Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

SIMULTANEOUS DETERMINATION AND VALIDATION OF PARACETAMOL AND CODEINE PHOSPHATE IN PHARMACEUTICAL PREPARATION BY RP-HPLC

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Received: 30 Jan 2013, Revised and Accepted: 26 Mar 2013

ABSTRACT

An accurate, simple, reproducible and sensitive method for the determination of Paracetamol and Codeine phosphate was developed and validated. Paracetamol and Codeine were separated by HPLC using a LiChrospher® RP-18 column and isocratic elution with a flow rate of 1.0 ml/min. Mixture of Acetonitile with Buffer solution (pH=2.5) (15:85) was used as mobile phase. The detection was at 210 nm wavelength. The proposed method was validated for linearity, accuracy, precision, limit of detection and limit of quantification. The linear range of determination for Paracetamol and Codeine phosphate were 100-1000µg/ml and 6-60 µg/ml, respectively. The percentage recovery of Paracetamol and Codeine phosphate were obtained form range of 99.88-100.2% and 99.33-100.3% respectively. The developed method is suitable for routine quality control analysis of titled drugs in combination of tablet formulation.

Keywords: Paracetamol, Acetaminophen, Codeine phosphate, RP-HPLC.

INTRODUCTION

Paracetamol (acetaminophen), N-(4-Hydroxyphenil)-acetamide (Fig.1) is a widely used analgesic and antipyretic agent for the relief of fever, headaches, minor pains, etc. It is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs and opioid analgesics, Paracetamol is used also in the management of severe pain (such as post operative pain). Paracetamol alone or in combination with other drugs is reported to be estimated by titrimetry [1-2], spectrophotometric method [3-5], HPLC [6-7], TLC [8], HPTLC [9], UHPLC [10], LC-MS [11], FT-IR [12], amperometric determination [13] and fluorimetry [14].

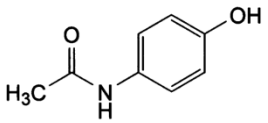

Fig. 1: Structure of Paracetamol

Codeine phosphate (7,8-Didehydro-4,5a-epoxy-3-methoxy-17-methylmorphinan-6a-ol) phosphate is predominant alkaloid in opium [15-16]. It is considered as a pro-drug, metabolized to active compounds of morphine and codeine-6-glucoronide [17]. Codeine (Fig.2) is the traditional choice for the treatment of moderate opioid-sensitive pains. Codeine phosphate in combination with other compounds has been determined in different pharmaceutical preparations by GLC [18], TLC [19], UV [20-21] and HPLC [22-26]. Combinations of Codeine with Paracetamol produce a significant increase in analgesia compared with Paracetamol alone. These pharmaceutical formulations accounted for 20% of total non-opiate analgesics during the last decade [27]. Their quality control is thus of paramount importance, especially the determination of Paracetamol in pharmaceuticals has been critically reviewed since its overdose can cause fulminating hepatic necrosis and other toxic effects [28].

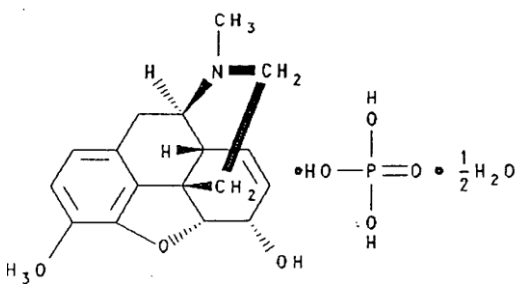

Fig. 2: Structure of Codeine Phosphate

In an effort to develop a simple and accurate method for routine analysis, this study describes HPLC-UV method, for the simultaneously determination of these drugs. Validation of the current method is performed according to the requirements of International Conference on Harmonization (ICH) guideline [29].

MATERIALS AND METHODS

Materials and Chemicals

Paracetamol and Codeine phosphate reference standards were obtained from Sigma Aldrich. Tablet formulation containing Codeine phosphate hemihydrates 30 mg and Paracetamol 500 mg were obtained commercially. HPLC grade Methanol was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation and Chromatographic Conditions

HPLC analysis was performed by isocratic elution with a flow rate 1.0 ml/min. A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD quaternary pump) with an auto sampler, Shimadzu DGU-20A_5 vacuum degasser and a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software. Separation was carried out at 30ºC, using LiChrospher® RP-18 (250 x 4.6 mm) column packed with octadecylsilyl silica gel 5 µm,. The detector was set at 210 nm. The chromatogram is shown in Fig. 3.

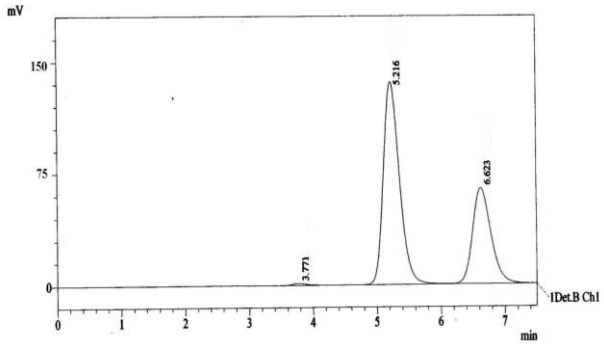

Fig. 3: Typical chromatogram of Paracetamol and Codeine phosphate

Preparation of Mobile Phase

The mobile phase (1000 ml) was prepared by mixing of Acetonitile, 1 ml triethylamine and Buffer solution (pH=2.5) (15:85). The mobile phase was sonicated for 10 min and then it was filtered through a 0.45 µm filter paper.

Preparation of Buffer Solution

2.04 g of monobasic potassium phosphate were dissolved into 1000 ml of water, and adjusted pH to 2.5 with o-phosphoric acid.

Preparation of Standard Solution

500 mg of Paracetamol and 30 mg Codeine phosphate working standards (accurately weighed) were transferred into a 100 ml volumetric flask. After addition of about 50 ml mobile phase, the mixture was sonicated for about 10 min and after made up to the volume. The stock solution was suitably diluted to produce a concentration of 0.5 mg/ml of Paracetamol and 0.03 mg/ml of Codeine phosphate respectively.

Sample Preparation

Twenty tablets were weighed, finely powdered and the average weight was determined. A portion of powder equivalent to 500 mg Paracetamol and 30 mg Codeine phosphate is transferred into 100 ml volumetric flask and 50 ml of mobile phase was added and sonicated for 10 minutes to effect complete dissolution of both substances. The suspension was then made up to volume with mobile phase and after filtered. The aliquot portion of the filtrate was further diluted to get final concentration of 500 µg/ml of Paracetamol and 30 µg/ml of Codeine phosphate. 20 µl of the test solution were injected, chromatogram was recorded and the amounts of the drugs were calculated.

RESULTS AND DISCUSSION

The developed method for determination of Paracetamol and Codeine phosphate was further validated by using the following parameters:

Selectivity

Selectivity of the current method was demonstrated by good separation of the two active ingredients (Paracetamol and Codeine phosphate). Furthermore, matrix components, e.g. excipients, do not interfere with the two analytes.

Linearity

Standard solutions containing Paracetamol (100-1000 µg/ml) and Codeine phosphate (6-60 µg/ml) were prepared in the mobile phase. Triplicate 20 µl injections were made for each standard solution to estimate the reproducibility of the detector response at each concentration level and chromatographed under the conditions described above. The area of each peak was plotted against the concentration to obtain the calibration graph (Fig.4 and 5). The five concentrations of each compound were subjected to regression analysis to calculate the calibration equation and correlation coefficients.

The results obtained are shown in the Tables 1-2 and show that the current method was linear for the two analytes in the range specified above with a correlation coefficients better than 0.999.

Table 1: Linearity data for Paracetamol

Linearity Level	Concentartion (µg/ml)	Area
1	100	194811
2	250	397621
3	500	795244
4	750	1192864
5	1000	1590483
Correlation Coefficient		0.999

Table 2: Linearity data for Codeine phosphate

Linearity Level	Concentartion (µg/ml)	Area
1	6	10977
2	15	23953
3	30	47906
4	45	71859
5	60	95812
Correlation Coefficient		0.999

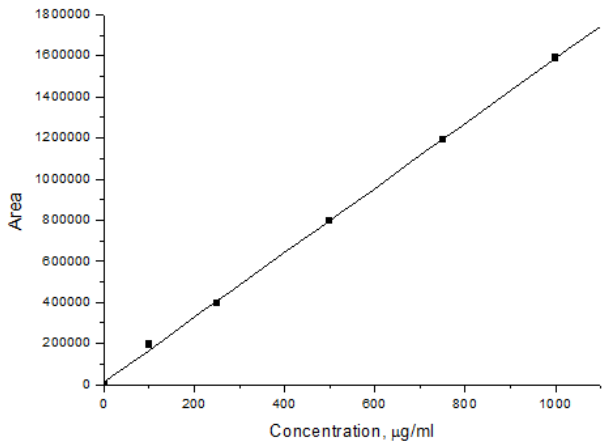

Fig. 4: Linearity graph of Paracetamol

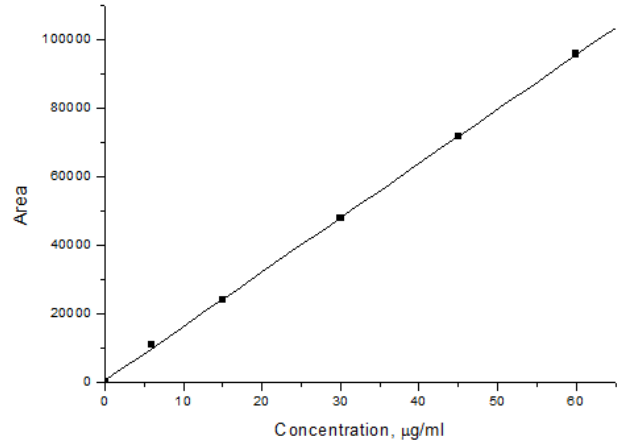

Fig. 5: Linearity graph of Codeine phosphate

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were experimentally verified by six injections of Paracetamol and Codeine phosphate at the appropriate concentrations. The LOD was calculated to be 1.0 and 0.06 µg/ml and the LOQ was calculated to be 10.0 and 0.6 µg/ml for Paracetamol and Codeine phosphate, respectively.

Precision

The system precision of this method was evaluated by calculating the %RSD of the peak areas of six replicate injections of the standard solution, which were found to be 0.39% and 0.37%. For method precision evaluated with six sample replicate injections were found to be 0.41% and 0.39% for Paracetamol and Codeine phosphate respectively and it was found to be less than 1.0% shown in the Table 3.

Table 3: Results of precision for Paracetamol and Codeine phosphate

	Paracetamol	Codeine phosphate
System Precision		
% RSD	0.39	0.37
Method Precision		
%RSD	0.41	0.39

Accuracy

Accuracy of the method was calculated by recovery studies. It is carried out by preparing the samples of 50%, 100% and 150% of target concentration. The samples were prepared in triplicate in each level. The results of studies along with its evaluation are given in the Table 4.

Table 4: Results of % recovery studies for Paracetamol and Codeine phosphate

Sample	Recovery	Amount present	Amount recovered	% recovered	SD	%RSD
	50%	15 mg	14.80	99.66	1.728	1.731
Codeine phasphate	100%	30 mg	29.80	99.33	1.099	1.102
Paracetamol	150%	45 mg	45.13	100.3	0.868	0.865
	50%	250 mg	249.7	99.88	1.022	1.023
	100%	500 mg	501.1	100.2	0.543	0.544
	150%	750 mg	748.4	99.79	1.031	1.029

CONCLUSION

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Paracetamol and Codeine phosphate was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for both drugs are less than 8 min and shows high degree of accuracy and precision with less than 2 % RSD. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

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