

Validated stability indicating HPLC method for estimation of theophylline from a novel microsphere formulation

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A new, simple, specific, precise and robust isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for determination of theophylline from a novel formulation. The liquid chromatographic separation was achieved isocratically using a mobile phase of acetonitrile: 50 mM sodium acetate buffer (15:85) adjusted to pH 6.5 using dilute hydrochloric acid. The analysis was carried out using Hi-Q-Sil C18 column [250 mm x 4.6 mm, 5 μ m] at flow rate of 1 ml/min and the UV detection at 270 nm. The method was validated for accuracy, precision, linearity, range, selectivity, and robustness. The linearity of the proposed method was investigated in the range of 1 to 24 μ g/ml ($r^2 = 0.9995$). The drug was subjected to oxidation, hydrolysis, heat, and photolysis to apply stress conditions. The method provided good peak parameters with retention time of 8.6 ± 0.3 min. Degradation products resulting from stress studies did not interfere with the detection of theophylline and the assay can thus be considered as stability-indicating.

Key words: Assay, high performance liquid chromatography, reverse phase, theophylline, validation

INTRODUCTION

Theophylline is used to prevent and treat wheezing, shortness of breath, and difficulty breathing caused by asthma, chronic bronchitis, emphysema, and other lung diseases. It relaxes and opens air passages in the lungs, making it easier to breathe. Theophylline is well absorbed throughout the gastrointestinal tract, half-life in adults varies considerably. In healthy adults, it ranges from 3 to 12 h. In children, the half-life is usually significantly shorter than in adults, averaging about 3.5 h. Thus, the drug finds wide application in pediatrics in immediate release as well as sustained release dosage forms.^[1-9] For pediatric application the dosage form should be palatable. Hence, microspheres were formulated by solvent evaporation method for masking the bitter taste of the drug.

Chromatographic methods have been described for the quantitative determination of theophylline in formulations as well as biological fluids.^[10-15] However, the methods which are reported do not give an

exhaustive validated study. The previously published methods also make use of complicated mobile phase systems and are not directly applicable for this type of formulation and need more investigation for method development and validation. Therefore, the main aim of this work was to develop and validate a stability indicating RP HPLC method for estimation of theophylline from a novel microsphere formulation.

MATERIALS AND METHODS

Theophylline was provided as gift sample from Cipla Pharmaceuticals. All the other ingredients used in formulation were obtained from S.D. Fine Chemicals. Acetonitrile used was of high-performance liquid chromatographic (HPLC) grade and was obtained from Merck (Darmstadt, Germany). Water was deionized and then doubly distilled. Sodium acetate was of A.R. grade purchased from S.D. Fine chemicals Limited, India.

Instrumentation and chromatographic conditions

A JASCO HPLC system 2000 Series with Jasco PU-2080 Plus HPLC pump (Jasco, Japan), equipped with a Rheodyne injection system with a 20 μ l loop was used for the study. Detection was accomplished with an UV 2075 detector at 270 nm. Borwin software was used to record and evaluate the data collected during and following chromatographic analysis.

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The chromatographic separation was achieved at room temperature on a reverse phase column Hi-Q-Sil C18 [250 mm x 4.6 mm, 5 μ m]. The mobile phase comprising of acetonitrile: 50 mM sodium acetate buffer (15:85) adjusted to pH 6.5 was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 μ m membrane filter and degassed using an ultrasonicator and the separations were achieved by isocratic elution with a flow rate of 1.0 ml/min.

Preparation of stock and standard solutions

A stock solution of theophylline (100 μ g/ml) was prepared by accurately weighing approximately 10 mg of theophylline into a 100 ml A-grade volumetric flask and making up to volume with double distilled water. Aliquots of the standard stock solution of theophylline were prepared with mobile phase to give the required final concentrations.

Method development and validation

A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of theophylline in the bulk drug and in the formulation. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis, ease of preparation, and use of readily available cost-effective solvents. The method was validated according to ICH^[16-19] and USP guidelines.^[20] The validation parameters addressed were specificity, precision, accuracy, linearity, and robustness.^[21- 28]

Stress testing of theophylline

Oxidation studies

Solutions for oxidation studies were prepared in 5% H₂O₂ and the resultant solutions were allowed to stand for 4 h to facilitate oxidation of the drug.

Acid degradation studies

Solutions for acid degradation studies were prepared in 0.1 N hydrochloric acid and the resultant solutions were refluxed for 8 h.

Alkali degradation studies

Solutions for alkali degradation studies were prepared in 0.1 N sodium hydroxide and the resultant solutions were refluxed for 8 h.

Neutral degradation studies

Solutions for neutral degradation studies were prepared in water and the resultant solutions were refluxed for 8 h.

Temperature stress studies

The drug was exposed to dry heat (80°C) in a hot air oven for 48 h. The drug solution was prepared and subjected to analysis.

Photostability studies

The drug was exposed to light for 48 h. The drug solution

was prepared and subjected to analysis.

Preparation of microspheres

The microspheres of theophylline were prepared using solvent evaporation method. The drug was dispersed in polymeric dispersion of Eudragit in acetone. This solution was poured into liquid paraffin under continuous stirring at 1600 rpm. The stirring was done for 90 min till all the acetone evaporated and the polymer precipitated out encapsulating the drug. Sweetener, sodium saccharin, and superdisintegrant Ac-Di-Sol was added to the resulting microspheres and were compressed to form tablet.

Assay

Tablets subjected to stability studies for a period of three months were assayed. Tablets in a number of 5 were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 10 mg of theophylline was transferred to a 100 ml volumetric flask. Purified water was added to make up the volume, sonicated for 15 min and filtered. From the filtrate, further dilutions were made with mobile phase to get a concentration of 10 μ g/ml. The resulting solutions were then injected into the column and chromatographed using the conditions mentioned above. The percent drug content was determined from the area of the peak using the regression equation obtained in the calibration experiments. The same procedure as described above was used for the assay of marketed capsule of theophylline.

RESULTS AND DISCUSSIONS

Method development

Of several solvents and solvent mixtures investigated the mobile phase consisting of acetonitrile: 50 mM sodium acetate buffer (15:85) adjusted to pH 6.5 was found to furnish sharp, well-defined peaks with very good symmetry. The pH of 6.5 helped in better resolution of the peak. The retention time for the drug was 8.6 min. A typical representative chromatogram is shown in Figure 1.

Method validation

Specificity and stress studies

The results of the stress studies indicated the specificity of the method that has been developed. After exposure of theophylline solutions to stress conditions, an assay of the drug was performed on the resultant solutions. Typical chromatograms obtained for these analyses are shown in Figures 2 and 3. It is clearly evident that when exposed to acidic conditions and alkaline conditions the drug undergoes degradation. The peaks of the resultant degradants were well resolved from the drug peak. However, the drug was found to be thermostable, photostable, and stable in oxidative conditions.

The assay of a product must also show specificity with regard to the potential interference that might be a result of the

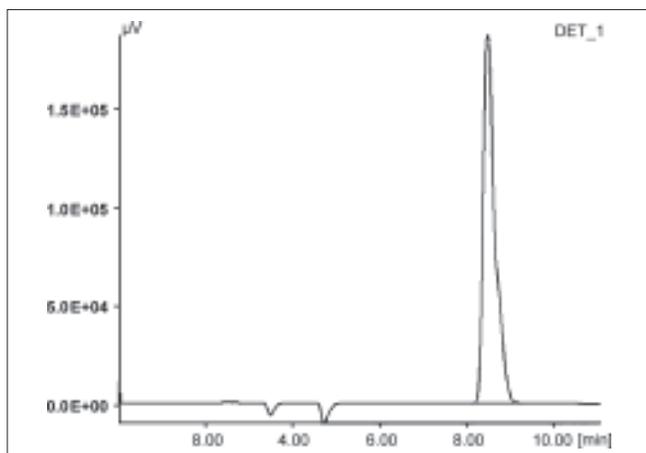


Figure 1: Typical standard chromatogram of theophylline

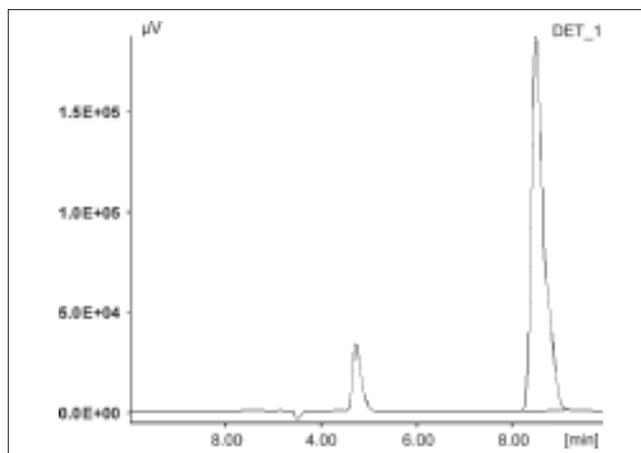


Figure 2: Chromatogram obtained following the exposure of drug to acidic conditions (0.1N HCl)

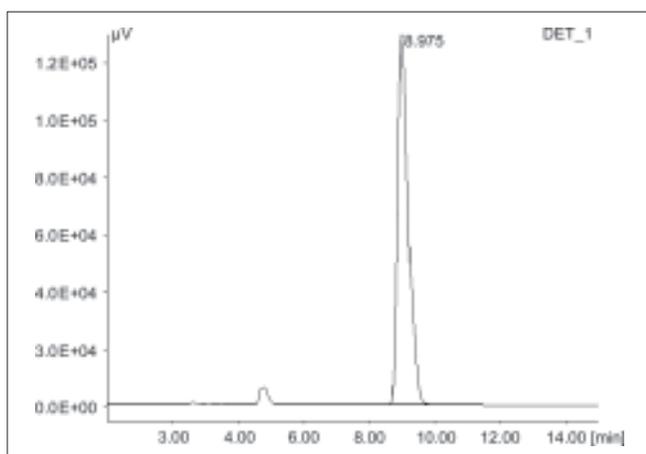


Figure 3: Chromatogram obtained following the exposure of drug to alkaline conditions (0.1N NaOH)

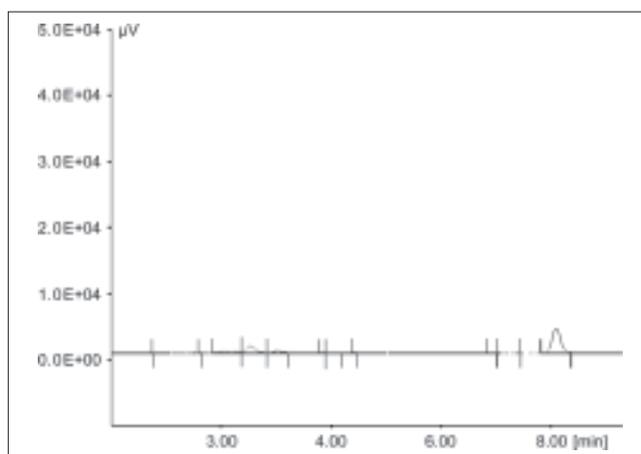


Figure 4: Typical chromatogram obtained following analysis of excipients

presence of excipients in a formulation. The specificity studies showed that there were no extra peaks due to the excipients which indicated lack of interference from the excipients. A typical chromatogram developed during the analysis of plain excipients and of the drug solution in presence of excipients is as shown in Figures 4 and 5 respectively.

Linearity and range

A calibration curve was constructed from ten non-zero samples covering the total range of 1-24 $\mu\text{g/ml}$. The peak area was plotted versus the concentration. The equation for the resultant calibration curve was $y = 326799x + 67577$ with a linear regression coefficient of 0.9995.

Precision

Intra-assay precision (repeatability) and inter-day (intermediate) precision were determined. The analyses were performed using concentrations at three levels, 8, 10, and 12 $\mu\text{g/ml}$. Each concentration was analyzed in triplicate

($n = 3$) and intra-assay precision was found to be less than 2 % relative standard deviation (RSD) for all samples on all days [Table 1]. Inter-day precision % RSD for analyses conducted on three separate days was found to be 1.81, 0.08, and 0.74% RSD for the low, middle, and high concentrations studied, respectively [Table 2].

Accuracy

Accuracy was determined at three concentrations, similar to those used to assess the precision of the method. Each of the solutions was analyzed ($n = 5$) and the percentage standard deviation was determined. The method showed percentage RSD of less than 2 for all solutions tested which indicated good accuracy of the method and the results are summarized in the Table 3.

Robustness

Robustness was determined by varying the mobile phase flow

Table 1: Intra-day precision

Levels ($\mu\text{g/ml}$)	Intra Day					
	Day 1		Day 2		Day 3	
	Average concentration recovered ($\mu\text{g/ml}$)	% RSD	Average concentration recovered ($\mu\text{g/ml}$)	% RSD	Average concentration recovered ($\mu\text{g/ml}$)	% RSD
8	7.82	0.06	7.69	1.42	7.79	1.79
10	10.29	0.01	10.28	0.01	10.34	0.99
12	11.80	0.05	11.82	0.08	11.89	0.67

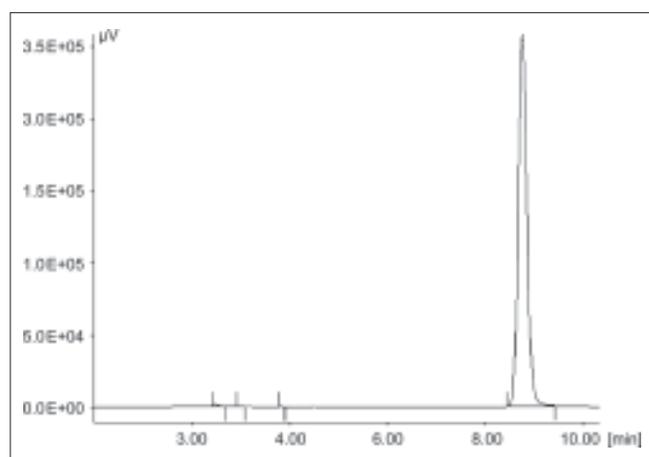
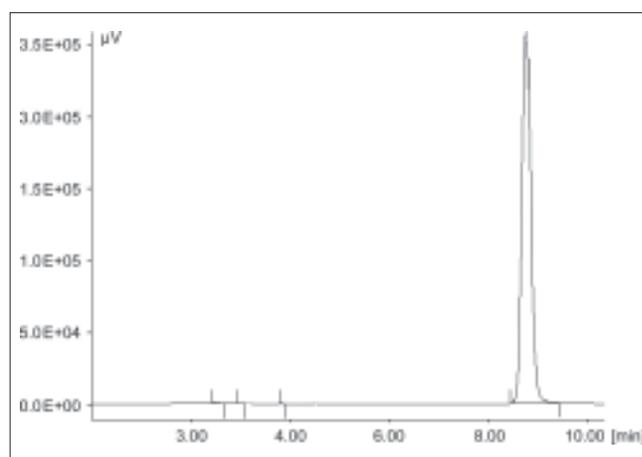
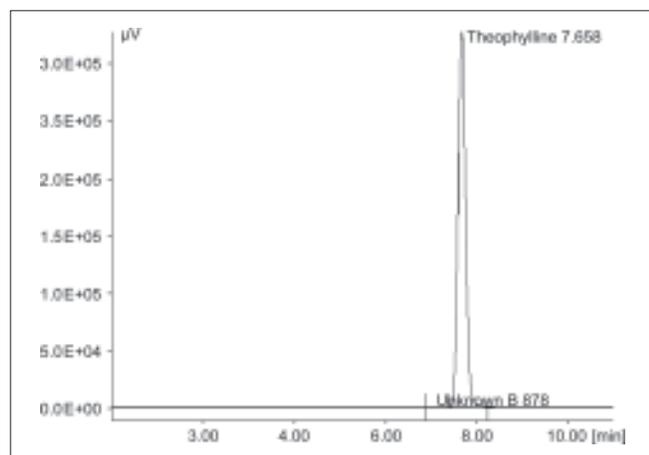
Table 2: Inter-day precision

Levels ($\mu\text{g/ml}$)	Inter Day		
	Amount recovered (mg)	S.D	% RSD
8	7.78	0.14	1.81
10	10.28	0.01	0.08
12	11.90	0.08	0.74

Table 3: Accuracy

Amount added (mg) per tablet	% Amount recovered mean \pm SD	% RSD
80	97.58 \pm 0.17	0.22
100	103.33 \pm 0.70	0.67
120	98.33 \pm 0.14	0.12

All the values are average of three determinations, SD: Standard Deviation, RSD: Relative standard deviation

**Figure 5:** Typical chromatogram obtained following analysis of theophylline in presence of excipients**Figure 6:** Chromatogram of theophylline in tablet assay at the end of three months**Figure 7:** Chromatogram of theophylline in marketed tablet

rate to ± 2 min (i.e., 0.8, 1, and 1.2 ml/min), the ratio in the mobile phase to ± 2 ml (Acn: sodium acetate buffer as 13:87, 15:85, and 17:83) and pH of the mobile phase to ± 0.5 (5.5, 6.5, and 7.0). The deliberate changes in the flow rate, mobile phase ratio and the change in pH did not affect the recovery

of the drug which indicated the robustness of the method.

Assay

The proposed method was applied to the determination of theophylline in formulated dosage form and in a marketed preparation of the same. A typical chromatogram obtained following the assay of formulation and marketed preparations are depicted in Figures 6 and 7, respectively. The result of the assay yielded $99.34 \pm 2\%$ of label claim and is comparable to the marketed preparation. The results of the assay indicate that the method is selective for the assay of theophylline without interference from the excipients used in the dosage form.

CONCLUSION

This developed RP-HPLC method for estimation of theophylline from the microspheres is accurate, precise, robust, specific, and stability-indicating. The method has been found to be better than previously reported methods, because of use of an economical and readily available mobile phase, UV detection,

and better resolution of peaks. The retention time of the drug is such that it distinguishes well from the degradants peaks. All these factors make this method suitable for quantification of theophylline in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

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