DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SERDEXMETHYLPHENIDATE AND DEXMETHYLPHENIDATE IN BULK AND THEIR COMBINED DOSAGE FORM

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Abstract

A simple, precise, accurate and cost affective ultra-pressure liquid chromatographic technique was established for concurrent assessment of serdexmethylphenidate as well as dexmethylphenidate in pure drug and formulation. The process has shown acceptable separation of serdexmethylphenidate and dexmethylphenidate. The retention time for serdexmethylphenidate and dexmethylphenidate were 1.476 minutes and 1.806 minutes correspondingly. The drug formulation was exposed to basic, acidic, photolytic, thermal and hydrolysis stress environment. Consequently, stressed samples were analysed by means of projected analytical technique. Quantitation was achieved through ultra violet detection at 245 nm depending on peak area with linear calibration curve. The concentration ranges were 6.525-39.15 µg/ml for serdexmethylphenidate and 1.3-7.8 µg/ml for dexmethylphenidate. The LOD's were 0.17 µg/ml and 0.03 µg/ml for serdexmethylphenidate and dexmethylphenidate correspondingly. The LOQ's were 0.53 µg/ml for serdexmethylphenidate and 0.09 µg/ml for dexmethylphenidate. The projected technique was recognized to be precise and stability representing as there were not any intervening peaks of degradants and excipients. As a result, this intended approach was ideal for use in QC laboratories for quantitative analysis of both individual and mixed dosage forms of pharmaceuticals, as it was simple, quick, and precise.

Keywords: *UPLC, Serdexmethylphenidate, Dexmethylphenidate, Na_2HPO_4*

1. Introduction

Chromatography is a critical biophysical technique for qualitative and quantitative analysis that permits the separation, identification, and refinement of mixture components. HPLC and UPLC are two of the most used chromatographic techniques [1]. When comparing UPLC to HPLC, the smaller column particle size and column dimensions result in a reduction in analysis time and a significant decrease in solvent consumption, lowering the cost. Peaks from UPLC have less noise and a higher signal-to-noise ratio. Sharp and narrow peaks with clearer information can be obtained through UPLC when compared to the peaks obtained through HPLC. As a result, this technology presents a new possibility for high-efficiency corporate profitability [2]. UPLC is a rising chromatographic separation technique. UPLC uses the most up-to-date peak recognition algorithms and specific formulas to optimise data processing and presenting, allowing for the reliable detection of contaminants in components at trace levels. MS and MS/MS data are required to characterise contaminants. The great resolving power of the UPLC system and mouldability of precise mass time-of-flight (TOF) mass spectrometry, enables for quick profiling and detection of contaminants. UPLC improves chemical and instrumentation productivity by providing extra data per unit of effort through enhanced liquid chromatography resolution, speed, and sensitivity**.** UPLC is utilised in QA/QC laboratories for highly regulated, quantitative analyses [3].

The IUPAC name of serdexmethylphenidate is 3-{[(1S)-1-carboxylato-2-hydroxyethyl] carbamoyl}-1-{[(2R)-2-[(1R)-2-methoxy-2-oxo-1-phenylethyl] piperidine-1-carbonyloxy] methyl} pyridin-1-ium [1]. The molecular formula of serdexmethylphenidate is $C_{25}H_{30}ClN_3O_8$ and it has a molecular weight of 535.98 g/mol. Serdexmethylphenidate is a prodrug of the CNS stimulant dexmethylphenidate (MPH), which is commonly used to treat ADHD. MPH's major function is to raise dopamine and norepinephrine levels in the extracellular space, which might have a variety of consequences. MPH's capacity to block the equivalent dopamine and norepinephrine monoamine transporters is mostly responsible for this [4]. The IUPAC name of dexmethylphenidate is methyl (2R)-2-phenyl-2-[(2R)-piperidin-2-yl] acetate. It has a molecular formula of $C_{14}H_{19}NO_2$ and molecular weight of 233.311 g mol−1. Dopamine and norepinephrine reuptake transporters in synapses, particularly in the thalamus and striatum, are inhibited by methylphenidate [5].

A comprehensive review of the literature on serdexmethylphenidate and dexmethylphenidate revealed only a few methods like HPLC [6,7,8,9] for determining these compounds in pharmaceutical formulations and bulk medicines. However, the majority of these analytical approaches appear to be of limited utility, particularly at the industrial level, where simple, economic, and specialised approaches are required. There are no works that have been developed on these drugs by UPLC. The goal of this study was to create and evaluate a stability-indicating RP-UPLC technique for determining serdexmethylphenidate and dexmethylphenidate in bulk and dosage forms.

2. Materials and Methods

Materials

Chemicals and solvents

Serdexmethylphenidate (Figure 1) and dexmethylphenidate (Figure 2) pure bulk drugs (API), serdexmethylphenidate and dexmethylphenidate mixed dosage form tablets (Azstarys***)***, distilled water, acetonitrile, ortho-phosphoric acid, sodium hydrogen phosphate buffer. All the chemicals and reagents listed above were obtained from Rankem.

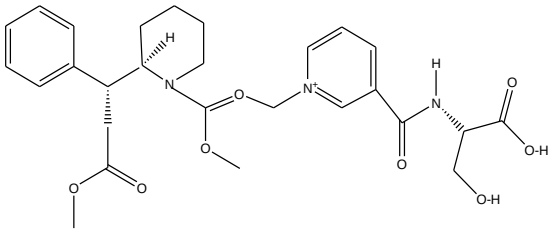

Figure 1. Structure of serdexmethylphenidate

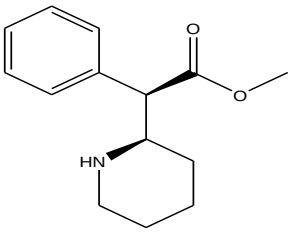

Figure 2. Structure of dexmethylphenidate

Instrumentation and equipment

The chemicals were weighed on electronics balance-Denver. The pH of the buffer was measured by means of pH meter -BVK enterprises. The solutions were sonicated or degassed using ultrasonicator-BVK enterprises. The chromatography analysis was performed using Waters UPLC auto sampler and the signal was detected using tunable ultra violet (TUV) detector with empower 2 software. The absorbances of drugs were measured using UV-VIS spectrophotometer PG instruments T60 with UV win 6 software programs.

Preparation of solutions

Buffer- 0.01N Na_2HPO_4

Precisely weighed 1.42 grams of Na_2HPO_4 was transferred into thousand millilitres volumetric flask. To degas, nine hundred millilitres milli-Q water was added and sonicated. Lastly, dilute orthophosphoric acid solution was used to correct the pH to 3.5 and the volume was made up with water.

Mobile phase

Mobile phase used for elution was acetonitrile: 0.01N Na_2HPO_4 in the proportion of 60:40 v/v.

Diluents

The diluent was chosen based on the drugs' solubility. A 50:50 mixture of acetonitrile and water was used.

Preparation of standard stock solution

Precisely weighed 26.1mg of serdexmethylphenidate and 5.2mg of dexmethylphenidate were taken into a 100ml volumetric flask. 75% of the dilutants were put in the flask. Then, the flask was sonicated for ten minutes. The flask was then filled with dilutants and marked as standard stock solution after sonication.

Preparation of standard working solutions

1 mL of the stock solution was pipetted out into a new volumetric flask with a capacity of 10 ml. The solution was then diluted with dilutant to yield 26.1 µg/ml serdexmethylphenidate and 5.2 µg/ml dexmethylphenidate concentrations.

Preparation of sample stock solutions

The average weight of ten pills were determined. The weight comparable to 1 pill was weighed after the tablets were crushed into powder. The powder was then placed to a hundred millilitres volumetric flask. 5 mL acetonitrile was assimilated and sonicated. Diluents were used to make up to 100ml of volume, which was then filtered using a 0.45 µm porosity membrane filter.

Preparation of sample working solution

1 mL of filtrated sample stock solution was moved into a volumetric flask with a capacity of 10 ml. Then, the solution was prepared with dilutant to produce a concentration of 26.1 µg/ml of serdexmethylphenidate and 5.2 µg/ml of dexmethylphenidate.

Chromatographic conditions

UPLC method development and validation was carried out on an Inertsil ODS 250 (4.6 x 150 mm, 5 µm) column, using a mobile phase of acetonitrile: 0.01N Na_2HPO_4 (60:40 v/v) with a flow rate of 1.0 ml/min. The column temperature was 30°C. The sample injection volume was 10 µl. From the UV spectrum of serdexmethylphenidate and dexmethylphenidate, 245 nm was chosen which displays the isosbestic wavelength. The eluted compounds were monitored at 245 nm.

Method development

Mobile phase was pumped for around 30 minutes to saturate the column and correct the base line. Various mobile phase ratios, buffers, and other parameters were changed to create the method.

Validation parameters

Assay of serdexmethylphenidate and dexmethylphenidate

A total of 20 pills were weighed and ground to powder. Fill a 100 ml volumetric flask with powder corresponding to 26.1 mg serdexmethylphenidate and 5.2 mg dexmethylphenidate. 70 mL diluents were added, sonicated to dissolve, diluted to volume using dilutants, and filtered by means of a 0.45 µm porosity membrane filter.

Linearity

Six distinct concentrations of serdexmethylphenidate and dexmethylphenidate were prepared in the concentration ranges of 6.525-39.15 µg/ml and 1.3-7.8 µg/ml consecutively, to test the method's linearity. Peak area versus concentration was plotted to create the calibration curves.

Accuracy

By spiking known amounts of the drug analyte and calculating percent recovery, the method's accuracy was assessed at 3 dissimilar concentration levels: 50 percent, 100 percent, and 150 percent.

Precision

Method precision (repeatability)- Six working standard solutions were used to determine the method precision. All the injections' peak areas were measured, and the standard deviation SD and % RSD were calculated.

Intermediate precision- Six working standard solutions were injected on separate days by diverse analysts or with dissimilar instruments to ascertain the intermediate precision. The SD and %RSD of all the injections were calculated.

LOD and LOQ

The lower limit of quantification and the limit of detection were derived by means of the subsequent equations based on the slope of the calibration curve and the SD of responses.

LOD = 3.3 × Standard deviation (SD) / slope

LOQ = 10 × Standard deviation (SD) / slope

System suitability parameters

Six duplicates of working standards samples of serdexmethylphenidate and dexmethylphenidate were administered to assess system suitability, and characteristics such as plate number, tailing factor, resolution, and peak asymmetry of solutions were examined.

Robustness

Small adjustments in chromatographic settings such as temperature (25°C, 35°C), flow rate (0.9 ml/minute and 1.1 ml/minute), and mobile phase (65:35, 55:45) were used to test the method's robustness.

Specificity and selectivity

The absence of adjuvant interference during the application of the planned approach to the study of pharmaceutical formulations demonstrated its selectivity. The method's specificity was assessed in terms of interference caused by the occurrence of any additional placebos. Two dissimilar samples were administered and compared to their placebo counterparts.

Forced degradation studies

Oxidation

1 ml of twenty percent hydrogen peroxide (H_2O_2) was mixed separately to one ml of stock solution of serdexmethylphenidate and dexmethylphenidate. The solutions were maintained at 60°C for thirty minutes. The subsequent composition was diluted to get 26.1 µg/ml and 5.2 µg/ml solutions, and 10 µl was administered into the system, with chromatograms collected to evaluate the sample's stability.

Acid degradation

1ml of 2N HCl was mixed to one ml of stock solution serdexmethylphenidate and dexmethylphenidate, and the mixture was refluxed for thirty minutes at 60°C. The resulting composition was diluted to get 26.1 µg/ml and 5.2 µg/ml solutions, respectively, and 10 µl solutions were administered in the system, with chromatograms documented to evaluate sample stability.

Base degradation

1 ml of stock solution of serdexmethylphenidate and dexmethylphenidate was mixed to 1 ml of 2N NaOH and refluxed at 60°C for 30 minutes. The resulting mixture was diluted to get 26.1 µg/ml and 5.2 µg/ml solutions, and 10 µl was administered into the system, with chromatograms recorded to determine sample stability.

Thermal degradation

To evaluate thermal degradation, the standard drug solution was heated in an oven at 105°C for 1 hour. The resulting solution was diluted to 26.1 µg/ml and 5.2 µg/ml solution for the UPLC analysis, and 10 µl was injected into the system, with the chromatograms verified to evaluate the sample's stability.

Photo stability

The drug's photo stability was examined by subjecting the 261 µg/ml and 52 µg/ml solutions to ultra violet luminescence for one day in a UV chamber or 200-Wh/m^2 in a photo stability chamber. The obtained mixture was diluted to get 26.1 µg/ml and 5.2 µg/ml solutions for the UPLC investigation, and 10µl were administered into the system and the chromatograms were documented to analyse the sample's stability.

Hydrolytic degradation

The medication was refluxed in water about for 1 hour at a temperature of 60°C for stress testing under neutral conditions. For the UPLC investigation, the resulting composition was diluted to 26.1 µg/ml and 5.2 µg/ml, and 10 µl was administered into the system, with chromatograms recorded to determine the sample's stability.

3. Results and Discussion

Optimized process

After a series of trials, the mobile phase of acetonitrile: 0.01N Na_2HPO_4 in the proportion of 60:40 had shown both peaks with good theoretical plate count, resolution, tailing factor. Hence this method was optimized and validated. The chief objective of the chromatographic technique was the separation and simultaneous assessment of serdexmethylphenidate and dexmethylphenidate in dosage form. Waters UPLC auto sampler enabled the separation, method development and validation of serdexmethylphenidate and dexmethylphenidate.

Assay

According to the label claim, the drug content obtained from the values of sample solutions was found to be in the permissible range of 90–110 percent. The study confirmed that the created UPLC method was accurate and easy enough to be used on a daily basis. The % content of the serdexmethylphenidate and dexmethylphenidate were found to be 99.87 and 99.87 respectively. The suggested assay method's high content results indicate that this technique can be engaged for quantitative regular quality control study of pharmaceutical dosage forms. The results were displayed in table 1.

Table 1. Results of marketed formulation analysis.

Compound name	Brand name	Label claim	% Content
Serdexmethylphenidate	Azstarys	26.1	99.87
Dexmethylphenidate	Azstarys	5.2	99.87

Linearity

Six linear concentrations of serdexmethylphenidate (6.525-39.15 µg/ml) and dexmethylphenidate (1.3-7.8 µg/ml) were administered in a twofold manner. The linearity equation for serdexmethylphenidate was y = 48688x + 6324 and for dexmethylphenidate was y = 54735x + 2319, and the peak areas were previously indicated. For the two drugs, serdexmethylphenidate and dexmethylphenidate the correlation coefficients were 0.9998 and 0.9999, respectively. The correlation coefficient was more than 0.98, which was within the allowed ranges (NLT 0.99). As a result, the findings revealed that the peak area and analyte concentration showed a strong correlation. The R^2 high value indicated good linearity. The results were shown in tables 2-4 and obtained graphs were shown in fig 3-4.

Table 2. Linearity studies of serdexmethylphenidate

S. No	Concentration (µg/ml)	Peak area
1	0	0
2	6.525	322318
3	13.05	647721
4	19.575	967752
5	26.1	1284728
6	32.625	1576736
7	39.15	1916482

Table 3. Linearity studies of dexmethylphenidate

S. No	Concentration (µg/ml)	Peak area
1	0	0
2	1.3	73660
3	2.6	146111
4	3.9	217996

5	5.2	288418
6	6.5	355603
7	7.8	428727

Table 4. Optical characteristics of serdexmethylphenidate and dexmethylphenidate

Parameters	serdexmethylphenidate	dexmethylphenidate
Linearity (µg/ml)	6.525-39.15 µg/ml	1.3-7.8 µg/ml
Regression equation	y = 48688x + 6324.3	y = 54736x + 2319.2
Slope	48688	54736
Intercept	6324.3	2319.2
Correlation coefficient (R^2)	0.9998	0.9999

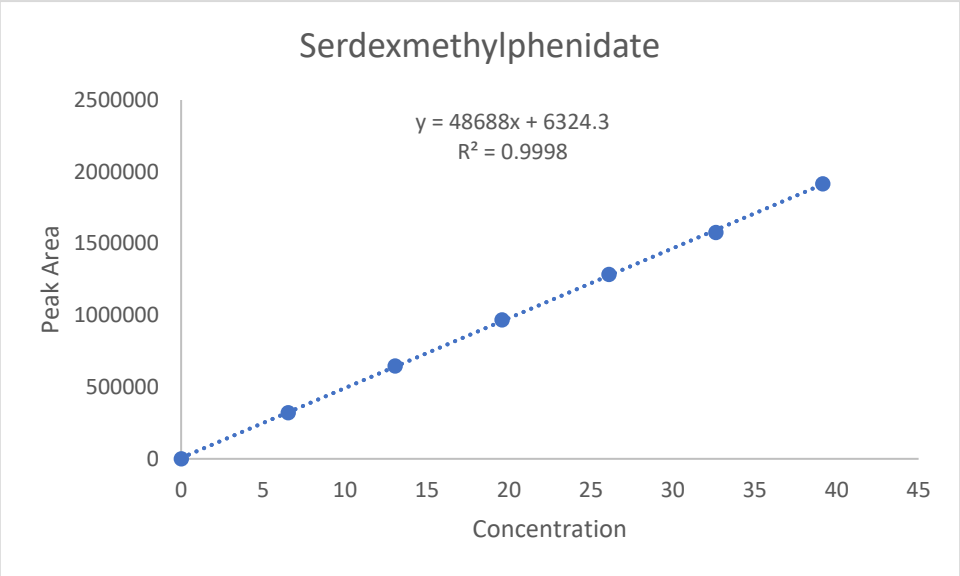

Figure 3. Linearity plot for serdexmethylphenidate

Accuracy

For each level of accuracy, three injections were given, and the mean percent recovery for serdexmethylphenidate and dexmethylphenidate was 99.68 percent and 99.91 percent, respectively. The method's accuracy was demonstrated using standard addition and recovery studies. The recovery was in the range of 98-102%. The results were shown in tables 5-6.

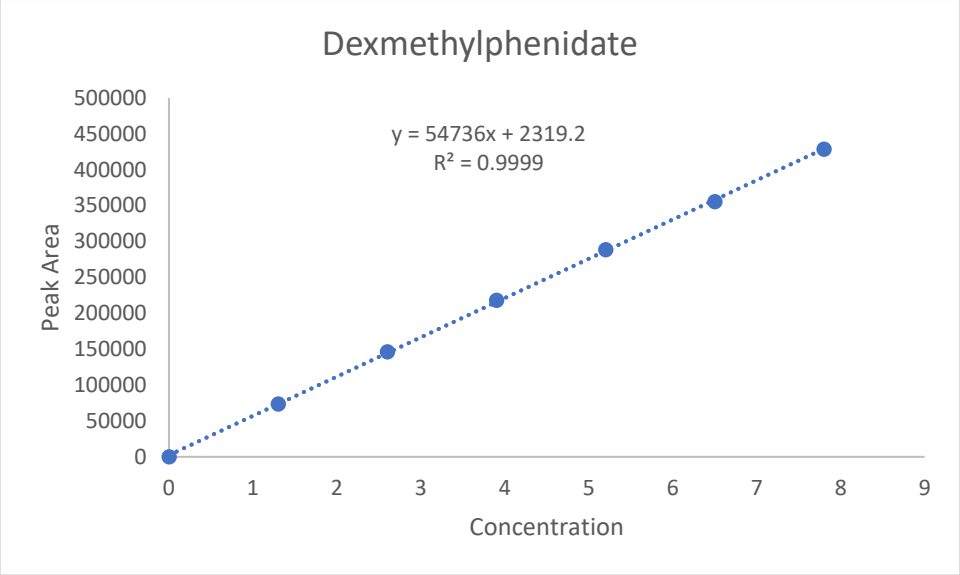

Figure 4. Linearity plot for dexmethylphenidate

Table 5. Recovery studies of serdexmethylphenidate

Recovery level	Accuracy of serdexmethylphenidate			Mean % Recovery
	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	
50%	13.05	12.9	99.0	99.68%
	13.05	13.1	100.3	
	13.05	13.1	100.3	
100%	26.1	26.0	99.6	
	26.1	26.0	99.5	
	26.1	26.0	99.8	
150%	39.15	38.8	99.0	
	39.15	39.2	100.1	
	39.15	39.0	99.6	

Table 6. Recovery studies of dexmethylphenidate.

Recovery level	Accuracy of dexmethylphenidate			Mean % Recovery
	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	
50%	2.6	2.62	100.59	
	2.6	2.61	100.26	
	2.6	2.58	99.27	

100%	5.2	5.12	98.38	99.91%
	5.2	5.18	99.68	
	5.2	5.21	100.14	
150%	7.8	7.78	99.69	
	7.8	7.86	100.72	
	7.8	7.84	100.47	

Precision

Method precision

Six working sample solutions of similar concentrations from a sample stock solution were created using multiple sampling. Each injection was injected from each working sample solution, and the areas acquired were listed in the table below. For two drugs, the average area, SD, and percent RSD were calculated. % RSD yielded 0.7 percent and 0.4 percent for serdexmethylphenidate and dexmethylphenidate, respectively. The closeness of agreement amid a sequence of measurements of the same sample is determined by the precision of a method. The results were well within the usually accepted 2-percentage-point range. As a result, the test method's precision was confirmed. The results were shown in table 7.

Intermediate precision

Six working sample solutions of similar concentrations from a sample stock solution were created using multiple sampling. The areas acquired were listed in the table below of each administration from each working sample solution which were given on the next day of the sample preparation. The average area, SD, and percent RSD for two medications were calculated. % RSD were 0.9 percent and 0.5 percent for serdexmethylphenidate and dexmethylphenidate, respectively. The results were well within the usually accepted 2-percentage-point range. As a result, the test method's precision was confirmed. The results were shown in table 8.

Table 7. Method precision studies of serdexmethylphenidate and dexmethylphenidate

S. No	Area of serdexmethylphenidate	Area of dexmethylphenidate
1.	1266665	286173
2.	1258826	285203
3.	1274906	285064
4.	1269137	285212
5.	1255932	288016
6.	1276569	286003
Mean	1267006	285945
S.D	8343.4	1114.3
%RSD	0.7	0.4

Table 8. Intermediate precision studies of serdexmethylphenidate and dexmethylphenidate

S. No	Area of serdexmethylphenidate	Area of dexmethylphenidate
1.	1277041	283416
2.	1246671	282921
3.	1272039	281591
4.	1261220	284781
5.	1252879	283014
6.	1262029	285608
Mean	1261980	283555
S. D	11364.8	1434.5
%RSD	0.9	0.5

LOD and LOQ

A compound's limit of detection is the lowermost concentration at which it can be detected. The lowermost concentration of a substance that can be measured is known as the limit of quantification. The method's sensitivity was determined by calculating the detection and quantification limits. The residual standard deviation and slope average of the calibration curve were used to compute the LOD and LOQ of serdexmethylphenidate and dexmethylphenidate, which ranged from 6.525 to 39.15 µg/ml and 1.3-7.8 µg/ml. The results were tabulated in table 9.

Table 9. LOD and LOQ of serdexmethylphenidate and dexmethylphenidate

Drug	LOD (µg/ml)	LOQ (µg/ml)
Serdexmethylphenidate	0.17	0.53
Dexmethylphenidate	0.03	0.09

System suitability parameters

System suitability parameters were tested by injecting multiple sampling of same concentrations of serdexmethylphenidate and dexmethylphenidate. Plate count should be greater than two thousand, tailing factor should be < 2, and resolution should be > 2. All of the system's appropriate parameters were accepted, and they were all within acceptable bounds. The results were tabulated in table 10.

Table 10. System suitability parameters for serdexmethylphenidate and dexmethylphenidate

S. No	Serdexmethylphenidate			Dexmethylphenidate		
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing
1	1.479	2855	1.34	1.803	8756	1.46
2	1.483	2912	1.35	1.805	8729	1.47
3	1.483	3029	1.35	1.807	8668	1.43
4	1.484	3073	1.34	1.807	8666	1.43
5	1.485	3060	1.33	1.808	8769	1.47
6	1.487	3195	1.33	1.811	8868	1.46

Robustness

Samples were injected under conditions of flow (-) (0.9ml/min), flow (+) (1.1ml/min), mobile phase (-) (65B:35A), mobile phase (+) (75B:25A), temperature (–) (25°C), and temperature (+) (35°C). The robustness of an analytical process is a measure of its capacity to persist uninfluenced by modest but purposeful changes in method parameters, and it serves as a marker of its dependability in routine use. The tailing factor of serdexmethylphenidate and dexmethylphenidate was less than 2.0 in all of the deliberately changed chromatographic settings. In all of the robustness scenarios, there was a very minimal variance in the resolution and tailing factor values, demonstrating the method's robustness. The results were tabulated in table 11.

Specificity and selectivity

In the retention time ranges, the UPLC chromatograms for the drug matrix (combination of the medicine and placebos) revealed nearly no interference peaks. As a result, the proposed UPLC approach in this study was selective. The method's specificity and selectivity were tested by looking for interference peaks in the chromatograms of blank and placebo samples. Because of the excipients, there were no interfering peaks. As a result, the procedure was specific and selective. Fig. 5 and 6 show the chromatograms of blank and working placebo solution respectively.

Table 11. Robustness studies of serdexmethylphenidate and dexmethylphenidate

Condition	%RSD of Serdexmethylphenidate	%RSD of Dexmethylphenidate
Flow rate (-) 0.9ml/min	0.4	0.6
Flow rate (+) 1.1ml/min	0.2	0.9
Mobile phase (-) 65B:35A	0.7	1.1
Mobile phase (+) 55B:45A	0.4	0.8
Temperature (-) 25°C	0.9	0.4
Temperature (+) 35°C	0.8	0.9

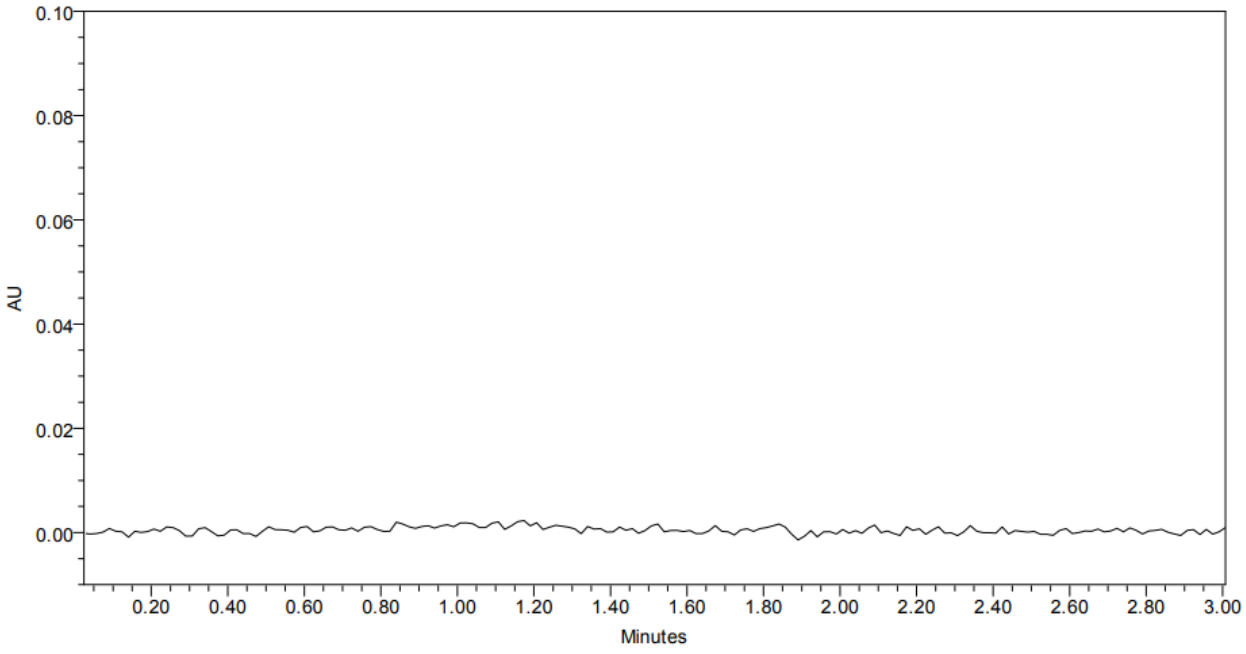

Figure 5. Chromatogram of blank

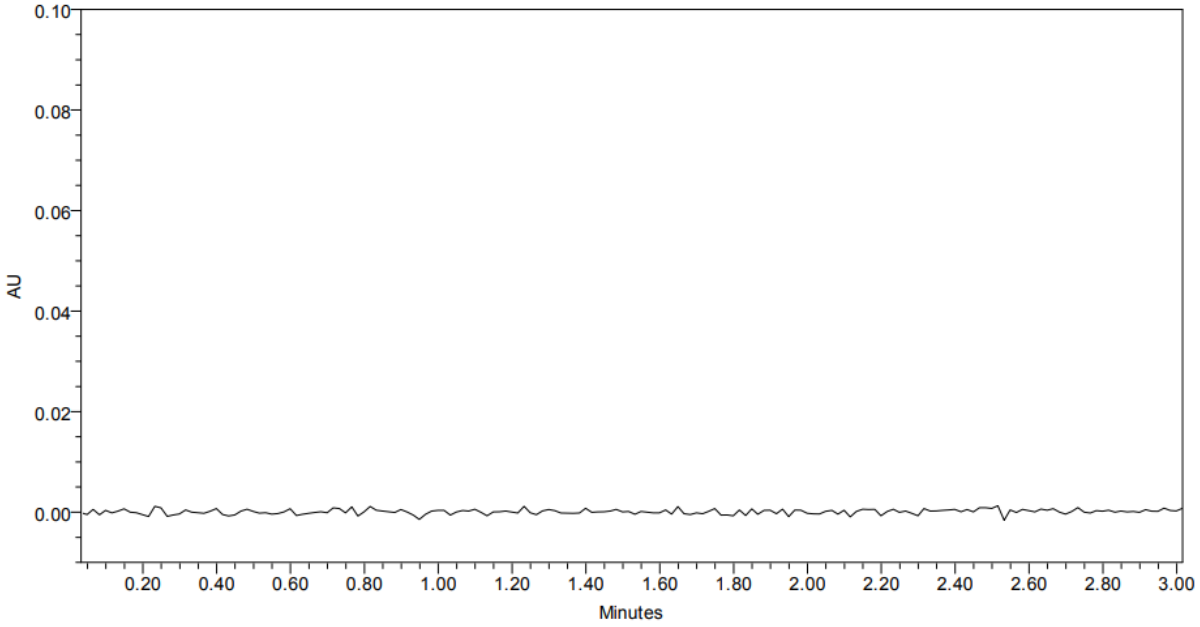

Figure 6. Chromatogram of placebo

Forced degradation studies

Serdexmethylphenidate and dexmethylphenidate were subjected to a variety of stress conditions, which include hydrolysis, base, oxidative, acid, photo stability, and thermal degradation, as per ICH guidelines. The proposed UPLC approach was used to monitor degradation behaviour on a regular basis. The TUV detector results from the forced deterioration results revealed that the serdexmethylphenidate and dexmethylphenidate peaks were pure and homogenous in all of the stressful conditions studied. This demonstrates that the approach is both particular and stable. All the results of stability studies were displayed in table 12.

Table 12. Stability studies of serdexmethylphenidate and dexmethylphenidate

Type of degradation	Serdexmethylphenidate		Dexmethylphenidate	
	%Recovered	% Degraded	%Recovered	% Degraded
Acid	95.55	4.45	95.68	4.32
Base	96.23	3.77	96.93	3.07
Peroxide	96.20	3.80	96.66	3.34
Thermal	97.19	2.81	98.17	1.83
Uv	98.45	1.55	99.02	0.98
Water	99.13	0.87	99.40	0.60

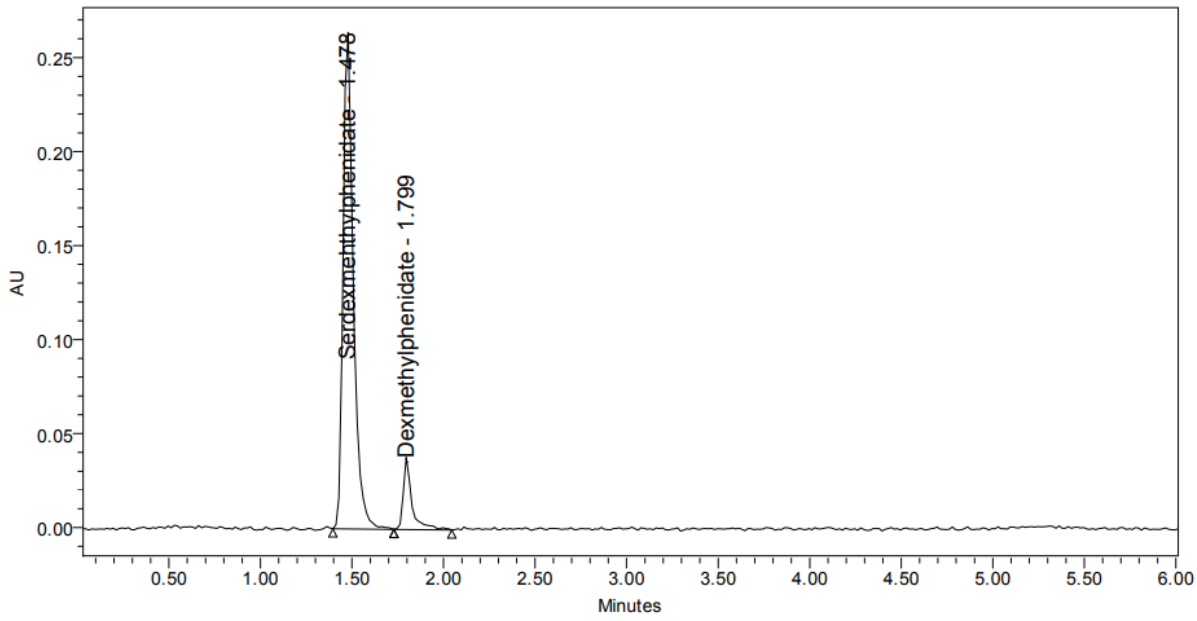

Figure 7. Acid degradation chromatogram

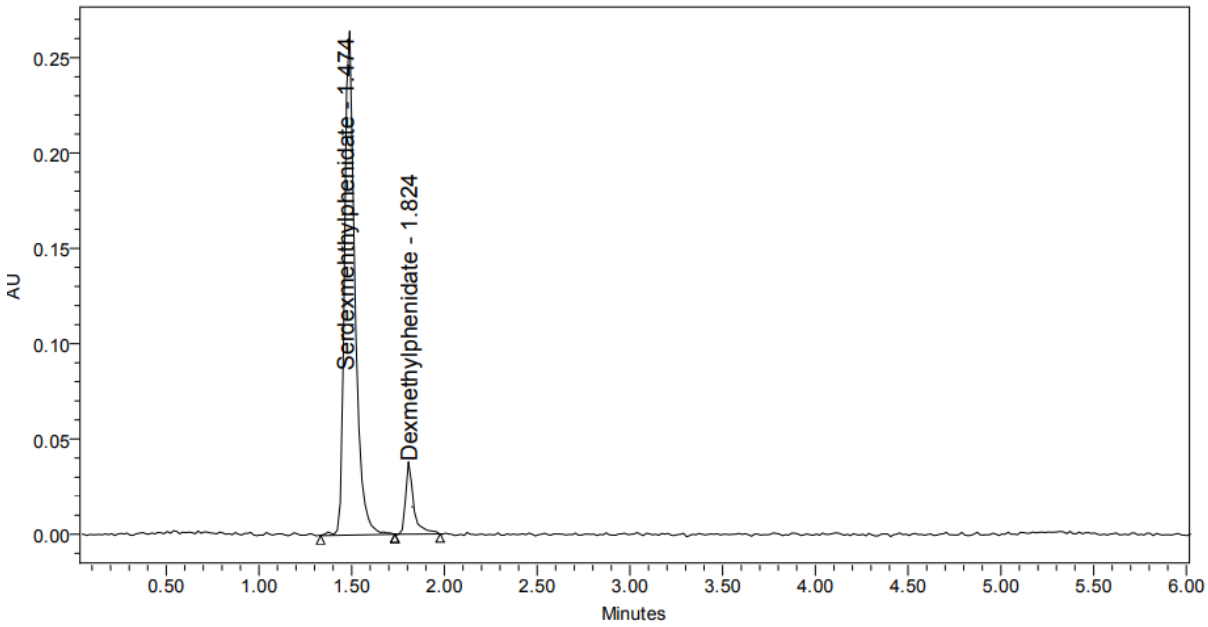

Figure 8. Base degradation chromatogram

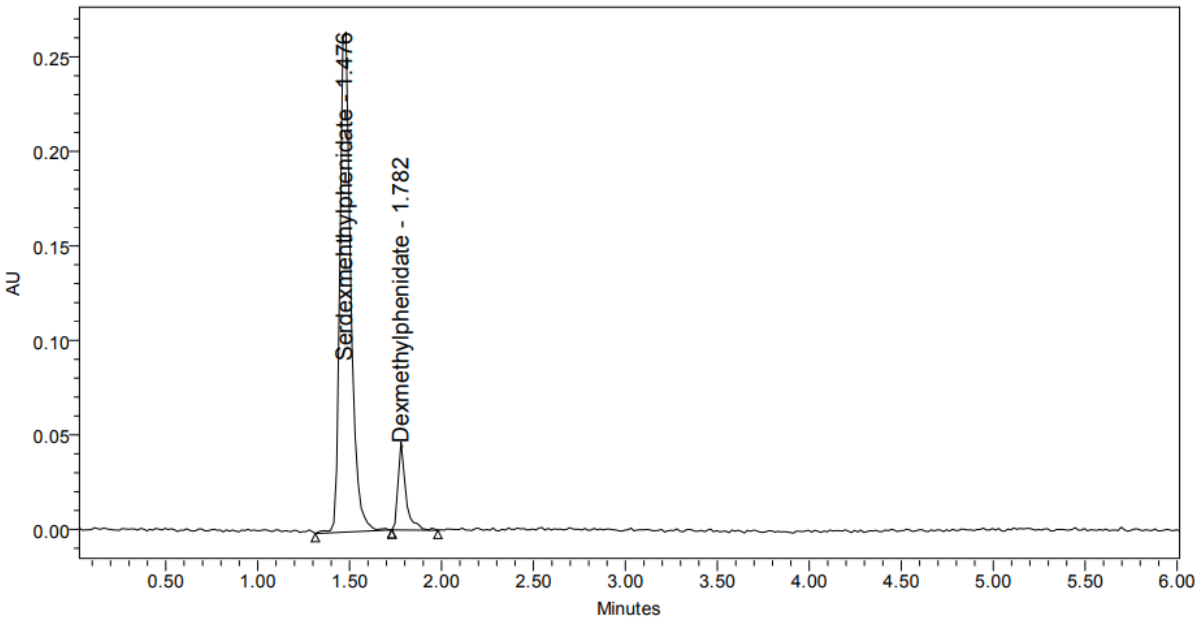

Figure 9. Peroxide degradation chromatogram

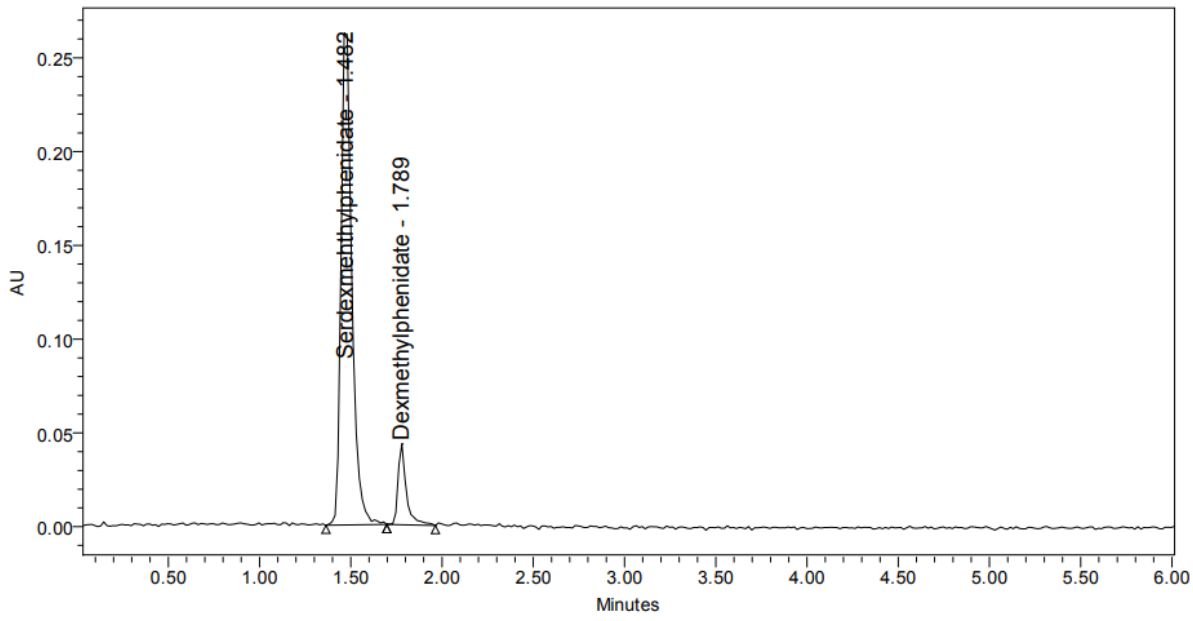

Figure 10. Thermal degradation chromatogram

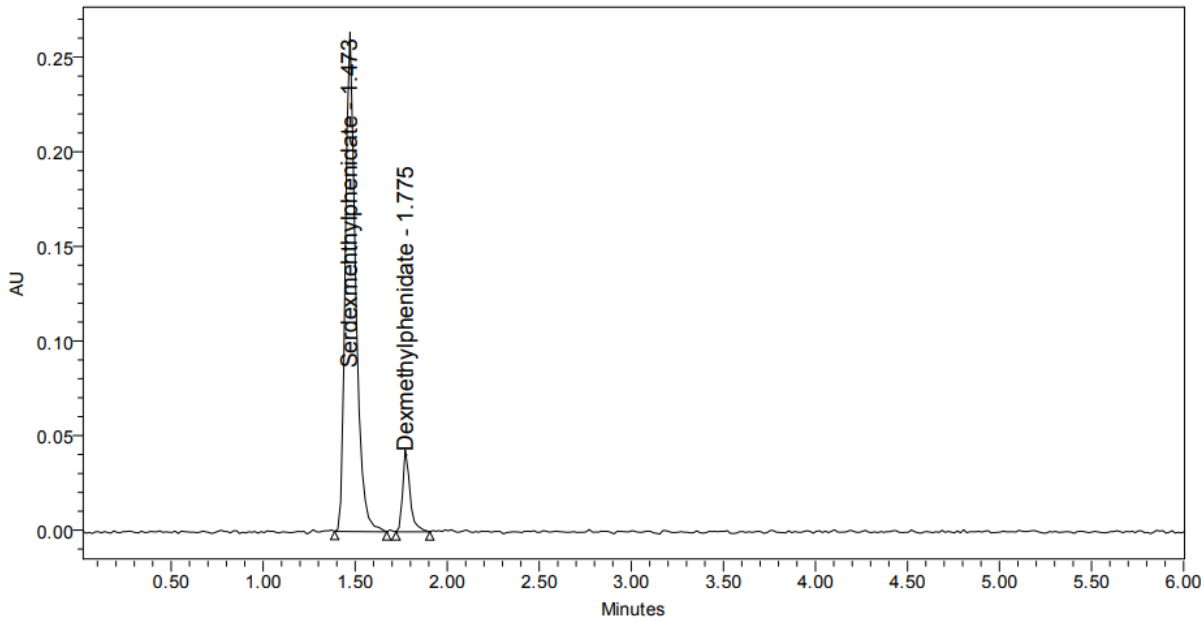

Figure 11. Photo degradation chromatogram

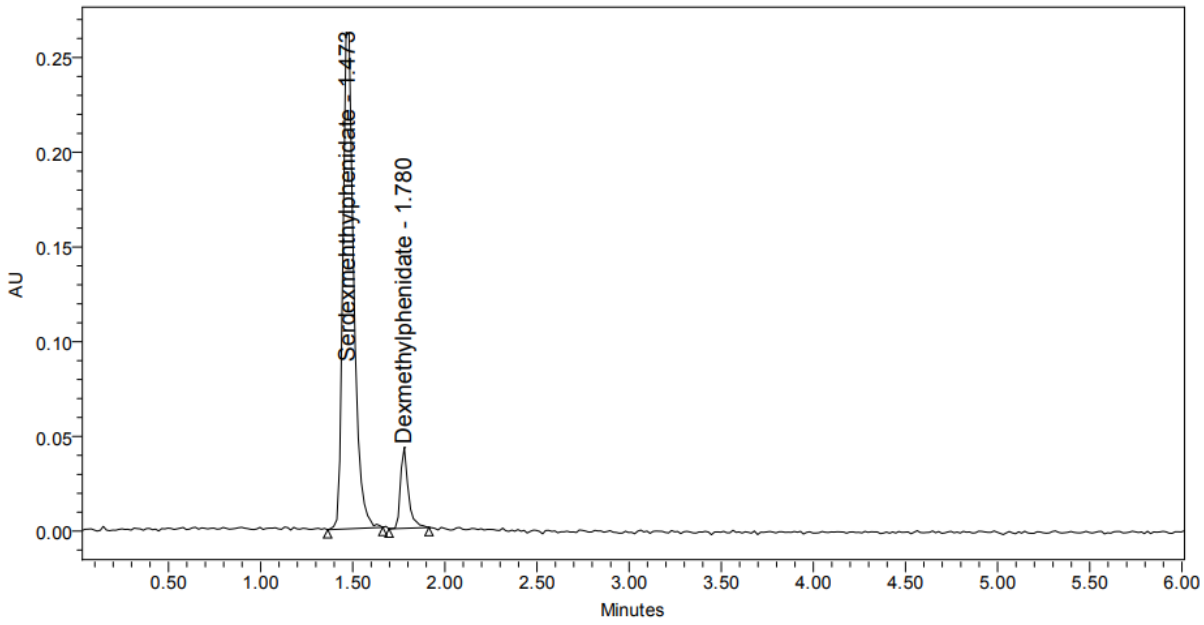

Figure 12. Hydrolytic degradation chromatogram

4. Conclusion

For the simultaneous estimate of serdexmethylphenidate and dexmethylphenidate in tablet dose form, a simple, accurate, and specific approach was established. Serdexmethylphenidate and dexmethylphenidate had retention times of 1.476 and 1.806 minutes, respectively. The percent RSD of serdexmethylphenidate and dexmethylphenidate, respectively, was found to be 0.6 and 0.7. a percentage. For serdexmethylphenidate and dexmethylphenidate, percent recoveries were 99.68 percent and 99.91 percent, respectively. The LOD and LOQ values for

serdexmethylphenidate and dexmethylphenidate calculated from regression equations were 0.17 µg/ml, 0.53 µg/ml, and 0.03 µg/ml, 0.09 µg/ml, consecutively. Regression equation of serdexmethylphenidate is y = 48688x + 6324, and y = 54735x + 2319.of dexmethylphenidate. The peaks were pure in all stressed conditions, according to the results of the forced degradation test. Because retention times and run times were reduced, the method created was simple and economic, and it might be utilized in frequent quality control tests in industries.

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Conflict of interest

The authors declare that there is no conflict of competing financial interests.

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