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A New Validated Stability-Indicating RP-HPLC Assay Method for Pentoxifylline In Bulk Drug

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ABSTRACT

An isocratic reversed phase stability-indicating reverse phase high-performance liquid chromatographic (HPLC) assay method have been developed and validated for the determination of pentoxifylline in bulk drugs. Separation of pentoxifylline from the degradation products was carried out using an Inertsil ODS C_{18} (250cm x 4.6)mm,5u column with mobile phase consisting a mixture of acetonitrile and KH_2PO_4 buffer (pH 4.0) in the ratio of (60:40v/v). The detection was carried out at wavelength 275nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity prove the stability indicating ability of the method.

Keywords: RP-HPLC, Stability indicating and Pentoxifylline.

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INTRODUCTION

Pentoxifylline[1-3], 1-(5-oxohexyl)-3,7-dimethylxanthine, is an active haemorheological drug routinely used for the treatment of intermittent claudicating and other circulatory disorders [4-6]. Several analytical methods for the assay of pentoxifylline in pharmaceuticals have been reported, that include high performance liquid chromatography[7,8], spectrophotometry[9-11] mostly in plasma and few in pharmaceutical formulations. The main goal of this present study is to develop, a simple, rapid, sensitive, precise, accurate and specific RP-HPLC method and its applications to bulk drug with high sensitivity and selectivity.

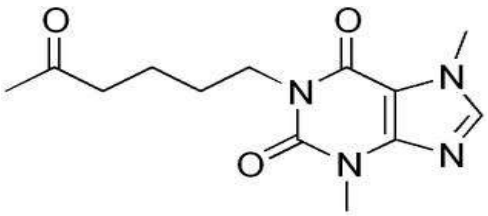

Figure.1: Structure of Pentoxifylline

MATERIALS AND METHOD

Material and reagents:

Pentoxifylline bulk drug was made available from Merck Ltd. India (purity 99.8%). Potassium dihydrogen ortho phosphate, Orthophosphoric acid, hydrochloric acid was obtained from S.D fine chemicals, India Limited. Acetonitrile, hydrogen peroxide, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent used were of HPLC grade; milli-Q-water was used throughout the experiment.

Chromatographic conditions:

A chromatographic system (Waters) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and equipped with photodiode UV detector 10A-VP series with waters software. The chromatographic column of 250cm length and internal diameter of 4.6 mm filled with Octadecyl silane chemically bonded to silica [Inertsil ODS C_{18}] stationary phase with particle size 3.0 micron was used. The instrumental settings were a flow of 1.0 mL/min; the injection volume was 5.0µL.

Mobile phase:

The mobile phase contains the KH_2PO_4 buffer (pH 4.0) and acetonitrile. The buffer was degassed and filtered through a 0.45µm nylon filter.

Preparation of standard stock solutions:

Standard stock solution of 200µg/mL of pentoxifylline in mobile phase containing the

acetonitrile and KH_2PO_4 buffer (pH 4.0) in the ratio of (60:40v/v) was prepared in volumetric flask. Working solutions (range 10-50µg/mL) were prepared by diluting the stock solution in 100ml with the same solvent.

Sample solution:

The content of the flask was shaken few minutes and diluted to volume with same solvent. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure.

Selectivity:

The selectivity of the developed LC method for Pentoxifylline was carried out in the presence of its degradation products. Stress studies were performed for Pentoxifylline bulk drug to study the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.1N Hydrochloric acid), alkali (0.02 N NaOH, hydrogen peroxide (30%), and heat (60^oC) to evaluate the ability of the proposed method to separate Pentoxifylline from its degraded products. For thermal degradation, study period was 3 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Pentoxifylline reference standard and the mass balance was calculated (Table 1).

Table 1: Results of forced degradation studies

Stress condition	Time	Assay of degradative substance	Remarks
Thermal degradation (80^0C)	3days	99.92	No degradation
Oxidation (H_2O_2)	48hrs	99.80	No degradation
Acid(0.1M)	48hrs	99.61	No degradation
BACE(0.2M)	2hrs	99.85	No degradation

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:

The main target for the development of chromatographic method was to get the reliable method for the quantification of pentoxifylline from bulk drug and which will be also applicable for the degradable products 9. Initially, efforts were made for the development of HPLC method quantification of standard pentoxifylline from bulk. For this purpose, assy wsa carried out on various columns such as Kromasil C_{18} (250 x 4.6) mm, 5µ column, Kromasil C_{18} (150 x 4.6) mm, 5 µ and Inertsil ODS 3V C_{18} (250 x 4.6) mm, 5 µ. Out of these used HPLC column, Inertsil ODS 3V C_{18} (250 x 4.6) mm, 5 µ, column found to comparatively better and gave the graph with better Gaussian shape at retention time 10.87 min. To improve the shape and width of the graph,

for the above column different ratios of solvents and buffer trials were carried with Acetonitrile and KH_2PO_4 (i.e 40:60,50:50 and 60:40)in these trials mobile phase mixture Acetonitrile and KH_2PO_4 in the ratio of 60:40 gave good and sharp peak (Table 2) .

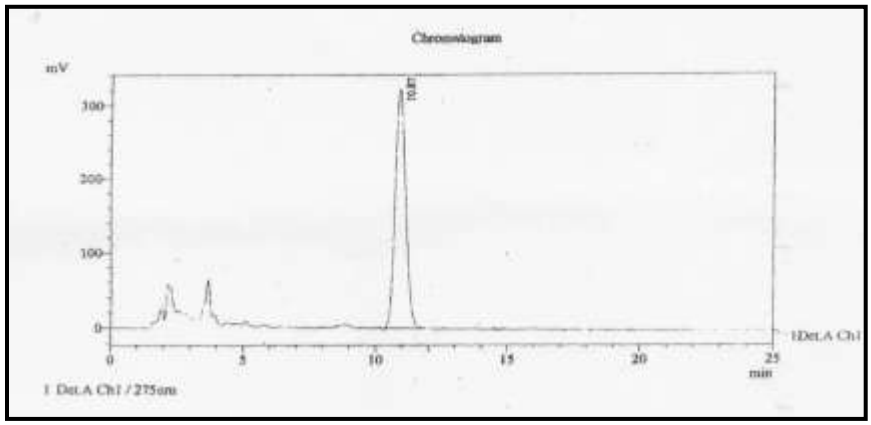

Figure 2: HPLC Chromtogram of Pentoxifylline

Table 2: Optimized chromatographic conditions

Parameters	RP- HPLC
Elution	Isocratic
Mobile phase	Acetonitrile and KH_2PO_4 buffer (pH 4.0) in the ratio of (60:40v/v)
Column	Agilent C_8 (250 x 4.6 mm) column
Flow rate	1.0 $mL.min^{-1}$
Detection	UV at 275nm
Injection volume	5.0µL
Temperature	Ambient
Retention time	10.87 minutes
Run time	25 minutes
Area	8862410 mAU

Calibration and linearity:

The linearity of the calibration graph and conformity of RP-HPLC value to Beer's Law were proven by the high correlation coefficients (r) for the regression equations. Standard solutions containing 10-50µg/mL of pentoxifylline in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear (0.9999) in the mentioned concentrations. The regression equation calculated from calibration curves given with the standard deviations of slope (Sb) and intercept (Sa) on the ordinate are given in Table 3.

LOD and LOQ (Sensitivity):

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for pentoxifylline was found to be 0.291µg.mL^{-1} and 0.972µg.mL^{-1} respectively. The results are shown in Table 3.

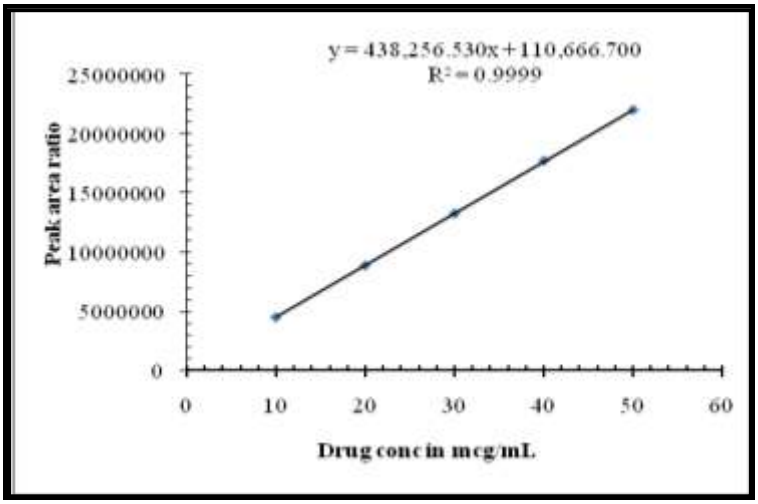

Figure 3: Calibartion Curve of Pentoxifylline

Table 3:Calibration of the RP HPLC for the estimation of Metoprolol Succinate

Concentration in µg.mL^{-1}	Area (mAU)
10	4491205
20	8862410
30	13253615
40	17698693
50	21985890
Regression equation	Y = a X + b
Slope (a)	110666
Intercept (b)	438256
Correlation coefficient	0.9999
LOD	0.2918
LOQ	0.9727

Precision:

The precision of the method was studied by determining the concentrations of the drug pentoxifylline in the tablet for six times. The results of the precision study (Table 4) indicate the reliability of the method (RSD % < 2).

Table 4: Precision data

Injection No.	Area response
1	8862410
2	8798614
3	8862458
4	8799658
5	8799986
	8882345
Mean	8839245
%RSD	0.5054
STDEV	44681.84

All the values are the averages of three determinations

Accuracy (Recovery test):

The accuracy of an analytical procedure expresses the closeness of agreement between the values, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 25%, 75% and 125%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for pentoxifylline ranged from 100.10% to 101.17% (Table 5). The average recoveries of three levelsnine determinations for pentoxifylline were 100.22-100.21%.

Table 5: Results of Recovery studies

Level of addition (%)	Amount added(n=3)µg	%Recovery
25	10	100.10
75	30	100.52
125	50	101.17

Assay studies of pentoxifylline from its formulation:

The proposed method was applied for the determination of valsartan in tablets. The results of these assays was 99.48% (% RSD = 0.84%) of the label claim for the formulation. The results of the assay indicated that, the developed method is selective for the assay of valsartan without interference from excipients used in the tablets (**Table 6**) respectively.

Table6: Assay studies

Formulation	Labeled amount(mg)	Recovered amount(mg)	% Recovery
Tablet	400	399.92	99.98

Average of five determinations

CONCLUSION:

The method developed for the assay of pentoxifylline is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method validated parameters tested. The developed method was stability indicating and can be used for assessing the stability of pentoxifylline as bulk drugs. In summary, the proposed RP-HPLC method can be used for the routine quality control analysis of pentoxifylline in bulk drugs.

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