Int. J. Chem. Sci.: 9(1), 2011, 337-352
ISSN 0972-768X

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF DOXOFYLLINE

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ABSTRACT

A simple, selective, rapid, specific and stability indicating reversed phase high performance liquid chromatography (RP-HPLC) method for the determination of doxofylline in the pharmaceutical dosage form has been developed and validated. The separation and quantification were achieved on a Supelco C18 DB (150 × 4.6 mm) column using a mobile phase of water : methanol : acetonitrile (75 : 20 : 5 v/v), at a flow rate of 1 mL/min with detection of analyte at 278 nm. The method was validated according to the regulatory guidelines with respect to precision, accuracy, linearity, specificity and robustness. All the validation parameters were within acceptance range.

Key words: Doxofylline, Stability indicating reverse phase HPLC, COPD.

INTRODUCTION

The technique of HPLC is developed from advances made in column chromatography. The technique is based on the same modes of separation mentioned above. It differs from conventional column chromatography in the sense that the mobile phase is pumped through the packed column under high pressure. Because of the relatively high pressure necessary to perform this type of chromatography, a more elaborate experimental set up is required[1-8].

Efficacy and safety of drug formulation is of prime importance in health care system. The drug products may undergo decomposition due to exposure to various environmental conditions, which leads to its contamination with its degradation products. This degradation not only leads to decreased therapeutic efficacy of the product but may also adversely affect the safety due to generation of toxic degradants. To overcome this, a thorough investigation of stability of drug and its vulnerability to environmental condition is necessary. This will

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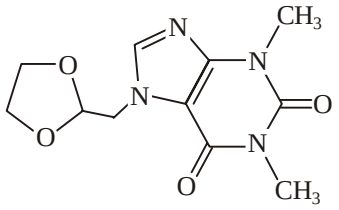
also help to design a suitable packaging and storage conditions as well as shelf life of drug product[9-13].

Doxofylline is a theophylline derivative, which is used as a bronchodilator in reversible airways obstruction with less side effects. Doxofylline is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD), and chronic bronchitis.

Structure of doxofylline



EXPERIMENTAL

Materials and method

Instruments

(i) Shimadzu HPLC System

(ii) Shimadzu HPLC pump LC-10ATvp

(iii) On line degasser DGU-14A

(iv) Low pressure gradient flow control unit FCV-10ALvp

(v) Rheodyne injector 7725 I with 20 µL loop

(vi) Column oven CTO-10ASvp

(vii) Variable UV-VIS detector SPD-10AVp with Shimadzu class CSW software

(viii) Supelco ODS 5 µ C18 DB column (150 x 4.6 mm)

Standard solutions

Solution A (Stock standard solution): An accurately weighed quantity of about 50.0 mg of doxofylline was dissolved in methanol and diluted to 50.0 mL.

Solution B (Working standard solution): A 1.0 mL of solution A was diluted to 10.0 mL with mobile phase (Conc.: 100 µg/mL).

Solution C (Working standard solution): A 1.0 mL of Solution B was diluted to 10.0 mL with mobile phase (Conc.: 10 µg/mL).

Preliminary optimization of mobile phase and other chromatographic conditions

Using following chromatographic parameters, different mobile phases were tried to get retention time for parent drug.

Column : Supelco C18 DB (150 x 4.6 mm)

Detection wavelength : 278 nm

Injection volume : 20 µL

Procedure

The chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Solution C was injected in the Rheodyne injector (20 µL) and the chromatograms were recorded for the drug. Various mobile phases were tried by permutation and combination and also by varying the flow rate and column temperature.

The mobile phase containing mixture of water : methanol : acetonitrile in the ratio of 75 : 20 : 5 (v/v/v) at a flow rate of 1.0 mL/min was found to yield satisfactory retention time of about 6 min with sharp symmetrical peak.

Force degradation (Stress studies) of doxofylline

The stress studies were performed by using 1 mg/mL methanolic solution of doxofylline and applying it to various stress conditions to study the effect over wide range of pH, heat, and oxidation and photodegradation using the following approach -

Acid degradation

Fifty mg of doxofylline was dissolved in 50 mL of 0.1N methanolic hydrochloric acid (1 mg/mL) and 25 mL of it was refluxed in round bottom flask on a boiling water bath for 8 h. The remaining solution was kept at room temperature.

Alkali degradation

Fifty mg of doxofylline was dissolved in 50 mL of 0.1N methanolic sodium hydroxide (1 mg/mL) and 25 mL of it was refluxed in round bottom flask on a boiling water bath for 8 h. The remaining solution was kept at room temperature.

Neutral degradation

Fifty mg of doxofylline was dissolved in 50 mL of distilled water (1 mg/mL) and 25 mL of the above solution was refluxed in round bottom flask on a boiling water bath for 8 h. The remaining solution was kept at room temperature.

Oxidative degradation

Fifty mg of doxofylline was dissolved in 50 mL 3 % H_2O_2 (1 mg/mL) and 25 mL of the above solution was refluxed in round bottom flask on a boiling water bath for 8 h. The remaining solution was kept at room temperature.

Sample preparation and HPLC resolution

The samples (1 mL each) were withdrawn during stress studies (1- 4 above) every 1st, 3rd, 5th and 8th hour to study the extent of degradation and to stop forced degradation after obtaining degradation of about 20%. To compare the effect of various stress conditions at elevated temperature and R. T., corresponding blanks were kept at R. T. and the samples were withdrawn simultaneously. The withdrawn samples were diluted to 10 mL with methanol (the samples of acid and alkali degradation were neutralized prior to dilution). The resultant solutions (1 mL each) were further diluted to 10 mL with mobile phase. (Conc. 10 μg/mL w.r.t. to parent drug)

In case of thermal degradation studies, the samples were withdrawn every 7th, 14th, 30th and 60th day whereas in case of photodegradation, the samples were withdrawn every 7th, 14th and 30th day along with a blank kept in dark. In all these cases, the total quantity of each withdrawn sample was dissolved in methanol and diluted to 100 mL. The resultant solutions (1 mL each) were further diluted to 10 mL with mobile phase. (Conc. 10 μg/mL w.r.t. to parent drug)

The stressed samples so prepared were injected in HPLC column and chromatograms were obtained using preliminarily optimized mobile phase (water : methanol : acetonitrile : 75 : 20 : 5 (v/v).

Study of system suitability parameters

Standard solution: Standard solution C was prepared.

Procedure

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady

baseline. Five replicate injections were made separately and the chromatograms were recorded.

Thus, the preliminarily optimized mobile phase is capable of resolving the parent drug (Doxofylline) and all the degradation products with adequate system suitability parameters.

Finally optimized chromatographic parameters were as follows:

Column	: Supelco C18 DB (150 x 4.6 mm)
Mobile phase	: Water : Methanol : Acetonitrile (75 : 20 : 5 v/v /v)
Detection wavelength	: 278 nm
Flow rate	: 1 mL/minute
Temperature	: 25^{o}C
Injection volume	: 20 µL

Linearity of response

Aliquot portions of stock standard solution B (0.2, 0.6, 0.8, 1.0, 1.2, 1.6 and 2.0 mL) were diluted to 10.0 mL with mobile phase. (Conc. : 2-20 µg/mL)

Procedure

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Standard solutions of different concentrations were injected separately and the chromatograms were recorded. A graph was plotted as peak area vs. concentration of drug (Fig. 6).

Estimation of doxofylline in tablets by proposed HPLC method

Standard solution: Standard solution C was prepared (10 µg/mL)

Sample solution: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 25 mg was sonicated with 15 mL methanol for 15 minutes and the volume was made to 25.0 mL with methanol. It was filtered and 1.0 mL of clear filtrate was diluted to 100.0 mL with mobile phase (conc.10 µg/mL). Five replicate sample solutions were prepared in similar manner.

Procedure

After equilibration of stationary phase, three replicate injections of standard solution and each of five sample solutions were made separately. The chromatograms were recorded and amount of drug estimated in sample weight was calculated using formula -

$$E_w = \frac{A_u}{A_s} \times Cs \times 2.5 \quad \ldots(1)$$

Where E_w = Drug estimated in sample weight (mg),

Cs = Concentration of standard (µg/mL),

Au = Peak area of unknown and

As = Peak area of standard.

Amount of drug present in average weight of tablet as per of labeled claim was calculated using following formula -

$$\% \text{ of Labeled claim} = \frac{E_w \times W_{AV}}{L_c \times W_s} \times 100 \quad \ldots(2)$$

Where,

E_w = Amount estimated in sample weight (mg),

W_{AV} = Average weight of tablet (mg),

W_s = Sample weight (mg) and

L_c = Labeled claim (mg/tablet).

The results are shown in Table 2.

Validation of proposed HPLC method

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

Standard solution: Standard solution C (conc. 10 µg/mL) was prepared.

Sample solution: Accurately weighed quantities of preanalyzed tablet powder

equivalent to about 18 mg of doxofylline were transferred to five different 25.0 mL volumetric flasks and accurately known amount of standard doxofylline were added followed by addition of 15.0 mL of methanol. The flasks were sonicated for 15 min and volumes were made up to the mark with methanol (the drug contents in different flasks represent 70%-130% of the labeled claim with fixed amount of excipients). The solutions were filtered and 1.0 mL of clear filtrate was diluted to 100.0 mL with methanol.

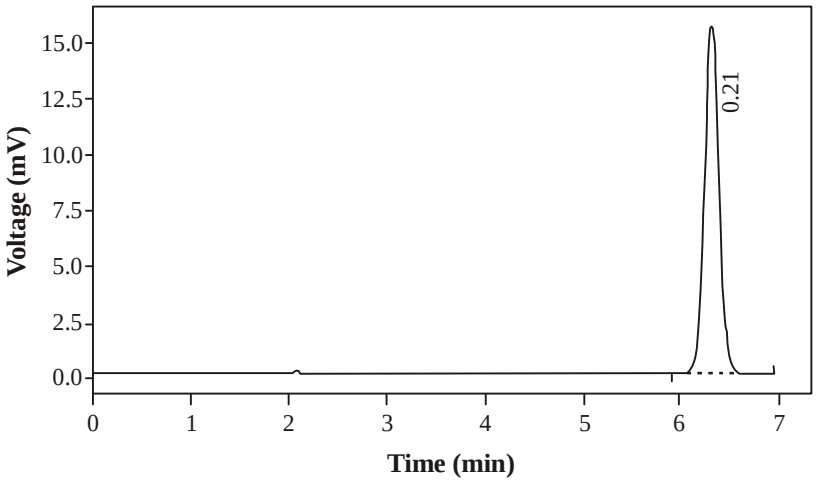
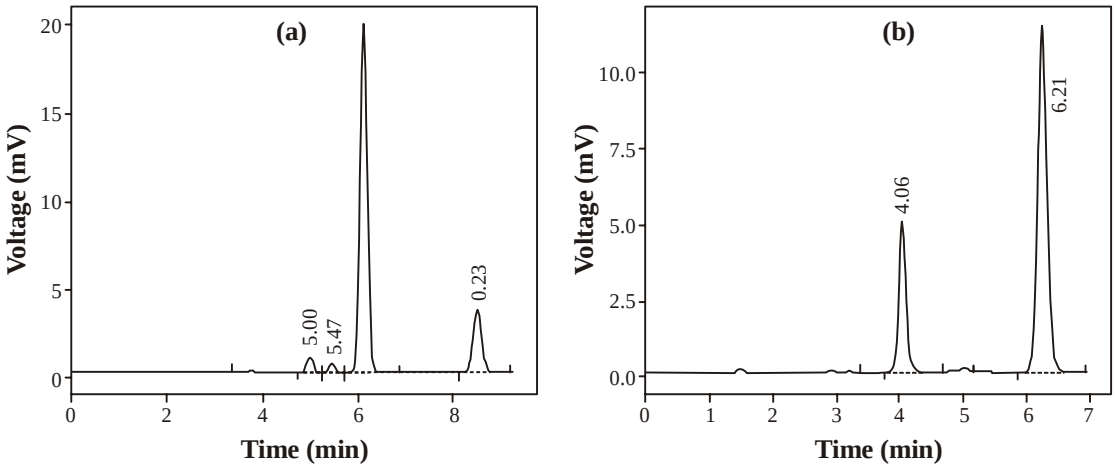


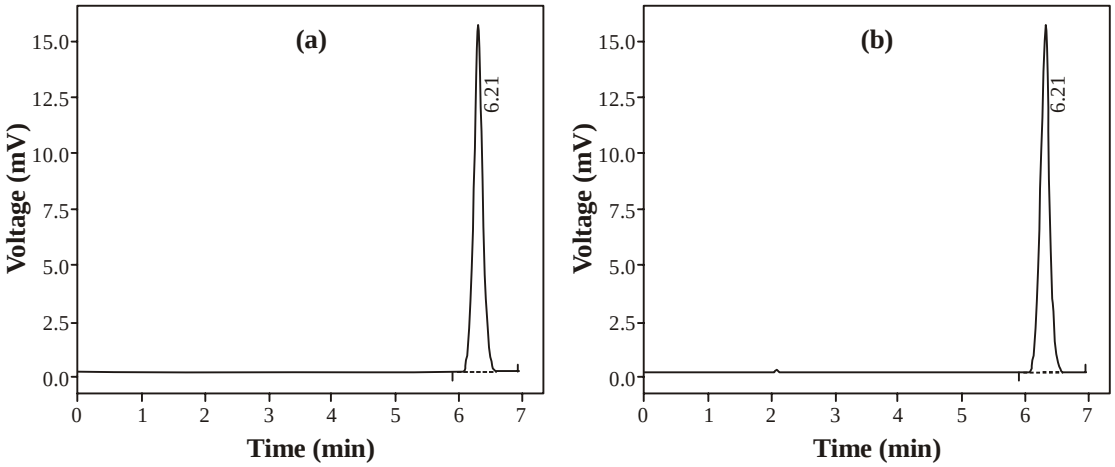
Fig. 1: HPLC Chromatogram of doxofylline

Force degradation (Stress studies) of doxofylline



(a) In 0.1 N HCl (3 h) (b) in 0.1 N NaOH (5 h)

Fig. 2: HPLC Chromatogram of doxofylline in (a) 0.1 N HCl and (b) 0.1 N NaOH



(a) In neutral (8 h) (b) In H_2O_2 (8 h)

Fig. 3: HPLC Chromatogram of doxofylline in (a) Neutral and (b) H_2O_2

Study of system suitability parameters

Table 1: Study of system suitability parameters

S. No.	Retention time (min)	Asymmetry	No. of theoretical plate	Capacity factor	Peak area
1	6.27	1.14	75429	5.27	215.1
2	6.27	1.14	75425	5.27	215.2
3	6.31	1.067	81658	5.31	214.8
4	6.27	1.14	75439	5.27	215.1
5	6.27	1.14	75429	5.27	214.8
Mean	6.278	1.1254	76676	5.278	215
± SD	0.017889	0.032647	2785.027	0.017889	0.187083
% RSD	0.28494	2.900888	3.632202	0.338927	0.087015

Procedure: Same as described under estimation of doxofylline in tablet.

The percent recovery was then calculated by using formula;

$$\% \text{ Recovery} = \frac{W_w - B}{C} \times 100 \quad \ldots(3)$$

where,

E_w = Total drug estimated (mg),

B = Amount of drug contributed by preanalyzed tablet powder (mg) and

C = Weight of pure drug added (mg).

Linearity and range

From the data obtained under accuracy studies, a graph was plotted as percent labelled claim vs. peak area.

Precision

Precision of analytical method is expressed as SD and RSD of series of replicate measurements. Precision of estimation of doxofylline by proposed method was ascertained by replicate analysis of homogeneous samples of tablet powder.

Intermediate precision

The intermediate precision was done according to ICH guidelines by using three standard concentrations.

Stability of standard solution

The chromatograms of the same standard solution C were obtained periodically over a period of 30 h.

Stability of sample solution

The chromatograms of the sample solution as prepared in accuracy studies were obtained periodically over a period of 30 h.

Specificity

Standard solution: Standard solution C (conc. 10 μg/mL) was prepared.

Sample solution: Accurately weighed quantities of tablet powdered equivalent to about of 25 mg doxofylline were transferred to five different 25.0 mL volumetric flasks. The samples were then exposed to stress conditions for 24 h as follows;

(i) Normal (control),

(ii) At 60^{o}C, after addition of 1.0 mL of 0.1N HCl (Acid),

(iii) At 60°C, after addition of 1.0 mL of 0.1N NaOH (Alkali),

(iv) At 60°C, after addition of 1.0 mL of 3% H_2O_2 (Oxide),

(v) At 60°C, (Heat) and

(vi) Sunlight

After 24 h, the flasks were cooled to room temperature, sonicated with 20.0 mL methanol for 15 min and volumes were made up to 25.0 mL. The solutions were then analyzed in similar manner as described under estimation of doxofylline in tablets. The results of specificity study are shown in Table 7 and no degradation products were observed.

Ruggedness

The studies were carried out for two different parameters i.e. Days (Intra-day and Inter-day) and analysts. **Intra-day and Inter-day :** The samples were analyzed at different times on same day and three different days. **Different Analyst :** The samples were analyzed by two different analysts by the proposed method.

Robustness

Change in wavelength (± 2 nm)

The tablet sample of doxofylline was analyzed using proposed method after a deliberate change in detection wavelength for estimation by ± 2 nm.

RESULTS AND DISCUSSION

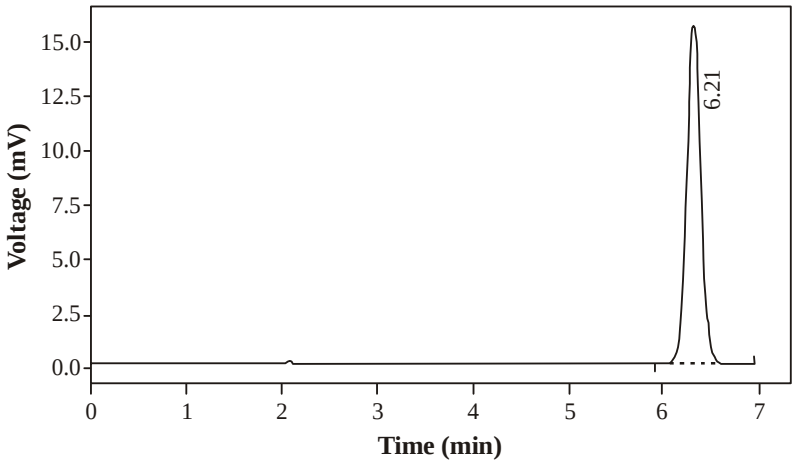


Fig. 5: HPLC Chromatogram of doxofylline

Linearity of response

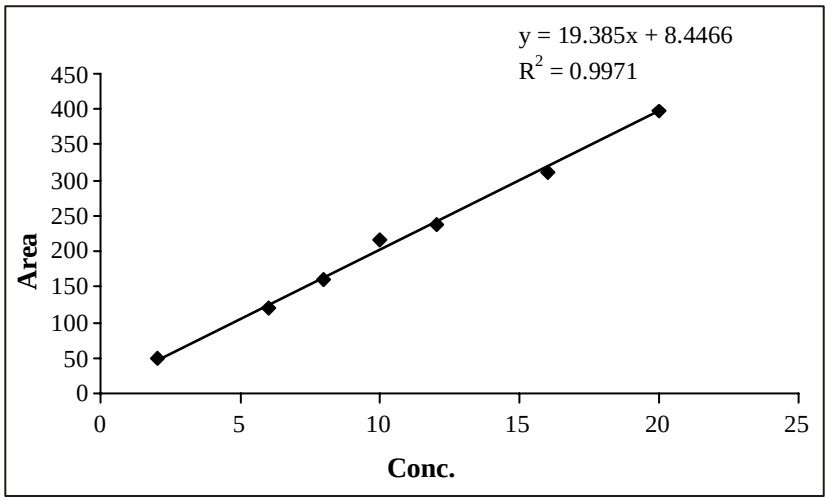


Fig. 6: Estimation of doxofylline in tablets by proposed HPLC method

Table 2: Results of estimation of doxofylline in table

Doxobid	**Wt. = 619.8 mg**			**Avg.**
Wt. of tablet powder (mg)	**Detector response (Peak area)**		**Amount estimated (mg)**	**% of Labeled claim**
	Standard*	**Sample**		
34.2		188.03	21.87	99.06
36.4		199.66	23.22	98.83
38.8	215	213.1	24.78	98.96
41.1		229.39	26.67	100.56
43.4		238.8	27.77	99.14
			Mean	99.31
			± SD	0.7092
			% RSD	0.7142

Validation of proposed HPLC method

Accuracy

Table 3: Results of recovery studies of doxofylline

Wt. of tablet powder (mg)	Detector response		Wt. of total drug estimated (mg)	Wt. of std. drug recovered of the added	% of Labeled claim
	Std. area	Peak area			
27.1 + 0 (70 %)		151	17.56	0.19	-
27.1 + 3.8 (85 %)		183.24	21.31	3.94	103.65
27.1 + 7.5 (100 %)	215	214.23	24.91	7.48	99.71
27.1 + 11.4 (115 %)		249.16	28.97	11.54	101.23
27.1 + 15(130 %)		280.75	32.65	15.21	101.42
				Mean	101.50
				SD	1.4061
				% RSD	1.3853

Linearity and range

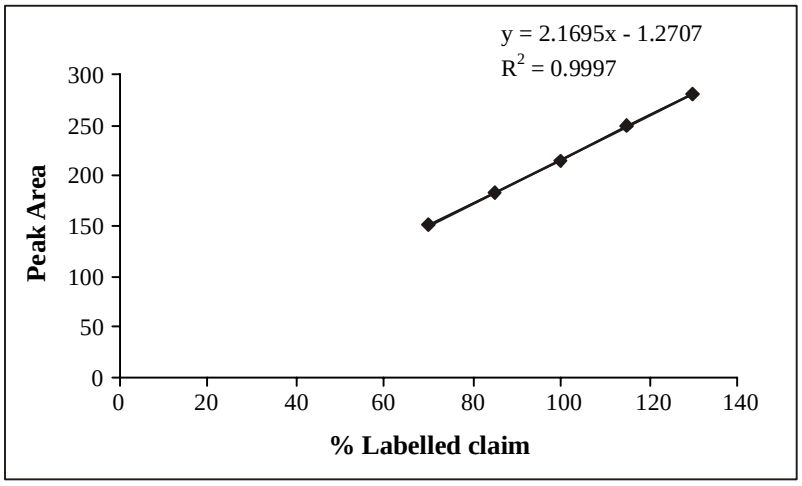


Fig. 7: Study of linearity and range

Table 4: Results of intermediate precision

Actual conc. (μg/mL)	Area		Measured conc. (μg/mL)		Mean	S.D	% R.S.D
	Intra-day	Inter-day	Intra-day	Inter-day			
2.1	48.78	49.21	2.27	2.29	2.28	0.0141	0.6206
10.1	213.31	213.51	9.92	9.93	9.93	0.0066	0.0663
19.8	398.95	399.12	18.56	18.56	18.56	0.0056	0.0301

Table 5: Results of stability of standard solution

Time (h)	Area	% Estimation
1	214.31	99.5185
6	215.61	100.1222
24	215.79	100.2058
30	215.24	99.95037

Table 6: Results of satability of sample solution

Time (h)	Area	% Estimation
1	99.5185	99.88535
6	100.1222	99.74604
24	100.2058	100.0247
30	99.95037	99.97823

Table 7: Results of specificity studies

Sample	% Labelled claim
Normal	99.91
Acid	99.51
Alkali	100.61
Oxide	99.34
Heat	99.87
Sunlight	99.34

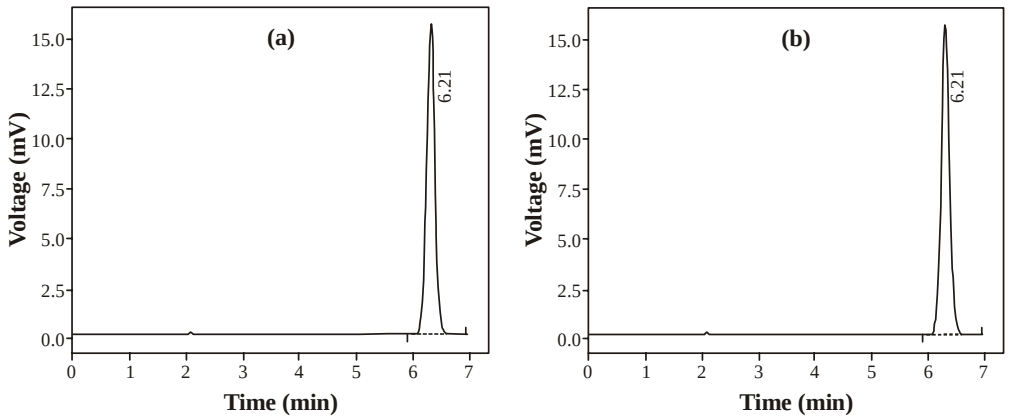


Fig. 8: HPLC Chromatogram of doxofylline in (a) Acid and (b) Alkali

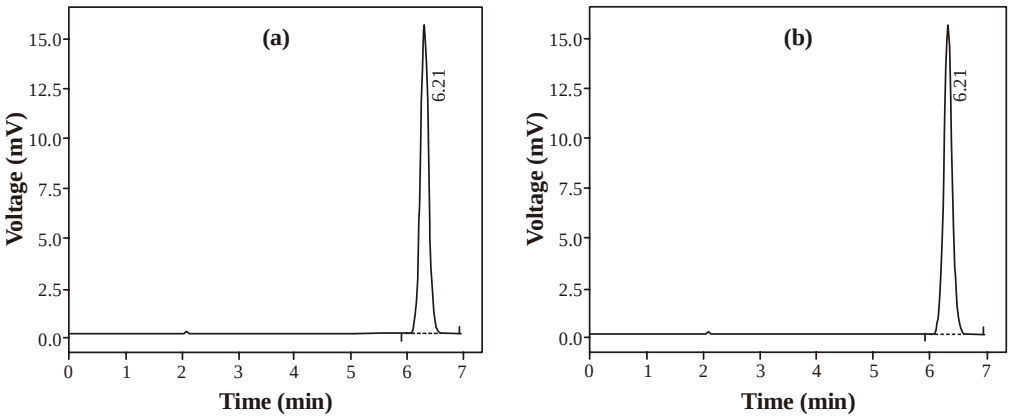


Fig. 9: HPLC Chromatogram of doxofylline in (a) Sunlight and (b) Oxide

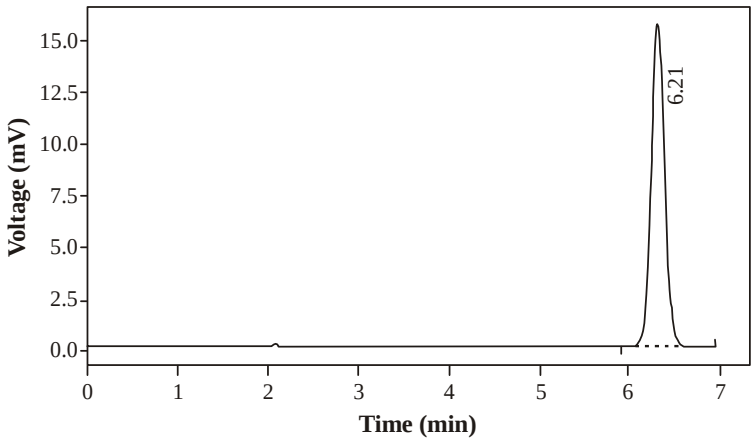


Fig. 10: HPLC Chromatogram of doxofylline in Heat

Ruggedness

Table 8: Results of ruggedness study (Intra-day and Inter-day)

(a) Intra-day	% of Labeled claim
T1	99.05
T2	99.94
T3	100.16
Mean	99.2
± SD	0.5845
% RSD	0.5861
(b) Inter-day	
D1	99.23
D2	98.94
D3	100.50
Mean	99.56
± SD	0.8345
% RSD	0.5458

Summary and discussion

Few HPLC methods are reported for its estimation in biological samples. None of the method could be located in literature as stability indicating assay method for estimation of doxofylline and its degradation products. Hence, the project was undertaken to develop precise, accurate, reliable, rapid, simple and specific method for estimation of doxofylline well resolved from its probable degradation products, by following relevant ICH guidelines. Results of estimation of doxofylline in tablet formulation were accurate and precise with standard deviation < 2.

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Revised : 20.12.2010 *Accepted : 22.12.2010*