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Research Article

Validation of Liquid Chromatographic Analytical Method for Determination of Cephalexin and Aspirin in Pure and Pharmaceutical Preparations

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ABSTRACT
In this research, an accurate, efficient and reproducible isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been modified and set for the determination of Cephalexin (CEP) and Aspirin (ASP) as pure and in formulated form. A mixture of drugs as standard material and in formulation tablets were separated using an phenomenex C18 column, (L, 15 cm, I.D, 0.46 cm, and Size of particle, 5 µm), with the shimadzu RP – 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 (H_2O) and acetonitrile (ACN) mixture have a ratio of (60: 40 V/V), with adjusted pH of 4.0 with acetic acid, have a pumped flow rate of 0.8 mL/min. The separation of drugs was done using a UV-VIS - detector at 270 nm for 4 min. The time of elution for the drugs was recorded at (1.981 and 3.072 min) for ASP and CEP respectively. The optimum conditions such as the composition of the mobile phase, flow rate, wavelength and pH were studied. Calibration graph was in the range of concentration (1 - 50 μg/mL) for the two drugs. While, R^2 values within (0.9996 and 0.9993), and the means of recovery were found within (99.77 – 100.16) for ASP and CEP. The LOD were found to be 0.05 μg/mL for ASP and CEP. The values of LOQ were 0.165 μg/mL for ASP and CEP.
Keywords: Determination, RP - HPLC, recovery, isocratic.

INTRODUCTION

Acetylsalicylic acid known as Aspirin is one of the widely use as anti-inflammatory and analgesic drugs [1]. Chemically is 2-(acetyloxy) benzoic acid [Figure1]. Its ability to inhibiting the production of thromboxane make the drug a good choice as antiplatelet., which under normal circumstances binds platelet molecules together to create a patch over damaged walls of blood vessels [2]. However, since the platelet patch can become big in size and this might lead to block the blood flow, aspirin can be used for long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at the risk of having blood clots [3]. Aspirin is official in Indian Pharmacopeia, British Pharmacopoeia and United States Pharmacopoeia, which describe acid-base titration for assay of aspirin [4]. Several methods such as UV [5-6] RP-HPLC [7-9] HPTLC [10-11] spectrofluorometric [12] spectrometric method [13-14] have been utilized to estimate the aspirin either in pure form or mixed with other drugs. Cephalexin [Figure 2] (also called Cefalexin) is chemically known as (6R,7R)-7-[(2R)-2-amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclooct-2-ene-2-carboxylic acid is an semisynthetic derivative of the cephalosporin nucleus (7-amino cephalosporanic acid) which is orally active broad spectrum antibiotic. Cephalexin is suitable treatment of many infections such as respiratory tract, genitourinary system, skin inflammation, bones and certain other bacterial infections. It can be given in relatively high oral doses with no gastrointestinal irritation, this is because its absorption occurs in the upper intestine [15,16]. A number of methods have been described in the literature for the determination of cephalexin. These include chromatographic methods [17], fluorimetry [18], flow injection analysis [19], atomic absorption [20] electroanalytical methods [21], RP-HPLC [22]. Limited spectrophotometric techniques are presented for the determination of cephalexin [23].

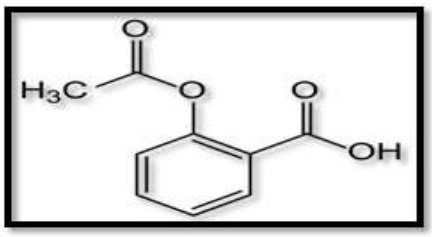

Fig.1: Chemical Structure of Aspirin

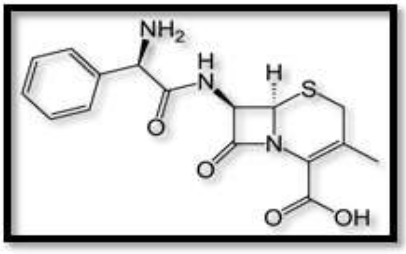

Fig.2: Chemical Structure of Cephalexin

MATERIALS AND METHODS

Materials

ASP and CEP standard powder was from SDI-Iraq. Different tablets samples were used as marketed formulation. Acetonitrile (HPLC-grade) is from BDH, acetic acid BDH and freshly prepared deionized water was used throughout the experiment.

Instrumentation and chromatographic conditions

HPLC (shimadzu - LC – 20 - A, Japan), Germany Sartorius - balance, Karl – Kolb - Ultrasonic bath - Germany), Shaking bath water (Taiwan) and Memmert - oven – Germany, were used in this study. ASP and CEP were separated on column type phenomenex - C-18 (250mm, 4.6mm - I.D, and 5- μm size of particle). Separation was utter at room - temperature (~25°C) and the run time was 10 min under Reversed Phase conditions. The elution phase was Acetonitrile (ACN) and water in the ratio of (40:60 V/V) adjusted pH with acetic acid at 4.0. The rate of flow was 0.8 mL / min, and an 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 ASP and CEP selecting the detection working wavelength were taken by the Jasco - V-650 – Japan, double - beam UV-VIS - spectrophotometer has 10 mm length path quartz cells, which was used for the analytical object. 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.

Preparation of drug stock solution (100 mg/L)

A 0.01 g of the standard drug was dissolved in water and in (water: acetonitrile 60:40 V/V) in 100 mL volumetric flask, and the volum was compelted to the mark using same solvent. Simple dilution of stock solution of drugs was uesd to prepare more diluted solutions.

Diluent

By using the stock solution of 10µg/mL, additional dilutions were conducted through withdrawing a different volume (0.1 – 5 mL) from the standard solution of ASP and CEP 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 – 50µg/mL).

Procedure for drugs assay in pharmaceutical tablets

Three pills of the ASP and CEP drug's, formula were accurately weighed and finely powdered. An accurately quantity weighed of tablets, powder which equivalent to (100 mg) of ASP and CEP drugs were moved to a 100 mL volumetric flask, then this was diluted using (water: acetonitrile 60: 40 V/V), the content were ultra - sonicated for 25 min. The drugs solutions volume were 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 ASP and CEP present in the solution sample was estimated using the standard calibration graphs.

RESULTS AND DISCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution of ASP and CEP 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: 40 V/V) gave a good and satisfactory resolution of ASP and CEP. The pH value (4.0) 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 0.8

mL/min. The volume of injection was 10 µL. The retention time of sample and standard for ASP and CEP 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 ASP and CEP and mixture of standard drugs applied optimum condition are revealed in (Figure 3).

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

Predicate optimum parameters results	
Compassion of eluent phase, H_2O: ACN 60: 40 V/V pH= 4	Column Type, ODS, (15 – 0.46) cm, 5μm
	Sample Temperature, ambient
Rate of flow, 0.8 mL/ min.	Column Temperature, 25 ±2 °C
Injection volume, 10 μL	Run Time min, 4.00
Detection wavelength, nm 270	Retention Time min, 1.981 PCM and 3.072 ASP

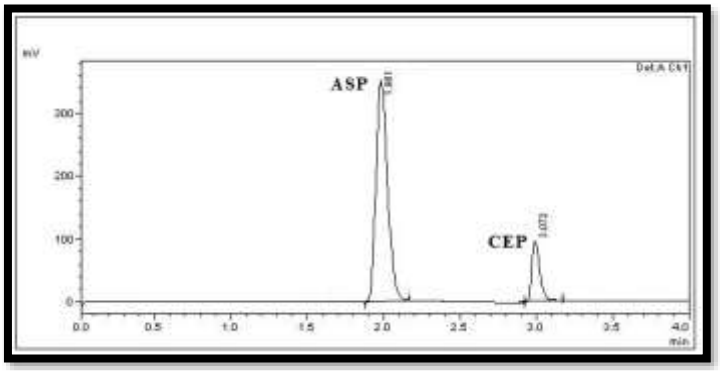

Fig.3: HPLC chromatograms for standard drugs mixture.

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 (1-50 µg/mL) of drugs. The injection of the solutions was carried out using injector loop of 10 µL and chromatograms were recorded. A graph was plotted by taking a concentration on X-axis and the area under the peak on Y-axis which gave a straight line.

Analytical method validation

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

Linearity and range

The suggested RP-HPLC method gave a good linearity in the concentration range of (1-50 µg/mL) for ASP and CEP respectively were represented in (Figure 4). The linear equations of the straight lines are y= 4294.4[X] + 499.6 (R^2 = 0.9996) for ASP and y = y = 15658[X] -7493.4 (R^2 = 0.9993) for CEP. The results are satisfactory, because there is a significant correlation between concentration of drugs and response factor within the concentration range.

Precision

The intraday reliability of the developed method was demonstrated through analysing ASP and CEP samples of different concentrations, this was repeated three times and carried out on the same day and %RSD was estimated. The interday accuracy was demonstrated by analyzing samples at different concentrations of ASP and CEP in three different days and %RSD was calculated. Repeatability was evaluated by injecting the standard drugs solutions of (4 µg / mL) four time on the same day and the value of %RSD were studied. The results obtained are shown in Table 2.

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.

Accuracy

This study was carried out to assure the closeness of the test results obtained by the analytical method to the true value [25]. For this method, ASP and CEP were measured at three selected different concentrations within the limits of Beer's law 5, 25, 35 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 98.80 – 100.23%.

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 CEP. The chromatogram of placebo was compared with those acquired from the CEP standard solution. The correlation was good (in terms of t_R and area) indicates the specificity of the method. Chromatograms of specificity for the CEP depicted in (Figures 5 and 6).

Ruggedness and robustness

The ruggedness of the proposed method was determined by analysis of aliquots of sample ASP solution (8µg/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. (0.9 mL/min and 0.7 mL/min), changing the pH by ± 0.2 % (3.8 and 4.2 %) for eluent phase and the wavelength detection changing by ± 2 nm (272 nm and 268 nm). The results obtained are shown in table 4.

Analytical assay

Three formulated samples were analysed for ASP, and CEP 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: ACN (60: 40 V/V) was delivered at a flow rate of 0.8 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 8 min. The results obtained are tabulated in Table 5. Figure 7, was shown the separation chromatograms of the drugs in different formulating samples. The recoveries of drugs in samples were between 99.77 – 100.16%.

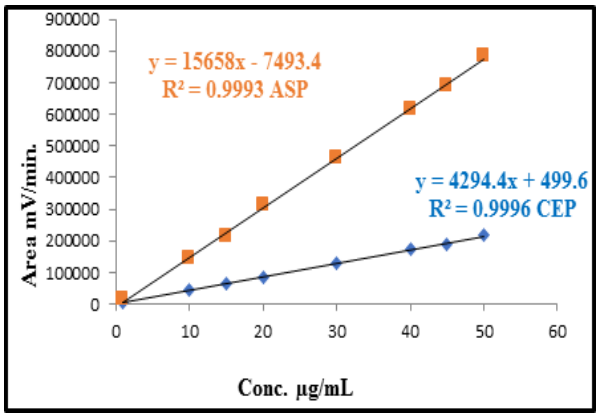

Fig.4: Calibration graph for ASP and CEP using HPLC method.

Table 2: Parameters validation summery

Sr. No.	Validation parameters	Results	Standard values
1	Linearity Range	1-50 ASP, CEP µg/L	-
2	Straight line equation	y= 4294.4[X] + 499.6 ASP y = 15658[X] -7493.4 CEP	-
3	Correlation Coefficient	0.9996 ASP, 0.9993 CEP	≥ 0.9990
4	Precision (% R.S.D.)		≤ 2.0 % R.S.D.
	Repeatability Intraday Interday	0.13 ASP, 0.11 CEP 0.37 ASP, 26 CEP 0.68 ASP, 0.55 CEP	
5	Mean % Recovery	99.64% ASP, 99.98% CEP	95 – 100%

6	Specificity	Specific	
7	LOD (µg/mL)	0.05 ASP, 0.05 CEP	-
8	LOQ (µg/mL)	0.165ASP, 0.165 CEP	-
9	Ruggedness	Complies	≤ 2.0 % R.S.D.
10	Robustness		
	Flow rate change Wavelength change Solution pH change	Complies	≤ 2.0 % R.S.D.

Table 3: Proposed method accuracy of drugs determination

ASP µg/mL		% Recovery		CEP µg/mL		% Recovery	
Taken	Found			Taken	Found		
5	4.94	98.8	Mean = 99.64	5	5.01	100.2	Mean = 99.98
25	24.99	99.96	SD = 0.74	25	24.95	99.8	SD = 0.20
35	35.06	100.17	R.S.D. 0.743	35	35.08	100.23	R.S.D. 0.200

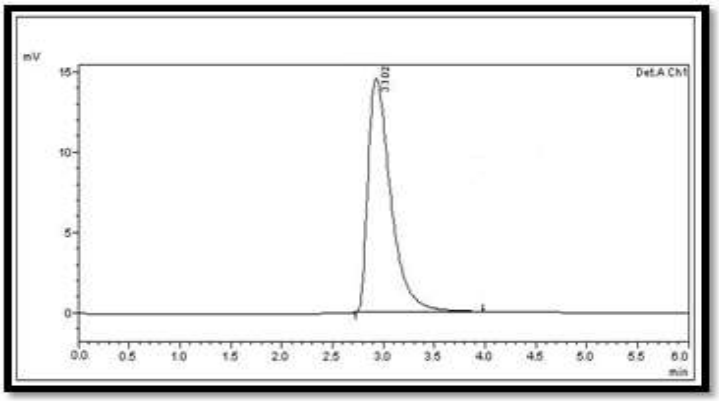

Fig.5: Specificity chromatogram of blank standard CEP (10 µg/ L)

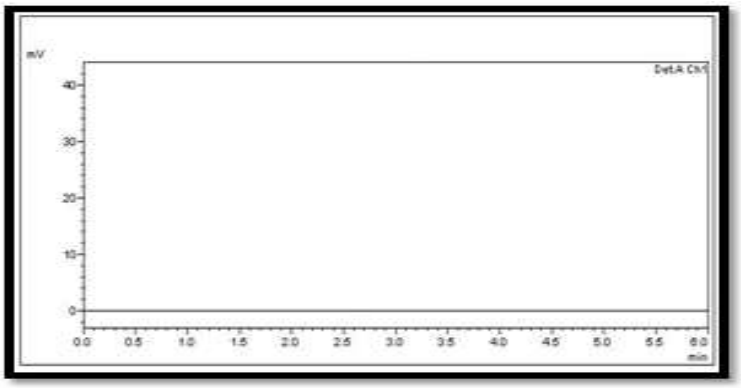

Fig.6: Specificity chromatogram of placebo in H2O: ACN 60:40

Table 4: The ruggedness and robustness 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 change 0.8 ± 0.1 mL/min.	99.92	0.45	0.45
Mobile phase pH change 4.0 ± 0.2	99.73	0.34	0.34
Detection wavelength change 270 ± 2 nm	100.15	0.21	0.199

* n = 3

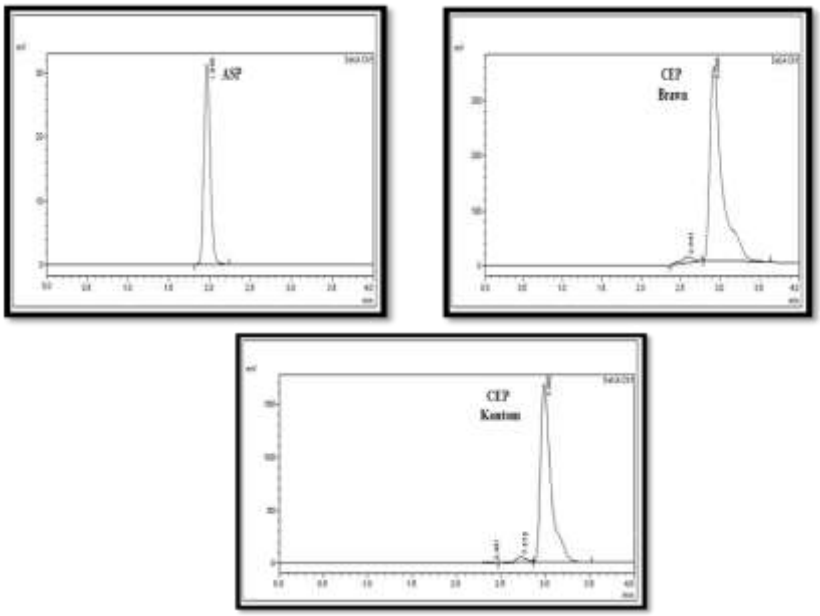

Fig.7: Separation chromatogram of drugs in different formulating samples

Table 5: Estimated quantity of drugs in different formulating samples

Drugs name	Drug Type	Label Claim mg/ tab.	Mean amount found mg/tab.	%Mean amount Found	R.S.D n=3
Aspirin Avenzop Syria	ASP	81	80.814	99.77	0.28
Brawn	CEP	500	500.8	100.16	0.15
Kontam	CEP	250	249.625	99.85	0.23

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 ASP and CEP as standard and in formulating form.

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