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A New Approach for Developing Spectrophotometric Determination of Phenylephrine Drug in Pure, Pharmaceutics and Serum Samples Using Sodium Periodate as Oxidizing Agent via a Green Method of CFIA/Merging Zone Technique

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Abstract:

The research involved a rapid, automated and highly accurate developed CFIA/MZ technique for estimation of phenylephrine hydrochloride (PHE) in pure, dosage forms and biological sample. This method is based on oxidative coupling reaction of 2,4-dinitrophenylhydrazine (DNPH) with PHE in existence of sodium periodate as oxidizing agent in alkaline medium to form a red colored product at λ_{max}(520 nm) . A flow rate of 4.3 mL.min^{-1} using distilled water as a carrier, the method of FIA proved to be as a sensitive and economic analytical tool for estimation of PHE.

Within the concentration range of 5-300 µg.mL^{-1}, a calibration curve was rectilinear, where the detection limit was 3.252 µg.mL^{-1}, and the RSD% was 4.09. Recovery was 100.55% with a sample throughput of 62 sample/hr. The proposed method was successfully applied for determining PHE in serum samples and pharmaceutical formulations, the interference of excipients was studied. The mechanism of the chemical reaction has been proposed. When the suggested method's results were compared to those provided by a British Pharmacopeia reference method, there was no discernible difference between the two at the 95 percent confidence level.

Keywords: CFIA/MZ technique, DNPH, Pharmaceutics, Phenylephrine. HCl, Serum samples.

Introduction:

Phenylephrine hydrochloride $C_9H_{14}ClNO_2$ (PHE.HCl) is (1R)-1-(3-Hydroxyphenyl)-2-(methyl amino) ethanol hydrochloride, as one can see in Fig.1, is a white crystalline powder, belongs to phenethylamine class, with direct action at α-adrenergic receptor agonists [1]. It is used topically as a decongestant and given orally, as drops or spray for the nose, in many cases including, for example: allergies, colds, flu, sinusitis, and nasal tumors benign [2, 3]. There are different analytical techniques for determination of PHE in bulk form and dosage forms including, ion pair chromatography [4], electrochemical [5], titrimetry [6], capillary zone electrophoresis [7], high-performance liquid chromatography [8-9], fluorescence[10], voltammetry[11], and spectrophotometry [12–14]. Spectrophotometry is paired with Flow Injection Analysis (FIA) and utilized as detectors in FIA system to detect certain samples and elements [15]. The proposed method of flow-injection analysis (FIA) is a technique that has high throughput sample performance with short time for analysis, economical, user-friendliness, green chemistry, accurate with remarkable reproducibility of the outcomes found, does not need further treatment for the samples as well as does not require an expensive or toxic reagent. PHE may be detected in many samples using inexpensive, automated, and user-friendly analytical techniques [16-18]. A green approach of CFIA /MZ technique for determining of PHE in pure, pharmaceutical formulations and biological samples is described in this manuscript based on oxidative coupling reaction with DNPH as organic reagent using $NaIO_4$ as oxidizing agent, colored product is measured at λ_{max} (520nm) .

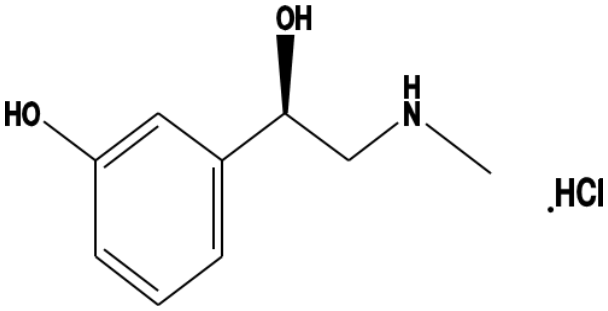


Figure 1. Structural formula of phenylephrine hydrochloride molecule.

Experimental

Material and Reagents

All of the chemicals and reagents used were of analytical grade and they have been used to prepare all of the solutions.

- A stock solution of pure material of PHE.HCl 1000 µg. mL^{-1} kindly provided from State Company for Drug Industries and Medical Appliance, SDI, Samara, Iraq. It was prepared by dissolving 0.1gm in 100 mL with distilled water in a standard flask. The diluted solutions were made by diluting the stock standard solution with distilled water to the desired concentration.
- A2,4-dinitrophenylhydrazine solution (DNPH), 1×10^{-2} M, M. wt =198.14 $g.mol^{-1}$ (BDH), was prepared by dissolving 0.19 gm in 1 mL concentrated H_2SO_4, and stirred, then the compound was completed to 100 mL with distilled water in a standard flask and further dilution was made to these solutions to obtain desired concentrations.
- An oxidizing agent ($NaIO_4$) solution 9.3×10^{-3} M, M. wt =214 $g.mol^{-1}$ (BDH), was prepared by dissolving 0.19 gm in 100 mL distilled water in a standard flask, more dilute solutions were prepared by dilutions with distilled water.
- Sodium hydroxide (BDH) 1M solution was prepared by dissolving 4 gm in 100 mL distilled water. More dilute solutions were prepared by dilutions with distilled water.

The Developed CFIA system

All absorbance in the batch procedure was measured by a Shimadzu UV- 1800, (Japan) UV-Visible Spectrophotometer doubled ray and quartz cuvette with an optical longitude of 1 cm. The suggested FI manifold was developed as a simple type with one channel numerous in the FIA/merging zones system proposed approach[19], as shown in Fig.2. The carrier stream (distilled water) was pumped through the injection valve (six three-way injection valve, handmade) by peristaltic pump (Master flex C/L, two channel, USA), which travels at 90° and three Teflon loops (I.d =0.5 mm) into which the sample (L_1), the reagent (L_2), and the oxidizing agent and basic medium (L_3) were loaded. The reaction coil is made of glass, and it is used to mix the ingredients (2 mm, I.D.). All absorbance and spectrum measurements were performed during the FIA processes using the modified Optima photometer 301-D+ (VIS-Spectro one beam) (Japan). The responses were expressed as peak height mV (n=3) and then were measured using a Kompensograph C1032 (Siemens) or a Chinese optical multimeter (DT9205A, OVA) for measuring absorbance. A flow cell quartz silica (QS, 1 cm) with an inner volume of 80 µL was placed inside in modified detection unit.

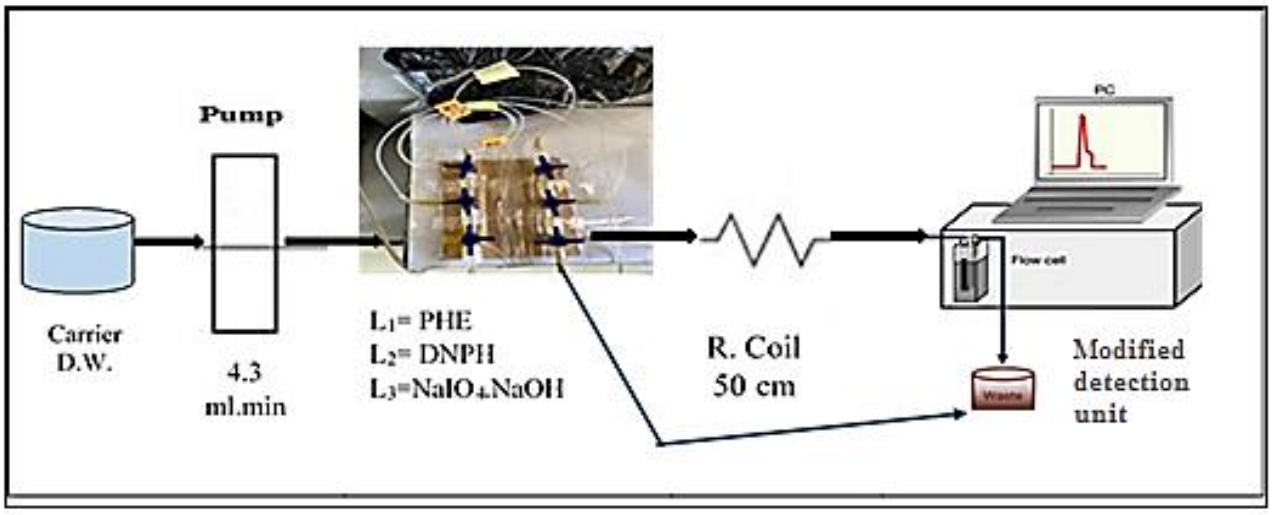


Figure 2. The developed of FI system for determination of PHE.HCl in dosage forms and biological samples.

Preparation of Pharmaceutical Samples

Four pharmaceutical preparations were taken in the form of (syrup, tablet). **1**/Tussilet (syrup) 25 µg.mL^{-1} , which contains 2.5mg of PHE, made by (General Company for the Manufacture of Medicines and Medical Supplies / Samarra – Iraq). **2**/Astulet (syrup) 25 µg. mL^{-1}, which contains 2.5 mg of PHE, manufactured by: Aswar AL-khaleej company Samarra –Iraq (SDI). **3**/ Tussiram (syrup) 50 µg.mL^{-1},which contains 5mg of PHE, General Company for the Manufacture of Medicines and Medical Supplies / Samarra – Iraq. A stock solution (25,50 µg. mL^{-1}) is generated by drawing up a certain volume (5,10 mL) from syrup solution that has been made up to 100 mL with distilled water. Pharmaceutical tablet solution was produced by weighing 20 tablets of drug, then crushed via motor. **4**/Nogrippin plus (pills) 100 µg. mL^{-1} (0.01 gm), that contains 10 mg of PHE, manufacturing: Biofarma ilac San. ve Tic. A.S. Sancaktepe / ISTANBUL. It was dissolved in 100 mL distilled water with whisking and then purified. The stock solution (50µg.mL^{-1}) was made in 100 mL volumetric vessel from the stock solution of dosage form and complemented to the marked with D.W.

Serum Samples Preparation

Serum samples were obtained from healthy volunteers and centrifuged at 3000 rpm for 15 minutes to separate the serum from the blood. The serum was then diluted with 1 ml of distilled water and acidified with 1 ml of HNO_3 (1M) to precipitate the proteins. For serum sample preparation, 10 µg. mL^{-1} was tested for accuracy and precision and analyzed thrice [20].

Result and Discussion:

Batch Method

Spectrophotometric determination of PHE based on oxidation of 1 ml 2,4-dinitrophenylhydrazine (1 ×10^{-2}) M with drug 1ml from 100 µg. mL^{-1} in presence of sodium periodate (1ml, 9.3×10^{-3} M) and 1mL NaOH (1N), were added in 10ml volumetric flask to form colored product (red), measured at λmax520 nm as seen in Fig.3. and Scheme 1.

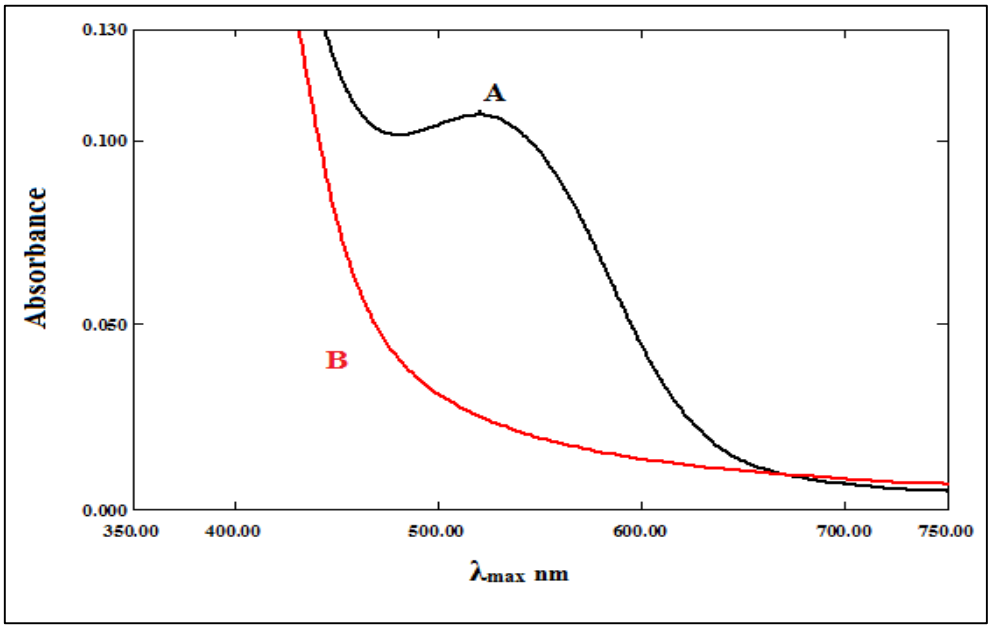
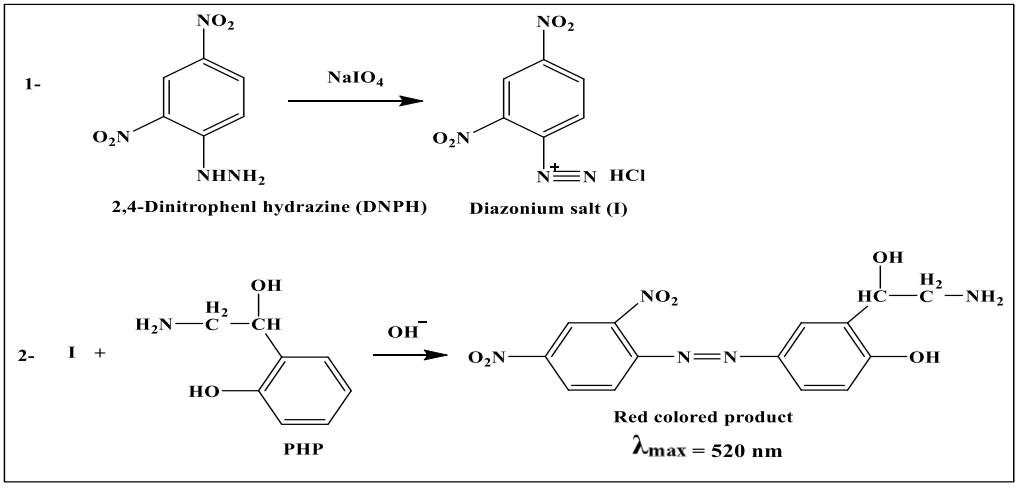


Figure 3. The Absorption spectrum of: A\ red color product against blank solution (10 µg. mL^{-1}) B\ blank solution against distilled water.



Scheme 1. Suggest mechanism of the reaction between PHE.HCl with DNPH.

The complexation ratio of reaction between drug with reagent at consistent sodium periodate and sodium hydroxide concentrations, using two ways was preceded by mole ratio and Job's method, it is clear that the PHE.HCl-DNPH complex link in is 1:1 ratio so the proposed mechanism below is likely to propose as be seen in Scheme1 and Fig.4.

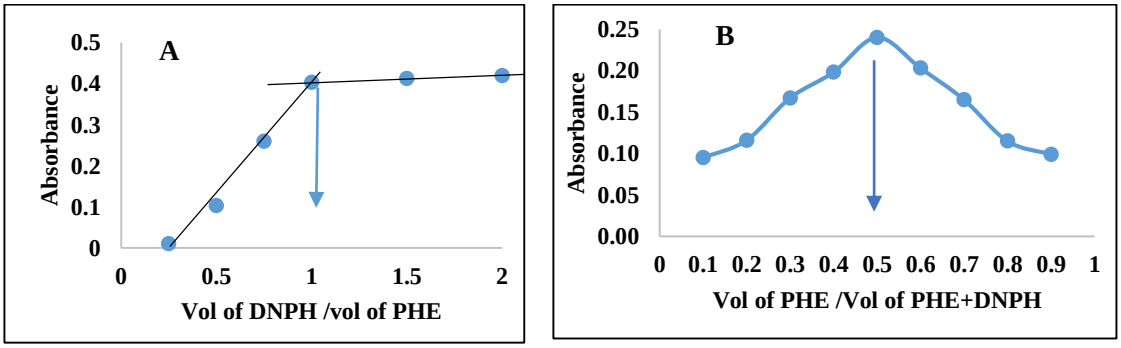


Figure 4. The complexation ratio between drug with reagent, A\ mole ratio for the complex, B\ job's method for the complex

Preliminary Investigation

The effects produced on the absorbance strength of the colored product were observed as each condition was investigated by changing one variable while holding the other constant. The effect of DNPH volume was examined with 10 µg. mL^{-1} PHE. The volume was monitored that gave the highest absorbance was 2 mL of 1×10^{-2} M DNPH and this volume was chosen for later adventures. The oxidative agent was examined and the best volume that gives the highest absorbance was 2 mL of 9.3×10^{-3} M $NaIO_4$, as shown in Fig.5A-B. The influence of volume and various alkaline solutions type, including KOH, $Na_2Cr_2O_7$, NH_4OH, and NaOH, were tested because the colorful dye product could only be produced in an alkaline environment, when the reaction was conducted in the existence of sodium hydroxide solution did maximum sensitivity and stability occur, as be seen in Fig.6A-B.

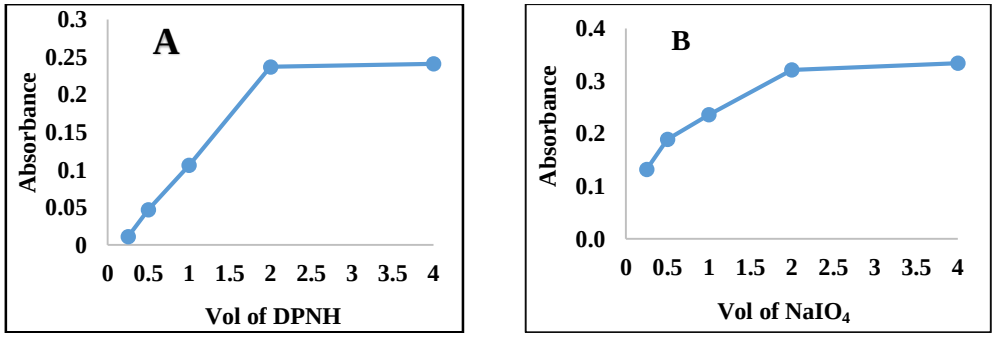


Figure 5. Chemical parameter for batch A/ volume of DNPH, B/volume of. $NaIO_4$.

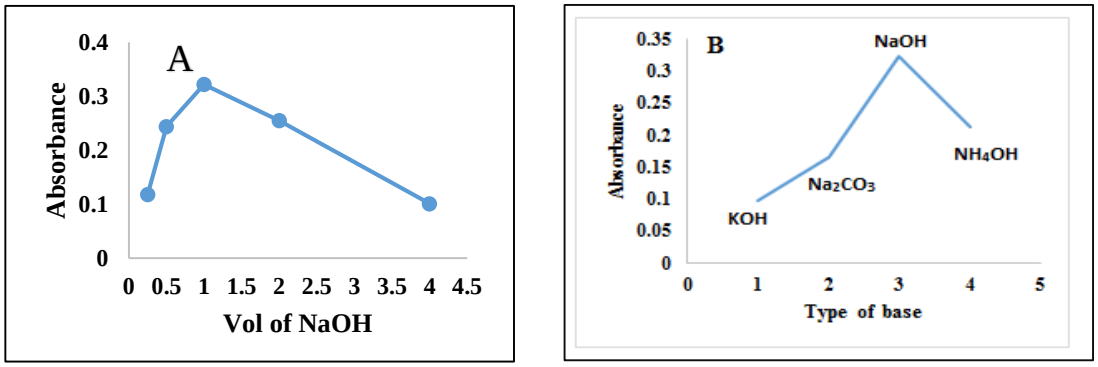


Figure 6. Chemical parameter for batch A/ volume of $NaIO_4$, B/ Type of base.

Calibration Curve of Classical Method

In order to estimate PHE, a standard curve with a linear range of 2–50 µg.mL^{-1} was created, as seen in Fig.7. Based on the ideal conditions outlined in the established method, these measurements were made using two different PHE levels for precision and accuracy. The results, which are shown in Table 1, demonstrate that the suggested method does have good precision and accuracy (five times determination).

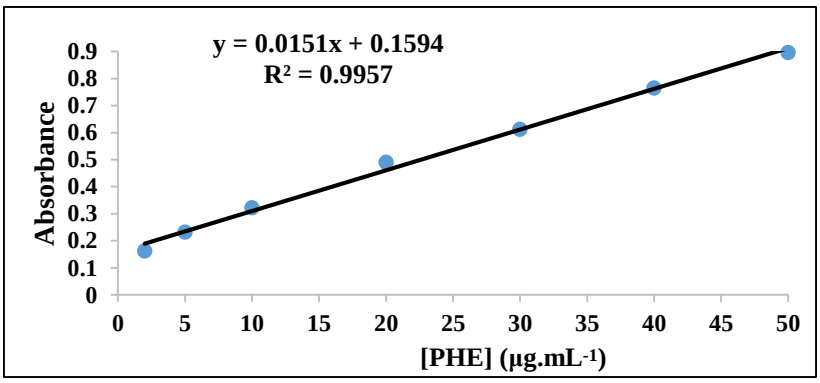


Figure 7. Linear calibration curve for the batch method of PHE drug determination.

Table 1. Precision and accuracy of the batch process.

PHE (µg. mL^{-1})		Error	Rec%	Erel%	RSD%
Present µ	***Found x̄**				
10	10.20	0.201	102.01	2.013	2.623
40	40.29	0.291	100.73	0.728	1.413

*Rec%, Mean of recovery%
*Erel%, Average of Relative Error %
*RSD%, Average of Relative standard deviation%

Calculations of Stability Constant

Two groups of solutions were used to determine an observed stability constant [21, 22] for the suggested interaction (PHE: DNPH) the first group contained a stoichiometric quantity of PHE to DNPH (As), while the second contained a two-fold excess of DNPH (Am). The proposed mechanism and the drug-to-reagent stoichiometry ratio 1:1. According to the following Eq. 1, the interaction between PHE and DNPH takes place:

$$\underset{\alpha C}{D} + \underset{\alpha C}{R} \rightleftharpoons \underset{(1-\alpha)C}{DR} \qquad K = \frac{[DR]}{[D][R]} \qquad K = \frac{(1-\alpha)}{\alpha\, C} \qquad \alpha = \frac{Am - As}{Am}$$

----- 1

While K is the stability constant, C is the product's molar concentration (M), which is the same as the concentration of PHE (1×10^{-2} M), (α) is the degree of dissociation. The absorbance readings of the aqueous solution, which contains an adequate and equivalent amount of reagent, are represented by Am and As. as be seen in Table 2.

Table 2. Stability constants of the reaction and Gibbs free energy of the reaction.

	Am	As	α	K (L.mol^{-1})	ΔG (J.mol^{-1})
1	0.413	0.403	0.024	1664390	-48693
2	0.419	0.408	0.026	1412826	-48286
Average				1538608	-48490

The spontaneous of complex formation reaction (ΔG value) was obtained based on K evaluation by using the Eq. 2.

$$\Delta G = -RTLnK \quad \text{---- 2}$$

(CFIA/ MZ) Spectrophotometric determination

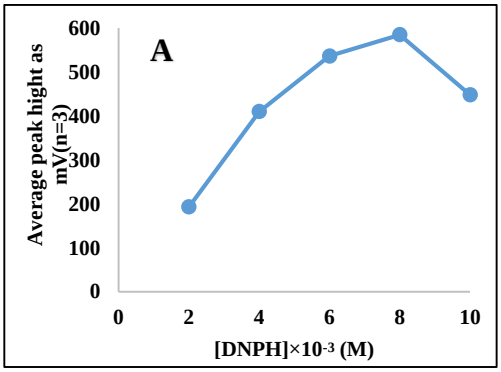
After determining the ideal cases for the reaction of PHE with DNPH using the traditional spectrophotometric method, the flow injection-merging zones methodology was utilized to automate the spectrophotometric reaction in order to analyze the best practical settings and obtain automated spectra to estimate PHE drug. As a result, flow injection analysis technique was developed and employed for determining of PHE.HCl in pure, dosage forms and serum samples

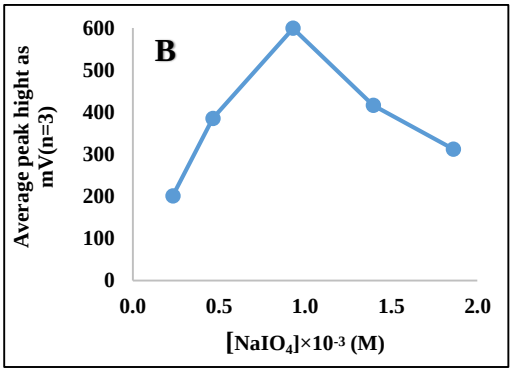
Manifold of the Proposed FI System

The optimal design for a Homemade FIA system was investigated once the system and its linked components were installed, Fig. 2. depicts the developed system which consists of three loops (varying loop lengths with 0.5mm I.D.) that are filled with drug, reagent, oxidizing agent, and base using one line as carrier (distilled water) to a homemade injection valve according to merge materials and chemical reagents and in sequence PHE in L1, DNPH in L2 and $NaIO_4$; NaOH in L3.

Chemical Parameters

- By administering several concentrations 2×10^{-3}-1×10^{-2} M, the ideal concentration of the reagent DNPH was examined. Fig. 8-A illustrates the optimum amount of absorbance represented as peak height in mV (n = 3) at the concentration of 8×10^{-3} M, which also exhibited great repeatability.
- In order to determine the optimal concentration of the oxidized agent $NaIO_4$ several concentrations 2×10^{-4}-1.9×10^{-3} M were injected into a homemade injection valve. Fig. 8-B, demonstrates that, with good repeatability, the concentration of 9×10^{-4} M created the highest absorbance value possible, which is shown as the peak height in mV (n=3).
- Fig. 8-C shows the best concentration of the base, where several concentrations were taken 3×10^{-2}-2×10^{-1} M, and it was found that the best concentration 5×10^{-2} M gave the highest absorbance represented by the height of the peak in mV (n=3).
- The types of base were studied and the best base type was found is sodium hydroxide, which provided the greatest absorption, as be seen in Fig. 8-D.
- The results in Fig. 8-E indicated that the best sequence is (D in L1 + R in L2 + O, B in L3) where D is PHE, R is DNPH, O is $NaIO_4$ and B is NaOH.





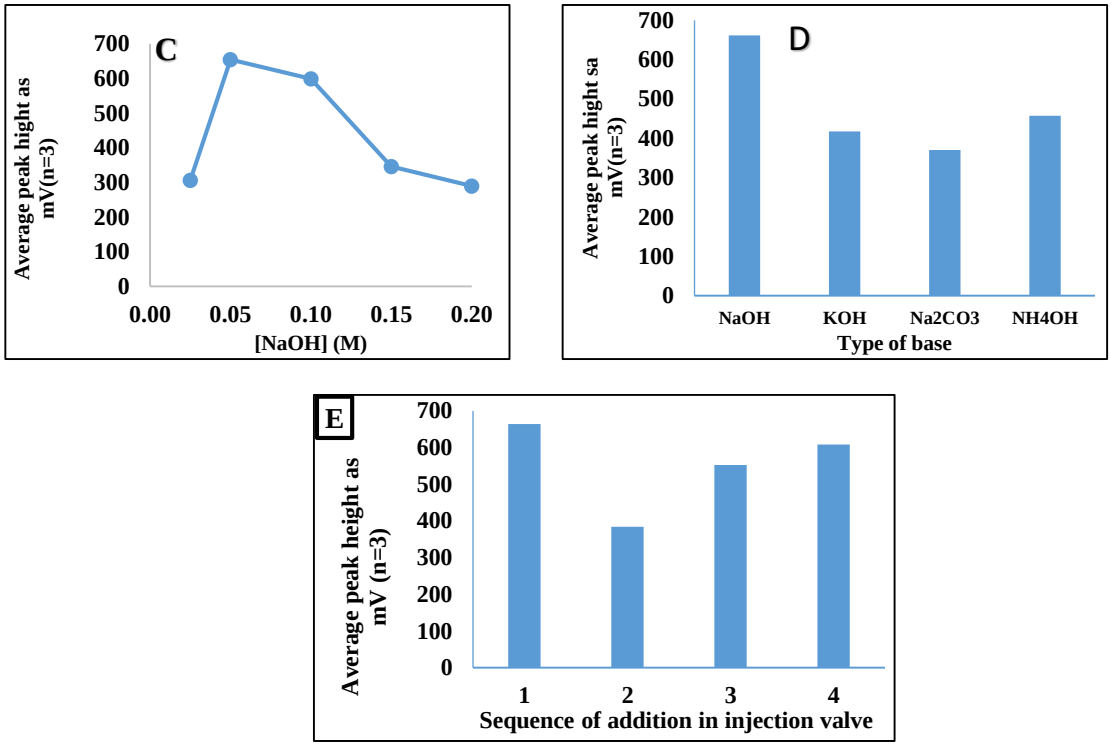


Figure 8. Effect of A\ DNPH concentration, B\ $NaIO_4$ concentration, C\ NaOH concentration, D\ Type of base, E\ sequence of chemicals.

Physical Variables

For the reaction, the best loop volumes for the drug, reagent, oxidizing agent and base were (117.7-78.5-117.7) µL equal to 60-40-60 cm as shown in Fig. 9-A, the best reaction coil extent was 50 cm as appears in Fig. 9-B. The flow rates of available system were all examined, and the results indicated that 4.3 $mL.min^{-1}$, with an average sample throughput of 62, was the optimal flow rate hour $^{-1}$, as displayed in Fig.9-C. The time it took to inject the solutions into each of the six-three-way valve loops 15 sec and the time it took for the highest peak height to appear 43 sec were used to compute the sampling rate, which came to 62 samples $hour^{-1}$.

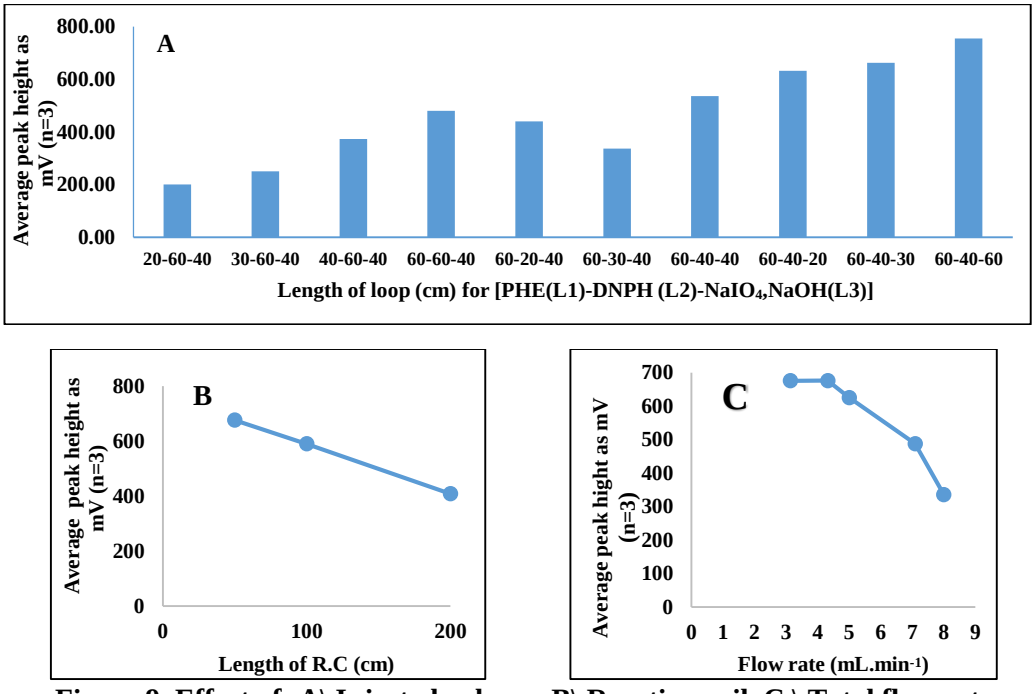


Figure 9. Effect of: A\ Injected volume, B\ Reaction coil, C \ Total flow rate. Purge Time

The purge time was calculated using optimal chemical and physical conditions, where different times were chosen, such as 5-10-15-20-25 seconds and an open valve [23]. By adding the sample segment, the purge time was estimated. Fig.10 shows that the open valve was chosen as the best purge time for completing sample movement between the flow cell and the sample loop with minimal dispersion.

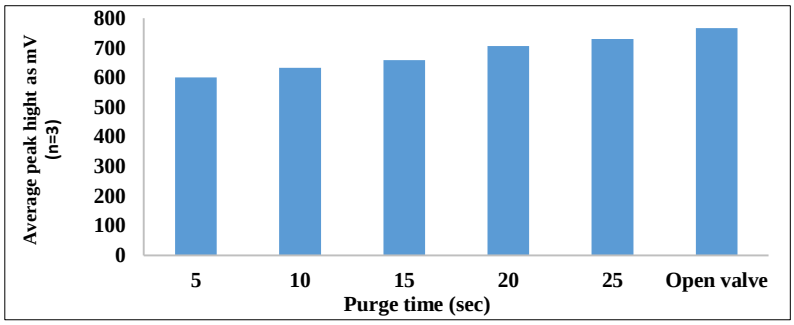


Figure 10. Effect of purge time.

Dispersion of Sample Zone

In the FIA method, the sample interacts with several solutions and becomes scattered throughout the solution, which is a physical phenomenon known as dispersion. The success of the FIA analytical technique is founded on three ideas [24]. (Control of sample zone dispersion, reproducible injection time, and repeatable injection volume) are displayed in Fig.11 and Table 3. The reaction's dispersion was 1.2 according to the formula D = Co/C, the dispersion was computed reaching the top when performing contact outside of the flow injection system, the peak without dilution is Co, but the peak after dilution is C. (interactivity inside the flow injection system). To get a fixed response stated as a percentage in the first experiment, all of the components were combined in the appropriate beaker, and the final solution was then administered by the flow injection mechanism (as carrier stream) (Co). The second experiment involved the injection of PHE into L1, DNPH into L2, and $NaIO_4$, NaOH into L3. Distilled water is employed in the system as a carrier, and the injected component forces the ingredients into the reaction coil before pushing them toward the detector, creating a response symbolized by C.

Table 3. Dispersion value of PHE drug using the suggested FI system.

PHE µg.ml^{-1}	Conc.	C_o (cm)	C (cm)	D
50		8.1	6.5	1.246
100		11.6	9.3	1.247

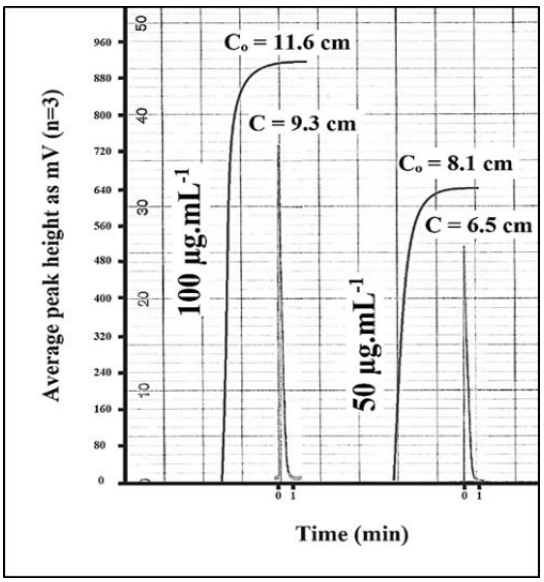


Figure 11. Dispersion of PHE in the developed CFIA system

Calibration Curve

A series of PHE concentrations from 1-300 µg.mL^{-1} were made by diluting stock solution (1000)µg.mL^{-1} and injecting it into the FI system with DNPH, $NaIO_4$ and NaOH in order to determine the appropriate range of PHE concentration. It shows that the concentration range extends 5-300 µg.mL^{-1}, as shown in Fig.12 and Table 4.

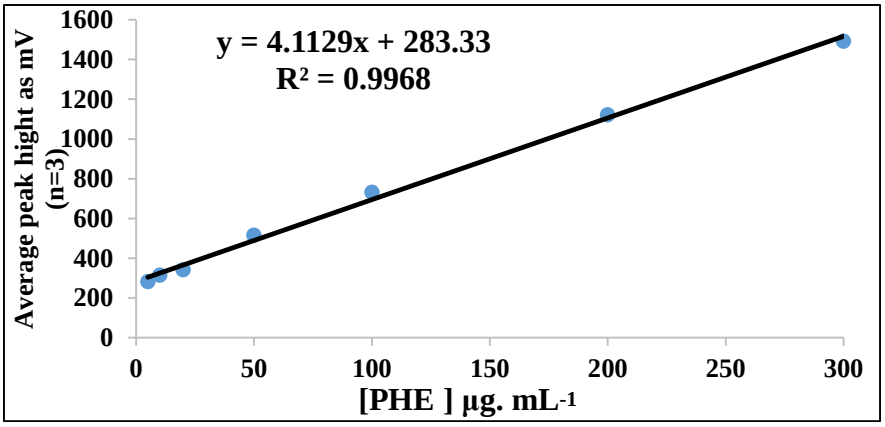


Figure 12. Linear dynamic range for spectrophotometric determination of PHE using the developed CFIA system

Table 4. Calibration table as S.E.M for PHE-DNPH-$NaIO_4$, NaOH system.

conc. of PHE (µg.mL^{-1})	Average response ($\bar{y}$) (mV)	RSD %	S.E.M	*E/y%
5	283	1.61	283 ± 11.3	3.99
10	315	1.47	315± 11.5	3.64
20	342	1.42	342± 12.1	3.54
50	516	1.34	516± 17.2	3.33
100	731	1.61	731± 29.3	4.00
200	1122	0.37	1122± 10.3	0.92
300	1492	1.30	1492± 48.3	3.24

$$*\frac{E}{y}\% = t_{tab}\frac{SD}{\sqrt{n}} \times \frac{100\%}{\bar{y}}$$

Analysis of Variation and Repeatability

The assumed error was calculated, called-for regression, and the sum of the squares of the difference between the response's (yi) and the appraiser's ŷi values to obtain $(yi - \hat{y}i)^2$ for (n-2) degrees of freedom to yield the sum of squares $(S_2)^2$. The sum of squares of the variance was calculated of values ŷi from the average value ȳ (due to regression), and for 1 of degrees of freedom, get sum of squares $(S_1)^2$, then divide by $(S_2)^2$, to get value (F) [25, 26], as shown in Table 5.

Table 5. ANOVA for the developed FIA technique.

Source of Variation	Sum. of Squares (SS)	Df	Mean of Squares (MS)	F ($\frac{s_1^2}{s_2^2}$)	F crit
Between Groups (Error) Σ(ȳi-ŷi)2	1646157.6	1	1646157.63	15.2724	4.7472
Within Groups (Regression) **Σ(ŷi − ȳi)2**	1293435.1	12	107786.2574		
Total	2939592.7	13			

The repeatability of the proposed method was acceptable as shown in Table 6.

Table 6. Repeatability of consecutive measurement of PHE (n=7).

Con. of PHE(µg.mL^{-1})	Found	Error	Rec%	Erel%	RSD%
50	50.22	0.224	100.449	0.449	4.430
100	100.66	0.655	100.655	0.655	3.740

Methods Validation

The analytical characteristics of each approach, including the detection limit, correlation coefficient (r), relative standard deviation, and linear range, were determined at the optimal condition [27, 28] as shown in Table 7. Numerous PHE standard solutions were used to construct calibration curves, and the technique's main analytical figure of deserts was used. Fig.12. A, statistical treatment of the regression line provided the standard deviation for the residuals (Sy/x), slope (Sb), and intercept (Sa) within 95% confidence intervals for (n-2) degrees of freedom. The proposed CFIA technique superior reproducibility in comparison with batch method was demonstrated to the small subjects because of the current study was completed quickly with high sensitivity (62 samples were analyzed in one hour), as well as simple and easier.

Table 7. Analytical characteristic of calibration curve for the reaction between PHE and DNPH using $NaIO_4$ as oxidizing agent.

Parameters	FIA method	Batch method
λmax (nm)	520	520
Regression equation; y = bx+ a	y = 4.1129x + 283.33	y = 0.0151x + 0.1594
Linear range (µg mL^{-1})	5- 300 ppm	2 - 50 ppm
Mean of recovery (Rec%)	100.55	101.37
Average of Relative Error % (Erel %)	0.55	1.37
Average of Relative standard deviation (RSD %)	4.09	2.02
Slope (b); (mL. μg^{-1}) $\boldsymbol{b} = \frac{\sum_i[(xi-\bar{x})(yi-\bar{y})}{\sum_i(xi-\bar{x})^2}$	4.11	0.02
Intercept (a); (a = y– b x)	283.33	0.16
Linearity R^2	0.997	0.9957
Correlation coefficient (r): $r=\frac{\sum_i[(xi-\bar{x})(yi-\bar{y})]}{\sqrt{(\sum_i(xi-\bar{x})^2)\,(\sum_i(yi-\bar{y})^2}}$	0.998	0.9978
Standard deviation of slope (Sb) $Sb=\frac{S_{\frac{y}{x}}}{\sqrt{\sum_i(xi-\bar{x})^2}}$	0.103	0.0004
Standard deviation of intercept (Sa) $Sa=S_{\frac{y}{x}}\sqrt{n\sum_i(xi-\bar{x})^2}$	14.745	0.012
Limit of detection (LOD) = (3.3 SD)/b	3.252	0.637
Limit of quantification (LOQ)= (10 SD)/b	10.841	2.124
Molar absorptivity Ɛ (L/mol.cm) Ɛ = b×M.Wt×1000	-----	3067.725
Sandel's sensitivity (S) (µg.cm^{-2}) $S=\frac{M.Wt}{\varepsilon}$	-----	0.066
Sample through put (h^{-1})	62	4
Standard deviation of the residuals; $S_{\frac{y}{x}}=\sqrt{\frac{\sum_i(yi-\hat{y}_i)^2}{n-2}}$ $\hat{y}_i = bxi + a$	28.4365	0.0198
Confidence limit of slope (b) $CL_b = b \pm t \times Sb$	4.113± 0.2527	------
Confidence limit of intercept (a) $CL_a = a \pm t \times Sa$	283.3 ± 36.1254	-------

Study of interferences

CFIA/MZ approach was investigated and by using this method, the effects of (cellulose, glucose, sucrose, lactose and sodium citrate) on the accuracy of PHE determination were evaluated. A pure 20 µg.mL^{-1} PHE sample was spiked with a half, equal, and double excess of a number of interference excipients before analysis was performed on it. The outcomes represented in Table 8, showed that there were no interferences during the determination of PHE utilizing the new CFIA system, as indicated by the acceptable recovery values 95-105%.

Table 8. Interferences effect on the reaction using the developed FI system between PHE with DNPH.

Interference	Interferences (µg mL^{-1})	Average response ($\bar{y}$) (mV)	*Erel%	*Rec%
Standard	20	365	-0.31	99.69
Sucrose	10	366	0.41	100.41
	20	365	-0.46	99.54
	40	364	-1.87	98.13
Cellulose	10	367	2.25	102.25
	20	368	2.40	102.40
	40	364	-2.50	97.50
Lactose	10	369	4.07	104.07
	20	362	-4.62	95.38
	40	370	4.86	104.86
Glucose	10	362	-3.88	96.12
	20	368	2.54	102.54
	40	365	-0.70	99.30
Sodium citrate	10	364	-2.11	97.89
	20	363	-2.71	97.29
	40	367	1.86	101.86

*Average three determination

Applications and Assessment of Suggested Method

The suggested methodology has been applied to the examination of a number of PHE-containing pharmaceutical products that have separate sources and follow the traditional adding process. The calculated F-test values were 0.8661 and 1.9192, and the t-test values were 0.4229 and 0.8026 less than the theoretical F-test (9.28) and t-test (2.45) via CFIA/MZ technique as shown in Table 9. According to a statistical comparison [29] between the proposed technique with official method (British pharmacopeia) [1].A spiked human serum sample's PHE can be calculated with success using the FIA method. A 10 µg. mL^{-1} PHE tested for accuracy and precision. There were three examinations of each concentration. The serum samples' repeatability is shown in Table 10 to be adequate.

Table 9. Application of the suggested techniques and compared with official method for estimating PHE in pharmaceutical formulations.

Dosage form	Proposed FIA method					Official method (theoretical)				
	conc. of PHE (µg.mL^{-1})		Ere %	Rec %	RSD %	conc. of PHE (µg.mL^{-1})		Erel %	Rec %	RSD %
	Present	Found				Present	Found			
ASTULET(syrup) 2.5 mg, (SDI)	10	10.24	2.40	102.4	2.20	10	9.74	-2.60	97.40	2.49
	15	15.18	1.20	101.2	0.90	15	15.02	0.13	100.13	0.66
TUSSIRAM (syrup) 5 mg, (SDI)	10	10.13	1.30	101.3	2.23	10	9.85	-1.50	98.50	2.46
	15	14.89	-0.73	99.27	0.92	15	15.12	0.80	100.80	0.65
NOGRIPPIN Plus (tablets) 10 mg , (Turkey)	10	9.73	-2.70	97.30	2.32	10	10.09	0.90	100.90	2.40
	15	15.16	1.07	101.07	0.90	15	14.92	-0.53	99.47	066
TUSSILET2.5 mg, (SDI)	10	10.14	1.40	101.4	2.22	10	10.28	2.80	102.80	2.36
	15	15.01	0.07	100.07	0.91	15	14.91	-0.60	99.40	0.66

$t_{tab} = 2.45$ for $n_1 = n_2 = 4, n_1 + n_2 - 2 = 6, at\ 95\%\ confidence\ level$

$F_{tab} = 9.28$ for $n_1 - 1 = n_2 - 1 = 3, at\ 95\%\ confidence\ level$

Table 10. Determination of PHE in serum samples using suggest CFIA system.

Sample	Conc. (µ) µg.mL^{-1}	Found Conc. ($\bar{x}$) µg.mL^{-1}	Erel %	Rec. (%)	RSD (%)
1	10	9.75	-2.4508	97.55	0.80
2	10	9.75	-2.5384	97.46	1.22
3	10	9.54	-4.6293	95.37	0.90
4	10	9.62	-3.8472	96.15	1.49
5	10	9.63	-3.6572	96.34	1.83
6	10	9.91	-0.8729	99.13	2.07
7	10	10.17	1.6801	101.68	2.49

Conclusions:

Through the search in scientific journals specialized in the field of continuous flow injection analysis, no researcher determined the drug PHE in the pure form, pharmaceutical, and biological samples, so the idea of completing the research for the semi-automated determination of phenylephrine hydrochloride using the developed system of CFIA/MZ technology, which is one of the methods of green chemistry. The sensitive spectrophotometric quantification of anti-allergic drugs in medications and serum samples via CFIA design is recommended as a study approach for our work, which is why a research plan was provided for this manuscript. It is distinguished by having a larger calibration range and a high sample rate (s/h). These methods can be used to calculate the amount of PHE in µg.mL^{-1}indigence for the prior divorce action, heating or prepping the piece, or extraction using a solid phase. The capital benefits of the CFIA technique include it's a wide working range, reasonable sensitivity, and applicability for routine assessment in pharmaceutical quality control laboratories. This is because, in comparison with other methods and the official standard methods, they reduce reagent waste and the toxicity of organic reagents [30].

Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given the permission for republication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad

Authors' Contributions:

Sh. L. H collected and analyzed the data, and interpreted the results. B. B. Q designed and planned the research plan, following up the results, and proofread the manuscript. Both authors contributed to produced the final draft.

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نهج جديد لتطوير التقدير الطيفي لأدويه الفنيلفيرين في المادة النقية والنماذج الصيدلانية و مصل الدم باستخدام بيرايودات الصوديوم كعامل مؤكسد عبر طريقة خضراء لتقنية التحليل بالحقن الجرياني المستمر /اندماج المناطق المتلاقية

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الخلاصة:

يتضمن البحث طريقة اوتوماتية، سريعة ، ذات توافقية عالية مطورة لتقنيه التحليل بالحقن الجرياني لتقدير الفينيلفرين هيدروكلورايد بالشكل النقي والنماذج الصيدلانية و مصل الدم . اعتمدت الطريقة على تفاعل الازدواج التأكسدي لكاشف 2,4- داي نايترو فنيل هيدرازين مع PHE بوجود بيرايودات الصوديوم كعامل مؤكسد في وسط قاعدي ، يتكون معقد احمر يقاس عند اعلى طول موجي 520 نانومتر بمعدل جريان 4.3 مل .دقيقة $^{-1}$ باستخدام الماء المقطر كناقل للمتفاعلات.

برهنت تقنية التحليل بالحقن الجرياني على أنها أداة تحليلية حساسة واقتصادية لتقدير دواء الفنيليفرين.

وبمدى خطي 5- 300 مايكروغرام.مل$^{-1}$ لمنحني المعايرة و كان حد الكشف3.309 مايكروغرام .مل$^{-1}$ و الانحراف القياسي النسبي4.09% و استعاديه 100.55% بمعدل نمذجة 62 انموذج بالساعه ..طبقت طريقة البحث المطوّرة بنجاح لتقدير PHE في نماذج مصل الدم و المستحضرات الدوائيه .تمت دراسه المتداخلات و اقتراح ميكانيكية التفاعل ..قورنت النتائج المستحصلةمع الطريقه القياسيه المعتمدة (دستور الادويه البريطاني) لم تظهر فروق جوهريه بالنتائج التي حصل عليها باستخدام الإحصاء الحديث على مستوى الثقة 95%.

الكلمات المفتاحية: تقنية التحليل بالحقن الجرياني المستمر/اندماج المناطق المتلاقية ، ثنائي نيتروفينيل هيدرازين ، مستحضرات صيدلانية ،فينيليفرين هيدروكلوريد ، عينات بايولوجية.