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Reversed-Phase High-Performance Liquid Chromatography and High-Performance Liquid Chromatography Methods for simultaneous Determination of Theophylline in Their crude Powders and in Dosages pharmaceutical.

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Abstract

Context; in this manuscript high performance liquid chromatography method for the determination of theophylline in pharmaceuticals was described and developed. Method; the HPLC method was developed and the results obtained to determine the form of theophylline. results; the method was validated for accuracy , precision , specificity , linearity, and sensitivity. A simple, precise and accurate high-performance liquid chromatography–ultraviolet methodwas developed and validated for the quantification of theophylline . Separation was performed on Waters® C18 column (µBondapak™5 µm, 150 × 3.9 mm) using a mobile phase consisting of water–acetonitrile (96:4 v/v) at a flow rate of 1 mL/min. Validation of the method was performed in order to demonstrate its selectivity, linearity,
precision, accuracy and stability. The calibration curves of theophylline were linear over a concentration.The extraction recoveries of theophylline at the three levels of quality control sampleswere 63.1, 69.4 and69.7%. The method was rapid with retention time of theophylline and the internal standard observed at ~5.2 and 6.5 min, respectively. The developed method was applied successfully for studying the pharmacokinetics of theophylline .

INTRODUCTION

Theophylline (1,3-dimethyl-7H-purine-2,6-dioneis also known as dimethyl xanthine and belongs to the methyl xanthine group of drugs. It is used for the treatment of respiratory diseases such as chronic obstructive pulmonary diseases and asthma. Previous attempts and popular Pharmacopoeia methods for the analysis of theophylline used buffer as aqueous solvent and acetonitrile as organic solvent .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 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 Salts used in buffer solution may precipitate in the presence of organic solvents and increase the maintenance cost of HPLC pumps Quantity of organic solvents , The immediate challenge for analytical laboratories is to reduce their consumption of HPLC solvent as much as possible. Regulated laboratories that work with validated method shave 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).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.

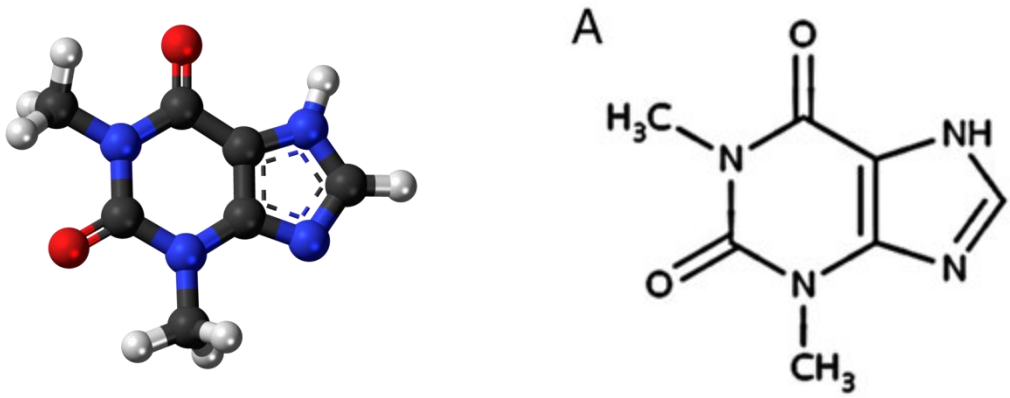

Figure 1. Chemical structures of theophylline

Various analytical methods have been reported for the analysis of theophylline in food, beverages, plants and biological fluids . Recently, Plonka reviewed the preparation stage and extraction methods and provides universal guidance on establishing a common procedures across laboratories to facilitate the preparation and analysis of methylxanthine compounds such as theophylline.For the analysis of theophylline in plasma samples, spectrophotometry (13), immunoassays (14) capillary electrophoresis (15), chromatographic methods (16, 17) and liquid chromatography–mass spectrometry (18–21) have been introduced. The immunological and spectrophotometric methods would be inappropriate for determination of the combination of different methylxanthines. Gas chromatographic procedures involve either multiple solvent extractions or chemical derivatization. Chromatographic and capillary electrophoresis methods can be applied to differentiate and measure these drugs, simultaneously. But most of the clinical laboratories are not equipped with capillary electrophoresis methods. Among the methods listed, the chromatographic methods are more widely accessible and capable of being implemented in clinical laboratories with standard high-performance liquid chromatography (HPLC) instrumentation.HPLC seems to be the most popular analytical method for the determination of theophylline in biological samples. Gradient elution can resolve much interference but, in general, it is more time consuming than isocratic methods. In the present study, we demonstrated and validated a sensitive and selective HPLC method for the quantitative determination of theophylline i with a small sample volume and simple sample preparation procedure. Melting point of theophylline was found to be in the range of 272°C to 274°C as reported in pharmacopoeia, thus indicating purity of the drug sample. The absorbance reading of theophylline standard solution containing 10- 100 µg/ml of drug in pH 1.2, pH 6.8 and pH 7.4 at the maximum wavelength of 272nm. Figure 4 shows the standard calibration curve for theophylline with slope, intercept and regression co-efficient. The calculations of drug contents and in-vitro drug release study are based on this standard curve.

Objective;

The Objective of the study was to develop and verify the RP-HPLC method with an UV detector for quantitative determination of theophylline in pharmaceuticals

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 ± 0.05) and filtered with 0.45 µm membrane filter (Millipore, Bedford, MA,USA) using Millipore vacuum filtration unit. All the reagents were used without any further purification. Generic and branded pharma ceutical 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 inTable 1.

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-10MVP) 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 µm membrane filter(Millipore, Bedford, MA, USA). The mobile phase was pumped isocratic ally at a flow rate of 1.0 mLmin-1 at 250 C. The injection volume was 10 µL.

Linearity assay

About 100 mg of theophylline anhydrous (99.9%) powder was accurately weighed into a dry 100 mL volumetric flask (25 ± 0.5 0C, RH 20 ±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 µ membrane filter. The filtrate (10 mL) was diluted to 100 mL with water to obtain standard solution of 100 µg/mL of theophylline. This standard solution (10 mL) was diluted to 100 mL with HPLC grade water to obtain the standard solution of 10 µg/mL of theophylline. To obtain the working solution, aliquots of standard theophylline solution were diluted to a concentration of 0.1 ug mL-1. The working standard solutions were prepared induplicate, filtered and degassed by passing them through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA). All the standard solutions were prepared at 25 ± 0.5 0C and 25 ± 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 *Trop J Pharm Res, January 2014; 13(1): 151*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 ug 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).

Validation of the bioanalytical method

Method validation was carried out according to European Medicines Agency (EMA) guidelines(24) in order to evaluate the method for selectivity, linearity of response, accuracy, precision, recovery and stability of analytes during processing and storage

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±0.5 0C and 25 ± 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 calibration curve of theophylline:

The UV scanning of drug sample was carried out using a solution of drug dissolved in 6.8 pH phosphate buffer solution. The lambda max was observed at 272nm. The calibration curve of Theophylline was obtained by dissolving the drug in 6.8 pH phosphate buffer solution and the absorbance was measured at 272nm by keeping 6.8

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 μg mL−1. Working standard solutions were prepared in triplicate

X-Ray Diffraction study:

Powder X-ray diffraction patterns where traced employing X-ray diffractometer (Seiferd,Model NO.3000,Germany) for samples, using Ni filtered CuK radiation, a voltage of 40 KV, a current of 30mA radiation scattered in the crystalline regions of the sample was measured . Patterns where obtained by using a step width of 0.04o C with a detector resolution in 2θ (diffraction angle) between 10o and 80o at ambient temperature 2

Statistical analysis

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

RESULTS

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 2.

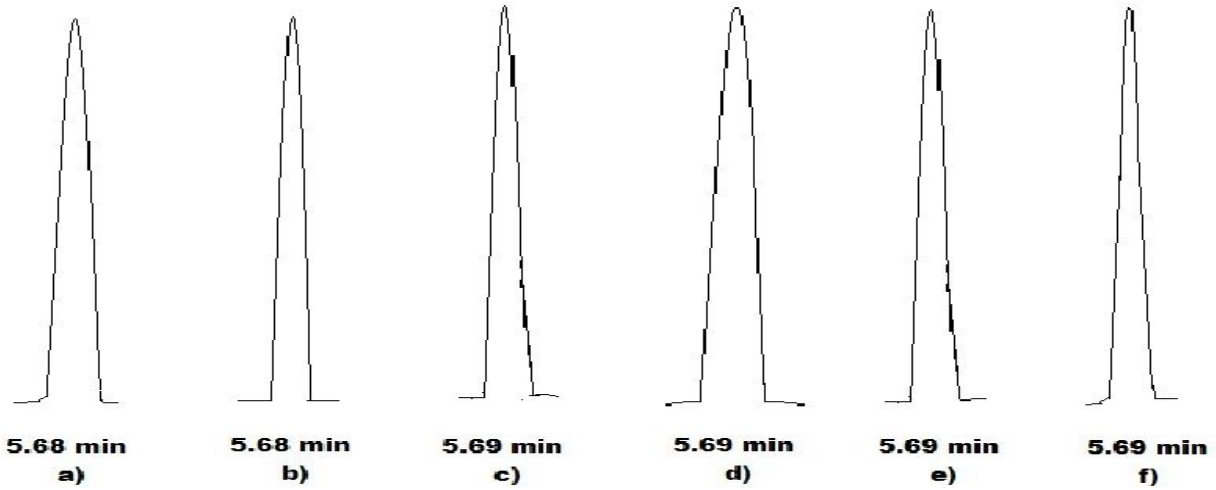

Fig 1: Representative chromatograms of (a) standard theophylline (anhydrous) (b) cyclodextrins inclusion
complex (c) Asmapax depot (d) Pharmaniaga Theophylline (e) Asmatide-BR and (f) Biryth

Table 2: Assay results for the determination of theophylline in commercial pharmaceutical preparations

Product	Labeled content (mg)	Actual content ±SD (mg)	RSD (%)
Cyclodextrin Complex	5	5.90±0.88	1.46
Asmapax depot	65	65.99±0.88	1.87
Pharmaniaga Theophylline	125	121.70±0.75	1.36
Asmatide-BR	200	198.50±0.55	1.46
Biryth	300	297.02±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 . 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 these is a sudden shortage of particular organic solvents due to technical or non-technical reasons at solvent manufacturing plants. In January 2009 the price 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 (R2) of0.994, 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.

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