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Research Article

VALIDATED LC METHOD FOR SIMULTANEOUS ANALYSIS OF PARACETAMOL AND CAFFEINE IN MODEL TABLET FORMULATION

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ABSTRACT

This paper describes development and validation of a high-performance liquid chromatographic analytical procedure for simultaneously determination of paracetamol and caffeine in a tablet formulation. The separation was achieved on a C18 column at a flow rate of 1.5 ml/min with UV detection at 220 nm. The mobile phase was composed of 1mM phosphate buffer pH 3.0 – acetonitrile (85:15 *v/v*) containing 0.2 % triethylamine (*v/v*). The method was validated for analytical parameters specificity, linearity, precision, accuracy, LOD, LOQ and robustness. The linearity of the method was investigated in the concentration ranges 31.25-250 µg/ml (r = 0.9999) for paracetamol and 4.06-32.50 µg/ml (r = 0.9986) for caffeine. Mean recoveries for paracetamol and caffeine were 99.37 and 99.12 %, respectively. The analytical procedure was applied to identification, purity and assay tests on model drug formulation. It was established that the developed analytical procedure was successfully used for routine analysis of paracetamol and caffeine in model drug dosage form without any interference from included excipients.

Keywords: Liquid chromatography, Validation, Paracetamol, Caffeine

INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular over-the-counter analgesic and antipyretic drug. Paracetamol is available in different dosage forms - tablets, capsules, suspensions, syrups, drops, elixirs and suppositories. Acetaminophen is generally administered in tablets containing 500 mg of active drug formulated with excipients. The usage of paracetamol alone or in combination with other drugs, such as caffeine is well established in the pharmaceutical formulations. Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) is an alkaloid from N-methyl derivatives of xanthine widely distributed in natural products, commonly used in beverages. It possess many physiological effects, such as stimulation of central nervous system, diuresis and gastric acid secretion. Caffeine is used therapeutically in combination with ergotamine in the treatment of migraine or in combination with nonsteroidal anti-inflammatory drugs in analgesic formulations.

Because paracetamol as well as caffeine are being increasingly used for therapeutic purposes, their determination and quality control are of significant importance.

Several methods for simultaneous determination of paracetamol and caffeine have been recently reported, such as chromatographic [1-15] fluorescent [16], spectrophotometric [17-33] and electrochemical techniques [34].

The aim of this study is to develop an analytical procedure for simultaneously determination of acetaminophen and caffeine in a model tablet formulation containing 500 mg paracetamol and 65 mg caffeine using isocratic reversed phased liquid chromatographic method. The study includes determination of analytical parameters in identification, purity and assay tests in accordance to European Pharmacopoeia (Eur. Ph.) and ICH requirements.

MATERIALS AND METHODS

Materials

Paracetamol RS and caffeine RS were used as standards. HPLC grade acetonitrile was used to prepare the mobile phase. All other chemicals for chromatographic experiments were of reagent grade. A model tablets containing 500 mg paracetamol and 65 mg caffeine were prepared. Povidone (type Kollidon 30) K 30, lactose monohydrate, microcrystalline cellulose (type Avicel PH101), corn starch, magnesium stearate and talk were used as excipients.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µl loop, column oven CTO-10A, SPD-M10A UV detector with fixed wavelength and communication bus module CBM-10A. A LiChrosorb C18, 250 mm x 4.6 mm, 5 µm particle size column was used as a stationary phase. The components were separated isocratically with a mobile phase consisting of 1mM phosphate buffer pH 3.0 – acetonitrile (85:15 *v/v*) containing 0.2 % triethylamine (*v/v*) at a flow rate of 1.5 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed. The analysis was carried out at an ambient temperature and injection volume was 20 µl. The UV detector was set at 220 nm.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving of accurately weighed 50.0 mg paracetamol CRS and 6.50 mg caffeine CRS in methanol in a 100.0 ml volumetric flask. Reference solution (b): The solution was prepared by diluting of 5.0 ml from reference solution (a) into a 20.0 ml volumetric flask with methanol.

Sample preparation

The homogenized powder from twenty tablets with average weight equivalent to amount of 50 mg paracetamol and 6.5 mg caffeine was transferred to a 100.0 ml volumetric flask. Approximately 70 ml of methanol were added and the obtained mixture was sonicated for 20 min with intermittent shaking. The contents were restored to room temperature and diluted to volume with methanol to furnish stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 5.0 ml was diluted into a 20.0 ml volumetric flask to give test solution containing 125 µg/ml paracetamol and 16.25 µg/ml caffeine.

Validation Procedure

The analytical method described is validated [35-38] and also applied to routine analysis of paracetamol and caffeine in combined dosage form.

Specificity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated

by evaluating specificity. The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 31.25 to 250 µg/ml for paracetamol and from 4.06 to 32.50 µg/ml for caffeine. The calibration curves were constructed by plotting peak areas versus concentrations of paracetamol and caffeine, and the regression equations were calculated. Each response was the average of three determinations.

Precision

The precision of the method, as intra-day repeatability, was evaluated by performing six independent assays of the test sample preparation and calculating RSD (%). The intermediate (inter-day) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions.

Accuracy

Accuracy was studied by adding three different amounts (corresponding to 50, 100, and 150% of the test preparation concentrations) of paracetamol and caffeine to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was injected in duplicate.

Robustness

The robustness of the method is a measure of the capacity to remain unaffected by small variations in method parameters and provides indication of its reliability during normal usage. Robustness of the analytical procedure was studied by deliberately varying parameters like mobile phase composition (±2% of organic solvent) and flow rate (±0.2 ml/min). Column-to-column reproducibility was also checked by using a C18 column of different make (Nucleosil) with same dimension.

Solution Stability

Sample solution stability was evaluated by storing the solution at ambient temperature and at 2-5ºC and analysis after 12, 24, 36, and 48 h. The responses from the aged solutions were compared with those from freshly prepared standard solutions.

Preparation of model tablets

Tablets were prepared by compression after wet granulation with a single punch tablet press (EK 0, Korsch, Berlin, Germany). A set of 13 mm diameter standard concave tooling was used for the preparation of tablets. All model tablets containing 500 mg paracetamol and 65 mg caffeine. Povidone (type Kollidon 30) K 30, lactose monohydrate, microcrystalline cellulose (type Avicel PH101), corn starch, magnesium stearate and talk were used as excipients. The uniformity of mass of obtained tablets was 654 mg ± 5%, friability of tablets was 0.6 %, and mechanical strength in range 70-80 N.

Determination of mechanical strength of the model tablets

It was performed by the method of the progressive loading according Eur. Ph. 7.0 (2.9.8), apparatus - Erweka type TBH 30, Germany.

Determination of friability

It was performed according Eur. Ph. 7.0 (2.9.7), in friabilitor– Erweka TAR 20, Germany.

In vitro drug dissolution studies

Drug release profiles were evaluated using a dissolution test apparatus (Erweka DT 600, Hensenstmm, Germany). The USP paddle method was selected. The test was carried out at a paddle rotation speed of 50 rpm, maintained at 37 ± 0.5 °C, in 900 ml dissolution medium at 5.8 pH value. Withdrawing 1.0 ml filtered (0.45µm) samples at preselected intervals of 5 minutes (up to 30 minutes). The quantity of paracetamol and caffeine in sample solutions was analyzed by described above isocratic reversed phased liquid chromatographic method. The cumulative percentage of drug release was calculated and the average of six determinations was used in the data analysis. The statistical analysis of the dissolution data of the tablets showed a statistical difference ($p < 0.05$) using t-test by Origin Plot software.

RESULTS AND DISCUSSION

From the chromatogram shown in Fig. 1, it is evident, that under the proposed chromatographic conditions, paracetamol and caffeine were completely separated, which indicated that the method is selective and could be used for their simultaneous identification and quantification.

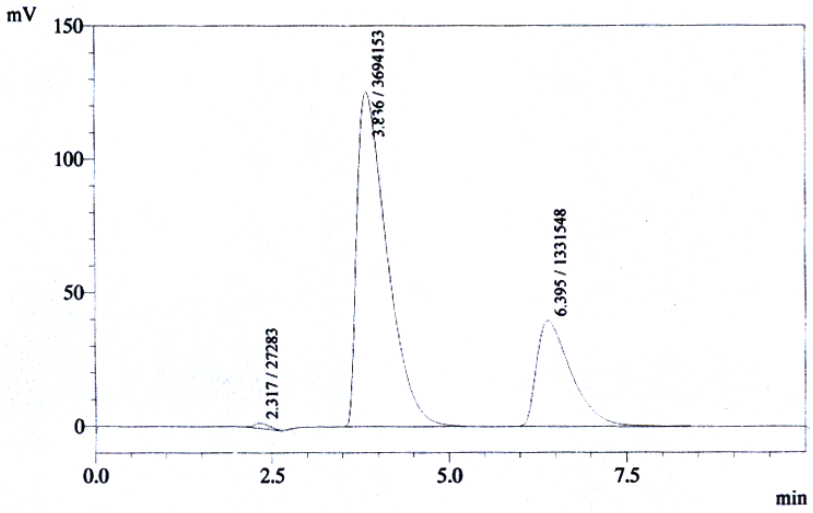

Fig. 1: Chromatogram obtained from paracetamol RS and caffeine RS

Retention times, number of theoretical plates and tailing factors obtained by used of the HPLC method were listed in Table 1.

Table 1: System suitability test parameters for paracetamol and caffeine

Parameter	Paracetamol	Caffeine
Retention time (min)	3.84	6.39
Tailing factor	0.85	0.82
Theoretical plates	6584	7560

Validation of analytical procedure

The analytical method was validated to furnish evidence it was suitable for purpose.

Specificity

The specificity of the method was determined by checking for interference with the analytes from placebo components. No interference from tablet excipients was found.

Linearity and range

Linear correlation was obtained between peak area and concentration in the range from 31.25 to 250 µg/ml for paracetamol and 4.06 to 32.50 µg/ml for caffeine. Linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r). The regression analysis of the calibration curves is shown in Table 2.

Precision

The RSD values measured during assessment of intraday and interday precision were <2.0% for both paracetamol and caffeine, confirming the method is precise (Table 3).

Accuracy

Recovery of paracetamol and caffeine from placebo was determined at three different concentrations. Mean recovery was 99.26-99.43 % for paracetamol and 98.65-99.40 % for caffeine (Table 4).

Robustness

It was found that the elution order and resolution for both components were not significantly affected by small variation of the conditions. Results from study of the robustness of the method were listed in Table 5.

Table 2: Linear regression data for calibration curves

Drugs	Paracetamol	Caffeine
Concentration range (µg/ml)	31.25-250.0	4.06-32.50
Slope	29342.6	816916
Intercept	37241.5	35531.2
Correlation coefficient (r)	0.9999	0.9986

Table 3: Intra-day and inter-day precision of the method described

Paracetamol			Caffeine		
Amount claimed (mg/tablet)	Amount found (mg/tablet)		Amount claimed (mg/tablet)	Amount found (mg/tablet)	
	Intra-day repeatability	Inter-day repeatability		Intra-day repeatability	Inter-day repeatability
	498.2	498.1		64.85	64.32
500.0	499.1	498.5	65.00	63.98	63.87
	499.3	497.9		65.03	64.87
	501.0	498.9		64.56	64.35
	498.9	498.6		64.12	65.01
	499.6	498.1		65.10	64.84
Mean	499.3	498.9	Mean	64.61	64.32
SD	0.93	1.00	SD	0.47	0.44
%RSD	0.19	0.20	%RSD	0.73	0.68

Table 4: Results from study of accuracy

Drug	Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	Mean recovery (%) ± SD	RSD (%)
	50	61.92	61.78		
Paracetamol			61.56	99.42±0.355	0.36
			61.34		
	100	125.6	124.8		
			124.2	99.26±0.355	0.33
			125.0		
	150	187.8	187.2		
			186.9	99.43±0.305	0.31
			186.1		
	50	8.12	8.05		
Caffeine			8.00	98.65±0.444	0.45
			7.98		
	100	16.20	16.08		
			16.05	99.30±0.252	0.25
			16.13		
	150	24.32	24.16		
			24.11	99.40±0.289	0.29
			24.25		

Table 5: Robustness parameters of LC method

Factor	Level	Paracetamol		Caffeine	
Mobile phase composition		**Assay, %**	**% RSD**	**Assay, %**	**% RSD**
83:17	-2	100.0	0.65	98.8	0.98
85:15	0	99.65	0.93	99.6	1.05
87:13	+2	99.48	0.92	99.1	0.87
Flow rate of mobile phase					
1.3	-0.2	98.70	0.64	98.2	0.56
1.5	0	101.0	0.89	99.6	0.85
1.7	+0.2	99.82	0.97	99.8	1.21
Stationary phase					
Nucleosil C 18	-	99.81	0.91	99.1	1.04
LiChrosorb C18	-	99.55	0.98	99.6	0.95

Limit of Detection and Limit of Quantification

The limits of quantification (LOQ) and limit of detection (LOD) were evaluated based on signal-to-noise ratios by serial dilution of paracetamol and caffeine reference solution (b). The LOQs for paracetamol and caffeine were found to be 5.0 and 2.5 µg/ml respectively; the LODs - 2.0 and 0.5 µg/ml, respectively.

Solution stability

Table 6 showed the results obtained from study of the stability of the test preparation. It was concluded the test solution was stable for up to 48 h at 2-5ºC and at ambient temperature, because differences between measured and original values were <2.0%.

Table 6: Results from study of solution stability

Time (h)	Assay (%), test solution stored at 2-5ºC		Assay (%), test solution stored at ambient temperature	
	Paracetamol	Caffeine	Paracetamol	Caffeine
Initial	99.98	98.91	99.97	98.91
12	99.94	98.82	99.98	98.90
24	99.81	98.80	99.91	98.83
36	99.81	98.81	99.90	98.80
48	99.65	98.80	99.89	98.80

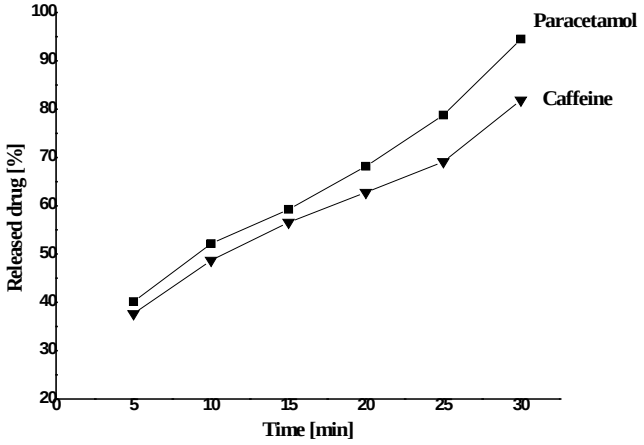

Fig. 2: Release kinetics of paracetamol and caffeine from model tablets (n = 6)

Release kinetics of paracetamol and caffeine from model drug tablets

The release profiles of paracetamol and caffeine from model tablets at pH 5.8 were presented in Fig. 2.

From the figure it can be clearly seen that quantity of drugs released on the fifth minute was 40.16% of included paracetamol and 37.64% of included caffeine. The kinetic of drug release is closest to zero order (R for paracetamol is 0.992 and for caffeine – 0.993). The time of release of 80% (Q) of included amount of drugs is 25.5 min and 28.9 min for paracetamol and caffeine, respectively. The results show that the described method has required accuracy and robustness for analysis of paracetamol and caffeine in combined dosage in real conditions form without any interference from common excipients.

CONCLUSION

The validated RP-LC method developed in this study proved to be simple, specific, accurate, precise, sensitive and robust. It can successfully used for routine analysis of paracetamol and caffeine in combined dosage form without any interference from common excipients.

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