Research Article

Method Development and Validation of RP-HPLC Method for Assay of Extended-release Pharmaceutical Solid Dosage Form for the Treatment of CNS-acting Drug Product

Ashish Ashok Gawai*, Vaibhav Shikare, Pallavi S. Tidke, Snehal Sunkare, Dipak Bhusari, Shirish P. Jain
Dept. of Quality Assurance, Rajarshi Shahua College of Pharmacy, Buldhana, Dist Buldana, Maharashtra, India.
***Corresponding author's E-mail:** drashishgawai@gmail.com

Received: 06-12-2024; **Revised:** 23-02-2025; **Accepted:** 10-03-2025; **Published online:** 20-03-2025.

ABSTRACT

Introduction: Dexmethylphenidate is a CNS stimulant agent and from the literature search, the available methods were Spectrophotometric and HPTLC.

Objectives: The objectives of this work were to develop an accurate, simple, time-efficient, as well as precise (Fast or rapid) reverse-phase analytical HPLC method.

Materials and Methods: On a SHISHA-DO, C18, 250 x 4.6mm, 5µ size column, the separation of this drug was obtained with a mobile phase made up of 0.5 percent orthophosphoric acid, acetonitrile, and methanol (20:10:70v/v) at a flow rate of 1ml/m, with UV detection at 226nm.

Results: Retention time was measured at 1.2, and linearity between 50 and 150µg/ml was discovered. For recovery, linearity, LOD, LOQ, precision, and accuracy, this technique has been statistically verified.

Conclusion: The findings showed that the linearity, precision, accuracy, LOD, and LOQ of the technique were satisfactorily verified.

Keywords: Dexmethylphenidate, RP-HPLC, Validation, CNS stimulant, Method development.

INTRODUCTION

The Dexmethylphenidate is chemically (R)-Phenyl-[(R)-piperidin-2-yl]e Bis-aure methyl-ester (Figure 1), it is a CNS stimulant agent, and a thorough literature review found that analytical techniques including spectrophotometric analysis and HPTLC were available for estimating the presence of these medications alone or in combination with other substances.[1,2] To estimate such medicines, only a few RP-HPLC techniques were available.[3,4] Our goal was to create a basic RP-HPLC technique to estimate this medication. The designed technique was approved by ICH norms (ICH Q2b, Q2A, Q1B, Q1A (R2)).[5-8]

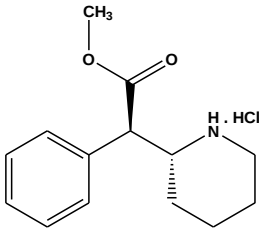

Figure 1: Structure of Dexmethylphenidate.

Dexmethylphenidate is a first-line agent and central nervous system stimulant used to treat ADHD i.e Attention Deficit Hyperactivity Disorder in patients aged six years and older.[9] This agent increases extracellular levels of dopamine and norepinephrine in the CNS. As a CNS agent, it carries a greater risk of recreational use, abuse, misuse, and dependency which need to be monitored with its use. As it is a CNS stimulant agent, it increases heart rate and causes serious adverse reactions in the heart, which is why it also needs monitoring after administration. Dexmethylphenidate is the d-enantiomer of methylphenidate. This enantiomer is more pharmacologically active than the racemic mixture.[10, 11]

MATERIALS AND METHODS

Chemicals and reagents

The 1-Heptane sulfonic acid sodium salt, Ortho-phosphoric acid (88%), Potassium dihydrogen phosphate, Tri-ethylamine, Acetonitrile, and Methanol were procured from "Dept. of Quality Assurance, Rajarshi Shahu College of Pharmacy, Buldhana Dist-Buldana, Maharashtra, India".

Instrumentation and analytical conditions

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump and UV detector (LC-20AD) was employed for analysis and Rheo-dyne injector with 10µl fixed loop was used for the present study.

Preparation of solutions

Standard solution preparation

Approximately 60mg of the working standard were properly weighed, put into a 100ml dry volumetric flask, along with 50ml of diluent 1, and then sonicated until the diluent was completely dissolved. With diluent 1, adjust the volume and blend.[12]

Sample solution preparation

In a 200 ml volumetric flask, the sample powder was accurately weighed and transferred. Then, 50ml of

methanol and 10ml of diluent 2 was added, and the mixture was sonicated for approximately 45m with occasional shaking. Combine with diluents 2 to add the necessary amount to bring the mixture to room temperature. The mixture was centrifuged for about 10m at a speed of 4000rpm before being filtered through a 0.45µm nylon syringe filter, with the first 5ml of the filtrate being discarded.[13]

Optimized analytical methods

The stationary phase was an Eclipse XDB C18 column, 150x4.6mm, 5µm. With a mobile phase consisting of buffer and acetonitrile in a ratio of (75:25 percent v/v), the dexmethylphenidate was gradient-eluted at a flow rate of 1.0ml/m. The UV detector was calibrated to operate at a wavelength of 210nm. The mobile phase was produced by passing it through a 0.45µm membrane filter (PVDF) and then sonicating it.

Method Validation

Guidelines established by the ICH: "International Conference on Harmonization" for the validation of analytical techniques were used to validate the accuracy of the designed method. We validated according to Q2 of the ICH guidelines (R1). The designed procedure was checked for its precision, specificity, and accuracy, as well as its linearity, LOQ, LOD, and other measurable qualities.[14]

System suitability

Six replicate injections of newly generated standard solutions were used to evaluate the HPLC system's appropriateness by measuring the Theoretical Plate Number (N), Retention Duration, and Tailing Factors (T) of each solute.

Linearity

For an analytical method to be considered linear, it must provide findings that are proportionate to the analyte concentration in the sample across a certain concentration range. The analytical approach should be used over the range of a linear connection. It can be shown on the drug compound itself (through dilution of a standard stock solution) or on the individual components of a synthetic combination used to make a drug product. A mathematical adjustment of the test outcomes can be vital to establish linearity between sample and assay concentration.[14]

Stock solution preparation

Transferred 60.30mg of the working standard by precise weighing to a volumetric flask with a capacity of 50 ml. Used a sonic cleaner and roughly 40ml of solvent to achieve total dissolution. Diluted it to the proper level and stirred it well. Linearity solutions of all concentrations were injected in duplicate and plotted a curve of concentration (%) vs. mean area. The residual sum of squares, regression line slope, y-intercept, and correlation coefficient were determined.[14]

Accuracy

It is the ratio between the theoretical rate and the observed value. In blind research, this is expressed as the percentage of analyte retrieved by assay or sample spiking. The analytical process should be shown accurately over the whole operating range. According to ICH Guideline Q2B, accuracy should be evaluated throughout a range of three concentrations (i.e., total concentration and three replicates at each concentration level).[15]

Assay analysis included triplicate measurements of a known quantity of medication or standard spiked with placebo at approx. 50%, 100%, and 150% of the test concentration. The percentage of recovery is determined by comparing the quantity of substance discovered with the total amount recovered.

Level-1: Into a 200 mL volumetric flask, we weighed and carefully transferred 60.42mg of working standard/API and 1287.44mg of placebo; then we added 50mL of methanol and 10 mL of Buffer, sonicated for 30 min while shaking rapidly, and then cooled to room temperature. Adjust the volume using Buffer and blend the two. Using a 0.45µm nylon filter, we filtered the solution and discarded the first few milliliters.

Level-2: Sonicated for about 30 min at intermediate shaking temperature, cooled to room temperature, and then added approximately 50ml of methanol and 10ml of buffer to a 200ml volumetric flask containing precisely 120.55mg of working standard/API and 1287.44mg of placebo. Adjust the volume using Buffer and blend the two. Using a 0.45µm nylon filter, we filtered the solution and discarded the first few milliliters.

Level-3: Sonicated for about 30 min at intermediate shaking temperature, cooled to room temperature, and then added approximately 50ml of methanol and 10ml of buffer to a 200ml volumetric flask containing 180.34mg of working standard/API and 1287.44mg of placebo. Adjust the volume using Buffer and blend the two. Using a 0.45 m nylon filter, we filtered the solution and discarded the first few milliliters.[15]

Procedure

Each dose was made in triplicate and injected twice. We determined the total quantity and percentage of recovery at each stage. RSD and mean recovery percentages were computed.[15] Table 1 indicates the preparation of the solution with recovery.

Precision

The accuracy of the analysis is the degree to which a set of measurements taken from independently obtained subsamples of the same homogeneous sample agree with one another under the same test conditions. There are three main levels of accuracy: Reproducibility, Repeatability, and Intermediate precision.[16]

Table 1: Preparation of Accuracy Solution and Recovery

Level	Replicate	Amount (mg/ml)	Recovery				
		Actual Amount added	Mean Area	Amount found	% Recovery	Mean	% RSD
Recovery-50%	1	60.42	5014058	0.30	99.30	**99.04**	**0.28**
	2	60.50	5014061	0.30	99.06		
	3	60.76	5014014	0.30	98.75		
Recovery-100%	1	120.55	9919069	0.60	99.54	**99.66**	**0.15**
	2	120.20	9925604	0.60	99.82		
	3	120.47	9906042	0.60	99.61		
Recovery-150%	1	180.34	14732925	0.90	99.81	**99.75**	**0.20**
	2	180.86	14772925	0.90	99.52		
	3	180.17	14832925	0.90	99.91		

Repeatability

Over a short period, and with the same operating conditions, repeatability indicates accuracy. It's also known as "intra" precision. One blank and six standard preparation injections were performed, and the peak area count of the analyte peak was collected from the standard chromatogram to ensure the system's accuracy (Repeatability). Percent relative standard deviation (%RSD) calculated.

Intermediate Precision

Variation in intermediate accuracy is to be expected within labs due to factors such as day of the week, analyst, lab equipment, etc. The chromatographic system was injected with one blank injection, five standard preparation injections, and six sample preparation injections (two injections of each sample). RSD (relative standard deviation) and percent assay results were computed.

Reproducibility

The ability to reliably reproduce results in different labs is known as reproducibility (collaborative studies, generally supplied to standard methodology). For Method precision of the analytical approach to determine the assay was performed by preparing six sample preparation from the same homogenous of a single batch and analyzing as per test procedure. The chromatographic system was injected with one blank injection, five standard preparation injections, and six sample preparation injections (two injections of each sample). Percent assay and %RSD computed.

Detection Limit

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.[17]

Based on visual evaluation

The detection of the limit is determined by the analysis of a sample with the known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on single-to-noise Ratio

A single-to-noise ratio between 3 and 2:1 is generally considered acceptable for estimating the detection limit.

Based on the slope and the response standard deviation

The LOD can be written as

$$LOD = 3.3 \times \sigma / S$$

Where, σ = response SD,

S = Calibration curve slope.

Quantitation Limit

The quantitation limit of a given analytical method is described as the smallest analyte sample concentration that can be reliably measured quantitatively. We may consider the possibility that some of the following methods are suitable.[18]

Based on Visual Evaluation

Analysis of the sample in relation to the concentration of the analyte determines the Quantitation limit.

Based on the S/N ratio

The ratio of signal to noise must be at least 10:1.

Based on the response SD and slope

The LOQ can be written as:

$$LOQ = 10 \times \sigma / S$$

Where, σ = response Standard Deviation,

S = calibration curve slope

Specificity

It's the capacity to assess the analyte consistently in the presence of a component that could be present. Impurities, degradants, excipients, matrices, etc. may fall under this category. The other supporting analytical processes may

make up for the lack of specificity in a single analytical method.[19]

It can be used for the following application:

Identification

To ensure the identity of the analyte.

Purity test

To ensure that all the analytical procedure performed allows an accurate statement of the content of impurities of an analyte, i.e., related substances, heavy metals, residual solvent content, etc.

Assay (content or potency)

To provide an exact result, which allows an accurate statement on the content or potency of the analyte in a sample. It has been shown in Table 2.

Table 2: Assay result of Dexmethylphenidate.

Sl. No	Avg. Area	% Assay
1	10045359	101.8
2	10032295	101.7
	Mean	**101.8**
	±SD	**0.07**
	% RSD	**0.07**

Range

The range of an analytical method is defined as the analyte concentration (amounts) in a given sample between which the analytical method has been proved to have appropriate precision, linearity, and accuracy.[20]

Robustness

Analytical procedures may be judged by their robustness based on how well they hold up under typical conditions when subjected to slight but intentional changes to one or more of the parameters of the analysis process.[21]

Ruggedness

The term "ruggedness" refers to the extent to which outcomes produced under different settings may be replicated. The robustness of an analytical technique is defined as the consistency with which results are produced when the same sample is analyzed several times using the same or different laboratory, analyst, equipment, reagent lot, elapsed assay time, assay temperature, and day conditions.[22,23]

RESULTS

Method validation results

Linearity

Table 3: Spiked level and mean peak area count of Linearity of prepared Dexmethylphenidate

Linearity level	Spiked level (%)	Mean peak area count
1	20	1985607
2	50	4964018
3	80	7942429
4	100	9928036
5	150	14892054

Figure 2: Standard linearity curve for a developed method

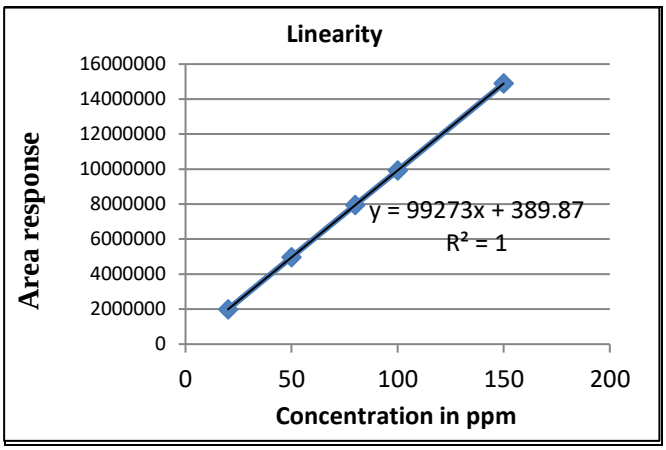

Table 4: Linearity regression data for the calibration curve.

Sl. No	Parameters	Result
1	Correlation Coefficient (r)	1.0000
2	Slope	99273
3	Y-intercept	0.99993

Accuracy

Table 5: Result of Accuracy with replicate and recovery.

Level	Replicate	Recovery		
		% Recovery	Mean	% RSD
Recovery-50%	1	99.30	**99.04**	**0.28**
	2	99.06		
	3	98.75		
Recovery-100%	1	99.54	**99.66**	**0.15**
	2	99.82		
	3	99.61		
Recovery-150%	1	99.81	**99.75**	**0.20**
	2	99.52		
	3	99.91		

Results of Precision like Method Precision (Reproducibility), Intermediate Precision (Ruggedness) and Percent of Assay comparisons between Reproducibility and Ruggedness.

Table 6: Peak area count, Avg Peak area count, and percent assay and difference of Assay of method precision.

No. of Injection	Peak area count	Average Peak area count (Reproducibility)	% Assay	Average Peak area count (Ruggedness)	% Assay	Reproducibility (% Assay)	Ruggedness (% Assay)
1	9917459	10011542	101.3	10021542	101.4	101.3	101.4
2	9930526	10062161	101.8	10052161	101.7	101.8	101.7
3	9918187	10066578	101.8	10056578	101.7	101.8	101.7
4	9897047	10049456	101.7	10029456	101.5	101.7	101.5
5	9979893	10041261	101.6	10021261	101.4	101.6	101.4
6	9945515	10031111	101.5	10041111	101.6	101.5	101.6
Mean	**9931438**	-	**101.6**		**101.5**		**101.6**
±SD	**28637.82**	-	**0.21**		**0.15**		**0.18**
%RSD	**0.29**	-	**0.20**		**0.15**		**0.18**

Specificity

Table 7: Result of specificity in terms of Avg. Area and % assay.

Sl. No	Avg. Area	% Assay
1	10045359	101.8
2	10032295	101.7
	Mean	**101.8**
	±SD	**0.07**
	%RSD	**0.07**

DISCUSSION

The acceptance criteria for the assay of the drug Dexmethylphenidate should ideally fall between 98 and 102%. The average percentage assay result for Dexmethylphenidate was found to be 101.8% w/v as the result was mentioned in Table 2. The result indicates the drug complies with the assay parameter. In the method validation, all validation parameters were tested. The linearity is determined over the range of 50% w.r.t. lowest sample concentration to 150% w.r.t. highest sample concentration as the result was mentioned in Table 3. Table 4 shows that the value of the correlation coefficient is lower than the critical threshold of 0.995. The analyte concentration in a sample has a linear relationship with the obtained areas. From the obtained result it indicated that in the given range, the approach is linear. The result of accuracy was obtained by preparing triplicate preparations for each level, and injected each preparation in duplicate, to test accuracy throughout the range of 50% w. r. t. to the lowest sample concentration of 150% w. r. t. to the highest sample concentration. At each level, the total quantity discovered and the percent recoveries were determined. Calculations were made for the average percent recovery and percent RSD. Acceptance Criteria for system suitability criteria were confirmed from these results as mentioned in Table 5. The parameters of the system's suitability fell well within the allowable range. As a result, each validation parameter was appropriate for the system and chromatography. The precision parameter was explained with its results in Table 6. Precision to RSD of assay of six replicate sample preparations really shouldn't exceed 2.0, since this is the threshold for approval. The combined RSD and technique precision should be less than 2.0 percent. The outcomes were entirely consistent with the expected range. Thus, the procedure achieved accurate results. According to the acceptance criteria for specificity as mentioned in Table 7, the primary peak shouldn't be interfered with by any placebo or blank peak. When preparing standards and samples, the primary peak's purity index should be equal to or greater than 0.995. The findings showed that there was no interference between the primary peak and the blank, placebo, or known impurities. The acquired peak purity readings were well within the parameters for acceptability. So, the approach is particular.

SUMMARY AND CONCLUSION

The data from the results and discussion demonstrated that an effective RP-HPLC technique for the determination of pharmaceutical dosages had been prepared. The Waters symmetry C8, 150*3.9mm, 5µm particle size was subjected to an isocratic RP-HPLC analysis at a column oven temperature of 300°C using the mobile phase Buffer: Acetonitrile (75:25v/v) with an adjusted flow rate of 1ml/m. At 210nm, the detection was performed. It was discovered that the drug had an average retention time of 7.0m. The concentration range of 50-150µg/ml showed linearity (r2=0.999). The technique has been used repeatedly to identify pharmaceuticals in tablet formulations. The excipients in the dosage had no adverse effects. It was discovered that the drug content was 101.6%. Recovery studies looked at the accuracy of the approach at three distinct levels: 50%, 100%, and 150%. With an average recovery of 98–102, it was determined that the % recovery was within the range of the acceptance requirements. System suitability assessments are an important factor in chromatographic techniques, according to USP. They help the chromatographic system be more reproducible. The data obtained from the proposed RP-HPLC method and validation was summarized.

The proposed approach of the assay for the substance was determined to have been validated under the ICH guideline with the following goals based on the findings. By adopting the RP-HPLC approach, a basic, accurate, robust,

economical, precise, and reliable analysis for the drug test has been devised. It was determined that the proposed technique is appropriate for usage after it had been validated under ICH standards and fulfilled all of the recognized criteria listed in those recommendations.

Acknowledgment

The authors are thankful to Wockhardt Research Centre Ltd. Aurangabad for providing API Dexmethylphenidate to carry out necessary research and continuous support in troubleshooting any problem during the research work.

ABBREVIATIONS

UV: Ultraviolet; **LOD:** Limit of detection; **µl:** Micro liter; **RSD:** Relative standard deviation; **LOQ:** Limit of quantification; **ICH:** International conference of harmonization; **SD:** Standard deviation.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Chatwal G, Anand S. Instrumental method of chemical analysis. 2nd ed. Bombay: Himalaya Publishing House; 2004. p. 2.107-10.
2. Gawai A. A et al, RP-HPLC method development and validation for hyperlipidemic agent Atorvastatin in Pharmaceutical dosage form. Res J Pharm Tech. 2017; 10:6:1780-1787.
3. Christian GD. Analytical chemistry. 4th ed. United Kingdom: John Wiley and Sons Inc. p. 1-6.1986.
4. Synder LR, Kirkland JJ, Glajch LJ. Practical: HPLC method development. 2nd ed. United Kingdom: John Wiley and Sons Inc. p. 653-660.1997.
5. International Conference on Harmonization (ICH), Q2b: Validation of Analytical Procedures: Methodology. Vol. 62. Federal, Register: US FDA; May 1997. p. 27463.
6. International Conference on Harmonization (ICH), Q2A: Text on validation of Analytical Procedures. Vol. 60. Federal, Register: US FDA; May 1995. p. 11260.
7. International Conference on Harmonization (ICH), Q1B: Stability Testing: Photo Stability testing of New Drug Substances and Products Q1B; 1996. p. 1-8.
8. International Conference on Harmonization (ICH). Stability testing of new drug substances and products. Vol. Q1A; February 2003. p. 1-6.
9. Moen MD, Keam SJ. Dexmethylphenidate extended release: a review of its use in the treatment of attention-deficit hyperactivity disorder. CNS Drugs. 2009 December;23(12):1057-83. doi: 10.2165/11201140-000000000-00000, PMID 19958043.
10. Orr K, Taylor D. Psychostimulants in the treatment of depression: a review of the evidence. CNS Drugs. 2007;21(3):239-57. doi: 10.2165/00023210-200721030-00004, PMID 17338594.
11. Minozzi S, Saulle R, De Crescenzo F, Amato L. Psychosocial interventions for psychostimulant misuse. Cochrane Database Syst Rev. 2016;9(9):CD011866. doi: 10.1002/14651858.CD011866.pub2, PMID 27684277.
12. Gawai A. A et al., RP-HPLC analytical method validation of oral solid dosage form of tablet for anti-spasmodic action. Int J Pharm Eng. 2017; 5:3, 731-740.
13. Elumalai S, Aher K. Development and validation of RP-HPLC method for determination of content uniformity of rabeprazole sodium in its tablets dosage form. J Appl Pharm Sci. 2011;01(06):165-70.
14. Ritihaas C, Prakash B. RP-HPLC method development and validation for simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide. Int J Pharm Biol Sci. 2015;6(1):180-7.
15. Gawai A. A., Faisal A. Shaikh. Method development and validation of stability indicating RP-HPLC method for estimation of Metformin and Miglitol in pharmaceutical dosage form. Int J Sci Res Sci Tech. 2018; 4:1, 423-442.
16. Sarma E, Babu K. Simple and stability indicating RP-HPLC assay method development and validation for enalapril maleate by RP-HPLC in bulk and dosage form. Caribbean J Sci Tech. 2015;3:767-73.
17. Neupane Y, Srivastava M. Stability indicating RP-HPLC method for the estimation of decitabine in bulk drug and lipid based Nanoparticles. Int J Pharm Sci Res. 2011:294-302.
18. Mishra G, Vikas S. Analytical method development and validation for assay of diosmin and Hesperidin in combined tablet dosage form by RP- HPLC. Int J Pharm Life Sci. 2013;4:2834-9.
19. Gawai A. A et al, RP-HPLC method development, and validation for determination of an Anti-hypertensive agent. Int J Chemtech Res. 2018;11, 02, :228-239.
20. Skoog D, Holler F, Stanley C. Principles of instrumental analysis. 6th ed. Thomson: Brooks/Cole. CA: Belmont; 2007. p. 762-816.
21. Cecchi T, Pucciarelli F, Passamonti P. Extended thermodynamic approach to ion interaction chromatography. Anal Chem. 2001;73(11):2632-9. doi: 10.1021/ac001341y, PMID 11403310.
22. Harvey D. Modern analytical chemistry. 1st ed. McGraw-Hill Publication; 2000. p. 578-89.
23. Sethi PD. HPLC quantitative analysis of pharmaceutical formulation. 1st ed, CBS Publication; 2015.