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Full Length Research Paper

Simultaneous high-performance liquid chromatography determination of paracetamol and ascorbic acid in tablet dosage forms

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A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method has been developed for the simultaneous determination of paracetamol and ascorbic acid in tablet dosage form. The chromatographic separation was achieved on a LiChrosorb C18, 250 mm × 4.6 mm, 5 µm column at a detector wavelength of 254 nm and a flow rate of 1.0 ml min^{-1}. The mobile phase was 1 mM sodium pentane sulphonate in a mixture of 0.4 volume of formic acid, 25 volume of methanol and 75 volume of water. The retention times for ascorbic acid and paracetamol were found to be 3.53 and 6.09 min, respectively. The method was validated for parameters like specificity, linearity, precision, accuracy, robustness, limit of quantitation and limit of detection. The method was found to be specific as no other peaks of impurities and excipients were observed. The square of correlation coefficients (R^2) for paracetamol and ascorbic acid were 0.9999 and 0.9998 while percentage mean recoveries were 99.16 and 98.76%, respectively. Intra- and inter-day relative standard deviations for both the components were <2.0%. The proposed RP-LC method can be applied for the routine analysis of commercially available formulations of these drugs either as such or in combination.

Key words: Liquid chromatography, validation, paracetamol, ascorbic acid.

INTRODUCTION

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug. It is well tolerated and lacks many of the side effects of aspirin, so it is commonly used for the relief of fever, headaches, and minor aches and pains as well as for the management of more severe pain, where it allows lower dosages of additional nonsteroidal anti-inflammatory drugs to be used, thereby minimizing overall side effects (Goyal and Singh, 2006; Dongre et al., 2009; Graham and Scott, 2005).

Vitamin C or ascorbic acid, a water-soluble vitamin, is widely present in many biological systems and in multivitamin preparations. Ascorbic acid is commonly used to supplement inadequate dietary intake and as an antioxidant (Kleszczewski and Kleszczewska, 2002). The synergetic and protective effects conferred during the use of paracetamol for therapeutic purposes by its combination with vitamin C and/or other pharmacological and biologically active compounds should also be mentioned (Raghavendran et al., 2004). The use of complementary presence of ascorbic acid intensifies the main favorable effect of paracetamol concomitantly with the compensation of the potential toxicity in the function of the liver (Grundman et al., 2006). On the other hand, most of scientific literature reports about the major interferences between acetaminophen and/or vitamin C and a great number of chemical species in a variety of matrices (Sandulescu et al., 2000).

Although many articles on individual determination of the two analytes, especially ascorbic acid, have been published, few articles reported the simultaneous determination of both compounds in which liquid chromatography (Gioia et al., 2008; Akay et al., 1999; Thomis et al., 1984), electrophoresis (Wang et al., 2000),

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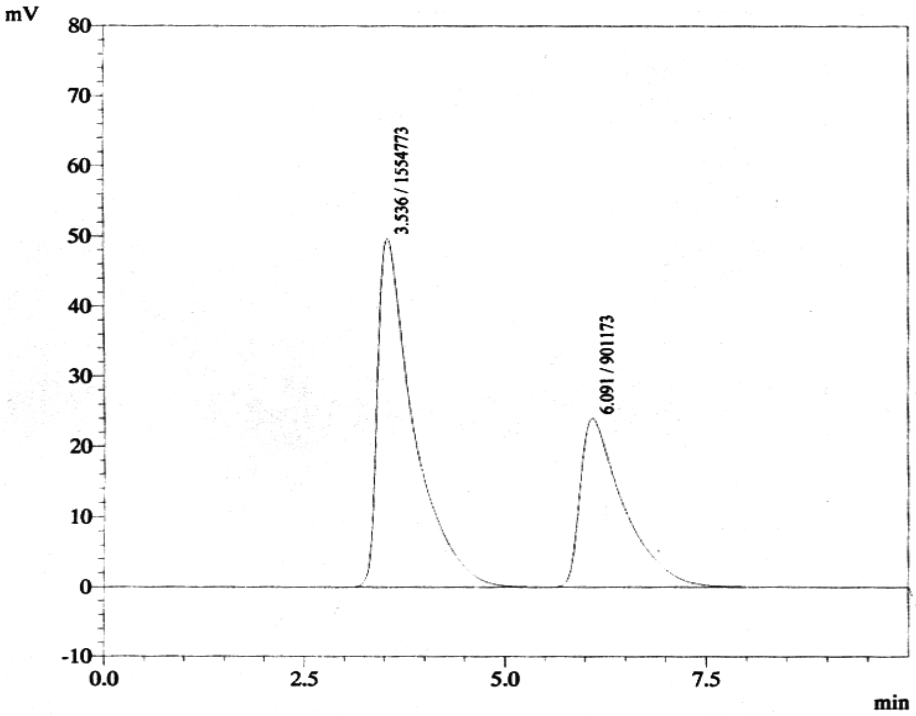

Figure 1. Chromatogram obtained from paracetamol and ascorbic acid RS.

UV-spectrophotometry (Dogan and Duran, 1998; Khajehsharifi et al., 2010) and electrochemical methods (Habibi et al., 2011; Muszalska et al., 2000) were employed for the determination of both drugs in pharmaceutical preparations.

The aim of this paper is to develop a specific, precise and accurate chromatographic method that could be applied in quality control for the simultaneously determination of drugs in respect of European Pharmacopoeia and ICH requirements (ICH, 2005).

MATERIALS AND METHODS

Material and reagents

Paracetamol RS and ascorbic acid RS were used as standards. A tablet containing 330 mg paracetamol and 200 mg ascorbic acid were obtained commercially. LC-grade methanol was supplied from Merck (Germany). All other chemical reagents were of analytical grade.

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, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. A LiChrosorb C18, 250 mm × 4.6 mm, 5 µm column was used as a stationary phase. The components were separated isocratically with a mobile phase consisting of 1 mM sodium pentane sulphonate in a mixture of 0.4 volume of formic acid, 25 volume of methanol and 75 volume of water at a flow rate of 1.0 ml min^{-1}. The analysis was carried out at an ambient temperature and injection volume was 20 µl. The UV detector was set at 254 nm.

Preparation of reference solutions

Reference stock solutions of paracetamol and ascorbic acid were prepared at 330 and 200 µg/ml in mobile phase as a diluent. The working solution of paracetamol and ascorbic acid was prepared at concentration of 33.00 and 20.00 µg/ml, respectively, by diluting the stock solutions in mobile phase.

Sample preparation

A commercially available tablet formulation containing paracetamol 330 mg and ascorbic acid 200 mg was analyzed using this method. Twenty tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to average mass of one tablet was transferred into a 100.0 ml volumetric flask. Approximately 70 ml of mobile phase were added, sonicated for 10 min and shaken for 15 min. The volume was diluted to the mark with mobile phase and mixed thoroughly. The solution was filtered through a 0.45 µm membrane filter and was further diluted with mobile phase to achieve a final concentration of 33.00 and 20.00 µg/ml of paracetamol and ascorbic acid, respectively.

RESULTS AND DISCUSSION

From the chromatogram shown in Figure 1, it is evident that under the proposed chromatographic conditions,

Table 1. System suitability parameters.

Parameter	Ascorbic acid	Paracetamol
Retention time (min) ± % RSD	3.53 ± 0.09	6.09 ± 0.08
Tailing factor ± % RSD	0.81 ± 0.10	0.83 ± 0.11
Theoretical plates ± % RSD	1963 ± 0.58	2914 ± 0.61
Resolution (Rs)	2.95	-
Limit of detections (µg/ml)	0.4	0.5

Table 2. Statistical data for precision.

Statistical parameter	Paracetamol			Ascorbic acid		
	Recovery (%)	SD	RSD (%)	Recovery (%)	SD	RSD (%)
Repeatability	99.02	0.45	0.46	98.98	0.88	0.89
Intermediate precision						
Day to day	98.93	0.59	0.60	98.38	0.98	0.99
Analyst to analyst	99.06	0.72	0.73	99.20	0.58	0.59

ascorbic acid and paracetamol were completely separated, which indicated that the method is selective and could be used for their simultaneously identification, quantification and purity tests.

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

Validation study

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

Specificity

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

Calibration and linearity

Calibration curves were constructed in the range of 8.25 to 66.00 µg/ml for paracetamol and 5.00 to 40.00 µg/ml for ascorbic acid, to encompass the expected concentration in measured samples. The corresponding linear regression equations were y = 15214.9x - 1340.1 with square of correlation coefficient R^2 of 0.9999 for paracetamol and y = 26476.1x - 513.2 with R^2 of 0.9998 for ascorbic acid, respectively. An excellent correlation existed between the peak areas and concentration of both compounds.

Precision

Repeatability

Dilutions of different concentrations were prepared and triplicates of each dilution were analysed in same day for repeatability and the results were subjected to statistical analysis. The %RSD was 0.46 for paracetamol and 0.89 for ascorbic acid which is according to ICH norms.

Intermediate precision

In this study, triplicate of each dilution was analysed in different days and by different analysts. In all conditions, %RSD was near to 1 which shows that the method is precise (Table 2).

Accuracy

To determine the accuracy of the method, the recovery was checked at three different concentration levels: 50, 100 and 150%. Values of analytical recovery experiments were listed in Table 3.

Robustness

The robustness was studied by evaluating the effect of

Table 3. Results from study of accuracy.

Drug	Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	Mean recovery ± SD (%)	RSD (%)
Paracetamol	50	16.62	16.33	99.04 ± 0.491	0.50
	100	33.24	33.08	99.53 ± 0.301	0.30
	150	49.86	49.31	98.90 ± 0.331	0.33
Ascorbic acid	50	10.34	10.13	98.00 ± 0.498	0.51
	100	20.69	20.45	98.84 ± 0.559	0.57
	150	31.04	30.87	99.45 ± 0.367	0.37

Table 4. Results from evaluation of robustness.

Condition	Assay (%)	
	Paracetamol	Ascorbic acid
Flow rate 0.9 ml/min	99.15	98.16
Flow rate 1.1 ml/min	98.58	98.40
Methanol: water, 22:78 (v/v)	98.87	98.51
Methanol: water, 28:72 (v/v)	99.12	98.80
Column change	98.45	98.79

small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.1 ml/min), mobile phase composition (% organic solvent) and use of LC columns from different batches. Results from study of the robustness of the method were listed in Table 4. Results for the test preparation were not affected by small variation of the conditions and were, therefore, accurate, that is, in agreement with those obtained under the optimum conditions.

Limit of quantitation and limit of detection

The limit of quantitation and limit of detection were calculated from the standard deviation of responses and slopes using signal-to-noise ratio as per ICH guidelines. The LOQs for paracetamol and ascorbic acid were found to be 2 and 1 µg/ml, while the LODs were 0.5 and 0.4 µg/ml, respectively.

System-suitability test

System-suitability test was performed in respect of European Pharmacopoeia from the determined validation procedure parameters: LODs, resolution, number of theoretical plates and tailing factors (Table 1).

Conclusion

The newly developed LC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing paracetamol and ascorbic acid either as such or in combination.

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