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RESEARCH ARTICLE

Application of H-Point Standard Addition Method in Kinetic-Spectrophotometric Determination of Phenylephrine in Nasal Drops and Tetracycline in Capsule

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Abstract

A new simultaneous spectrophotometric-kinetic method was developed to determine phenylephrine (PHEN) and tetracycline (TETR) via H-point standard addition method (HPSAM). The proposed procedures rely on the measurements of the difference in the rate of charge-transfer (CT) reaction between each of PHEN and TETR as electron donors with p-Bromanil (p-Br) as an electron acceptor. Different experimental factors which affect the extent of the complex formation were investigated by monitoring the value of absorbance at 446 nm. Time pair of 50 -100 sec was selected and employed, among different examined pairs since it results in the highest accuracy for HPSAM-plot. Linear calibration graphs in the concentration ranges of 10.0-40.0 and 10.0–50.0 mg/L were obtained for PHEN and TETR, respectively and the proposed method was applied successfully in the determination of PHEN and TETR in their pure form and in commercial formulations.

Keywords: *Spectrophotometry, Kinetic H-point standard addition method, Phenylephrine, Tetracycline, Simultaneous analysis.*

Introduction

Tetracycline and phenylephrine have the IUPAC name (4*S*, 4a*S*, 5a*S*, 6*S*, 12a*S*)-4-(Dimethylamino)-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydrotetracene-2-carboxamide and (1*R*)-1-(3-Hydroxyphenyl)-2-(methylamino) ethanol respectively [1] Fig.1 and 2.

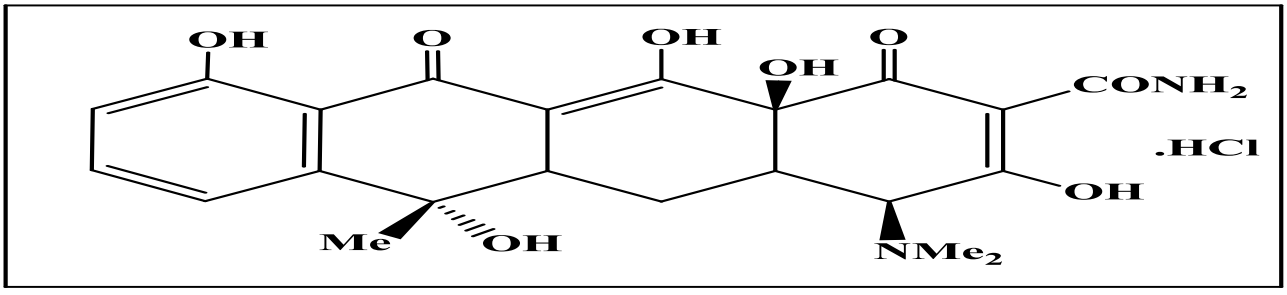

Fig 1: Chemical structure of TETR

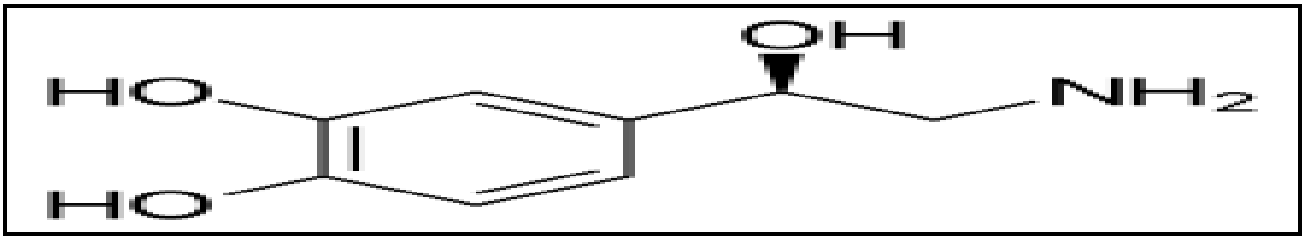

Fig 2: Chemical structure of PHEN

The tetracycline is antibiotic medication and wide range anti-toxin that is strong against about all gram-positive and gram-negative creatures [2]. While phenylephrine has a place with the group of drugs called sympathomimetic. It acts animating the alpha receptors in specific territories of the body [3]. Various spectrophotometric techniques have been investigated for the determination of tetracycline and phenylephrine [4, 11].

Hence, there is a wide-spread requirement for a specific, simple, and fast for simultaneous determination of multicomponent blends has dependably been fascinating fields for analysts, such a significant number of selectively analytical methods have recently been advanced. Differential kinetic techniques utilize various rates, at which at least two species react, with a typical reagent, to determine blends with no earlier detachment.

Multivariate calibration methods are as a rule effectively connected to the multicomponent kinetic assurance to defeat a portion of the disadvantages of traditional techniques. As of late, delicate calculations, for example, principle component regression (PCR), partial least squares (PLS) and artificial neural network (ANN), which evade the co-linearity issues, have been utilized for the simultaneous determination of the analyses having a similar chemical property that can't be settled with normal techniques [12,13].

The H-point standard addition method (HPSAM) which is a simple bivariate chemometric technique that is an adjustment of the standard addition method that changes the incorrigible mistake coming about as a result of the proximity of a direct interfering in the confirmation of an analyte into a consistent precise blunder .This technique (HPSAM) is applied to work at two chose wavelengths where the analytical signals because of one of the types (interferent) is steady and for another (analyte) to be distinctive however much as possible [14].

Experimental

Apparatus

A Shimadzu 1800 UV-Visible spectrophotometry equipped with a 1 cm matched cells was used for UV-Vis spectra acquisition. A Sartorius BL 210S balance, hot plate with magnetic stirrer (Germany) and water bath (Memmert W-200 RING-Germany).

Reagents

All chemicals were of analytical reagent grade were used throughout the experiments. TETR HCl and PHNE .HCl were donated by Samara Co., Iraq (SDI). Tetracycline capsules and phenylephrine as nasal drops (Samara-Iraq) labeled to contain 250 mg and 0.25% of TETR and PHEN respectively were purchased from local pharmacies. A standard solution of TETR and PHNE (500 mg/L) were prepared by dissolving accurately 0.1250 g in distilled water in a 250 mL volumetric flask. 0.35×10^{-3} M *p*-Br (Merck) was prepared by dissolving 0.1484 g of the compound in 100 mL of acetonitrile and 1.006 g of sodium tetraborate pH 9 solution was prepared in 100 mL distilled water.

Assay Procedure

The content of ten capsules was grinded and finely powdered. A quantity of powder containing 100 mg of the drug TETR was dissolved in 25 mL of DW and left to stand for 5 minutes. The total volume of the formed solution was made to 100 mL in a volumetric flask with DW to obtain 1000 mg/L TETR solution. The undissolved materials were filtered-off via Whatman filter paper No.41 before use. The content of one bottle of nasal drops (5 mg PHEN.HCl /10 mL) was diluted to 50 mL with DW in a volumetric flask and stepwise dilution with distilled water was made to obtain the requisite working solutions.

Procedure

Individual Calibration

One milliliter aliquots of 0.35×10^{-3} M *p*-Br and of appropriate concentration of TETR or PHEN standard solutions were transfered into a series of five milliliters volumetric flasks, the mixture kept for 5 minutes at room temperature in the dark, followed by addition 1.0 mL of sodium borate buffer pH 9 to the mixture. The volume was made up to the mark with acetonitrile.

The value of the absorbance against the relative blank vs. time was followed by transferring appropriate volume of each

sample to 1-cm quartz cuvette at 446 nm at interval of 1.0 min.

The concentration of TETR and PHEN in the measured samples ranged between 10.0-50.0 and 10.0-40.0 mg/L respectively.

Kinetic-HPSAM

1.0 mL of 0.35 × 10^{-3} M *p*-Br and 1.0 mL of the suitable concentration TETR and PHEN standard solutions were transferred into 4 mL cuvette and kept it for 5 minutes at room temperature in the dark. 1.0 mL of sodium borate buffer pH 9 was added to the mixture and then the absorbance values of these solutions were measured at 446 nm at a time 50.0 and 100.0 second to carry-out the proposed kinetic-HPSAM in the given system.

Results and Discussion

Optimizing the Experimental Parameters

The experimental conditions affecting the chromogenic reaction were optimized. The variables were found to influence the arrangement of the coloured species; 0.35 × 10^{-3} M for reagent concentration was utilized in all absorption estimation to ensure the full color development. The effect of various value of pH on the reaction yield was investigated by using various buffer media. Sodium borate buffer solution at pH 9 was the optimal one since the color development in this medium was complete. The absorbance of the developed colored complex with respect to different time intervals was investigated. The absorbance value reaches to its maximum after 5 minutes. And all studies were performed at room temperature.

Principle of the Method

The method is based on charge transfer reaction between TETR and PHEN with *p*-Bromanil (*p*-Br) to form a colored complex. Moreover, the abilities of the studied drugs toward the formation of the charge transfer complexes are kinetically different which enables the mathematical treatment of measured data to perform the kinetic HPSAM for simultaneous determination of the two drugs.

Fig 3 illustrates the low-rate of TETR reaction with *p*-Br and shows a small difference in the values of the measured absorbance, but on the contrary the rate of PHEN reaction is much faster. Beer's law characteristics of linear graphs for TETR and PHEN are given in Table 1 under optimized conditions

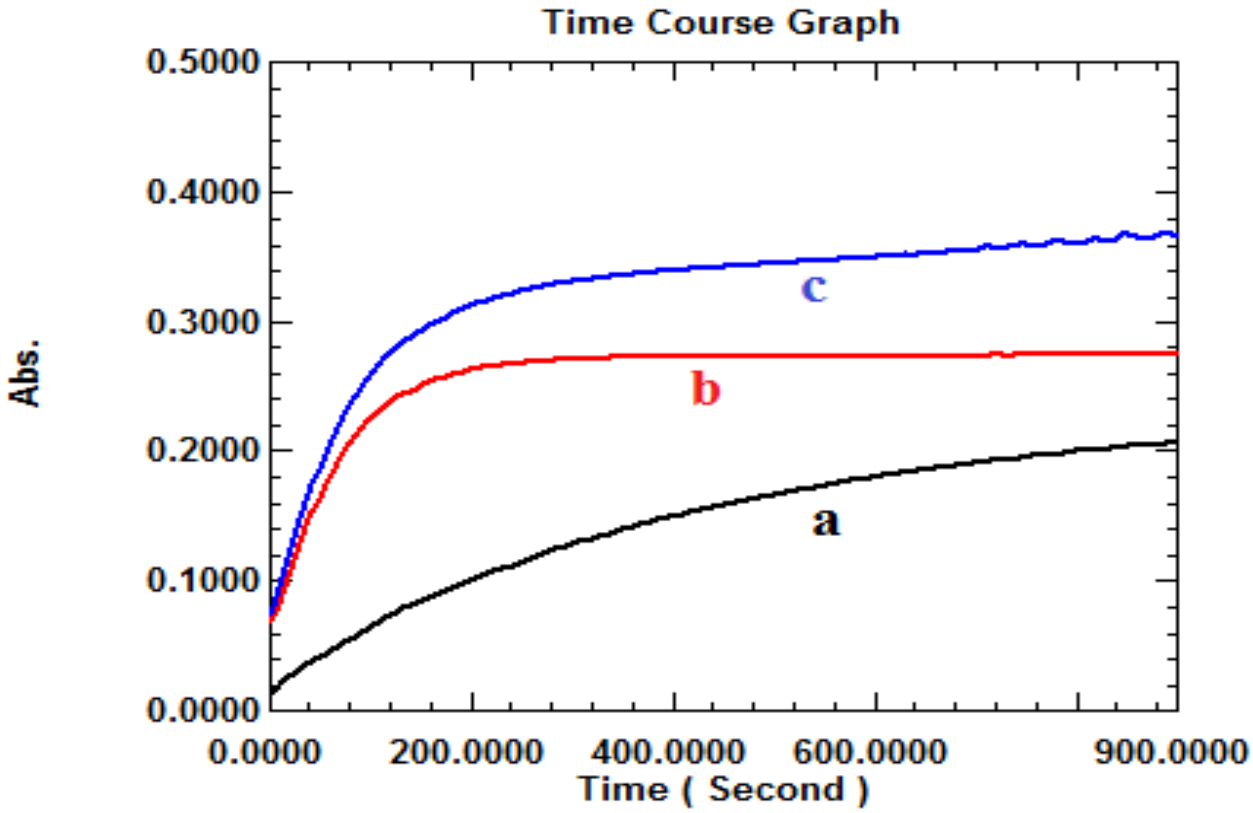

Fig. 3: Measured absorbance versus time plots of (a) 10.0 mg/L TRTR; (b) 30.0 mg/L PHEN; (c) mixture of 10.0 mg/L TETR and 30.0 mg/L PHEN.

Table 1: Characteristics of the constructed calibration curves for TETR and PHEN

Compound	Linearity (mg/L)	Slope	Intercept	R^2
Tetracycline	10.0 – 50.0	0.0053	0.0104	0.9968
Phenylephrine	10.0 - 40.0	0.0043	0.0323	0.9990

Application of Kinetic-HPSAM

In the proposed system TETR was considered as analyte while PHEN assumed as interference. Therefore, the application of HPSAM for the simultaneous kinetic analysis of the two drugs was based on the difference in the rates of the reaction of the two studied drugs with *p*-Br.

Selection of Appropriate Time for Applying HPSAM

The selection of the appropriate pair of time (i.e. t_1, and t_2) is accomplished by selecting the conditions at which the difference in values of slopes of the two straight lines is as great as possible. The best pair of time (t_1 and t_2) when the slope difference of two straight lines must be as large as possible to achieve good accuracy .For this reason, sometime pairs such as, 5-100, 50-100, 50-150, 50-200, 50-300, 100-200 and 100-300 sec were examined .Results in table 2 and the plot in Fig 4 show that the highest accuracy was obtained when a time pair of 50-100 sec was used.

On the other hand, theoretically at the coordinates of H-point (i.e. $-C_H$, S_H), at the abscissa the analyte (TERT) concentration is independent on the interference (PHEN) concentration (Fig 5), and at the ordinate the value of the measured absorbance due to the interference (PHEN) is independent on the analyte concentration (TERT), Fig 6.

Table 2: Application of the proposed HPSAM for the determination of Tetracycline in a synthetic mixture of 10.0 mg/L TETR and 10.0 mg/L PHEN

Time interval (Sec.)	Tetracycline found ±SD (mg/L)	Recovery%	RSD% (n=3)
5.0-100.0	9.56 ± 0.046	95.9	0.479
50.0-100.0	9.96 ± 0.0153	99.6	0.153
50.0-150.0	9.53 ± 0.021	95.3	0.218
50.0-200.0	9.06 ± 0.071	90.6	0.780
50.0-300.0	9.51 ± 0.087	95.1	0.917
100.0-200.0	9.04 ± 0.025	90.4	0.278
100.0-300.0	9.36 ± 0.047	93.6	0.505

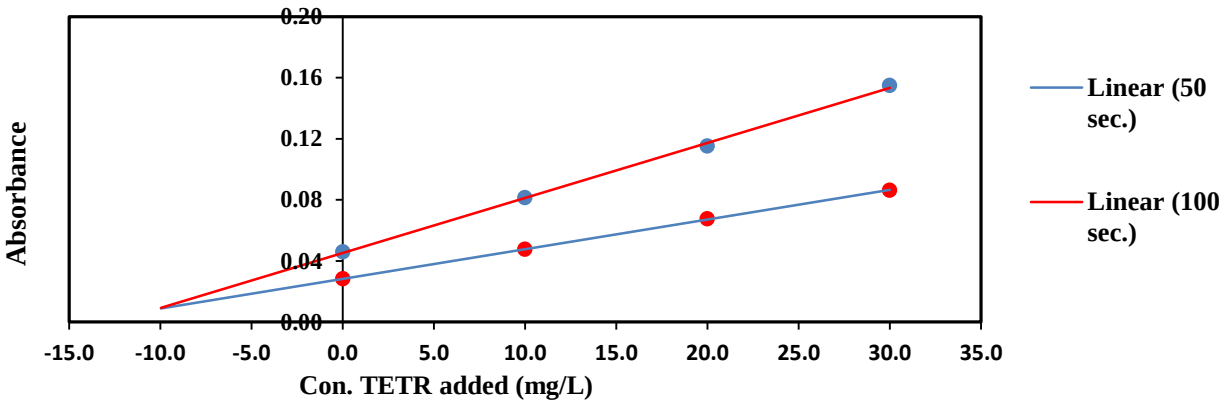

Fig 4: HPSAM plot for the simultaneous determination of TETR (10.0 mg/L) and PHEN (10.0 mg/L) simultaneously in their binary mixture

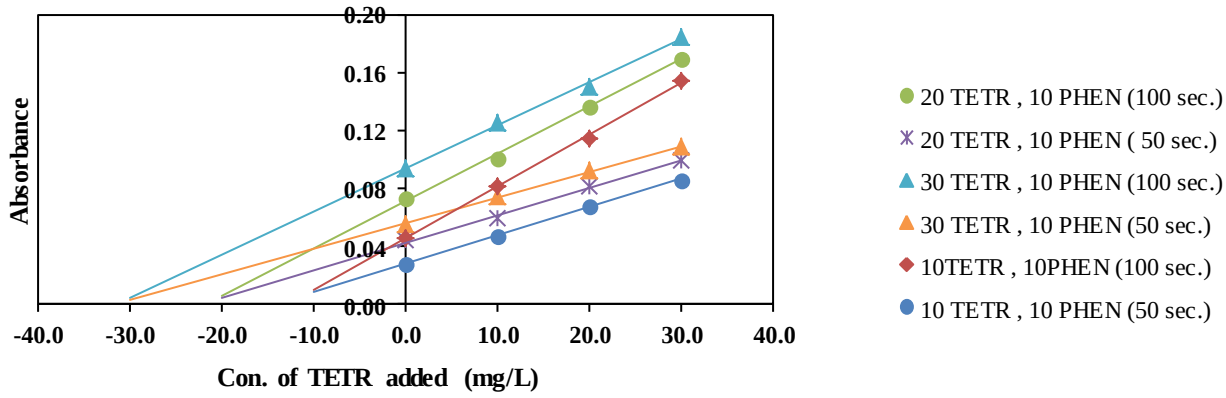

Fig 5: HPSAM plot for mixtures of fixed PHEN (10.0 mg/L) and TETR (10.0, 20.0 and 30.0 mg/L)

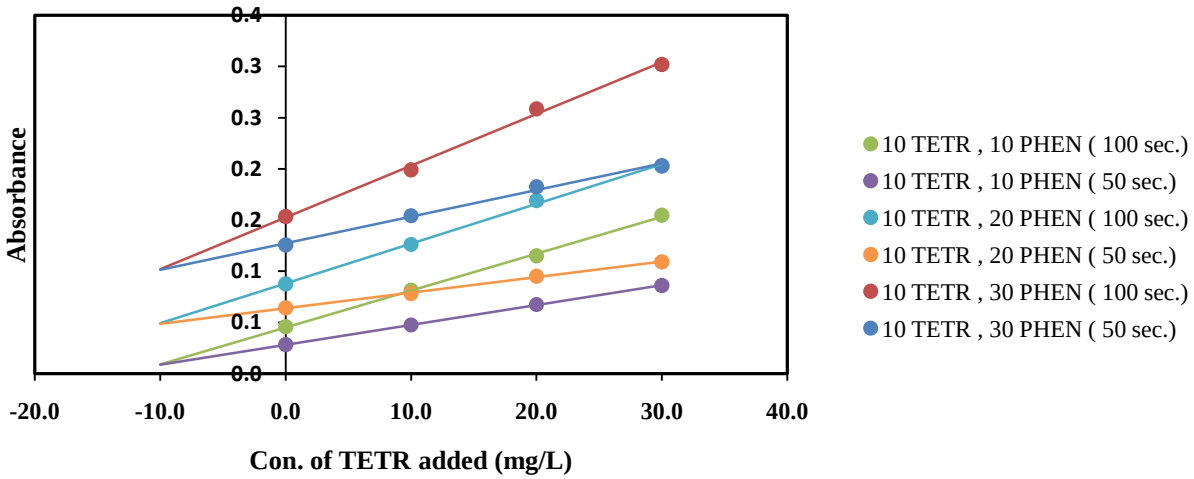

Fig 6: HPSAM plot for mixtures of fixed TETR (10.0 mg/L) and PHEN (10.0, 20.0 and 30.0 mg/L)

Accuracy of the Method

The analytical results obtained from proposed HPSAM can be seen in Table 3.

The accuracy and precision of the method are considered very satisfactory, that was adequate for the quality control analysis of the studied drugs.

Table 3: Result of four experiments for the analysis of TETR and PHEN mixture in different concentration ratios by HPSAM

A-C equation	R^2	Taken (mg/L)		Found (mg/L)	
		TETR	PHEN	TETR	PHEN
A_{50}=0.0019C + 0.0283	0.9998	10	10	10.101	9.837
A_{100}=0.0036C + 0.0452	0.9989				
A_{50}=0.0019C + 0.0428	0.9968	20	10	20.052	9.488
A_{100}=0.0033C + 0.0709	0.9973				
A_{50}=0.0018C + 0.0563	0.9981	30	10	30.251	9.535
A_{100}=0.003C + 0.0934	0.9973				
A_{50}=0.0015C + 0.0639	0.9987	10	20	10.029	19.140
A_{100}=0039C + 0.0880	0.9980				
A_{50}=0.0026C + 0.1274	0.9948	10	30	10.022	30.767
A_{100}=0.0051C + 0.1525	0.9963				

Application

To determine the accuracy (in terms of recovery) of the recommended method, standard addition procedure was followed by performing three replicates analyses TETR and PHEN in two commercially available pharmaceutical samples. The obtained results were in good agreement with the spiked values indicating the capability of the developed HPSAM for the determination of TETR and PHEN simultaneously in commercial dosage, Table 4.

Table 4: Determination of TETR and PHEN in several real sample solutions

Sample	Added		Found		Recovery%	
	TETR (mg)	PHEN%	TETR (mg)	PHEN%	TETR	PHEN
1	50	0.10	50.505	0.095	101.010	95.350
2	100	0.15	100.260	0.144	100.260	95.701
3	150	0.20	151.255	0.205	100.837	102.557

Conclusion

The suggested kinetic-HPSAM was successfully applied for simultaneous determination of phenylephrine and tetracycline in bulk and dosage forms.

The procedure is simple, reliable and sufficiently sensitive to be applied for determination of small amounts of TETR and PHEN, so it could be used for the analysis of the cited drugs in real samples and in quality control of pharmaceutical dosages.

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