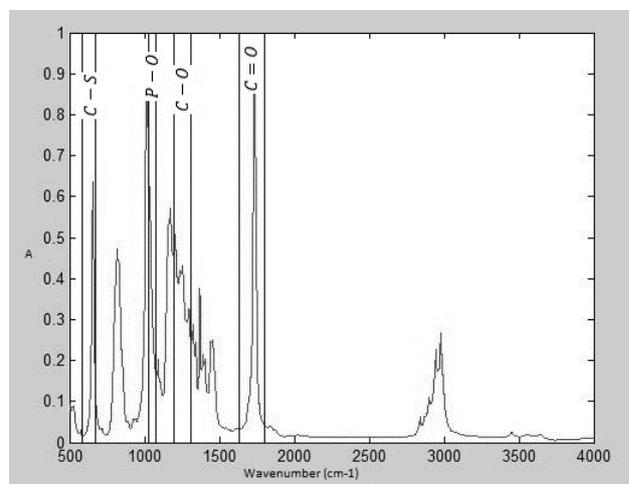


Figure-2. Malathion absorbance spectrum. Area under the curve in ranges of 620-715 cm<sup>-1</sup>, 1018-1047 cm<sup>-1</sup>, 1200-1300 cm<sup>-1</sup> and 1700-1800 cm<sup>-1</sup>

The areas under the curve, in the ranges of interest were stored in the database as a function of the amount and the type of bond, present in the matrix analyzed. Thus, the result is a function (F (a)) for each area relating to the amount of Malathion in millimoles (mmol).

### Development of the quantization algorithm

Once the database was obtained, an algorithm in Matlab® was performed, with which the amount of Malathion in the sample was quantified. In Figure 1, the input to the algorithm is an IR spectrum, which by interacting with the user defines if was transmittance or absorbance. It was transmittance, the digital image processing was used to generate the absorbance curve, but if was an absorbance spectrum, the data were collected and plotted, obtaining as output the replica of the entrance, but in Matlab®.

To obtain the number of *mmol* present in the sample, the area under the curve was determined in the vibrations mentioned (C-S, P-O, C-O and C=O) using the absorbance spectrum to enter these values into the functions F (a) for each bond.

Finally the algorithm, take the amount of Malathion |the type of bond. The results were compared with each other, to see if the input spectrum corresponds to the pesticide, or a different one, the value found have to be the same in all the four cases.

### Algorithm verification

Two Malathion IR spectra were used, the first from the base SDBS data and the other from a commercial



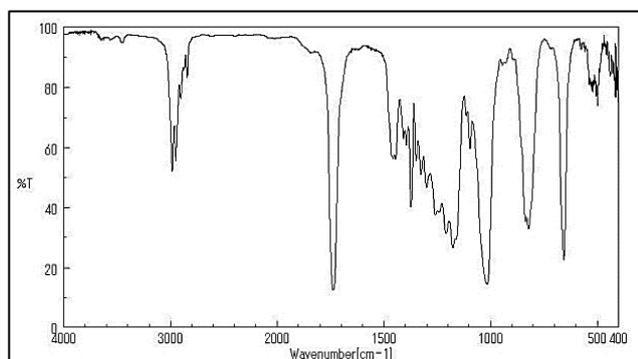
sample of 3.5 $\mu$ L of 57% w/v. Which was analyzed in a spectrometer Thermo Nicolet iS10.

## RESULTS AND DISCUSSIONS

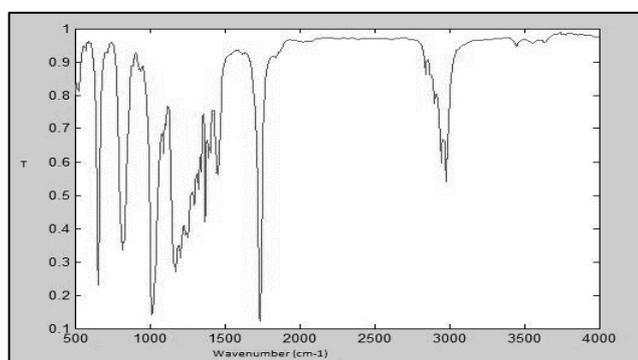
### Image processing algorithm

It was developed in MATLAB®, an algorithm to analyze the IR spectral images of the seven compounds selected from the data base SDDBS. Its function was to analyze the pixels of the images by determining the spectral domain and range of the spectrum, and thus associate the position of a pixel in the plane (x, y), with the wavelength (x axis) and the transmittance or absorbance (y axis), according to the case. In a further step, this identifies the black dots on the image for each value of (x) and thus selects the first one it finds its value (y) associated within the graph. In the same process, it saved into Matlab® vectors forming a wavelength vs. transmittance or absorbance table.

The image processing algorithm finally makes a mirror image of the spectrum as presented in Figure-3, because this organizes the scale on the x axis, ascending and descending image.



A



B

**Figure-3.** IR spectrum analysis (Malathion). A) Input to the algorithm [15]. B) IR spectrum generated by the algorithm.

The magnitude of the relative error ( $\epsilon_r$ ) depends on the image size and the number of pixels found in the values of (x). In the first case, if the image input was very small, the value of each pixel on the axis (y) was higher. Therefore, it increases the error when the actual value was

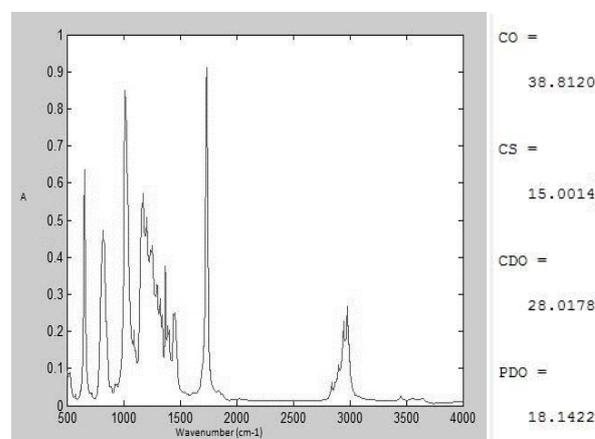
not one of the discrete numbers of the sum of pixels. In the second, the more points you have, the greater the probability that the first black point found this out the actual value. Table-1 shows the IR to certain points Malathion SDDBS base spectrum data.

**Table-1.** Relative error of the algorithm. Comparison algorithm data with SDDBS.

$\lambda$ ( $\text{cm}^{-1}$ )	T(SDBS)	T(algorithm)	% $\epsilon_r$
2983	0,52	0,61	17,31
2950	0,56	0,66	17,86
1738	0,13	0,15	15,38
1446	0,56	0,57	1,79
1258	0,38	0,39	2,63
1176	0,27	0,27	0,00
1016	0,15	0,15	0,00
822	0,33	0,36	9,09
<b>Average</b>	<b>0,3625</b>	<b>0,395</b>	<b>8,01</b>

According to the above, an average of 8.01% relative error was presented, because in the spectrum there are places where several consecutive black pixels. This made the noise of the input image, so that information between the input and the output is wrong, in some cases.

With this, the accuracy of the algorithm in quantifying Malathion depends on the quality of the input image, which should be as large as possible and well defined. The result of image processing in the absorbance curve of the IR spectrum and the areas under the curve in the four intervals of interest: C-S (620-715  $\text{cm}^{-1}$ ), P-O (1018-1047  $\text{cm}^{-1}$ ), C-O (1200-1300  $\text{cm}^{-1}$ ) and C=O (1700-1800  $\text{cm}^{-1}$ ), were shown in Figure-4.



**Figure-4.** Results of image analysis.

### Database

Table-2 shows the results obtained for the construction of the database. During the preparation, organophosphorus pesticides were used. This pretends to reduce the error caused by the molecular dynamics of the



compound. For each pesticide, the number of times of a certain type of bond was presented, by counting on the molecular structure as shown in Figure-5, where for example, 6 C-O bonds in a molecule Malathion equivalent to  $3,63 \times 10^{-2}$  mmol of the bond in  $6,05 \times 10^{-3}$  mmol of compound (see Table-2).

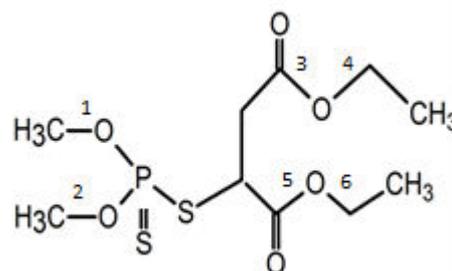


Figure-5. C-O Bond on the molecular structure of Malathion.

Table-2. Database built with the digital processing of data analysis. The number of bonds per molecule was determined in the figure (see Figure-5).

Molecule	mmol	Bond per molecule				Mmol bond				Area			
		P-O	C-O	C=O	C=S	P-O	C-O	C=O	C=S	P-O	C-O	C=O	C=S
Malathion	$6,05 \times 10^{-3}$	2	6	2	1	$1,21 \times 10^{-2}$	$3,63 \times 10^{-2}$	$1,21 \times 10^{-2}$	$6,05 \times 10^{-3}$	18,1	38,8	28,02	15,0
Acephate	$5,46 \times 10^{-3}$	1	1	1	1	$5,46 \times 10^{-3}$	$5,46 \times 10^{-3}$	$5,46 \times 10^{-3}$	$5,46 \times 10^{-3}$	7,05	20,6	7,24	6,56
Dimethoate	$4,36 \times 10^{-3}$	2	2	1	1	$8,71 \times 10^{-3}$	$8,71 \times 10^{-3}$	$4,36 \times 10^{-3}$	$4,36 \times 10^{-3}$	13,5	7,47	3,30	18,3
Formothion	$3,89 \times 10^{-3}$	2	0	2	1	$7,77 \times 10^{-3}$	0	$7,77 \times 10^{-3}$	$3,89 \times 10^{-3}$	12,9	13,4	8,97	12,8
Disulfoton	$5,73 \times 10^{-3}$	2	2	0	3	$1,15 \times 10^{-2}$	$1,15 \times 10^{-2}$	0	$1,72 \times 10^{-2}$	18,2	6,29	1,79	16,5
Imidan	$3,15 \times 10^{-3}$	2	2	2	1	$6,30 \times 10^{-3}$	$6,30 \times 10^{-3}$	$6,30 \times 10^{-3}$	$3,15 \times 10^{-3}$	3,19	7,75	15,78	8,07
Metidathion	$6,62 \times 10^{-3}$	2	4	1	1	$1,32 \times 10^{-2}$	$2,65 \times 10^{-2}$	$6,62 \times 10^{-3}$	$6,62 \times 10^{-3}$	16,5	31,5	10,64	15,1

The objective of find the area that represents some moles of bond in the database, it was to determine a mathematical relationship, function  $F(a)$ , between the area and the moles of the bond. This relationship should be ideally linear, where the slope of the line corresponds to the amount of area that represents a mol of bond.

Figure-6 shows the area ratio vs. the mmol of bond P-O, where the linear regression of these data correspond to the function  $F(a)$ . In this case was observed a linearity with a correlation of 0.77, which is due to the molecular dynamics of each chemical species, that affects the signal obtained from the bond measured, where the change in the structure makes that exist different interactions intermoleculars and with other nearby molecules, where noise was generated.

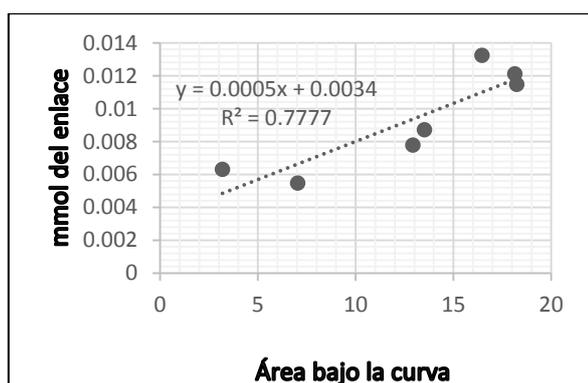


Figure 6. Linearization Area vs Bond/mol. Bond P-O.

In Table-3, the results for all bonds were show. It should be noted that the average correlation in the 4 types of bonds was 0.76, indicating that the present noise between compound and compound was the same, so these can be considered in future research within the  $F(a)$  function, enhancing the correlation and hence the reliability of algorithm for quantification of Malathion developed in this article.

Table-3. Correlation  $R^2$  found for the peaks (ranges of interest).

	Outstanding	Intercept	Correlation
P-O	0,0005	0,0034	0,77
C-O	0,0008	0,0016	0,69
C=O	0,0004	0,0022	0,77
C-S	0,0005	0,0009	0,80
Average	0,00055	0,002025	0,76

#### Algorithm for quantify Malathion

Malathion direct quantification was performed using the stoichiometric ratio of bond types and the number of times that was located within the molecule (B), as shown in ec.1.

$$[M]_i = \frac{[i]}{B} \quad (1)$$



Four values for the amount of Malathion were compared to each other with a margin of reliability of 95%, which allows to consider the errors caused by the correlation with which  $F(a)$  was determined and deviations from the image processing were obtained. Subsequently, if the values found meet this condition, the algorithm makes an average of them, and returns this as the solution of the problem. Otherwise the answer is wrong, because this way it was confirmed that the input spectrum corresponds to one of Malathion, and believes that the entry was incorrect.

#### Algorithm evaluation

It was found that the relative error for the commercial sample was 11.76% and for the pure sample of SDBS was 4.62%. The cause of the high error in the first was due to the matrix effect, i.e. by interference of different compounds to be analyzed. This indicates that the higher the purity of the sample, the better the results presented by the quantification algorithm. Table-4 shows the results obtained.

**Table-4.** Results obtained, verification test of the algorithm.

	mmol sample	mmol algorithm	% $\epsilon_r$
Malathion SDBS	0,0065	0,0062	4,62
Commercial Malathion	0,0034	0,0038	11,76

Finally, the algorithm did not introduce any error in the identification of the IR spectra of Malathion, because the erroneous entries were identified, with the spectra of Acephate, Dimethoate, Formothion, Disulfoton, Imidan and Methidathion. Therefore there was a very high precision in the spectral identification of each compound, thanks to the way the program makes the comparison.

#### CONCLUSIONS

It was performed the development of an algorithm in Matlab®, able to make image analysis of IR spectra and quantify the amount of mmol of Malathion in a sample. Due to errors in obtaining information from the spectra and the function  $F(a)$ , it displays an error of 4.62% in the quantification of a sample obtained from a database and 11.76% on a commercial sample 57% w/v.

The direct influence of the amount of a bond, present in a sample and the signal obtained was confirmed, which can be exploited to achieve a universal mathematical expression to quantify Malathion without the need for calibration curves. It must solve the problems produced by the matrix and the molecular dynamics effect of each compound used in the construction of the database.

The algorithm generated good results, which was capable of performing immediate identification and quantification of Malathion by IR spectrum.

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