Analytical method for identification and determination of indoxacarb content in Suspension concentrate SC pesticide formulations using high performance liquid chromatography

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Abstract: This study described an analytical and validated method for the determination of indoxacarb in suspension concentrate (SC) pesticide formulation product with concentration 300 g a.i./L. Indoxacarb belongs to oxadiazine insecticides and a sodium channel blocker. The product under study is a readymade formulation produced under its manufacturing conditions and it is under registration process in Egypt. The active ingredient content is quantified using a high performance liquid chromatography (HPLC) equipped with Diode-Array Detection (DAD), with external standard of high and known purity. Validation parameters used for the current study based on the Australian Pesticides and Veterinary Medicines Authority (APVMA) guidelines and ISO/IEC 17025 definition. The parameters evaluated included taking into consideration method specificity, linearity, precision, and accuracy. Under the optimum conditions, the linearity was linear between 0.1 mg/ml and 3.2 mg/ml. The correlation coefficient value was found to be high (R^2> 0.99998) for the target indoxacarb SC formulation. The results showed precise and accurate method where the RSD% obtained was about 0.35% from 9 replicates with two injections each. Selectivity showed no interference with the active ingredient peak from any adjuvants or components included in the sample. The results of this study indicated that the HPLC - DAD described method is reliable, suitable and successfully applied to the estimation of the target indoxacarb determination and can be confirmed. GC- MS spectroscopy was used to ascertain the presence of indoxacarb in the SC solution.

Keywords: HPLC - DAD, indoxacarb, SC formulation, validation, GC - MS, APVMA

1.Introduction:

Indoxacarb is an insecticide used for control of certain lepidopteran pests including beet armyworm and also has activity on selected sucking insect pests **(Dinter & Wiles 2000).** In Egypt it is recommended on different fruits and vegetables of the target insects of these insect families according to the Agriculture Pesticide Committee **(APC, 2020),** the responsible authority of the registration of agricultural pesticides. It is mode of action is based on blocking of sodium channel resulting in paralysis and death with reduced risk **(Wing *et al.* 2000; McKinley *et al.* 2002).** It has been classified by the EPA as a reduced-risk pesticide and is considered an organophosphate (OP) replacement **(USEPA, 2000).**

Indoxacarb produced by DuPont and marketed as StewardTM, AvauntTM, and Technical indoxacarbTM. Indoxacarb was formulated as a 30% a.i. water dispersible granule (WG) and as a 15% a.i. suspension concentrate (SC) liquid. A newly introduced formulation as a 30 % a.i. SC of Chinese origin which under the current study and under the registration process in Egypt. In this research, the insecticide analysed is indoxacarb. It is primarily available in emulsifiable concentrate (EC), water dispersible granules (WG) and suspension concentrate (SC) formulations. The formulation under analysis study is 300 g a.i. /L as mention in its declared label. The aim was to develop and validate a simultaneous rapid method for the determination of the active ingredient indoxacarb in the commercial formulation products in our routine laboratory analysis. The method performance for the determination meets the required criteria (**European Guide, 1998; European Commission SANCO, 2000 and APVMA, 2004).**

The results of validation of the HPLC - DAD method for the measurement were based on the ISO/IEC 17025 definition and (APVMA, 2004), with emphasized on the following validation parameters; method Specificity, linearity, precision (repeatability) and accuracy.

2.Materials and Methods:

2.1.Indoxacarb 30 % SC (w/v) sample.

The Suspension concentrate (SC) formulation sample with concentrations 300 g (a.i.) /L (Farotida 30 % w/v – Chinese origin) which under registration process was provided from the Research Department of Pesticide Analysis at the Central Laboratory of Agricultural Pesticides (CAPL) with the analytical standard.

2.2.Reagents and Standards

Methanol & Acetonitrile HPLC grade

Deionized Water, HPLC grade

Analytical standard of known purity 97 – 99.6% as certified by manufacturer(s). The indoxacarb identity is shown as follows.

2.3.Identity of Indoxacarb

2.3.1.ISO Common name Indoxacarb

2.3.2.Chemical name methyl (S)-7-chloro-2,3,4a,5-tetrahydro-2-[methoxy-carbonyl(4-trifluoromethoxy-phenyl)-carbamoyl]- indeno[1,2-e][1,3,4]oxadiazine-4a-carboxylate (IUPAC)

methyl (4aS)-7-chloro-2,5-dihydro-2-[[(methoxy- carbonyl)[4-(trifluoromethoxy) phenyl]amino]- carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)- carboxylate (CA; 173584-44-6)

2.3.3.Empirical formula $C_{22}H_{17}ClF_3N_3O_7$

2.3.4.Chemical Abstracts Service (CAS) No.: 173584-44-6

2.3.5.Pesticide Type: Insecticide Chemical

2.3.6.Family: oxadiazines

2.3.7.Formulations Suspension concentrates, water dispersible granules, dry flowables and emulsifiable concentrates

The ISO common name indoxacarb refers to the S - enantiomer solely being the carrier of insecticidal activity. The R-enantiomer does not carry insecticidal activity **(FAO, 2000).**

2.4.Equipment

nalytical balance, capable of measuring to 0.1 mg

Ultrasonic bath – model UCS - 05

2.4.1.Instruments /Apparatus

2.4.1.1.HPLC – DAD

High performance liquid chromatograph (HPLC) – 1260 Agilent model equipped with a DAD - detector and a constant temperature column with an autosampler is used for this assay.

2.4.1.2.GC-MS, Agilent model

GC-MS, Agilent model 7890B equipped with 5977 A MSD.

2.5.Preparation of the Standard and Sample Solution:

Weigh out 0.0104 g (10.4 mg) of standard indoxacarb and dissolve in 25 ml volumetric flask using methanol. This will give a solution containing 0.4 mg/ml of the standard indoxacarb, (solution A). For preparation solution B, weigh out 0.3445 g (344.5 mg) of the indoxacarb 30 % SC (w/v) sample and proceed exactly as in standard solution sample. The weight is multiplied by density; the results will be in weight per volume). To homogenize the two mixed solutions, place the flasks of solutions A and B in an ultrasonic bath for few mins. Remove the flask from the bath and allow to stand at room. shake for homogenize.

2.6.Determination and data handling

Equilibrate the column with mobile phase until a stable baseline is obtained. Introduce 5 µl of the solution A and solution B into the HPLC individually on the optimum conditions of apparatus as mentioned and observe the formation of peak of indoxacarb.

Indoxacarb content, percent m/m = (M_1 X A_2 X P)/ (M_2 X A_1)

Where

M_1 = mass in g of standard indoxacarb in standard solution.

M_2 = mass in g of sample taken for test

A_1 = peak area of indoxacarb in the chromatogram of standard solution.

A_2 = peak area of indoxacarb in the chromatogram of sample solution.

P = percent purity of indoxacarb standard.

2.6.1.HPLC Procedures

2.6.1.1.Operating conditions:

Column	Prepure BU CHI - C18 Aº 100 ,10 □m, 150 x 10 mm
Mobile phase	Acetonitrile (70 %) + methanol (20 %) + 10 % deionized water
Flow rate	1.0ml/min
Column temperature	ambient
Injection volume	5 µl
Detection wavelength (bandwidth 100nm)	285 nm
Run time minutes	16

2.6.2.GC-MS analysis and identification

The procedures have been carried out and performed using GC-MS, Agilent model 7890B equipped with 5977 A MSD, with a fused silica capillary column HP-5MS (30 m x 0.25 mm x 0.25 µm film thickness). Helium was the carrier gas used with 1.0 ml/min pulsed split mode. The injection volume applied 1 µl. The temperature program was as follows; held at 50℃ for 0.5 min, ramp 10℃ /min to 190℃ for 1 min. followed by ramp 10℃ /min to 300 - held for 2 min (total run time 28.5 min). The injector temperature set at 280℃. The mass spectra were identified using Wiley mass spectral data base and the National Institute of Standards and Technology (NIST) library. **Fig. (1)** shows a typical GC – MS chromatograms and fragments of indoxacarb.

3.Results and discussion:

3.1.High performance liquid chromatography (HPLC - DAD) analysis

HPLC is used for analysis and determination of inxoxacarb in technical and in its different formulation type. The out signal was monitored using HPLC – Photo Diode Array Detector (DAD) - 1260 Agilent model using the analytical conditions as for the active substance determination. Chromatographic conditions for confirmation of peak identity identification under the conditions selected was based on a retention time and concentration based on Area. The peak area for each injected was recorded and compared with reference standard. **Fig. (2)** shows typical chromatograms for the indoxacarb formulation in two different concentrations

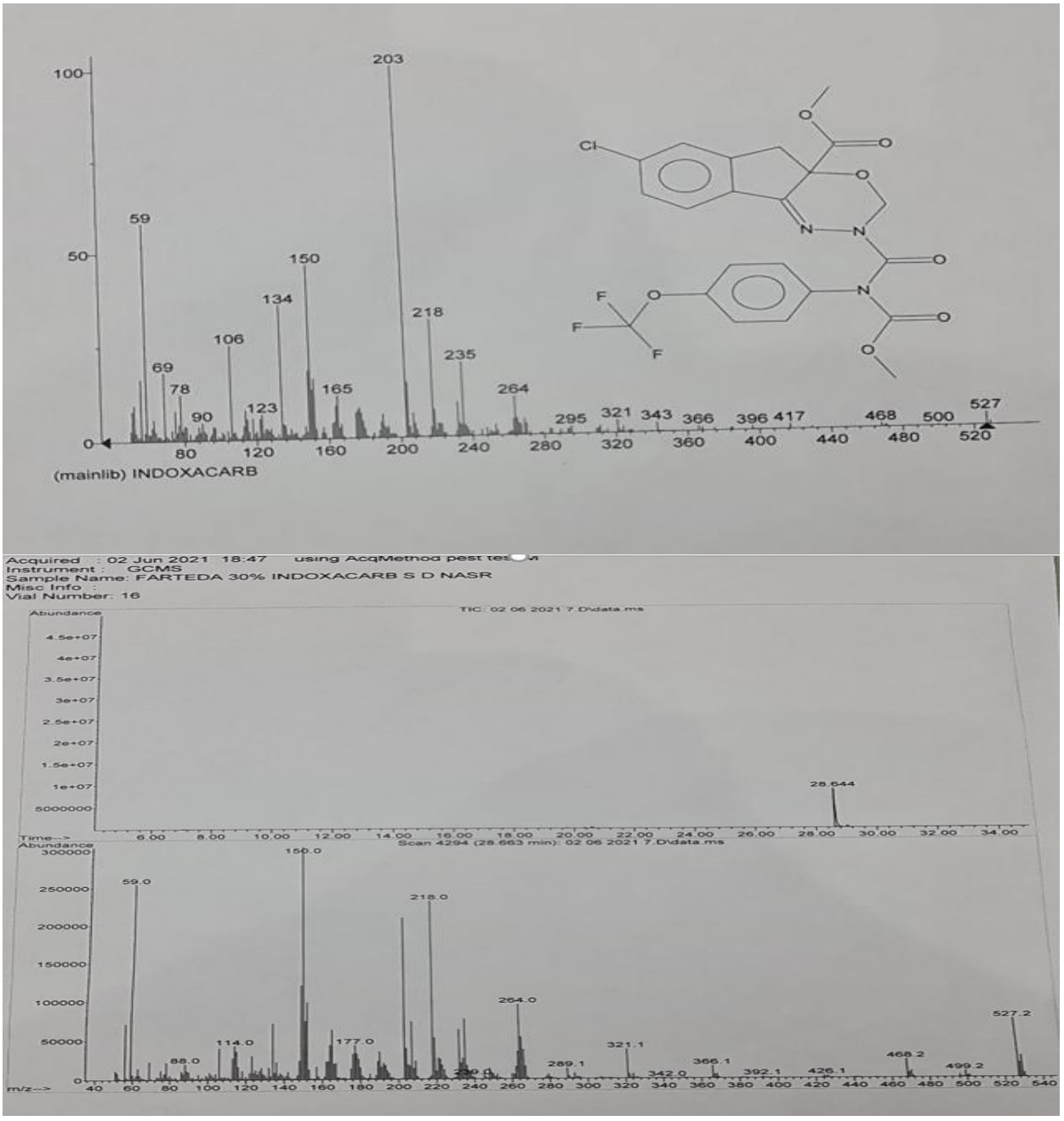

Fig. (1): Typical GC – MS chromatograms and fragments and indoxacarb

3.2.Method Validation Parameters

Validation of the method was performed according to APVAMA, 2004(Australian Pesticides and Veterinary Medicines Authority) guidelines, and ISO 17025 definitions.

3.2.1.Specificity

Specificity was stablished from the optimized method for this study. The spectra of the peak of indoxacarb from sample analysis and the external standard are identical. The data indicate that active substance peak is free from any coeluent. Examinations of chromatograms showed no impurities interfered and no significant matrix peaks observed in the retention times. **Fig. (3)** shows a typical chromatogram of blank sample where no peaks found **Precision.**

3.2.2.Repeatability

Data in **Table (1**) indicated the relative standard deviation values obtained using the method under study. The numbers were calculated from nine (9) replicates and average of two injections of concentration 400 ppm of the active ingredient in methanol solution and from of analyses of a single sample solution during a 1-day period and duplicate injections. The data show very closeness of the series of the measurements obtained where the RSD obtained was 0.35% from the multiple sampling injections under the prescribed conditions.

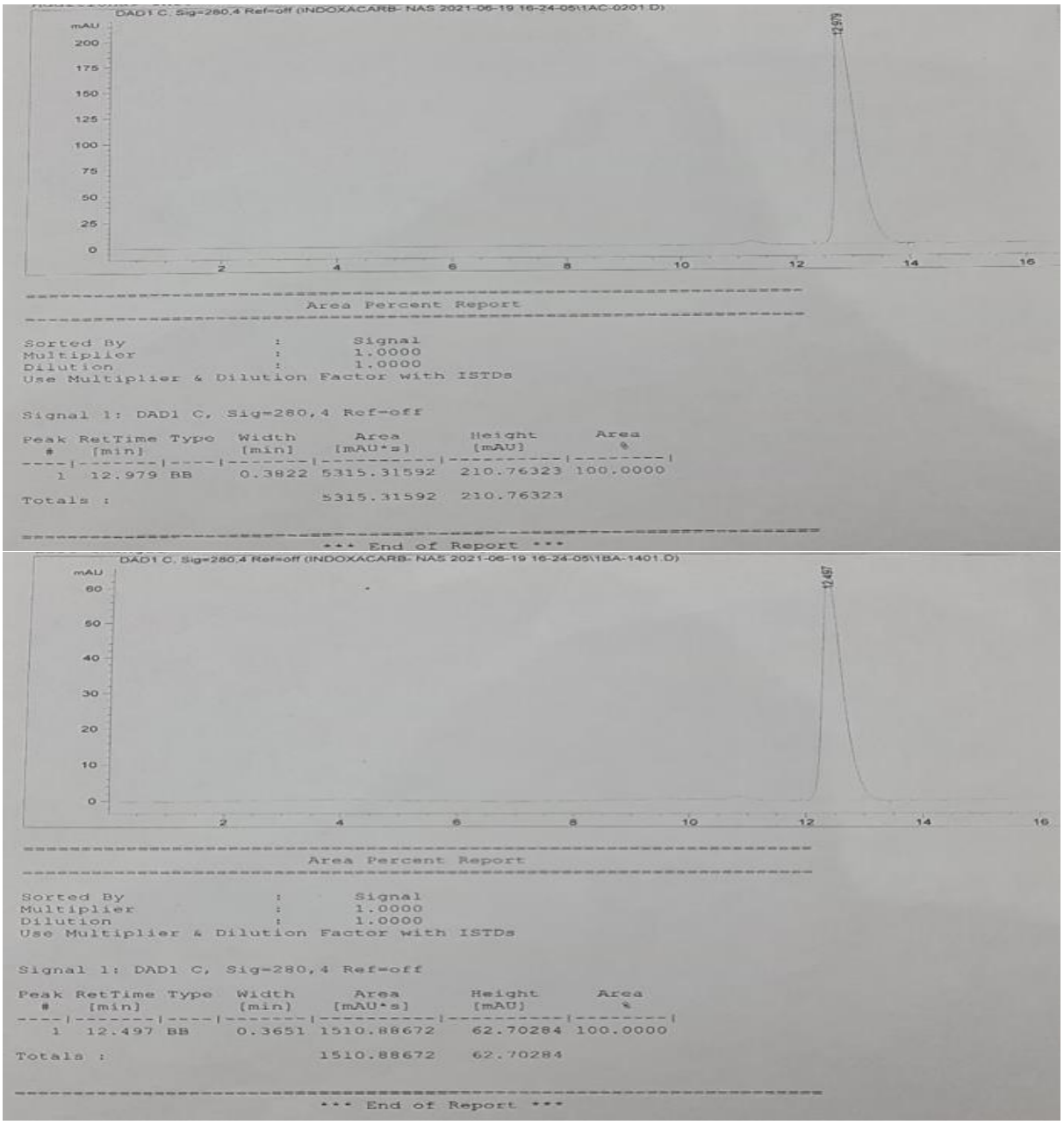

Fig. (2): Typical chromatograms for the indoxacarb formulation in two different concentrations.

3.2.3.Reproducibility:

The determination of indoxacarb content in the formulation under study was carried out in the way and the same optimized condition on a second day but in a different concentration used 100 ppm and the data as shown in table (2) indicated a very close of RSD% about 0.26 %.

3.2.4.Linearity:

The data indicated that the response or test results is directly proportional to the concentration of the given analytical parameter. In this study, the standard calibration curves established with six different concentrations levels 100, 200, 400, 800 and 1600 mg (a.i.) / L with three replicates for each level. Linearity of the method is usually expressed in terms of the results (area and absorbance).

The response of indoxcarb was found to be linear with six concentrations used in the range (0.1 – 3.2 mg/ml) with a determination of correlation coefficient (R^2> 0.99998). **Fig. (4)** indicates the linearity data with its correlation coefficient.

3.2.5. Accuracy:

The data generated in Tables (1) was also used to calculate the accuracy of the method where the accuracy was expressed as the recovery determined as the percentage of ratio of the concentration of indoxacarb detected relative to the analyte concentrate. The results showed that recoveries lie between 98.3 and 99.6 % recovery.

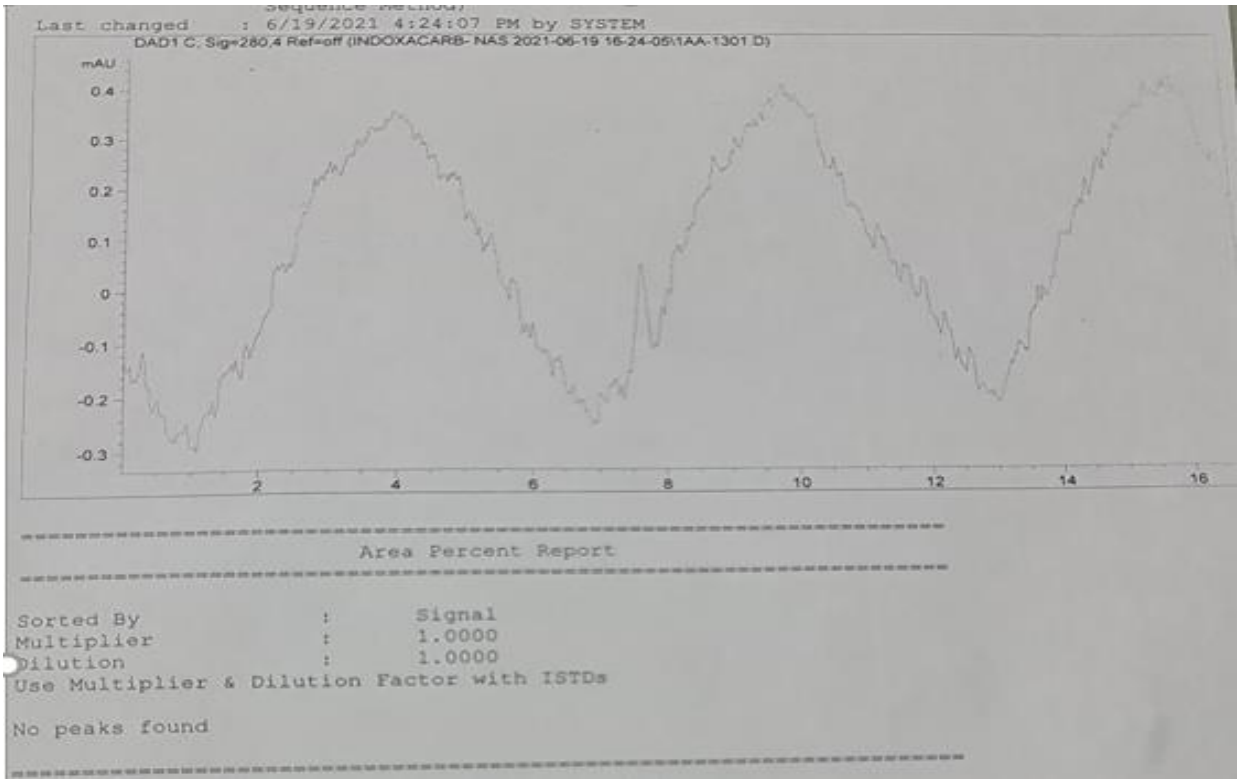

Fig. (3): Typical chromatogram of blank indoxacarb sample where no peaks found.

Table (1): Repeatability, STDEV and RSD% of indoxacarb 300 g a.i. /L SC with concentration 400 mg a.i./L of EC formulation

Prepared concentration 400 mg a.i. /L	Retention time RT: min	Area Average of two injections	Estimated concentration g/L
Rep. 1	12.552	5287.402345	298.8736278
Rep. 2	12.518	5234.578855	295.8877479
Rep. 3	12.523	5262.57129	297.4700373
Rep. 4	12.511	5240.399361	296.2167556
Rep. 5	12.512	5260.09106	297.3298408
Rep. 6	12.493	5240.100095	296.1998394
Rep. 7	12.473	5243.67041	296.4016536
Rep. 8	12.477	5235.8501	295.9596058
Rep. 9	12.478	5229.399415	295.594977
Mean		5248.229215	296.6593428
SDEV.		18.45007011	1.042901415
RSD%		0.351548482	0.351548482
Prepared concentration 100 mg a.i. /L	**Retention time RT: min**	**Area Average of two injections**	
Rep. 1	12.497	1510.88672	
Rep. 2	12.504	1519.1239	
Rep. 3	12.497	1515.4895	
Rep. 4	12.477	1509.54419	
Rep. 5	12.509	1511.14001	
Mean		1513.236864	
SDEV.		3.97766818	
RSD%		0.262858266	

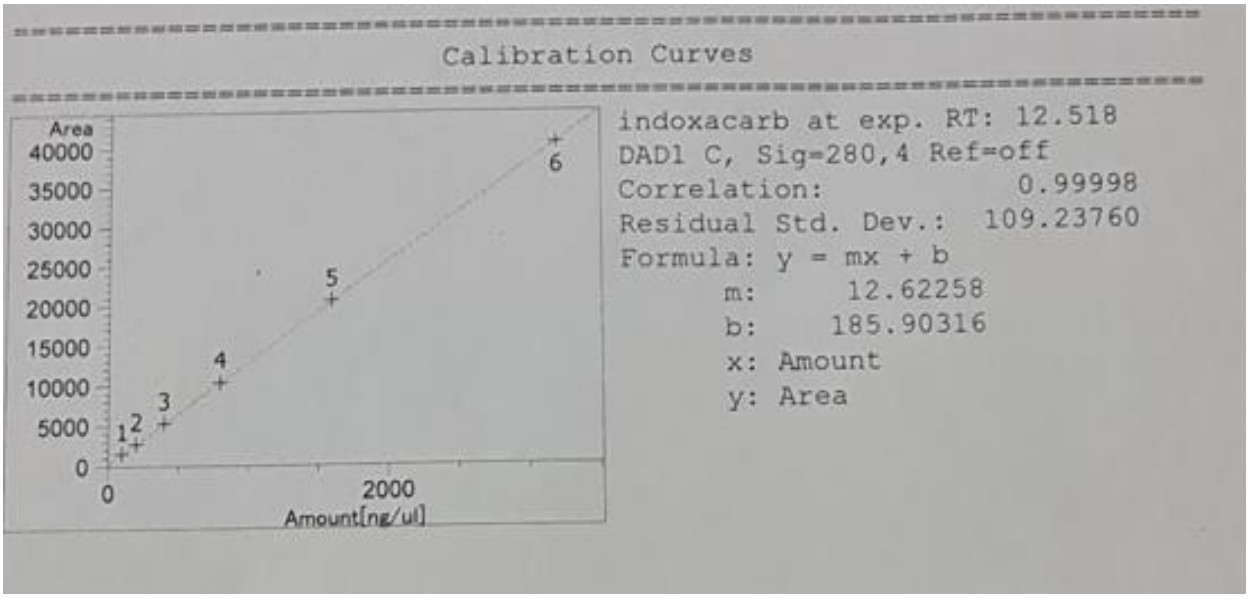

Fig. (4): The linearity data of indoxacarb with its correlation coefficient (R^2> 0.99998)

Conclusions:

The method described is a simple and fast HPLC-DAD method for the determination of indoxacarb insecticide in suspension concentrate formulation. The procedure described is simple, precise, and applicable for routine pesticides analysis laboratories. The proposed method has been validated with good and accepted validation parameters according to the references guidelines.

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طريقة تحليل لتقدير مبيد اندوكسارب في بعض مستحضرات المبيدات في صورة مركز معلق بإستخدام جهاز كرومانوجرافي السائل عالي الكفاءة

الملخص العربي

في هذا البحث تم وصف طريقة لتحليل وتقدير محتوي المادة الفعالة اندوكسارب (مبيد حشري) في بعض مستحضرات المبيدات في صورة مركز معلق (Suspension Concentrate (SC) بتركيز ٣٠٠ جم مادة فعالة / لتر. ينتمي مبيد إندوكساكارب إلى المجموعة الكيماوية أوكساديازين ويعتمد في طريقة عمله علي غلق قنوات الصوديوم. المبيد المستخدم موضع البحث قيد اجراءات التسجيل في مصر حاليا وينتج في بلد المنشأ الخاص به (الصين) تحت ظروف التصنيع الخاصة بالشركة المنتجة. تم التعرف و تقدير محتوى المادة الفعالة اندوكسارب باستخدام كروماتوغرافي السائل عالية الكفاءة (HPLC - DAD) وبإستخدام المادة القياسية للمبيد ذات النقاوة العالية والمعروفة (Reference St). تم تقييم وإثبات صحة الطريقة بإستخدام معايير التحقق المستندة إلى إرشادات هيئة مبيدات الآفات والأدوية البيطرية الإسترالية (APVMA) وتعريف معايير الإعتماد (أيزو) ISO / IEC 17025. تضمنت المعايير التي شملها البحث، الاختصاص النوعي أو خصوصية الطريقة (Specificity)، الخطية وتشمل المدي (Linearity)، الإحكام – تكرار القياس (Precision)، الدقة (accuracy). أظهرت النتائج للطريقة المستخدمة أن معامل الارتباط الخاص بالخطية (linearity) (R2> 0.99998) لمستحضر مبيد اندوكسارب تحت الدراسة بإستخدام ٦ تركيزات ما بين ٠,١ مجم / مل و ٣,٢ مجم / مل ، كما أظهرت النتائج أن الإنحراف النسبي RSD ٪ في حدود ٠,٣٥ % وذلك بدراسة التكرارية لعدد ٩ مكررات مع ازدواج المكرر الواحد. لم تظهر النتائج أي تداخل من أي مواد مساعدة أو مكونات أخرى محتملة في مستحضر مبيدات مع المادة الفعالة وتم استخدام جهازي GC-MS للتأكد من وجود المادة الفعالة اندوكسارب في المستحضر مع عدم وجود مواد فعالة او محظورة أخري. والمستخاص أن الطريقة موضع الدراسة. أوضحت الطريقة موضع البحث بإستخدام جهاز HPLC - DADمناسبة، دقيقة ويمكن تطبيقها بنجاح في المختبرات الروتينية لتحليل المبيدات للغرض المبين.