ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 4, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR DEFERIPRONE ESTIMATION IN PHARMACEUTICAL DOSAGE FORM

SACHIN N KOTHAWADE*, VISHAL V PANDE

Department of Pharmaceutics, RSM's N.N Sattha College of Pharmacy, Ahmednagar, Maharashtra, India.
E-mail: sachin.kothawade23@gmail.com

Received: 06 December 2022, Revised and Accepted: 18 January 2023

ABSTRACT

Objectives: For the simultaneous estimation of deferiprone in pharmaceutical formulations, a high-performance liquid chromatographic technique that is straightforward, reproducible, and effective was developed.

Methods: The Advanced Technologies Ltd HPLC system with a UV-2075 UV-Vis detector, P-2080 HPLC pump, and a Hypersil C18 column (250 × 4.6 mm) was used to perform the chromatographic separation. ACN and water make up the mobile phase (55:45 v/v). The solvent system was flowing at a rate of 1.0 mL/min. 4.7 g of sodium dihydrogen orthophosphate and 1 mL of triethyl amine were used as the buffer, and orthophosphoric acid was used to bring the pH of the solution to 4.0 ± 0.05. The sample volume was 20 µL, as well as the temperature range, was kept at room temperature.

Results: The method was approved for the rapid screening and confirmed in conformance with ICH guidelines. For the purpose of determining the presence of deferiprone in active ingredients, an RP-HPLC analysis model with Uv spectrophotometer has been developed. The drug determination retention time (4.960 min), which is crucial for routine analysis. The method's high sensitivity was indicated by the low identification and qualifying limits.

Conclusion: The developed method for simultaneously determining Deferiprone in pharmaceutical formulations was found to be more accurate, precise, and selective.

Keywords: ICH Guidelines, Deferiprone, Process development, Validation, High-performance liquid chromatography.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i4.47000. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Pharmaceutical impurities are unwanted chemicals that persist with active pharmaceutical ingredients (APIs), develop during formulation, or appear after the formulation and API have aged. Even trace amounts of these unwanted synthetic substances can affect a pharmaceutical's effectiveness and safety [1]. The chemical name for deferiprone is 3-hydroxy-1,2-dimethylpyridin-4(1H)-one (Fig. 1). An iron chelator called deferiprone capable of binding to ferric ions (iron II) and forms a stable 3:1 (deferiprone: iron) complex that is then excreted in the urine [2]. Deferiprone has a lower affinity for other metals like zinc, copper, and aluminium due to its higher selective for iron [3-5].

Reference material and a general survey show that similar work is constantly being done in so many academic organizations to develop analysis tools for new medicines and their combinations that are introduced to the market [6]. Plans for the current work follow a similar pattern. The goal of the study is to develop sensitive and precise methods for calculating iron chelator drug dosages in pharmaceutical formulations.

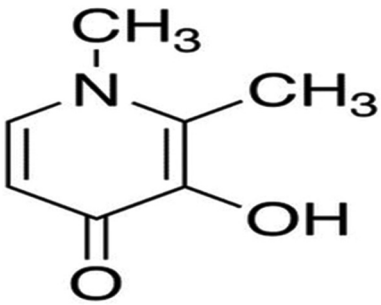

Fig. 1: Chemical structure of Deferiprone

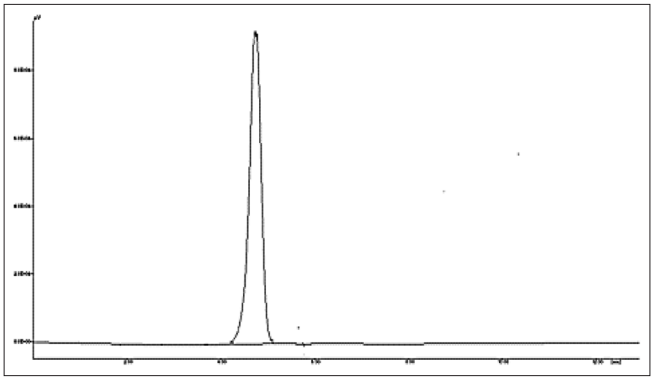

Fig. 2: HPLC chromatogram of DFP in mobile phase – ACN: Water (55:45V/V)

METHODS

Reagents and chemicals

Deferiprone drug sample was procured from Emcure Pharmaceutical Industry in Bhosari, Pune. The following ingredients were purchased from Merck, Hyderabad: acetonitrile (HPLC Grade), water (HPLC Grade), acetic acid (AR Grade), sulfuric acid (AR Grade), sodium oxide (AR Grade), methanol (HPLC Grade), and sodium dihydrogen phosphate (AR Grade).

Selection of mobile phase

Based on the HPLC separation parameter, a different mobile phase proportion was chosen. A mobile phase containing acetonitrile:

water (55:45 v/v) was chosen from among the various mobile phases tested [7-9].

Determination of detection wavelength

The chosen mobile phase, acetonitrile: water (55:45 v/v), was used to dissolve 100 mg of DFP in 100 mL to create the standard stock solution of DFP. The standard stock solution aliquot was appropriately diluted to achieve a concentration of 100 µg/mL using the same mobile phase. Using mobile phase as a blank, the λ_{max} was determined using a Shimadzu UV-Visible spectrophotometer in the 200–400 nm range. The chosen detection wavelength is 280.0 nm [10].

Instrumentation and analytical conditions

Used was an HPLC system from JASCO (Made in Japan) with a manual injector of a 20-µL loop, a UV-Visible detector, and a Hypersil C18 column (250 × 4.6 mm). Both an ultra sonicator and a Shimadzu (BL-220H) electronic balance were employed. The procedures were carried out utilizing a gradient reverse phase method. Acetonitrile and water were the components of the mobile phase, which had a rate of flow of 1 mL/min. Before use, the mobile phase was degassed and filtered through a 0.45 m nylon membrane filter. The measurement was made at a wavelength of 280.0 nm. The overall duration time was 10 min and the sample volume was 20 µL [11-14].

Preparation of DFP standard stock solutions

Transfer 100 mg of standard DFP to a volumetric flask after precisely weighing it. DFP was dissolved in 50 mL of acetonitrile: water (55:45, v/v) mobile phase using sonication for 15 min and the volume was then increased with mobile phase to the required amount. A final concentration of 100 µg/mL of the standard stock solution was prepared by diluting the stock solutions further. This stock solution was filtered using membrane filter paper with a 0.4 mesh size [15].

Preparation of sample solution

Powder 20 tablets after weighing them. Accurately weighing a quantity of powder equal to 100 mg, it was then transferred to a volumetric flask of 100 mL, dissolved in 50 mL of solvent system, and sonicated for 15 min. Further dilutions were made using solvent system as a diluent to make up to the mark [16-19].

Method validation

In accordance with ICH guideline Q2, the following parameters were validated for the methods (R1) [20].

Linearity

For DFP, the linear relationship in pure solutions was tested over concentration ranges of approximately 10–50 g/mL. Regression analysis was used to derive the regression line pertaining standard drug concentrations, this same calibration curves have been linear in the studied range, and the regression analysis equations were obtained: y=165.2×(−74.30), R^2=0.9991 [21].

Accuracy

Recovery studies were conducted by adding reference drug solution to pre-analyzed stock solution at three distinct levels 80%, 100%, and 120% to evaluate the method's accuracy. Recovery studies determined the method's accuracy. The reference standards for the drugs were added to the formulation (pre-analyzed sample) at levels of 80%, 100%, and 120%. Three recovery studies were conducted and the percentage of recovery and the percentage of mean recovery for drugs were computed [22].

Precision

Without altering the proposed chromatographic method's parameter, the instrument's accuracy was tested through repeated

Table 1: System suitability data

Parameter	Experimental value	Standard limits
Retention time (R_T)	4.960	-
Tailing factor (T)	1.641	T=2
No of theoretical plate (N)	4207	N>2000
Asymmetric factor (As)	0.88	As=2
Capacity factor	0.092	2–10

Table 2: Result and statistical data for tablet analysis

Parameters	Standard area	sample area
Injection-1	3323.906	3315.154
Injection -2	3320.770	2958.636
Injection- 3	3293.679	3099.476
Injection- 4	3274.548	3304.544
Injection -5	3193.687	3067.165
Average area	3281.318	3148.901
Tablet average weight	0.30132g	
Standard weight	0.010 g	
Sample weight	0.0120 g	
Label amount	0.500 g	
Assay (% purity)	99.65	

Table 3: Summary of calibration data for DFP

Concentration (µg/mL)	Peak area
10	1656229
20	3312459
30	4868688
40	6624918
50	8281147

Table 4: DFP linear regression data

S. No.	Parameters	DFP
1	Detection of wavelength (nm)	280.0
2	Linear range (µg/mL)	10-50
3	Correlation coefficient (R^2)	0.999
4	Linear regression equation (y=mx+c)	y=16507×(+74.03)
5	LOD (µg/mL)	24.340
6	LOQ (µg/mL)	73.750

Table 5: Accuracy study of DFP

Recovery Level	Amount taken (mg/mL)	Area	Average area	Amount recovered (mg/ml)	% Recovery	Average % recovery
80%	80	3404.393	3256.777	73.60	98.14	
	80	3069.834				
	80	3296.104				
100%	100	3838.430	3483.758	99.98	98.98	98.40
	100	3285.170				
	100	3327.673				
120%	120	4838.317	4760.862	122.63	98.10	
	120	4781.051				
	120	4663.219				

n*=3

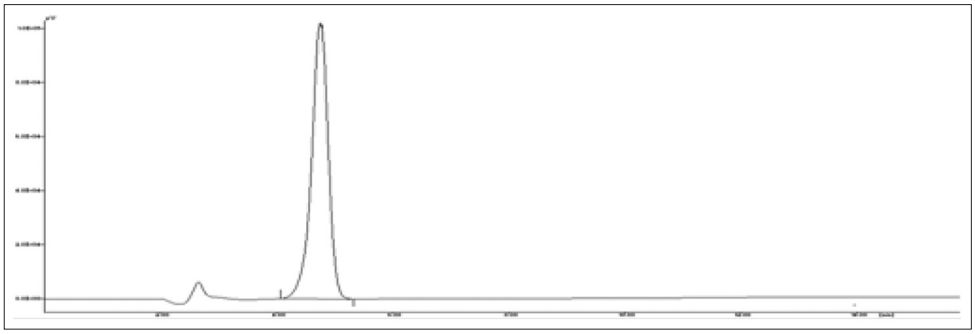

Fig. 3: HPLC chromatogram tablet (DFP 500 mg)

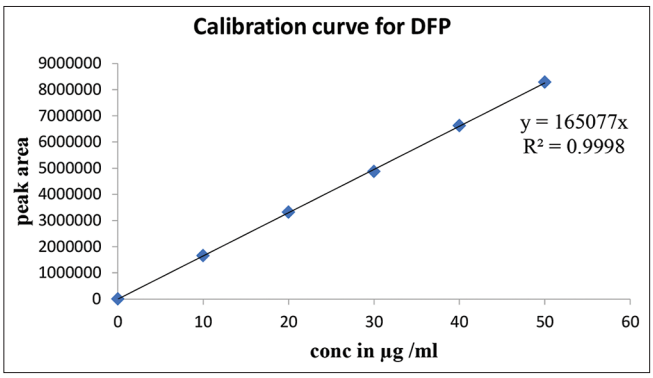

Fig. 4: Calibration curve of DFP by HPLC method

Table 6: Precision study of DFP

S. No.	Inter-day precision area	Intra-day precision area
1	3059.296	3059.296
2	3091.557	3068.996
3	3314.064	3432.659
4	3293.679	3697.550
5	3067.164	3249.340
6	3315.152	3093.479
Avg	3190.152	3266.886
S.D	23.204	22.410
%RSD	0.71	0.69

Table 7: Robustness and ruggedness of DFP by RP-HPLC method

Parameter	Retention time	Peak area	S.D	% R.S.D
Wavelength in nm				
278	4.973	318760	±0.4967	0.5759
280	4.950	326476		
282	4.233	317612		
Mobile phase flow rate (mL/min)				
0.7	4.080	320950	±0.9118	1.1847
1.1	4.950	331804		
1.2	4.233	326377		

scanning and measurements of the absorption of solutions (n=6) for Deferiprone [20].

Intermediate precision

By examining the corresponding responses 3 times on the same day and 3 times on 3 different days over the course of 1 week for three different concentrations of standard solutions of Deferiprone, the intraday and interday precision of the suggested protocol was assessed. The relative standard deviation (% RSD) was used to express the outcome The data are given in Table 6 [22].

Detection limit (LOD) and limit of quantification (LOQ)

LOD and LOQ estimates were used to determine the measurement sensitivity of Deferiprone (LOQ) [21].

Robustness and ruggedness

By adjusting the experimental conditions (flow rate, wavelength, and mobile phase), the robustness of a HPLC method is investigated. By switching analysts, the robustness of a HPLC method is investigated [22].

RESULTS AND DISCUSSION

System suitability

By altering the mobile phase's composition, the HPLC procedure was made more effective. After testing a number of mobile phases, the one that contained acetonitrile, water, and mL in a proportion of 55:45 (v/v) has been chosen. The drug concentration of 100 µg/mL of DFP was used for optimization of chromatographic conditions. Good resolution of 4.960 min at 280.0 nm with retention time 4.960 min for DFP, respectively, was achieved with C18 column. The chromatogram was given in Fig. 2. Chromatographic analysis time was found to be < 10 min. Analysis of DFP was used to determine the system suitability parameters, including retention time (RT), number of theoretical plates (N), tailing factor (T), and resolution (RS), and it was discovered that all of the parameters were within the acceptable range, making the method acceptable and suitable for routine analysis. The results are given in Table 1.

Analysis of DFP in marketed formulation

Label claim – Deferiprone –500 mg as shown in Fig. 3.

Method validation

The method was tested in terms of repeatability, accurateness, precision, LOQ, LOD, ruggedness, and robustness in accordance with ICH guidelines [Q2B]. The retention time and peak area of both drugs remain unchanged, proving the specificity of the method. The data are given in Table 2.. Under a UV detector, the method's linearity was seen for DFP concentrations of 10, 20, 30, 40, and 50 µg/mL, respectively (Table 4). In the chosen range at the chosen wavelength (280.0 nm), the linearity of the signal intensity was observed with a regression coefficient (r^2) >0.9998 for drugs as shown in Fig. 4. The calibration curve of peak area versus concentration give the regression equation y=165077×(−74.30) for DFP, respectively. The data are given in Table 3.

Using the standard addition method to calculate the mean recovery from the sample, the accuracy of the method was verified. Results showed a DFP with a %RSD of <2% at 98.40%. Table 5 displays these outcomes. To LOD for DFP 24.340, the suggested method was discovered to be sensitive. For DFP, the LOQ was discovered to be 73.750. Because it is unaffected by slight changes in operating conditions, the method was robust. To test this method's robustness and ruggedness by varying the flow and wavelength. The fact that the method's chromatographic response and retention time show no discernible effects shows how reliable and tough the method was. The results are given in Table 7.

CONCLUSION

The proposed research project is based on the analysis of Deferiprone using the UV method and RP-HPLC method in tablet dosage form and bulk. The method was approved for the rapid screening and confirmed in conformance with ICH guidelines. For the purpose of determining the presence of deferiprone in active ingredients, an RP-HPLC analysis model with Uv spectrophotometer has been developed. The drug determination retention time (4.960 min) is crucial for routine analysis. The method's high sensitivity was indicated by the low identification and qualifying limits.

ACKNOWLEDGMENT

The authors thankful to management of RSM's N. N. Sattha College of Pharmacy, Ahmednagar for providing resources to perform this work.

CONFLICTS OF INTEREST

The researchers have no competing interests.

AUTHORS' CONTRIBUTION

Dr. Sachin Kothawade: Contributed significantly to the article's concept, interpretation of data for the article, and drafted the article; Dr. Vishal Pande: Authorized the version to be published.

AUTHORS' FUNDING

Nil

REFERENCES

1. Abbas M, Nawaz R, Iqbal T, Alim M, Asi MR. Quantitative determination of deferiprone in human plasma by reverse phase high performance liquid chromatography and its application to pharmacokinetic study. Pak J Pharm Sci 2012;25:343-8. PMID 22459459
2. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis: (For Hons. and Post-graduate Students of Indian and Foreign Universities). New Delhi: Himalaya Publishing House; 1979.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed., part 2. New Delhi: CBS Publishers and Distributors; 1997. p. 275-95.
4. Ohannesian L, Streeter AJ. Handbook of Pharmaceutical Analysis: Dekker Series. New York City: Marcel Dekker; 2002. p. 219-21.
5. Kolhe S, Khose Y, Kale A. Simultaneous estimation of cetirizine hydrochloride, phenylpropanolamine hydrochloride and paracetamol by RP-HPLC method. Int J Pharm Life Sci 2013;4:3122-32.
6. Skoog DA, West DM, Holler FJ, Crouch SR. Fundamentals of Analytical Chemistry. 8th ed. Philadelphia, PA: Saunders College Pub; 2004. p. 973-85.
7. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Method of Analysis. 7th ed. New Delhi: CBS Publishers and Distributors; 1986. p. 600-7.
8. Nadukuru A, Kumar TS. Development, validation of a stability indicating method for the simultaneous determination of levofloxacin hemihydrate and ornidazole by high-performance liquid chromatography. Int J Pharm Drug Anal 2013;1:37-48.
9. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical Formulation. 1st ed. CBS Publication; 2001. p. 3-10, 11-4, 59-63, 101-3.
10. Fong GW, Lam SK. HPLC in Pharmaceutical Industry. Vol. 47. New York City: Marcel Dekker; 1991. p. 1.
11. Mali AS, Salunke PA, Barhate SD, Bari MM. Development and validation of simultaneous RP-HPLC method for estimation of tenofovir and lamivudine in bulk and pharmaceutical formulations. J World World J Pharm Res 2015;4:995-1006.
12. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's Textbook of Quantitative Chemical Analysis: ELBS. 5th ed. England: Longman Pubication; 1996. p. 224-8, 647-9.
13. Blum F. High performance liquid chromatography. Br J Hosp Med (Lond) 2014;75(Suppl 2):C18-21. doi: 10.12968/hmed.2014.75.Sup2.C18, PMID 24521830
14. Synder R. Practical HPLC Method Development. 2nd ed. Hoboken, New Jersey: Wiley Inter Science Publication; 1997. p. 120.
15. Indian Pharmacopoeia Commission. Government of India Ministry of Health and Family Welfare. 6th ed., Vol. 1. Gaziabad: Indian Pharmacopoeia Commission; 2010. p. 133-5.
16. ICH. Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization. Geneva: ICH; 1994. p. 1-5.
17. ICH. Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization. Geneva: ICH; 1996. p. 1-8.
18. Ahmed M, Jangade VK, Shetty S, Aradhya VC, Kuppast IJ. RP-HPLC method development and validation for estimation of deferiprone in capsule dosage form. World J Pharm Pharm Sci 2014;4:831-8.
19. Kothawade SN, Pande VV, Albhar SN, Bole SS, Raut KG, Wagh VS. A high-performance stability-indicating liquid chromatographic novel method for determining recombinant human erythropoietin in bulk and dosage form. Preprints 2022;1:1-22. doi: 10.20944/preprints202211.0577.v1
20. Sireesha KS, kiran BS, Sekhar KB, Muneer S. A new RP-HPLC method development and validation of deferiprone in bulk and its pharmaceutical dosage form. Int J Adv Res 2016;4:2174-9. doi: 10.21474/IJAR01/1435
21. Padmaja N, Ramathilagam N. Method development and validation of RP-HPLC method for the determination of deferasirox in tablets. Int J Pharm Biol Sci 2012;2:338-43.
22. Dash RN, Habibuddin M, Sahoo A, Kothawade SN, Chaudhari MR, Mahadik KR. Factorial approach for the development of stability indicating HPLC assay of recombinant human insulin: Application to its stability study. Curr Pharm Anal 2013;9:318-29. doi: 10.2174/1573412911309030010