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Research Article

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY AND SPECTROSCOPIC METHODS FOR THE ANALYSIS OF DOXOFYLLINE IN PHARMACEUTICAL DOSAGE FORMS

Ethiraj Thiruvengadam[1]*, Revathi Ramadoss[1], Ganesan Vellaichamy[2]

[1]Dept of Pharmaceutics & Pharmaceutical Analysis. The Erode College of Pharmacy, Erode, Tamilnadu, 638112, India
[2]Dept of Pharmaceutics, The Erode College of Pharmacy,Erode, Tamilnadu, 638112, India

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*Corresponding author
revathi ethiraj

Email :
revathethiraj@gmail.com

ABSTRACT

A high performance liquid chromatography (HPLC) and ultraviolet spectroscopic (UV) methods were developed and validated for the quantitative estimation of doxofylline (DF) in pharmaceutical dosage forms. HPLC was carried out using reverse-phase technique on RP-8 column with a mobile phase composed of 0.05M phosphate buffer pH 6 and acetonitrile (60:40, v/v). The mobile phase was pumped at a flow rate of 1mL/min, and detection was made at 230nm with PDA detector. UV method was performed with λ max at 270nm with apparent molar absorptive of $0.878x10^3 L\ mol^{-1}\ cm^{-1}$. Both the methods showed good linearity, recovery and precision. No spectral or chromatographic interferences from the tablet excipients were found in UV and HPLC methods. The various parameters such as linearity, precision, accuracy, specificity, and robustness, limit of detection and limit of quantization were studied according to ICH guidelines. Statistical analysis was done by student's *t*-test and *F*-test, which showed no significant difference between the results of both methods. So the proposed methods could be applicable for routine analysis of DF and monitoring of the quality of marketed drugs.

Key words: Doxofylline, Validation, HPLC, UV spectroscopy, Comparison studies, Student's *t*-test, *F*-test.

INTRODUCTION

Doxofylline (DF) is chemically 7-(1, 3-Dioxolan-2-yl-methyl)-3, 7-dihydro-1, 3-dimethyl-1H-purine-2, 6-dione (Figure 1) belongs to the class of antiasthmatic/ bronchodilators. It is a new generation of methyl xanthine derivative. Literature survey revealed that doxofylline was estimated by few RP-HPLC methods in dosage forms (Akilesh *et al.*, 2011; Ashu *et al.*, 2010 and Venkatesan *et al., 2010)* and in biological samples (Gannu *et al.*, 2007). Some spectrophotometric methods also have been reported for estimation of DF (Kamila *et al.*, 2007; Sagar *et al.,* 2011; and kumar *et al.*, 2011). Joshi *et al.* (2010) has been reported for the development of UV and HPLC methods for estimation of doxofylline in pharmaceutical formulation. The huge amounts of costlier solvents were used as mobile phase solvent system and time of analysis also more in some reported methods.

The aim of the present work was to develop simple, fast and reliable isocratic RP-HPLC, UV methods for the determination of DF in pharmaceutical dosage forms by using less cost solvents. The results of analysis using the UV and RP-HPLC method were found to be satisfactory, such that the proposed methods can be utilized for routine analysis of drug.

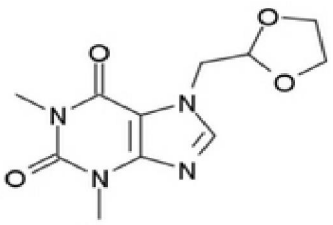

Figure 1. Chemical structure of doxofylline

METHODOLOGY

Chemicals

HPLC grade acetonitrile and potassium dihydrogen phosphate (AR grade) were purchased from Rankem Fine Chemicals Ltd. Water (HPLC grade) was obtained from a Milli-Q water purification system (Millipore, Milford, USA). Acetone and ortho- phosphoric acid were purchased from S.D.Fine Chemicals Ltd. DF was kindly supplied by Fourrt's India, Chennai. Pharmaceutical dosage forms (T.Doxobid, T.Synasma) containing 400mg of DF were obtained commercially.

Instrumentation and analytical condition

HPLC chromatographic separation was performed on a Shimadzu Prominence Liquid Chromatographic System equipped with a LC-10AT-VP solvent delivery system (pump), SPD M-10 AVP photo diode array detector and rheodyne injector with LC solution software version 1.2. The HPLC was carried out at a flow rate of 1mL/min using a mobile phase consisted of 0.05M phosphate buffer pH 6: acetonitrile (60:40, v/v) and detection was made at 230nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter and sonicated before being used for analysis. Intersil C8 column (5µm, 4.6×250mm) was used for separation. UV method was performed on UV-Visible spectrophotometer (UV-2201, Systronics) with the λ max at 270nm using 10mm matched quartz cells.

Standard solution

For HPLC: Accurately weighed 100mg of DF reference standard was transferred to 100mL volumetric flask, dissolved and made up to the mark with mobile phase (1mg/mL concentration). The concentrations of 5-50µg/mL were prepared individually from the above stock solution using mobile phase.

UV: Accurately weighed 100mg of DF reference standard was transferred to 100mL volumetric flask and dissolved in 10mL of acetone. Then, the volume was made up to the mark with 15% phosphoric acid to get final concentration of 1mg/mL. From this stock solution, the concentration ranges from 5-30µg/mL were made in 10 mL volumetric flasks and volume was adjusted with distilled water.

Sample solution

HPLC: Twenty tablets of each brand, each containing 400 mg of DF were weighed and finely powdered; a quantity of powder equivalent to 100mg of DF was transferred to a 100mL flask and made the volume up to the mark with mobile phase. It was kept in ultrasonication for 45 minute and filtered through 0.45µm membrane filter. Furthermore, it was diluted with same to get 10µg/mL concentrations.

UV: For estimation of DF in tablet dosage formulations, twenty tablets of each brands were weighed and triturated to fine powder. Tablet powder equivalent to 100mg of DF was weighed and dissolved in 10mL acetone and further diluted with 15% phosphoric acid. It was kept for ultrasonication for 45min and filtered through Whatmann filter paper No.41 to get the stock solution of 1mg/mL. Then, the solution was diluted with distilled water to get the final concentration of 10µg/mL.

Method validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose, as it is stated in ICH guidelines (ICH guidelines, 2005). The method was validated for linearity, precision, accuracy and system suitability. Student's *t*-test and *F*-test were used to verify the validity of the methods.

Linearity: The calibration curve was obtained with various concentrations of standard solution, as 5-50µg/mL for HPLC method and 5-30µg/mL for UV method, respectively. The solutions were prepared in triplicate and linearity was evaluated by linear regression analysis. LOD and LOQ were determined on the basis of slope and intercept values from regression equation.

Precision: The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from three replicate injections of freshly prepared DF solution in the same equipment at a concentration of 20µg/mL on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision.

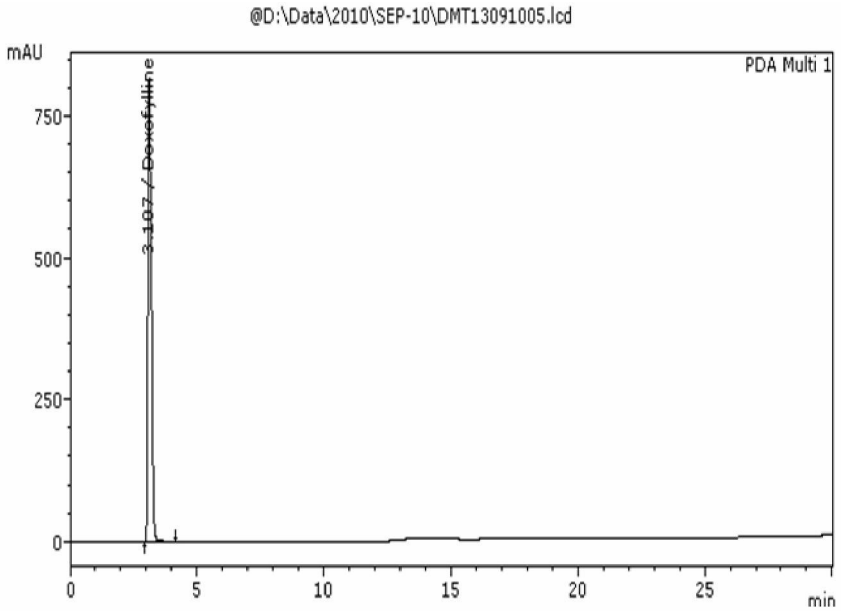

Figure 2. Chromatogram for doxofylline

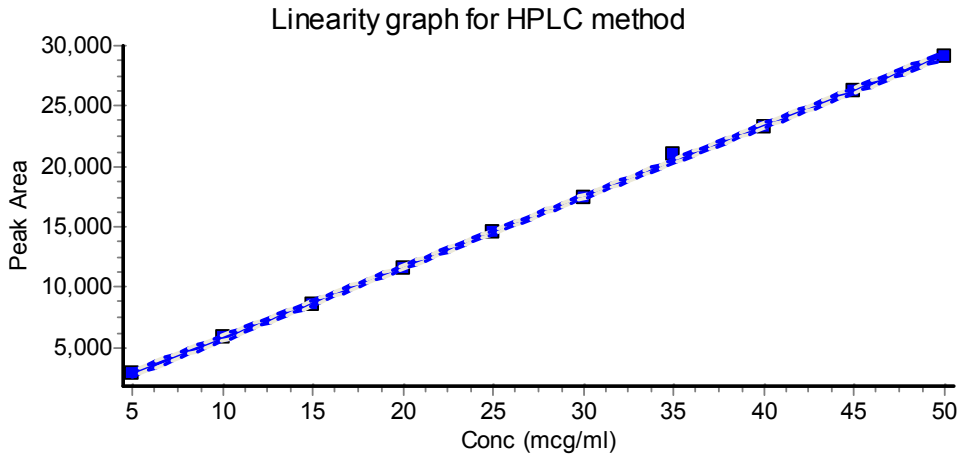

Figure 3. Calibration graph for HPLC method

Accuracy: Percentage recovery was analyzed by adding reference drug of DF at three different levels to the powdered drug product using three preparations at each level. The results were expressed as the percentage of DF recovered in the sample and %RSD.

Specificity: Specificity of the HPLC method was assessed by comparing the chromatograms obtained from standard and sample preparations with those obtained from excipients which take part in the commercial tablet preparation.

Robustness: the robustness of the HPLC method was determined by analysis of samples under various conditions like changes in the pH (5.8-6.2), changes in the percentage of organic phase (±10%) in the mobile phase and changes in the flow rate (0.9mL/min-1.1 mL/min). The effect of retention time and system suitability parameters was observed. For UV, the drug content was analysed under the experimental variables like changes in the concentration of the reagent and stability of the sample solution.

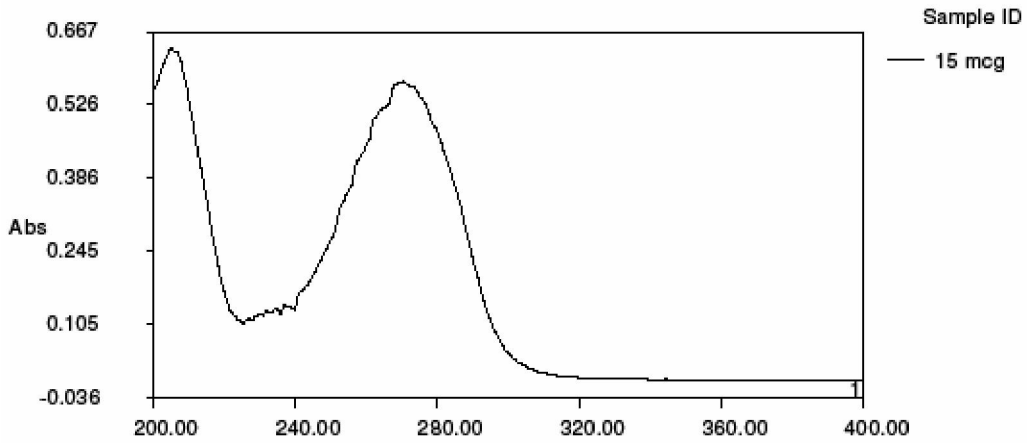

Figure 4. UV spectrum for doxofylline (15 μg/mL).

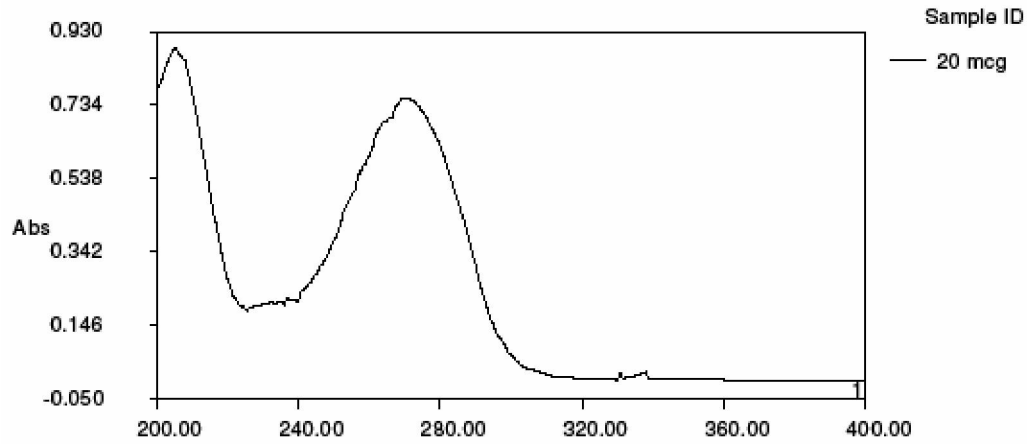

Figure 5. UV spectrum for doxofylline (20 μg/mL).

RESULTS AND DISCUSSION

HPLC method: A reversed phase HPLC method was developed to analyze DF in pharmaceutical preparation as a suitable method. The chromatographic conditions were adjusted in order to provide a better result for assay. The mobile phase (0.05M phosphate buffer pH 6 and acetonitrile (60:40,v/v)) was selected by taking into account some peak parameters like tailing factor, number of theoretical plates, run time, easy of preparation and cost. A typical chromatogram (Figure 2) was obtained with retention time of 3.107min which showed a rapid determination of drug for frequent analysis. In this proposed method, the peak was eluted with a tailing factor (T) 1.55 and number of theoretical plates (N) 4003. From the calibration curve (Figure 3), DF showed good linearity in the range of 5-50μg/mL with regression equation of y=587.71x-119.31 and correlation coefficient of r= 0.9995, indicating a high sensitivity of the method (Table I). The precision of the method was ascertained by repeatability (*Intra-day*) and intermediate precision (*Inter-day*). The results of % purity and % RSD were tabulated in table II showing good precision. The recovery of the drug was analyzed by adding different levels (10%, 20% and 30%) of standard drug to the predetermined tablet powder. The results of % RSD, SE were showed in table III.

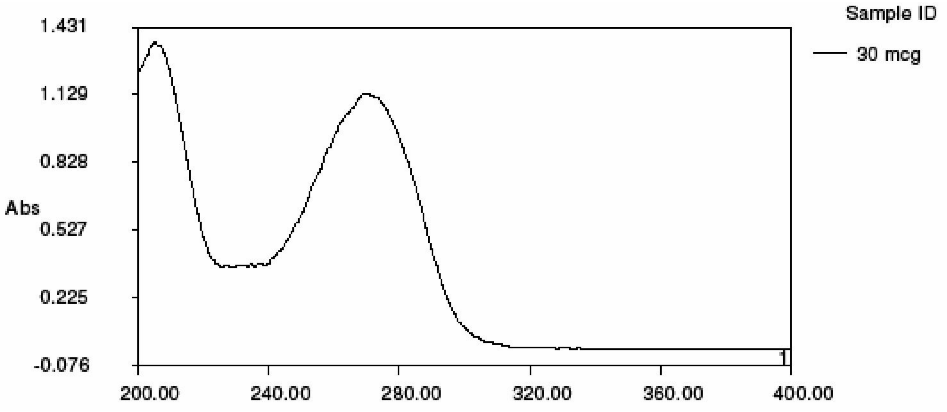

Figure 6. UV spectrum for doxofylline (30µg/mL)

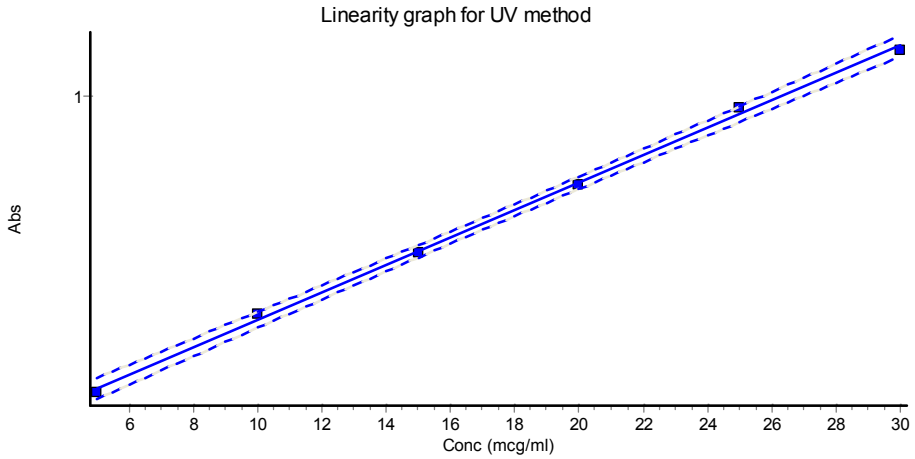

Figure 7. Calibration graph for UV method

There was no significant change in the system suitability factors of DF when the organic composition, flow rate and column temperature were changed. The low values of the % RSD indicated that the method was robust (Table IV).

UV method: The spectrum of DF standard solution with concentrations of 15, 20 and 30µg/mL (Figure 4-6) showed intense absorbance peak with λ max at 270nm. The good linearity (Figure 7) was obtained on standard solutions of DF over the concentration range of 5-30µg/mL. The precision of the method was assessed with % RSD values of 0.1680 for repeatability and 0.1126 for intermediate precision which were found for three replicates at a concentration of 20µg/mL (Table II). Accuracy of the method was done as per HPLC method and the mean percentage content obtained was between 99.60% and 100.46% with 95% confidence interval of 97.964 and 102.72 (Table III).

Robustness of the method was assessed by evaluating the influence of small variations of experimental variables like changes in the concentration of the reagent and stability of the sample solution on the analytical performance. The small variations in any of the variables did not significantly affect the results. This provided an indication for the reliability of the proposed method during routine analysis (Table IV).

Table I. Results for linearity study

Parameters	HPLC method	UV method
Linearity Range (µg/mL)	5-50	5-30
LOD (µg/mL)	0.6699	0.8247
LOQ (µg/mL)	2.0300	2.499
Slope	587.71	0.03841
Standard Error on Slope	4.511	0.0007065
Confidence Limit of Slope	577.31 to 598.12	0.03644 to 0.04037
Intercept	-119.31	-0.009600
Standard Error on Intercept	139.95	0.01376
Confidence Limit of Intercept	-441.87 to 203.60	0.04779 to 0.02859
Correlation Co-efficient	0.9995	0.9986
Standard deviation of Residuals	204.87	0.01478

µg/mL = Micro gram per Milli litre, LOD = Limit of Detection,
LOQ = Limit of Quantitation

Table II. Results for precision study data

Methods	Precision parameters	Amount found (mg)	Percentage purity (%w/w)	% RSD
HPLC	Repeatability	400.66	100.16	0.1212
	Intermediate Precision	400.74	100.18	0.1046
UV	Repeatability	401.29	100.32	0.1680
	Intermediate Precision	400.71	100.17	0.1126

Table III. Results for recovery study data

Brands	Levels of std drug added	Mean percent recovery*	% RSD*	S.E	95 % CI
HPLC method					
T. Doxobid	10 %	99.60	0.1601	0.092	99.20-100.00
	20 %	100.04	0.4915	0.2839	98.92-101.26
	30 %	99.85	0.7616	0.4391	97.96-101.74
T. Synasma	10 %	100.46	0.3866	0.2242	99.49-101.42
	20 %	100.39	0.9348	0.5419	98.06-102.72
	30 %	100.18	0.5845	0.3381	98.72-101.63
UV method					
T. Doxobid	10 %	100.10	0.3727	0.2154	99.170-101.02
	20 %	100.31	0.8260	0.4784	98.255-102.37
	30 %	100.20	0.0654	0.0378	100.04-100.36
T. Synasma	10 %	100.29	0.9261	0.5362	97.97-102.59
	20 %	99.65	0.4990	0.2871	98.41-100.89
	30 %	100.31	0.8335	0.4827	98.23-102.38

*(n=3), % RSD = Percentage Relative Standard Deviation, S.E = Standard Error, 95 % CI = 95 Percent Confidence Interval

Table IV. Data for robustness study

	Parameters		USP tailing factor	Theoretical plates	% content
HPLC	pH of organic phase	5.8	1.54	4068	99.67
		6.2	1.50	5102	101.24
	Organic phase composition (%)	90	1.54	4625	100.65
		110	1.55	4005	100.43
	Flow rate (mL/min)	0.9	1.53	5862	100.64
		1.1	1.57	4236	99.35
UV	Conc of reagent (phosphoric acid)	12 %	-	-	100.54
		18 %			99.94
	Stability of sample solution	After 6 h	-		100.48
		After 12 h			100.98

% content = Percentage Drug Content

Table V. Results for statistical comparison on UV and HPLC methods

Brands	% RSD for % content		*F*-test[a]	*t*-test[b]
	HPLC	UV		
T. Doxobid	0.4078	0.6475	2.536	0.774
T. Synasma	0.5139	0.2086	6.006	1.860

[a,b] = Limits of 95 % confidence Interval

Comparison and application of the methods to the analysis of tablets: From the validation results, the above developed methods were suitable for routine quality control analysis of DF in marketed formulations. They were applied for two different brands of DF and the results were statistically compared (Table V). In the t-test and F-tests, no significant differences were found between the calculated and theoretical values of both methods at 95% confidence interval. From this report, it was evident that the proposed UV and HPLC methods were applicable to the DF in tablets in convenient manner.

CONCLUSION

The above proposed methods are very simple, precise and sensitive. It was concluded that, the spectroscopic method requires only wavelength scan, while the HPLC method was less time consuming method (within 4 min). Further more, the developed spectroscopic and liquid chromatography methods are cheaper when compared to other reported methods. No need of any extraction procedure to separate the active constituents from formulation and time of analysis was very less. Hence, the above said methods can be utilized for routine analysis of DF in bulk and pharmaceutical dosage forms.

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