www.revistageintec.net
ISSN: 2237-0722
revista GEINTEC Gestão, Inovação e Tecnologias

A New Indirect Sensitive and Green CFIA Method for Assay of α-cypermethrin Insecticide in Pure Form, Biological and Industrial Samples Using N-Bromo Succinamide as Oxidizing Agent in Acidic Medium

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Abstract
A new simple, sensistive and green environmental method of Flow-injection/merging zones technique was developed for indirect determination of α-cypermethrin pesticide in pure form, industrial and biological samples. This method based on oxidation reaction of pesticide (α-cypermethrin) with a calculated increase of N-bromosuccinamide (NBS) in acidic medium then react with Tartrazine dye (TART) and estimation of the excess of the oxidizing agent by measuring the residual absorption of TART (yellow color) at λ_{max} *= 430nm, this method is a green chemistry, low cost and less consumption of poisonous chemical reagents. The limit of detection and limit of quantification for this method are 0.05 µg.ml*$^{-1}$ *and 0.17 µg.ml*$^{-1}$ *respectively, RSD% = 0.69% and the recovery is about = 102.15%. Chemical and physical parameters was optimized that effect on the pesticide reaction. The calibration curve was linear within concentration range (0.5 – 30) µg.mL*$^{-1}$ *with sampling through put is about 65 sample.hour*$^{-1}$*; the results of determination were compared with those given by trusted method (HPLC). No significant differences between both methods regarding in terms of accuracy and precision at 95% dependability level.*

Keywords: N-Bromo Succinamide, α-cypermethrin, CFIA Technique, Green Chemistry, TART.

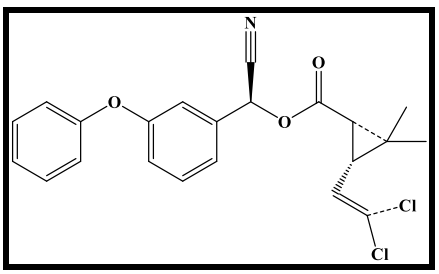
1. Introduction

A α-cypermethrin **(CYM)** known chemically [Cyano-(3-phenoxyphenyl)-methyl] 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate as shown in figure (1) **[1,2].** Molecular formula ($C_{22}H_{19}Cl_2NO_3$), Molecular weight (416.30 g.mol^{-1}). It is a synthetic pyrethroid that contains a group of cyanide used as an insecticide in agricultural applications to control pests that

affect cotton, fruits and vegetables **[3,4].** It is also used to control cockroaches, fleas and termites. Generally considered safe for humans. However, excessive use may cause environmental and health problems**.** The half-life in different conditions ranges from (4-100) days. It acts on the nervous system of insects by binding with sodium channels **[5,6]**. It is a highly toxic pesticide to fish, bees and aquatic invertebrates **[7],** Cypermethrin is stable in light and has a very low volatility and water solubility. Cypermethrin action is due to its action in disrupting normal functioning of the nervous system**.** Cypermethrin is not considered as irritate compound to the skin, but orally can cause coughing, difficulty in breathing, skin and eye irritation, nausea and vomiting **[8-10].** Cypermethrin is a mixture of eight stereoisomers, four of them in cis and four in trans configuration. The most effective pair of cypermethrin isomer are 1R, cis and 1S, cis which they make up cypermethrin **[11,12].** Several analytical methods have been reported for determination α-cypermethrin in different organic samples such as: Liquid-liquid extraction (LLE) **[13].** Supercritical-fluid extraction (SFE) **[14,15]** Microwave-assisted extraction (MAE) **[16-17]**, Solid phase extraction (SPE) **[18]**, Molecularly imprinted polymers (MIPs) **[19]**, Cloud point extraction (CPE) **[20]**, High performance liquid chromatography **[21,22]**, Gas chromatography **[23,24]**, Spectrophotometer **[25-27].** In the present work, a new CFIA technique was suggested for indirect spectrophotometric determination of pyrethroid insecticide (α-CYM) in pure form, industrial and biological samples through the oxidation reaction of CYM using NBS as oxidizing agent in acidic medium and estimation of the excess of NBS through the determination of residual absorption (unbleached yellow color) of tartrazine dye at λ_{max} = 430nm.

The work was applied in six-three way margining zone programming at flow rate (3.03 $mL.min^{-1}$) applied to individual assay steps allows optimization of a high frequency **[28-32].** Miniaturized and automation of analytical techniques based on flow techniques offers many benefits, such as a reduction in human exposure to toxic substances and the production of more environmentally friendly products **[33,34]**. It's simple rapid and sensitive, moderate experimental condition, wide linear range, reproducibility and repeatability of the results obtained, low time consumption and cost **[35]** for determination α-cypermethrin.

Figure 1 - Chemical Structure of α-Cypermethrin



Received: 19.06.2021 – Accepted: 20.07.2021

Chemicals and Reagents

A standard solution of pesticide (Cypermethrin) (M.Wt =416.30 $g.mol^{-1}$) 1000 $\mu g.ml^{-1}$ (2.4×10^{-3}M) was prepared by dissolving 0.1g in 10mL of methanol using volumetric flask 100 mL and complete to the mark by distilled water. A standard solution of N-bromosucssinamide (NBS) (M.Wt =177.9 $g.mol^{-1}$) 200 $\mu g.mL^{-1}$ (1.7×10^{-3}M) was prepared by dissolving 0.02 gm in volumetric flask 100 ml using distilled water. A standard solution of the tartrazine dye (TART) (M.Wt =534.30 $g.mol^{-1}$) 100 $\mu g.mL^{-1}$ (1.12×10^{-3}M) was prepared by dissolving 0.01g in 100 mL using distilled water. Standard solution of hydrochloric acid 1M (HCl) was prepared by taken 16.72 mL of concentrated acid in volumetric flask 200 mL and complete to the mark with distilled water.

Preparation of Biological Samples

1- Human Serum

Human serum samples were collected from healthy persons, in plastic tubes separated from blood at 3000 rpm for 15 minutes by centrifugation and acidified with 1 ml of HNO_3 (1M) to precipitate proteins. A 0.5 ml of the supernatant was pipette in a glass tube and stored at 4°C until they used **[36,37].**

2- Plasma Samples

Blood samples were collected from healthy persons in glass tubes [contain EDTA] and centrifuged for 20 min at 3000 rpm. Precisely 0.5 mL of plasma was pipetted into a 10 mL plastic tube and stored at 4 C° until it was used **[38].**

3- Urine Samples

The samples were collected from different healthy people (male), directly used after added 5 drops of $HClO_4$ acid (to precipitate the protein) **[39]** and then centrifuge at 3000 rpm.

Preparation of Industrial Samples

According to the standard addition method, two types of the industrial preparation containing Cypermethrin Alpha cypermethrin EC10%(India), Alpha 10% (China) have been analyzed under the

developed FIA method as shown in table (3-16), stock solution (1000 ppm) of industrial preparation was prepared from the main bottle, One ml was withdrawn from the main vial of concentration of 25000 ppm into a 250 ml volumetric flask and the volume was completed to the mark with distilled water.

The statistical comparison between the proposed method and trusted method (HPLC) using the student t-test and F-test **[19]** showed that the calculated F-test values and t-test less than the theoretical values for trusted method.

Instrumentation of the Suggested CFIA Manifold

The measurements in batch method procedure were made by shimatzu UV-1800(Japan) UV-Visible spectrophotometer double beam with quartz cuvette (1cm). The suggested FI manifold was developed in this study using a single channel manifold/FIA system for spectrophotometric determination of CYM pesticide. A peristaltic pump (Shenchen, LabM1) was used to pump the carrier stream (distilled water) at flow rate 3.03 $mL.min^{-1}$ through the injection valve (six-three way home made) which contain three loops made of Teflon (I.d=0.5 mm), the sample(CYM) in loop 1 and NBS, HCl in loop 2 and TART in loop 3 were loaded and then mixing in the reaction coil which is made of glass (I.d =2mm). The detection unit was modified (photometer 301-D^{+}, VIS-spectro, single beam) (Japan). Kompensograph C1032 (siemens) was used for convert the absorption measurement as average peak Hight expressed in mV. (n=3) or optical multimeter absorption (DT9205A, OVA, China) for the absorption measurement. The detection unit containing a flow cell (a quartz silica,1cm) with internal volume of 80µL.

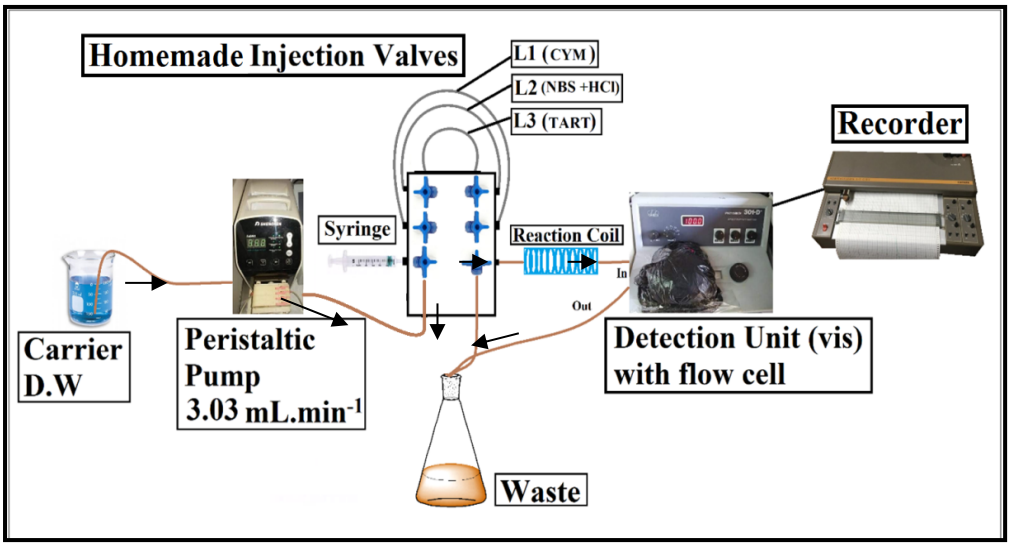
Batch Method

In 10 mL volumetric flask containing concentration of cypermethrin (0.1-20) $\mu g.mL^{-1}$, added 0.5ml of NBS and 0.3mL of HCl (1M) and waiting for 5 min to complete the oxidation reaction of pesticide then added 1.5 mL of TART dye and complete to the mark using distilled water, the residual absorbance of unbleached TART dye (yellow product) was measured at λ_{max}=430nm against the blank.

Flow Injection Method

In the developed flow injection, 10 mL of volumetric flask was used which contain concentration of cypermethrin (0.1-20) µg.mL^{-1} which injected in loop 1 (40 cm) equal to 79 µL a mixture of 1mL HCl and 3ml of NBS which equal 0.1 ml HCl and 0.3 ml NBS was inject in loop 2 (65cm) equal to 128 µL and the TART was inject in the loop 3 (50 cm) equal to 98 µL. After loading the loops, reopened the valves to be ready to inject the reactant to reaction coil (85 cm) using the peristaltic pump in the speed 35rpm (flow rate = 3.03 mL.min^{-1}), the absorbance result of the yellow product recorded in chart as average peak height (mV) as showing in figure (2).

Figure 2 - Single Channel Manifold of FIA/Merging Zones System for Determination of α-Cypermethrin in Pure Form, Industrial and Biological Samples

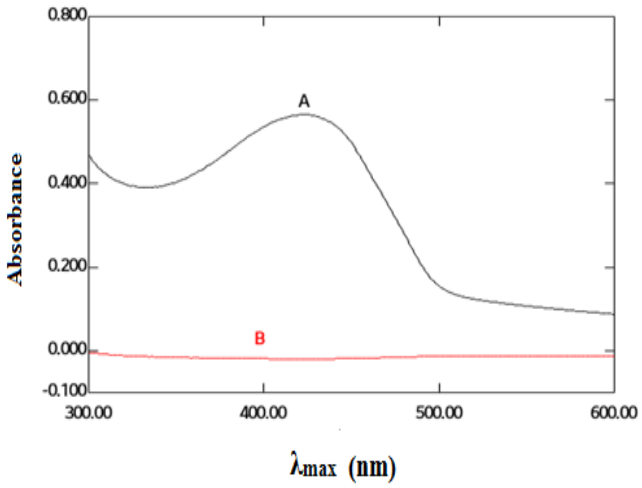


2. Result and Discussion

Absorption Spectra

A final concentration of 20 µg.mL^{-1} CYM (0.48×10^{-4}M) was reacted with (3.36×10^{-5}M) NBS in acidic medium (0.01M), then adding (3.6×10^{-5}M) TART, to calculate the concentration of unbleached TART dye as absorbance which was examined in visible region (λ_{max} = 430 nm) for indirect estimation of CYM.

Figure 3 - Absorption Spectra of Residual Tartrazine Dye (Yellow Product) 20 µg. mL^{-1} for Indirect Estimation α-Cypermethrin at 430nm Opposite the Blank Solution (NBS and HCl)



Optimum Conditions for Batch Method

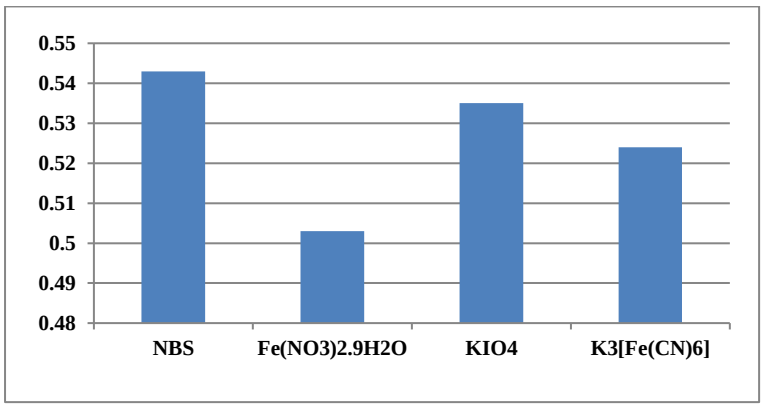
Effect of Type of Oxidizing Agent

The effect of a number of oxidizing agents was studied by react 6ppm of CYM with concentration of NBS (10^{-3} M) to find out the extent of their influence, as the results showed in table 1 and figure (4), the best oxidizing agent for the oxidation of the pesticide in the acidic medium is (NBS), as it was confirmed in subsequent experiments.

Table 1 - Effect of Type of Oxidizing Agent

Type of oxidation agent	Absorbance
NBS	0.543
$Fe(NO_3)_2.9H_2O$	0.462
KIO_4	0.497
$K_3[Fe(CN)_6]$	0.478

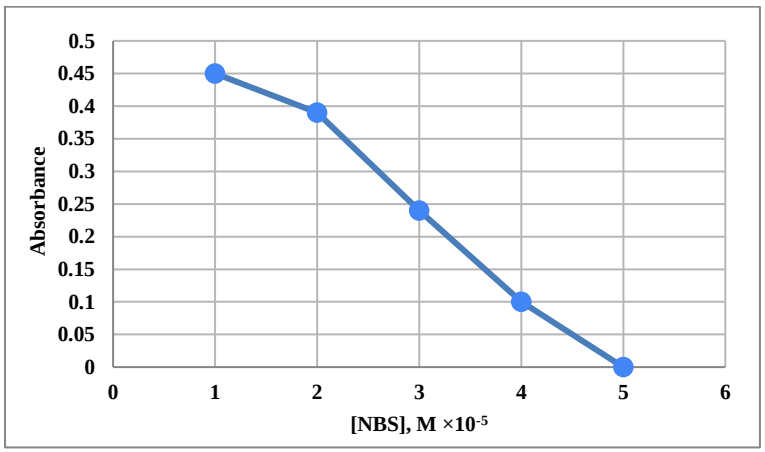
Figure 4 - Effect of Type of Oxidizing Agent



Effect of conc. of NBS

Different concentration of NBS were studies to determined the optimum concentration of NBS by different volume from the stock solution (0.05, 0.1, 0.2, 0.3,0.4) mL which is equal to (5.6×10^{-6} to 4.48×10^{-5} M), The optimum amount of NBS that works to bleach the color of the TART dye has been determined exactly. As shown in the figure (5), the best amount of NBS is 0.4 mL (4.48×10^{-5} M) which is the optimal amount for bleaching the color of the TART dye.

Figure 5 - Effect of Concentration of NBS



Effect of Acidic Medium and Concentration

Different type of acidic medium were used to determine α-Cypermethrin by preparing 1M of (HCl, H_2SO_4, HNO_3, CH_3COOH). The results showed in (figure 6) and (table 2) that the best type of acid was HCl, as the remaining dye was in a stable state, which gave the best absorption of the pesticide (10 µg.mL^{-1}) at 430 nm. The effect of the concentration of 1M HCl by adding different volumes of it to the reaction (0.1, 0.2, 0.3,0.4, 0.5) mL was studied. The results showed that the best concentration of the acid is by adding 0.1 ml, which is equivalent to 0.01M as the acidic function PH is equal to 1.8, in which the dye is In a stable condition, it gave the best absorption of the pesticide, and this amount was adopted in subsequent experiments.

Table 2 - Effect of Medium in Absorption of CYM

Type of acidic meduim	Abs.
HCl	0.551
HNO_3	0.46
H_2SO_4	0.41
CH_3COOH	0.4

Figure 6 - Effect of Acidic Medium

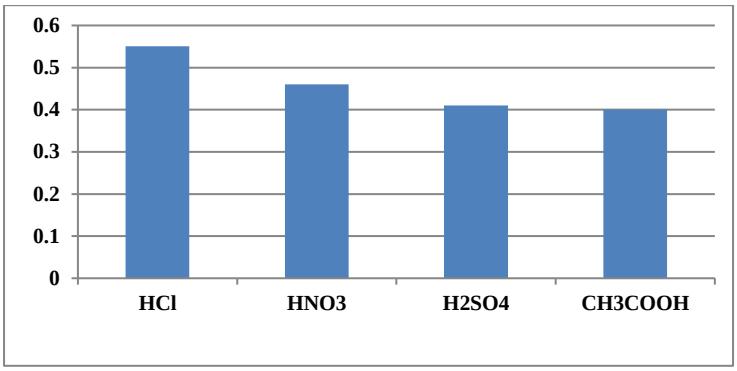
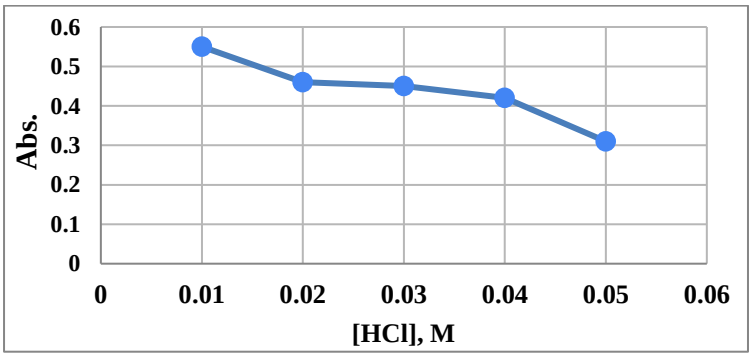


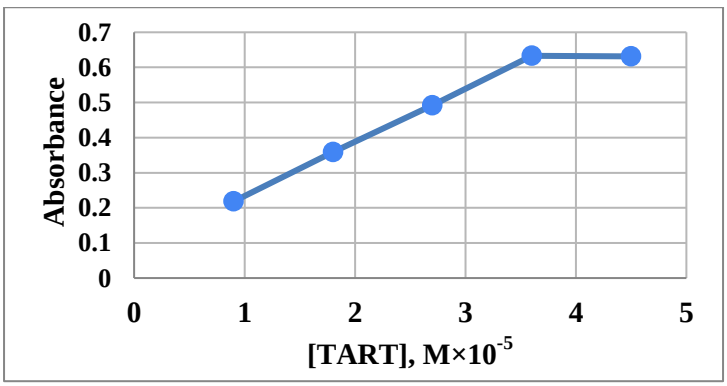
Figure 7 - Effect of Concentration of HCl



Effect of conc. of TART

In order to find the best amount of TART dye that can be used in the estimation of the pesticide. The range of linear dye concentrations that obey with Lambert-Beer law were studied. Increased concentrations of dye solution (0.9×10^{-5} M to 4.5×10^{-5} M) were prepared, as the results showed at figure(8) that the best dye concentration was (3.6×10^{-5} M) which equal 2 ml for absorption of the residual unbleached of the TART dye by the excess of NBS at λ_{max} =430nm.

Figure 8 - Effect of Concentration of TART

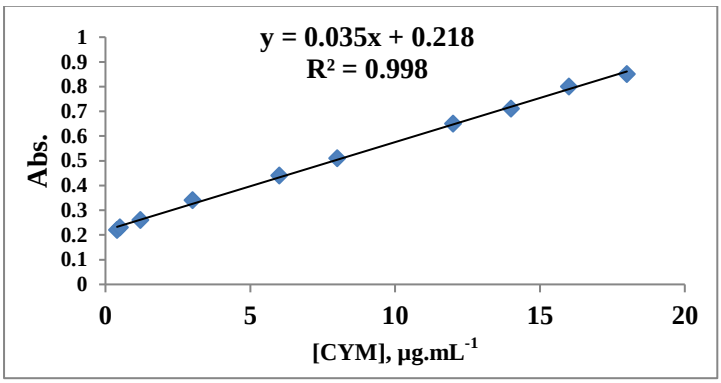


Received: 19.06.2021 – Accepted: 20.07.2021

Calibration Curve

After fixing the best conditions for the estimation process, a calibration curve was prepared to find the equation of the straight line, as well as the limits of the concentration of the final colored compound of the pesticide and the value of molar absorbance. Concentration Standard curves were obtained, which show that the method follows the Lambert-Beer law within the ranges (0.4-18µg.mL^{-1}) shown in the figure (9) and that there is a negative deviation from the Lambert-Beer law after the estimated upper limits as shown in the figure (9).

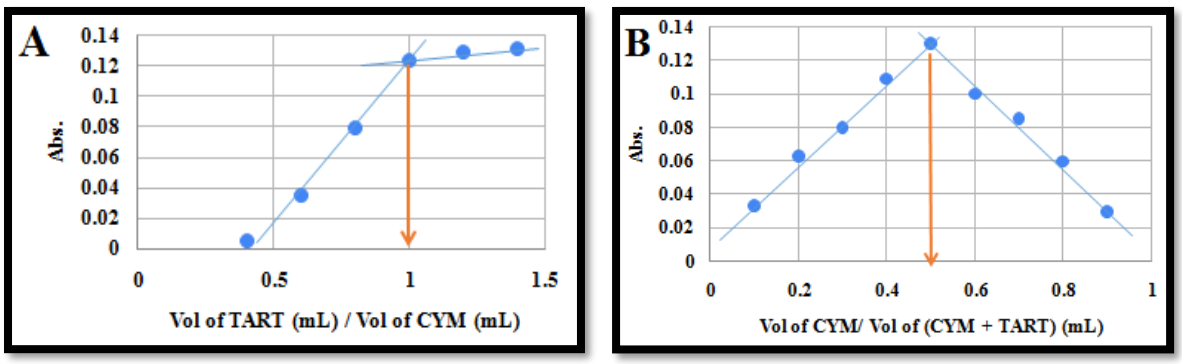
Figure 9 - Calibration Curve for Determination α-Cypermethrin with TART Dye Using NBS as Oxidizing Agent



Stady of Stoichiometry Complex between CYM with TART

In order to know the ratio of reaction that occur between the reagent with pesticide, two important way were proceed which is mole ratio method and continuous variation methods at λ_{max} =430nm, the results shown that CYM produced a 1:1 complex with TART as shown in figure (10A,B):1/A (5×10^{-6} M of each CYM and TART mix in order to the procedure of mole ratio and complete to 10 ml with distilled water. 2/B (5×10^{-6} M of each CYM and TART mix in order of Job's method and complete to 10 ml with distilled water.

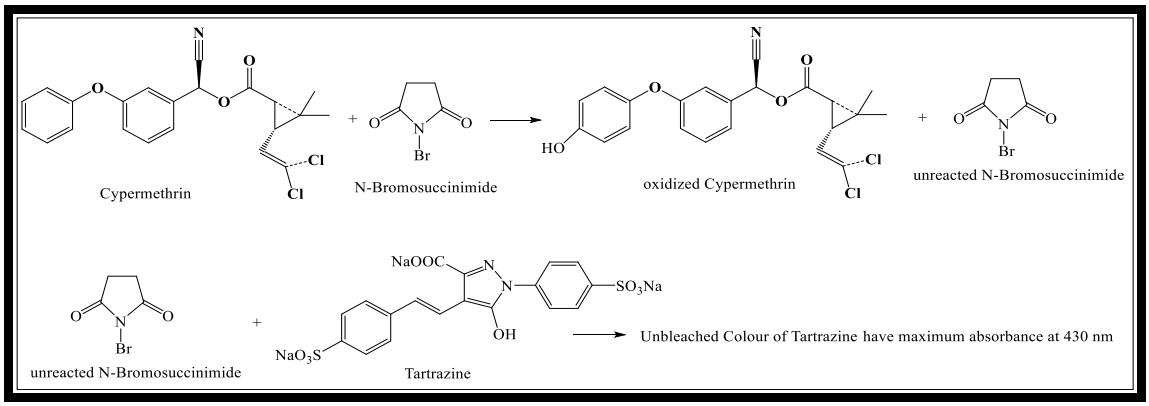
Figure 10 - Absorbance Versus Concentration of CYM with TART
A- Molar Ratio, B- Jobs Method



Mechanism of the Reaction

Depending on the results that collected from the mole ratio and Job's methods it is clear that the CYM-TART complex associate in 1:1 ratio so the proposed below mechanism is likely to suggest as shown in (Figure 11).

Figure 11 - The Proposed Mechanism of Complex between CYM and TART



Accuracy and Precision

Under the ideal condition that describe in established method, accuracy and precision was studied through measuring two concentration of CYM and according to the result that has been reached as shown in (table 3) the classical method have good with high accuracy and precision; each measurement is repeated for three times.

Table 3 - Accuracy and Precision for Batch Method

[CYM], µg.mL^{-1}		Error	Rec%	Erel%	RSD%	SD
Present µ	Found $\bar{x}$					
4.0	3.924	-0.076	98.095	-1.905	0.430	0.002
8.0	8.095	0.095	101.190	1.190	0.461	0.002

Average fine determination

Calculation of Stability Constant

Calculated static stability for the proposed interaction (CYM+TART) with concentration (5×10^{-5}M) for both was calculated based on two groups of solutions were prepared; first group of solution were placed to include stoichiometric lot of CYM to TART, and the second group were

placed to include two-fold excess of TART. According to the mechanism and stoichiometry ratio between TART and CYM. The stability constant can writing as following: K=**1- α/ α^2 C**

While **(α)** (degree of dissociation) can be wrote as follows: **α= Am – As /Am**

Where Am; As are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the TRAT as shown in table 4.

Table 4 - Stability Constant for the Oxidization Reaction of CYM Using NBS as Oxidizing Agent

Am	As	α	K(L.mol^{-1}) M^{-1}
0.12	0.1	0.166	5.95714×10^5

Continuous Flow Injection Analysis Technique

After choosing the optimum condition for the reaction between CYM and TART using classical spectrophotometric method. The spectrophotometric reaction was automated with flow injection/merging zone method to recognize the optimum parameters and to obtain spectracl automated with fast way to for determination of α-CYM. So the batch procedure was employed to develop FIA method.

The Suggested Manifold of Flow Injection System

After install the system and connect the portions to study of optimal design of system, the developed system as shown in (figure 2) is contain a single line represented one channel which supply as carrier (distilled water) passing by injection valve (six three way, homemade), which contain three loops (different length with same inside diameter 0.5mm) filled with the sample, reagent, acidic medium and oxidizing agent.

Optimization of the Developed FIA System

Effect of chemical variables such as (CYM, NBS, TART and acidic medium) and the physical variables (flow rate, sample and reagent volumes, reaction coil length, purge time, dispersion and sampling/h).

Effect of Oxidizing Agent (NBS)

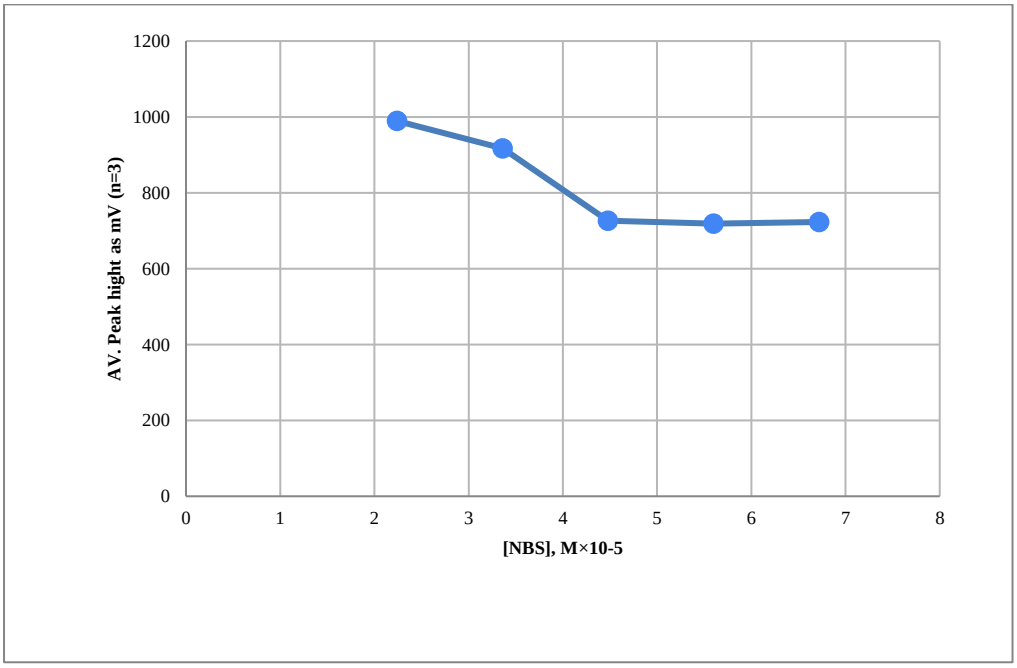
Different concentration of NBS was studied (2.24×10^{-5} to 6.72×10^{-5} M) and injected in loop 2 using homemade injection valve (figure 2) (CYM 10ppm), the results as shown in (figure 12) and table (5) that the optimum concentration of NBS for residual unbleached TART dye was 3.36×10^{-5}M which is gave the highest value of absorbance in mV (n=3).

Table 5 - Effect of NBS Concentration

conc. of NBS (M)	Average response ($\bar{y}$) (mV)	SD	*RSD%	S.E.M
2.24×10^{-5}	989	4.62	0.47	989 ± 11.47
3.36×10^{-5}	917	9.24	1.01	917 ± 22.93
4.48×10^{-5}	726	6.55	0.90	726 ± 16.26
5.6×10^{-5}	719	6.11	0.85	719 ± 15.17
6.72×10^{-5}	723	4.62	0.64	723 ± 11.47

Average fine determination

Figure 12 - Effect of NBS Concentration



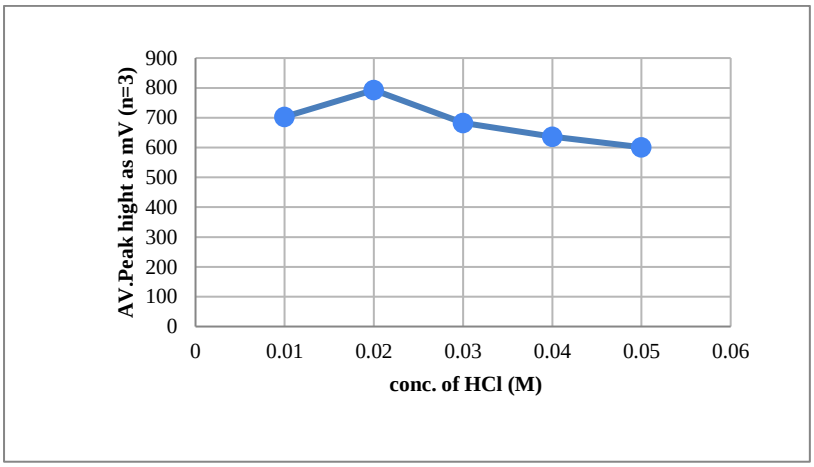
Effect of concentration of HCl

Different concentration of HCl was used (0.01 to 0.05 M) as shown in figure (13) and table (6) that the best concentration of HCl was 0.02 M, as the acidic function PH is equal to 1.8, in which the dye is In a stable condition, it gave the best absorption of CYM pesticide.

Table 6 – Effect of HCl Concentration

conc.of HCl (M)	Average response ($\bar{y}$) (mV)	SD	RSD%	S.E.M
0.01	703	8.33	1.19	703 ± 20.67
0.02	793	0.80	0.10	793 ± 1.99
0.03	682	2.12	0.31	682 ± 5.25
0.04	636	4.00	0.63	636 ± 9.93
0.05	601	1.22	0.20	601 ± 3.03

Figure 13 - Effect of Concentration of HCl Using [CYM-NBS-HCl-TART] CFIA System



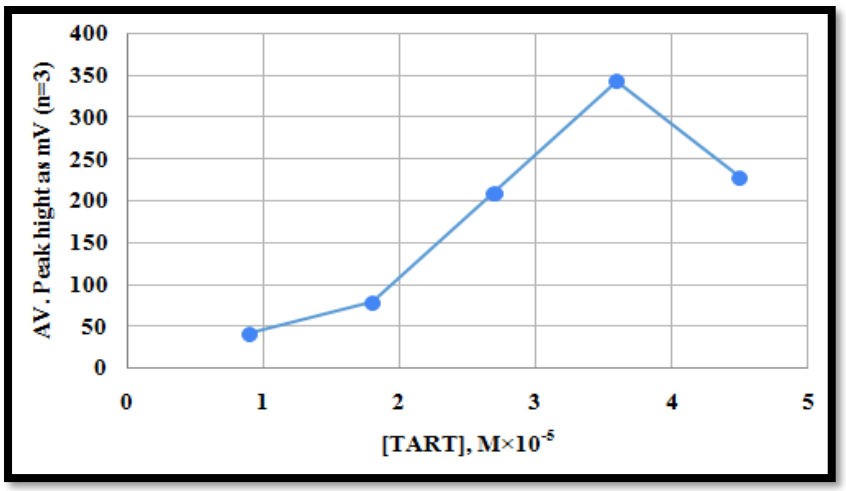
Residual of Unbleached Dye

Different concentration of TART was used (0.9×10^{-5} to 4.5×10^{-5} M) as shown in figure (14) and table (7) the optimum concentration of TART was 3.6×10^{-5} M for absorption of the residual unbleached of the TART dye using excess of NBS as oxidizing agent at λmax =430nm.

Table 7 - Effect of TART Concentration

[TART], M	Average response ($\bar{y}$) (mV)	SD	RSD%	S.E.M
0.9×10^{-5}	41	0.92	2.28	41 ± 2.29
1.8×10^{-5}	77	4.62	5.97	77 ± 11.47
2.7×10^{-5}	208	8.00	3.85	208 ± 19.86
3.6×10^{-5}	341	12.22	3.58	341 ± 30.34
4.5×10^{-5}	227	12.22	5.39	227 ± 30.34

Figure 14 - Effect of Concentration of TART



Received: 19.06.2021 – Accepted: 20.07.2021

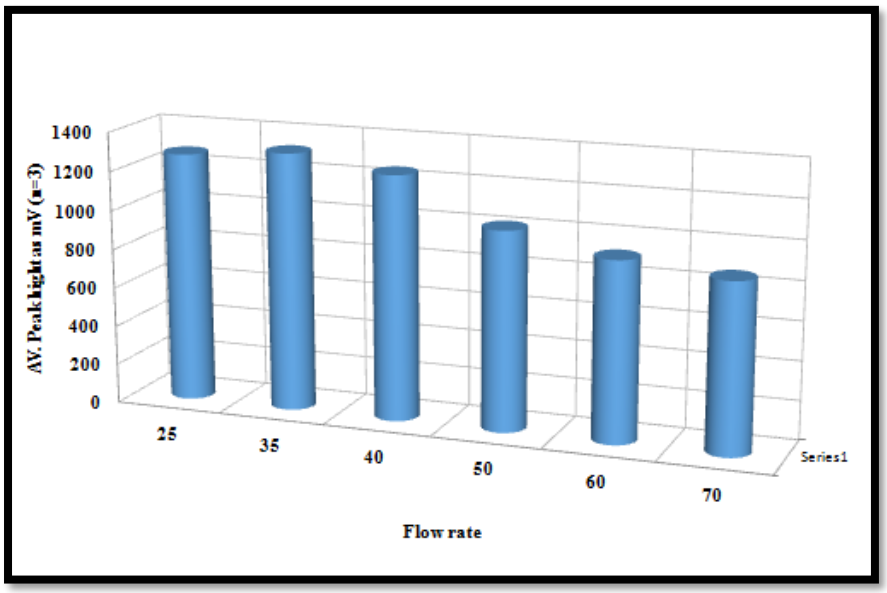
Effect of Flow Rate

Different flow rate was studied (25, 35, 40, 50, 60 and 70) rpm of the pump which are equal to (2.33-4.48) mL.min^{-1}to get the optimum condition for the oxidizing reaction of CYM pesticide with NBS in acidic medium. The best speed is 35rpm equal to 3.03 mL.min^{-1} as flow rate which found a high intensity of absorbance of the suggest reaction as shown in figure (15), and table (8).

Table 8 - Effect of Flow Rate on Reaction of CYM with TART Dye

Pump (rpm)	Flow rate (mL.min^{-1})	Average response ($\bar{y}$) (mV)	SD	RSD%	S.E.M
25	2.33	1277	4.62	0.36	1277 ± 11.47
35	3.03	1320	8	0.60	1320 ± 19.86
40	3.22	1251	12.22	0.97	1251 ± 30.34
50	4.10	1019	4.62	0.45	1019 ± 11.47
60	4.39	920	8	0.86	920 ± 19.86
70	4.48	867	4.62	0.53	867 ± 11.47

Figure 15 - The Best Flow Rate for the Reaction between CYM Pesticide with TART Dye Using [CYM-NBS-HCl-TART] CFIA System



Effect of Injected Volume of Sample and Reagent, Reaction Coil

For the CFIA system [CYM-NBS-HCl-TART], the best volume of CYM (loop 1) was 40µl, HCl with NBS (loop 2) was 65 µl, TART dye (loop 3) was 50 µl and the reaction coil length was (85cm).as shown in table (9).

Table 9 - Optimization of Sample and Reagents Volumes Using New [CYM-NBS-HCl-TART] CFIA System

length of loop (cm)			peak hight mV			CYM(L1)/NBS-HCL (L2)/ TART (L3)	Average response ($\bar{y}$) (mV)	SD	RSD%	S.E.M
L1	L2	L3								
35	**65**	**50**	**1080**	**1048**	**1080**	**35-65-50**	**1069**	**18.48**	**1.73**	**1069±45.87**
40	**65**	**50**	**1280**	**1264**	**1272**	**40-65-50**	**1272**	**8**	**0.63**	**1272±19.86**
50	**65**	**50**	**1064**	**1056**	**1048**	**50-65-50**	**1056**	**8**	**0.76**	**1056±19.86**
65	**65**	**50**	**1256**	**1240**	**1240**	**65-65-50**	**1245**	**9.24**	**0.74**	**1245±22.93**
40	**35**	**50**	**1184**	**1176**	**1176**	**40-35-50**	**1179**	**4.62**	**0.39**	**1179±11.47**
40	**40**	**50**	**1032**	**1008**	**1008**	**40-40-50**	**1016**	**13.86**	**1.36**	**1016±34.40**
40	**50**	**50**	**1104**	**1088**	**1088**	**40-50-50**	**1093**	**9.24**	**0.84**	**1093±22.93**
40	**65**	**50**	**1280**	**1264**	**1272**	**40-65-50**	**1272**	**8**	**0.63**	**1272±19.86**
40	**65**	**35**	**952**	**936**	**920**	**40-65-35**	**936**	**16**	**1.71**	**936±39.72**
40	**65**	**40**	**848**	**840**	**824**	**40-65-40**	**837**	**12.22**	**1.46**	**837±30.34**
40	**65**	**50**	**1280**	**1264**	**1272**	**40-65-50**	**1272**	**8**	**0.63**	**1272±19.86**
40	**65**	**65**	**600**	**592**	**584**	**40-65-65**	**592**	**8**	**1.35**	**592±19.86**

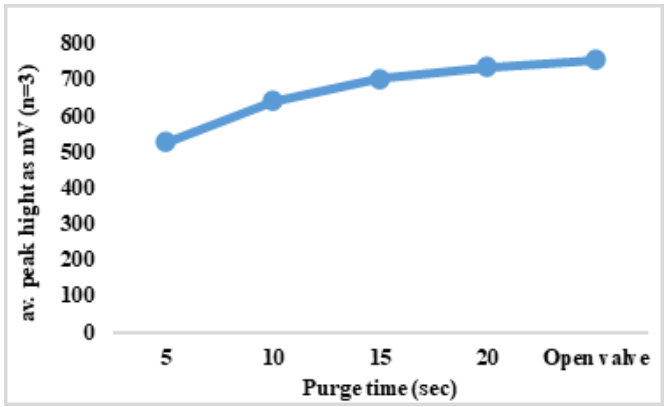
Table 10 - Effect of Reaction Coil Length

Length of R.C (cm)	Average response ($\bar{y}$) (mV)	SD	RSD%	S.E.M
75	661	20.13	3.04	661±49.98
85	688	8.00	1.16	688±19.86
70	547	12.22	2.23	547±30.34
125	488	13.85	2.83	488±34.40

Purge Time

Purge time for the sample segment to be injected via the carrier stream (distilled water) was studied, using the ideal chemical and physical parameters were studied previously [**39**]. For CYM-TART system time like (5,10,15,20) sec and open valve (injected mode) were used, and showed that the purge time more than 20 sec giving a highest response intensity. For this reason, open valve was selected as an optimum purge time to complete transportation of sample from sample loop to flow cell, the results obtained were summarized in figure (8).

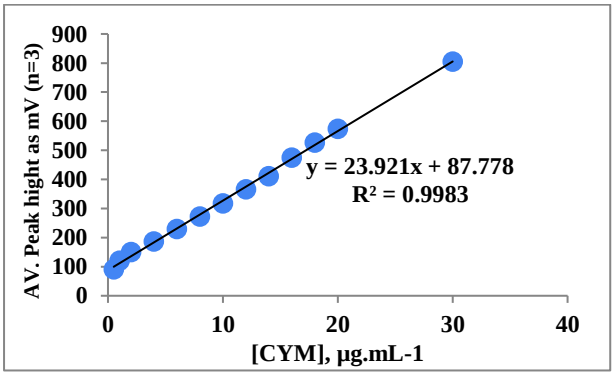
Figure 16 - Effect of Purge Time on Peak Height in mV for CYM-TART (6 μg.mL^{-1})



Calibration Curve of the Proposed Method and Calculation of Sampling

After verbal and verification all optimum condition, a series concentration of CYM from (0.05 to 30) ppm were prepared and injected in the developed FIA system with NBS, HCl and TART to know the liner range of CYM pesticide which can be applicable for this method as shown in table (11) and figure (17).

Figure 17 - Linear Calibration Curve for Estimation of CYM Using the Suggested CFIA System



Sampling was calculated by measure the time it need to load the sample and reagent (chemicals) in to their loops which is equal to 15 sec. and measure the time that need to move the chemicals from injection valve across the reaction coil to the detector to appear analytical signal which is equal to 40 sec, the sampling is 65 sample/hour.

Table 11 - Statistical Treatment of Calibration Curve for Determination of CYM Pesticide Using New [CYM-NBS-HCl-TART] CFIA System

[CYM]. µg.mL^{-1}	peak hight (mV)			Average response ($\bar{y}$) (mV)	ŷ	SD	RSD%	S.E.M	E/y%
0.5	91.2	89.6	91.2	91	99.73	0.92	1.02	91±2.29	2.53
1	120	122	120	121	111.69	1.15	0.96	121±2.87	2.38
2	150	151.2	148.8	150	135.61	1.20	0.80	150±2.98	1.99
4	187.6	187.6	184.8	187	183.45	1.62	0.87	187±4.01	2.15
6	231	228	229.5	230	231.29	1.52	0.66	230±3.77	1.64
8	275.2	272	268.8	272	279.13	3.20	1.18	272±7.94	2.92
10	320.3	316.8	316.8	318	326.97	2.03	0.64	318±5.05	1.59
12	368.5	364.7	364.7	366	374.81	2.17	0.59	366±5.39	1.47
14	412	412	410	411	422.65	1.15	0.28	411±2.87	0.70
16	475.2	473	475.2	474	470.49	1.27	0.27	474±3.15	0.66
18	523.9	528.6	528.6	527	518.33	2.73	0.52	527±6.77	1.28
20	576	571.2	576	574	566.17	2.77	0.48	574±6.88	1.20
30	806	806	803.4	805	805.37	1.50	0.19	805±3.7267	0.46

Study of Dispersion

Dispersion is a physical phenomenon that occurs in FI technique as a producer of confluence of different concentration solution, the sample mixed with the carrier and then spread the sample in the solution. Success of the analysis process by FIA is depence on some points **[40]:**

1-reproducible injection time.

2-reproducible sample injection volume.

3- control on the dispersion of the sample zone.

The dispersion of [CYM-NBS-HCl –TART] was 1.13 and it was calculate by the low

$D = C_o / Cmax$, As shown in table (12).

While, C_o is the peak height without dilution (conducting interaction outside the flow injection system), Cmax is peak height with dilution (conducting interaction inside the flow injection system) as explain in figure (18). The study was conducted with two experiments, in the first experiment, mixed all the ingredients interact in a suitable beaker and then pass the solution through the flow injection system (as carrier stream) to get fixed response represented (C_o). In the second experiment, CYM into L1, NBS and HCl in L2 and TART in L3. Distilled water pass through the system as carrier (3.03mL.min-1) and the component injected, works to push the components to reaction coil and then to detector to get response represented by (C).

Figure 18 - Dispersion of CYM and TART

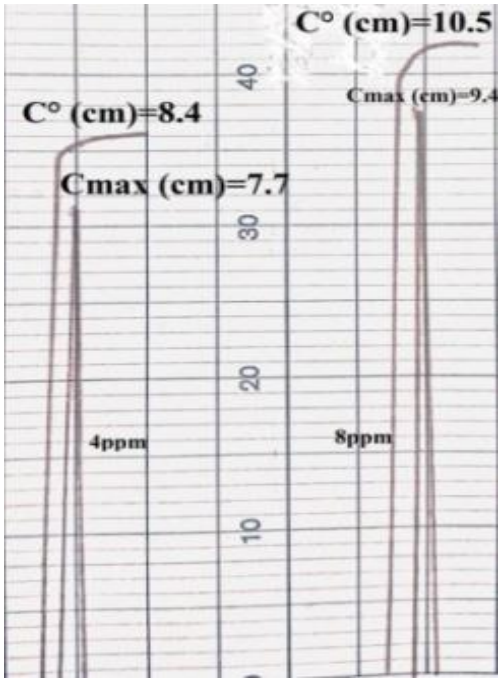


Table 12 - Dispersion Value of CYM

[CYM], µg.mL^{-1}	C° (cm)	Cmax (cm)	D
4	**8.7**	**7.7**	**1.13**
8	**10.5**	**9.4**	**1.117**

Analysis of Variation (ANOVA) [38] of Linear Equation

Analysis of variation (ANOVA) of linear equation is Calculate sum of squares of the difference of values yi (response) from ŷi (appraiser response), (imply error) and called (about regression) to obtain $\sum (yi-\hat{y}i)^2$ for (n- 2) freedom degrees to get sum of squares $(S_o)^2$.

Calculate the sum of squares of the variance of values yi from average value $\bar{y}$ (due to regression) to obtain E $(\hat{y}i - \tilde{y})^2$ and for (1) of degrees of freedom to obtain sum of squares $(S1)^2$ when dividing the $(S1)^2$ on $(So)^2$ get the value(F) as shown in Table (13).

Table 13 - Analysis of Variation for Developed FIA Method

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	347377.3536	1	347377.3536	41.46418254	4.64209E-06	4.413873405
Within Groups	150799.8466	18	8377.769253			
Total	498177.2002	19				

From above table (13), (F critical=4.413 << F calculate = 41.46), so it may be complete which there is an important relation between the concentration of CYM pesticide and the response obtained.

Method Validation

The analytical characteristics just as correlation coefficient (r), detection limit, linear range and relative standard deviation of each procedure were estimated [**30, 31]** at the improved conditions; as shown in the (Table 14). A calibration curve was constructed (Figure 13) for a set of CYM standard solution and the basic analytical figure of deserts of the proposed method. Statistical assessment of regression line presented result of standard deviation for residuals (Sy/x); intercept (Sa) and slope (Sb) under 95% confidence limits for (n-2) freedom degrees were clarified in the table. The result obtained shows that $t_{tab} >> t_{cal}$, where the calculated value was 0.59 and tabulated value for (n-2) at confidence 95% =4.3 Therefore, there was a meaningful relationship between CYM and peak

height in (mV.) with time (min) and from the value of Correlation coefficient (r), possible to know that congruence be more and better bonding, the result were summarized in table (14).

Table 14 - Analytical Characteristic of Calibration Curve for Estimation of CYM Pesticide Using [CYM-NBS-HCl-TART] CFIA System

Parameters	FIA method	Batch method
λmax (nm)	430	430
Regression equation; y = bx+ a; y = absorbance; x = concentration (µg. mL^{-1})	y = 23.92x + 87.77	y = 0.035x + 0.218
Linear range (µg mL^{-1})	0.5 - 30	0.4 - 18
Average of recovery (%)	99.4675	99.643
Average of Relative Error % Erel %	-0.533	-0.357
Average of Relative standard deviation (RSD %)	1.277	0.445
Slope (b); (mL. μg^{-1}) $b = \Sigma i [(xi - \bar{x})(yi - \bar{y})]/\Sigma i(xi - \bar{x})^2$	23.92	0.035
Intercept (a); (a = y– b x)	87.77	0.218
Linearity R2	0.998	0.998
Correlation coefficient (r): $r=\Sigma i [(xi - \bar{x}) (yi - \bar{y})] [(\Sigma i (xi - \bar{x})^2) (\Sigma i (yi - \bar{y})^2)]^{0.5}$	0.999	0.999
Standard deviation of slope (Sb) $Sb = Sy/x /[\Sigma i(xi - \bar{x})^2]^{0.5}$	0.2691	0.0005
Standard deviation of intercept (Sa) $Sa = Sy/x[\Sigma i\, xi^2 / (n\Sigma i(xi - \bar{x})^2)]^{0.5}$	4.0858	0.0051
Limit of detection (LOD)*	0.05	0.016
Limit of quantification (LOQ)**	0.18	0.05
Molar absorptivity Ɛ (L/mol.cm) Ɛ = b*M.Wt*1000	9957896	14570.5
Sandell s sensitivity (S) (µg.cm-2) S=M/Ɛ	4.1806E-05	0.028571429
Sample through put (h-1)	65	5
Standard deviation of the residuals; $Sy/x = [\Sigma i (yi - \hat{y}i)^2 / (n - 2)]^{0.5}$; $\hat{y}i = bxi+a$	8.95	0.39
Confidence limit of slope (b) = b ± tSb	23.92 ± 0.576	0.035 ± 0.0011
Confidence limit of intercept (a) = a ± tSa	87.7 ± 8.744	0.218 ± 0.0115

Study of Interferences

In order to examine the selectivity of the method, the interference likely to be introduced from excipients (as cellulose, sucrose, fructose, glucose and some of ions) were studied. A sample of pure CYM (10ppm) spiked with half, equal and double fold excess concentration of selected interferences (5-10-20) ppm, three excipients were analyzed. The acceptable recovery values (83.56-104.4%) demonstrated that, there were no interferences during the determination of CYM pesticide using proposed MZ-FIA methods, the results summarized in Table (15).

Table 15 - Effect of Interferences on CYM-TART (10µg/ml)

Type of Interference	Conc. of Interferences (ppm)	Present conc. of CYM (ppm)	Average response ($\bar{y}$) (mV)	Found conc. Of CYM (ppm)	Erel%	Rec%
Sucrose	5	10	328	10.04	0.431	100.4
	10	10	336	10.38	3.775	103.8
	20	10	320	9.71	-2.914	97.09
Cellulose	5	10	335	10.33	3.329	103.3
	10	10	318	9.63	-3.694	96.31
	20	10	303	9.02	-9.826	90.17
Lactose	5	10	336	10.39	3.887	103.9
	10	10	342	10.63	6.317	106.3
	20	10	325	9.92	-0.796	99.2
Glucose	5	10	327	10.03	0.319	100.3
	10	10	337	10.42	4.221	104.2
	20	10	319	9.68	-3.248	96.75
NO^{3-}	5	10	339	10.52	5.224	105.2
	10	10	344	10.72	7.231	107.2
	20	10	318	9.61	-3.917	96.08
Cl^{-}	5	10	334	10.28	2.772	102.8
	10	10	339	10.52	5.224	105.2
	20	10	325	9.93	-0.684	99.32
SO^{4-}	5	10	345	10.74	7.398	107.4
	10	10	350	10.95	9.461	109.5
	20	10	324	9.88	-1.242	98.76
Na^{+}	5	10	337	10.41	4.054	104.1
	10	10	339	10.51	5.046	105
	20	10	324	9.89	-1.13	98.87
Ba^{+2}	5	10	336	10.39	3.887	103.9
	10	10	336	10.37	3.664	103.7
	20	10	322	9.80	-2.022	97.98
Fe^{+2}	5	10	335	10.32	3.218	103.2
	10	10	329	10.08	0.765	100.8
	20	10	326	9.95	-0.461	99.54

Biological Samples Applications

The FIA/MZ technique was applied for estimation of CYM pesticide concentration in spiked human biological samples **[37],[39]** according to the standard addition method, three types of biological samples (plasma - serum – urine) have been analyzed under proposed method which come from three different patient's samples. Three different concentrations of biological samples (for serum [5,10 and 20] µg.mL^{-1}, for plasma and urine [4,6 and 8]µg.mL^{-1} were examined for accuracy and precision. Each concentration was analyzed (n=3). Acceptable accuracy with high

repeatability of the results obtained for determination of CYM in biological samples were observed, as shown in Tables (16a,b,c).

Table 16a - Application of the Suggest FIA Method for Determination of CYM in Biological Samples (Spiked Serum Samples)

type of app.	CYM (ppm)		Error	Rec%	Erel%	RSD%	SD
	Present μ	Found $\bar{x}$					
Serum1	5	4.69	-0.31	93.84	-6.16	0.00	0.00
	10	10.10	0.10	101.00	1.00	1.87	6.11
	15	14.50	-0.50	96.68	-3.32	1.06	4.62
Serum 2	5	5.42	0.42	108.33	8.33	1.06	2.31
	10	9.99	-0.01	99.87	-0.13	1.87	6.11
	15	14.89	-0.11	99.28	-0.72	0.90	4.00
Serum 3	5	5.13	0.13	102.53	2.53	1.01	2.12
	10	10.10	0.10	100.99	0.99	0.70	2.31
	15	14.79	-0.21	98.62	-1.38	0.63	2.77

Table 16b - Application of the Suggest FIA Method for Determination of CYM in Biological Samples (Spiked Plasma Samples)

type of app.	CYM (ppm)		Error	Rec%	Erel%	RSD%	SD
	Present μ	Found $\bar{x}$					
Plasma 1	4	3.53	-0.47	88.31	-11.69	2.56	4.41
	6	5.75	-0.25	95.85	-4.15	1.03	2.31
	8	7.76	-0.24	96.97	-3.03	0.85	2.31
Plasma2	4	3.83	-0.17	95.84	-4.16	2.20	3.95
	6	5.68	-0.32	94.74	-5.27	0.20	0.46
	8	7.75	-0.25	96.90	-3.10	0.15	0.40
Plasma3	4	3.66	-0.35	91.38	-8.62	0.23	0.40
	6	5.66	-0.34	94.27	-5.73	0.37	0.83
	8	7.59	-0.41	94.88	-5.12	0.86	2.31

Table 16c - Application of the Suggest FIA Method for Determination of CYM in Biological Samples (Spiked Urine Samples)

type of app.	CYM (ppm)		Error	Rec%	Erel%	RSD%	SD
	Present μ	Found $\bar{x}$					
Urine1	4	3.58	-0.42	89.57	-10.43	1.27	2.20
	6	5.36	-0.64	89.35	-10.65	1.85	4.00
	8	7.69	-0.31	96.07	-3.94	0.15	0.40
Urine2	4	3.62	-0.38	90.54	-9.46	1.21	2.12
	6	5.39	-0.61	89.77	-10.23	0.25	0.55
	8	7.65	-0.35	95.69	-4.31	0.15	0.42
Urine3	4	4.03	0.03	100.85	0.85	0.25	0.46
	6	5.31	-0.69	88.42	-11.58	1.08	2.31
	8	7.67	-0.33	95.86	-4.14	0.30	0.80

Assessment of the Suggested Method

To assessment the success and efficiency of a new proposed method, two types of industrial preparation containing CYM were analyzed under proposed method which is come from different origins (India, china). The industrial preparations were prepared as mentioned procedure shown in practical paragraph. After preparing the solution of these industrials, the proposed FIA/MZ method was successfully applied for estimation CYM in it and the results are listed in Table (17) **[22]**. In the direction of assessing the proficiency of the developed method, the obtained results were compared with trusted method HPLC method (Table 17). The statistical comparison between proposed and trusted methods using the student t- test and F-test [**40]** indicated that the calculated values were less than the theoretical one, which referred to insignificant difference between both methods regarding accuracy and repeatability.

Table 17 **-** Comparison of the Proposed Method with Trusted Method

Industrial application	**Classical method**						**Trusted method**					
	conc. of CYM (ppm)		**Erel %**	**Rec %**	**Mean Rec %**	**RSD %**	**conc. of CYM (ppm)**		**Erel %**	**Rec %**	**Mean Rec %**	**RSD %**
	Present	**Found**					**Present**	**Found**				
Alpha cypermethrin EC10%	**6**	**5.95**	**-0.83**	**99.17**	**100.08**	**2.97**	**6**	**6.02**	**0.33**	**100.33**	**100.02**	**0.470**
	10	**10.10**	**1**	**101**		**1.33**	**10**	**9.97**	**-0.3**	**99.7**		**0.426**
Alpha 10%	**6**	**6.20**	**3.33**	**103.33**	**101.22**	**2.85**	**6**	**5.98**	**-0.33**	**99.67**	**99.98**	**0.473**
	10	**9.91**	**-0.9**	**99.1**		**1.36**	**10**	**10.03**	**0.3**	**100.3**		**0.423**
t_{tab}=4.30 $for\ n1$=$n2$=2,$n1$+$n2$−2=2,at 95% $confidence$ F_{tab}=161.40 $for\ n1$−1=$n2$−1=1,at 95% $confidence$											**t cal=0.59** **F cal=39.06**	

3. Conclusion

By reviewing the literature survey in the field of injection analysis, few researchers found that used this automation technique depence on merging zone of chemicals to determine of α-cypermethrin pesticide in pure form, biological and industrial samples, that is why a researcher plan for this manuscript was suggested for the sensitive spectrophotometric determination of CYM pesticide using the proposed CFIA design which is characterized by a wider calibration range, high sampling rate. Moreover, it was conduct out in watery intermediate and did not demand any sample pretreatment or transmutation. A new design homemade CFI /Merging zones analytical process are strong, purchase and compassionate for the spectrophotometric of CYM.in elegant system and industrial formulations with lowest consumption of toxic reagent and chemicals for the regard of

μg.ml^{-1}amount of CYM and without indigence for prior divorce action, temperature or pretreatment of specimen and solid phase extraction. The capital benefit of the methods are its huge workings range; suitable sensitivity and its proper for appropriate in routine examination laboratories due to their expertness and their result in industrial specify control decrease reagents waste. The suggested procedures has fit linearity, exalted for analyzation the search into of CYM at major of when comparison with batch methods and official HPLC analytical frequency with throughput 65 sample.h^{-1}. In addition, the broad applicability of improved progress microgram even (μg.ml^{-1}) in industrial preparations samples.

References

Sekhon BS. Chiral pesticides. *Journal of Pesticide Science.* 2009; 34(1): 1-12.

Soundararajan R. Pesticides: advances in chemical and botanical pesticides. *BoD-Books on Demand;* 2012. p. 39-44.

Maguire RJ, Chemical and photochemical isomerization of deltamethrin. *Journal of agricultural and food chemistry.* 1990; 38(7): 1613-7.

Feo ML, Eljarrat E, Barceló D. A rapid and sensitive analytical method for the determination of 14 pyrethroids in water samples. *Journal of Chromatography A.* 2010; 1217(15): 2248-53.

Deng, W., Lin, D., Yao, K., Yuan, H., Wang, Z., Li, J., & Hu, X. (2015). Characterization of a novel B - cypermethrin - degrading Aspergillus niger YAT strain and the biochemical degradation pathway of B - cypermethrin. *Applied microbiology and biotechnology,* 99 (19), 8187-8198.

Akbar, S., Sultan, S., & Kertesz, M. (2015). Determination of cypermethrin degradation potential of soil bacteria along with plant growth - promoting characteristics. *Current microbiology,* 70(1), 75-84.

Acikkol M, Semen S, Turkmen 2, Mercan S. Determination of a- cypermethrin from soil by using HPTLC. *JPC-Journal of Planar Chromatography-Modern TLC.* 2012; 25(1): 48-53.

Agency. UEP. *Reregistration eligibility decision for cypermethrin (revised 01/14/08). US.* Environmental Protection Agency Washington, DC; 2008.

Seyler L. EXTOXNET: Extension Toxicology Network: pesticide information notebook (; H00 a). 1994.

Hongsibsong S, Stuetz W, Sus N, Prapamontol T, Grune T, Frank J. Dietary exposure to continuous small doses of a-cypermethrin in the presence or absence of dietary curcumin does not induce oxidative stress in male Wistar rats. *Toxicology reports.* 2014; 1: 1106-14.

Nguyen, T.D., M.H. Lee, and G.H. Lee, Rapid determination of 95 pesticides in soybean oil using liquid–liquid extraction followed by centrifugation, freezing and dispersive solid phase extraction as cleanup steps and gas chromatography with mass spectrometric detection. *Microchemical Journal,* 2010, 95(1): p. 113-119.

Liu, Guozhu, R., Lei, G., Bin, Z., Mingshan L. and Shengjun W., Development of an improved method to extract pesticide residues in foods using acetontrile with magnesium sulfate and chloroform. *Journal of Chromatography A*, 2011, 1218(11): p. 1429-1436.

Martín, L., Luis, F., Ana, M., and Jesus, M., Comparative chemistry and insect antifeedant action of traditional (Clevenger and Soxhlet) and supercritical extracts (CO 2) of two cultivated wormwood (Artemisia absinthium L.) populations. *Industrial Crops and Products,* 2011, 34(3): p. 1615-1621.

De Castro, M.L. and Jiménez-Carmona, M., Where is supercritical fluid extraction going?. *Trends in Analytical Chemistry,* 2000,19(4): p. 223-228.

Barriada-Pereira, González-Castro, M., Muniategui-Lorenzo, MJ. and López-Mahía, S., Comparison of pressurized liquid extraction and microwave assisted extraction for the determination of organochlorine pesticides in vegetables. *Talanta,* 2007, 71(3): p. 1345-1351.

Ji, J., Chunhui, Z., Huiqin, W. and Yunyun, Z., Microwave-assisted steam distillation for the determination of organochlorine pesticides and pyrethroids in Chinese teas. *Talanta*, 2007, 71(3): p. 1068-1074.

Beyer, A. and Biziuk, M., Comparison of efficiency of different sorbents used during clean-up of extracts for determination of polychlorinated biphenyls and pesticide residues in low-fat food. *Food Research International*, 2010, 43(3): p. 831-837.

De Pinho, Gevany, P. N., Antônio, A. Q., Maria, E. L., Ribeiro, S. and Flaviano, O., Pesticide determination in tomatoes by solid– liquid extraction with purification at low temperature and gas chromatography. *Food Chemistry,* 2010, 121(1): p. 251-256.

Li J, Lin D, Ji R, Yao K, Deng W, Yuan H, Wu Q, Jia Q, Luo P and Zhou K. Simultaneous Determination of B-Cypermethrin and Its Metabolite 3-Phenoxybenzoic Acid in Microbial Degradation Systems by HPLC-UV. *Journal of chromatographic science.* 2016; 54(9): 1584-92.

Albaseera SS, Raob RN, Swamyc Y, Mukkantia K. Optimization of dispersive liquid-liquid microextraction of pyrethroid insecticides from aqueous samples for determination by reversed-phase high performance liquid chromatography. *Global Journal of Analytical Chemistry,* Volume. 2011; 2(5).

Abdulra'uf LB, Adeyemo F, Atanda F, Lawal R. Detection of Pendimethalin and Cypermetrin Residues in Locally Produced Tomato Using QUECHERS-HPLC Analysis. *Nigerian Journal of Basic and Applied Sciences.* 2019; 27(1): 34-40.

Ihsan M.S., Ph.D thesis, University of Baghdad, 2020.

Tankiewicz M. Determination of selected priority pesticides in high water fruits and vegetables by modified QUECHERS and GC-ECD with GC- MS/MS confirmation. *Molecules.* 2019: 24(3): 417.

Muhamad H, Zainudin BH, Zulhilmi Z, Bakar NKA. A rapid and cost effective ultrasonic solvent extraction method for determination of 2- cyhalothrin and cypermethrin residues in soil. *Journal of Oil Palm Research.* 2015; 27(4): 377-86.

K. Suresh Kumar', B. Lokanath Swaroop', K. Suvardhan', D. Rekha', B. Jayaraj² and P. Chiranjeevi'. Facile and Sensitive Spectrophotometric Determination of Synthetic Pyrithroids in Their Formulations, Water and Grain Samples. *Environmental Monitoring and Assessment* (2006) 122: 1-8.

Urmila Tamrakar, V.K. Gupta®, and Ajai K. Pillai. A Spectrophotometric Method for the Determination of Fenvalerate and Cypermethrin in Presence of Each Other. *Journal of Analytical Chemistry,* 2012, Vol. 67, No. 5, pp. 437–442. © Pleiades Publishing, Lt., 2012.

Gouda, A. A., & Al Mazroai, L. S. (2014). Sensitive spectrophotometric determination of cypermethrin in its formulations, water and environmental samples. *Main Group Chemistry,* 13(3), 233-242.

Bushra B. Q*.,* Asmaa A. Z. (2020). Sensitive simultaneous estimation of Atorvastatin. Ca in pure and dosage forms via developed CFIA using 1,2 Naphthoquinone-4-sulfonate as a suitable organic agent, *Indian Journal of Forensic Medicine & Toxicology,* 14(2), PP: 2109-2116.

Assaf H.T. & Bushra B. Q*.,* (2020). A green method for assay of doxycycline hyclate using continuous flow injection/merging zones technique via coupling with azo metol in aqueous medium, *Chemistry and chemical technology issues*, No. 4, pp: 31-37.

Suhair M.Y. & Bushra B. Q., (2020). Spectrophotometric determination of Co(II) in Vitamin B12 using 2-(biphenyl-4-yl)-3-((2-(2,4-dinitro phenyl)Hydrazone)methyl) imidazo[1,2-a]pyridine as ligand by Flow Injection-Merging Zone Analysis, *AL-Nahrain journal of science*, 23(3), pp:24-38.

Luma L. Hamed, Bushra B. Qassim, (2020). Direct and new flow injection method for assay of Iron as ferrous sulfate in pure and dosage forms through the complexation with 2,2dipyridyl reagent, *International Journal of Pharmaceutical Research*, vol.12 (2), pp: 1329-1338.

Bushra B. Qassim, Luma L. Hamed, (2020). Simple green method high throughput flow injection technique for spectrophotometry determination of Fe (III) in Iron drugs through the reaction between DPA-4-Sulfonat with hydrogen peroxide using a modified detection unit. *International Journal of Drug Delivery Technology;* 10(4): 563-570.

janghel, E. K., Rai, J. K., Rai, M. K., & Gupta, V. K. (2007). A new sensitive spectrophotometric determination of cypermethrin insecticide in environmental and biological samples. *Journal of the Brazilian Chemical Society*, 18(3), 590-594.

Ahmed A. A. M.Sc thesis, university of Baghdad, 2018.

S.A.A. Elsuccary, Alaa A. Salem. (2015). Novel flow injection analysis methods for the determination of total iron in blood serum and water. *Talanta,* vol (131), pp: 108-115.

Cláudio L. Donnici, Carolina C. Souza, Mark A. Beinnerc and José Bento B. da Silva. Fast Determination of Iron and Zinc in Hair and Human Serum Samples After Alkaline Solubilization by GF AAS. *J. Braz. Chem. Soc.*, Vol. 27, No. 1, 119-126, (2016).

T. H. Bothwel and Barbara Mallett. (1955). The determination of iron in plasma or serum. *Biochem J.;* 59(4): 599–602.

Asmaa, A. Z., M.Sc. thesis, University of Baghdad, 2020.

Assaf H.T. & Bushra B. Q*.,* (2020). A novel method of CFIA/Merging zones technique for assay of Doxycycline in bulk and pharmaceutical preparation depending on azo dye formation, *Research Journal of pharmacy and Technology*, 14(1), pp: 67-74.

Asmaa A. Z*.,* Bushra B. Q*.,* (2020). Novel approach of oxidation-reduction reaction with KMnO4 for simultaneous determination of Simvastatin Drug in either pharmaceutics preparation or human urine using Homemade FIA-Stopped-flow/Merging Zone technique. *Biochem. Cell. Arch.,* 20(1), pp: 2147-2156.