RESEARCH ARTICLE

High-Performance Liquid Chromatographic Method Development and Validation for Analysis of Expired and Marketed Doxofylline Tablets

Manisha P Puranik[1*], Debarshi Kar Mahapatra[2], Rajendra O Ganjiwale[1], Shital D Tiple[1]

[1]*Department of Quality Assurance, Institute of Pharmaceutical Education and Research, Wardha, Maharashtra, India.*
[2]*Department of Pharmaceutical Chemistry, Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India.*

Received: 25th March, 2024; Revised: 10th July, 2024; Accepted: 25th July, 2024; Available Online: 31st August, 2024

ABSTRACT

The objectives of the study were to develop a simple, reliable, sensitive and accurate high-performance liquid chromatography (HPLC) method for doxofylline pure and pharmaceutical dosage forms. HPLC method was developed for the estimation of doxofylline in pure and marketed, recently expired, and 1-year expired tablet formulations. Chromatographic separation of the drug was achieved on a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), Cosmosil® C_{18}, column using a mobile phase of Potassium dihydrogen orthophosphate buffer: Acetonitrile pH 5.1 (60:40 v/v) at a flow rate of 1-mL/min. The drug eluted was monitored at 274 nm and the retention time of doxofylline was found to be 3.575 minutes, respectively. The validation of developed methods was done according to ICH Q2 (R1) guidelines. The calibration curve was linear over the range of 5 to 50 µg/mL. The correlation coefficient was found to be 0.9943 for doxofylline. The average retention time was found to be 3.538 minutes. The results were reproducible, showing the effectiveness of the system. The plate number was found to be 4909, which is also within the limit, *i.e.* ≥ 2000 prescribed in I.P. The dissolution studies of all three marketed, recently expired and 1-year expired formulations were carried out by using 0.01 M HCl and the results showed almost a similar rate of release profile and all three formulations accomplished the drug release within the limit. The developed and validated HPLC method offers a precise, accurate, and efficient analytical tool for the determination of doxofylline in pharmaceutical formulations.

Keywords: High-performance liquid chromatography, Validation, Estimation, Doxofylline, Dissolution, Expired.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.82

How to cite this article: Puranik MP, Mahapatra DK, Ganjiwale RO, Tiple SD. High-Performance Liquid Chromatographic Method Development and Validation for Analysis of Expired and Marketed Doxofylline Tablets. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1638-1645.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In the therapy of asthma, theophylline (1,3-dimethylxanthine), particularly in sustained-release forms, is commonly used. Doxofylline (2-[7'theophylline methyl]-1,3-dioxolane) (Figure 1), one of the theophylline analogs recently introduced for such therapy, is said to retain the theophylline's therapeutic characteristics while having a lower adverse effect.[1] Doxofylline is a derivative of methyl xanthine; it is a bronchodilator and directly leads to the relaxing of bronchial smooth muscle.[2] Doxofylline inhibits phosphodiesterase enzyme[3] and is soluble in acetone, water, ethyl acetate, benzene, chloroform, dioxane, hot methanol, hot ether, and practically insoluble in ethyl ether reported in I.P.[4] By preventing the smooth muscle cells' phosphodiesterase from functioning and causing smooth muscle relaxation, doxofylline suppresses asthma. Doxofylline is a novel xanthine bronchodilator that varies from theophylline through the presence of a dioxalane group in position C-7.[5] Doxofylline has a molecular weight of 266.257 g/mol with a chemical formula $C_{11}H_{14}N_4O_4$.[6]

Pharmaceutical analysis is defined as a branch of practical chemistry that deals with the resolution, separation, identification, determination, and purification of a specific sample of a pharmaceutical dosage form. It also includes the detection and estimation of any impurities that may be present in the sample.[7] High performance liquid chromatography (HPLC) is one of the most effective tools in analytical chemistry nowadays is chromatography. Any material that can dissolve in a liquid can have its constituents separated, identified, and quantified using this technique. The most precise analytical techniques, such as HPLC, are commonly used to analyze drug products both quantitatively and qualitatively, as well as to evaluate their stability.[8]

Development and validation of analytical methods are essential steps in the development and manufacturing of

**Author for Correspondence: manisha68_12@yahoo.com*

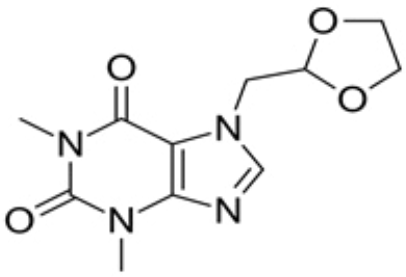

Figure 1: Structure of doxofylline

pharmaceuticals. These techniques are used to ensure the identification, purity, potency, and effectiveness of drug products. When developing techniques, many factors must be taken into consideration. The first step is to collect data on the physiochemical characteristics of the analyte (pKa, log P, and solubility), and then determine which mode of detection would be appropriate for analysis in the case of UV detection. The validation of a stability-indicating HPLC method utilizes the majority of the analytical development effort.[9] The manufacturer's assurance of a medication's complete efficacy and safety expires on the day of expiration. The majority of drug labels, including those for prescription, over-the-counter (OTC), and dietary (herbal) supplements, include expiration dates.[10]

Literature reveals that various determinations of doxofylline on rat-dried blood spots and urine[11] RP-HPLC: ESI-MS/MS, ^{1}H-NMR, and ^{13}C-NMR spectroscopic characterization of degradation products and process-related impurities of doxofylline.[12] The LESDA study[13] FTIR-ATR and UV/Visible techniques[14] have been reported for the determination of doxofylline in pharmaceutical preparations. Hence, in the present communication, we would like to report a simple, economical, feasible, rapid, sensitive and validated specific HPLC method for the simultaneous determination of doxofylline in the marketed and expired tablets.

The main aim and objective of the work was to develop and validate the HPLC method for the determination of doxofylline marketed, recently expired, and 1-year expired tablet dosage form.

MATERIALS AND METHODS

Chemicals

An analytically pure sample of doxofylline was acquired as a gifted sample from Cadila Healthcare Ltd., Indore, Madhya Pradesh, India. Pharmaceutical tablet dosage form of doxofylline (Doxolin^{TM} – 400) marketed Exp. Date of June 2025, expired June 2024, 1 year expired on May 2023 was manufactured by Zydus Healthcare Ltd., Daman, Daman and Diu, India and the label claim was found to be 400 mg. Acetonitrile (HPLC grade), methanol (HPLC grade), potassium dihydrogen orthophosphate, water (HPLC grade), and chloroform were purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India.

Identification of drug

Performed as per I.P. 2018, Infrared Absorption Spectrophotometric procedure by comparing the spectrum obtained of marketed, recently expired, and 1-year expired samples with reference doxofylline. Finely powdered marketed and expired tablets containing about 0.1 g of doxofylline was extracted vigorously with 40 mL of chloroform, and the contents were filtered and evaporated to dryness. On the dried residue, infrared absorption spectrophotometry was performed.[15]

Determination of detection wavelength

A 10 µg/mL dilution of doxofylline was scanned on a double beam UV-vis spectrophotometer, in the range of 200 to 400 nm, against the water. Doxofylline showed maximum absorption at 274 nm.[16]

Method development and optimization

Initial set of chromatographic conditions was defined based on analyte molecules. Stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), cosmosil C_{18}, the column was selected since it has noble separation capability. The mobile phase for HPLC comprises Potassium dihydrogen orthophosphate buffer: Acetonitrile (pH 5.1) (60:40 v/v). The overall run time was found to be 3.575 minutes. The flow rate of about 1 mL/min was selected since it was taken as the optimum flow rate for the selected column conditions. The detection wavelength of 274 nm and injection volume of 20 µL was selected to elevate detection capability.[17]

Preparation of the mobile phase

To prepare the mobile phase, 60 mL of potassium dihydrogen orthophosphate buffer and 40 mL of acetonitrile were taken in a 100 mL measuring cylinder. The mobile phase was filtered through Whatman Filter paper No. 42 and degassed by sonication for 20 minutes using an ultrasonicator bath and later transferred into a solvent reservoir, avoiding bubbles.[18]

Preparation of Buffer

About 0.68 g of potassium dihydrogen orthophosphate was exactly weighed, transferred into a 100 mL volumetric flask, dissolved by the addition of distilled water and finally diluted to the mark with water.[19]

Preparation of standard solution

Weight accurately 20 mg of standard drug transfer in 50 mL of volumetric flask. Add 25 mL of mobile phase sonicate for 20 minutes volume make up to 50 mL shake well (400 µg/mL). The solution was filtered through Whatman filter paper No. 42. 1 mL of this solution was diluted to 10 mL of mobile phase (40 µg/mL).[20]

Preparation of test solution

Weigh and powder 20 marketed, recently expired, and 1-year expired tablets of doxofylline. Disperse a quantity of each (marketed, recently expired, and 1 year expired) powder containing about 20 mg of doxofylline in 50 mL of mobile phase sonicate for 20 minutes volume make up to 50 mL shake

well (400 µg/mL). The solution was filtered through Whatman filter paper No. 42. 1 mL of this solution was diluted to 10 mL of mobile phase (40 µg/mL). The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution and sample solution was injected and the chromatogram and peak area were recorded at 274 nm.[21]

Preparation of standard calibration curve of doxofylline

Accurately weight 25 mg of doxofylline was taken in 25 mL volumetric flask and dissolved in the mobile phase and volume was made up to mark with mobile phase to make a concentration of 1000 µg/mL. The aliquot portion of the standard stock of doxofylline was further diluted with mobile phase to get a series of concentrations as 5, 10, 20, 40, and 50 µg/mL. The mobile phase was allowed to equilibrate with the stationary phase until a steady baseline was obtained. 20 µL of the prepared solution was injected into the column and the peak area of various dilutions was recorded at 274 nm. Under the optimized chromatographic conditions described, the graph peak area *vs*. concentration µg/mL was plotted and data was subjected to linear regression analysis using Microsoft Excel.[22]

Dissolution test

The release profiles were examined using dissolution tests for both marketed, recently expired, and 1 year expired tablets of doxofylline. It was performed using apparatus No. 1 (paddle), medium was taken of 0.01 M HCl, and the time of the system was fixed to 50 RPM and 45 minutes, respectively. The medium was maintained at 37±0.5ºC. The sample of marketed, recently expired, and 1 year expired formulations under dissolution testing were taken at appropriate time intervals of 10, 20, 30, 40, and 45 minutes and assayed spectrophotometrically at 274 nm.[23]

Method validation

The method was validated as per the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.[24] The method was validated for system suitability, linearity, precision, accuracy, and ruggedness.

System suitability test

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of a standard solution of the drug.

Preparation of Standard Drug Solution

An accurately weighed quantity of 20 mg of doxofylline was dissolved in mobile phase and volume was made up to 50 mL mark to get a standard stock solution having a concentration of 400 µg/mL of a drug. It was sonicated for 20 minutes. The stock standard solution was diluted further with the mobile phase to get a final concentration of about 40 µg/mL. The previously filtered and degassed mobile phase was allowed to equilibrate with the stationary phase until a baseline was obtained. 20 µL standard solution of the drug was injected in five replicates and the system suitability parameters were recorded.

Accuracy

Accuracy in the analytical method is the closeness of test results obtained by the method to the true value. Accuracy was determined on the basis of a recovery study performed by the proposed method.

Recovery studies

The recovery study was done by the proposed method. These studies were carried out at three levels, i.e., multiple level recovery studies. An accurately weighed quantity of pre-analyzed doxofylline 25 mg was taken in 25 mL. Volumetric flasks and it 80, 100, and 120% of doxofylline pure drug and mobile phase was added and sonicated for 20 minutes. Finally, volume was made to mark with mobile phase and filtered through Whatman filter paper No. 42 and required dilutions were made. The percent recovery was then calculated using the following formula:

$$\%\text{Recovery} = \frac{A-B}{C} \times 100$$

Where A - % Total amount of drug estimated; B - % Amount of drug found on a pre-analyzed basis; and C - % Amount of pure drug added.

Precision [25, 26]

The precision of an analytical method is the degree of agreement among individual test results. It was ascertained by replicate estimation of marketed formulation (five times) and expressed as the SD and %RSD of the series of measurements.

Linearity and range[25, 26]

Accurately weighed quantities of tabled powder equivalent to 80, 90, 100, and 120% were taken and dissolved in the mobile phase and diluted appropriately with the mobile phase to obtain a concentration in the range of 80 to 120% of the test concentration. The chromatogram of the resulting solution was recorded.

Ruggedness[25, 26]

The study of ruggedness was carried out under two different conditions:

Inter-day study[27, 28]

The samples were prepared as described under the marketed, recently expired, and 1 year expired formulations. The inter-day study was performed by applying the proposed method on same sample of tablets on different days.

Intra-day study[27, 28]

The sample was prepared as described under the marketed, recently expired, and 1 year expired formulation. The intraday study was performed by applying the proposed method on the same sample of tablets on the same day at 2-hours intervals.

Different analysts

The samples were prepared as described under the marketed and expired formulation. Different analyst and analyses

prepared for the sample solutions was carried out by the proposed method.

Dissolution study

A dissolution study was performed to conclude the release profile of the drug in *in-vitro* conditions.

Standard Calibration Curve

Standard stock solution

An accurately weighted quantity of doxofylline equivalent to 100 mg was taken in 100 mL volumetric flask and dissolved with 0.01 M HCl and volume was made up to the mark with 0.01 M HCl (1000 µg/mL).

Working stock solution

The 1 mL portion of the standard stock solution was diluted with 0.01 M HCl to get 100 µg/mL. The aliquot portions of working stock solutions of doxofylline were further diluted with 0.01 M HCl to get a series of concentrations ranging from 10 to 70 µg/mL and absorbances were noted at 274 nm by UV-visible spectrophotometry.

Assay

Standard solution and sample solution were injected distinctly into the HPLC system from which peak area response for the analyte was measured. The standard was prepared from active pharmaceutical ingredient (API) and a sample was prepared from the formulation. Five replications analyzed both standard and sample. Marketed doxofylline and expired doxofylline were assessed (formulation) by taking the standard as a reference.[29]

RESULTS AND DISCUSSION

The present study deals with the development and validation of analytical methods for quality evaluation of recently expired, 1 year expired and marketed doxofylline tablets by the HPLC method.

Identification

The FTIR spectrum obtained from reference doxofylline is concordant with the spectrum obtained with marketed, recently expired, and 1 year expired tablets.

Selection of wavelength

During preliminary studies, it was observed that the standard stock solution was stable in water; thus, water was used as a solvent. From the spectral study, it was found that the λ_{max} of the doxofylline was 274 nm. Hence, the wavelength selected for the estimation of doxofylline was 274 nm for the HPLC method. The standard calibration curve was plotted in a concentration range of 5 to 50 µg/mL, and it was found to be linear with the regression coefficient (r^2) of 0.9941.

Chromatographic condition

Different chromatographic trials were done to achieve better efficacy of the chromatographic system. The trial-1 showed retention time is more (9.517 minutes) in the chromatogram (Figure 2A). The trial 2, the chromatogram showed a retention time of 7.608 min (Figure 2B). The trial-3 chromatogram (doxofylline) eluted with a good peak shape. The tailing factor was found to be < 2 and theoretical plates were found to be >2000 (Figure 2C). Potassium dihydrogen orthophosphate buffer: Acetonitrile (pH 5.1) (60:40 v/v) was fixed as a mobile phase.

System Suitability Parameters

A system suitability test was used to verify the reproducibility of the chromatographic system. System suitability tests were carried out on freshly prepared standard solution of concentration 40 µg/mL. The average retention time was found to be 3.538 minutes. The results were reproducible, showing the effectiveness of the system. The theoretical plate number was found to be 4909, which is also within the limit, *i.e.,* ≥ 2000 prescribed in I.P.

Recovery (Accuracy)

The quantitative recovery calculated from marketed, recently expired, and 1-year expired were indicated in Tables 1, 2, and 3, respectively. The samples at concentration levels 80, 100, 120% are found to be 99.45, 99.97, and 99.71%, respectively, which is within the limit, *i.e.,* 98 to 101% prescribed in USP, indicating that the method is free from interference of excipients.

Precision

Precision of the method according to ICH guidelines was ascertained by replicate estimation of the marketed formulation (n = 5) and it was expressed as the ±SD and %RSD. The precision for marketed, recently expired, and 1-year expired

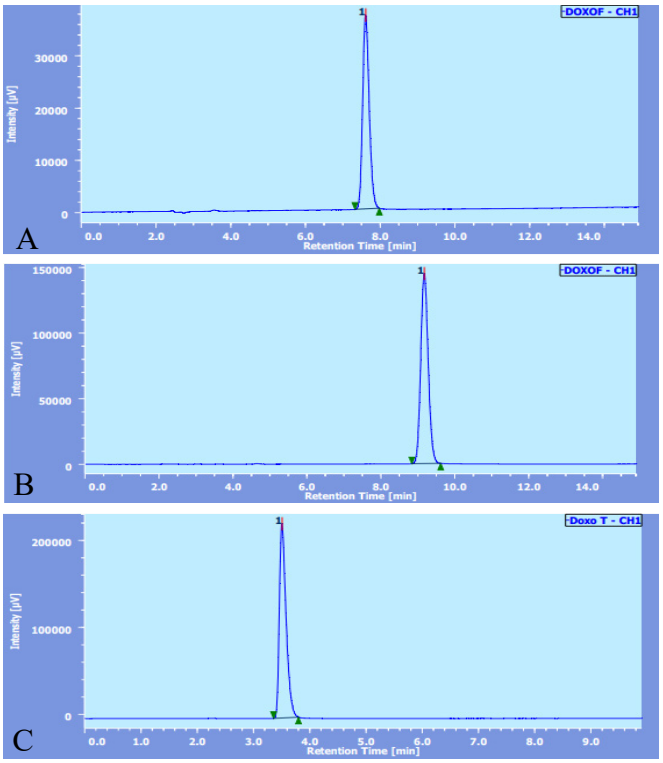

Figure 2: (A) First trial chromatogram run of doxofylline; (B) Second trial chromatogram run of doxofylline; (C) Optimized chromatogram of doxofylline by proposed method

Table 1: Results of recovery study of marketed formulation

S. No.	*Level of recovery (%)*	*Wt. of tablet powder taken (g)*	*Amount of pure drug added*	*%Amount of drug found on a pre-analyzed basis*	*Peak area*	*%Recovery*
1	80	0.0269	0.016	100.58	3358528	100.90
2	100	0.0269	0.02	100.58	3697438	98.65
3	120	0.0269	0.024	100.58	4062476	98.80
					Mean	99.45
					±SD	1.257
					%RSD	0.01265
					CV	1.265

Table 2: Recovery study of recently expired formulation

S. No.	*Level of recovery (%)*	*Wt. of tablet powder taken*	*Amount of pure drug added*	*%Amount of drug found on pre-analyzed basis*	*Peak area*	*% Recovery*
1	80	0.0271	0.016	97.74	3302439	100.88
2	100	0.0271	0.02	97.74	3636420	98.53
3	120	0.0271	0.024	97.74	4053221	100.36
					Mean	99.92
					±SD	1.23
					%RSD	0.01235
					CV	1.235

Table 3: Recovery study of 1 year expired formulation

S. No.	*Level of Recovery (%)*	*Wt. of tablet powder taken*	*Amount of pure Drug added*	*% Amount of drug found on pre-analyzed basis*	*Peak area*	*% Recovery*
1	80	0.0270	0.016	98.65	3317630	100.42
2	100	0.0270	0.02	98.65	3656431	99.28
3	120	0.0270	0.024	98.65	4031631	99.44
					Mean	99.71
					±SD	0.617
					%RSD	0.00619
					CV	0.619

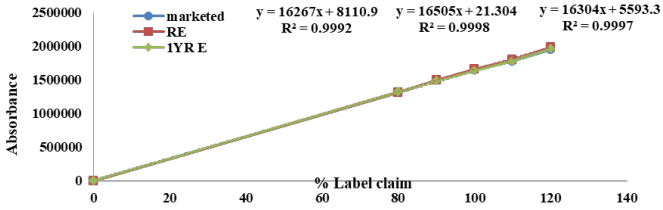

Figure 3: Linearity and range for marketed, recently expired, and 1-year expired formulation

formulations was found to be 0.6229 and 0.88409, 1.06402 with %RSD values of 0.00624 and 0.00894, 0.01094, respectively from series of measurements. The statistical evaluation of the results establishes good precision. The results were indicated in Tables 4, 5, and 6, respectively.

Table 4: Precision study of drug in marketed formulation

S. No.	*Sample*	*Wt. taken (g)*	*Peak Area*	*Estimation (%)*
1	Standard	0.02	1843751	-
2	Sample	0.0291	1853754	100.58
		0.0291	1834754	99.49
		0.0291	1835782	99.61
		0.0291	1843827	100.47
		0.0291	182899	99.18
			Mean	99.86
			±SD	0.6229
			%RSD	0.00624
			CV	0.624

Linearity and range

Accurately weighed quantities of tabled powder equivalent to 80, 90, 100, and 120% was taken and dissolved in the mobile phase and diluted appropriately with the mobile phase to obtain a concentration in the range of 80 to 120% of the test concentration. The chromatogram of the resulting solution was recorded. The results are indicated in Table 7 and Figure 3, respectively.

Table 5: Precision study of drug in recently expired formulation

S. No.	*Sample*	*Wt. taken (g)*	*Peak Area*	*Estimation (%)*
1	Standard	0.02	1843751	-
2	Sample	0.0271	1802103	97.74
		0.0271	1842391	99.92
		0.0271	1812101	98.35
		0.0271	1834340	99.56
		0.0271	1823121	98.95
			Mean	98.90
			±SD	0.88409
			%RSD	0.00894
			CV	0.894

Table 6: Precision study of drug in 1 year expired formulation

S. No.	*Sample*	*Wt. taken (g)*	*Peak Area*	*Estimation (%)*
1	Standard	0.02	1843751	-
2	Sample	0.0270	1802172	97.73
		0.0270	1813251	98.34
		0.0270	1798521	97.54
		0.0270	1792931	97.23
		0.0270	1761235	95.51
			Mean	97.27
			±SD	1.06402
			%RSD	0.01094
			CV	1.094

Table 7: Linearity and range study

S. No.	*Wt. of tablet powder taken*	*Peak area*		
		Marketed	*Recently expired*	*1 Year expired*
1	0	0	0	0
2	80	1310589	1311473	1321497
3	90	1502421	1496903	1482301
4	100	1648031	1659845	1642595
5	110	1773231	1798561	1778716
6	120	1947989	1985781	1960531

Table 8: Ruggedness study of marketing, recently expired, and 1-year expired formulations

1	Interday study	Marketed	Mean	99.46
			± SD	0.7135
			%RSD	0.00717
		Recently Expired	Mean	98.61
			± SD	0.335
			%RSD	0.0034
		1 Year Expired	Mean	97.70
			± SD	0.963
			%RSD	0.00986
2	Intraday study	Marketed	Mean	99.81
			± SD	0.4864
			%RSD	0.00487
		Recently Expired	Mean	98.35
			± SD	0.4782
			%RSD	0.00486
		1 Year Expired	Mean	97.61
			± SD	0.6308
			%RSD	0.00646
3	Different analysts	Marketed	Mean	99.76
			± SD	0.0424
			%RSD	0.00043
		Recently Expired	Mean	98.45
			± SD	0.7778
			%RSD	0.0079
		1 Year Expired	Mean	96.90
			± SD	0.770
			%RSD	0.0079

Table 9: Assay Data

Parameters	*Marketed*	*Recently expired*	*1 year expired*
Mean peak area	1839423	1822811	1787841
Wt. taken (mg)	20	20	20
Label claim (mg)	400	400	400
Average weight (mg)	26.91	27.135	27.011
Amount recovered	99	98	97
%Assay	99.86	98.90	97.27

Ruggedness

Intra-day and inter-day studies were performed and the mean concentration did not deviate from the nominal concentration. The interday, intraday, and different analysts study show variation with %RSD values of marketed sample 0.00717, 0.00487, and 0.00043, respectively, for recently expired formulation %RSD values are 0.0034, 0.00486, and 0.0079, respectively, for 1-year expired formulation %RSD values are 0.00986, 0.00646, and 0.00795, respectively, which are within the limit as prescribed by ICH and USP which signifies that the method is rugged. The results are indicated in Table 8.

Dissolution study

The release profile was examined using dissolution tests for marketed, recently expired, and 1 year expired tablets of doxofylline. The %cumulative drug release of marketed, recently expired, and 1 year-expired doxofylline tablets was found to be 79.86, 77.54, and 76.90 within the 45 mins. This shows that marketed, recently expired, and 1-year expired tablets were found to have an almost similar rate of release profile in 0.01 M HCl, which is within the limit; *i.e.,* NLT 75% release in 45 minutes as per I.P. The assay data is described in Table 9.

CONCLUSION

In this study, we successfully developed and validated a robust HPLC method for the analysis of doxofylline in both expired and marketed tablet formulations. The method was meticulously designed to meet the requirements of sensitivity, specificity, accuracy, and precision as per the ICH guidelines. The retention time, peak symmetry, and reproducibility of the method were optimized to ensure reliable quantification of doxofylline under various conditions. Our findings demonstrated that the developed HPLC method could effectively distinguish doxofylline from its potential degradation products, allowing for a comprehensive assessment of the drug's stability in expired tablets. This capability is crucial for understanding the degradation profile of doxofylline and ensuring the quality and safety of the medication even when subjected to expiration conditions. The method exhibited excellent linearity over a broad concentration range, with a correlation coefficient (r^2) close to 1. Additionally, the method proved to be highly reproducible with intra- and inter-day precision values well within the acceptable range, indicating its suitability for routine analysis in quality control laboratories. The accuracy of the method, as evidenced by recovery studies, was consistently within the range of 98 to 102%, underscoring its reliability for quantifying doxofylline in both marketed and expired tablets. The degradation studies conducted under stress conditions (acidic, basic, oxidative, thermal, and photolytic) provided insights into the stability of doxofylline. The method could effectively monitor the degradation process, making it a valuable tool for stability-indicating studies. The application of the method to expired tablet formulations revealed significant differences in the degradation patterns compared to marketed formulations, highlighting the importance of monitoring drug stability beyond the expiration date. In conclusion, the developed and validated HPLC method offers a precise, accurate, and efficient analytical tool for the determination of doxofylline in pharmaceutical formulations. Its application extends beyond quality control to stability studies, ensuring that the marketed and expired products meet the necessary safety and efficacy standards. The method's capability to detect degradation products and quantify doxofylline accurately makes it an essential addition to the analytical methodologies available for this important bronchodilator. This study not only contributes to the pharmaceutical analysis field but also reinforces the importance of continuous monitoring of drug quality throughout its shelf life, ensuring patient safety and therapeutic efficacy. Future studies could explore the application of this method to other dosage forms and further investigate the degradation kinetics of doxofylline under various storage conditions.

ACKNOWLEDGMENT

The authors are thankful to the management of the Institute of Pharmaceutical Education and Research Borgoan (Meghe) Wardha, Maharashtra, India, for providing the necessary facilities to carry out the research work. Further, the authors are highly thankful to Cadila Healthcare Limited, Sarkhej-Bavla N.H. No.8A, Moraiya, Tal. Sanand, Dist. Ahmedabad for providing the standard doxofylline (API).

REFERENCES

1. Sindhuri J, Panneerselvam P, Karthikeyan M, Rajeswari C. Development and validation of RP-HPLC method for the estimation of doxofylline in bulk and pharmaceutical dosage forms. International journal of chemtech research. 2011;3(3):1049-1053.
2. Radhika N, Venkateswara Rao J, Raju V. Method development and validation of doxofylline in pure and pharmaceutical dosage forms by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;2(4):859-863.
3. Srivastava A, Swain SP, Pandey S, Singhai A. Development and validation of stability-indicating RP-HPLC method for the estimation of doxofylline in pharmaceutical formulationIndian journal of pharmaceutical sciences. 2013;75(5):591-595.
4. Murthy M, Srinivasa Rao Y, Rambabu C. Development and validation of stability-indicating RP-HPLC method for the determination of doxofylline in pharmaceutical dosage form. International Journal of Pharmaceutical Sciences and Drug Research. 2015;7(2):173-178.
5. Gowri Sankar D, Lakshmana Rao A, Rajendra Prasad Y. A stability-indicating RP-HPLC method for the determination of doxofylline in bulk and dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(6):392-395.
6. Chaitanya B, Pradeepthi K, Sudhakar M, Rao KNV, Rajendra Prasad Y. RP-HPLC method development and validation for the simultaneous estimation of doxofylline and montelukast in pharmaceutical dosage form. J Pharm Sci Res. 2015;7(11):927-392.
7. Kandikere VN, Phadke M, Bothara KG, Shinde DB. Simultaneous determination of doxofylline and terbutaline sulphate in human plasma by HPLC. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2008;869(1-2):189-194.
8. Yunoos M, Sankar DG. A validated stability-indicating RP-HPLC method for the estimation of doxofylline in bulk drug and pharmaceutical dosage form. Journal of Chemical and Pharmaceutical Research. 2013;5(11):317-323.
9. Sonawane SS, Potale LV, Shinde VN. Simultaneous determination of doxofylline and terbutaline sulphate in pharmaceutical dosage form by RP-HPLC method. International journal of pharmtech research. 2010;2(1):348-352.
10. Madhavi B, Varaprasad V, Srinivasulu D. Development and validation of new analytical method for the determination of doxofylline in bulk and pharmaceutical dosage forms by RP-HPLC. International Journal of Pharmaceutical Sciences and Research. 2015;6(9):4007-4012.
11. Tamizh MT, Sathish Kumar T, Arul Kumar S, Swaroop KK, Srinivas Rao D. Analytical method development and validation of doxofylline by RP-HPLC method in bulk and dosage form. Journal of Chemical and Pharmaceutical Research. 2012;4(7):3378-3382.
12. Samyuktha R, Elphinstone S. Stability-indicating RP-HPLC method development and validation for the simultaneous estimation of montelukast sodium and doxofylline in bulk and pharmaceutical dosage form. International Journal of Pharmaceutical Sciences and Research. 2016;7(2):757-762.

13. Tejaswini J, Suneetha A. A validated RP-HPLC method for the estimation of doxofylline in pharmaceutical formulations. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(10):1695-1705.
14. Chandran S, Singh RSP. Comparison of various international guidelines for analytical method validation. Pharmazie. 2007;62(1):4-14.
15. Jha SK, Bhaskaran S, Kamble MA, Mahapatra DK. A novel RPHPLC based assay for the estimation of Tramadol HCl content in tablets: Development and Validation. Inventi Impact Pharm Anal Qual Assur. 2017;2017(4):142-146.
16. Puranik M, Shambharkar S, Nimbalkar S, Mahapatra DK. Comparison of UV-spectrophotometric and RP-HPLC methods for estimation of deflazacort in solid dosage form. Journal of Applied Pharmaceutical Science. 2020;10(7):082-088. DOI: 10.7324/JAPS.2020.1071
17. Murarka BG, Mahapatra DK, Bagde SM, Mahajan UN, Pise AG. Saxagliptin and Metformin Estimation in Tablet Formulation. Int J Pharm Phytopharmacol Res. 2020;10(2):27-36.
18. Sawale V, Dhabarde DM, Mahapatra DK. Development and validation of UV spectrophotometric method for simultaneous estimation of olmesartan medoxomil and chlorthalidone in bulk and pharmaceutical dosage form. European Journal of Chemistry. 2017;12(1):55-66.
19. Sabale PM, Bhagwat D, Sabale VP, Mahapatra DK. Development and Validation of RP-HPLC method for Simultaneous Estimation of Esomeprazole and Domperidone in Capsule Formulation. Eurasian Journal of Analytical Chemistry. 2018;13(6):57-66.
20. Kanthale SB, Thonte SS, Mahapatra DK. Development of validated stability indicating RP-HPLC method for the estimation of glecaprevir and pibrentasvir in bulk and pharmaceutical dosage form. Journal of Applied Pharmaceutical Science. 2019;9(6):052-60. DOI: 10.7324/JAPS.2019.90607
21. Kanthale SB, Thonte SS, Mahapatra DK. Stability indicating RP-HPLC method for the simultaneous estimation of ivabradine and metoprolol in bulk and tablet formulation. Journal of Applied Pharmaceutical Science. 2019;9(4):137-144. DOI: 10.7324/JAPS.2019.90418
22. Deodhe ST, Dhabarde DM, Kamble MA, Mahapatra DK. Novel stability indicating RP-HPLC method for the estimation of Pinaverium bromide in tablet formulation: assay development and validation. Eurasian Journal of Analytical Chemistry. 2017;12(2):3–16. DOI 10.12973/ejac.2017.00150a
23. Deodhe ST, Dhabarde DM, Kamble MA, Mahapatra DK. Development and validation of a novel stability indicating RP-HPLC method for the estimation of Entecavir in tablet formulation. Eurasian Journal of Analytical Chemistry. 2017;12(3):223–235.DOI: 10.12973/ejac.2017.00165a
24. Kanthale SB, Thonte SS, Pekamwar SS, Mahapatra DK. Development and validation of a stability indicating RP-HPLC method for the determination of prucalopride succinate in Bulk and Tablet. International Journal of Pharmaceutical Sciences and Drug Research. 2020:166-174. https://doi.org/10.25004/IJPSDR.2020.120211
25. Pund S, Changediya V, Rajurkar V. Design and Development of Analytical Method for Deflazacort Estimation: A Robust HPLC Approach. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):363-368. DOI: 10.25258/ijpqa.14.2.21
26. Pranita J, Agrawal R, Chambhare N, Sahare AY. Development and Validation of a Stability-Indicating RP-HPLC Method for Estimation of Glibenclamidei Bulk and Pharmaceutical Formulation. International Journal of Drug Delivery Technology. 2023;13(3):875-883. DOI: 10.25258/ijddt.13.3.17
27. Patil DJ, Godge RK, Jahagirdar SN, Mandhare SB. Validated Stability Indicating RP-HPLC Method for the Quantification of Process Related Impurities of Solifenacin and Mirabegron in Pharmaceutical Formulations. International Journal of Drug Delivery Technology. 2024;14(1):55-60. DOI: 10.25258/ijddt.14.1.10
28. Ahmad S, Aakanksha D, Patil M, Bhise M, Barde L, Tare H. Development and Validation of HPLC And HPTLC for Simultaneous Analysis of E and Z Guggulsterone, A-11–KBA And 11–KBA from Herbal Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):393-396. DOI: 10.25258/ijpqa.14.2.25
29. Prakash O, Mahapatra DK, Singh R, Singh N, Verma N, Ved A. Development of a New Isolation Technique and Validated RP-HPLC method for Quercetin and Kaempferol from Azadirachta indica leaves. Asian Journal of Pharmaceutical Analysis. 2018;8(3):164-168. http://dx.doi.org/10.5958/2231-5675.2018.00030.3