ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIOPHARMACEUTICS

USE OF SIMPLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF THEOPHYLLINE (TH) IN SALIVA AND URINE OF HEALTHY HUMAN VOLUNTEER

VIKAS KUMAR[2*], ARVIND SHARMA[1], SANDEEP ARORA[1] AND VIPASHA DHILLON[2]

[1]Chitkara College of Pharmacy, Chitkara University, Punjab
[2]Chitkara School of Pharmaceutical Sciences, Chitkara University, Himachal Pradesh

VIKAS KUMAR
[2]Chitkara School of Pharmaceutical Sciences, Chitkara University, Himachal Pradesh

*Corresponding author

ABSTRACT

A simple and sensitive spectrophotometric method was developed and validated for the determination of Theophylline (TH) in pharmaceutical dosage form as well as in human saliva and urine samples. This method is based on the measurement of absorbance of TH in Phosphate Buffer 6.8 at 270 nm. Beer's law is obeyed over the range of 2-10 µg/ml. This method was used successfully for the quality assessment of Theophylline drug in human urine and saliva samples with good precision and accuracy. The Sandell sensitivity, limit of detection (LOD) and limit quantification (LOQ) values are also reported. All the methods were validated in accordance with current ICH guidelines. The developed methods were employed with high degree of precision and accuracy for the estimation of total drug content in commercial tablet formulations of TH. The results obtained from human spiked urine and saliva are satisfactory and recovery values were in limits.

KEYWORDS

Absorbance, Buffer, Theophylline, UV anaylsis

INTRODUCTION

Systemic drugs administered orally or parenterally must reach the general circulation in their pharmacologically active form to be distributed throughout the body and to exert therapeutic effect. The intensity of the therapeutic actions of many drugs correlate well with the concentration of the drug in the biological fluid, Koch-weser et al (1982). The rate of absorption is therapeutically important with single doses of drugs, especially in case of narrow therapeutic index drugs, Benet et al (1995). where relatively small changes in the concentration can lead to marked changes in pharmacodynamic response. Theophylline is a narrow therapeutic index drug and exhibits dose-dependent pharmacokinetics, Dahlquist et al (1984). It is commonly used for the control and management of acute asthma and acute exacerbations of chronic obstructive lung disease, Markham et al (1998).

The determination in biological fluids normally requires the use of trace analysis techniques such as HPLC, LC, capillary electrophoresis (CE), cyclic voltametry, LC-MS, gas chromatography– mass spectrophotometry (GC-MS), inductively coupled plasma – mass spectrophotometry. These methods require long and tedious pretreatment of the samples and laborious clean up procedures prior to analysis. Therefore, it is very imperative to develop a simple and suitable analytical method for the determination of theophylline in saliva and urine. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity, Rajinder et al (2009).

Experimental Procedure

Materials

Pure Theophylline was obtained as a gift sample from Cipla Ltd. TH-1, TH-2 are Phylobid & Theobid 200mg respectively. All the chemicals were used of analytical grade. All Absorbance measurements were made on Shimadzu model UV 1601 double beam UV Visible spectrophotometer with matched quartz cuvettes.

Method

Calibration curve of Theophylline:

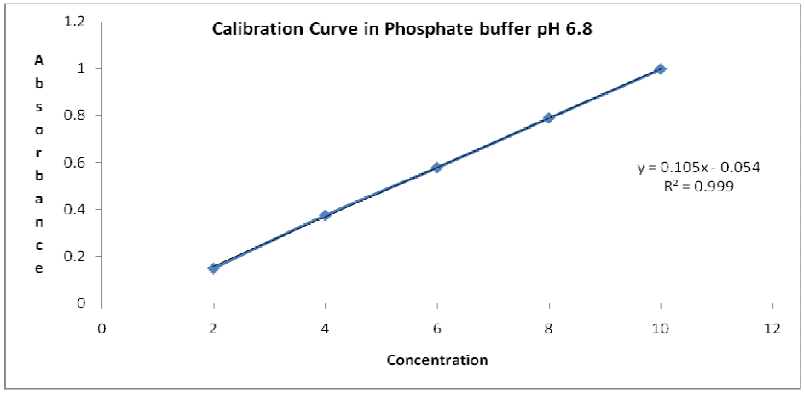

Fig - 1
Calibration curve of TH in phosphate buffer pH 6.8

Aliquots of stock solution (50 µg/ml) were pipette into a series of 10 ml volumetric flask and diluted to volume with phosphate buffer pH (6.8). The reaction was allowed to proceed at room temperature. The calibration curve was constructed by plotting absorbance against the initial concentration. The linearity range or Beer's range follows in between 2 to 10 µg/ml (Fig.1). The content of TH was calculated either from the calibration curve or corresponding regression equation and found that the absorbance is stable for at least ten days at room temperature. Assay of tablet was done as per method prescribed in IP (2007).

Study design for urine and salivary analysis

The study was conducted as an open label, balanced, randomized, two treatment, two period, two sequence single-dose crossover study in 8 healthy adult, male, human subjects, under fasting conditions, comparing the concentration marketed formulation in saliva and urine . Each subject received 200mg theophylline (two different brands TH-1,TH-2 of same drug) formulation with 240 ml of drinking water after an overnight fast during each of the study day. There was a washout period of seven days between each study period for estimation of drug saliva and urine. Informed consent was obtained from all subjects and the project was approved by the Ethical Committee of our Institution (Chitkara University).

Procedure for determination of theophylline in human urine samples

Eight healthy adult male volunteers between the ages of 21-25, weighing between 55 to 70 kg body-weight were selected for the study. Prior to initiation of the study the participants were subjected to thorough physical examination and their medical history taken. Basic tests like liver function test (LFT), urine analysis, full blood count (FBC) and blood sugar levels were conducted for each subject, to certify that they were medically fit for the test. All the subjects abstained from any xanthine-containing food or beverages or alcoholic products for 48 h prior to dosing and throughout the sampling schedule during each period. Aliquot volumes of human urine samples were transferred into small separating funnel 10 ml of phosphate buffer pH-6.8 were added and mixed well. A total of 64 Urine samples (5 ml each) were collected pre dose and at 0.5, 1.0, 2, 3, 4, 6, 8, and 10 h post dose. The total volume of each interval was recorded and a 10 ml sample each was frozen until assay. Spectrophotometric method was employed to determine the elimination half life from the calibration graphs or corresponding regression equation

Procedure for determination of theophylline in human saliva samples

Following overnight fast one tablet containing 200mg of Theophylline was given orally along with 150 ml of water. The mouth was rinsed promptly with another 100 ml of water which was also swallowed. Food was with held for a further period of 2 h to ensure complete absorption of the drug. Saliva samples were collected simultaneously at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min. The salivary sample was collected by placing citric acid (about 10 mg) over the tongue and held in the mouth for one to two minutes after which the contents were spit into a centrifuge tube. (Preliminary experiments showed that citric acid did not interfere with the estimation). Samples were centrifuged to remove mucous and particulate matter from saliva. The salivary supernatants samples were stored at -20°C until analyzed. The Theophylline concentration in saliva was estimated by a modified method of Miceli et al (1979). For the determination of theophylline concentration in saliva: 1 ml of 20% trichloracetic acid was added drop by drop to 1 ml saliva with constant shaking. It was then centrifuged at 4000 rpm for 5 min followed by 2000 rpm for 3 min. The supernatant (1.75 ml) was transferred to a screw-capped 10 ml test-tube followed by dilution with phosphate buffer

(pH 6.8). The remaining procedure salivary estimation was the same as Miceli et al. (1979). With the above modification concentration up to 1µg/ml could be detected in saliva. The salivary concentrations of theophylline were plotted against time on a semi logarithmic graph paper and the elimination half life ($t^{1/2}$%) was calculated. Both protocol was in accordance to our previous study a comparative bioequivalence study of some brands of ofloxacin by urine and salivary analysis in India, Sharma et al (2010).

RESULT AND DISCUSSION

Method Validation

The method was validated for selectivity, linearity, precision, accuracy, recovery and stability according to the principles of the Food and Drug Administration (FDA) industry guidance. The specificity and selectivity of the proposed method was evaluated by estimating the amount of TH in the presence of common excipients. The linearity of the proposed method was constructed for TH standard stock solution by plotting concentration of the compound versus the absorbance. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The accuracy and precision of the method was evaluated within the linear range based on the analysis of TH samples and pharmaceutical formulations at 2.0, 4.0 and 6.0 µg/ml. Five independent analysis were performed at each concentrations level within one day (Intra-day precision) as well as for five consecutive days (Inter-day precision). The accuracy was ascertained by recovery studies using the standard addition method. The proposed method was used for estimation of TH from tablets after spiking with 50, 150 and 250 % additional pure drug. The amount of TH was determined from the regression equation. The absorbance-concentration plot for the proposed method was found to be rectilinear over the range of 2- 10 µg/ml. Statistical analysis of regression lines were made regarding the standard deviation of slopes (Sb) and standard deviation of intercepts (Sa) and the values are summarized in Table 1.

Table 1
Summary of optical and regression characteristics of the proposed method

Parameters	Theophlline
Linear dynamic range (µg/ml)	2-20
Regression equation	$Y=4.5x10^{-2}+0.157$
Correlation coefficient (r)	0.998
LOD (µg/ml)	$2.17x10^{-1}$
LOQ (µg/ml)	$7.895x10^{-2}$

Estimation of drug in urine and saliva

The proposed methods were found to be simple, accurate and reproducible for routine estimation of TH in urine and saliva. The average values for % cumulative amount of TH excreted, rate of excretion (dAU/dt) and log-transformed rate of excretion (log(dAU/dt)) for both urine and saliva with respect to mid-point of time are given in (Table.2). The plots of average % cumulative of TH excreted over a period of 12 h versus mid-point of time and average (log(dAU/dt)) versus mid-point of time, are shown in (Fig. 1& Fig. 2) respectively. From these figures it is evident that analysis of TH can be done for a period of 12 hrs in urine and saliva respectively.

Table 2

The average values for % cumulative amount of TH excreted, rate of excretion (dAU/dt) and log-transformed rate of excretion (log(dAU/dt))

Mid Point of time (h)	%age Cumulative Release		Rate of Excretion		log(dAU/dt) (mg/h)	
	Urine	Saliva	Urine	Saliva	Urine	Saliva
0.5	1.76	1.86	7.05	15.32	1.02	1.04
1	5.12	4.32	14.87	24.5	1.36	1.31
1.5	8.99	7.76	16.032	21.08	1.33	1.35
2	11.87	9.77	17.33	19.02	1.41	1.29
2.5	13.76	12.34	15.76	16.6	1.29	1.22
3	16.98	14.98	13.97	15.19	1.21	1.21
3.5	22.12	16.09	11.023	14.6	1.16	1.18
4	26.65	21.77	9.87	12.33	1.11	1.09
4.5	35.66	33.53	8.13	10.78	1.03	1.03
5	39.31	42.64	7.34	9.87	0.85	0.71
5.5	40.23	44.03	6.81	8.05	0.77	0.54
6	42.47	45.76	5.76	6.69	0.59	0.33

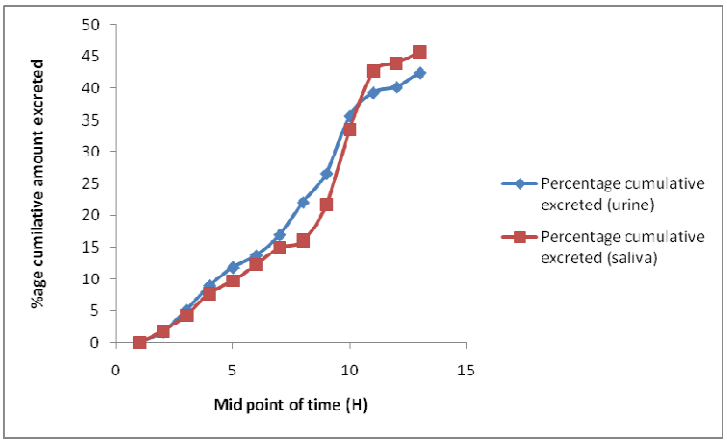

Fig.1

Average % cumulative TH excreted, versus mid-point of time plots after administration to eight healthy male volunteers

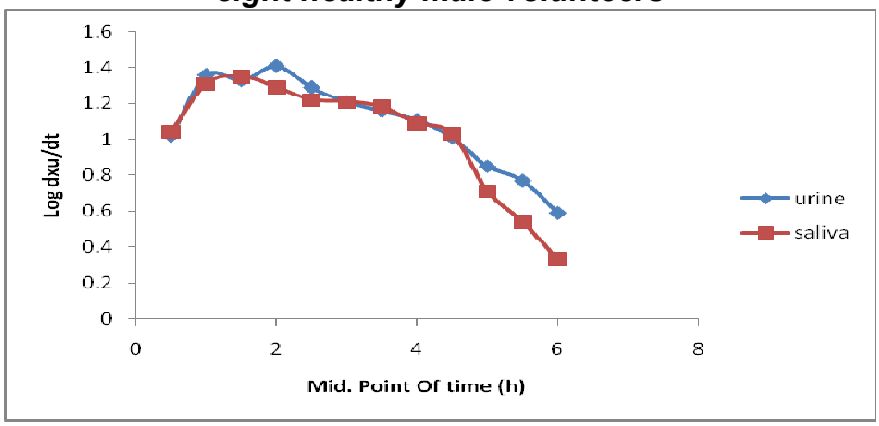

Fig 2

Average log excretion rate (log (dAU/dt)) versus mid-point of time plots for TH after administration to eight healthy male volunteers in urine and saliva

Urinary excretion levels of TH after administration Formulation TH-1&TH-2 (different brands) equivalent to 200mg of TH in eight volunteers was estimated using proposed spectrophotometric method. The proposed method was used for estimating of TH from tablets after spiking with 50, 150 and 250 % of additional pure drug. The selectivity of the proposed method was ascertained by analyzing standard TH in the presence of excipients. It was observed that the excipients did not interfere with the proposed method. The proposed method was further extended to the in vitro determination of TH in spiked human urine samples.TH undergoes minimal metabolism in human with unchanged parent representing ≥90% of drug derived in urine. This concentration fell well in within working range of proposed method. The calibration graphs were constructed by plotting absorbance versus increasing concentrations of TH in spiked human urine samples over the concentration range 2-10 µg/ml. The results (Table.1) are satisfactorily accurate and precise. The performance of the proposed method was studied with other existing method, Gujral et al (2009). In case of proposed method and reported method, the reported method has comparable LOD and LOQ value as in earlier reported method. Analysis of TH concentration in 160 paired samples of saliva collected at different time intervals showed significant correlation ($r = 0.64$, $P<0.05$). We could not find any significant difference between the mean urine and salivary TH half lives.

CONCLUSIONS

The proposed method does not require any laborious clean up procedure before measurement. In addition, the method has wider linear dynamic range with good accuracy and precision. The method shows no interference from the common excipients and additives. This may help in analyzing affectivity of this drug in human beings during treatment. Therefore, it is concluded that the proposed method is simple, sensitive and rapid for the determination of in bulk, pharmaceutical formulations and in human urine and salivary samples.

ACKNOWLEDGEMENT

The authors wish to express their sincere thanks to all the volunteers and Chitkara Educational Trust for their help in the earlier stage of this research work.

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