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Research Article

A VALIDATED REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF DOXOFYLINE IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT
A novel reverse phase high performance liquid chromatographic method (RP-HPLC) was developed and validated for the determination of doxofylline (DXF) in pharmaceutical formulations. Agilent 1100 series HPLC instrument equipped with variable wavelength programmable UV-Visible detector and a chromosil C18 (250mm x 4.6mm, 5µm) column with an auto injector was used for the present investigation. A volume of 20µL of working standard of concentration 25µg/mL was injected into the chromatographic system, the component was separated by using the mobile phase of the mixture of potassium dihydrogen phosphate buffer of pH 5.5 and acetonitrile in the ratio 75:25(v/v) at a flow rate of 1.0 mL/min and the detection of the components was carried out at a wavelength of 275nm. Chemstation software was used for the data acquisition through out the analysis. The system suitable parameters such as number of theoretical plates, tailing factor and resolution were found to be satisfactory. The retention time of the component was found to be at 4.814 minutes. The proposed method was validated in terms of precision, accuracy, linearity, limit of detection, limit of quantification, robustness and ruggedness. The developed method was found to be rapid, simple and sensitive hence it could be used as an alternative method in assay of the doxofylline in any pharmaceutical industries.
KEYWORDS: Assay, Method development, RP-HPLC, System suitability, Validation

INTRODUCTION

Doxofylline (DXF) is a new generation long acting oral methyxanthine derivative drug used in the treatment of asthma.[1] It is mainly used for maintenance therapy in patients suffering with Asthma and Chronic Obstructive Pulmonary Disease (COPD). DXF is a theophylline derivative. It has antitussive and bronchodilator[2] effects, and acts as a phosphodiesterase inhibitor[3]. In animal and human studies, it has shown similar efficacy to theophylline but with significantly fewer side effects[4]. 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. It is chemically known as 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione with molecular formula and molecular weight $C_{11}H_{14}N_4O_4$ and 266.25 g/mol respectively. The chemical structure of the DXF is given in Figure 1.

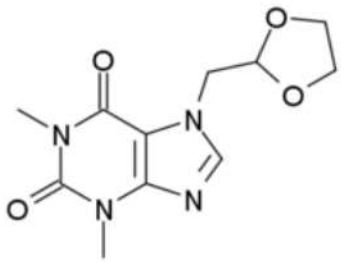


Figure 1: The chemical structure of doxofylline molecule

An extensive literature survey was carried out and found that one LC-MS/MS method[5] and four RP-HPLC methods [6-9] for the determination of DXF in human serum and plasma respectively. Several RP-HPLC methods [10-16] and UV-Spectrophotometric methods[17-19] were reported for the determination of DXF in commercial dosage form, raw material and in tablets. Acid degradation study of doxofylline by RP-HPLC and LC-MS/MS[20] was studied by Gupta et al. The main objective of the present investigation is to develop a simple, precise and accurate reverse phase isocratic liquid chromatographic method and can be applied this method for the determination of the amount of doxofylline present in different pharmaceutical formulations.

METHODS AND MATERIALS

Experimental

Agilent 1100 series HPLC instrument equipped with variable wavelength programmable UV-Visible detector and a Chromosil C18 column (250 mm x 4.6 mm, 5µm) was used for the present investigation. Mettler Toledo (XS1O5) balance and Loba ultrasonic bath sonicator were also used for weighing and degassing respectively. Elico SL 159 UV-Visible spectrophotometer was used for spectral studies. Chemstation software was used for the data acquisition throughout the analysis

Chemicals and reagents

A reference sample of DXF was obtained from Cipra Labs Hyderabad. The pharmaceutical formulations were purchased from the local market. The chemicals and solvents such as potassium dihydrogen phosphate, acetonitrile and water used for the present investigation were of AR and HPLC grade respectively. About 1.36 gm of potassium dihydrogen phosphate was accurately weighed, dissolved in 1000mL HPLC grade water, sonicated for five minutes, filtered through a 0.45µm filter and finally the pH of the solution was adjusted to 5.5 by adding 0.1N potassium hydroxide solution drop wise using calibrated pH meter. A mixture of buffer and acetonitrile in the ratio 75:25 (v/v) was prepared by adding 250 mL of acetonitrile to 750 mL of buffer, the resulting solution was sonicated and filtered through 0.45µm filter and used as mobile phase.

Preparation of standard solution

About 25 mg of DXF reference standard was accurately weighed, transfer into a 100 mL volumetric flask and dissolved in 70 mL of mobile phase. The solution was made up to mark with mobile phase and sonicated. Further about 4 mL of the above solution was accurately transferred into a 10 mL volumetric flask and make up to the mark with mobile phase. The concentration of the resulting solution was 100µg/mL. A series of different concentrated solutions were prepared from the stock solution.

Preparation of sample solution

Doxofylline is available in different brand names like Synasma (Ranbaxy), Doxobid (Dr.Reddy's), Doxosal (Salveolife) and Doxoril (Macleods). The average weight of two tablets of DXF (Synasma 400mg of each tablet) was determined, grounded to fine powdered and amount of the powder equivalent 25 mg of DXF was accurately weighed, transferred to 100 mL volumetric flask and dissolved in minimum amount of methanol then made up to mark with mobile phase, sonicated for 3 minutes and filtered through 0.45µm filter. About 4mL of the filtrate was further diluted to 10 mL with mobile phase to get the final concentration of the drug 100µg/mL.

Method development

The chromatographic analysis was carried out under the isocratic mode. The data were acquired at 275nm and processed by use of Chemstation software. A volume of 20µL of working standard solution (100µg/mL) was injected into the column and the mobile phase was allowed to flow a rate of 1.0 mL/min and the chromatograms were recorded. Different trails were performed by changing one parameter (i.e. concentration of the working standard, injection volume, composition of the mobile phase, flow rate and detection wavelength) at a time keeping other parameters as constant. The chromatograms were recorded in each and every trail. Finally the chromatogram obtained under the chromatographic conditions such as 20µL volume of the working standard 100 µg/mL, mobile phase of composition of 75:25 (v/v) of buffer and acetonitrile at a flow rate of 1.0 mL/min and detection wavelength at 275nm was found to obey the system suitable conditions.

Table-1: System suitability parameters

	Concentration (µg/mL)	Retention Time (min)	Theoretical Plates	Tailing Factor	Area of Peak
Blank	0.00	0.00	0.00	0.00	0.00
Standard	100.00	4.81	2514.00	0.97	4343308.89

†Acceptability criteria: Number of theoretical plates should be more than 2000, tailing factor should be less than 2.0 and resolution (not applicable for the present method) should be more than 2.0

Table-2: Precision of the developed method

Sample ID	Retention Time (min)	Area of Peak	Tailing Factor
1	4.81	4343308.89	0.99
2	4.82	4341404.35	1.00
3	4.82	4363600.27	0.98
4	4.82	4346388.74	1.02
5	4.81	4355416.52	1.00
6	4.81	4350673.06	1.00
Mean	4.82	4350131.97	1.00
S.D.	0.004	8322.883	0.013
%R.S.D†.	0.077	0.191	1.331

†Acceptability criteria for precision: %R.S.D. should be less than 2.0

Table-3: Intra –Day and Inter-Day Precision (100 µg/mL)

Statistical Parameters	Intra-Day Precision*	Inter-Day Precision*
Mean	4350131	4356246
S.D.	8322	10126
%R.S.D.	0.191	0.232

*Statistical parameters were evaluated for n=6

Table-4: Linearity between the area of the peak and concentration of the doxofylline

S.No.	Concentration (µg/mL)	Mean Peak Area
1	10	415956.78
2	25	896670.99
3	50	1957171.58
4	100	4372852.23
5	200	8042749.88
6	300	12335156.95
Slope		41064.00
Intercept		-15497.00
Squared Correlation Coefficient		0.9997

†Acceptability criteria: Correlation coefficient should be more than 0.828

Table-5: Accuracy studies at three different concentrations

Spike Level	Sample-ID	Amount of Drug Taken	Amount of Drug Found	% of Recovery	%Mean Recovery	%RSD
50	1	50	49.72	99.44		
	2	50	49.89	99.78		
	3	50	50.84	101.68	100.3	1.203
100	1	100	99.87	99.87		
	2	100	100.57	100.57		
	3	100	99.82	99.82	100.08	0.419
150	1	150	148.82	99.21		
	2	150	151.04	100.69		
	3	150	150.83	100.55	100.15	0.0716

†Acceptability criteria: Mean percent of recovery should be between 98.0 -102.0

Table-6: Study of robustness of the proposed method

Parameter	Value of the Parameter	Retention Time (min)	Tailing Factor	Number of Theoretical Plates
Flow rate	0.8mL/min	4.82	1.15	2641
	1.0mL/min**	4.81	0.97	2514
	1.2mL/min	4.76	1.12	2145
Mobile Phase Composition (Buffer: Acetonitrile)	80:20	4.87	1.02	2427
	75:25**	4.81	0.97	2514
	70:30	4.74	1.11	2018
Wavelength	270nm	4.81	1.1	2419
	275nm**	4.81	0.97	2514
	280nm	4.79	1.0	2549

** System suitable parameters under the optimized chromatographic conditions

Table-7: A report on study of ruggedness

Parameter	Retention Time	Peak Area	Tailing Factor	Theoretical Plates
Laboratory-I††	4.81	4343308	0.97	2514
Laboratory-II	4.85	4345545	1.0	2505
Agilent HPLC System ††	4.81	4343308	0.97	2514
Shimatzu HPLC System	4.76	4339782	1.1	2417
Chromosil C18††	4.81	4343308	0.97	2514
Symmetric C18	4.82	4342977	0.95	2518

†† Laboratory, HPLC System and Column actually followed in the analysis

Table-8: Results of pharmaceutical formulation analysis

Sample-ID	Area of Peak	% of Assay†
1	4409038.8	100.82
2	4331218.7	99.05
3	4322151.4	98.84
Mean	4354136	99.572
SD	47762	1.092
%RSD	1.097	1.097

†Acceptability criteria: Mean percent of assay should be present in between 98.0 – 102.0%

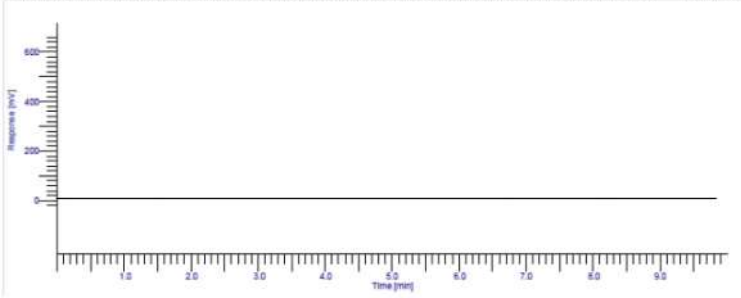


Figure 2: Typical chromatogram of blank at detection wavelength 275nm

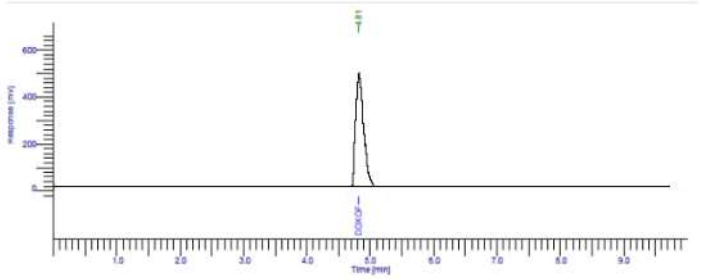


Figure 3: Typical chromatogram of working standard at detection wavelength 275nm

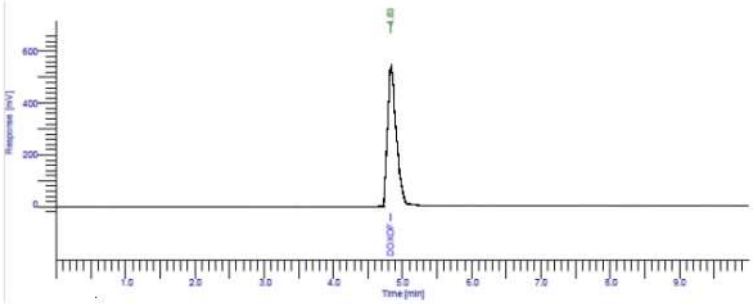


Figure 4: Typical chromatogram of formulation at detection wavelength 275nm

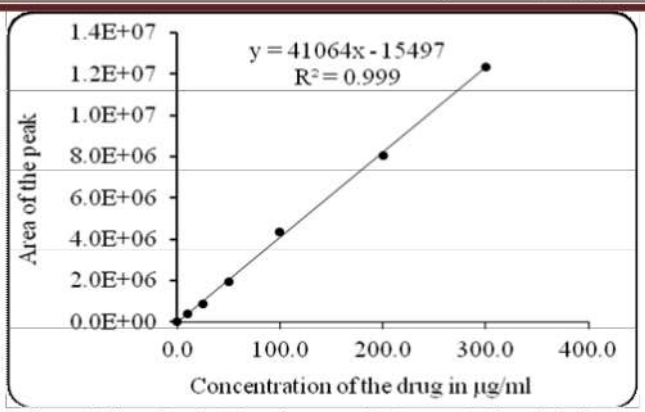


Figure 5: Linearity plot of peak area against concentration of the drug.

RESULTS AND DISCUSSION

System suitability

A volume of 20µL of working standard solution (100µg/mL) was injected into the chromatographic system, chromatogram was recorded and system suitability parameters like number of theoretical plates and tailing factor were obtained from the analysis of chromatogram using Chemstation software, and found to be 2514 and 0.97 respectively. Retention time of the component and area of the peak were found to be 4.814 min., 4343308.89 respectively. Typical chromatograms for blank and working standard solution were presented in Figure 2 and Figure 3 respectively. The results were summarized in Table-1.

Precision

Precision was expressed in terms of percent of relative standard deviation (%R.S.D.) and was calculated from the mean and standard deviation of area of six peaks. A volume of 20µL of working standard of concentration 100 µg/mL was injected into the column six times, the chromatograms was recorded. Statistical parameters such as mean, standard deviation (S.D.) and percent of relative standard deviation (%R.S.D.) were calculated and presented in Table-2

Intra Day and Inter-Day Precision

Intraday precision is the degree of repeatability of an analytical method in the same day under normal operational conditions. Whereas inter day precision of the method was reproducibility of the method on different days, different laboratories, between two different analysts. To study the intraday precision, six replicate measurements for working standard solution of doxofylline were made and the respective chromatograms were obtained. To find inter day precision, six replicate measurements were made for working standard solution on two different days and result was expressed in terms of relative standard deviation (% RSD). The % RSD was calculated in each case and found to be 0.191 and 0.232 respectively. The results were found to be within the acceptable criteria and were shown in Table-3.

Linearity

To determine the linearity of the proposed method, a series of six different concentrated solutions of the standard DXF including working concentration were prepared and about 20µL of each solution was injected in duplicate into the HPLC system, chromatograms were recorded under the optimum chromatographic conditions. A plot (Figure 5) between mean peak area and concentration was found to be linear in the range of concentration 10-300 µg/mL. The slope, intercept and correlation coefficient were calculated by least square regression method and were presented in Table-4.

Accuracy

To find out the accuracy of the developed method a known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution at three different concentration levels in triplicate. Percent recoveries were calculated by comparing the area before and after the addition of the standard drug. Satisfactory results were obtained presented in Table-5.

Limit of detection and Limit of quantification

Limit of detection (LOD) and limit of quantitiation (LOQ) of the developed method was evaluated from the study of signal by noise ratio method. To determine LOD the sample was dissolved in mobile phase and injected into the system until peak was diapered. After 0.25 µg/mL dilution the peak was not clearly observed. So it confirms that 0.25 µg/mL was the limit of detection. The limit of quantification was evaluated by determining the least concentration of the drug that can be quantified and found to be 0.825µg/mL.

Robustness

The study of robustness was performed by slight modification in chromatographic conditions such as flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. The working standard solution of DXF was analyzed under these new experimental conditions. Only one parameter was changed while the others were kept unaltered. The system suitability parameters were evaluated as per the test method in all the cases and found to be within limits shown in Table-6

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions. Ruggedness of the proposed method was performed by injecting six replicate injections of sample solution and the percent of assay was determined under different columns, different systems, and different labs over a period of one week. The results were presented in Table-7.

Analysis of pharmaceutical formulations

Pharmaceutical formulations were analyzed by the developed method by preparing a sample solution of 100.0µg/mL. The assay of the drug present in the each tablet was calculated by comparing the area of the peak of test with the standard. A typical chromatogram for the sample was given in Figure 4. The results were presented in Table-8.

CONCLUSIONS

To estimate the amount of DXF in pure and pharmaceutical formulations by reverse phase high performance liquid chromatography under optimized chromatographic conditions the chromatograms were recorded for standard and test

solutions (Figure 3, 4). The system suitability parameters were evaluated and found within the limits. A plot was drawn between concentration of the component and the instrument response, it was found to be linear (Figure 5) in the concentration range 10µg/mL to 300µg/mL with good correlation coefficient (r=0.9997). Precision and accuracy of the developed method were expressed in %RSD and % of recovery of the active pharmaceutical ingredient respectively. The proposed RP-HPLC method for the estimation of DXF in dosage forms was found to be more sensitive and highly precise. The percent of recovery of the drug (accuracy) indicates that the proposed method was selective. Hence the developed RP –HPLC method may be used as an alternative method for routine analysis of the raw materials and formulations.

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