Original Research Article

REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD FOR ANALYSIS OF DOXOFYLLINE IN PRESENCE OF ITS DEGRADATION PRODUCTS

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ABSTRACT

A simple, precise and novel reverse phase liquid chromatographic method for analysis of doxofylline in bulk drug as well as in pharmaceutical preparations in the presence of potential degradation products of doxofylline has been developed and validated. Forced degradation studies were carried on doxofylline in acidic, neutral and alkaline hydrolytic conditions in addition to oxidative, thermal and photolytic conditions. Optimum separation among doxofylline and its degradation products was achieved using a ternary mixture of water: methanol: ethyl acetate in the ratio of 80:10: 10 % v/v/v as the mobile phase at a flow rate of 1.0 ml/min on a Supelco C18 DB 150 mm X 4.6 mm column as the stationary phase when scanned at a wavelength of 277 nm. The retention time for the various degradation products were found to be sufficiently different with each other as well as with the parent drug at the optimized chromatographic conditions to permit their accurate quantitative estimation. The method was found to be linear at least in the range of 5-25 µg/ml. The developed method was then validated for precision, accuracy, specificity, robustness and ruggedness in accordance with the ICH guidelines and other available regulatory guidelines.

Keywords: Doxofylline, Forced degradation studies, Method development, Validation

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INTRODUCTION

Doxofylline is chemically, 7-(1, 3-Dioxolon-2-yl methyl)-3, 7-dihydro- 1, 3-dimethyl-1H purine- 2, 6-dione [Figure 1]. Unlike other xanthines, doxofylline lacks any significant affinity for adenosine receptors and does not produce stimulant effects. This suggests that its antiasthmatic effects are mediated by another mechanism, perhaps its actions on phosphodiesterase [1]. It has a better safety profile, because of reduced affinity for adenosine A_1 and A_2 receptors. Moreover, unlike theophylline, doxofylline does not antagonize calcium-channel-blocker receptors nor does it interfere with the influx of calcium into cells. Doxofylline is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD), and chronic bronchitis. It is given orally in doses of up to 1,200 mg daily either as tablets or syrup. It may also be given by slow intravenous injection.

An extensive literature survey revealed analytical methods [2-20] reported for estimation of doxofylline. Most of the methods reported are for estimation of doxofylline alone or along with its metabolites in biological fluids such [2-11]. The reported methods for estimation of doxofylline in pharmaceutical formulations available are [11-20] of which methods [11-15] are for estimation of doxofylline in single component formulations whereas methods [16-20] are reported for estimation of doxofylline in multi-component formulations. Most of these methods are based on chromatographic and spectrophotometric methods. However, only few stability indicating analytical methods for estimation of doxofylline [15-17] are reported in literature so far. In this paper we report a novel analytical method which provides a simple,

rapid, precise and accurate method for determination of doxofylline in the presence of its potential degradation products.

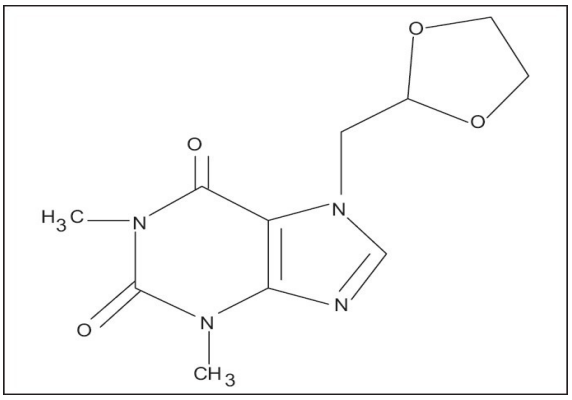

Figure 1: Structural formula of Doxofylline

EXPERIMENTAL

Instrumentation

Shimadzu HPLC system provided with Shimadzu HPLC pump LC-10AT Vp, on line degasser DGU-14A, low pressure gradient flow control unit FCV-10AL Vp, universal injector 2E 7725 (Rheodyne) with 20 µl loop, Hamilton micro liter syringe 25 µl, column oven CTO-10AS Vp, variable wavelength UV-VIS detector SPD-10AVp with Shimadzu class CSW software, C_{18} -column (Supelco, 4.6 mm × 150 mm) was used for method development.

Chemicals, Reagents and Solutions

Pure doxofylline was generously gifted by Dr. Reddy's Laboratory, Hyderabad, India. The marketed preparations of doxofylline used in the study, *Doxobid* was purchased from the local market. All the chemicals and solvents used were of HPLC grade. Double distilled water and Whatmann filter paper Grade-I, 0.45-µm filter paper were used throughout the experimental work.

Preparation of Standard Solution

An accurately weighed quantity of doxofylline (10 mg) was taken in 100 ml volumetric flask and dissolved in about 20-25 ml of methanol and made up to the volume to obtain a standard stock solution of doxofylline of 1 mg/ml concentration. One milliliter of this standard stock solution was transferred to a 100 ml volumetric flask and the volume was made up to the mark with mobile phase so as to obtain a working standard of 10 µg/ml concentration.

Preparation of Sample Solution

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 5 mg of doxofylline was transferred into a 50 ml volumetric flask containing little methanol. The flask content was agitated for 30 min on a wrist shaker to release the drug from the tablet matrix. Then, the volume was adjusted to the mark with methanol and sonicated for 15 min to make the content homogeneous. The solution was then filtered through Whatmann Grade I filter paper to obtain a resultant sample solution of 100 µg/ml concentration. Five milliliters of the filtrate was then transferred to 50 ml volumetric flask and the volume was made up to the mark with mobile phase to get a final concentration of 10 µg/ml of doxofylline.

Force Degradation (Stress Studies) of Doxofylline

Stress studies were performed on pure doxofylline and one of its marketed preparations *Doxobid* by exposing them to various stress conditions over wide range of pH, heat, oxidation and photo degradation separately.

Hydrolytic degradation under acidic, neutral and alkaline condition was carried by transferring 50 mg of Doxofylline in each of the three round bottom flask and dissolving them separately using 50ml of 0.1N aqueous hydrochloric acid, 50 ml of double distilled water and 50 ml of 0.1N aqueous sodium hydroxide respectively followed by refluxing them on a thermostatic water bath at 70˚C.

Oxidative degradation was carried out both at ambient temperature and by refluxing at 70˚C. This was carried taking into consideration the facts that – (a) many of the oxidative degradation products formed are thermally unstable and may decompose at higher temperatures, (b) the observed oxidative rates and pathways may be different than those observed at higher temperatures and this may lead to different oxidation degradation product as the O–O bond of hydrogen peroxide is a weak bond that will readily cleave at elevated temperatures to form hydroxyl radicals which is a much harsher oxidative reagent and (c) the reaction rate in solution may actually be reduced at higher temperatures because of the decrease in oxygen content of the solvent. For oxidative degradation, 50 mg each of doxofylline were dissolved in 50 ml of 3 % H_2O_2 (1 mg/ml) in each of the two round bottom flasks separately. One of the round bottom flasks was refluxed on water bath maintained at 70˚C. The other round bottom flask was kept at room temperature (RT).

Photolytic degradation was carried by evenly spreading doxofylline as thin layer in a covered petridish and exposing in sunlight. Thermolytic degradation was carried by transferring doxofylline in covered petridish kept in an oven maintained at a temperature of 80˚C.

Similarly, the various degradation products of *Doxobid* were prepared by exposing equivalent quantity of *Doxobid* tablet powder and treating in similar manner as in the case of preparation of forced degradation samples of pure doxofylline.

The samples which showed no degradation at the initial stress conditions were subjected to increasingly more severe stress conditions till a maximum limit was reached. The maximum stress conditions to be subjected were determined based on the available regulatory guidelines, the current pharmaceutical stress testing trends and/or practical constraints imposed by the physicochemical properties of the molecule.

Sampling of Force Degradation Products

Five milliliters of all hydrolytic and refluxed oxidative degradation samples (1 mg/ml) were withdrawn during hydrolysis under acidic and alkaline conditions every 1st, 3rd, 5th and 8th hour and stored under refrigeration. For oxidative degradation sample at RT, 5 ml samples were sampled at intervals of 1, 3, 5 & 7 days. In case of thermal and photo degradation studies, 10 mg samples were withdrawn after 7th, 14th and 30th day and dissolved in 10 ml of methanol to obtain a resultant concentration of 1 mg/ml each.

Preparation of Degradation Sample for HPLC

All the stock degradation samples collected (1 mg/ml each) were diluted with mobile phase so that the final working solutions of all forced degradation products were of the concentration 10 μg/ml with respect to the parent drug.

Chromatographic Conditions

All the experimental work was performed on Shimadzu HPLC system using C_{18} -column (Supelco, 4.6 mm × 150 mm). The mobile phase containing mixture of water: methanol: ethyl acetate in the ratio of 80:10: 10 % v/v/v, was found to be most satisfactory as it gave good resolution of drug and degradation products peaks with

reasonable symmetry. Figure 2 shows HPLC chromatogram of doxofylline. The wavelength of 277 nm, the λmax of pure doxofylline itself in the said mobile phase as determined by its UV spectroscopy was found to be sensitive enough to detect both the parent drug and its degradation products. A flow rate of 1 ml/min with 25^{o}C column oven temperature was found to be optimum. The retention times under the optimized chromatographic conditions was found to be 6.28 ± 0.02 min with an asymmetry factor of 1.15 ± 0.03. A total run time of about 9 min of the chromatogram was able to depict the peaks for doxofylline and all of its potential degradation products.

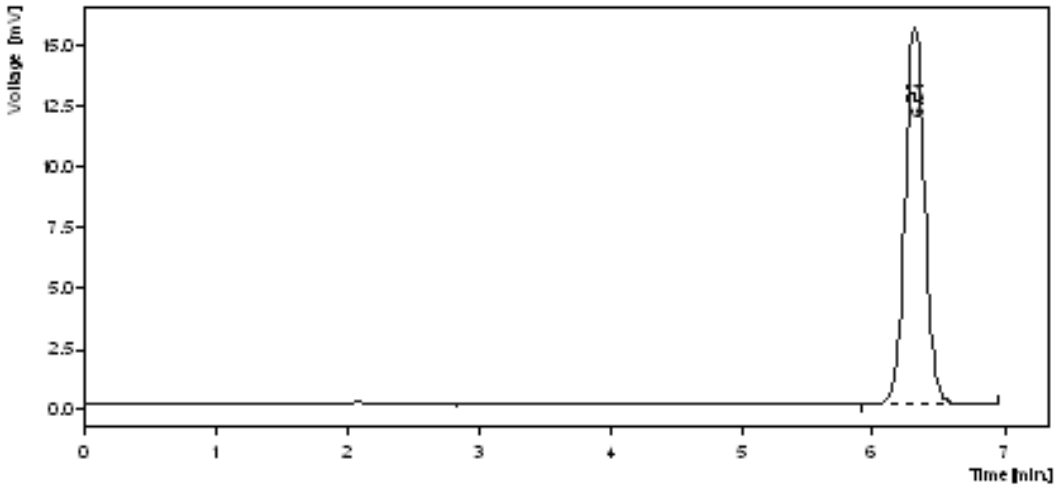

Figure 2: HPLC chromatogram of Doxofylline

Stability of Standard and Sample Solutions

The chromatograms of the same working standard and sample solution were obtained periodically over a period of 24 hours and the solutions of both the standard drug and sample were found to be stable in the tested duration. The results are shown in following Table 1.

Table 1: Results of stability of standard and sample solutions

Time (hrs)	% Estimation	
	Standard	*Doxobid*
0	-	99.89
6	99.60	99.75
12	99.43	100.02
24	100.30	99.98

System Suitability Test (SST)

SST is commonly used to verify resolution, column efficiency, and repeatability of the chromatographic system to ensure its adequacy for a particular analysis. Results of SST carried are shown in Table 2.

Table 2: Result of system suitability studies

S. No	Retention Time (min)	Asymmetry	No. of Theoretical plates	Capacity Factor	Peak Area
1	6.32	1.15	80355	5.81	223.6
2	6.29	1.12	78261	5.74	221.9
3	6.28	1.16	76514	5.78	224.7
4	6.31	1.15	77902	5.69	224.1
5	6.29	1.17	81003	5.83	223.2
Mean	6.30	1.15	78807	5.77	223.5
± SD	0.16	0.19	2295.52	0.056	1.056
% RSD	2.6	0.17	2.91	0.97	0.47

SD = Standard deviation, RSD = Relative standard deviation

Linearity study

A plot of peak area versus concentration [Figure 3] showed that the method was found to be linear at least in the range of 5-25 µg/ml. The regression equation was found to be y = 13.754x + 2.9881 and the coefficient of regression was 0.9991.

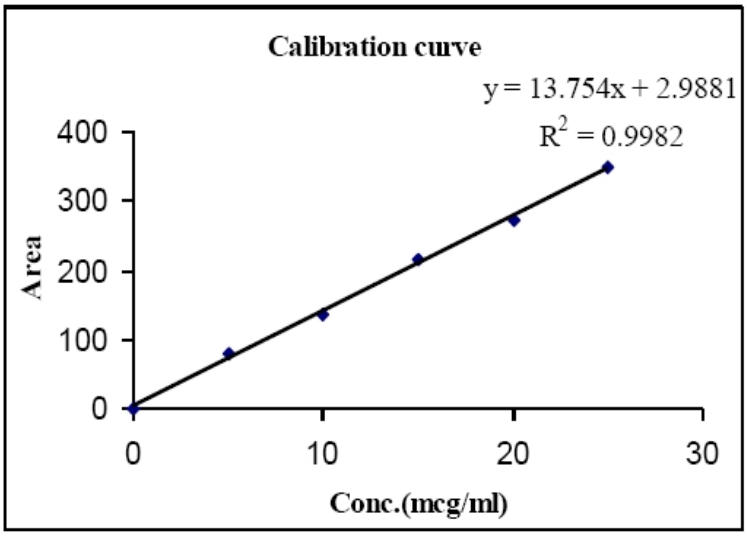

Figure 3: Calibration curve of Doxofylline

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

The chromatographic separation of doxofylline and its degradation products was done on reverse phase C_{18} column. The mobile phase containing mixture of water: methanol: ethyl acetate in the ratio of 80:10: 10 %

v/v/v was found to be most satisfactory as it gave good resolution of drug and degradation products with reasonably symmetrical sharp peaks. The detection wavelength of 277 nm, the λmax of pure doxofylline itself was optimum as the degradation products of doxofylline showed substantial absorbance to be detected at this wavelength. A flow rate of 1 ml/min with 25ºC column oven temperature was found to be optimum. The retention time for the parent drug under the optimised chromatographic conditions was found to be 6.28 ± 0.02 min with an asymmetry of 1.15 ± 0.03. A total run time of 9 min was sufficient to depict all the degradation products of doxofylline [Figure 4(a-h)]. Stress studies on one of the marketed formulation *Doxobid*, showed similar results to that of the bulk drug with no any additional peaks or interference indicating that the proposed method could be used for estimation of doxofylline in the presence of its potential degradation products in pharmaceutical preparations as well.

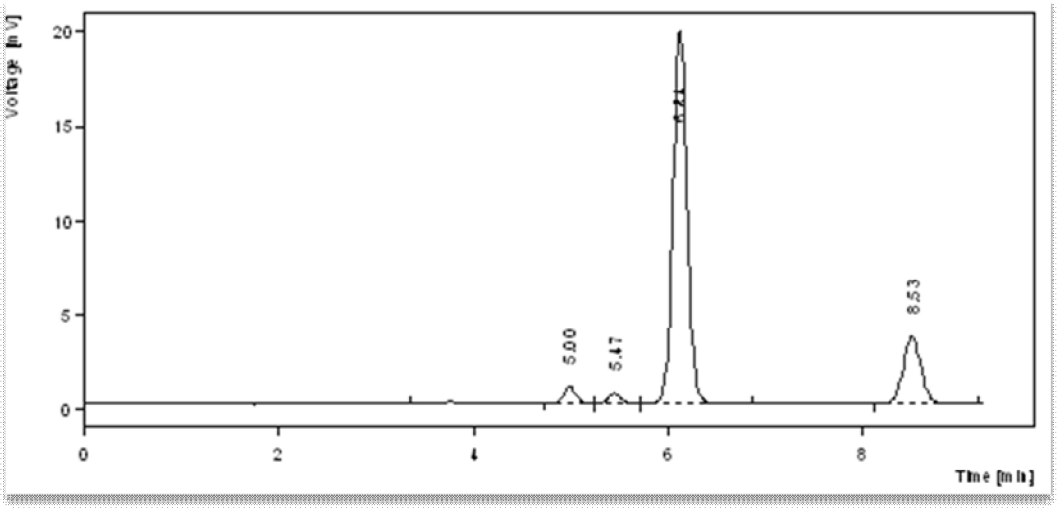

Figure 4(a): Chromatogram - Acidic hydrolysis in 0.1 N aq. HCl at 70˚C (3 hrs)

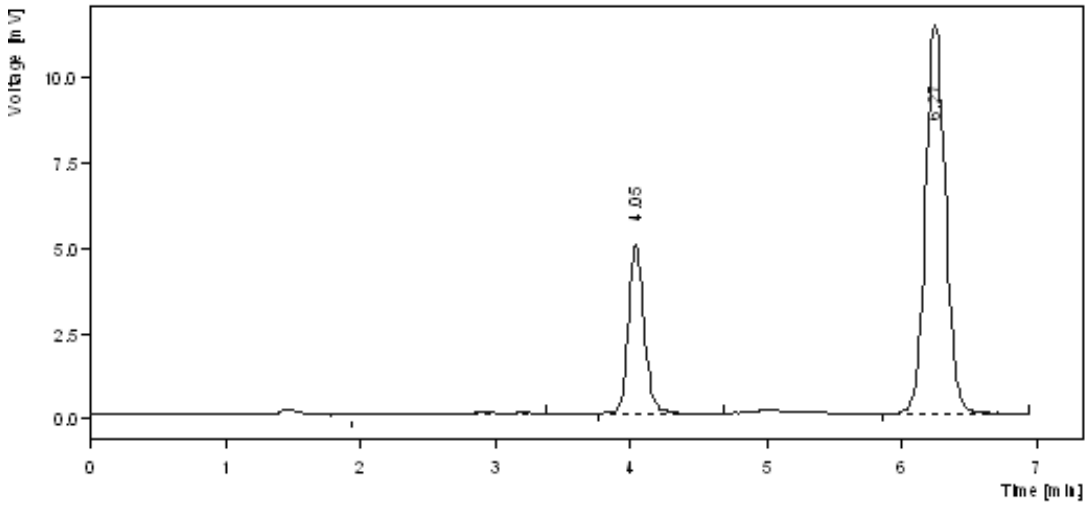

Figure 4(b): Chromatogram - Alkaline hydrolysis in 0.1 N aq. NaOH at 70˚C (5 hrs)

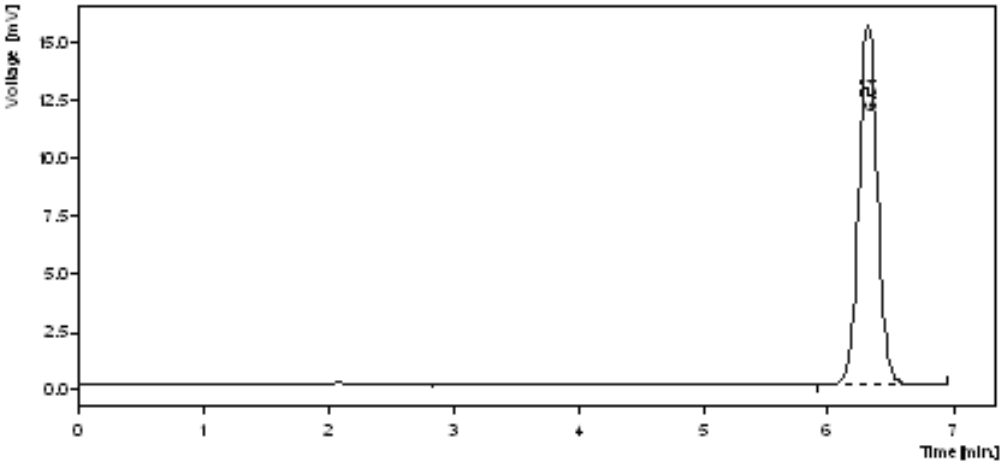

Figure 4(c): Chromatogram - Neutral hydrolysis in water at 70˚C (8 hrs)

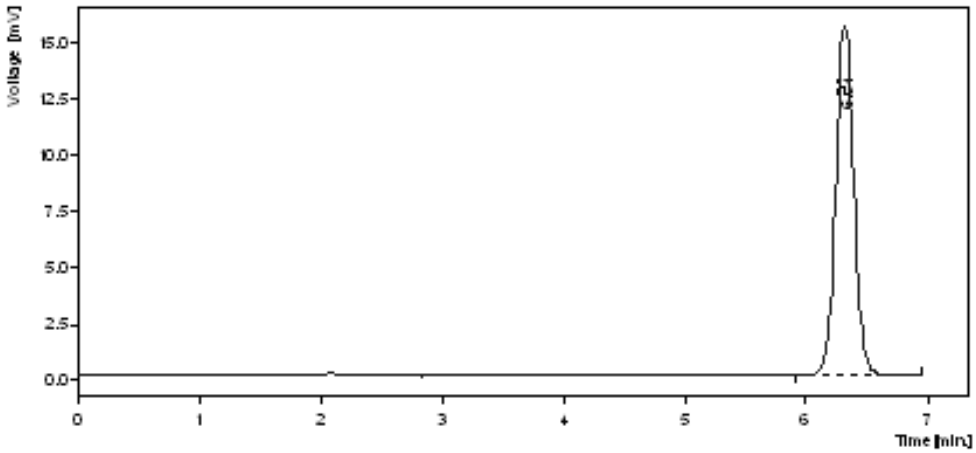

Figure 4(d): Chromatogram - Oxidative degradation in 3% H_2O_2 at RT for 7 days

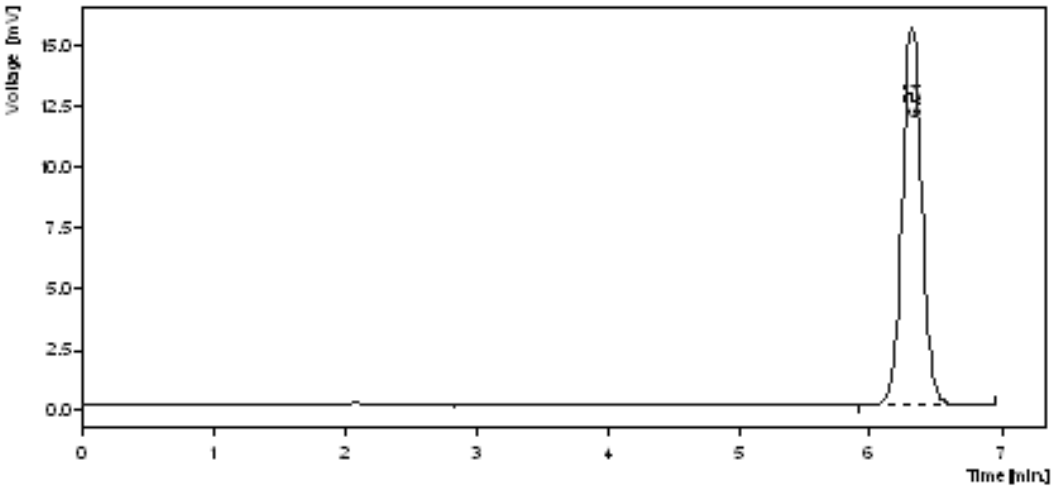

Figure 4(e): Chromatogram - Oxidative degradation in 3% H_2O_2 at 70°C for 7 days

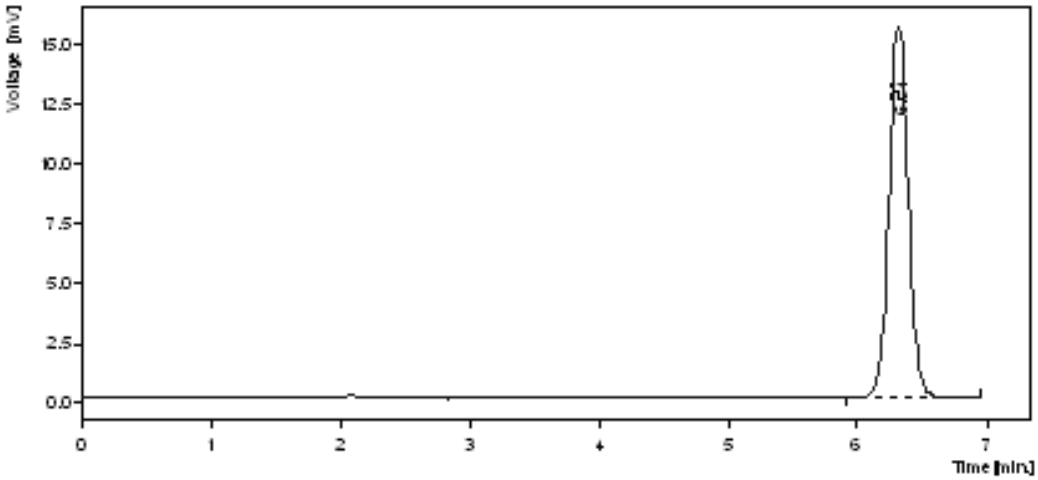

Figure 4(f): Chromatogram- Thermal degradation at 80°C (30 days)

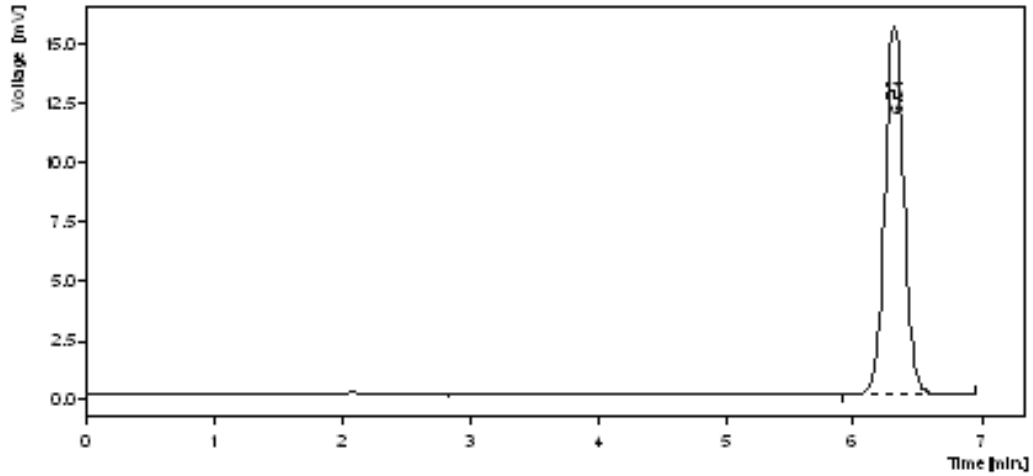

Figure 4(g): Chromatogram- Photodegradation in sunlight (30 days)

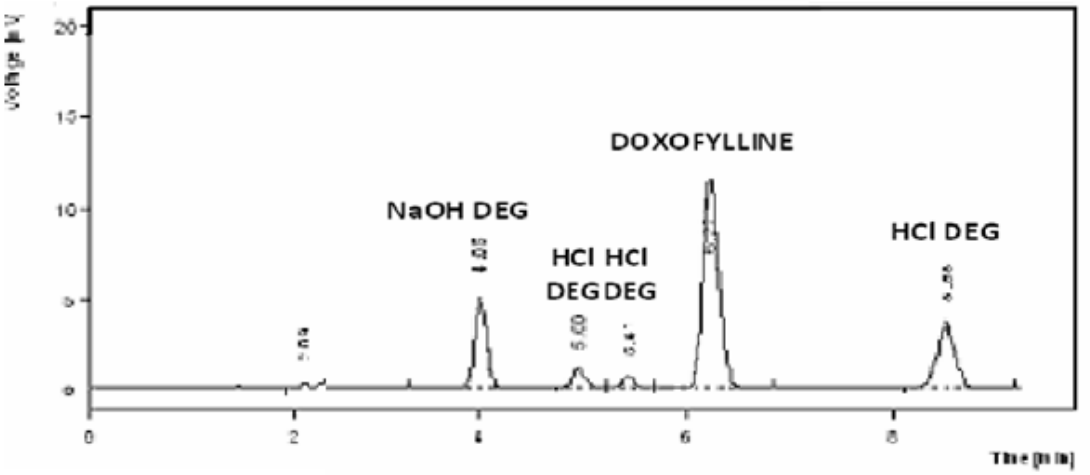

Figure 4(h): Chromatogram - Mixture of Doxofylline Degradation Products
Figure 4(a-h): Results of various forced degradation study

Validation of Proposed Method

As recommended in ICH guidelines [21, 22], all validation studies were performed during development of the procedure. The proposed method was validated for linearity & range, limit of detection & limit of quantitation, precision, accuracy, specificity and ruggedness. The results of precision, accuracy, specificity and ruggedness studies are shown in **Tables 3, 4**, **5** & **6** respectively.

Table 3: Results of system, method and intermediate precision

Parameters	System Precision*	Method Precision*	Intermediate precision*		
			Interday	Intraday	Different analyst
Mean ± SD	99.89 ± 0.11	99.56 ± 0.37	99.48 ± 0.28	99.27 ± 0.71	98.97 ± 0.84
% RSD	0.11	0.37	0.28	0.72	0.85

* Each value is a mean of six observations.

Table 4: Results of recovery (accuracy) studies

% Spiking level	Wt. of sample (mg)	Added concentration (mg)	% Recovery*	Mean ± SD	% RSD
80	15.5	8.0	99.58	99.87 ± 0.25	0.25
100	15.5	10.0	100.04		
120	15.5	12.0	99.99		

* Each value is a mean of three observations.

Table 5: Results of specificity study

S No.	Sample	% Labeled claim
1.	Normal	99.91
2.	Acid	99.51
3.	Alkali	100.61
4.	Oxidation	99.34
5.	Heat	99.87
6.	UV	99.34

Table 6: Results of robustness study

S. No.	Wavelength (± 2 nm)	% Estimation
1.	275	100.36
2.	277	101.82
3.	279	98.96
	Mean	100.38
	± SD	1.1676
	% RSD	1.1632

CONCLUSION

The method is simple, precise and accurate for the determination of doxofylline and its degradation product in bulk drug and pharmaceutical preparations. It was validated for parameters like precision, accuracy, specificity, ruggedness and robustness and was found to yield good results. The method can therefore be applied for routine quality control analysis of doxofylline in pharmaceutical preparations.

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