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Method validation in the determination of Carbaryl pesticide in water samples using sonication and liquid chromatography–tandem mass spectrometry

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Abstract

The application of pesticides in agricultural activities is well known; however, their extensive or improper application may pollute water resources. As a consequence, monitoring of their residue levels in the water is a necessity. In this study, a rapid and sensitive analytical method has been validated according to the European Union SANCO/12495/2011 guidelines and the requirements of the norm V03-110 and those of the NF T 90-210 for the determination of Carbaryl pesticide in water samples using liquid chromatography-tandem mass spectrometry (LC–MS/MS). This work will help us to quantify in an exact way our samples and to be able to ensure the quality and the healthiness of the water. The linear calibration function is considered acceptable in the area studied [10 – 160 µg/Kg] for the pesticide studied. The accuracy of the quantification limit presupposed 10 µg/Kg was checked and the average recovery obtained by the LC–MS/MS method for both MRM Carbaryl pesticides ranged from 89.53 to 101.72 % which is in the acceptance range of the LAB GTA 26. The trueness, fidelity and accuracy of the method were verified for three levels of concentrations 10, 30 and 120µg/Kg. The method is easy, with low consumption of reagents, is characterized by reliability, sensitivity and therefore is suitable for the monitoring the levels of Carbaryl pesticide residues in water.

1. Introduction

In order to ensure sufficient food production all over the world pesticides are widely applied to crops at various stages of cultivation to provide protection against insects, weeds and pathogens [1]. However the use of pesticides may generate residues which involve a risk for both the environment and human health [2]. In recent years, traces of active ingredients from phytosanitary products and other products used in treating crops have been isolated from many phases of the environment, including water supplies. [3].

This presence of pesticides residues in the aqueous environment has raised increasing concerns recently due to their recalcitrance, toxicity, mutagenicity, carcinogenicity and tumorigenicity [4-6]. For these reasons, the residues of pesticides in water constitute an important parameter of its quality; they must be monitored regularly and kept as low possible to ensure consumer protection. Therefore, standards fixing the maximum residue limits have been established. According to the European Union Water Framework Directive and Groundwater Directive, the concentration of pesticides in drinking water and groundwater should not exceed 0,1 µg/L for a single compound, or 0,5 µg/L for the sum of all pesticides [7-8]. These maximum residue limits of pesticides are becoming weaker and this requires having reliable analytical methods capable of identifying and quantifying pesticide residues at very low levels. A reliable analytical method requires validation [9].

In this context, the aim of this study is to characterize the performance of a simple and rapid method developed by Fenoll et al. [10] for the determination of Carbaryl pesticides in water using high performance liquid chromatography tandem mass spectrometry.

Carbaryl (1-naphthalenyl-N-methylcarbamate) widely used carbamate insecticide, was chosen as the target molecule for the present study. This carbamate pesticide is known to present a wide spectrum of activity and is extensively used for pest control on fruit, vegetables and other crops [11] and it has been detected in the aquatic environment [12]. U.S. Geological Survey's National Water Quality Assessment (NAWQA) Program reported carbaryl as the second most frequently found insecticide in water [13].

Carbaryl has attractive features from an environmental point of view, such as low mammalian toxicity and low bioaccumulation potential. However, indiscriminate use can lead to bioaccumulation in food or waters, leading to bio-concentration through the food chain [14].

At present, there is increased concern about the environmental fate and toxicity of carbaryl because of its high toxicity to both humans and wildlife. [15]. In humans, acute and chronic occupational exposure inhibit the cholinesterase enzyme, impairing the function of central nervous system and can cause nausea, vomit, bronco-constriction, blurred vision, convulsions, coma and respiratory failure, in addition to its teratogenic characteristics [16].

Taking into account these effects, monitoring the residue levels of Carbaryl pesticide in water is a necessity for the protection of human health. Therefore, it was highly desirable to have an effective analytical method to quantitatively evaluate the fate of this pesticide and ensure the quality of drinking water. Because the concentrations of pesticides in water are usually very low (ng/L or lower), it is necessary to incorporate a concentration step in to the analytical procedure prior to gas chromatographic or liquid chromatographic determinations. Solid phase extraction (SPE) is the most commonly used extraction method to extract multi residue compounds from water samples [17]; however, new extraction techniques, aiming at reducing the overall analytical time and solvent consumption, have been recently proposed.

A rapid and efficient method based on sample pretreatment using initial single phase extraction of samples with acetonitrile by sonication, followed by liquid–liquid partition aided by "salting out" process using NaCl coupled with liquid chromatography-tandem mass spectrometry (LC–MS/ MS) was developed and successfully applied to multiresidue pesticide extraction and determination in real water samples. This method presents advantages compared with other conventional methods given the use of a low volume of organic solvent in the sample extraction and the fact that a cleanup step is not required [10].

When the developed methods are not standardized, the quality standards ISO 17025 type [9] require validation. The validation of a method implies providing evidence by the study and evaluation of different characteristics that the developed method meets the goals set. So to use the developed method employing sonication and liquid chromatography–tandem mass spectrometry to quantify the Carbaryl pesticide in water, we validated this method according to the requirements of the norm V03-110 [18] and those of the NF T 90-210 [19]. The different characteristics of the method was studied : the adequacy of the calibration model and the presupposed limit of quantification was verified and the accuracy of the method was cheeked using the accuracy profile which is an graphical tool that offers the possibility to visually observe the ability of an analytical method to fulfill its objectives.

2. Materials and Methods

2.1. *Reagents and standards*

Carbaryl ($C_{12}H_{11}NO_2$) was the pesticide used in this stady, which was purchased from Sigma-Aldrich company (Casablanca, Morocco) and its technical grade was 99,8% purity. The chemical structure of Carbaryl is given in Figure 1. All solvents used for LCMSMS were HPLC grade. The reagents have been blank analysis before use and no residues of pesticides have identified.

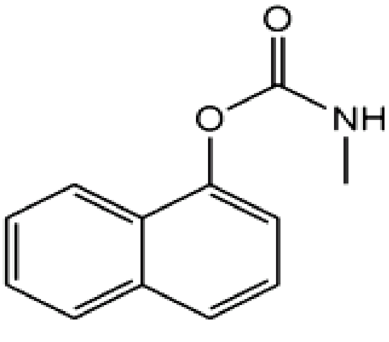


Figure 1: Chemical structure of Carbaryl

2.2. *Apparatus*

The concentrations of Carbaryl in water were determined using a liquid chromatography coupled with tandem mass spectrometry LC-MS/MS (API 3200 System, AB SCIEX /USA). For LC-MS/MS analysis the samples were transferred to an HPLC vial after filtration. Samples were kept at 25 °C in the autosampler, and 20 µL of subsamples were injected. Analytes were eluted through a colonne Gemini-NX C 18 (100 mm x 2 mm i.d, 3 µm) (Phenomenex/USA) using a gradient elution phase A / phase B (Phase A: ammonium formiate in ultra pure water at 5 mmol/L and Phase B: ammonium formate in methanol at 5 mmol / L) as described in table 1. Mass spectra were determined.

Table 1: Gradient LC using the phase A (5 mM ammonium formate in water) and phase B (5 mM ammonium formate in Methanol)

Step	Time (min)	Flow (µL/min)	Phase A (%)	Phase B (%)
0	**5**	**850**	**70**	**30**
1	**5**	**850**	**0**	**100**
2	**13**	**850**	**0**	**100**
3	**15**	**850**	**70**	**30**

Identification of Carbaryl was performed using MRM "multiple reaction monitoring" method. The MRM method is a mass spectrometric technique for quantifying one or more target molecules in a complex sample. The mass spectrometer measures the ratio mass/charge (m/z) of ionized molecules. The analytical specificity of the MRM method is afforded by the combination of the value of m/z of the molecule to be assayed (precursor) and the value of m/z of the fragment. Together these two parameters, intended to be specific for the molecule to be assayed, is called a transition. The parameters of the transition of the pesticide studied are presented in Table 2. The MRM 1 is afforded by the combination of the value of m/z of the Carbaryl pesticide (202.1) nominated precursor and the value of m/z of the fragment 1 (145.3), while the MRM2 is afforded by the combination of the value of m/z of the Carbaryl molecule (202.1) and the m/z value of the second fragment (127.1).

Table 2: LC-MS/MS parameters for Carbaryl

	Q1	Q2	Dwell	DP	EP	CE	CXP
Carbaryl 1	202.1	145.3	100	29.5	10	12	5
Carbaryl 2	202.1	127.1	100	31	10	35	5

2.3. *Preparation of standard solutions*

The stock solution of Carbaryl pesticide standard was prepared by accurately weighing appropriate amount of the pesticide in volumetric flasks and dissolving in adequate volume of methanol. This solution was stored at −20°C and before each use was left to reach room temperature.

The stock standard solution was used for all the experiments after necessary dilutions with distilled water. All glassware used was certified 'A' class and were cleaned with distilled water and dried at 110 °C before each use. Several standard solutions, with concentrations of 10–160 µg/Kg (10, 20, 40, 80, 160 µg/Kg), were injected to evaluate the Carbaryl linearity of the method.

2.4. *Preparation of the mobile phase*

The mobile phase was prepared by adding suitable aliquots of ammonium formiate to pure water or HPLC-grade methanol to obtain a final concentration of 5 mmol/L (Phase A: ammonium formiate in ultra pure water at 5 mmol/L / Phase B: ammonium formate in methanol at 5 mmol/L). The mobile phase was filtered under a vacuum through 0.45 mm nylon filters before use.

2.5. *Extraction procedure*

Water samples (10 mL) were added into a centrifuge tube and then extracted with 10 mL of acetonitrile by sonication (15 min at 0.5 cycles and 60% amplitude) using a Hielscher's sonic dismembrator 200 W generator equipped with standard titanium probe (Germany), followed by a salting-out step with 2 g NaCl. The tube was shaken and centrifuged for 10 min at 3000×g. Extract was filtered quantitatively through glass funnel containing a filter separation phase paper DP302, 150 mm diameter. The organic phase was concentrated to dryness using rotary vacuum evaporation. The residue was redissolved in 1 mL of acetonitrile, filtered through 0.45 µm filter and analyzed by LC–MS/MS under conditions described above [10].

2.6. *Method Validation*

The validation of analytical methods is used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. The validation of our method for quantitative analysis of Carbaryl pesticides in water was preformed according to the newest EU guidelines [20]. A novel approach using accuracy profile was applied to validate the ability of our method to quantify Carbaryl pesticide in water. It is based on β-expectation tolerance intervals for the total measurement error which includes trueness and fidelity. As a useful decision tool, this approach was found to be interesting since it allows controlling and evaluating the risk associated to the acceptance of unsuitable assay [21].

2.7. *Statistical tools*

To validate our method, we have used a Macro Excel developed by Mr CAILLAUD Philippe (Member of the General Committee of the food analysis methods AFNOR, EURL Oenologists from France).

3. Results and Discussion

3.1. Study of the calibration function

The linearity of the method should be tested in order to demonstrate the capacity of the method over the working range to obtain results of dosage directly proportional to the concentration or amount of analyte in the sample. The experiment plan A of the standard NF T 90-210 allows evaluating a calibration function in the field of linearity chosen either by comparing the model error observed to the observed experimental error or by comparing the relative biases observed at the Maximum Allowed Deviation (MAD).

To evaluate our calibration function in the field of linearity chosen (0.010 to 0.160 mg/kg) we opted for the comparison of relative biases observed at the Maximum Allowed Deviation (MAD).

To study the Linearity in the concentration range between 0.010 and 0.160 mg/Kg for the Carbaryl pesticide, we have prepared a five concentration levels (0.010, 0.020, 0.040, 0.80 and 0.160 mg/Kg) for five days under the same conditions with three repetitions per day and level. After analyzing our samples and obtaining a peak areas, we calculated the coefficient of variation, the intercept b and the slope a of our linear function y = ax + b. Table 3 shows the results obtained.

Table 3: Report of areas obtained on samples on different days of evaluation of a linear function of the type y = ax + b for Carbaryl pesticide

Pesticides	**Series**	**Area** **Level 1**	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**y = ax + b**		**CV**
		10 ppb	**20 ppb**	**40 ppb**	**80 ppb**	**160 ppb**	**a**	**b**	**residual**
Carbaryl MRM 1	D 1	7.19E+05	1.11E+06	1.79E+06	3.07E+06	5.66E+06	3.27E+04	4.44E+05	2.0%
	D 2	6.98E+05	9.98E+05	1.60E+06	2.71E+06	4.90E+06	2.79E+04	4.48E+05	1.8%
	D 3	8.10E+05	1.13E+06	1.66E+06	2.78E+06	4.84E+06	2.68E+04	5.84E+05	2.5%
	D 4	5.00E+05	8.18E+05	1.40E+06	2.52E+06	4.84E+06	2.88E+04	2.29E+05	0.9%
	D 5	1.18E+06	1.45E+06	2.12E+06	3.46E+06	6.00E+06	3.24E+04	8.36E+05	1.6%
Carbaryl MRM 2	D 1	4.72E+05	7.02E+05	1.16E+06	2.04E+06	3.74E+06	2.17E+04	2.74E+05	1.8%
	D 2	4.13E+05	5.90E+05	9.68E+05	1.67E+06	3.00E+06	1.72E+04	2.59E+05	2.5%
	D 3	5.08E+05	6.93E+05	1.04E+06	1.66E+06	2.99E+06	1.65E+04	3.59E+05	1.8%
	D 4	2.91E+05	4.63E+05	8.43E+05	1.55E+06	2.97E+06	1.79E+04	1.15E+05	0.9%
	D 5	5.42E+05	7.71E+05	1.28E+06	2.29E+06	4.20E+06	2.45E+04	2.99E+05	1.6%

Using these results, we calculated the amounts found using the same calibration function but reversed. For our linear function y = ax + b applied to our standard range, amounts found x was calculated using the following equation:

$$x = \frac{(\mathbf{y} - \mathbf{b})}{\mathbf{a}}$$

Table 4 shows the amounts of Carbaryl found using the reversed calibration function. Once we calculated the amounts of Carbaryl found we calculated the relative biases % associated with each standard which corresponds to the difference between the value found by the reversed calibration function and the theoretical value. The results for each MRM of Carbaryl are reported in Table 5.

Table 4: Amounts of Carbaryl pesticides found in µg/kg

Pesticides	Series	Quantification pesticides					Quantification pesticides				
		Level 10 ppb	**Level 20 ppb**	**Level 40 ppb**	**Level 80 ppb**	**Level 5 160 ppb**	**Level 10 ppb**	**Level 20 ppb**	**Level 40 ppb**	**Level 80 ppb**	**Level 5 160 ppb**
Carbaryl	**D 1**	8.40	20.37	41.28	80.43	159.52	9.08	19.69	40.59	81.37	159.26
	D 2	8.94	19.67	41.12	80.88	159.39	8.90	19.19	41.09	81.84	158.98
	D 3	8.45	20.28	40.33	81.93	159.02	9.08	20.30	41.41	79.09	160.12
	D 4	9.41	20.43	40.51	79.59	160.06	9.86	19.46	40.75	80.08	159.85
	D 5	10.60	18.88	39.82	81.10	159.60	9.95	19.29	39.96	81.39	159.41
		MRM 1					**MRM 2**				

The approach by comparing the relative biases observed to a set MAD calibration as described in the standard NF T 90-210 consist on checking that all biases observed are (±) lower than the set MAD calibration. The choice of MAD calibration depends on the simulation of the fault accepted in the field calibration as well as a correlation is greater than 0,999. Our calibration model is applied with a ± 20% for the concentration level of 0.010 mg/kg and with a ± 10% for the concentrations level ranging between 0.020 and 0.160 mg/kg. The results obtained are plotted in Table .5.

Table 5: Relative biases of Carbaryl pesticide

Pesticides	Series	relative biases					relative biases				
		Level 1 10 ppb	**Level 2 20 ppb**	**Level 3 40 ppb**	**Level 4 80 ppb**	**Level 5 160 ppb**	**Level 1 10 ppb**	**Level 2 20 ppb**	**Level 3 40 ppb**	**Level 4 80 ppb**	**Level 5 160 ppb**
Carbaryl	**D 1**	-16.0%	1.9%	3.2%	0.5%	-0.3%	-9.2%	-1.5%	1.5%	1.7%	-0.5%
	D 2	-10.6%	-1.6%	2.8%	1.1%	-0.4%	-11.0%	-4.0%	2.7%	2.3%	-0.6%
	D 3	-15.5%	1.4%	0.8%	2.4%	-0.6%	-9.2%	1.5%	3.5%	-1.1%	0.1%
	D 4	-5.9%	2.2%	1.3%	-0.5%	0.0%	-1.4%	-2.7%	1.9%	0.1%	-0.1%
	D 5	6.0%	-5.6%	-0.4%	1.4%	-0.3%	-0.5%	-3.5%	-0.1%	1.7%	-0.4%
	MAD fixed in %	**20%**	**10%**	**10%**	**10%**	**10%**	**20%**	**10%**	**10%**	**10%**	**10%**
	Conclusion	**Verified**					**Verified**				
	MRM	**MRM 1**					**MRM 2**				

The linear calibration function is considered acceptable in the field studied [0.010 à 0.160 mg/Kg] with the approach MADcalibration since all measured relative biases are less than the MADcalibration fixed. Figure 2 illustrates our results.

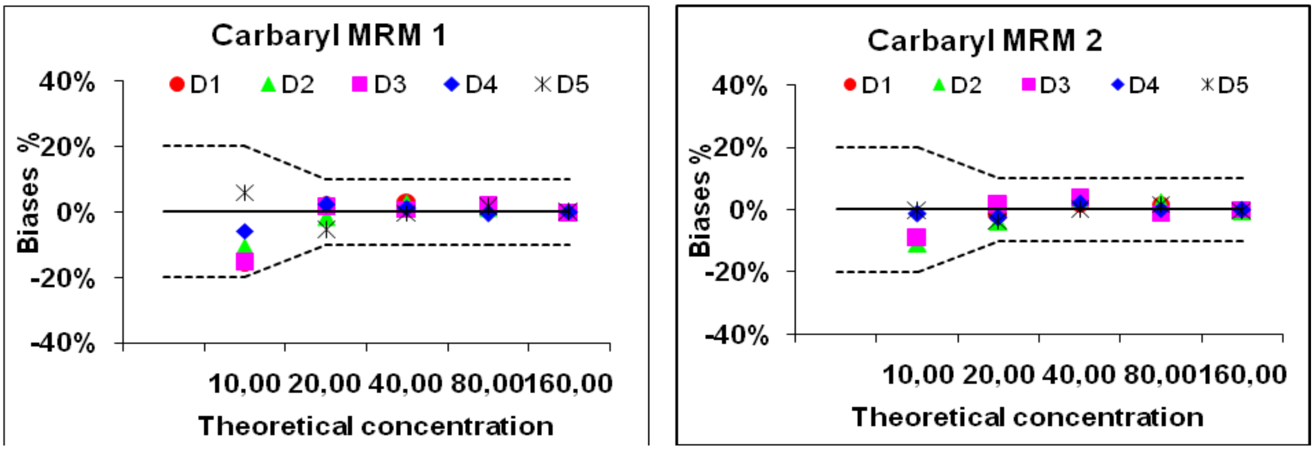


Figure 2: Distribution of relative biases % depending on the level of concentration compared to $MAD_{calibration}$ for Carbaryl pesticide

3.2. *Study of recovery*

The objective of this study is to characterize the influence of the preparation step when the latter is not considered in the study of the calibration function. It is advisable to approach this step before any study of accuracy and Limit of Quantification in order to assess the extraction efficiency of the method. For that, we have performed tests using two different fortification levels at 20% and 80% of the maximum concentration of application domain.

Five uncontaminated water samples were prepared at each level (128 µg/Kg and 32 µg/Kg), processed as described and analyzed in intermediate fidelity conditions. Each analysis was repeated twice in repeatability conditions. The results obtained are plotted in Tables 6 and 7.

Table 6: Study of recovery at 20 % of application domain

Pesticide	Series	Initial content before adding	Added content ppb	Found content (measured - initiale) ppb		Recovery %		Average recovery %	Variance series
				1	2	1	2		
Carbaryl 1	D 1	0	32	30.50	30.10	95.3	94.1	94.69	0.781
	D 2	0	32	30.20	30.60	94.4	95.6	95.00	0.781
	D 3	0	32	28.90	28.40	90.3	88.8	89.53	1.221
	D 4	0	32	29.70	29.50	92.8	92.2	92.50	0.195
	D 5	0	32	33.00	32.10	103.1	100.3	101.72	3.955
Carbaryl 2	D 1	0	32	28.90	29.50	90.3	92.2	91.25	1.758
	D 2	0	32	30.70	31.30	95.9	97.8	96.88	1.758
	D 3	0	32	32.10	32.80	100.3	102.5	101.41	2.393
	D 4	0	32	29.80	29.90	93.1	93.4	93.28	0.049
	D 5	0	32	29.60	29.70	92.5	92.8	92.66	0.049

Table 7: Study of recovery at 80 % of application domain

Pesticide	Series	Initial content before adding	Added content ppb	found content (measured - initiale) ppb		Recovery %		Average recovery %	Variance series
				1	2	1	2		
Carbaryl 1	D 1	0	128	130.40	129.90	101.9	101.5	101.68	0.076
	D 2	0	128	129.70	129.60	101.3	101.3	101.29	0.003
	D 3	0	128	126.20	126.50	98.6	98.8	98.71	0.027
	D 4	0	128	125.10	125.70	97.7	98.2	97.97	0.110
	D 5	0	128	124.60	123.20	97.3	96.3	96.80	0.598
Carbaryl 2	D 1	0	128	129.20	129.90	100.9	101.5	101.21	0.150
	D 2	0	128	128.30	128.40	100.2	100.3	100.27	0.003
	D 3	0	128	127.00	126.50	99.2	98.8	99.02	0.076
	D 4	0	128	126.70	126.20	99.0	98.6	98.79	0.076
	D 5	0	128	125.60	125.90	98.1	98.4	98.24	0.027

3.2.1. *Estimation of accuracy parameters of each addition level*

After quantifying Carbaryl pesticide in each sample, we calculated the following statistics on the recovery measured for each addition level using the formulas of accuracy in annex A of the standard NF T 90-210:

- R : Average recovery calculated.
- S_{FI} : Standard deviation of intermediate fidelity calculated.

The results obtained are plotted in Table 8.

3.2.2 *Interpretation of accuracy parameters of each addition level*

The recoveries obtained for both MRM Carbaryl pesticides ranged from 89.53 to 101.72%. These recoveries were in the acceptance range of the LAB GTA 26 [22]; the Technical Guide on pesticide accreditation who requires that the average recovery rate is between 70 and 120%.

Table 8: Accuracy parameters at each level (128 µg/Kg and 32 µg/Kg)

ADDITION LEVEL	**32 µg/Kg**		**128 µg/Kg**	
MRM	**MRM 1**	**MRM 2**	**MRM 1**	**MRM 2**
Number of series : n	5	5	5	5
Number of repetitions per serie : r	2	2	2	2
Repeatability variation: $s_{répét}^2$	1.387	1.201	0.163	0.067
Variance of average : s_R^2	20.227	16.755	4.501	1.460
Inter-series variance : s_B^2	19.534	16.155	4.419	1.427
Variance of intermediate fidélity : s_{FI}^2	20.920	17.356	4.582	1.493
Standard deviation of repeatability : $s_{répét}$	1.2	1.1	0.4	0.3
CV of repeatability $CV_{répét}$ in %	1.2%	1.2%	0.4%	0.3%
General average R	**94.7**	**95.1**	**99.3**	**99.5**
Standard deviation of intermediate fidelity : s_{FI}	**4.6**	**4.2**	**2.1**	**1.2**
CV of intermediate fidelity CV_{FI} in %	**4.8%**	**4.4%**	**2.2%**	**1.2%**

3.3. *Study of a presupposed Limit of Quantification*

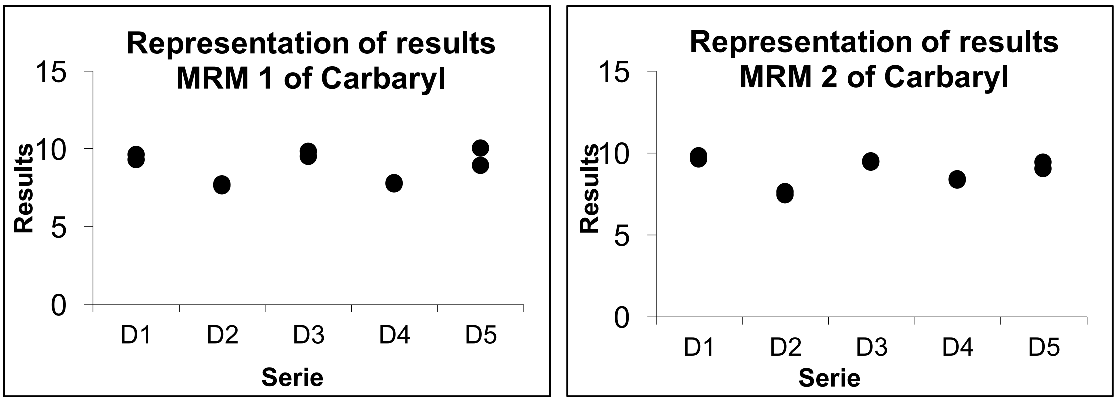
The Limit of Quantitation (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with a stated acceptable precision and accuracy, under stated experimental conditions. The ''B test'' of the AFNOR NF T90-210 standard describes a method to check whether a presupposed LOQ can be acceptable [19]. It presents the advantage to assess experimentally a LOQ value, which is to our point of view more realistic than to calculate one.

The objective of this study is to verify that a presupposed limit of quantification LOQ is acceptable. To realize this study, we prepared a sufficient volume of uncontaminated water with pesticide studied, then we doped samples with a quantity of Carbaryl pesticides corresponding to the limit of quantification presupposed to 10 µg/Kg.

This volume of water was fractionated in 5 identical test samples with two repetitions for each, after analyzing our samples we quantified Carbaryl pesticide under conditions of intermediate precision. Each analysis was repeated twice in repeatability conditions. Table .9 and Figure .3 successively present the results obtained after quantification of Carbaryl pesticide.

Table 9: Study of a presupposed LOQ of Carbaryl

Series	**CARBARYL – MRM 1**				**CARBARYL – MRM 2**			
	RESULTS in µg/kg		**Average**	**Variance series**	**RESULTS in µg/kg**		**Average**	**Variance series**
	Repetition				**Repetition**			
	1	**2**	**zi**	**si²**	**1**	**2**	**zi**	**si²**
D 1	9.60	9.30	9.45	0.045	9.80	9.60	9.70	0.020
D 2	7.60	7.70	7.65	0.005	7.40	7.60	7.50	0.020
D 3	9.80	9.50	9.65	0.045	9.40	9.50	9.45	0.005
D 4	7.70	7.80	7.75	0.005	8.30	8.40	8.35	0.005
D 5	8.90	10.00	9.45	0.605	9.00	9.40	9.20	0.080



Figue 3: Study of a presupposed LOQ of Carbaryl

3.3.1. *Estimation of accuracy parameters of a presupposed LOQ*

Once the Carbaryl pesticide was quantified in each sample, we calculated the following statistics on the measured quantities using the formulas in annex A of the standard NF T 90-210:

- z_{LQ} **:** Average quantity calculated.
- s_{LQ} **:** Standard deviation of intermediate fidelity calculated.

3.3.2. *Interpretation of accuracy parameters for a presupposed LOQ*

To interpret our results, we have to ensure the accuracy of the presupposed limit of quantification compared to an acceptable maximum deviation of 60% of the LOQ by checking the following two inequalities:

$$z_{LQ} + 2\, s_{LQ} < LQ + 60\% \times LQ$$

$$z_{LQ} - 2\, s_{LQ} > LQ - 60\% \times LQ$$

If at least one of the inequalities is not checked then the limit of quantification is not verified. Table .10. represents the results of this study. The presupposed limit of quantification to 10µg/kg is verified; it was checked by the two inequalities. Even if experimental methods are longer than calculation, LOQ values obtained are by far more reliable [23].

3.4. *Study of accuracy (NF T 90-210 and NF V 03-110)*

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (concentration) of the analyte [24]. It is an important concept in method validation because it represents the global performance of the method. In general, accuracy is quantified by combining trueness and fidelity. These two concepts are well known: the trueness is the difference between average and the true value while fidelity is the dispersion of individual results from the mean [25].

Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the studied concentration range is recommended.

Table 10: Accuracy parameters of a presupposed LQ to 0,010 mg/K

	CARBARYL	
	MRM 1	**MRM 2**
Number of series : n	5	5
Number of repetitions per serie : r	2	2
Repeatability variation : srép^2	0.141	0.026
Variance of average : s(zi)^2	0.998	0.819
Inter-series variance: sb^2	0.927	0.806
Variance of intermediate fidélity : slq^2	1.069	0.832
General average : zlq	8.790	8.840
Standard deviation of intermediate fidelity : slq	1.034	0.912
CV of intermediate fidelity in % : CVlq	0.118	0.103
Reference value : ref (in µg/Kg)	**10**	**10**
Acceptable Maximum Deviation	**6**	**6**
lq + 60% lq	**16**	**16**
zlq + 2 slq	**10.86**	**10.66**
zlq - 2 slq	**6.72**	**7.02**
lq - 60% lq	**4**	**4**
The accuracy of the method	**verified**	**verified**

The ‘‘D test’’ of the AFNOR NF T90-210 standard describes a method to verify the accuracy of an analytical method. To study the accuracy of the method in the concentration range between 0.010 and 0.160 mg/Kg for the Carbaryl pesticide, we have prepared a three concentration levels (0.010, 0.030 and 0.120 mg/Kg) for five days under the same conditions with two repetitions per day and level. Table .11 presents the results obtained after quantification of Carbaryl pesticide at each concentration levels.

Table 11: Study of accuracy of Carbaryl to 10, 30 and 120 µg/Kg

Level		Level 1 (10 µg/Kg)		Level 2 (30 µg/Kg)		Level 3 (120 µg/Kg)	
MRM	**Series**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
Carbaryl 1	**D1**	9.6	9.3	29.5	28.3	105.2	105.3
	D2	7.6	7.7	23.7	23.1	111.1	111.4
	D3	9.8	9.5	26.3	26.3	111.6	110.5
	D4	7.7	7.8	28.0	28.4	109.5	115.3
	D5	8.9	10.0	28.2	28.5	109.7	111.4
Carbaryl 2	**D1**	9.8	96	28.1	29.1	103.4	105.8
	D2	7.4	7.6	23.4	21.4	103.5	106.1
	D3	9.4	9.5	26.9	26.2	112.7	107.3
	D4	8.3	8.4	28.9	28.8	110.2	116.1
	D5	9.0	9.4	27.5	27.6	109.9	111.5

3.4.1. *Trueness and fidelity criterias at each concentration level*

Once the Carbaryl pesticide was quantified in each sample, we used data in table .11. to calculate the standard deviation of repeatability, inter-series and fidelity intermediate. This calculation was performed independently for each concentration level according to the principle of the standard ISO 5725-2 [26], as described in Annex A of the standard NF V 03-110. Table .12 presents the results obtained at each concentration levels.

Using these results, we calculated the fidelity criteria and the trueness criteria as described in the formulas in annex A of the standard NF T 90-210. Table .13 represents the results obtained at each concentration levels.

3.4.2. *Calculation of tolerance intervals according to NF V03-110*

The calculation method proposed by Mee [27] is the one that was chosen for the standard NF V03-110. It has also been adopted by a committee of the Society of Sciences and Pharmaceutical Technology (SFSTP) (Ref. [28] to [30]). The calculation is based on data in tables 12 and 13.

Table 12: Study of accuracy of Carbaryl to 10, 30 and 120 µg/Kg

MRM	CARBARYL 1			CARBARYL 2		
Theoretical concentration in µg/Kg	**10**	**30**	**120**	**10**	**30**	**120**
Number of series: n (I)	5	5	5	5	5	5
Number of measures (IJ)	10	10	10	10	10	10
Number of repetitions (J)	2	2	2	2	2	2
SSD residual	0.70	1.03	18.92	0.13	2.76	39.52
SSD total	8.69	41.66	81.40	6.68	57.61	154.53
SSD inter-series	7.98	40.64	62.48	6.55	54.85	115.00
Intermediate calculation s²B	0.93	4.98	5.92	0.81	6.58	10.42
Variance of repeatability (s²r)	0.14	0.20	3.78	0.03	0.55	7.90
Variance Inter Series (s²B)	0.93	4.98	5.92	0.81	6.58	10.42
Variance of fidelity (s²FI)	1.07	5.18	9.70	0.83	7.13	18.33

Table 13: Results of the study of accuracy - fidelity and trueness

MRM	CARBARYL 1			CARBARYL 2		
Theoretical concentration in µg/Kg	**10**	**30**	**120**	**10**	**30**	**120**
Fidelity						
Average concentration found	8.79	27.03	110.10	8.84	26.79	108.65
Standard deviation of repeatability (s_r)	0.38	0.45	1.95	0.16	0.74	2.81
Standard deviation inter Series (s_B)	0.96	2.23	2.43	0.90	2.57	3.23
Standard deviation of fidelity (s_{FI})	1.03	2.28	3.11	0.91	2.67	4.28
Trueness						
Biases (%)	-12.10	-9.90	-8.25	-11.60	-10.70	-9.46

For each level of concentration, we calculate the standard deviation of the tolerance interval sIT, coverage factor Ktol and the low and high tolerance interval limit using the formulas in annex A of the standard NF V03 -110 and taking into consideration the fact that this standard suggest a coverage factor that depends on a probability $\pi \geq 80\%$. All calculations are gathered in Table 14. The tolerance interval is expressed as a symmetrical interval around the mean concentration z found for each level:

$$z \pm k_{tol} \times s_{IT}$$

The standard deviation of the tolerance interval s_{IT} and the quantity k_{tol} which is the cover factor of the tolerance interval are calculated according to the formulas indicated in the standard PR NF V 03-110 [18].

Table 14: Tolerance intervals Limits by level

MRM	**CARBARYL 1**			**CARBARYL 2**		
Theoretical concentration in µg/Kg	**10**	**30**	**120**	**10**	**30**	**120**
Cover factor of the tolerance interval						
Variance ratio (R)	6.58	24.28	1.56	31.01	11.94	1.32
Coefficient B²	0.54	0.51	0.62	0.51	0.52	0.64
Number of degrees of freedom	4.57	4.16	5.90	4.13	4.32	6.13
Probability tolerance (pi)	0.80	0.80	0.80	0.80	0.80	0.80
t Student low	1.53	1.53	1.48	1.53	1.53	1.44
t Student high	1.48	1.48	1.44	1.48	1.48	1.41
Cover factor (k_{tol})	1.50	1.52	1.44	1.53	1.51	1.44
Standard deviation of tolerance (s_{IT})	1.13	2.49	3.36	1.00	2.92	4.60
Tolerance interval						
Low tolerance interval limit	7.10	23.24	105.26	7.32	22.37	102.04
High tolerance interval limit	10.48	30.82	114.94	10.36	31.21	115.26

Once we calculated the low and high tolerance interval limits, we calculate the average concentration found and the recovery rate for each level and for each MRM, then taking into consideration the absence of any official reference, we based on LAB GTA 26 [22] to fix the high and low acceptability limits of our method. This technical guide on pesticide accreditation requires that the average recovery rate is between 70 and 120%. Table 15 present the results obtained.

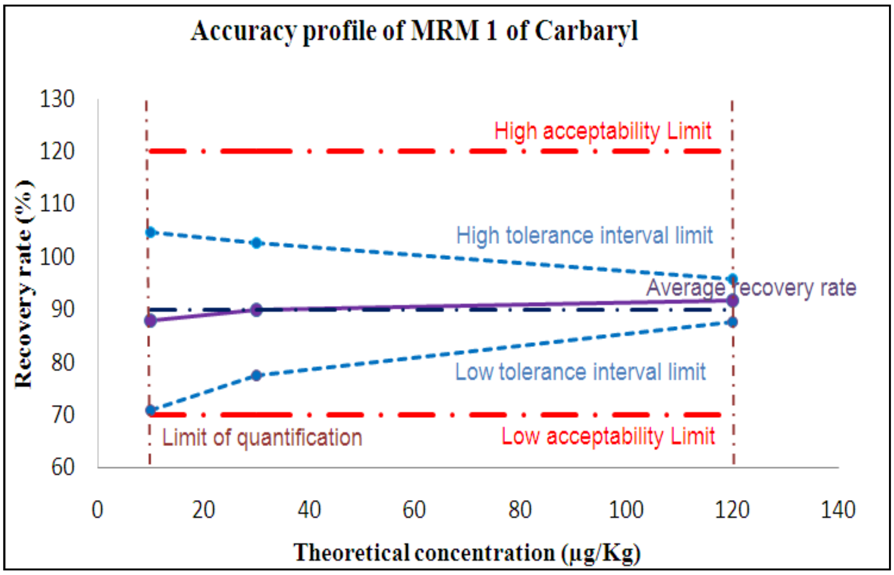
3.4.3. *Construction of « accuracy profile » of the method and its interpretation*

After fixing the acceptability limits and calculating the tolerance intervals limits of our method, recovery rate and the average concentration found at each concentration level we connected all this elements to construct the accuracy profile. An accuracy profile as a graphical tool offers the possibility to visually observe the ability of an analytical method to fulfill its objectives and to control the risks associated with its routine application [31].

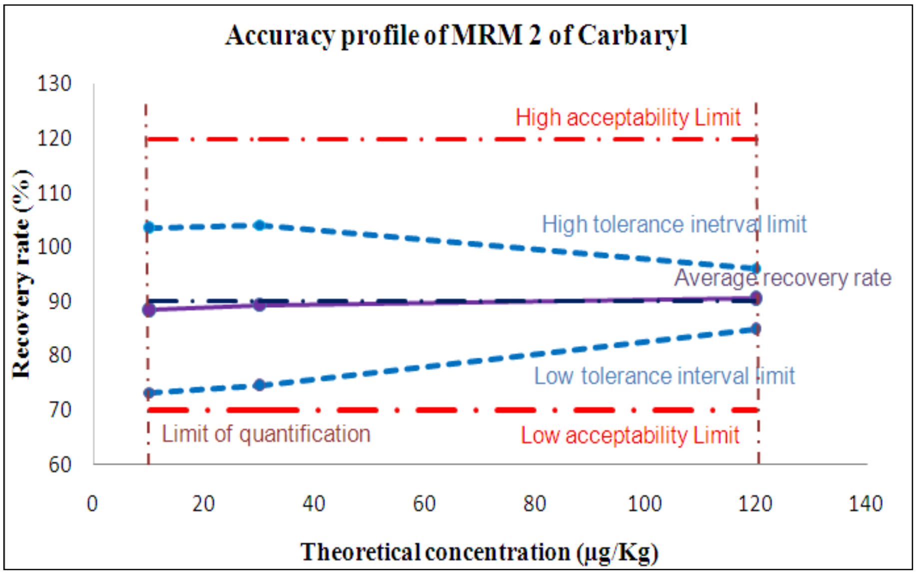
Table 15: Review of the study of accuracy with standard V03-110

MRM	**CARBARYL 1**			**CARBARYL 2**		
Theoretical concentration in µg/Kg	**10**	**30**	**120**	**10**	**30**	**120**
Average concentration found	8.79	27.03	110.10	8.84	26.79	108.65
Recovery rate (%)	87.90	90.10	91.75	88.40	89.30	90.54
Low tolerance interval limit	71.00	77.45	87.71	73.17	74.58	85.03
High tolerance interval limit	104.80	102.75	95.79	103.63	104.02	96.05
Low acceptability Limit	70.00	70.00	70.00	70.00	70.00	70.00
High acceptability Limit	120.00	120.00	120.00	120.00	120.00	120.00

To build the accuracy profile and allow visual interpretation of results, we selected the values of the line "theoretical concentration" in abscissa axis and the values of " Low and high tolerance limits relatives", "average recovery rates " and " Low and high acceptability Limits" in ordinate axis under the title "accuracy profile". The figures 4 and 5 present the accuracy profile obtained for each MRM of Carbaryl pesticide. For both MRM Carbaryl pesticide, we observe that the tolerance limits to 80% are between the acceptability limits in a valid domain that extends from 0.010 mg/kg to 0.160 mg/kg. We can then conclude that the method is valid in this field.



Figue 4 : Accuracy profile of MRM 1 of Carbaryl



Figue 5 : Accuracy profile of MRM 2 of Carbaryl

4. Conclusion

The validation of an analytical method is the ultimate step before its implementation in routine use. In this work, a method using sonication and liquid chromatography–tandem mass spectrometry for the analysis of residues of carbaryl pesticide in water by LC–MS/MS was validated according to European Union SANCO/12495/2011 guidelines and the requirements of the norm V03-110 and those of the NF T 90-210.

In order to guarantee the ability of the method to provide accurate results, a recent validation approach based on accuracy profile has been used. This alternative validation strategy uses the tolerance interval by providing a visual decision tool, it also provides in one statistic compiled information on the methods performance characteristics, compared to the classical approach in which each required parameter is evaluated individually.

The results of validation demonstrate the good accuracy of the method over a concentration range of 10-160 µg kg-1. Furthermore, the method proved to be simple and gave quantitative results for the pesticide studied, providing good validation parameters, such as linearity, recovery, limit of quantification, trueness, fidelity and accuracy.

An accuracy profile was drawn for each MRM of Carbaryl pesticide. In both cases, the tolerance limits were inside the acceptance limits over the whole defined concentration range(acceptability limits were fixed at 70 and 120% and β-expectation tolerance intervals was fixed at 80%). Thus, the method was validated over the whole defined concentration range.

References

1. Kazemi H.V., Anderson S.H., Goyne K.W., Gantzer C.J., *Chemosphere.* 74 (2009) 265–273.
2. Moreno-González D., Huertas-Pérez J.F., García-Campaña A.M., Gámiz-Gracia L., *Talanta*. 128 (2014) 299- 304.
3. Chattoraj S., Mondal N.K., Das B., Roy P., Sadhukhan B., *Appl Water Sci.* 4 (2014) 79–88.
4. Chelinho S., Lopes I., Natal-da-Luz T., Domene X., Nunes ME., Espíndola EL., Ribeiro R., Sousa J.P., *Environ. Toxicol. Chem*. 31(2) (2012) 437–445.
5. Lu C. S., Holbrook C. M., Andres L. M., *Environ. Health Perspect.* 118(1) (2010) 125–130.
6. Muhammetoglu A., Durmaz S., Uslu B., *Environ Forensics*. 11(1-2) (2010) 179–186.
7. The European Parliament and the Council of the European Union, Directive 2000/60/EC,. EFT L 327 of 22.12.2000. *OJEC*, (2000).
8. The European Parliament and the Council of the European Union. Directive 2006/118/EC,. EFT L 372 of 27.12.2006. *OJEC*, (2006).
9. ISO EN 17025, laboratories (2005).
10. Fenoll J., Hellin P., Martinez C.M., Flores P., Navarro S., *Talanta*. 85 (2011) 975–982.
11. Miwa D.W., Malpass G.R.P., Machado S.A.S., Motheo A.J., *Water Res.* 40 (2006) 3281 – 3289.
12. Wilsont P.C., Foos J.F., *Environ. Toxicol. Chem*. 25(11) (2006) 2847-2852.
13. Chattoraj S., Mondal N.K., Das B., Roy P., Sadhukhan B., *J. Environ. Chem. Eng*. 2 (2014) 1920–1928.
14. Ruiz-Medina A., Llorent-Martınez E.J., Fernandez-de Cordova M.L., Ortega-Barrales P., *J. Food Comp. Anal.* 26 (2012) 66–71.
15. Chattoraj S., Mondal N.K., Das B., Roy P., Sadhukhan B., *J. Environ. Chem. Eng.*2 (2014) 1920–1928.
16. Gupta N., Pillai A.K., Parmar P., *Spectrochim Acta A Mol Biomol Spectrosc*. 15 (139) (2015)471-476.
17. Primel E.G., Caldas S.S., Escarrone A.L.V., *Cent. Eur. J. Chem*. 10 (2012) 876–899.
18. French Standards Association (AFNOR),. NF V03-110 standard, Mai 2010.
19. French Standards Association (AFNOR),. NF T 90-210 standard, Mai 2009.
20. European Commission., Document N1 SANCO/12495/2011, 2011.
21. Djang'eing'a Marini R., Servais A.C., Rozet E., Chiap P., Boulanger B., Rudaz S., Crommen J., Hubert P., Fillet M., *J. Chromatogr. A.* 1120 (2006) 102–111.
22. Accreditation guideline, COFRAC, LAB GTA. 26 (2010).
23. Du Bois de Maquillé L., Wund P., Renaudin L., Gautier C., Jardy A., Vial J., Thiébaut D., Fichet P., Goutelar F., J. Radioanal. Nucl. Chem. 306 (2015) 213-220.
24. USP 25-NF 20, (1225), United States Pharmacopeal Convention, Rockville, MD, 2002, p. 2256.
25. Yardin C., *17th International Congress of Metrology*. EDP Sciences. 02016 (2015).
26. ISO 5725-2,. (1994) (reviewed in 2013).
27. Mee R.W., *Technometrics*. 26:3 (1984) 251-254.
28. Commission SFSTP., Hubert P., Nguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Cohen N., Compagnon P.A., Dewé W., Feinberg M., Lallier M., Laurentie M., Mercier N., Muzard G., Nivet C., Valat L., Part I. *STP Pharma Pratiques*. 13:3 (2003)101-138.
29. Commission SFSTP., Hubert P., Nguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Cohen N., Compagnon P.A., Dewé W., Feinberg M., Lallier M., Laurentie M., Mercier N., Muzard G., Nivet C., Valat L., Part II – Statistics. *STP Pharma Pratiques*. 16:1 (2006) 30-60.
30. Commission SFSTP., Hubert P., Nguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Cohen N., Compagnon P.A., Dewé W., Feinberg M., Lallier M., Laurentie M., Mercier N., Muzard G., Nivet C., Valat L., Part III- Application examples. *STP Pharma Pratiques*. 16:2(2006) 87-121.
31. Hubert P., Nguyen-Huu J.J., Boulanger B., Chapuzet E., Chiap P., Cohen N., Compagnon P.A., Dewé W., Feinberg M., Lallier M., Laurentie M., Mercier N., Muzard G., Nivet C., Valat L., *J. Pharm. Biomed. Anal.* 36 (2004) 579-86.

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