Journal of Pharmaceutical Research International

33(31B): 203-211, 2021; Article no.JPRI.68948
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

A Newly Developed Reverse Phase-High Performance Liquid Chromatography Method for the Assay of Dexmethylphenidate and Serdexmethylphenidate with PDA

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMT designed the study, performed the method development and validation, wrote the protocol, and wrote the first draft of the manuscript. Authors AV and SNMB helped the analyses of the study. Author KSPA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i31B31707
Editor(s):
(1) Dr. Prem K. Ramasamy, Brandeis University, USA.
Reviewers:
(1) Jean De Dieu Nzabonakuze, Lanzhou University, China.
(2) Charles Nyandwi, Lanzhou University, China.
(3) Atared Saad Jebur AL-Mashhadi, Al-Mustaqbal University College, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68948

Original Research Article

Received 01 April 2021
Accepted 09 June 2021
Published 15 June 2021

ABSTRACT

Aims: The present application is a Newly Validated Reverse Phase-High Performance Liquid Chromatography Method for the Assay of Dexmethylphenidate and Serdexmethylphenidate with PDA.
Study design: Mentioned study is a quick, rapid, economical, precise, and accurate reverse phase- high performance liquid chromatographic method for estimating Dexmethylphenidate and Serdexmethylphenidate.
Place and duration of study: The present assay was carried out at the Shree icon Pharma

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laboratories PVT.ltd, Vijayawada, AP, and India, from December 2020 to February 2021.
Methodology: The stationary phase Agilent C18 column with dimensions of 150x4.6mm, 3.5 was used for chromatography and pH-2.5 ammonium acetate buffer with orthophosphoric acid: acetonitrile in a 50:50 ratio used as a buffer. The detection wavelength was 265nm, and the flow rate was 1mL/min. The strategy was justified according to ICH guidelines
Results: Dexmethylphenidate and Serdexmethylphenidate had retention periods of 4.258 and 5.629 minutes, respectively. For the estimation of Dexmethylphenidate and Serdexmethylphenidate, the method has been validated for linearity, accuracy, precision, stability tests, and forced degradation studies including acid, base, hydrolysis, peroxide, and thermal degradation. By multiplying the quality six times, the system's suitability parameter was investigated, and they were well within reasonable limits. The regression coefficient of the two drugs was found to be 0.999 during the linearity study, which was performed at 10% to 150 percentage points. Precision results for Dexmethylphenidate and Serdexmethylphenidate were 0.54 and 1.24, respectively. The drugs were recovered at a rate of 98-102 percent, which is within the acceptable range.
Conclusion: The validation results were found to be satisfactory. It was clear that the proposed method was suitable for routine quality control and analysis of pharmaceutical preparations.

Keywords: Serdexmethy lphenidate; dexmethylphenidate; development; validation, ICH guidelines.

1. INTRODUCTION

Sudden cardiac death, mania [1], loss of appetite [2], Dexmethylphenidate, also known as Focalin, is a drug used to treat attention deficit hyperactivity disorder (ADHD) [3,4], in children and adolescents above the age of five. It is rational to stop using it after four weeks if no result is seen. It is taken orally. The immediate-release formulation has a five-hour shelf life, while the extended-release formulation has a twelve-hour shelf life. Dangerously prolonged erection [5] is all serious side effects, hysteria [6], Violence [7]. Because of the delayed onset and long-term effects of Serdexmethylphenidate, some dosage formulations containing Serdexmethylphenidate have been studied for use as long-acting psychostimulants [8,9] in the treatment of ADHD. Serdexmethylphenidate, also known as KP484, has been studied as a "super-extended length" psychostimulant, with therapeutic effectiveness lasting up to 16 hours after oral administration. MonoSol Rx and KenPharm formed a collaboration in 2011 to produce oral films containing KP415. Dexmethylphenidate is a stimulant for the Central Nervous System [10]. It is not tangible regarding how it functions in ADHD. It is methylphenidate's more active enantiomer. Common side effects anaphylaxis [11], seizures [12], Abdominal pain [13-14], and fever are all. It is not sure to note whether it is safe to use during pregnancy and breastfeeding. Serdexmethylphenidate is a dexmethylphenidate derivative developed by the KemPharm pharmaceutical business. As of 2020, the compound is being studied for the treatment of ADHD in infants, teenagers, and adults. The FDA approved the medication for medical use in March 2021. In comparison to its parent compound, dexmethylphenidate, dexmethylphenidate is a prodrug with slower onset of action and a longer period of impact. The combination of dexmethylphenidate and dexmethylphenidate allows for faster onset of action while also maintaining therapeutic effectiveness for up to 13 hours. Serdexmethylphenidate and Dexmethylphenidate structures are shown in Fig. 1.

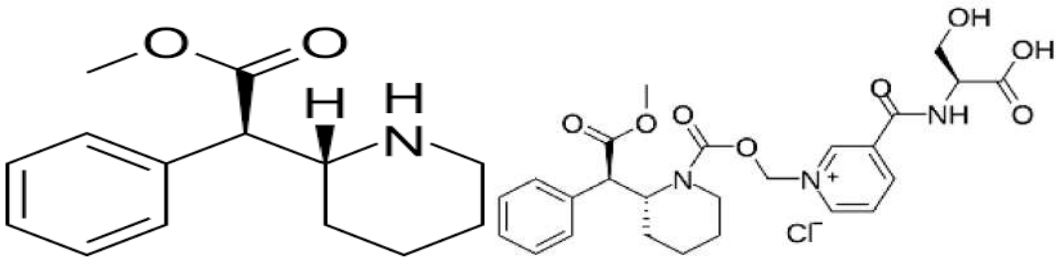

Fig. 1. Chemical structures of Dexmethylphenidate and Serdexmethylphenidate

2. MATERIALS AND METHODS

2.1 Chemicals

Merck (India) Ltd. Worli, Mumbai, India has given acetonitrile, orthophosphoric acid (OPA), ammonium acetate, and water (HPLC grade). Spectrum Pharma Research PVT. Ltd., Hyderabad, provided all APIs of Dexmethylphenidate and Serdexmethylphenidate as reference levels.

2.2 Equipment

The chromatographic system Waters alliance-2695, which included a PDA detector-2996, quaternary pump, and chromatographic software Empower-2.0 was used.

2.3 Buffer Preparation

7.71 mg of ammonium acetate in 1-liter water; change pH to 2.5 with OPA. Using 0.45 filter paper, filter the solution.

2.4 Mobile Phase

Acetonitrile is a 50:50 ratio of buffer was used as a mobile phase.

2.5 Diluent

Buffer is a 50:50 ratio of Acetonitrile used as diluent.

2.6 Preparation of Standard Solution

Weigh about 6 mg of Dexmethylphenidate; 28 mg of Serdexmethylphenidate was moved into a 100ml volumetric flask. Add 70ml of diluent, sonicated to disintegrate, and dilute with the diluent to volume. Dilute 5ml of the above solution to 50ml by utilizing diluent.

2.7 Preparation of Sample Solution

Move the weight equal to 59 mg of Dexmethylphenidate and Serdexmethylphenidate sample into a 100mL clean dry volumetric flask, apply the diluent, sonicated for 30 minutes to dissolve, centrifuge for 30 minutes to dissolve fully, and make volume up to the mark with the same solvent. After that, it goes through a 0.45-micron injection filter (Stock solution). Pipette 5 ml of the above stock solution into a 50mL volumetric flask and dilute with diluent to the desired concentration. Dexmethylphenidate (6 ppm) and Serdexmethylphenidate (28 ppm).

2.8 Chromatographic Conditions

The Agilent C18 (150x4.6mm, 3.5) Column was used for chromatographic separation in isocratic mode at room temperature. Change the pH of the ammonium acetate solution with OPA: The mobile phase was acetonitrile 50:50v/v with a flow rate of 1.0 mL/min. The injection volume was 10 µL, and the eluent was controlled using a PDA detector at 265 nm. The duration of the run was ten minutes.

2.9 Validation Procedure

Device suitability, accuracy, linearity, precision, specificity, robustness, limit of detection (LOD), the limit of quantification (LOQ), and stress degradation studies were all validated according to ICH Q2 (R1) guidelines.

2.10 System Suitability

To verify the system's accuracy, the system's suitability parameters were calculated. The parameters, USP tailing, USP plate count, and percent RSD are measured and found to be within the acceptable range.

2.11 Specificity

The capacity to test the analyte unequivocally in the presence of other segments (impurities, degradants, or excipients) that might be available in the sample and a standard solution is known as specificity. The chromatograms of blank samples and test samples spiked with Dexmethylphenidate and Serdexmethylphenidate were analyzed.

2.12 Accuracy

The degree to which the method's test results are accurate in comparison to the true value is referred to as accuracy. Recovery trials were conducted at three different concentration levels to determine its effectiveness. A minimum of three injections was given at each stage, and the content of drug available, percentage recovery, and standard deviation were examined.

2.13 Precision

The degree of agreement among individual test results is the precision of an analytical process.

Multiple sampling of a homogeneous sample was used to investigate it. The repeatability, intra-day, and inter-day variations of the current system were all evaluated. It was checked by examining samples taken at different times during the day as well as on others days.

2.14 Linearity

The capacity of an analytical technique to produce results that are corresponding to the concentration of the analyte in the sample within a specified range is known as linearity. For determining the linearity spectrum, six series of standard solutions were chosen. The regression equations were determined after plotting the calibration curve using peak area versus concentration of the standard solution. The intercept, slope, and coefficient of correlation were calculated utilizing the least-squares method.

2.15 LOD and LOQ

LOQ is the most minimal measure of analyte in a sample that can be measured with sufficient accuracy and precision, while LOD is the least measure of analyte in a sample that can be distinguished. The calibration curves were used to calculate LOD and LOQ separately. Using the established RP-HPLC process, the LOD and LOQ for Dexmethylphenidate and Serdexmethylphenidate were determined by injecting progressively lower concentrations of standard solutions. According to ICH guidelines, the LOD and LOQ were measured as 3.3s/n and 10s/n, individually, where s/n stands for signal-to-noise ratio.

2.16 Stress Degradation

No interference between the peaks acquired for the chromatogram of forced degradation preparations when forced degradation is available. Stress degradation tests were carried out by ICH Q1A guidelines (R2). The peak purity of the theory peaks must pass, and the deterioration peaks must be at least 1.0. Different forms of stress conditions were used to create forced degradation experiments that resulted in 20 percent degradation.

2.17 Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by minor but deliberate changes in process parameters, and it indicates the procedure's reliability in regular use. The standard solution was injected into the high performance liquid chromatography system, and chromatographic parameters such as flow rate (0.2 ml/min) and organic matter in the separation process (ten percent) were changed. The effect of the adjusted parameters was used to examine the, retention time, separation factor and peak asymmetry.

3. RESULTS AND DISCUSSION

3.1 Method Validation

By using Symmetry alignment C_{18} column we obtained the best chromatographic separation with a mobile phase consisting of acetonitrile and buffer in (50:50) at a flow rate of 1ml/min and PDA detection 265nm. Finally, the following conditions were found to be optimum after evaluating the column efficiency by parameters. The results were shown in Fig. 2 .and Table1.

The suitability, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation, and stability studies for the selected drugs Dexmethylphenidate, Serdexmethylphenidate are all validated in this process. Figs. 3 and 4 depict the proposed approach with chromatograms of the normal and sample solutions.

3.2 System Suitability

To obtain a stable baseline, the HPLC device was stabilized for 60 minutes. The system's suitability was tested using six duplicate injections of a standard solution containing 6g/ml Dexmethylphenidate and 28g/mL Serdexmethylphenidate. Dexmethylphenidate and Serdexmethylphenidate had 3471 and 13885 theoretical plate counts, respectively. Dexmethylphenidate and Serdexmethylphenidate have tailing factors of 1.02 and 1.06, respectively. All these variables were found to be within the permissible range. The results were shown in Table 2.

3.3 Linearity

The linearity of the strategy was determined by preparing a standard solution containing 6µg/mL of Dexmethylphenidate, 28µg/mL of Serdexmethylphenidate (100% of the targeted level of the assay concentration). Successive dilutions were performed to the given solutions at

10, 25, 50, 100, 125, and 150% of the target concentrations. These were injected and the peak areas were utilized to plot calibration curves against the concentration. The correlation coefficient values of these analytes were 0.999. The results were shown in Table 3 and the calibration curves were shown in Fig. 5 respectively.

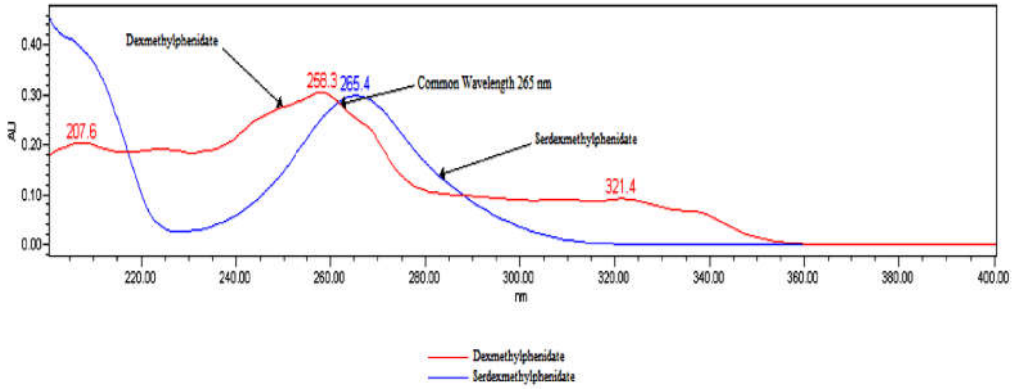

Fig. 2. PDA spectrum of Dexmethylphenidate and Serdexmethylphenidate

Table 1. Optimized chromatographic conditions

Stationary Phase	Agilent C_{18} (150x4.6mm, 3.5µ)
Mobile Phase	Acetonitrile: Buffer (50:50)
Injection volume	10 µl
Flow rate	1.0 ml/min
Column temperature	25°C
Wavelength	265 nm
Run time	10 min.
The retention time of Dexmethylphenidate	4.258 min.
The retention time of Serdexmethylphenidate	5.629 min.

Table 2. System suitability results

Parameter	Dexmethylphenidate	Serdexmethylphenidate
Theoretical plate count	3471	13885
Tailing factor	1.02	1.06
Resolution	-	7.73
Retention time	4.258	5.629

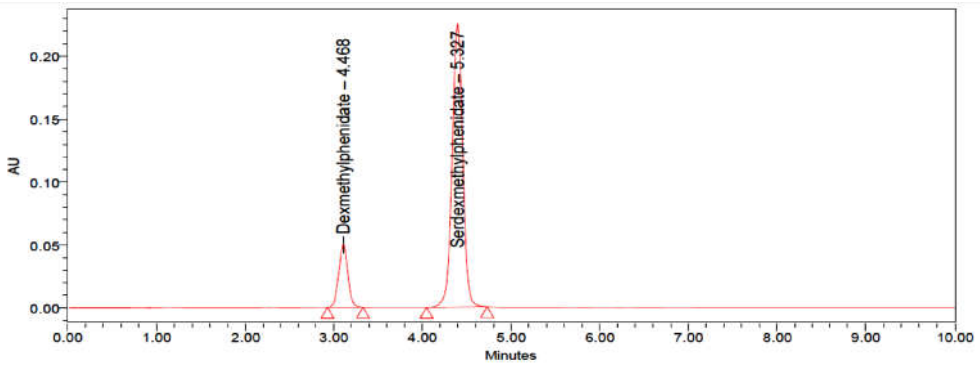

Fig. 3. Chromatogram of standard

Table 3. Linearity study results

Analyte	Linearity Range	Equation of calibration curve	Correlation coefficient
Dexmethylphenidate	0.6-9 µg/mL	Y=372168x+9287	0.9993
Serdexmethylphenidate	2.8-42µg/mL	Y=117068x+42617	0.9992

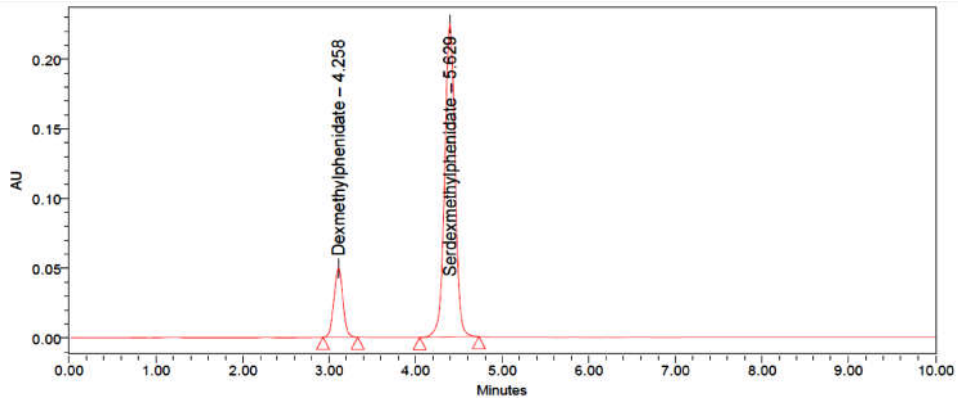

Fig. 4. Chromatogram of sample

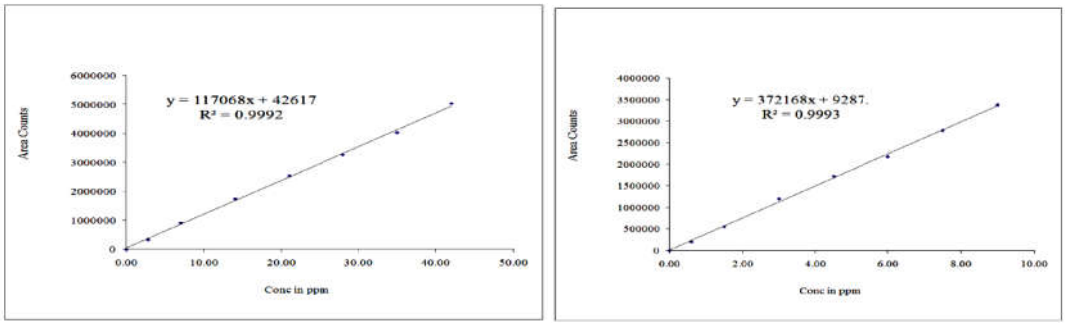

Fig. 5. Linearity plot for dexmethylphenidate and serdexmethylphenidate

3.4 Limit of Detection and Quantification

Limit of detection and quantification is the lowest concentration level at which the analyte can be accurately detected, as determined by standard formulas (3.3 times/s for LOD and 10 times/s for LOQ). Dexmethylphenidate and Serdexmethylphenidate had LOD values of 0.008g/mL and 0.035g/mL, respectively. Dexmethylphenidate and Serdexmethylphenidate had LOQ values of 0.025g/mL and 0.116g/mL, respectively.

3.5 Precision

The precision of the method was examined by analyzing six samples from the same batch that were prepared separately. The peak areas obtained from these six different sample solutions were used to measure mean and percentage RSD values. The current approach was found to be accurate, with a percent RSD of less than 2.0%. Table 4 summarizes the findings.

3.6 Accuracy

Recovery trials were conducted at three different concentration levels to determine accuracy (50 percent, 100 percent, and 150 percent). APIs were made with Dexmethylphenidate concentrations of 3, 6, and 9 g/ml, and Serdexmethylphenidate concentrations of 14, 28, and 42 g/ml. The test solution was injected into three preparations at each spike stage according to the test process, and the assay was performed. The percentage recovery values were discovered to be between 98 and 101 percent. Table 5 summarizes the findings.

Table 4. Method precision results

Analyte	Amount present	% RSD
Dexmethylphenidate	6	0.54
Serdexmethylphenidate	28	0.55

Table 5. Accuracy (recovery) study results

% of target concentration	Dexmethylphenidate (%recovery)	Serdexmethylphenidate (%recovery)
50%	100.3	98.3
100%	100.7	100.1
150%	99.8	100.6
Mean (% Recovery)	100.3	99.7

Table 6. Robustness results

Drug Name	Flow Plus (1.2mL/min)	Flow Minus (0.8mL/min)	Org Plus (55:45)	Org Minus (45:55)
	%RSD			
Dexmethylphenidate	0.65	1.11	0.98	0.75
Serdexmethylphenidate	0.48	1.72	0.64	1.16

Table 7. Forced degradation results

Degradation	Dexmethylphenidate (% of Degradation)	Serdexmethylphenidate (% of Degradation)
Control	0.01	0.02
Acid	13.72	15.37
Alkali	12.98	15.15
Peroxide	15.53	13.33
Thermal	14.3	14.06
Hydrolysis	13.47	11.73

3.7 Ruggedness

The method's robustness was investigated, and it was discovered that chromatographic patterns did not alter significantly by using various HPLC systems, analysts, and columns. The fact that the RSD percentage was less than 2% demonstrates the robustness of the established process.

3.8 Robustness

The robustness of the strategy was found to be 0% and RSD should be less than 2%. Slight variations were done in the optimized method conditions like flow rate (±20%) and Organic content in the mobile phase (±10%). The results are given in Table 6.

3.9 Forced Degradation

As per ICH Q1A, stress degradation studies such as, basic, acidic, peroxide, thermal, and hydrolysis stresses were examined (R2). Table 7 shows the impact of the assay on their performance.

4. CONCLUSION

The analysis of Dexmethylphenidate and Serdexmethylphenidate pharmaceutical formulation and bulk as per ICH guidelines. The developed method was precise, linear, and acceptable. The benefit of the present examination is the use of less costly reagents and the proposed HPLC requirements ensured that adequate resolution and accurate compound quantification. The precision and reproducibility data are satisfactory, according to statistical analysis of the experimental results. The developed chromatographic method can be used in drug testing for routine analysis.

DISCLAMAR

The products used for this research are commonly and predominantly using products in

our area of research and country there is no conflict of interest between the authors and producers of the products because we do not intend to use the products as an avenue for any litigation but the advancement of knowledge.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

ACKNOWLEDGEMENTS

The authors are thankful to the Sir C R Reddy college management, Eluru, AP, for financial assistance and the authors are grateful to the Acharya Nagarjuna University, Guntur, AP, India for providing all the facilities to done this research work successfully.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68948