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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF THEOPHYLLINE IN TABLET FORMULATION

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ABSTRACT

A simple, sensitive and accurate stability indicating HPTLC method has been developed and validated for estimation of theophylline as bulk drug and in tablet dosage form. The separation of drug was achieved by spotting drug on precoated silica gel 60 F_{254} aluminum plates using ethyl acetate: methanol: acetic acid (9:0.5:0.5, v/v/v) as mobile phase with densitometric detection at 271 nm. The retention factor was found to be 0.60 ± 0.003. The drug was subjected to hydrolytic, oxidative, thermal and photolytic stress conditions. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the concentration range of 250-1500 ng band^{-1} with high correlation coefficient. The method found to be accurate as results of the recovery studies are close to 100%. The developed method was found to be simple, sensitive, selective, accurate and repeatable and can be adopted for routine analysis of drug in bulk and tablet dosage form.

KEYWORDS: Theophylline, Stress degradation, HPTLC, Validation.

INTRODUCTION

Theophylline, chemically, 1, 3-dimethyl-3, 7-dihydro-1H-purine-2, 6-Dione is methylxanthine drug used in therapy for respiratory diseases such as chronic obstructive pulmonary disease and asthma. It competitively inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, possibly resulting in bronchodilation.[1] It is official in Indian Pharmacopeia.[2] Extensive literature survey revealed that methods such as spectrophotometry[3-5], High performance liquid chromatography (HPLC)[6-17] and (LC-MS)[18] has been reported in the literature for the determination of Theophylline in human serum and in pharmaceutical formulations either as single drug or in combination with other drugs.

To best of our information, no reports were found in the literature for the estimation of Thaophylline in tablet dosage form by HPTLC method. Therefore the aim of the present work is to develop and validate an accurate, specific, and reproducible stability indicating HPTLC method for determination of Theophylline as bulk drug and in tablet dosage form.

MATERIALS AND METHODS

Reagents and chemicals

Analytically pure standard theophylline was received from Spectrum Labs, (Hyderabad, India). The pharmaceutical dosage form Unicontin-E tablet which was labeled to contain 250 mg of theophylline was procured from the local pharmacy. Ethyl acetate, methanol, acetic acid (AR grade) was obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on aluminum plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate as a band of 4 mm width using Camag 100µL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The chromatographic resolution was achieved by linear ascending development in twin trough glass chamber (CAMAG, Muttenz, Switzerland) using ethyl acetate: methanol: acetic acid (9: 1: 0.5, v/v/v) as mobile phase. The chamber was saturated with mobile phase vapor for 15 min. The development distance was 9 cm and the development time approximately 15 min. The slit dimensions 6 mm × 0.30 mm and scanning speed of 20 mm sec^{-1} was employed. After chromatographic development, plates were dried and densitometric estimation was done on CAMAG thin layer chromatography scanner-3 at 271 nm for all developments operated by win CATS software version 1.4.2.

Selection of Detection Wavelength
From the standard stock solution (1000 µg mL^{-1}) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 271 nm.

Preparation of Standard stock solution
Accurately weighed 10 mg drug was dissolved in 10 mL solvent to have 1000 mg mL^{-1} concentration from which 2.5mL of solution was diluted with methanol to obtain final conc. of 250 ng μL^{-1} final concentration.

Preparation of sample solution
Twenty tablets were accurately weighed and then finely powdered. Powder quantity equivalent to 250 mg was taken and shifted to a 100 mL flask consisting 60 mL methanol. The content was sonicated for 15 min and filtered. The volume was adjusted up to the mark with methanol to attain the concentration 2500 ng μL^{-1}. One mililitre volume of solution was diluted with methanol to obtain 250 ng μL^{-1} as final concentration. Two µL volume of this solution was applied on TLC plate to get final sample concentration of 500 ng $band^{-1}$. Peak areas of the bands were measured at 271 nm after chromatographic development.

Stress degradation studies
Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, hydrolysis, and oxidation. Dry heat and photolytic degradation were carried out in the solid state. The hydrolytic studies were carried out by keeping the stock solution with 1 N HCl and 1 N NaOH at room temperature for 12 h, respectively. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 1000 ng $band^{-1}$. Neutral hydrolysis study was performed by treatment of drug with water at room temperature for 12 h. The oxidative degradation was carried out in 30 % H_2O_2 at room temperature for 12 h and sample was diluted with methanol. Thermal stress degradation was performed by keeping drugs in oven at 90ºC for period of 48 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square $meter^{-1}$. Thermal and photolytic samples were diluted with methanol to get concentration of 250 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method optimization
The aim of present research work was to develop stability indicating HPLTC method which would be capable to give the satisfactory resolution between theophylline and its degradation products. The separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using ethyl acetate: methanol: acetic acid (9: 1: 0.5, v/v/v) as mobile phase. Densitometric detection was performed at 271 nm. The drug was resolved adequately with RF value 0.60 ± 0.003 (Figure 1).

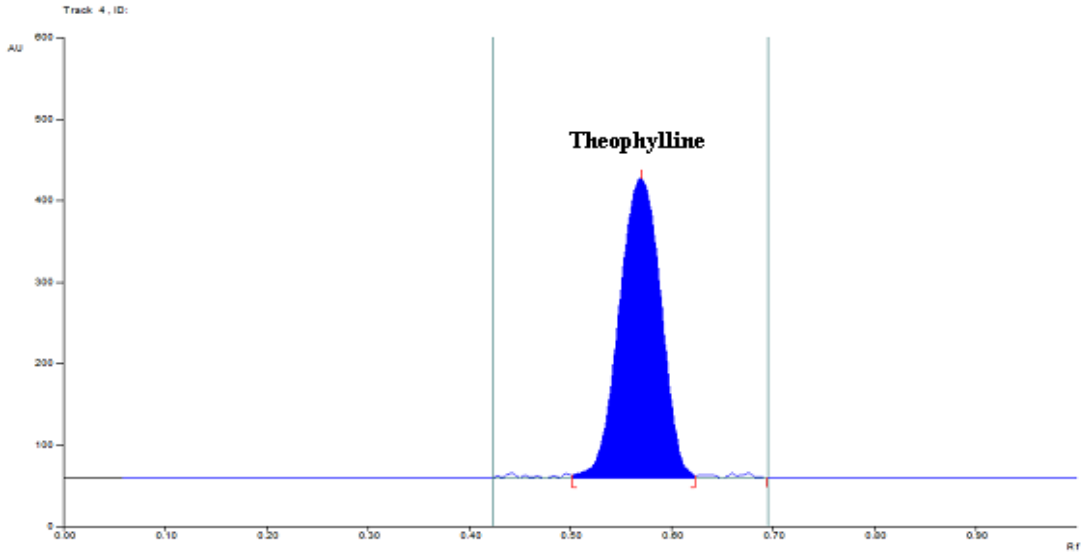

Figure 1: Densitogram for Theophylline reference standard (1000 ng $band^{-1}$, Rf = 0.60).

Stress degradation studies
The stress degradation results demonstrated susceptibility of theophylline to acid and base catalysed hydrolysis, neutral hydrolysis, and oxidative stress conditions. Theophylline was found stable under thermal and photolytic stress conditions. Figures 2-4 show the densitograms of acid, alkali and neutral hydrolytic degradation, while Figures 5 represents the densitogram of oxidative degradation. Marked degradation in the densitograms was observed but no additional degraded products were observed. The findings of degradation studies along with % recovery and % degradation are represented in Table 1.

Table 1: Results of stress degradation study.

Sr. No.	Stress degradation conditions used	% Assay of active substance	% Degradation
1.	Acid/ 1N HCl/ Kept at RT for 12 h	73.36	26.64
2.	Base/ 1N NaOH/ Kept at RT for 12 h	85.33	14.66
3.	Neutral/ H_2O/ Kept at RT for 12 h	94.96	5.03
4.	Oxidation/ 30 % H_2O_2 /Kept at RT for 12 h	85.77	14.22
5.	Dry heat/ 90ºC/ 48 h	98.57	1.42
6.	Photo stability UV light	83.87	16.12

RT: Room temperature

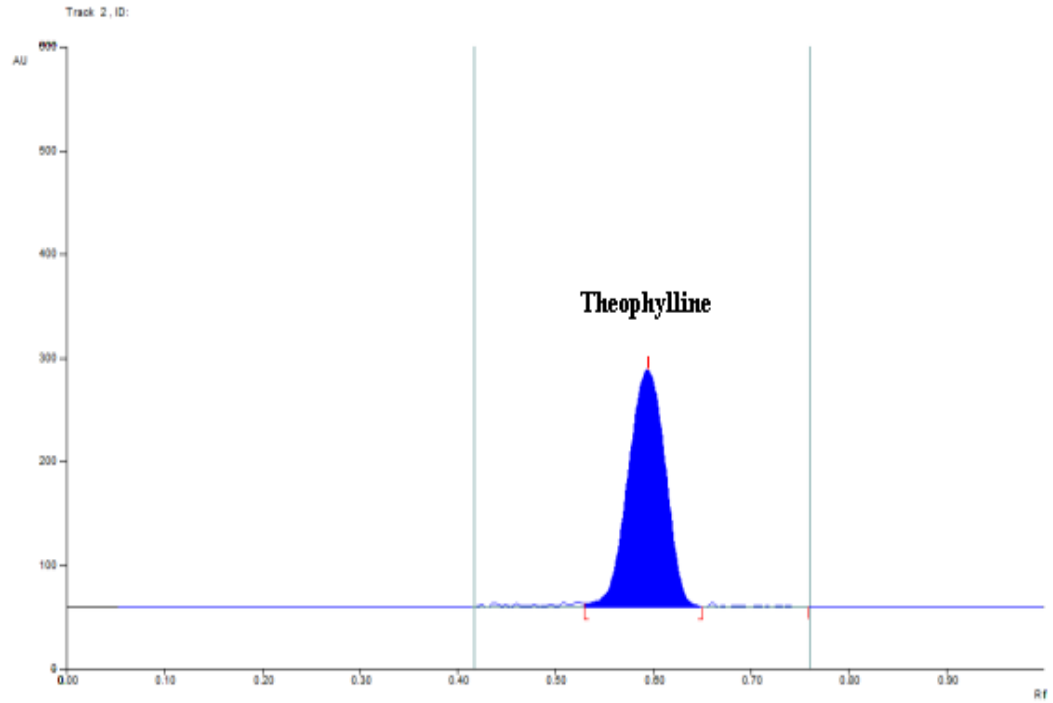

Figure 2: Representative densitogram of theophylline after acid treatment (1 N HCl, Kept at RT for 12 h).

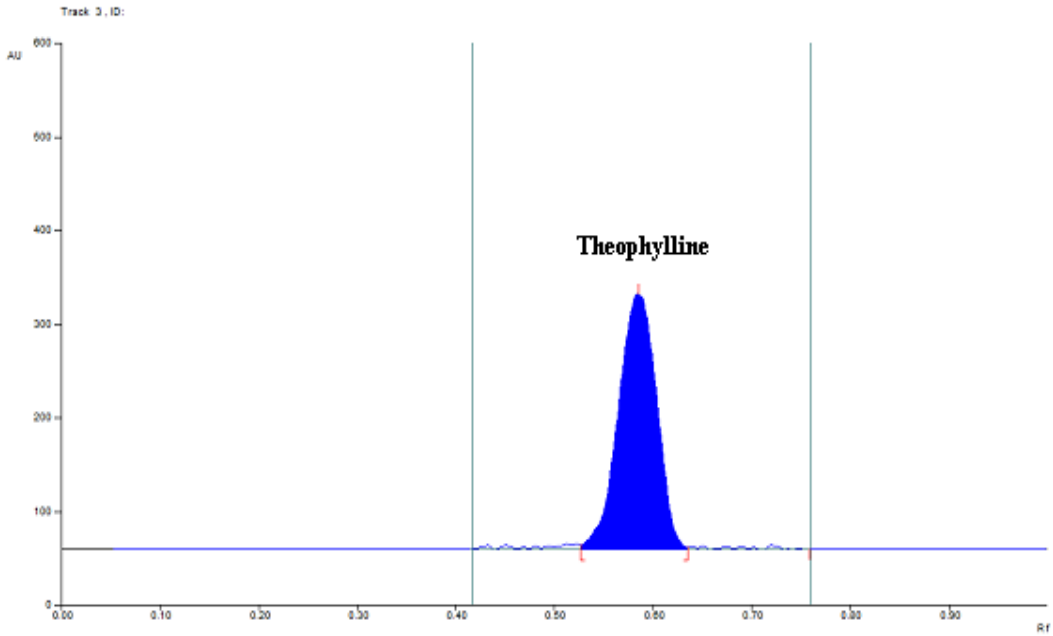

Figure 3: Densitogram after alkali treatment (1 N NaOH, Kept at RT for 12 h).

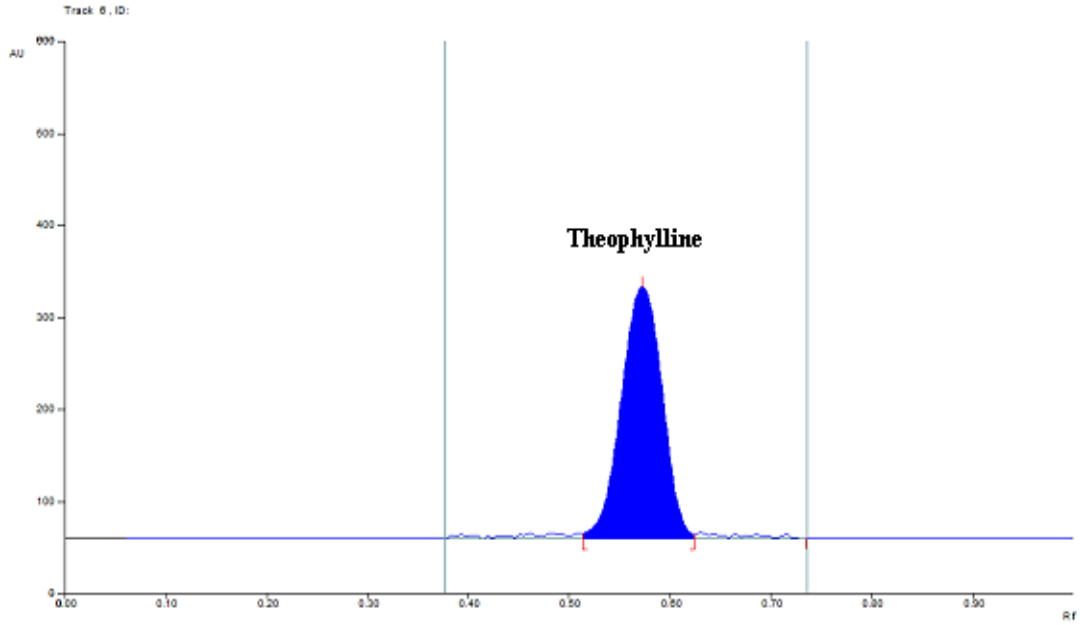

Figure 4: Densitogram of Theophylline after neutral hydrolysis.

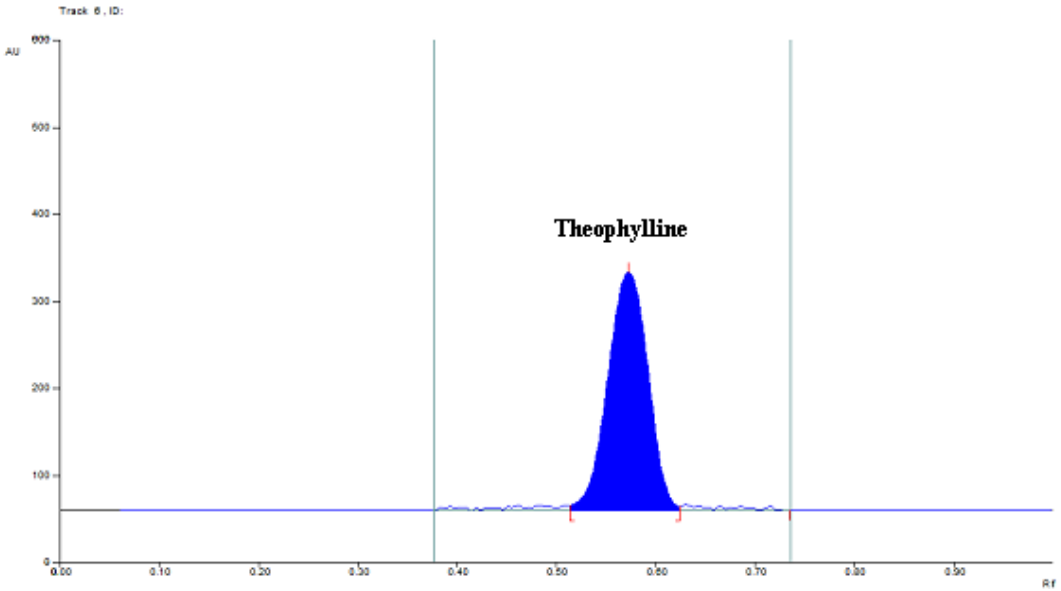

Figure 5: Densitogram after treatment with 30 % H_2O_2, Kept at RT for 12 h.

Analytical method validation

The method developed was validated in terms of linearity, accuracy, precision, robustness, limit of quantization (LOQ), limit of detection (LOD) to make sure the reliability of results of analysis as per International Conference on Harmonisation (ICH) guidelines for Validation of Analytical Procedures: Text and Methodology Q2 (R1)[19] and Stability testing of new drug substances and products, Q1A (R2) (ICH 2005; 2003).[20] The linearity of was determined by application of aliquots of 1, 2, 3, 4, 5 and 6 µL of standard solution of Theophylline (250 ng μL^{-1}) on TLC plate. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates (n = 6) was analyzed and peak areas were recorded. The linearity was observed in the range of 250-1500 ng $band^{-1}$ with correlation coefficient 0.969. The Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1. LOD value was found to be 33.95 ng $band^{-1}$ and 102.90 ng $band^{-1}$. The repeatability of separation was accessed by intra day and inter day precision studies with concentrations of 500, 750 and 1000 ng $band^{-1}$. The % RSD values were not more than 2 indicating the precision of developed method. Recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150%. At each level of the amount, three determinations were carried out. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % RSD not more than 2 (Table 2). Robustness of the method (n =

3) was examined at a concentration level of 1500 ng band^{-1} under the influence of small, deliberate variations of the analytical parameters. Parameters varied were wavelength (± 1 nm), chamber saturation time (± 10 min). The areas of peaks of interest remained unaffected by small changes of the operational parameters and % RSD was within the limit (< 2%) indicating the robustness of the developed method.

Table 2: Accuracy studies.

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount recovered (ng band^{-1})	% Recovery ± S.D.*
Theophylline	500	250	801.83	106.91±1.21
	500	500	1047.10	104.71±1.22
	500	750	1178.43	94.27±1.55

*n = 3

CONCLUSION

Stability indicating HPTLC method for the determination of Theophylline as bulk drug and in tablet dosage form has been developed and validated. The developed method is simple, precise, accurate, and reproducible and can be used for quantitative analysis of Theophylline in pharmaceutical dosage form as well as for routine analysis in quality control laboratories. The proposed method would be suitable for analysis of Theophylline without any interference from the excipients and can be successfully used to estimate the amount of drug in the formulation by easily available low cost materials.

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