Research Article

HPLC Assay and Stability Studies of Tablets Containing Paracetamol and Caffeine

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ABSTRACT

A high-performance liquid chromatography analytical procedure for the simultaneous determination of paracetamol and caffeine in a model tablet formulation has been developed. The separation was achieved on a C8 column at a flow rate of 1.0 ml/min with UV detection at 230 nm. The mobile phase was composed of 1mM phosphate buffer pH 7.0 – methanol (65:35 *v/v*). The method was validated through investigation of analytical parameters such as specificity, linearity, precision, accuracy, LOD and LOQ. 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 = 0.9998) for caffeine. The method was found to be accurate, with recoveries in the range 99.57% – 99.87%. Stability studies of a model tablet formulation containing 500 mg paracetamol and 65 mg caffeine were carried out at 25 ± 2°C and RH = 60 ± 5% (long term storage), for one year, and at 40 ± 2°C and RH = 75 ± 5% (accelerated storage). Essential physical-chemical properties of the tablets were evaluated such as: mechanical strength, disintegration time, drug release kinetics and drug content. No significant changes were found in any of the evaluated tablet characteristics. It was established that the produced tablets possess good stability characteristics which allows to proceed to clinical evaluation and scale up of the product.

Keywords: Stability studies, liquid chromatography, validation, paracetamol, caffeine.

INTRODUCTION

Paracetamol (acetaminophen, N-acetyl-p-aminophenol) is a safe and effective analgesic and antipyretic agent although its anti-inflammatory effect is weak [1]. Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) is an alkaloid N-methyl derivative of xanthine widely distributed in natural products, commonly used in beverages. Caffeine is used therapeutically in combination with ergotamine in the treatment of migraine or in combination with nonsteroidal anti-inflammatory drugs in analgesic formulations. The use of the mixture of paracetamol and caffeine as an analgesic and antipyretic is well established in pharmaceutical practice[2]. In order to achieve better therapeutic effect and lower toxicity, it is very important to control the content of paracetamol and caffeine in pharmaceutical formulations[3]. Recently, several methods for simultaneous determination of paracetamol and caffeine have been reported, including chromatographic[2,4-10], fluorescent[11], spectrophotometric[2, 12-24] and electrochemical techniques [25].

At the stage of development of a drug product, extensive stability evaluation must be performed. Preliminary (intermediate) stability testing is conducted to establish the maximum time for which a drug product can be stored in intermediate containers. The data could be used for further processing of the drug product, such as pre-clinical testing in humans and preliminary stability studies to evaluate the shelf life of the final product[26]. Stability studies should include testing of those attributes of the drug product that are susceptible to changes during storage. The testing should cover adequate physical-chemical characteristic of the drug dosage form.

In our previous study, an investigation of an optimized formulation of tablets containing 500 mg paracetamol and 65 mg caffeine[27] was reported. The aim of the present study is to (i) develop a HPLC method for simultaneous determination of paracetamol and caffeine; (ii) perform stability studies of the optimized tablet formulation.

MATERIALS AND METHODS

Materials

Paracetamol RS and caffeine RS were used as standards. HPLC grade methanol was used to prepare the mobile phase. All other chemicals used for the chromatographic experiments were of a reagent grade. For the preparation of the tablets, paracetamol and caffeine substances of Eur. Ph. grade were used. Povidone, microcrystalline cellulose, lactose monohydrate, corn starch, magnesium stearate and talk were used as excipients in the preparation of the tablets. All used excipients were of an analytical grade.

Methods

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a modular HPLC system LC-10AShimadzu (Japan) comprising 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 C8,

250 mm x 4.6 mm, 5 µm particle size column was used as a stationary phase. The components were separated isocratically using a mobile phase consisting of 1mM phosphate buffer pH 7.0 – methanol (65:35 v/v) at a flow rate of 1.0 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 the injection volume was 20 µl. The UV detector was set at 230 nm.

Preparation of reference solutions

Reference solution (a): The solution was prepared by dissolving 50.0 mg of precision-weighed 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 5.0 ml of reference solution (a) with methanol, into a 20.0 ml volumetric flask.

Sample preparation

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

Preparation of tablets

Tablets were produced through compression after wet granulation. A single punch tablet press (EK 0, Korsh, Germany) at 20 kN pressure and a set of 13 mm diameter standard concave tooling were used. The tablets containing 500 mg paracetamol and 65 mg caffeine had a total weight of 654 mg.

Determination of mechanical strength

The study was carried out by the progressive loading method according to Eur. Ph. 7.0 (2.9.8) using an Erweka type TBH 30 apparatus (Germany).

Determination of friability

The study was performed according to Eur. Ph. 7.0 (2.9.7) using an Erweka type TAR 20 friabilator (Germany).

Determination of disintegration time

The investigation was performed according to Eur. Ph 7.0 (2.9.1) in the basket-rack assemble using an Erweka, type ZT 3 apparatus (Germany).

In vitro drug dissolution studies

The USP Apparatus 2 (paddle) was chosen to evaluate drug release profiles, using Erweka type DT 60, Hensenstmm (Germany). The dissolution test was carried out in 900 ml of buffer solution with pH 5.8 (phosphate buffer according to Eur.Ph. 7.0), at a paddle speed of 50 rpm. Five milliliter samples were withdrawn and filtrated (through a 0.45 µm filter) at predetermined intervals of 5, 10, 15, 20, 25 and 30 minutes. The amounts of paracetamol and caffeine dissolved in the samples were determined by the above described HPLC analytical procedure.

Stability studies

Stability studies of the tablets were carried out for two sets of storage conditions according to ICH Q1A (R2) [28] (i) at 25 ± 2 °C and RH = 60 ± 5% (long term storage conditions), for one year, and (ii) at 40 ± 2 °C and RH = 75 ± 5% (accelerated storage conditions), for six months. For this purpose a sufficient number of tablets was packed in amber colored screw capped bottles and kept into a climatic chamber under the above conditions. Samples were taken out periodically, at every three months, and evaluated for: appearance, hardness, friability, disintegration, dissolution, and drug content. No intermediate test was included in this study because no "significant changes" were established in the drug product stored using the accelerated method [28].

RESULTS AND DISCUSSION

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

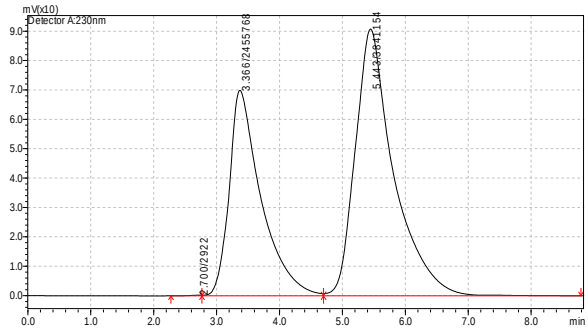

Figure 1: Chromatogram of paracetamol RS and caffeine RS

Method validation

The proposed method was validated as per ICH guidelines with respect to specificity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Specificity

The specificity of the method was determined by checking the interference of the components against placebo. No interference was observed for any of the excipients of both drugs.

Calibration and linearity

Calibration curves were plotted in the range of 31.25-250.0 µg/ml for paracetamol and 4.06-32.50 µg/ml for

caffeine. The corresponding linear regression equations were *y=21542.1x-32650.4* with a squared correlation coefficient R^2 0.9999 for paracetamol and *y=78023.1x-2918.4* with R^2 of 0.9998 for caffeine, respectively. An excellent correlation existed between the peak areas and the concentrations of both compounds.

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD values calculated for assessment of the precision were <2.0% for both analytes, confirming that the method is precise.

Accuracy

The accuracy of the method was determined by calculating the recoveries of paracetamol (PAR) and caffeine (CAF) by the standard addition method. Known amounts of standard solutions of both PAR and CAF (50, 100, and 150%) were added to prequantified sample solutions of tablets. The amounts of drugs were determined by substituting these values in the regression equation of the calibration curves.

The method was found to be accurate with recoveries of 99.57%–99.87% and an acceptable RSD of not more than 2% at each level. The recoveries obtained by the proposed method for PAR and CAF are shown in Table 1.

Limit of quantitation and limit of detection

The limit of quantitation and limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio as per ICH guidelines[29]. The LOQs for paracetamol and caffeine were found to be 4 µg/ml and 2 µg/ml, while the LODs were 0.5 µg/ml and 0.2 µg/ml, respectively.

Stability studies

The results of the conducted stability studies are presented in Tables 2 and 3. All compendia requirements for physicochemical stability were accomplished for both long term and accelerated storage conditions covering a one year period and a six month period, respectively. The data revealed no significant changes in all evaluated characteristics of the tablets. The uniformity of the tablet mass was 654 ± 5%.

Table 1: Results from study of accuracy

Amount of sample (µg/ml)		Sets	Amount drug of spiked (µg/ml)		Average amount recovered (µg/ml)		Mean recovery (%) ± SD		% RSD	
PAR	CAF		PAR	CAF	PAR	CAF	PAR	CAF	PAR	CAF
30	4	1	0	0						
30	4	2	0	0	29.88	3.99	99.61±0.92	99.68±1.04	0.92	1.04
30	4	3	0	0						
30	4	1	15	2						
30	4	2	15	2	44.82	5.98	99.61±0.65	99.68±1.34	0.65	1.34
30	4	3	15	2						
30	4	1	30	4						
30	4	2	30	4	59.79	7.99	99.64±0.59	99.84±0.52	0.59	0.52
30	4	3	30	4						
30	4	1	45	6						
30	4	2	45	6	74.67	9.99	99.57±0.80	99.87±0.70	0.80	0.70
30	4	3	45	6						

Table 2: Stability study results

Study ↓	Months ↓	Appearance	Uniformity of mass [mg]	Hardness [N] ± sd	Friability [%]	Disintegration [min]
Acceptance criteria →		White, round-concave tablets	654 ± 5%	70-90	< 1.0	< 15
Initial	0	complies	654 ± 5%	80 ± 3.4	0.62	7
Long-term T = 25 ± 2 °C RH =60 ± 5%	3	complies	complies	77 ± 4.1	0.59	6
	6	complies	complies	79 ± 3.7	0.65	6
	9	complies	complies	77 ± 4.6	0.63	7
	12	complies	complies	76 ± 4.4	0.67	5
Accelerated T = 40 ± 2 °C RH =75 ± 5%	3	complies	complies	79 ± 3.8	0.66	6
	6	complies	complies	77 ± 4.5	0.68	5

Table 3: Stability study results

Study	Months	Dissolution 30 min [%]		Drug content [%]	
		Paracetamol	Caffeine	Paracetamol	Caffeine
Initial	0	94.3	80.8	493.4	64.8
Long-term T=25 ± 2 °C RH = 60 ± 5%	3	-	-	490.5	64.6
	6	-	-	490.8	64.6
	9	-	-	487.4	64.4
	12	94.8	86.5	482.0	64.2
Accelerated T = 40 ± 2 °C RH = 75 ± 5%	3	-	-	491.3	64.2
	6	92.4	82.8	486.2	63.8
Acceptance criteria		≥ 80%	≥ 80%	NLT 95% and NMT 105%	

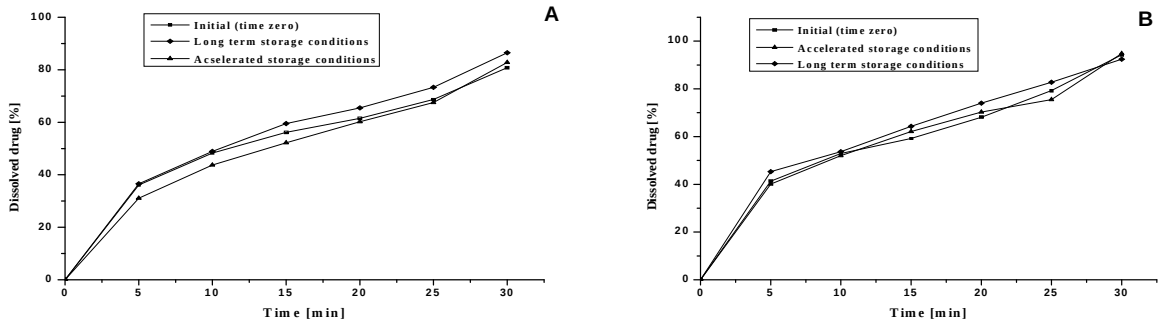

Figure 2: Release kinetics of paracetamol (A) and caffeine (B) for the model tablet formulation (i) Initial prepared model tablets (time zero); (ii) Long term storage conditions at 25 ± 2 °C and RH = 60 ± 5% for one year; (iii) Accelerated storage conditions at 40 ± 2 °C and RH = 75 ± 5% for six months.

The mechanical properties of the tablets were within the acceptance range and the disintegration time was from 5 to 7 minutes. The contents of paracetamol and caffeine were within the required range of 95-105% of the labeled amount, for all storage conditions.

The dissolution profiles of the tablets stored under long term and accelerated conditions were compared with the dissolution profiles of the initial prepared model tablets (time zero). Similarity was established of the kinetic dissolution profiles of the drugs between the initial prepared tablets and those after storage for one year at 25°C and for six months at 40°C. The results are presented in Fig. 2.

For paracetamol, the calculated factor of similarity (f_2) between the initial prepared tablets and those stored under long term conditions was 75.58, and f_2 between the initial prepared tablets and those stored under accelerated conditions was 69.57. For caffeine, f_2 between the initial prepared tablets and those stored under long term conditions was 71.06 and f_2 between the initial prepared tablets and those stored under accelerated conditions was 73.68.

CONCLUSION

A validated HPLC for simultaneous determination of paracetamol and caffeine in model tablets has been developed. The study proved that the method was specific, accurate, precise, sensitive and robust. Stability determination of an optimized tablet formulation containing 500 mg paracetamol and 65 mg caffeine was performed. It showed no significant changes in the main characteristics of the tablets. It could be concluded that the produced tablet formulation has good stability features which allows to proceed to clinical assessment and scale up of production.

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