H-point standard additions method for simultaneous determination of paracetamol and phenylephrine in pharmaceutical preparations

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Abstract

The development of H-Point Standard Additions Method (HPSAM) has been applied to resolve the corresponding overlapping spectra of paracetamol (PAR) and phenylephrine (PHEPH) by Ultraviolet–visible spectrophotometry. HPSAM is based on the coupling of PAR and PHEPH with diazotized of 1-aminopyrene reagent to form relatively stable water soluble colored azo compounds. The results of reactions show that maximum absorptions of PAR and PHEPH azo compounds are (490 nm and 420nm) respectively. The Individual calibration shows that the linear range was (10-90) and (5 -80) µg.ml-1 for PAR and PHEPH, respectively.The relative standard deviations RSD for the simultaneous determination of PAR and PHEPH by H-point standard addition method were 0.553 and 0.227, respectively. The results show that the proposed method is suitable for the simultaneous determination of PAR and PHEPHE in different pharmaceutical preparations.

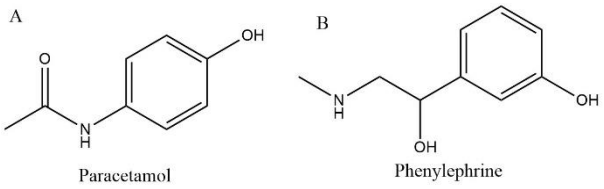
Keywords: *paracetamol, phenylephrine, 1-aminopyrine reagent and H-Point standard addition method.*

1. Introduction

Paracetamol (PAR)[1] is a medicine utilized For the treatment of fever and mild to moderate pain such as toothache, headaches or sprains, and reducing fever due to colds and flu [2]. Other names of PAR are N-acetyl-para-aminophenol and acetaminophen. The chemical formula of PAR is C8H9NO2, molar mass 151.165 g•mol−1 [3,4].

Phenylephrine (PHEPH) a medicine used to relieve congestion in the nasal passages and treat low blood pressure[5]. Trade names of PHEPH is Neo-synephrine, The chemical formula of PHEPH C9H13NO2 , molar mass 167.208 g•mol−1 [6,7] . Figure. 1 depicted the chemical structures of PAR and PHEPH

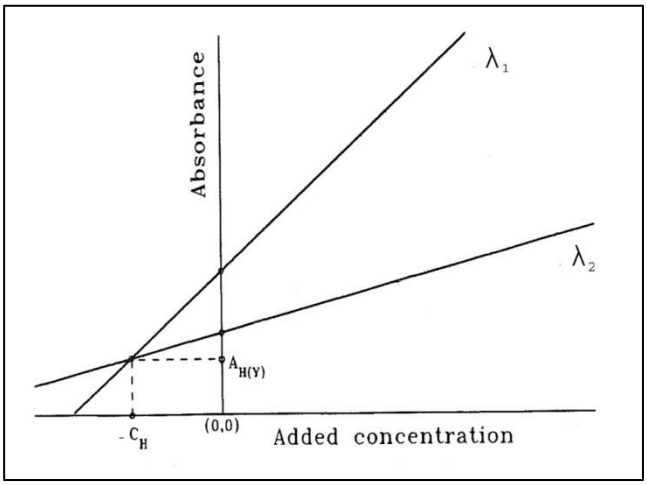
Figure 1. Structural formula of PAR (A) and PHEPH (B).



In the pharmaceutical formulations the combination of PAR, PHEPH and Chlorpheniramine Maleate used to relieve symptoms of cold such as runny nose, watery eyes, cough, fever and headache [8].Several methods in the literature report the simultaneous quantitative determination of PAR and PHEPH in pharmaceutical preparations including spectrophotometric

methods[9.10],electrochemical methods [11], HPLC [12].], H-point standard additions method (HPSAM) is modulation of traditional standard addition method suggested in 1988 by Reig and Falco[13] to obtain the concentration of the analyte when analyte and interferences are existence in the same solution. It also allows to identification of interference that is known to exist. The principle of dual-wavelength spectrophotometry and the standard addition method on which HPSAM depended. The best advantage of HPSAM is that it can eliminate errors caused by the existence of an interference and blank reagent. The principles of HPSAM method include that at the two specified wavelengths, the analyte concentration should be linear with its signal, while the signals of interference should remain similar, even if the analyte concentrations vary. In the plotting of the analytical signal against the concentration of the analyte added, Two straight lines intersecting in point with coordinates (–CH, AH) are obtained, where (–CH) is the concentration of the unknown analyte and (AH) is the interference analytical signal [14,15] as shown in figure 2. In the present work the development of H- point method for the simultaneous spectrophotometric quantitative determination of PAR and PHEPH in pharmaceutical preparations have been studied.

Figure2. general plot of H point standard addition method



2. Experimental

2.1. Apparatus

Shimadzu, Japan, UV-1800 PC double-beam Spectrophotometer, with 1cm quartz cuvette. A Sensitive balance with four decimal places, Switzerland type Mettler Toledo were used in this study.

2.2. Chemicals and reagents

A stock solution of each paracetamol and phenylephrine (1000ug.mL-1) was prepared by dissolving 0.10 g of PAR and PHEPH in 100 mL of distilled water. The working Working standard solutions were prepared by suitable dilution with distilled water to the appropriate concentration, 1.0 mol.L-1 HCl solution was prepared by diluting (8.8 mL) from concentrate acid to 50 mL of distilled water. The diazotized 1- aminopyrene (3x10-3 mol.L-1) by dissolving 0.16 g in 1 mL ethanol and 3 mL of 1.0 mol.L-1 HCl solution and the volume was completed to 250 mL with distilled water. 1.0 mol.L-1 NaOH solution was prepared by dissolving 4.00 g in 50 mL of distilled water., (0.02 mol.L-1) NaNO2 solution was prepared by dissolving (2.76)g in 50 ml of distilled water. Sulfamic acid solution 0.2mol.L-1 was

prepared by dissolving 1.940 g in 100 mL distilled water

2.3. Individual calibration carve

To two series of 10 mL volumetric flasks aliquots of (100 µg.ml-1) of PAR (first series) and (100 µg.ml-1) of PHEPH (second series) solutions were added to each flask equivalent to the concentrations range (10-90) µg.ml-1 and (5-80) µg.ml-1 for PAR and PHEPH for first and second series respectively, then added to each flask of (first series) PAR , 1 mL of (5 ×10-3 mol.L-1) diazotized 1-aminopyrine reagent solution, 2 mL of (1.0 mol.L-1) sodium hydroxide solution, and 0.5 mL of (0.2 mol.L-1) sulfamic acid ,and then the volume of each flask was made up to the mark with distilled water. While for each flask of (second series) PHEPH , added 2 mL of diazotized (5 ×10-3 mol.L-1) of 1-aminopyrine reagent solution, 2.5 mL of (1.00 mol.L-1) sodium hydroxide solution, and 0.75 mL of (0.2 mol.L-1) sulfamic acid were added and then the volume of each flask was made up to the mark with distilled water .The maximum wavelength 490 nm and 420 nm was applied for the determination of PAR and PHEPH respectively.

2.4. Procedure [16]

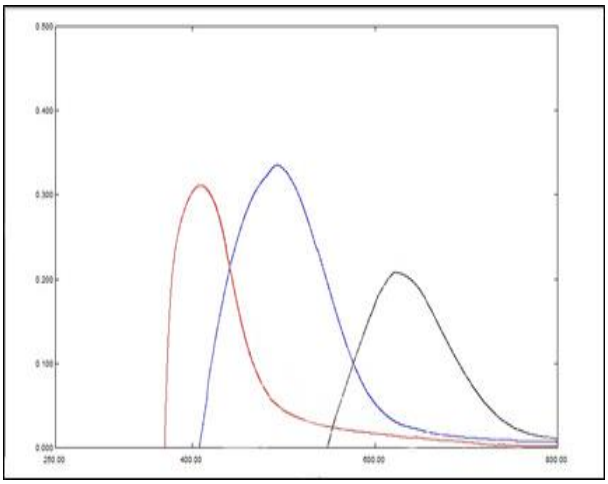
An aliquot of a solution containing 10-90 µg of PAR and 5-80 µg of PHEPH , by standard addition method , then 1 mL of (5 ×10-3 M) diazotized , 1- aminopyrine reagent ,solution, and 2 mL of 1.0 mol.L-1 sodium hydroxide solution were added to two series of a 10 mL volumetric flasks first containing standard addition PAR with fixed amount of PHEPH and second series containing standard addition PHEPH with fixed amount of PAR, and complete the volume to the mark with distilled water. Absorption spectra were recorded at 495 nm and 520 nm when PAR was considered an analyte. Furthermore, for PHEPH determination as analyte, at 350 nm and 365 nm were chosen to record the absorbance

3. Results and discussion

3.1. Preliminary study

The 1-aminopyrene is a good reagent for the determination of PAR and PHEPH by azo coupling reaction forms a reddish - orange color at maximum absorption wavelength of 490 nm for PAR and an orange color at a maximum absorption wavelength of 420 nm for PHEPH (Figure 1). The determination of PAR is hard in the existence of PHEPH by normal spectrophotometric measurements due to spectra overlapping. HPSAM is a good method to resolve this overlap and simultaneous determination of PAR and PHEPH in the same solution.

Figure 3.spectral absorption of (A) 50 µg.ml-1 PHPHE complex (B) 60 µg.ml-1 PAR complex and (C) blank solution absorption.



3.2. Optimization of conditions

The optimum conditions for the formation of azo – dye compounds of PAR and PHPHE with diazotized of 1- aminopyrine were optimized by studying the effect of acid type used , reagent concentration ,volume of

diazotization salt , the volume of hydrochloric acid, volume of sodium hydroxide, volume of sulfamic acid , and the time effect on the absorbance of both azo- day compounds formation. The results were summarized in table1.

Table 1. Optimum condition of PAR and PHEPH complexes.

Condition	PAR azo – compound	PHPHE azo - compound
Acid type	HCl	HCl
1 aminopyrene concentration	$3*10^{-3}$ mol.L^{-1}	$3*10^{-3}$ mol.L^{-1}
Diazonium salt volume	1 Ml	2 mL
HCl volume	2 Ml	2 mL
NaOH volume	2 mL	2.5 mL
Sulfamic acid volume	0.5 mL	0.75 mL
Time of stability	10 min.	15 min.

3.3. H point standard addition method

If the PAR is chosen as the analyte, two wavelengths (495nm and 520 nm) are selected which show the same absorbance for the PHEPH azo- dye compound . A samples series containing standard aliquots of PAR with different sets of PHEPH (Figure 4) or fixed amounts of PHEPH with different sets of PAR (Figure 5) was made by adding standard solutions of PAR

Figure 4. HPSAM for fixed concentration of PHEPH (10 µg mL −1) and different concentrations of PAR ( ) 20 µg ml−1 ( ) 30 µg mL −1 and ( ) 40 µg ml -1.

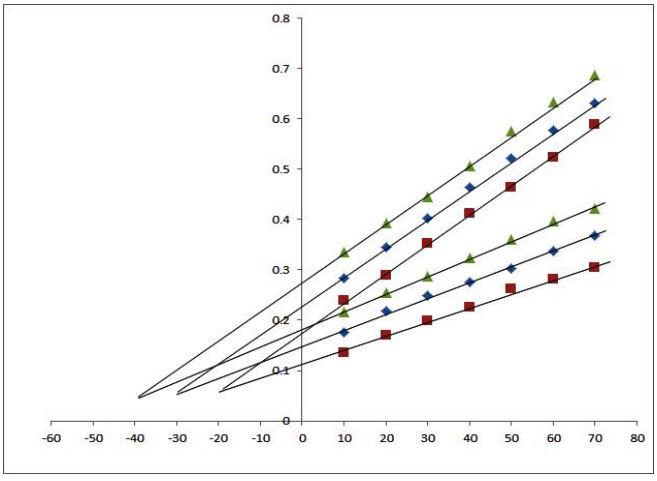
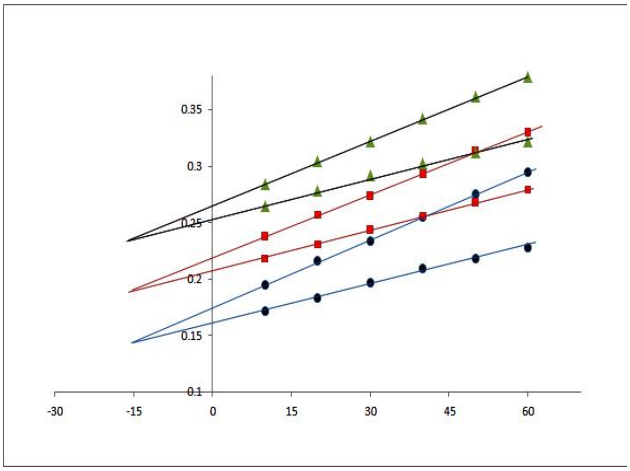


Figure 5. HPSAM for fixed PAR concentration (15 µg mL −1) and different concentrations of PHEPH ( )30 µg ml -1 ( ) 40 µg ml -1 ( ) 50 µg ml -1.



Through doing a standard addition of PHEPH, lthe applicability of HPSAM was tested in determining PAR and PHEPH in a series of solutions containing fixed aliquots of PHEPH together with different concentrations of PAR (Figure 4), or both fixed aliquots of PAR with different concentrations of PHEPH (Figure 5). The results showed that the contents of PAR and PHEPH in the samples were accurately determined at this development method [16] .

$$X_{495} = Uo + U + M_{495}C_{PAR} \quad (1)$$

$$X_{520} = Vo + V + M_{520}C_{PAR} \quad (2)$$

$$X_{(495)} = X_{(520)} \quad (3)$$
$$C_{PAR} = -C_H$$

Where $X_{(495)}$ and $X_{(520)}$ are the signals of analytical measured at 495 nm and 520 nm, respectively. $C_{PAR} = -C_H$ is the unknown PAR concentration.

$$U_o + U + M_{495}(-CH) = V_o + V + M_{520}(-CH) \quad (4)$$

$$-CH = [(U_o - V_o)+(U - V)]/(M_{495} - M_{520} \quad (5)$$

Uo and Vo are original PAR signals at 495 and 520 nm, respectively, U and V are analytical PHEPHE signals at 495 and 520 nm, respectively, M495 and M520 are calibration lines slopes of standard addition at 495 and 520, respectively.

If PHEPH concentration is known, and the analytical signal of PHPHE (U and V) at 495 and 520 nm doesn't effected by standard additions of PAR, X = U.

$$-CH = (U_o - V_o)/(M_{495} - M_{520}) = -U_o/M_{495} = -V_o/M_{520} \quad (6)$$

By substitution -CH value in equation (1), the analytical signal is

$$AH = Uo + U + M_{495}(-C_H) \quad (7)$$

From equation (6)

$$Uo = M_{495}(C_H) \quad (8)$$

Therefore, equation (7) becomes

AH = U

And

AH = Y

Thus, AH refers to the PHEPH signal at 495 and 520 nm.

3.4. Accuracy and precision

Several mixtures of PAR and PHEPH with different concentration ratios were prepared and analyzed by the suggested method. the results are given in Table(1) . The precision of the suggested method was verified by repeated the experiments of PAR and PHEPH in a mixture. The results were summarized in Table (2). The accuracy and precision of the method are acceptable.

Table 2. **Determination of PAR and PHEPH in some synthetic mixtures**

A-C equation	R^2	Added in sample.µg mL^{-1}		Found in sample. µg mL^{-1} (rec%)	
		PAR	**PHEPH**	**PAR**	**PHEPH**
A 1= 0.0029x + 0.1095	0.9981	20	10	30.21 (101.2)	10 (100)
A 2 = 0.0068x + 0.154	0.9996				
A1= 0.0032x + 0.1481	0.9985	30	10	30.16 (100.53)	9.88 (98.8)
A2 = 0.0058x + 0.2284	0.9995				
A1 = 0.0035x + 0.1846	0.9984	40	10	39.88 (99.7)	10.1 (101)
A2 = 0.0059x + 0.2758	0.9993				
A1=0.0028x + 0.0975	0.9984	15	30	15 .1 (100.66)	30.18 (100.76)
A2= 0.0056x + 0.1956	0.999				
A1=0.003x + 0.137	0.9989	15	40	14.9 (99.3)	40.2 (100.5)
A2=0.0057x + 0.2435	0.9991				
A1= = 0.0031x + 0.1848	0.9988	15	50	15.1 (100.66)	49.7 (99.4)
A2= 0.0056x + 0.2961	0.9993				

Table 3. Precision of the proposed method

Added in sample.µg mL^{-1}		Found in sample. µg mL^{-1} (rec%)	
PAR	**PHEPH**	**PAR**	**PHEPH**
45	20	44.8	20.35
45	20	45.5	19.8
45	20	44.85	20.37
45	20	45.4	20.43

45	20	45.45	20.4
Mean		45.2	20.27
S.D		0.25	0.046
R.S.D (n=5)		0.553	0.227
Re %		100.44	101.35

3.4. Application

To evaluate the applicability of the suggested method for the determination of PAR and PHEPH in pharmaceutical preparations (tablets), the results obtained are compared statically by the percent recovery, with those obtained by the HPLC standard method. In real pharmaceutical preparations, a low difference was found between the drugs ratio, due to the composition of real pharmaceutical formulations (500:10 or 325:5 for PAR and PHEPH, respectively). For that, standard addition of PHEPH was applied to the pharmaceutical formulations to increases their concentrations until they are within the linear range. The results of determination of PAR and PHEPH in real pharmaceutical preparation by using the suggested method were summarized in table (4). Table (5) shows the comparison of the suggested method and HPLC standard method with a 95% confidence, values of t-test and F-test shows that a good agreement between proposed method and official method. The results show that the suggested method can be effectively used to the determination of PAR and PHEPH simultaneously in a real pharmaceutical formulations (tablets).

Table4. Determination of PAR and PHEPH in different pharmaceutical preparations

Drug factory	**Approximate doses (mg**		**PAR**		**PHEPH**	
	PAR	**PHEPH**	**Found**	**Rec%**	**Found**	**Rec%**
India	500	10	490.5	98.10	9.2	92
Ireland	500	10	496.7	99.34	9.5	95
Iraq	325	5	319.6	98.33	4.5	90
Turkey	325	5	320.8	98.70	4.4	88

Table5. **Statistical comparison between the proposed and official method**

	PAR		**PHEPH**	
	HPSAM	**Official method[17]**	**HPSAM**	**Official method[18]**

Mean	100.44	100.16	101.35	100.18
S.D.	0.25	0.792	0.046	0.952
N	5	6	5	6
t-test	1.19 ((2.228)		1.87 (2.201)	
F test	1.404 (5.05)		1.4762 (4.39)	

4. Conclusions

PAR and PHEPH can react with diazotized of 1 aminopyrene reagent to give colored azo coupling products with maximam absorbance at wavelengths 490nm and 412 nm for PAR and PHEPH respectively.The present study explains the ability of HPSAM to determine of PAR and PHEPH simultaneously in a solution with overlapping spectra for pharmaceutical preparations where a suggested HPSAM can determine the unknown concentration of analyte from H- point give a high precision and accuracy and good reproducibility for simultaneous determination of PAR and PHEPH in a mixture

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Reference

[1] Jozwiak,M and Nowak, J,2014," Paracetamol: Mechanism of action, applications ans safety concern", Polish Pharmaceutical Society, 71(11-23)..

[2] Knochen, M, Giglio, J and Boaventura F ,2003," Flow-injection spectrophotometric determination of paracetamol in tablets and oral solutions", Journal of Pharmaceutical and Biomedical Analysis, 33 (191-197).

[3] Morelli B,1989," Spectrophotometric determination of paracetamol in pure form and in tablets", Journal of Pharmaceutical and Biomedical Analysis,5,7(577-584).

[4] Saeed A M and Ahmed N Q, 2018,"Simultanous spectrophotometric determination of paracetamol and Codeine", ,5(4), 11-15..

[5] Ahmed I S and Amin A S,2007," Spectrophotometric microdetermination of phenylephrine hydrochloride in pure and in pharmaceutical formulations using haematoxylin", Journal of Molecular Liquids,130(84-87).

[6] Al-Shaalan N H, 2010," Determination of phenylephrine hydrochloride and chlorpheniramine maleate in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-UV methods", Journal of Saudi Chemical Society, 14(15-21)..

[7] Negussie W. Beyene, Jacobus F. Staden V,2004," Sequential injection spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical preparations", Talanta,63,3(599-604).

[8] Ragab M, . El Yazbi F A, . Hassan E M, Khamisa E F and Hamdyb M M, 2018," Spectrophotometric analysis of two eye preparations, vial and drops, containing ketorolac tromethamine and phenylephrine hydrochloride binary mixture and their ternary mixture with chlorphenirmaine

maleate", Bulletin of Faculty of Pharmacy, Cairo University, 6 (91-100).

[9] Joliana F. Farid, Hebatallah M. Essam, Nadia M. Mostafa, Yasmin M. Fayez, 2021,"Spectral manipulation of ratio data for simultaneous determination of binary mixture of Paracetamol and Phenylephrine Hydrochloride", Spectrochimica Acta,260 , 119894.

[10] Muhammad W.Sabra and Diyar S.Ali, 2022," H-point standard addition method for simultaneous determination of phenylephrine hydrochloride, chlorpheniramine maleate, and paracetamol as a ternary mixture in pharmaceutical formulations", ournal of the Indian Chemical Society, 99, 100526.

[11] S. Lotfi, H. Veisi, 2019,"Pd nanoparticles decorated poly-methyldopa@ GO/Fe3O4 nanocomposite modified glassy carbon electrode as a new electrochemical sensor for simultaneous determination of acetaminophen and phenylephrine", Mater. Sci. Eng., C 105, 110–112.

[12] H. Senyuva, T. Ozden, 2002,¨ Simultaneous high-performance liquid chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms", J. Chromatogr. Sci. 40, 97–100.

[13] Reig, F.B. and Falco, P.C. 1988,"H-point standard additions method. Part 1. Fundamentals and application to analytical spectroscopy". Analyst, 113: 1011–1016

[14] P.Campins, J. Verdu-Andres and F.B Reig, 1995," H-Point standard additions method for resolution of binary mixtures with simultaneous addition of both analytes", Analytica Chimica Acta, 315, 267-278.

[15] T N. Al-Sabha, A A. Bunaciu and H Y. Aboul-Enein , 2011," H-Point Standard Addition Method (HPSAM) in Simultaneous Spectrophotometric Determination of Binary Mixtures: An Overview", Applied Spectroscopy Reviews, 46 , 607-623.

[16] T N. Al-Sabha, A A. Bunaciu and H Y. Aboul-Enein , 2011," H-Point Standard Addition Method (HPSAM) in Simultaneous Spectrophotometric Determination of Binary Mixtures: An Overview", Applied Spectroscopy Reviews, 46 , 607-623..

[17] British Pharmacopoeia, in: British Pharmacopoeia, Stationary Office, Appendix 1D, Buckingham Palace Road, London, UK, 2016.

[18] The United States of Pharmacopoeia-39/ National Formulary-34, in, United States Pharmacopoeial Convention, Inc., Rockville, Maryland, USA, 2016.