

## Original Research Article

# Buffer-Free High Performance Liquid Chromatography Method for the Determination of Theophylline in Pharmaceutical Dosage Forms

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Received: 15 January 2013

Revised accepted: 30 November 2013

## Abstract

**Purpose:** To develop and validate a simple, economical and reproducible high performance liquid chromatographic (HPLC) method for the determination of theophylline in pharmaceutical dosage forms.

**Method:** Caffeine was used as the internal standard and reversed phase C-18 column was used to elute the drug and standard. The mobile phase was prepared by mixing water:acetonitrile:methanol at the ratio of 90:03:07 and the pH set at 4.6. Flow rate and ultraviolet (UV) detector were set at 1.0 mLmin<sup>-1</sup> and 271 nm, respectively. The method was validated for linearity, recovery, accuracy, precision, specificity, and also for inter-day stability under laboratory conditions.

**Results:** Retention time was 5.5 min. The limits of detection and quantification were 12.5 ngmL<sup>-1</sup> and 100 ngmL<sup>-1</sup>, respectively. Recovery accuracy (%) for different concentrations ranged from 100.05 to 102.43; regression coefficient (R<sup>2</sup>) of 0.994; precision RSD < 2.0, and negligible interference from common excipients.

**Conclusion:** The method is simple, rapid, highly specific and suitable for the determination of theophylline. Absence of buffer and use of small quantity of organic solvents increase the life span of the column and reduce the cost of routine analysis of theophylline in industry.

**Keywords :** Buffer-free, High performance liquid chromatography, Theophylline, Dosage form.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Theophylline (1,3-dimethyl-7H-purine-2,6-dione) is also known as dimethylxanthine and belongs to the methylxanthine group of drugs. It is used for the treatment of respiratory diseases such as chronic obstructive pulmonary diseases and asthma [1,2]. Previous attempts and popular Pharmacopoeia methods for the analysis of theophylline used buffer as aqueous solvent and acetonitrile as organic solvent [3-6]. These

methods gave good results but are unsatisfactory due to high cost and time constraint in order to prepare the sample for routine analysis in industry [6].

A greater portion of routine HPLC analysis cost arise from the life span of column which is affected by salts and acid used in buffer solutions [7,8]. Salts used in buffer solution may precipitate in the presence of organic solvents and increase the maintenance cost of HPLC pumps [8].

Quantity of organic solvents used also impact on the total routine analysis cost [9,10] In January 2009, the price of acetonitrile was recorded at about 100 (US dollar) per liter with disposal costs of about double this price.

The immediate challenge for analytical laboratories is to reduce their consumption of HPLC solvent as much as possible. The degree to which each laboratory is able to reduce acetonitrile consumption depends on the individual operating environment. Regulated laboratories that work with validated methods have less flexibility to change these methods and therefore have less opportunity to reduce solvent consumption. Different strategies must be followed than in non-regulated environments, in which the opportunity to reduce solvent consumption is high [11].

Hence, there is a need for buffer-free HPLC method development and validation for the assay of theophylline using minimum quantity of organic solvents which this study seeks to address.

## EXPERIMENTAL

### Materials

Theophylline anhydrous with 99.9 % purity was a gift from Bukul Pharma Mumbai, India. All the solvents used were of HPLC grade obtained from Merck. HPLC grade water was produced from distilled water (pH:  $6.3 \pm 0.05$ ) and filtered with  $0.45 \mu\text{m}$  membrane filter (Millipore, Bedford, MA, USA) using Millipore vacuum filtration unit. All the reagents were used without any further purification. Generic and branded pharmaceutical formulations of theophylline in tablet form were obtained from commercial sources and used as received, without any further purification. The composition of the preparations is as shown in Table 1[12-15].

**Table 1:** Composition of the commercial preparations tested

Brand name	Manufacturer	Label claim (mg)	Other active ingredients
Asmapax Depot	AHPL, India	65	Ephedrine, Phenobarbitone
Pharmaniaga Theophylline	Pharmaniaga Manufacture Berhad	125	--
Asmatide-BR	Systopic, India	200	Albuterol sulfate, Bromhexine HCl
Biryth	Emcure, India	300	--

### HPLC apparatus and operating conditions

The HPLC system used for this experimental consisted of Shimadzu Corp, Japan (model HPLC class 10AT), a dual piston reciprocating two LC-10AT VP pumps, ultra-violet detector from Shimadzu Corp., Japan (model SPD-10M VP) and auto sampler of SIL-10AD series. Prior to performing the validation assay, chromatographic conditions for the HPLC method were studied in order to achieve appropriate system suitability. Mobile phase of various composition was tested with water: acetonitrile (87:13, v/v), water : acetonitrile (90:10, v/v), water : acetonitrile (93:7 v/v), water: acetonitrile: methanol (90:03:07), water : acetonitrile : methanol (90:5:5, v/v), water: acetonitrile : methanol (90:7:3, v/v) in C18 column at UV wavelength of 271 nm. Routine degassing of the mobile phase was carried out by passing it through a  $0.45 \mu\text{m}$  membrane filter (Millipore, Bedford, MA, USA). The mobile phase was pumped isocratically at a flow rate of  $1.0 \text{ mL min}^{-1}$  at  $25^\circ \text{C}$ . The injection volume was  $10 \mu\text{L}$ .

### Preparation of inclusion complex

Inclusion complex of theophylline with beta cyclodextrins was prepared using 1:1 molecular weight ratio by standard kneading method [16].

### Linearity assay

About 100 mg of theophylline anhydrous (99.9 %) powder was accurately weighed into a dry 100 mL volumetric flask ( $25 \pm 0.5^\circ \text{C}$ , RH  $20 \pm 0.5\%$ ) and sonicated with 50 mL of mobile phase and made up to 100 mL by mobile phase and filtered through a  $0.45 \mu\text{m}$  membrane filter. The filtrate (10 mL) was diluted to 100 mL with water to obtain standard solution of  $100 \mu\text{g/mL}$  of theophylline. This standard solution (10 mL) was diluted to 100 mL with HPLC grade water to obtain the standard solution of  $10 \mu\text{g/mL}$  of theophylline. To obtain the working solution, aliquots of standard theophylline solution were diluted to a concentration of  $0.1 \mu\text{g mL}^{-1}$ . The working standard solutions were prepared in duplicate, filtered and degassed by passing them through a  $0.45 \mu\text{m}$  membrane filter (Millipore, Bedford, MA, USA). All the standard solutions were prepared at  $25 \pm 0.5^\circ \text{C}$  and  $25 \pm 0.5\%$  RH. The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to the concentration. To establish linearity of the proposed methods, five separate series of theophylline solutions were prepared from the

stock solutions and analyzed. Least square regression analysis was carried out for the data obtained. The linearity was studied over a concentration range of 0.1 – 0.35  $\mu\text{g mL}^{-1}$ . Replicates of three injections were performed for each sample. Linearity data was computed on a personal computer using Microsoft Excel program (version 2003).

#### Determination of accuracy/recovery and precision

The accuracy of the method is a quantification of the closeness of the measured value to the true value for the sample. Accuracy was assessed as percent relative error and mean % recovery. Approximately 40, 50 and 60 mg of standard theophylline (anhydrous) were weighed precisely and dissolved, separately in 50mL of the mobile phase at  $25 \pm 0.5$  °C and  $25 \pm 0.5$  %RH. To achieve accuracy/recovery, aliquots of these samples were diluted to appropriate final concentrations of theophylline solution, (i.e. 2 ml of each solution diluted to 10 ml with mobile phase solution). The accuracy of the method was checked by determining the recovery values. The accuracy/ recovery were calculated for six runs of each solution. The precision was determined by measuring five sample probes under the same experimental conditions. To calculate precision, intra- and inter-day tests were performed and the results were expressed as relative standard deviation (RSD, %).

#### Determination of limits of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were determined by serial dilutions of theophylline solutions in order to obtain signal / noise ratios of equal to 3:1 for LOD and 10:1 for LOQ. Approximately 25 mg of standard theophylline was weighed precisely and dissolved in 50 mL of the mobile phase. Appropriate amounts of standard theophylline solution were diluted to the required concentrations of 0.0125, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3  $\mu\text{g mL}^{-1}$ . Working standard solutions were prepared in triplicate

#### Statistical analysis

All statistical calculations were performed using Statistical Package for Social Sciences (SPSS version 13.0®) software, SPSS Inc.,USA. Data were analysed using one-way analysis of variance (ANOVA), and differences were considered statistically significant at  $p < 0.05$ .

## RESULTS

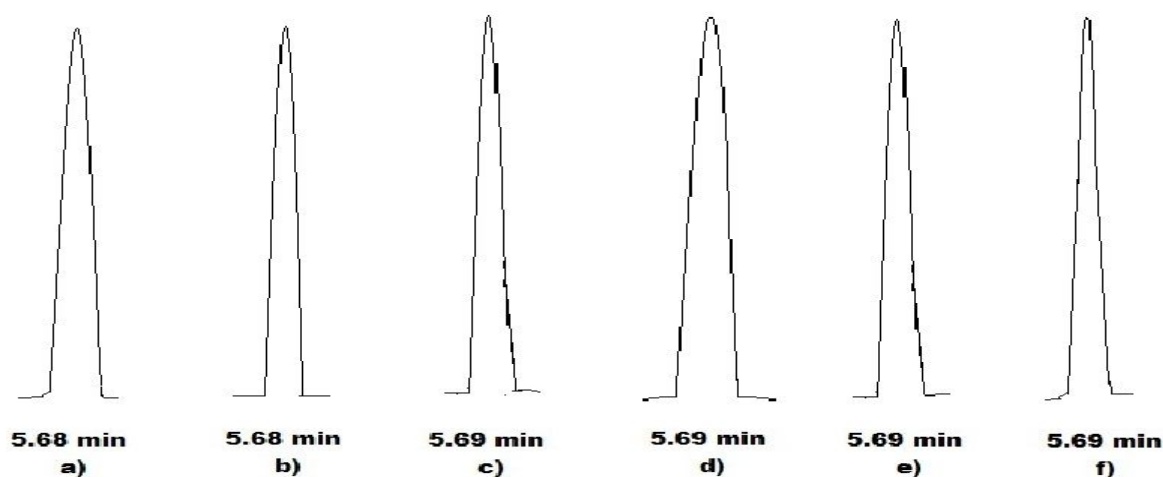
Combination of water: acetonitrile: methanol at ratio 90:03:07 selected as mobile phase in this method increased the retention time to 5.5 min. This gave a satisfactory resolution of theophylline from the other drugs and excipients present in selected formulations. Sharp and symmetrical peaks were detected with minimal interferences from equipment noise. The results demonstrated satisfactory and consistent performance of the HPLC method.

Least-squares regression analysis indicate excellent linearity over the concentration range studied ( $0.1 - 0.35 \mu\text{g mL}^{-1}$ ), with regression coefficient  $> 0.99$ . The low and maximum values of relative standard deviation (RSD) of 0.38 and 1.87%, respectively, are the evidence of the good precision of the proposed HPLC method. They also indicate the non-variability of the data. LOD and LOQ values of 12.5 ng and 100 ng  $\text{mL}^{-1}$ , respectively, indicate that the method is highly sensitive. The accuracy of the method is as summarized in Table 2.

**Table 2:** Accuracy of the HPLC method for theophylline determination (N = 3)

Concentration (mg/ml)	Recovery (%) Z	Z $\pm$ SD	RSD %
40	102.43	102.39 $\pm$ 0.05	0.036
	102.42		
	102.34		
50	102.03	101.70 $\pm$ 0.45	0.332
	101.89		
	101.19		
60	100.09	100.07 $\pm$ 0.02	0.018
	100.05		
	100.07		

Under the test conditions, theophylline was observed to be well resolved from the other components of the formulations and potential degradation products of theophylline. Thus, the method is specific for theophylline. Application of the developed method to determine theophylline in pharmaceutical formulations is shown in Figure 1. The representative chromatograms of the standard sample of theophylline and the test preparations shows identical retention times in Figure 1. Assay results for the determination of theophylline in pharmaceutical formulations and cyclodextrin complex are summarized in Table 3.



**Fig 1:** Representative chromatograms of (a) standard theophylline (anhydrous) (b) cyclodextrins inclusion complex (c) Asmapax depot (d) Pharmaniaga Theophylline (e) Asmatide-BR and (f) Biryth

**Table 3:** Assay results for the determination of theophylline in commercial pharmaceutical preparations

Product	Labeled content (mg)	Actual content $\pm$ SD (mg)	RSD (%)
Cyclodextrin Complex	5	5.90 $\pm$ 0.88	1.46
Asmapax depot	65	65.99 $\pm$ 0.88	1.87
Pharmaniaga Theophylline	125	121.70 $\pm$ 0.75	1.36
Asmatide-BR	200	198.50 $\pm$ 0.55	1.46
Biryth	300	297.02 $\pm$ 0.09	0.38

## DISCUSSION

Percent relative standard deviation data indicate the accuracy of the developed method for the determination of theophylline in pharmaceutical preparations. The specificity of the HPLC method for theophylline quantization in the pharmaceutical formulations is an indication of a possible lack of interference from excipients in the preparations. The presence of other ingredients, including active ingredients, in the formulations did not cause any interference with the theophylline peak.

The mobile phase combination selected helps in maintaining healthy column life due to absence of salts which is usually used in preparation of buffers used as mobile phase [8]. In addition to this, the developed method utilized possible minimum amount of organic solvents to achieve cost effective HPLC method for the routine analysis of theophylline. This could be highly beneficial in conditions where there is a sudden shortage of particular organic solvents due to technical or non technical reasons at solvent manufacturing plants. In January 2009 the price of acetonitrile was recorded at about US\$100 per

liter with disposal costs of about double this price. The immediate challenge for analytical laboratories is to reduce their consumption of this HPLC solvent as much as possible [10]. Hence, the proposed method would be less costly to carry out routine determination of theophylline in the industry.

The results of the validation parameters are within acceptable limits. Good linearity was observed in the concentration range employed in the test with a regression coefficient ( $R^2$ ) of 0.994, thus indicating a high degree of sensitivity. The presence of the other ingredients in the formulations, including other active ingredients did not cause any interference with theophylline peak. Theophylline was well resolved from the other components of the formulations and the potential degradation product of theophylline. Thus, the method is selective for theophylline. The relative standard deviation (RSD) of 1.87 % was low, being less than RSD max. Thus the method is precise. Overall, the results show that the proposed method can be successfully applied for the determination of theophylline in pharmaceutical preparations.

## CONCLUSION

The developed HPLC method for the determination of theophylline in pharmaceutical preparations containing various other active and inactive pharmaceutical ingredients has been validated. Validation parameters were linearity, sufficient accuracy/recovery and precision, as well as low values of limits of detection and quantification. The method is column and machine-friendly, rapid, sensitive, accurate, and provides a reproducible means of determining theophylline in marketed pharmaceutical preparations and cyclodextrin inclusion complex.

## ACKNOWLEDGEMENT

The authors would like to express their gratitude to Dr. Yusrida Darwis (Associate Professor) of the Department of Pharmaceutical Technology, University Sains Malaysia (USM) for her encouragement and support, and Research & Innovation Division, USM, for financing the research as well as to USM Fellowship for continuous support.

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