

# Continuous Flow Injection Analysis Method for the Determination of a Drug Diphenhydramine Hydrochloride by Using Phosphomolybdic Acid

Muntadhar M. Jabbar \*, Elham N. Mezaal 

Department of Chemistry, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

\*Corresponding Author.

Received 23/06/2023, Revised 03/07/2023, Accepted 05/07/2023, Published Online First 20/01/2024



© 2022 The Author(s). Published by College of Science for Women, University of Baghdad.

This is an Open Access article distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Abstract

The study presented a new method for determining diphenhydramine hydrochloride (DPH) in its pure form and some pharmaceutical preparations, within CFIA technique. The method is simple, fast, sensitive, easy to operate, and of a low cost. It is based on the reaction of DPH with phosphomolybdic acid (PMA), in aqueous media, forming a white, slightly yellowish precipitate. The precipitate formed was studied using an Ayah 6S×1-ST-2D solar cell CFI analyzer, by the reflection of incident light off the surfaces of the deposited particles at (0–180°), expressed as the response measured in (mV). Some chemical and physical variables were studied to provide optimal conditions for the study. The linear range was 0.07–9 mmol/L and it had a correlation coefficient (*r*) with a value of (0.9998). The limit of detection (L.O.D.) of the new method was 729.55 ng/sample, calculated by gradually diluting the lower limit of the concentration in the linear range (0.07 mmol/L). The % RSD was less than 0.2% for a concentration of 0.1, 4.0 and 10.0 mmol/L of DPH for *n* = 8. The method was successfully applied in the determination of DPH in three samples from three different pharmaceutical production companies. Using the standard addition method, the new method was compared with the UV-Vis spectrophotometry method at  $\lambda_{\max}$  = 258 nm. Both T-test and F-test. The results of the two tests showed no significant difference at a confidence level (95%).

**Keywords:** Allergy, Antihistamines, Diphenhydramine hydrochloride, Flow injection analysis, Ion pair complex.

## Introduction

Diphenhydramine hydrochloride (DPH), which is known by the chemical name 2-(Diphenylmethoxy)N,N-dimethylethanamine hydrochloride molecular structure as in Fig. 1, its chemical formula is  $C_{17}H_{22}ClNO$ , the general appearance of it in its pure form is a white crystalline powder. Highly soluble in water, it's

freely soluble in alcohol. It's offered commercially as a pharmaceutical preparation in several forms, including the form of tablets or syrup taken orally, or in the form of injections taken through intramuscular or intravenous injection<sup>1,2</sup>. It's one of the antihistamines and falls into the category of the first generation of antihistamines<sup>2,3</sup>. It's used alone

or in combination with other medicine<sup>4</sup>. Diphenhydramine hydrochloride is prescribed to relieve allergy symptoms, to treat motion sickness, as an anti-emetic and anti-cough, in cases of bee stings and skin itching and to treat some cases of runny nose and eyes<sup>5</sup>. There are several methods for estimating diphenhydramine hydrochloride in literature; we mention some of them: spectrophotometric methods<sup>6,7</sup>, chromatographic methods<sup>8-11</sup>, flow injection<sup>12,13</sup> and potentiometric methods<sup>14</sup>. In this study, a new method was introduced within the continuous flow injection analysis technique<sup>15-18</sup>, using homemade cells<sup>19-22</sup>, which depends on fluorescence or turbidity<sup>23-26</sup> as it is an easy, simple, sensitive, safe and inexpensive method to control the quality of DPH-containing drug tablets using homemade Ayah 6S×1-ST-2D

## Materials and Methods

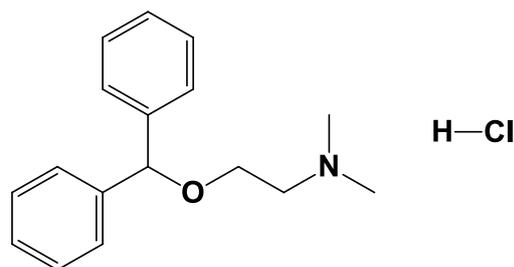
### Reagents and Chemicals

All analytical reagent class chemicals were used in this study, and solutions were prepared using distilled water as a solvent. A standard solution of diphenhydramine hydrochloride ( $C_{17}H_{21}NO.HCl$ ), M.Wt 291.82 g/mol, SDI, 0.01 mol/L) was prepared by dissolving 0.1459 g in 50 ml of distilled water. A stock solution (0.05 mol/L) of phosphomolybdic acid  $H_3[Mo_{12}PO_{40}]$  (M.Wt 1825.25 g/mol, Hopkin & Williams) the preparation was done by dissolving 45.6312 g in 500 ml of distilled water. A 0.1 mol/L from each of the following acids (supplied from BDH) were prepared:  $H_2SO_4$  solution (98% w/w, 1.84 g/mol), HCl solution (35% w/w, 1.19 g/ml),  $HNO_3$  solution (70% w/w, 1.42 g/mol),  $CH_3COOH$  solution (99.5% w/w, 1.05 g/mol). 0.1 mol/L from each of the following salts supplied from BDH) were prepared: NaCl (M.Wt 58.44 g/mol),  $NaNO_2$  (M.Wt 68.9953 g/mol),  $NaNO_3$  (M.Wt 84.9947 g/mol),  $NH_4Cl$  (M.Wt 53.491 g/mol),  $Na_2CO_3$  (M.Wt 105.99 g/mol).

### Sample Preparation

A quantity of twenty tablets was weighed with a four-stage sensitive scale, crushed, ground using a mortar, and used a 200 mesh sieve to sieving the formed. Each tablet has 25 mg of diphenhydramine hydrochloride (supplier from SDI –Iraq, Aswar Al-

solar cell CFI analyzer<sup>27</sup>, which has been successfully used in the determination of some drugs and some elements<sup>28</sup>. The study aims to present a simple, easy-to-work, environment-friendly, low-cost, repeatable and high recovery percentage analytical method for diphenhydramine hydrochloride (DPH).



**Figure 1. Molecular structure of diphenhydramine hydrochloride.**

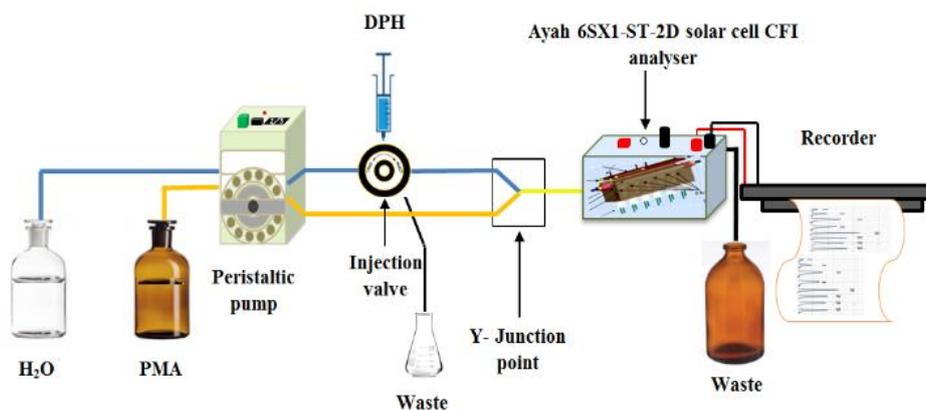
Khaleej-Iraq and Al-Kindi- Iraq) weight :0.6659, 0.6875, & 0.6968 g according to the sequence, which is equivalent to 0.14591 g of the active drug substance to get 10 mmol/L. Distilled water was used to dissolve the powder and to get rid of any undissolved residue that might affect transducer response, the solution was filtered. These solutions were prepared in a 50 ml volumetric flask (The volume was completed to the mark in a 50 ml volumetric flask with distilled water before the filtration process).

### Apparatus

The apparatus used in the new method for DPH determination consists of a two-channel variable speed peristaltic pump (supplied from Ismatec – Switzerland) and three pairs-hole medium pressure injection valve (IDEX Co.-U.S.A. supplied it) with a sample loop (0.7 mm i.d. Teflon, different length). The response was measured using Ayah 6S×1-ST-2D Solar cell CFI analyser (homemade), containing six snow white LEDs as a light source in a 2mm flow cell. Two solar cells were used as a detector to collect signals via a 60 mm travel-sample. The response is output in the form of peaks through the x-t potentiometric recorder (Kompenso GraphC-1032, supplied from Siemens-Germany, 1-500 volt, 1-500 mV) or Digital AVO-meter (auto range) (0.00-2000 mV) (China), the flow diagram for the

determination of DPH is shown in Fig. 2. For Uv/vis measurements, the Uv/Vis spectrophotometer (Shimadzu double beam) was

used. Its wavelengths scan ranges 190-1100 nm, the measuring cell is 1 ml (1000 $\mu$ l) made of quartz, model UV-1800 Kyoto-Japan).

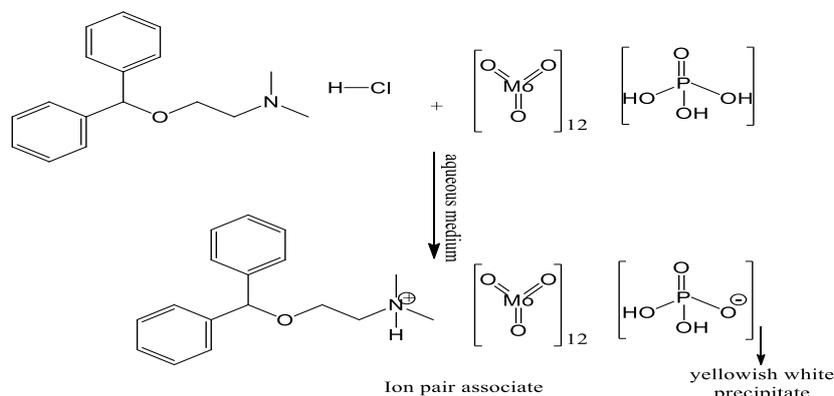


**Figure 2. Flow diagram manifold system used for determination of diphenhydramine hydrochloride(DPH)**

### Methodology

Two lines make up the manifold flow system Fig. 2 for determination of DPH by formation ion-pair complex with Phosphomolybdic acid (PMA) in the aqueous medium, where a white slightly yellowish precipitate was formed. The first line supplied distilled water, which is connected to the injection valve as a carrier stream moving at 2.0 ml/min for hold DPH (used sample volume 50  $\mu$ l), the second line carried PMA (20 mmol/L) at 2.0 ml/min. At Y-Junction, the reagent line and the sample current

line meet, where the starting point of the reaction is, after which the reaction product heads towards the measurement cell in Ayah 6S $\times$ 1-ST-2D Solar cell CFI analyser, each solution was injected three times successively. The responses were captured by x-t potentiometric recorder and the responses appeared in the form peaks whose height is proportional to the amount of light reflected from the surfaces of the precipitate particles after the light fell on them from the source. A mechanism has been suggested for the reaction between DPH and PMA in aqueous medium as shown in scheme 1<sup>14</sup>.



**Scheme 1. The suggested mechanism of the DPH and PMA reaction in aqueous media medium**

### Results and Discussion

The concentration of the PMA reagent and the selection of the type of reaction medium (carrier

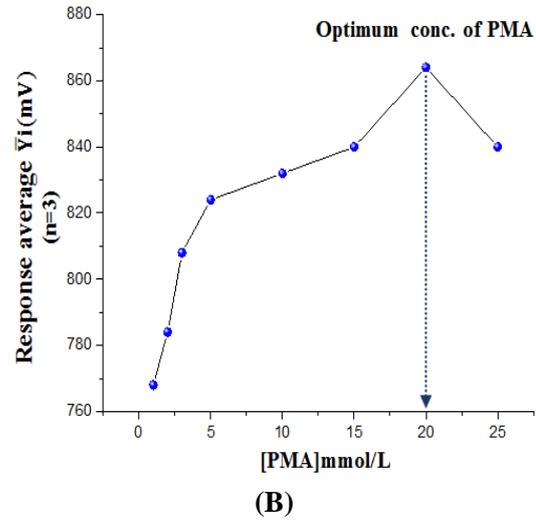
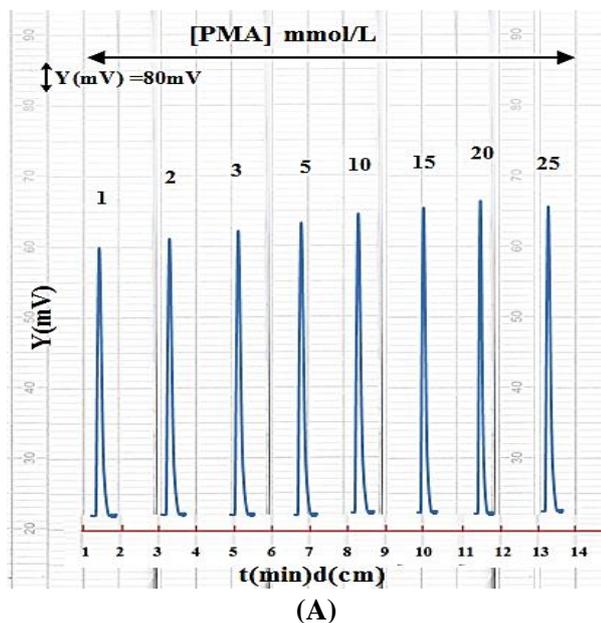
stream), were mainly studied to determine some of the optimal chemical parameters. While the flow rate, sample volume and purge time, were studied to

determine some of the optimal physical parameters. By making it constant and variable each time these variables were optimized.

### Chemical Variables

#### Effect of Phosphomolybdic Acid (PMA) Concentration

A range of PMA concentrations between 1 and 25 mmol/L was generated to determine the ideal reagent concentration. Distilled water was employed as a carrier stream with a sample volume of 25  $\mu$ l. A (6 mmol/L) DPH concentration was injected, both the carrier and reagent lines at a flow rate of 2.3 ml/min. Three times of each measurement were done. Fig. 3A` depicts the experiment's response diagram. Table.1 provides a summary of the findings. The plot of results using the Ayah 6S $\times$ 1-ST-2D solar cell -CFI Analyser is shown in Fig.3A. The responses outlined in Fig. 3A demonstrated that the response peak heights gradually increased until the concentration (20 mmol/L) at which the response peaked, where it showed a peak with the highest height and lowest base width of the other responses. As a result, the concentration of the reagent 20 mmol/L was determined to be the best in this experiment and will be utilized in further tests. Fig. 3B revealed the effect of PMA concentration on the transducer response's peak rising rate.



**Figure 3. PMA concentration's effect on: A: Response outline against time. B: Peak rise rate of transducer response in (mV).**

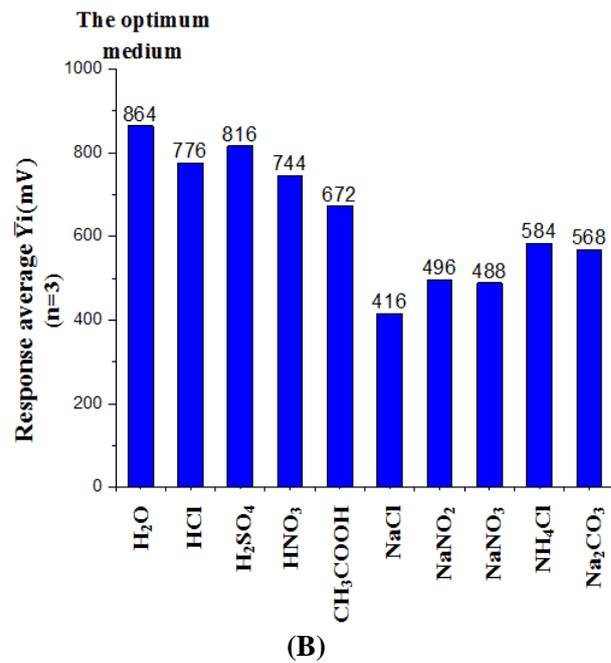
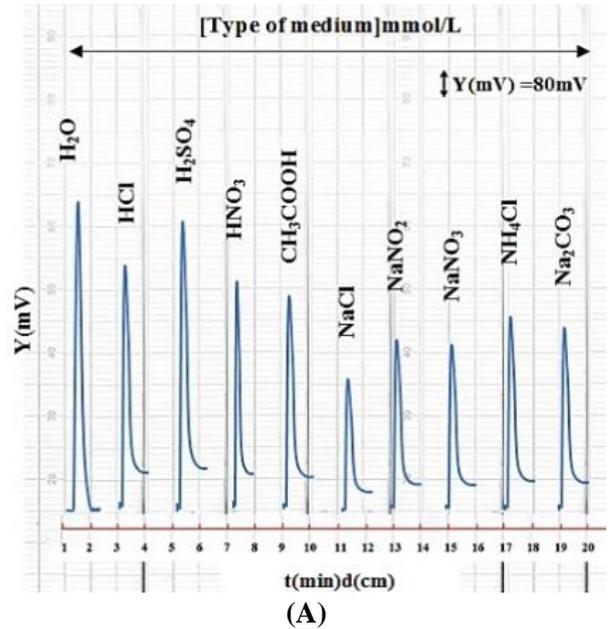
**Table 1. Synopsis of the results of effect of the concentration of PMA on the responses average of the transducer.**

[PMA] mmol/L	Response average $\bar{Y}_i$ (mV) (n=3)	RSD%	Interval of confidence at (95%) $\bar{Y}_i \pm t_{0.05/2, n-1} \cdot \frac{\sigma_{n-1}}{\sqrt{n}}$
1	768	0.1823	768 $\pm$ 3.4780
2	784	0.1811	784 $\pm$ 3.5277
3	808	0.1769	808 $\pm$ 3.5526
5	824	0.1747	824 $\pm$ 3.5774
10	832	0.1743	832 $\pm$ 3.6023
15	840	0.1738	840 $\pm$ 3.6271
20	864	0.1736	864 $\pm$ 3.7265
25	840	0.1750	840 $\pm$ 3.6520

**PMA: Phosphomolybdic acid .  $\bar{Y}_i(\text{mV})(\text{S/N})$  response of an energy transducer in (mV) for  $n=3, t_{\text{tab}0.05/2,2}=4.303$ . RSD%: relative standard deviation,  $\sigma_{n-1}$ : standard deviation,  $n$ : the number of repetitions of the measurement, Interval of confidence: A range of values above and below the point estimate where the true value is most likely to be found with a 95% confidence level**

### Effect of Different Medium

In order to determine the optimal medium for the interaction between diphenhydramine hydrochloride (6 mmol/L) with phosphomolybdic acid (20 mmol/L), this experiment was carried out using solutions of different acids and salts as a carrier stream, which were ( $\text{CH}_3\text{COOH}$ ,  $\text{HCl}$ ,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{NaNO}_2$ ,  $\text{NaNO}_3$ ,  $\text{Na}_2\text{CO}_3$ ) at a concentration of 0.1mol/L, as well as aqueous medium. Observed through the results of the experiment (shown in plot of the transducer response against time) in Fig. 4A. Different media except for the aqueous medium caused a decrease in the S/N response; This can be explained by the fact that these media caused more agglomeration of the sediment particles and fusion with each other, as part of the reflector is lost and a decrease in the amount of light reflected on the surfaces of the sediment particles and thus a decrease in the transducer response (decrease in peak height), while the distilled water caused the sediment particles to not agglomerate and to increase the regularity of their shape, and the amount of light reflected from their surfaces is greater. Thus, the higher response appeared when distilled water was used as a reaction medium (carrier stream). Therefore, subsequent experiments used distilled water as the optimal carrier stream. A summary of the experiment results is shown in Table 2. Fig. 4B reveals the effect of different media on the rate of increase of the transducer response peak.



**Figure 4.A:Medium type's effect on: Response outline against time. B:A graphical diagram showing the effect of medium change on the response average of a transducer**

**Table 2. Synopsis of the results of the effect of changing the mediums on the response of the energy transducer.**

[Type of media] mmol/L	Response of average $\bar{Y}_i$ (mV) (n=3)	RSD%	Interval of confidence at (95%),n-1 $\bar{Y}_i \pm t_{0.05/2,n-1} \cdot \frac{\sigma_{n-1}}{\sqrt{n}}$	
H <sub>2</sub> O	864	0.1736	864 3.7265	±
HCl	776	0.1830	776 3.5277	±
H <sub>2</sub> SO <sub>4</sub>	816	0.1790	816 3.6271	±
HNO <sub>3</sub>	744	0.1895	744 3.5030	±
CH <sub>3</sub> COOH	672	0.1994	672 3.3290	±
NaCl	416	0.1803	416 1.8632	±
NaNO <sub>2</sub>	496	0.1976	496 2.4346	±
NaNO <sub>3</sub>	488	0.1926	488 2.3353	±
NH <sub>4</sub> Cl	584	0.1883	584 2.7327	±
Na <sub>2</sub> CO <sub>3</sub>	568	0.1743	568 2.4595	±

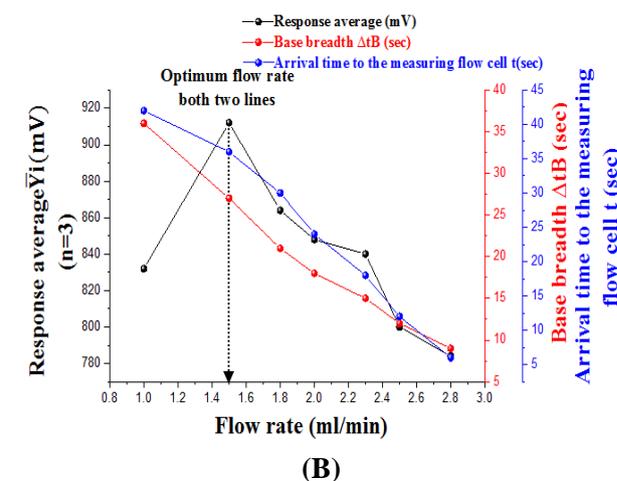
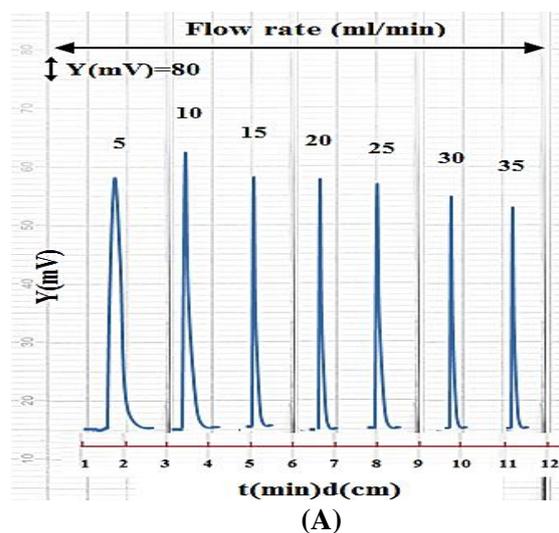
$\bar{Y}_i$ (mV)(S/N)response of an energy transducer in (mV) for  $n = 3$ ,  $t_{tab0.05/2,2} = 4.303$ .RSD%:relative standard deviation,  $\sigma_{n-1}$ :standard deviation, n:the number of repetitions of the measurement, Interval of confidence: A range of values above and below the point estimate where the true value is most likely to be found with a 95% confidence level

### Physical Variables

#### Flow Rate

Flow rate is one of the physical variables affecting the interaction of DPH determination. After examining the effects of flow rate on the transducer's response, the optimal flow rate for this experiment will be selected. The experiment was

within the extent (1.0-2.8 ml/min) for both the reagent and carrier streamlines. A peristaltic pump controls the flow rate, the concentration of DPH was 6 mmol/L, 25 $\mu$ l of the sample was utilized in this experiment and the reagent (PMA) was used at 16 mmol/L. The carrier stream was distilled water. Fig. 5A shows how varying the flow rate affects the transducer's response. The experiment's results are summarized in Table 3, which shows that the flow rate 1.5 ml/min for each of the two lines produced the highest response. . Therefore, it was adopted as an optimal flow rate in subsequent experiments. Fig. 5B emphasizes the effect of flow rate on the transducer response's peak rising rate, base breadth  $\Delta t_B$  and arrival time to the measuring flow cell.



**Figure 5. Flow rate's effect on: A: response outline against time. B: peak rise rate of transducer response in (mV), base breadth  $\Delta t_B$ (sec) and arrival time to the measuring flow cell (sec)**

**Table 3. Synopsis of the results of the effect of changing the flow rate on the response of the transducer.**

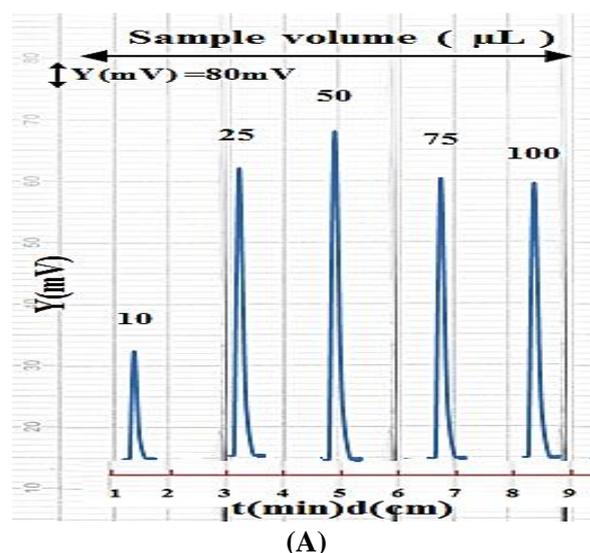
Pump speed	Flow rate both two lines (ml/min)	Response average		Interval of confidence (95%),n-1	t	Base breadth $\Delta t_B$ (sec)	$V_{add}$ (ml)	Concentration mmol/L	Df
		$\bar{Y}_i$ (mV) (n=3)	RSD %						
5	1.0	832	0.1706	$832 \pm 3.5277$	42	36	1.8250	0.0821	73.0816
10	1.5	912	0.1645	$912 \pm 3.7265$	36	27	1.3750	0.1090	55.0458
15	1.8	864	0.1736	$864 \pm 3.7265$	30	21	1.2850	0.1167	51.4138
20	2.0	848	0.1701	$848 \pm 3.6023$	24	18	1.2250	0.1224	49.0196
25	2.3	840	0.1702	$840 \pm 3.5526$	18	15	1.1750	0.1276	47.0219
30	2.5	800	0.1750	$800 \pm 3.4780$	12	12	1.0250	0.1463	41.0116
35	2.8	784	0.1760	$784 \pm 3.4284$	6	9	0.8650	0.1734	34.6020

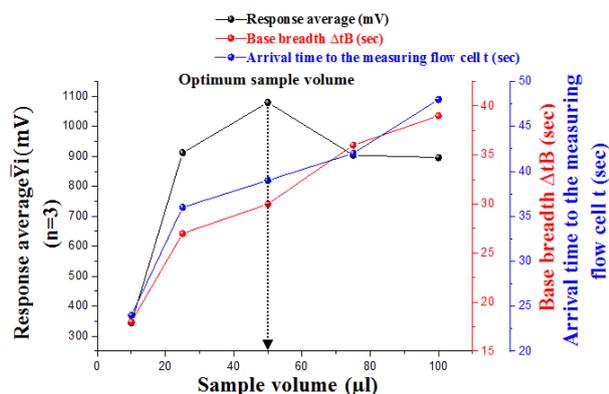
$\bar{Y}_i$ (mV) (S/N) response of an energy transducer in (mV) for  $n=3, t_{tab0.05/2,2}=4.303$ . RSD%: relative standard deviation,  $\sigma_{n-1}$ : standard deviation, n: the number of repetitions of the measurement, Interval of confidence: A range of values above and below the point estimate where the true value is most likely to be found with a 95% confidence level, t: Time arrival estimated from the injection valve to the measurement cell (sec),  $\Delta t$ : Base breadth of peak (sec),  $V_{add}$ : Addition volume (ml) in flow cell, Df: Dilution factor in flow cell

### Sample Volume

In this experiment, the concentration of DPH (6 mmol/L), the concentration of PMA (16 mmol/L) and distilled water were used as a carrier stream to test the effect of changing the sample volume on the intensity of the response, using the optimal parameters that were established in the previous experiments, such as (1.5 ml/min) the optimal flow rate for both the PMA line and the carrier stream line. It's different for the sample loop in the injection valve, it can be observed in the graph of the response against time Fig. 6A that as volume increases, the peak was sharp (needle peak), when

the sample volume (50  $\mu$ l), as explained in Fig. 6A and through Fig. 6B it was observed when using a sample volume greater than (50  $\mu$ l), there is a relatively constant rise in the response and breadth of the base, so the volume of (50  $\mu$ l) will be adopted as an optimal sample volume in subsequent experiments. A synopsis of the experiment's results shown in Table 4. Fig. 6B indicated that the sample volume affected the transducer response's peak increase rate and arrival time to the measuring flow cell.





(B)

Figure 6. Sample volume's effect on: A: Outline's response against time. B: Peak rise rate of transducer response in (mV), base breadth  $\Delta tB$  (sec) and arrival time to the measuring flow cell (sec).

Table 4. Synopsis of the effect of sample volume change on transducer response average results

Sample volume ( $\mu\text{l}$ )	Response average	RSD%	Interval of confidence	of at t	Base breadth $\Delta tB$ (sec)	$V_{\text{add}}$	Concentration		Df
	$\bar{Y}_i$ (mV) (n=3)		(95%), n-1			(ml)	In flow cell	In flow cell	
10	344	0.1337	$344 \pm 1.1428$	24	18	0.9100	0.0659	91.0470	
25	912	0.1645	$912 \pm 3.7265$	36	27	1.3750	0.1090	55.0458	
50	1080	0.1388	$1080 \pm 3.7265$	39	30	1.5500	0.1935	31.0077	
75	904	0.1615	$904 \pm 3.6271$	42	36	1.8750	0.2400	25.0000	
100	896	0.1607	$896 \pm 3.5774$	48	39	2.0500	0.2926	20.5058	

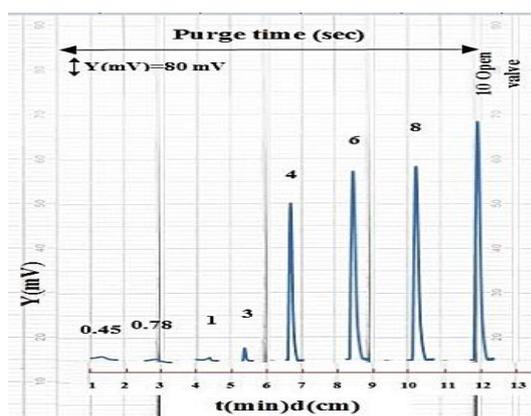
$\bar{Y}_i$  (mV) (S/N) response of an energy transducer in (mV) for  $n=3, t_{\text{tab}0.05/2,2}=4.303$ . RSD%: relative standard deviation,  $\sigma_{n-1}$ : standard deviation, n: the number of repetitions of the measurement, Interval of confidence: A range of values above and below the point estimate where the true value is most likely to be found with a 95% confidence level, t: Time arrival estimated from the injection valve to the measurement cell (sec),

$\Delta t$ : Base breadth of peak (sec),  $V_{\text{add}}$ : Addition volume (ml) in flow cell, Df: Dilution factor in flow cell

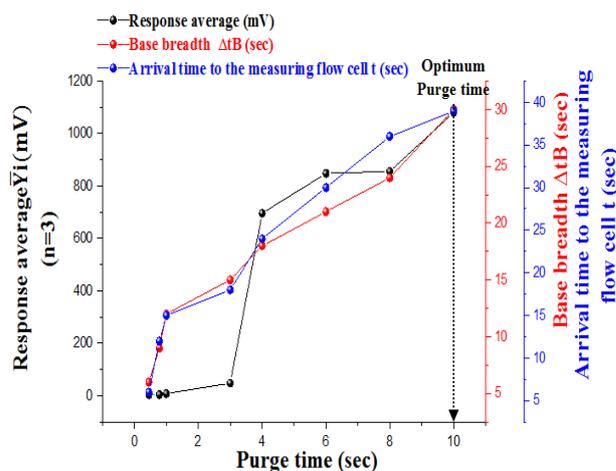
#### Purge Time

In this experiment, different times were used to purge the sample section (0.45-8 sec) and the opening position of the injection valve (10 sec). The carrier solution passes through the injection valve

when the valve is in the injection position, followed by turning the valve to the loading position. The experiment was carried out using the optimal parameters established in previous experiments. The concentration of DPH used in this experiment was 6 mmol/L. A synopsis of the results of the experiment is shown in Table 5, Fig. 7A shows the transducer's response against time, which shows a continuous increase in response with increased purge time up to the time of 10 sec which is the open valve mode. Therefore, it was chosen as the optimal time to purify the sample from the sample loop completely. Fig. 7B shows the effect of purge time on the peak rise rate of transducer response, base breadth  $\Delta t_B$  and arrival time to the measuring flow cell.



(A)



(B)

Figure 7. purge time's effect on: A:Response outline against time. B:peak rise rate of transducer response in (mV), base breadth  $\Delta t_B$ (sec) and arrival time to the measuring flow cell (sec)

Table 5. Synopsis of the results of the effect of changing the purge time on the response of the transducer

Purge time expressed (count)	Purge time by expressed by(sec)	Response average $\bar{Y}_i$ (mV) (n=3)	RSD%	Interval confidence (95%),n-1 $\bar{Y}_i \pm t_{0.05/2,n-1} \cdot \frac{\sigma_{n-1}}{\sqrt{n}}$	of at t (sec)	Base breadth $\Delta t_B$ (sec)
1	0.45	4	0.1050	$4 \pm 0.0104$	6	6
3	0.78	4	0.1025	$4 \pm 0.0102$	12	9
5	1	8	0.1125	$8 \pm 0.0223$	15	12
10	3	48	0.1041	$48 \pm 0.1242$	18	15
15	4	696	0.1911	$696 \pm 3.3041$	24	18
20	6	848	0.1701	$848 \pm 3.6023$	30	21
25	8	856	0.1717	$856 \pm 3.6520$	36	24
(30)Open valve	10	1080	0.1388	$1080 \pm 3.7265$	39	30

$\bar{Y}_i$ (mV) (S/N) response of an energy transducer in (mV) for  $n=3, t_{tab0.05/2,2}=4.303$ . RSD%: relative standard deviation,  $\sigma_{n-1}$ : standard deviation, n: the number of repetitions of the measurement, Interval of confidence: A range of values above

and below the point estimate where the true value is most likely to be found with a 95% confidence level, t: Time arrival estimated from the injection valve to the measurement cell (sec),  $\Delta t$ : Base breadth of peak(sec)

### A Calibration Curve (Scatter Plot) For the Variance of DPH Concentration against Energy Transducer Response

The optimal chemical with physical parameters were adopted to prepare a series of DPH solutions within the extent (0.07-10) mmol/L , the measurement was repeated for each concentration three consecutive times . Responses are shown as in Fig. 8A, which shows the response range and peak height of each concentration of DPH. As shown in Fig.8B, the linear calibration curve was within the range (0.07-9)mmol/L, accompanied by a correlation coefficient (r)=0.9998. The obtained results ( which briefly show the linear regression of the change of transducer response against the change of DPH concentration , the equation that was used in this part of the study was  $\hat{y}=a+bx$  <sup>29</sup> (first-degree equation). The value of t was calculated at a confidence level (95%), which was greater than the value of tabular t, which leads us to say that linearity versus nonlinearity is acceptable. Synopsis of results is shown in Table 6.

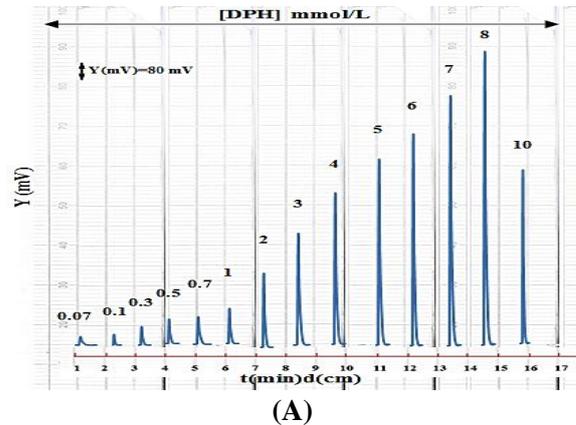


Figure 8. A: A calibration curve shows the effect of DPH concentration change against time on transducer response (for some responses)

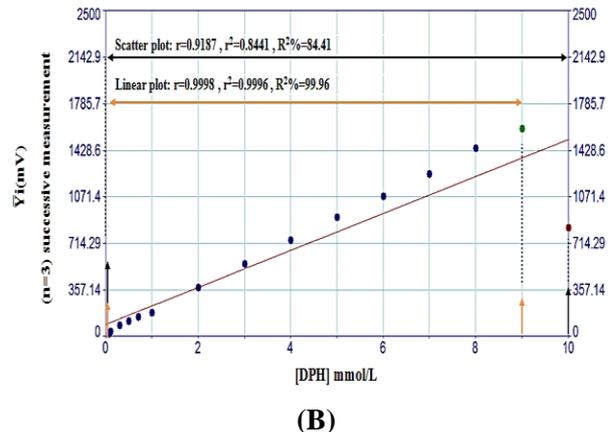


Figure 8. B: Scatter and linear plot of the new method expresses the transducer response against concentration by linear equation .

Table 6. Synopsis of results for the first-degree equation's application to the variation of the (DPH) energy transducer response

Type of mode	Extent of [DPH] mmol/L	$\hat{Y}$ (mV) = a mV $\pm$ S <sub>a</sub> t + b( $\Delta y$ (mV) / $\Delta x$ (mmol/L)) $\pm$ S <sub>b</sub> t at confidence level 95%,n-2	r r <sup>2</sup> R <sup>2</sup> %	t <sub>tab</sub> at 95%,n-2	t <sub>cal</sub> = $\frac{ r  \sqrt{n-2}}{\sqrt{1-r^2}}$
Scatter plot	0.07-10 (n=15)	91.8133 $\pm$ 185.4478 + 142.0469 $\pm$ 36.5644 [DPH]	0.9187 0.8441 84.41	2.160 < 8.3900	
Linear range	0.07-9 (n=14)	25.1449 $\pm$ 9.1947 + 176.4510 $\pm$ 2.0343 [DPH]	0.9998 0.9996 99.96	2.179 << 173.17	

n: number of measurement,  $\hat{Y}$ (mV): estimated value of cell in(mV) , r: Correlation coefficient, r<sup>2</sup>: Coefficient of determination, R<sup>2</sup>%: Explained

variation as a percentage / total variation, t<sub>tab</sub>=t<sub>0.05/2,n-2</sub>

### Limit of Detection (L.O.D)

This study was carried out to determine the detection limit for DPH using three different approaches.: practically based on gradual dilution of the minimum concentration in the calibration curve, it was calculated theoretically on the basis of the value of the slope and on the basis of the linear equation, and the results of this part of the work are summarized in Table 7. The minimum concentration was 0.07 mmol/L and the sample volume was 50 $\mu$ l.

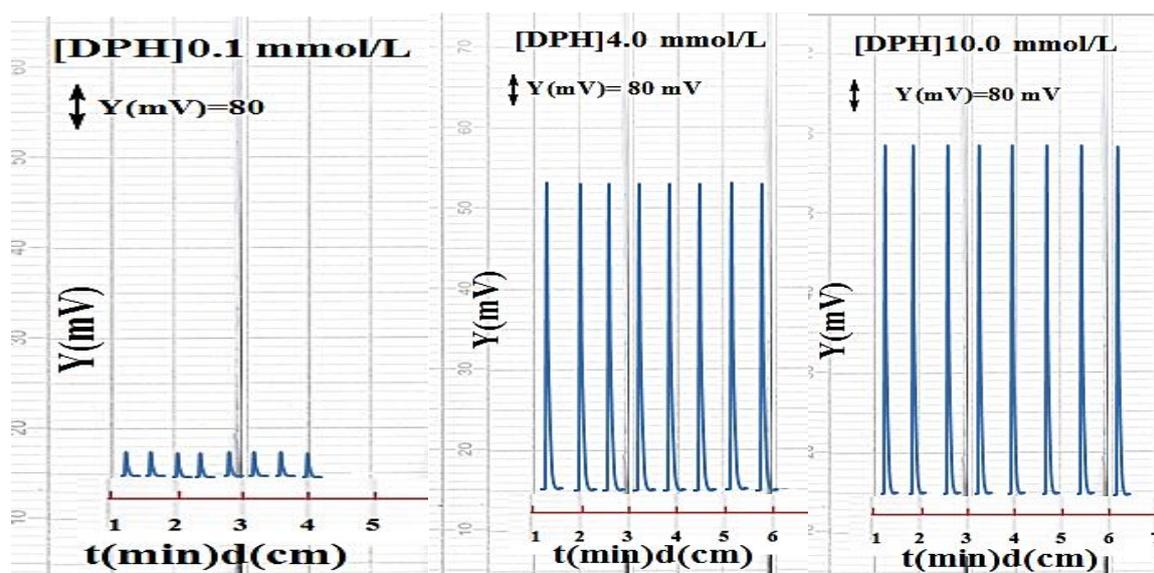
**Table 7. Detection limit values for determination of DPH , using 50 $\mu$ l as a sample volume .**

Practically based on the gradual dilution for the minimum concentration (0.07 mmol/L)	Theoretical based on the slope value $X = 3S_B/\text{slope}$	Theoretical based of linear equation $\hat{Y} = Y_b + 3S_b$
729.55 ng/50 $\mu$ l	74.421 ng/50 $\mu$ l	2.64 $\mu$ g/50 $\mu$ l

**X=** limit of detection value , **S<sub>B</sub>**=standard deviation value of blank refined for 16 times, **Y<sub>b</sub>**: average response for blank=intercept(a), **S<sub>b</sub>**: Standard deviation equal to  $S_y/x(\text{residual})$  from linear range , **$\hat{Y}$** :estimated response(mV).

### Repeatability

The percentage equivalent to the test-retest measurement reliability expresses the relative standard deviation. The responses were re-measured for each concentration through eight consecutive injections for three stable DPH concentrations (0.1, 4.0 and 10.0) mmol/L in optimum conditions for n=8. Response outline shown in Fig. 10, Table .8, indicates that the relative standard deviation as a percentage was less than 0.2%, making it abundantly evident that the suggested approach and instrument were suitable for determining DPH.



**Figure 9. Response against time outline for eight consecutive repeated measurements of DPH concentration(0.1 , 4.0 , 10.0 ) mmol/L**

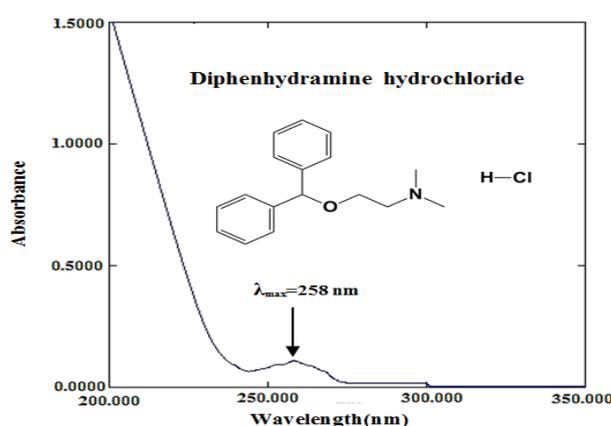
**Table 8. Synopsis of results for repeatability of DPH at optimal parameters.**

[DPH] mmol/L	Response average $\bar{Y}_i$ (mV) (n=8)	RSD%	Interval of confidence at (95%) $\bar{Y}_i \pm t_{0.05/2, n-1} \cdot \frac{\sigma_{n-1}}{\sqrt{n}}$
0.1	40	0.1250	$40 \pm 0.0418$
4.0	740	0.1891	$740 \pm 1.1706$
10.0	840	0.1904	$840 \pm 1.3378$

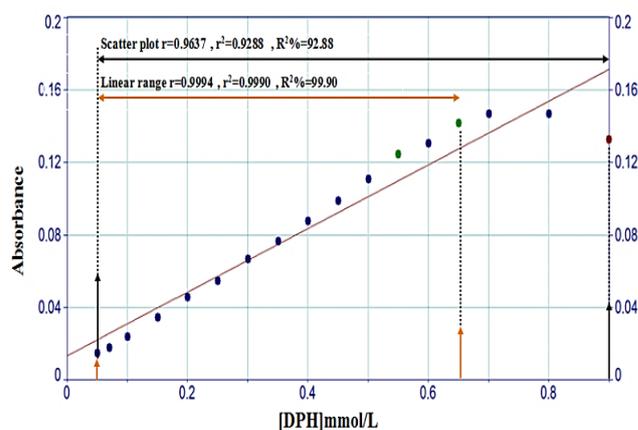
$\bar{Y}_i$ (mV)(S/N) response of an energy transducer in (mV) for n=8 n: number of injection,  $t_{tab0.05/2,7}=2.365$ , R.S.D%:relative standard deviation,  $\sigma_{n-1}$ :standard deviation, n: the number of repetitions of the measurement, Interval of confidence: A range of values above and below the point estimate where the true value is most likely to be found with a 95% confidence level

### The traditional method of UV-Spectrophotometric

In order to evaluate the new DPH determination method, a comparison was made between it and a UV-spectrophotometric method, on which absorbance measurements are based. The concentration extent of the method was 0.05-0.9 mmol/L at  $\lambda_{max}=258$  nm at 0.55 mmol/L, Fig. 10, using quartz cell(1ml) . From Fig. 11 , the scatter plot from 0.05-0.9 mmol/L, while the linear extent was (0.05-0.65 mmol/L) . Correlation coefficient (r) = 0.9994 and  $R^2\%=99.90$  , n=14 (n= number of measurements) . The detection limit was 8.7546  $\mu$ g/sample; it was calculated by gradually diluting the minimum concentration in the calibration curve (0.05 mmol/L). Synopsis of the results of the method is illustrated in Table.9



**Figure 10. Absorbance of UV-Spectrum of DPH at concentration 0.55 mmol/L that shows  $\lambda_{max}=258$  nm**



**Figure11. Scatter plot at (0.05-0.9 mmol/L) ,n=17 for DPH using classical method at 258 nm , in addition linear extent at (0.05-0.65 mmol/L)for n= 14**

**Table 9. Synopsis of linear regressive for the determination of DPH, using UV-Sp. method ( classical method) .**

Type of mode	Extent of calibration curve	atn	linear regressive at C.I. 95%,n-2 $\hat{Y}=a \pm Sa.t + b(\Delta y/\Delta x \text{mmol/L}) \pm Sb.tr^2$ [DPH]mmol/L	r $R^2\%$	$t_{tab}$ at 95%, n-	$t_{cal} = \frac{ r \sqrt{n-2}}{\sqrt{1-r^2}}$	L.O.D.
Scatter plot	0.05-0.9	17	$0.0134 \pm 0.0127 + 0.1754 \pm 0.0266$ [DPH]	0.9637 0.9288	2	2.131 < 13.9891	

<b>Linear</b>				0.9994		8.7546
<b>range</b>	0.05-0.65	14	0.0027±0.0015 +0.2153±0.0041[DPH]	0.9990	2.179 << 109	.5569 µg/Sample
				99.90		

### DPH-PMA-H<sub>2</sub>O System Evaluation for the CFIA Method (New Method) for Determining Diphenhydramine Hydrochloride (DPH) in Pharmaceutical Preparations

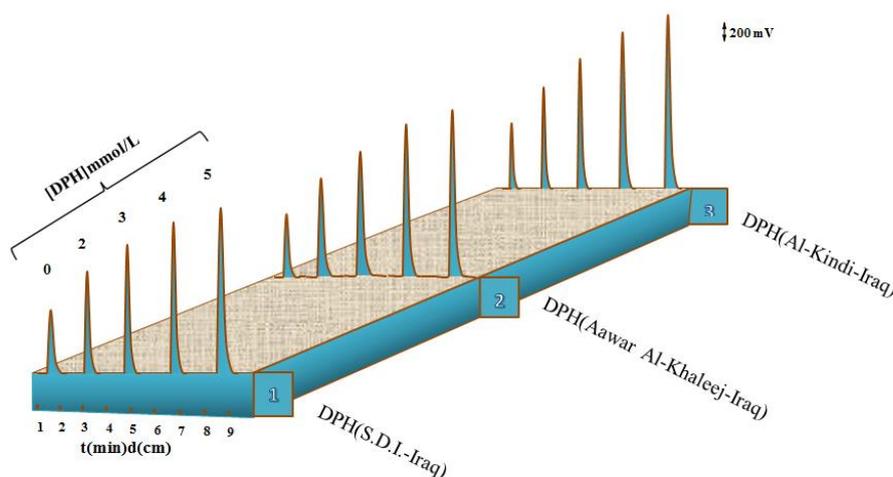
To determine DPH in pharmaceutical preparations, to evaluate the efficiency of the new method, which was made using Ayah 6S×1-ST-2D Solar cell CFI Analyser(homemade), five solutions were prepared for each drug for samples from three different companies for the production of pharmaceutical preparations (SDI-Iraq , Aswar Al-Khaleej-Iraq and Al-Kindi-Iraq) , where comparison was made with the UV-Spectrophotometric method (classical method) after applying the method of a standard addition to both the two methods are as follows:

For the new method: five volumetric flasks of 10 ml were prepared and 3.0 ml from 10 mmol/L was transferred into each of them, after which different volumes of the standard solution of DPH were gradually added (S.D.I.-Iraq) (0 , 2 , 3 , 4 , 5 )ml of 10 mmol/L to gain 0, 2 , 3 , 4 , 5 mmol/L.

For the classical method: five volumetric flasks of 10 ml were prepared and 1 ml from 1 mmol/L

was transferred into each of them, after which different volumes of standard solution of DPH were gradually added (S.D.I.-Iraq) (0, 1, 2, 3, 4 )ml from 1 mmol/L to gain 0 , 0.1 , 0.2 , 0.3 , 0.4 mmol/L.

Flask number 1 is sample. Measurements were made for both methods. The results obtained from the standard addition method were mathematically processed. The results are summarized in Table 10A, B at C.I. (95%) The t-test and F-test were used to evaluate whether there is a significant difference. The results were processed statistically<sup>29</sup> . The results of the t-test and F-test were summarized in Table 10B (columns 4 and 5). The findings made it very evident that, at a 95% level of confidence, there is no significant difference between the new method and the classical method, t-calculated (1.846) less than t-tabular (4.303), as well as the calculated F-value (1.1682) less than tabular F-value (39). Fig. 12 shows that, for three drug samples, the effect of changing DPH concentration on the S/N energy transducer response against time using the Ayah 6SX1-ST-2D solar cell CFI analyser.



**Figure 12. Effect of variation of DPH concentration (using standard addition method) on S/N energy transducer response versus time(min), distains(cm) for three sample drugs using Ayah 6SX1-ST-2D solar cell CFI analyser . 1-Iraq, Allermine, S.D.I., 2-Iraq, Alermen,Aswar Al-Khaleej , 3-Iraq,Kindiramin.Al-Kindi**



**Table 10 A . Synopsis of results standard addition in three pharmaceutical preparations samples for both the classical and new methods.**

No. of sample	Commercial name Company Content Country	Recovered weight (mg)	Type of method					standard addition equation at 95% for n-2	r r <sup>2</sup> R <sup>2</sup> %
			New method						
			UV- Sp. method measurement of absorbance at λ <sub>max</sub> =258 nm						
			Diphenhydramine hydrochloride						
			0 ml	2.0 ml	3.0 ml	4.0 ml	5.0 ml	Ŷ	
			0 mmo	2.0 mmo	3.0 mmo	4.0 mmol	5.0 mmo	(mV)=a <sub>mV</sub> ±S <sub>a</sub> t+b(Δy <sub>(mV)</sub> /Δx <sub>mmol/L</sub> )±S <sub>b</sub> t	
			1/L	/L	l/L	/L	l/L	[DPH]mmol/L	
			0 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml	Ŷ =a± S <sub>a</sub> t +b (Δ y / Δx <sub>mmol/L</sub> )±S <sub>b</sub> .t	
			0 ml	0.1 ml	0.2 ml	0.3 ml	0.4 ml	[DPH]mmol/L	
1	Allermine Al-S.D.I 25 mg Iraq	24.4359	550 mV	910 mV	1090 mV	1280 mV	1480 mV	543.2432±25.7353+185.2702±7.8309 [DPH ] mmol/L	0.9997 0.9994 99.94
		24.3002	0.022	0.042	0.063	0.082	0.109	0.0208±0.0057+0.2140±0.0241 [DPH] mmol/L	0.9980 0.9961 99.61
		24.9285	560 mV	940 mV	1100 mV	1300 mV	1500 mV	557.8378±34.9966+186.4865±10.6488 [DPH ] mmol/L	0.9995 0.9990 99.90
		24.6223	0.020	0.041	0.061	0.082	0.102	0.0202±0.0006+0.2050±0.0031[DPH] mmol/L	0.9999 0.9999 99.99
2	Alermen Aswar Khaleej 25 mg Iraq	24.7717	555 mV	930 mV	1090 mV	1290 mV	1490 mV	551.4865±35.9814+185.5405±10.9479 [DPH]mmol/L	0.9994 0.9989 99.89
		24.7523	0.020	0.040	0.059	0.080	0.100	0.0198±0.0012+0.2000±0.0051[DPH] mmol/L	0.9999 0.9998 99.98
3	Kindiramin Al-Kindi 25 mg Iraq								

Ŷ: Calculated response in (mV) for the new method and the UV-Sp. method absorbance value , r: correlation coefficient ,R<sup>2</sup> %: variance explained in percentage /total variation , UV-Sp.

:sign of UV-Spectrophotometric method, t<sub>tab</sub>= t<sub>0.05/2,∞</sub>=1.960 at 95%, t<sub>tab</sub>= t<sub>0.05/2,3</sub>= 3.182 for n=5, volume of cell (quartz) 1ml used in UV-Spectrophotometric method.

**Table 10.B: Synopsis of practical content results ,percentage recovery (Rec.%) for DPH determination in three pharmaceutical preparations ,paired t-test and F-test .**

No. of sample	Type of method	Paired t-test		F-test	
		t <sub>cal</sub> = $\bar{X}d/\sqrt{n}/\sigma_{n-1}$	t <sub>tab</sub> at 95% confidence level	F <sub>cal</sub> = S <sup>2</sup> <sub>2</sub> /S <sup>2</sup> <sub>1</sub>	F <sub>tab</sub>
	<b>New method</b>				
	<b>UV-Sp. method measurement at 258nm</b>				
	<b>Workable concentration (mmol/L)</b>				
	<b>In 10 ml</b>				
	-----				
	<b>In 50 ml</b>				

	2.9321			
	-----	97.7437		
	9.7739			
1	0.0972			
	-----	97.2008		
	9.7196			
	2.9913			
	-----	99.7142		$\sigma_{n-1}^{**}=0.2515, S^2_{1(CFIA)}=0.0632$
	9.9710		$\bar{X}_d=0.1537$	$\sigma_{n-1}^*=0.2327, S^2_{2(UV-Sp.)}=0.0541$
2	0.0985		$\sigma_{n-1}=0.1442$	
	-----	98.4893	$1.846 < 4.303$	$1.1682 < 39.0000$
	9.8536			
	2.9723			
	-----	99.0868		
	9.9077			
3	0.0990			
	-----	99.0090		
	9.9000			

$\bar{X}_d$  : Comparing two types of methods, on average (new & classical) ,(n) sample number sign=3,  $\sigma_{n-1}$ :standard deviation sign of different (paired t-test),  $t_{tab}=t_{0.05/2,2}=4.303$  (for paired t-test),  $F_{tab}=F_{0.95,V1,V2}=F_{0.95,2,2}=39, \sigma_{n-1}^{**}$  :

standard deviation sign(CFIA method),  $\sigma_{n-1}^*$  standard deviation sign(for UV-Sp. method) ( F-test), UV-Sp.: sign of UV-Spectrophotometric method

## Conclusion

The method described in this study is simple, sensitive, speedy, requires a few samples and reagents, and is simple to use. When distilled water was used as the reaction medium, it was successfully applied to the measurement of diphenhydramine hydrochloride in both pure and pharmaceutical preparations based on the formation of a white-slightly yellowish precipitate as a result

of the interaction of DPH with phosphomolybdic acid (PMA). The findings of the study demonstrated that the new method is a viable substitute for the classical method with which it was compared, i.e., an alternative analytical method was discovered through this study, which was carried out under simple conditions and with optimum parameters using an Ayah 6SX1-ST-2D solar cell CFI analyzer.

## Acknowledgment

After completing the study, we extend our thanks and appreciation to Prof. Dr. Issam Al-Hashemi and Prof. Dr. Nagham Shaker Al-Awadi, who have the patent for the measurement cell, through which the work methodology in this study was completed., the State Company For Drugs Industry And Medical

Appliances/Samarra (S.D.I.);for providing the pure sample of the drug for free, to the Department of Chemistry in College of Science / University of Baghdad ;to facilitate the task of completing some of the research requirements

## Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore,

any Figures and Images, that are not ours, have been included with the necessary permission for

re-publication, which is attached to the manuscript.

- The authors signed an animal welfare statement.
- Authors sign an ethical consideration approval.

- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

### Authors' Contribution Statement

Both of the authors M. M. J. and E. N. M. contributed to the design and implementation of the results and to the writing of the manuscript.

### References

1. British pharmacopoeia, London: The stationery office, 2020: 1823.
2. United State Pharmacopoeia and National Formulary USP41 NF36, 2018; 1: 1327.
3. Paul R M, Vasanth K P, Vikiran K, Deepak K. Analytical method development and validation for simultaneous estimation of diphenhydramine and naproxen in pharmaceutical dosage forms. *J Pharm Negat Results* 2022; 13(6): 792-798 .  
<https://doi.org/10.47750/pnr.2022.13.S06.109>
4. Sapna S, Kuldeep S, Suvarcha C, Kuldeep K. Volumetric compressibility and viscometric properties of diphenhydramine hydrochloride in aqueous solution of electrolytes: an insight into interactional characteristics. *J Mol Liq* 2021; 342(2): 1-12.  
<https://doi.org/10.1016/j.molliq.2021.117481>
5. Sanjay M, Timothy B, Emma F, Mary A H, Silas W S, Mark K. A Case of Massive Diphenhydramine and Naproxen Overdose. *J Emerg Med*. 2021; 61(3): 259-264.  
<https://doi.org/10.1016/j.jemermed.2021.04.020>
6. Noor Al-Huda A R, Khalid Al-Janabi Spectrophotometric determination of diphenhydramine HCL in pure and pharmaceutical formulations using thymol blue. *Ann Trop Med Public Health* 2021; 24(2): 242-253.  
<https://doi.org/10.36295/ASRO.2021.24244>
7. Cesar A S, Renato A S, Cristian A P, David R C, Diego P O, Maria I T. Sensitive kinetic spectrophotometric method for the determination of diphenhydramine in real samples. *J Chil Chem Soc*. 2019; 64(3): 4507-4512.  
<https://doi.org/10.4067/S0717-97072019000304507>
8. Nandeesh I M, Basppa C Y, Manjunatha D H. Liquid chromatographic method development and validation for the simultaneous determination of phenylephrine hydrochloride, paracetamol, caffeine and diphenhydramine hydrochloride in pure and formulations. *Research J Pharm Tech* 2022; 15(9): 3993-3998. <https://doi.org/10.52711/0974-360X.2022.00669>
9. Hina J, Syed Nisar H S, Nida J. Development and validation of HPLC method for diphenhydramine hydrochloride. *RADS J Pharm Pharm Sci*. 2020; 8(3): 154-160.  
<https://doi.org/10.37962/jpps.v8i3.390>
10. AL-Salman H N K, Erfan A S, Rajaa H F, Hussein H H, Ekhlas Q J. Development of the stable, reliable, fast and simple RP-HPLC analytical method for quantifying diphenhydramine –HCl(DPH) in pharmaceuticals. *Int J Pharm Res*. 2020; 12(4): 4457-4467.  
<https://doi.org/10.31838/ijpr/2020.12.04.608>
11. Revati S, Leena T, Vandana J. Novel rapid isocratic RP-HPLC method for simultaneous estimation of phenylephrine hydrochloride, paracetamol, caffeine, diphenhydramine hydrochloride. *Curr Pharm Anal* 2021; 17(6): 792-800.  
<https://doi.org/10.2174/1573412916999200430091855>
12. Chunling Y, Yuhai T, Xiaonian H. Flow injection chemiluminescence analysis of diphenhydramine hydrochloride and chlorpheniramine maleate. *Instrum Sci Technol* 2006; 34: 529-536.  
<https://doi.org/10.1080/10739140600809421>
13. Changzhi Z, Xiaoli C, Shengchen T, Minghua L, Kui J. Selective determination of diphenhydramine in compound pharmaceutical containing ephedrine by flow injection electrochemiluminescence . *Anal Sci*. 2008; 24: 535-538.  
<https://doi.org/10.2116/analsci.24.535>
14. Eman Y Z, Gehad G M, Wael G E. Potentiometric determination of antihistaminic diphenhydramine hydrochloride in pharmaceutical preparations and biological fluids using screen-printed electrode. *Bioelectrochemistry* 2011; 82(2): 79-86.  
<https://doi.org/10.1016/j.bioelechem.2011.05.006>
15. Issam M A Shakir, Bashaer A K. A novel online coupling of ion selective electrode with the flow injection system for the determination of vitamin B1.

- Baghdad Sci J. 2016; 13(2): 458-469.  
<https://doi.org/10.21123/bsj.2016.13.2.2NCC.0854>
16. Jasim M S Jamur, Sumayha M A, Zahra M A. Analytical Methods for Determination of Ketoprofen Drug: A review Ibn Al-Haitham J Pure Appl Sci. 2022; 35(3): 76-82.  
<https://doi.org/10.30526/35.3.2842>
  17. Jalal N Jeber, Nagham S Turkey An optoelectronic flow-through detectors for active ingredients determination in the pharmaceutical formulations. J Pharm Biomed Anal 2021; 201: 1-11.  
<https://doi.org/10.1016/j.jpba.2021.114128>
  18. Ghadah F H, Nagham S T. A new continuous flow injection analysis method with NAG-ADF-300-2 Analyzer for promethazine-HCl by cadmium iodide as a precipitating reagent. Chem Methodol. 2021; 5: 498-512.  
<https://doi.org/10.22034/chemm.2021.138807>
  19. Sarah F H, Nagham S T. Determination of Ferrous Ion in Pure & Pharmaceutical Preparation by Continuous Flow Injection Analysis Via Turbidimetric Utilizing NAG-4SX3-3D Analyzer. Egypt J Chem. 2022; 65(13): 871 - 886.  
<https://doi.org/10.21608/EJCHEM.2022.131307.5788>
  20. Zahraa D, Nagham S T. New Turbidimetric Method for Determination of Cefotaxime Sodium in Pharmaceutical Drugs Using Continuous Flow Injection Manifold Design with CFTS- Vanadium Oxide Sulfate System. Chem Methodol. 2022; 6: 91-102. <https://doi.org/10.22034/chemm.2022.2.2>
  21. Nagam S T, Elham N M. Assessment of long distance chasing photometer (NAG-ADF-300-2) by estimating the drug atenolol with ammonium molybdate via continuous flow injection analysis. Baghdad Sci J. 2020; 17(1): 78-92.  
<https://doi.org/10.21123/bsj.2020.17.1.0078>
  22. Elham N M, Nagam S T. Assessment of long distance chasing photometer (NAG-ADF-300-2) by estimating the drug Atenolol with povidone iodine via CFIA. Ibn Al-Haitham J Pure Appl Sci. 2020; 33(1): 65-83. <https://doi.org/10.30526/33.1.2383>
  23. Zahraa N A, Nagam S T. New turbidimetric method for determination of ciprofloxacin hydrochloride in pharmaceutical drugs using continuous flow injection manifold design with CIP.HCL-sodium nitro prusside system. Eurasian Chem Commun. 2021; 3: 743-754.  
<https://doi.org/10.22034/ecc.2021.300288.1222>
  24. Nagam Shakir Turkie, Hussein Fares Abd-Alrazack Newly developed method for determination Indomethacin using Phosphotungstic acid by continue flow injection analysis via homemade ISNAG-fluorimeter. IOSR-J Appl Chem. 2018; 11(2): 25-39. <https://doi.org/10.9790/5736-1102012539>
  25. Hashim J A, Bushra B Q. Development and validation CFIA/MZ system as a green method for determination of thiol drug(D-PEN). Egypt J Chem. 2022; 65(1): 259-270.  
<https://doi.org/10.21608/ejchem.2021.80167.3964>
  26. Nagham S T, Jalal N J. Flow injection analysis with turbidity detection for the quantitative determination of mebeverine hydrochloride in pharmaceutical formulations. Baghdad Sci J. 2022; 19(1): 141-154. <https://doi.org/10.21123/bsj.2022.19.1.0141>
  27. Nagam S T, Rana A K. CFIA-Turbidimetric and Photometric Determination of Vitamin B9 (Folic acid) Using LEDs as a Source of Irradiation and Two Solar Cells as an Energy Transducer. Baghdad Sci J. 2017; 14(4): 773-786.  
<https://doi.org/10.21123/bsj.2017.14.4.0773>
  28. Read F H. New mode semi-automated turbidimetric determination of mefenamic acid by Ayah 6SX1-ST-2D solar cell-CFI analyser. Res J Pharm Technol 2019; 12(12): 5773-5780.  
<https://doi.org/10.5958/0974-360X.2019.00999.5>
  29. Miler J C, Miler J N. Statistics for analytical chemistry .6<sup>th</sup> ed. St. John Wiley and N.Y.Sons 2010, p: 145

## طريقة تحليل بالحقن الجرياني المستمر لتقدير عقار داي فينهيدرامين هيدروكلوريد باستخدام حامض الفوسفوموليبيدك

منتظر محمد جبار، الهام نعيمش مزعل

قسم الكيمياء، كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد، بغداد، العراق.

### الخلاصة

قدمت الدراسة طريقة جديدة لتقدير ديفينهيدرامين هيدروكلوريد (DPH) في شكله النقي وبعض المستحضرات الصيدلانية، ضمن تقنية CFIA. الطريقة بسيطة وسريعة وحساسة وسهلة التشغيل ومنخفضة التكلفة. يعتمد على تفاعل DPH مع حمض الفوسفوموليبيدك (PMA)، في وسط مائي، مكوناً راسباً أبيض مائل إلى الصفرة قليلاً. تمت دراسة المادة المترسبة المتكونة باستخدام محلل الخلايا الشمسية Ayah 6S × 1-ST-2D CFI analyzer، عن طريق انعكاس الضوء الساقط عن أسطح الجسيمات المترسبة عند (0-180 درجة)، معيراً عنها بالاستجابة المقاسة بـ (الملي فولت). تمت دراسة بعض المتغيرات الكيميائية والفيزيائية لتوفير الظروف المثلى للدراسة. المدى الخطي كان (0.07-9) مليمول / لتر وكان له معامل ارتباط (r) بقيمة (0.9998). كان حد الكشف (L.O.D.) للطريقة الجديدة (729.55 نانوغرام / عينة)، محسوباً بالتخفيف التدريجي للحد الأدنى للتركيز في النطاق الخطي (0.07 مليمول / لتر). كان % RSD أقل من 0.2% لتركيز (0.1، 4.0 و 10.0) مليمول / لتر من DPH لـ n = 8. تم تطبيق الطريقة بنجاح في تحديد DPH في ثلاث عينات من ثلاث شركات إنتاج صيدلانية مختلفة. باستخدام طريقة الإضافة القياسية، تمت مقارنة الطريقة الجديدة مع طريقة القياس الطيفي للأشعة المرئية وفوق البنفسجية عند  $\lambda_{max} = 258$  نانومتر. لكل من اختبار t و F. أظهرت نتائج الاختبارين عدم وجود فرق معنوي عند مستوى الثقة (95%).

**الكلمات المفتاحية:** حساسية، مضادات الهستامين، داي فينهيدرامين هيدروكلوريد، التحليل بالحقن الجرياني، معقد الشحنة المزدوجة.