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A comparative analytical determination of phenylphrene and cephalexin in pure and pharmaceutical preparations

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Abstract

Validation of analytical methods is an important role in analytical analysis. In this research, an accurate, efficient and reproducible isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of phenylephrine hydrochloride (PHP) and cephalexin (CPH). A mixture of drugs as standard material and in formulation tablets were separated using a phenomenex C18 column, (L, 15 cm, 0.46 cm, I.D, and 5 µm Size of particle), with the Shimadzu HPLC, model LC–20 - A, Japan. The eluent phase was optimized through the design, experiment. Elution was done by an eluent phase composed of water (H2O), methanol (MOH) and acetonitrile (ACN) mixture have a ratio of (60: 20: 20, V/V), with adjusted pH of 3.0 with acetic acid, have a pumped flow rate of 1.0 mL/min. The drugs, separation were performed at 270 nm using a UV-VIS - detector for 10 min. The time of elution for the drugs was recorded at (3.685 and 6.134 min) for PHP and CPH respectively. The validation of the method was conducted in a range of concentration (1 - 60 µg/mL) for PHP and CPH. The method was found to be robust, specified and resisting against the small experimental changes in the value of the flow rate, pH, and wavelength. The LOD was found to be 0.5 µg/mL for PHP and CPH. The values of LOQ were 1.65µg/ for PHP and CPH.

Keywords: Determination; RP - HPLC; Robust; Isocratic

1. Introduction

Phenylephrine hydrochloride (PHP) Phenylephrine hydrochloride (PHP**)** is (R)-1-(3-hydroxyPHEPHnyl)-2- methyl amino ethanol hydrochloride, with chemical formula $C_9H_{13}NO_2$.HCl [1]. It is a nasal decongestant which helps to relieve a blocked nose [2]. It is a sympathomimetic (alphaadrenergic) agent who stimulates alphaadrenergic receptors, orally producing pronounced vasoconstriction. It is also a frequent constituent of administered nasal decongestant preparations [3]. The combination of drugs has ultimate effective when the agents act out of different analgesic mechanization and synergistically active [4]. Cephalexin (CPH) is a semisynthetic cephalosporin antibiotic for oral administration, belongs to the group of β-lactam antibiotics. It is chemically designated as (6R,7R)-7-[[(2R)-2-Amino-2-phenylacetyl] amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid monohydrate. The chemical formula for cephalexin is C16H17N3O4S. H2O and the molecular weight is 365.4 [5]. It is bactericidal and acts by inhibiting synthesis of the peptidoglycan layer of the bacterial cell wall by irreversibly bind to the active site of PBP, which is essential for the synthesis of the cell wall [6]. At least 70–90% of a cephalexin dose are eliminated in urine within 8–12 hours in adults with normal renal function without change. Various methods of analysis are announcing to determine these drugs in formulating drugs like HPLC [7- 9], SP-FT - Raman [10], electromagnetic [11], spectroscopy [12- 14], GC-Mass [15], Electrode ion [16] and GC - ion trap [17]. The work objective is to evaluate a new accurate and easy chromatography analytical method for the estimation of the drug content in tablet formulated samples manufactured by different pharmaceutical corporation which available in the pharmaceutical market in Iraq, to tool up information about the different products, which may enforce or not enforce with the requirements of the formal method or other standard methods.

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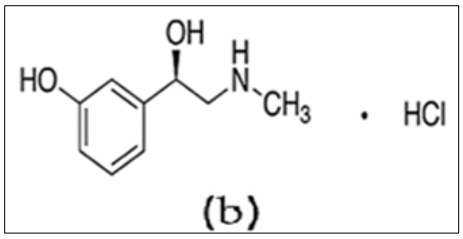

Figure 1 Chemical Structure of Phenylephrine hydrochloride (PHP)

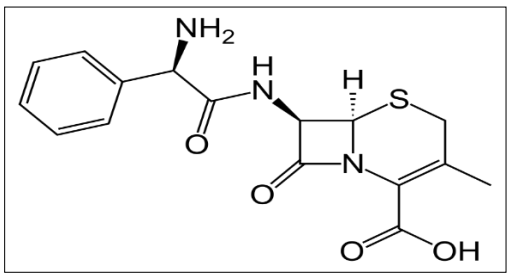

Figure 2 Chemical Structure of Cephalexin

2. Material and methods

PHP and CPH standard powder were from SDI- Iraq. Methanol and acetonitrile (HPLC-grade) were from BDH. Sodium hydrogen phosphate, sodium borate, sodium acetate, boric acid, acetic acid, and phosphoric acid were from BDH and water freshly distilled prepared were used.

2.1. Instrumentation and chromatographic conditions

LC – 20 A, Shimadzu, Japan, Germany Sartorius - balance, Karl – Kolb - Ultrasonic bath - Germany), Shaking bath water (Taiwan) and Memmert - oven – Germany, were used in this study. PHP and CPH were separated on column type C-18 phenomenex - (L, 15 cm, 0.46 cm, I.D, and 5 µm Size of particle). Separation was utter at room - temperature (~25 ^{o}C) and the run time was 10 min under Reversed - Phase conditions. The elution phase was methanol (MOH) acetonitrile (ACN) and water (H_2O) in the ratio of (20:20: 60 V/V) adjusted pH with acetic acid at 3.0. The rate of flow was 1.0 mL / min, and a 10 µL injector loop was used for injecting samples and detection was done at 270 nm. The eluent phase was degassing using the sonicator type - ultrasonic cleaner, power - sonic- 420, and then filtered over a 0.45 µm filter of nylon. The identity established of the compound was done through the comparing of the standard compound solution retention time with those of a sample compound solution. Chromatography was complete in temperature column that maintained at 25 ±2 °C. The UV- spectrums of PHP and CPH selecting the detection working wavelength were taken by the V-650, Jasco – Japan, UV-VIS - spectrophotometer double – beam with quartz cells has 1 cm path length, which were used in the analytical object.

2.2. Preparation of drug stock solutions (60 mg/L)

A 0.006 g of the standard drug was weighed and dissolved in water and in (H_2O: MOH: ACN 60: 20: 20 V/V), transferred to a 60 mL volumetric flask, then completed to the mark with the same solvent. More diluted solutions were prepared by simple dilution of stock solution of drugs.

2.3. Diluent

By using the stock solution of 60 µg/mL, additional dilutions were conducted through withdrawing a different volume (0.2 – 10 mL) from the standard solution of PHP and CPH into the series of 10 mL volumetric calibrated flasks and all were complete to the mark with eluent phase to prepare standard working solutions have concentrations of (1 – 60 µg/mL).

2.4. Procedure for the drugs assay in pharmaceuticals tablets

Tow tablets of the PHP and CPH drug's, formula was accurately weighed and finely powdered. An accurately quantity weighed of tablets, powder which equivalent to (100 mg) of PHP and CPH drugs were conveyed to a (100 mL) volumetric flask and then diluted with (H_2O: MOH: ACN 60: 20: 20 V/V), the content was ultra - sonicated for 25 min. The drugs solutions volume was completed to the mark and mixed well with solvent. The solutions were filtered again using no.1 whatman filter paper for the removing of unwanted materials particulate. A filtered solution was appropriately further diluted with the elution phase to produce a sample solution for analysis. The amount of the PHP and CPH present in the solution sample was estimated using the standard calibration graphs

3. Results and discussion

3.1. Method Development

Several tests were performed in order to get satisfactory separation-resolution of PHP and CPH using different eluent phases with different ratios of water and organic phases. An ideal eluent phase was found to be the mixture of water and acetonitrile. This eluent phase used in ratio (60: 20: 20 V/V) gave a good and satisfactory resolution of PHP and CPH. The pH value (3.0) of the eluent phase, increasing or decreasing by ± 0.2, did not indicate a worthy change in the analyte retention time. The time of retention using analytical column was estimated at a rate of flow with 1.0 mL/min. The volume of injection was 10 µL. The retention time of sample and standard for PHP and CPH was well pleased with high resolution in formulating sample. This labour was converging on optimization of the conditions for the rapid, simple, low cost, and effective analysis, involving a selection of the eluent phase to take out satisfactory results. Solvent strength, solvent type (organic solvent volume fraction in the eluent phase and pH of the mobile phase solution), the wavelength of detection and rate of flow were varied to estimation the Chromatographic conditions which were given the good separation. The optimized of eluent phase conditions was conducted so there no solvent interference and excipients. The entire predicate chromatographic optimum conditions and the notice values of column efficiency, resolution and factor tailing were mentioned in Table 1. The chromatograms of standard PHP and CPH and mixture of standard drugs applied optimum condition are revealed in (Fig. 3).

Table 1 The predicate optimum parameters and system suitability of HPLC method

Predicate optimum parameters results	
Compassion of eluent phase, H_2O: MOH: ACN 60: 20:20 V/V pH=3	Column Type, ODS, (15 – 0.46) cm, 5µm
	Sample Temperature, ambient
Rate of flow, 1.0 mL/ min.	Column Temperature, 25 ±2 °C
Injection volume, 10 µL	Run Time min, 10.00
Detection wavelength, nm 270	Retention Time min, 3.685 PHP and 6.143 CPH

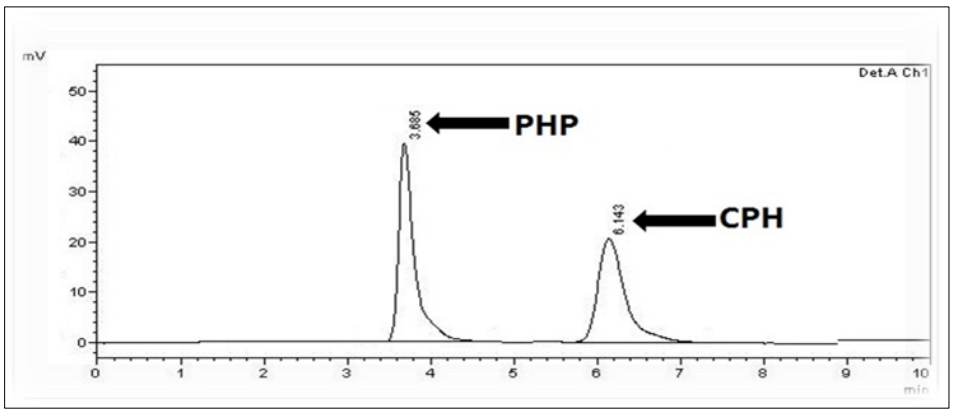

Figure 3 HPLC chromatograms for standard drugs mixture

3.2. Preparation of Calibration graph

From the standard stock solution, posterior dilutions were done with eluent phase to gain a series of standard solutions have a range of concentration with (1-60 µg/mL) of drugs. The solutions were injected using injector loop of 10 µL and chromatograms were recorded. Graphs were plotted by taking a concentration on X-axis and the area under the peak on Y-axis which gave a straight line.

3.3. Analytical method validation

Validation of progress method was conducted as per ICH Q_2 R_1 guideline [24]. Parameters such as accuracy, linearity, precision, specificity, LOD and LOQ, robustness and ruggedness were taken in considering testing for the analytical validation method.

3.4. Linearity and Range

The proposed RP-HPLC method was shown a good linearity in the concentration range of (1-60 µg/mL) for PHP and CPH respectively were represented in (fig. 4). The linear equations of the straight lines are y= 5233.8[X] – 2194.3 (R^2 = 0.9995) for PHP and y = 13089[X] -6223.8 (R^2 = 0.9993) for CPH. The results are satisfactory, because there is a significant correlation between concentration of drugs and response factor within the concentration range.

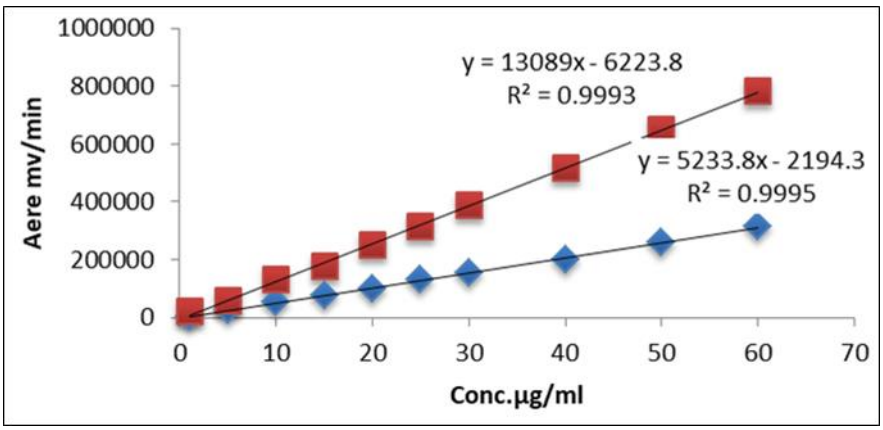

Figure 4 Calibration graph for CPF and PHP using HPLC method

3.5. Precision

The intraday precision of the developed method was evaluated by analysing PHP and CPH samples of different concentrations three times on the same day and %RSD was estimated. The inter day precision was evaluated by analyzing samples of different concentrations of PHP and CPH in different three days and %RSD was calculated. Repeatability was evaluated by injecting the standard drugs solutions of (6 µg / mL) four time on the same day and the value of %RSD were calculated. The results obtained are shown in Table 2.

3.6. LOD and LOQ

LOD and LOQ were estimated by the gradual dilution for lowest concentration, and 3.3 LOD respectively. The obtained results are tabulated in table 2.

Table 2 Parameters validation summery

Sr. No.	Validation parameters	Results	Standard values
1	Linearity Range	1-60 PHP, CPH µg/L	-
2	Straight line equation	y= 5233.8 [X] - 2194.3 PHP y = 13089 [X] -6223.8CPH	-
3	Correlation Coefficient	0.9995 PHP, 0.9993 CPH	≥ 0.9990
4	Precision (% R.S.D.)		≤ 2.0 % R.S.D.
	Repeatability Intraday Interday	0.14 PHP, 0.12 CPH 0.36 PHP, 24 CPH 0.67 PHP, 0.56 CPH	
5	Mean % Recovery	99.95% PHP, 99.93% CPH	95 – 100%
6	Specificity	Specific	
7	LOD (µg/mL)	0. 5 PHP, 0. 5 CPH	-
8	LOQ (µg/mL)	1.65 PHP, 1.65 CPH	-
9	Ruggedness	Complies	≤ 2.0 % R.S.D.
10	Robustness		≤ 2.0 % R.S.D.
	Flow rate change Wavelength change Solution pH change	Complies	

3.7. Accuracy

This study was carried out to assure the closeness of the test results obtained by the analytical method to the true value [18]. For this method, PHP and CPH were measured at three selected different concentrations within the limits of Beer's law 4, 17, 34 µg/mL. The results are tabulated in table 3, which revealed that the suggested method for detection of interesting and quite convenient with respect to the methods and parameters calculated. The recoveries of standard drugs are between 98.33 – 102.80%.

Table 3 Standard Drug Recovery Rate

PHP µg/mL		% Recovery		CPH µg/mL		% Recovery	
Taken	Found			Taken	Found		
4	4.03	100.75	Mean =100.29 S.D. =0.42 R.S.D. 0.153	4	3.98	99.50	Mean =99.89 S.D=0.34 R.S.D. 0.120
17	16.99	99.94		17	17.01	100.06	
34	34.06	100.18		34	34.04	100.12	

3.8. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of placebo in mobile phase was injected and the chromatogram showed no inferring peaks at retention time of the CPH. The chromatogram of placebo was compared with those acquired from the CPH standard solution. The correlation was good (in terms of t_R and area) indicates the specificity of the method. Chromatograms of specificity for the CPH depicted in Fig. 5 and 6).

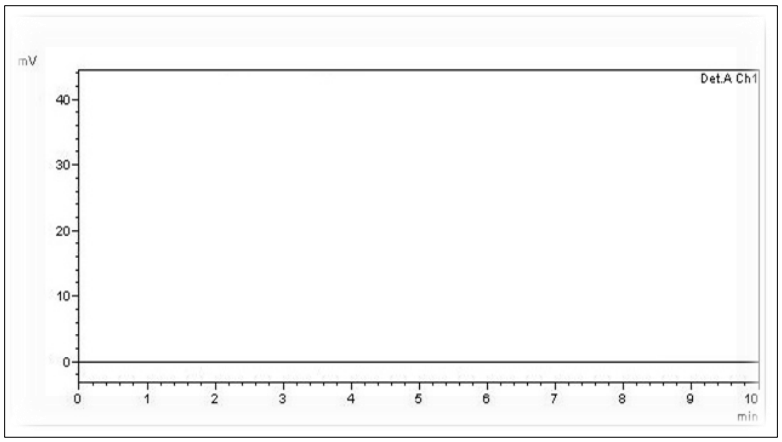

Figure 5 Specify chromatogram of blank placebo in H2O: MOH: ACN 60:20:20

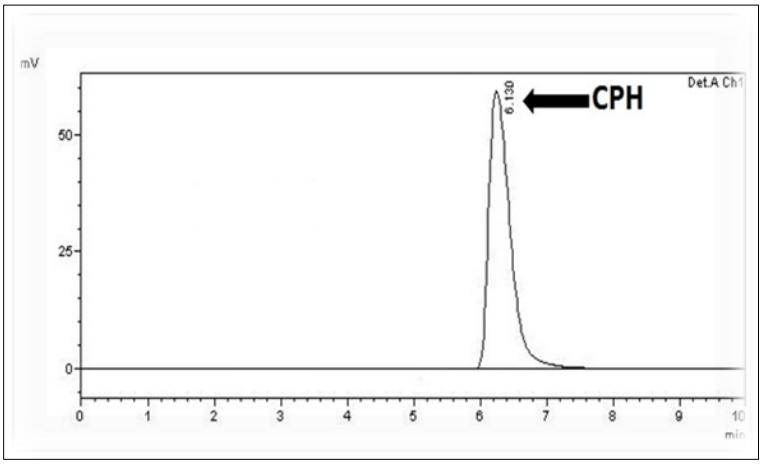

Figure 6 Specificity chromatogram of standard CPH (50 µg/ L)

3.9. Ruggedness and Robustness

The ruggedness of the proposed method was determined by analysis of aliquots of sample CPH solution (8 µg/mL) by two analysts using same operational and environmental conditions. The method robustness was evaluated by changing the rate of flow by ± 0.1 mL/min. (1.1 mL/min and 0.8 mL/min), changing the pH by ± 0.2 % (2.8 and 3.2 %) for eluent phase and the wavelength detection changing by ± 2 nm (272 nm and 268 nm). The results obtained are shown in table 4.

Table 4 The ruggedness results of the proposed method

Ruggedness results			
	Analyst 1	**Analyst 2**	
Mean % Assay* ± SD	99.82 ± 0.28	98.93 ± 0.21	
% R.S.D.	0.280	0.212	
Robustness results			
Method Robustness Parameters	Mean*	S.D.	%R.S.D.
Flow rate changes 1.0 ± 0.1 mL/min.	99.92	0.45	0.45
Mobile phase pH changes 4.0 ± 0.2	99.73	0.34	0.34
Detection wavelength changes 270 ± 2 nm	100.15	0.21	0.199

* n = 3

Three formulated samples were analysed for PHP and CPH uses a validated high-performance liquid chromatography (HPLC) method with UV detection at 270 nm. A 10 µL of sample were injected to HPLC analysis under the optimum separation conditions. Eluent phase H_2O: MOH: ACN (60: 20: 20 V/V) was delivered at a flow rate of 1.0 mL/ min with UV detection at 270 nm. The column was Phenomenex C-18 (15 cm × 0.46 cm I.D) and 5 µm particle size. Analysis was performed at room temperature (~25 °C) and the total run time was 10 min. The results obtained are tabulated in Table 5. Fig. 5, A and B were shown the separation chromatograms of the drugs in different formulating samples. The recoveries of drugs in samples were between 99.85– 100.26%.

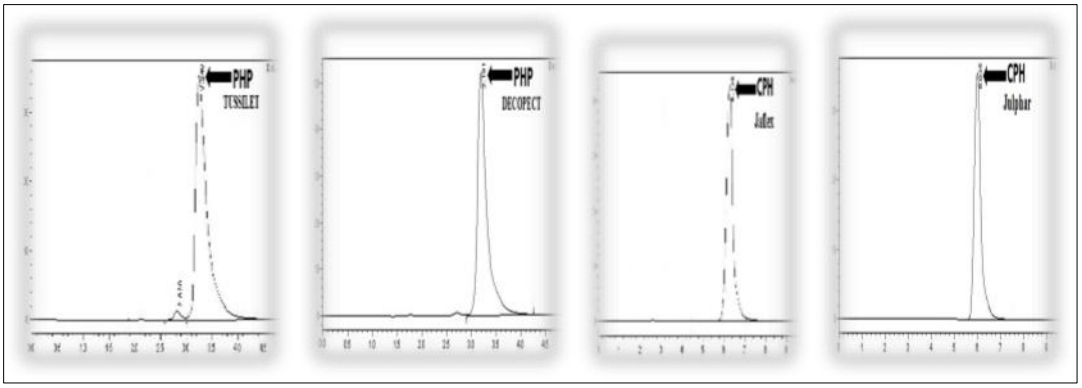

Figure 7 Separation chromatogram of drugs in different formulating samples

Table 5 Estimated quantity of drugs in different formulating samples

PHP / **Company**	**Amount taken mg/ 5 ml.**	**Mean amount found mg/ 5 ml.**	**%Mean amount Found**	**R.S.D n = 3**
TUSSILET	2.5	2.49	99.99	0.17
DECOPECT	2.5	2.51	100.26	0.13
CPH / Company	Label Claim mg/ tab.	Mean amount found mg/tab.	% Mean amount found	R.S.D n = 3
Jaflex	500	500.65	100.13	0.15
Julphar	500	499.25	99.85	0.21

4. Conclusion

The RP – HPLC validated methods appoint here steady to be accurate, fast, simple, robust, and precise, so it can used in the routine analysis of PHP and CPH as standard and in formulating form.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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