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RP-UPLC Accelerated Degradation Method Development and Validation for Determination of Dexmethylphenidate and Serdexmethylphenidate in Bulk and Fixed Dosage Form of Tablet

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ABSTRACT

Methylphenidate (MPH) FDA-approved CNS stimulant used to cure Attention-deficit/hyperactivity disorder (ADHD) and narcolepsy. A straightforward, accurate, precise and economical RP-UPLC method was established for the synchronous evaluation of dexmethylphenidate and serdexmethylphenidate in both pure drug and marketed tablets. Acceptable separation of dexmethylphenidate and serdexmethylphenidate has been demonstrated using the method. An Acquity UPLC system, by using Agilent Eclipse Plus C18 (50 x 4.6mm, 2.1µm) column and the mobile phase containing 0.1% TEA pH-2.5/OPA & ACN in the ratio of 50:50% v/v. Both Dexmethylphenidate and Serdexmethylphenidate had retention times of 1.024 and 1.673 minutes, correspondingly. A selection of stress conditions, including basic, acidic, photolytic, thermal, and hydrolytic, were applied to the drug formulation. As a result, stressed samples were examined using the planned analytical method. Quantification was accomplished using a linear calibration curve and ultraviolet detection at 236 nm based on peak area. Dexmethylphenidate's concentration ranged from 7.50 to 45.00 µg/ml, while serdexmethylphenidate's ranged from 35.00 to 210.00 µg/ml. For serdexmethylphenidate and dexmethylphenidate, the LODs were 0.30 µg/ml and 1.40 µg/ml, correspondingly. The LOQs for dexmethylphenidate and serdexmethylphenidate were 0.42 µg/ml and 0.09 µg/ml, accordingly. The absence of any intervening peaks of excipients and degradants indicated that the proposed approach was accurate and stable. This established method was therefore straightforward, rapid, and accurate, making it suitable for use in QC labs for quantitative analysis of pharmaceuticals in both individual and marketed tablet forms.

Keywords: *Dexmethylphenidate, Serdexmethylphenidate, Forced Degradation, RP-UPLC.*

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INTRODUCTION

Methylphenidate (MPH) FDA-approved CNS stimulant used to cure Attention-deficit/hyperactivity disorder (ADHD) and narcolepsy. Because of its ability to reduce the primary symptoms of ADHD in children and its generally good safety record, it is the drug that is most frequently given to treat individuals with ADHD. The advanced treatment (ADHD), a common behavioral disease, is a mix of medication and behavioral therapy. This is a first line of treatment for ADHD. A prodrug of the widely used stimulant drug

dexmethylphenidate (d-MPH), serdexmethylphenidate (SDX), was just approved and is available for sale in the United States [1]. Figure 1 Chemical structure of dexmethylphenidate.

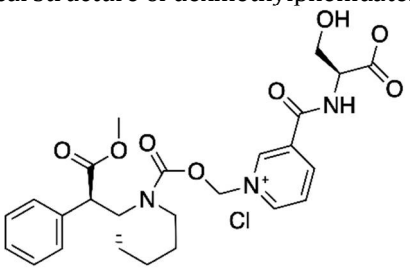

Figure 1 Structure of Serdexmethylphenidate

Dexmethylphenidate (d-MPH), serdexmethylphenidate is a prodrug of the CNS stimulant which is frequently used to treat ADHD. Norepinephrine and Dopamine levels are enhanced in the extracellular space is MPH's primary function, and this could have a number of effects. This is primarily due to MPH's ability to block the corresponding monoamine transporters for dopamine and norepinephrine. The mol. formula for (d-MPH) is $C_{14}H_{19}NO_2$ and (SDX) mol. formula is $C_{25}H_{29}N_3O_8$. Specifically in the thalamus and striatum, methylphenidate inhibits synaptic dopamine and norepinephrine reuptake transporters. Figure 2 Chemical structure of serdexmethylphenidate [2, 3].

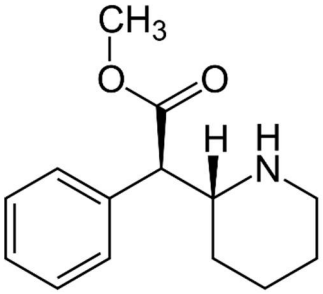

Figure 2 Structure of Dexmethylphenidate

According to a comprehensive review of literature (d-MPH) and (SDX), revealed only a few methods like RP-HPLC & UPLC for evaluating these molecules in marketed tablets and pure forms. However, these analytical approaches appear to be of limited use, particularly at the industries, where specific, affordable and specialised approaches are required. The aim of this current research was to establish and evaluate a stability indicating RP-UPLC methodology for estimating serdexmethylphenidate and dexmethylphenidate in pure form and marketed tablet forms [4-13].

MATERIAL AND METHODS

Instruments

Agilent Eclipse Plus C18 (50 x 4.6mm, 2.1µm) UPLC ACQUITY Waters - Empower software 2.0 versions, UV/VIS spectroscopy UV-1700, Ultra sonicator UCA 701 Unichrome were utilized for the study.

Chemicals

ACN HPLC grade, OPA AR grade, TEA AR grade, Merck Water HPLC, House Production (Milli Q).

Preparation of standard solution

Put 30 mg of Dexmethylphenidate and 140 mg of Serdexmethylphenidate reference standard into a 100 mL VF after precise weighing. Add 70 mL of diluent and blend using sonication to completely solubilize the standard and adjust the volume to the appropriate level. Take 5 ml of each stock solution to a 50 ml VF using a pipette. Fill the flask up to the mark with diluents. (30 ppm of Dexmethylphenidate and 140 ppm of Serdexmethylphenidate)

Sample Solution Preparation

Measure out 31 mg of Dexmethylphenidate and Serdexmethylphenidate sample, then transfer the sample into a 10 mL VF. After sonication for 30 minutes to solubilize the sample solution. After 30 mins centrifugation to thoroughly solubilize the mixture. Finally, increase the liquid up to the mark with same liquid. filter through Injection filter (0.22-micron). Pour 5 ml of each stock solution to a 50 ml VF. Fill the flask up to the mark with diluents. (30 ppm of Dexmethylphenidate, 140 ppm of Serdexmethylphenidate).

Preparation of 0.1% TEA solution

1ml of TEA is dissolved in 1 litre of water (HPLC), adjust its pH-2.5 with OPA. Filter through 0.22µ nylon filter.

Movable Phase Preparation:
Movable phase was treated by mixing 0.1% TEA pH-2.5/OPA and ACN considering the ratio 50:50. Use membrane filter (0.22µ) to filter to remove the impurities which may produce fronting and tailing peaks.

Chromatographic conditions
Development of RP-UPLC method and validation was carried out on an Agilent Eclipse Plus C_{18} (50 x 4.6mm, 2.1µm) column, using a MP of ACN and 0.1% TEA pH-2.5/OPA (50:50) with a 0.5ml/min flow rate. From the UV spectrum of serdexmethylphenidate and dexmethylphenidate, the isosbestic wavelength was chosen 236 nm.

Validation parameters

Linearity
Six distinct concentrations of dexmethylphenidate and serdexmethylphenidate were prepared in the concentration levels of 7.50-45.00 µg/ml and 35.00-210.00 µg/ml consecutively, to test the method's linearity. Peak area versus concentration was plotted to create the calibration curves.

Accuracy
To evaluate the accuracy of the approach, three distinct concentration levels 50%, 100%, and 150% were used to spike required amounts of the drug analyte and compute the % recovery.

Precision
Repeatability- Six times injections were used to determine the method precision. All the chromatogram peak areas were analysed, and the SD and % RSD were analysed.
Ruggedness- Six times injections were introduced on separate days by diverse analysts or with dissimilar equipment's to ascertain the Ruggedness. The SD and %RSD of all the injections were calculated.

LOD and LOQ
The LOD and LOQ were described by means of the regression equations based on the slope of the regression line and the SD of responses. LOD = 3.3 × Standard deviation (SD) / slope LOQ = 10 × Standard deviation (SD) / slope.

System suitability parameters
Six duplicates of standards of dexmethylphenidate and serdexmethylphenidate were delivered to analyze system suitability, and characteristics such as N, TF, distance between peaks and peak asymmetry of solutions were assessed.

Robustness
To verify the robustness of the approach, minor chromatographic setting changes were made, including temperature (25°C, 35°C), flow rate (0.9 ml/min and 1.1 ml/min) and mobile phase (55:45).

Forced degradation studies

Acid degradation:
A 10 ml vacuum flask was filled with 1 ml of the prior prepared solution and 1 ml of 1N HCl. After an hour of being kept at 60°C, the VF was neutralized with 1 N NaOH and mixed with liquid diluent to 10 ml. Transfer the solution to bottles after filtering it via 0.22-micron syringe filters.

Alkali degradation:
A 10 ml VF was filled with 1 ml and previously rendered with 1 ml of 1N NaOH. After exposure to 60ºC for 1 hour, the VF was neutralized with 1N HCl and diluted to a level of 10 ml. Fill the solution in vials after filtering it using 0.22-micron syringe filters.

Thermal degradation
The (d-MPH) and (SDX) standards were placed in a petri dish and heated to 105^0 C for three hours. A small amount of sample was mixed, diluted with diluents, introduced into a UPLC, and analysed.

Peroxide degradation
A 10 ml VF was filled with 1 ml of the stock solution, 1 ml of 3% w/v H_2O_2, add diluent to bring the volume up to the desired level. After that, the VF was kept at 60°C for an hour. The VF was then exposed to room temperature for 15 minutes. Use 0.22-micron syringe filters to filter the mixture, then pour it into container.

Reduction degradation
One ml of the above-stock solution was filled in a 10 ml VF. One ml of 10% sodium bisulphite was then added, and the liquid was increased using diluent to reach the desired amount. After that, the VF was kept at 60°C for an hour. The VF was then exposed to room temperature for 15 minutes. Use 0.22-micron syringe filters to filter the mixture, then pour it into containers.

Photolytic degradation
Samples of (d-MPH) and (SDX) were kept in a photo stability chamber for 3 hours. After being solubilised, a small amount of sample was mixed with diluents, introduced into a UPLC, and analysed.

Hydrolysis degradation
One mL of the above-stock solution was pipetted into a 10 mL VF. 1 mL of water (HPLC-grade) was then added, and the amount was increased with diluent to reach the desired volume. After that, keep the VF at

60°C for one hour. The vacuum flask was allowed to stand at room temperature for 15 minutes. Finally filtering the solution via 0.22-micron syringe filters, pour it into containers.

RESULTS

Optimized process

After taking several trials of series of mobile phase of ACN and 0.1% TEA pH-2.5/OPA (50:50) has exhibited two peaks with good N, resolution, TF. 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 validation and development of serdexmethylphenidate and dexmethylphenidate.

System suitability parameters

System appropriateness was tested by taking multiple sampling of same concentrations of serdexmethylphenidate and dexmethylphenidate. Plate count must be higher than 2000, TF must be < 2, and resolution > 2. All of the system's appropriate parameters were accepted, and they were all within stipulated bounds. The results were tabulated in table 1, Figure 3 Blank Chromatogram & Optimized chromatogram of (d-MPH) and (SDX).

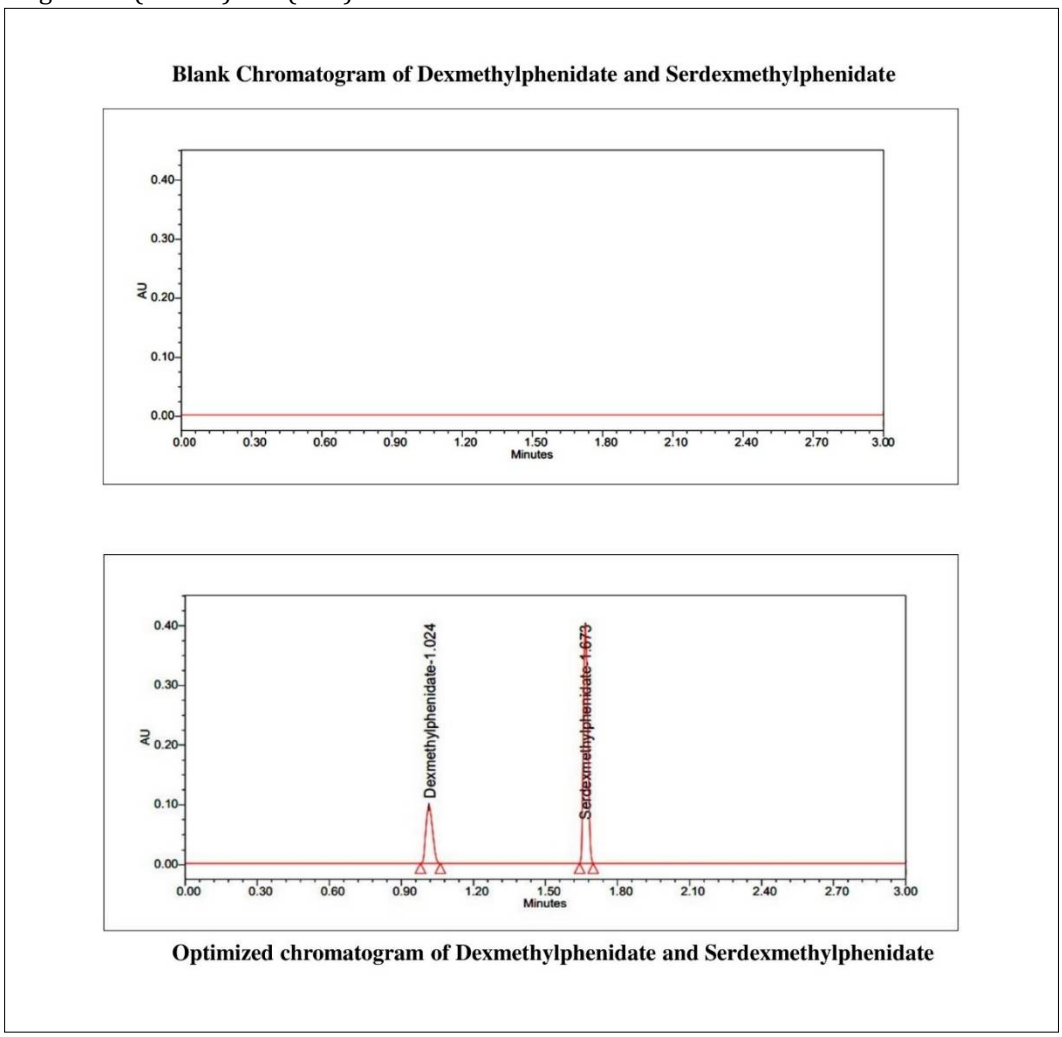

Figure 3 Blank and Optimized Chromatogram

Linearity

Six linear concentrations of serdexmethylphenidate (35.00-210.00 µg/ml) and dexmethylphenidate (7.50-45.00 µg/ml) were estimated in a twofold manner. The linearity method for serdexmethylphenidate was y = 26905.45x + 38928.57 and for dexmethylphenidate was y =28635.39x + 571.43, and the peak areas were previously indicated. For the two drugs, serdexmethylphenidate and dexmethylphenidate the correlation coefficients were 0.99968 and 0.99968, respectively. The values are displayed in table 2 and obtained graphs were shown in fig 4.

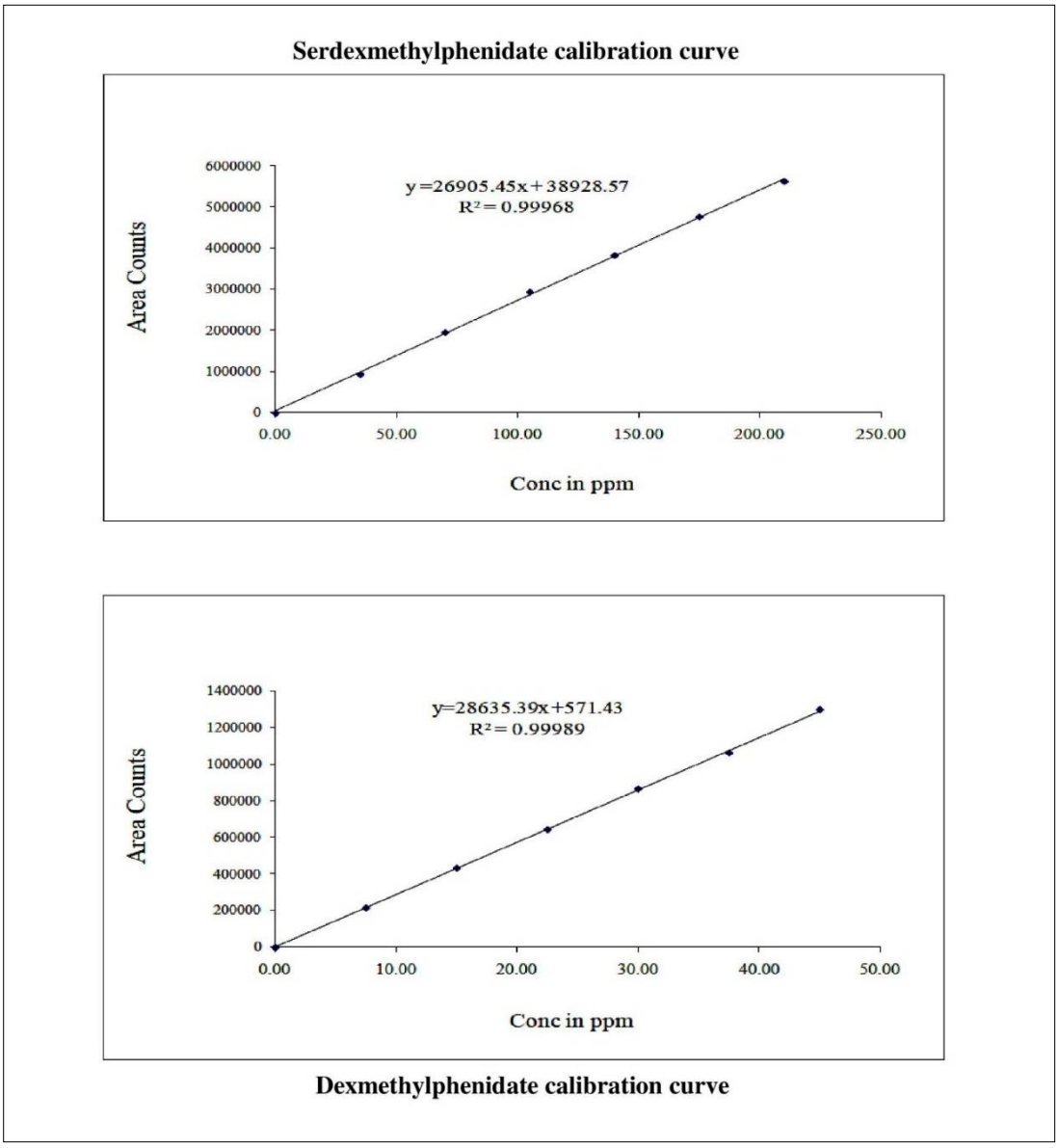

Figure 4 Calibration curve of serdexmethylphenidate and dexmethylphenidate

Accuracy

For each level of accuracy, three injections were given, and the mean recovery % for serdexmethylphenidate and dexmethylphenidate was 99.68 % and 99.91 %, respectively. The accuracy was evaluated using standard addition and recovery %. The recovery was in the range of 98-102%. The values are represented in table 3.

Precision

Method precision

Six sample of similar concentrations from a sample stock solution were created using multiple sampling. Each liquid was injected from each sample, and the areas acquired were listed in the table below. SD, the average area, and % RSD were evaluated for both the drugs. % RSD yielded 0.21 % and 0.17 % for serdexmethylphenidate and dexmethylphenidate, considerably. The values were well within the usually acceptable 2 %-point range. As a result, the test repeatability was examined.

Intermediate precision

Six injections from a sample stock solution were created using multiple sampling. The areas acquired were listed in the table below of each administration from each sample liquid which were tested on the alternate days of the sample preparation. The average area, SD, and % RSD for two medications were calculated. % RSD were 0.9 % and 0.5 % for serdexmethylphenidate and dexmethylphenidate, respectively. The results were well within the usually accepted 2 %. As a result, the test ruggedness was analysed.

LOD and LOQ

A compound's LOD is the lowermost concentration at which it can be detected. The lowermost concentration of a substance that can be quantified is known as the LOQ. The sensitivity was evaluated by analysing the detection and quantification limits. The residual standard deviation and slope average of the regression line 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 acquired results are listed in the Table 4.

Robustness

Samples were injected under conditions of flow (-) (0.45 ml/min), flow (+) (0.55 ml/min), mobile phase (-) (45) and (+) (55), temperature (–) (25°C), and temperature (+) (35°C). The tailing factor of serdexmethylphenidate and dexmethylphenidate was less than 2.0. The results were tabulated in table 5.

Forced degradation studies

Serdexmethylphenidate and dexmethylphenidate were exposed to a series of stress parameters, which include acidic, basic, hydrolytic, oxidative, reduction, photo stability and thermal degradation, as per ICH criteria. The results of stress degradation parameters are represented in table 6. The degradation study peaks are displayed in Figures 5 & 6

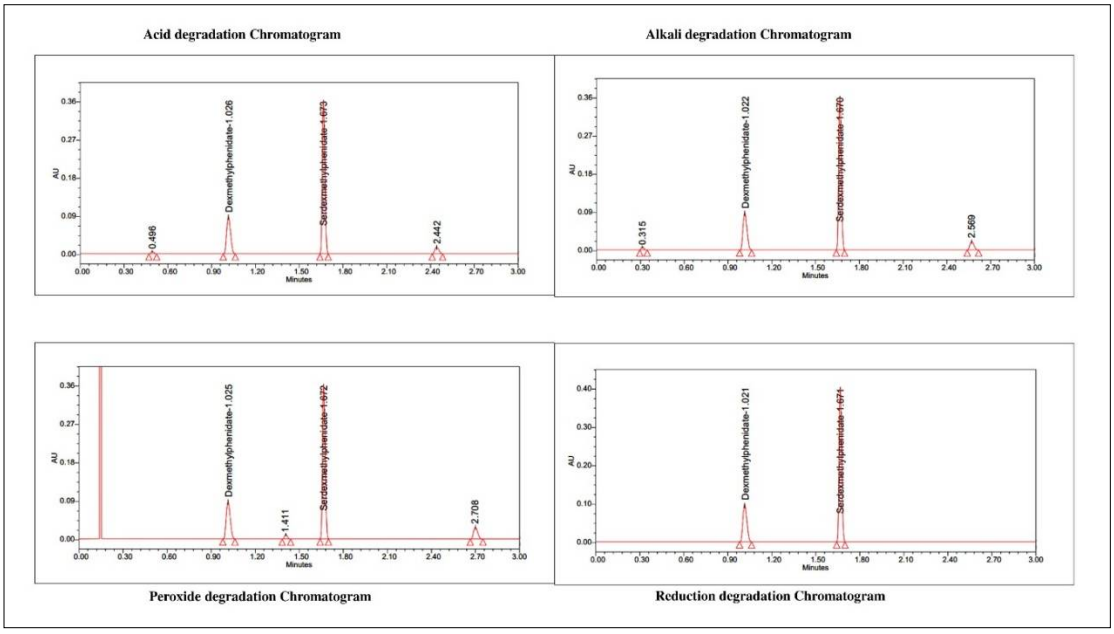

Figure 5 Forced degradation chromatograms of serdexmethylphenidate and dexmethylphenidate

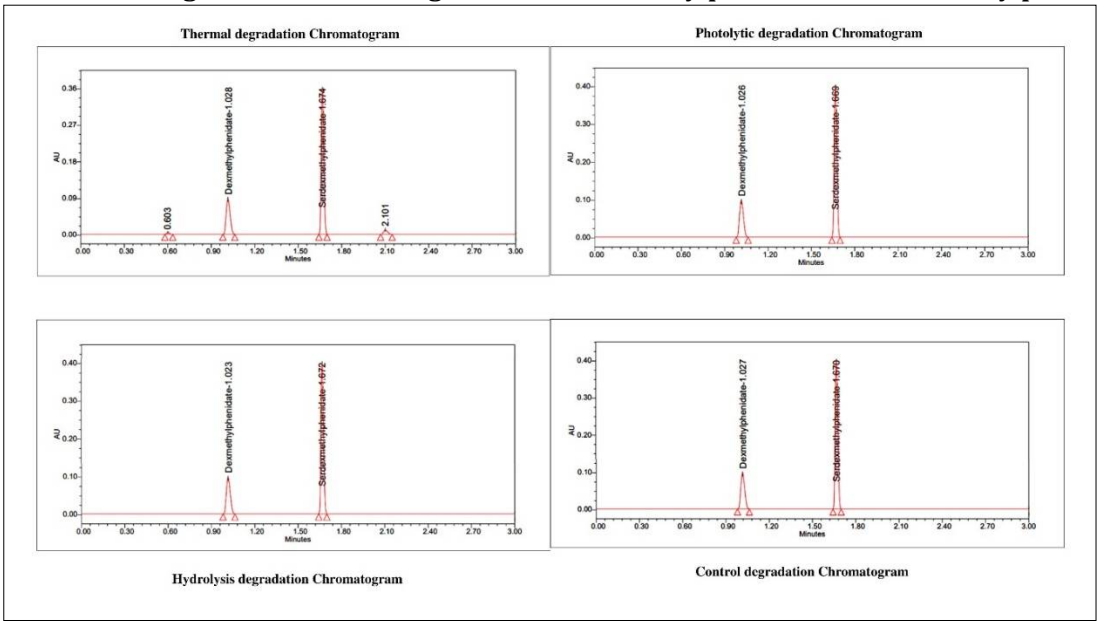

Figure 6 Forced degradation chromatograms of serdexmethylphenidate and dexmethylphenidate

Table: 1 System suitability parameters for Dexmethylphenidate and Serdexmethylphenidate

S.no	Parameter	Dexmethylphenidate	Serdexmethylphenidate
1	Retention time	1.024	1.673
2	Theoretical plates	9654	11735
3	Tailing factor	0.87	1.08
4	Resolution	--	5.78
5	%RSD	0.21	0.17

Table 2. Results of linearity for Dexmethylphenidate and Serdexmethylphenidate

S.NO	Dexmethylphenidate		Serdexmethylphenidate	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	7.50	216194	35.00	939905
2	15.00	432388	70.00	1959810
3	22.50	642582	105.00	2939715
4	30.00	864776	140.00	3829620
5	37.50	1060970	175.00	4759525
6	45.00	1297164	210.00	5619430
Regression equation	y =28635.39x + 571.43		y = 26905.45x + 38928.57	
Slope	28635.39		26905.45	
Intercept	571.43		38928.57	
R^2	0.99989		0.99968	

Table 3. Accuracy results of Dexmethylphenidate

% Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	431410	1.5	1.50	100.0	100.4
	436971	1.5	1.52	101.3	
	430541	1.5	1.50	100.0	
100%	861580	3.0	3.00	100.0	100.1
	860287	3.0	2.99	99.7	
	869251	3.0	3.02	100.7	
150%	1295870	4.5	4.51	100.2	100.0
	1294128	4.5	4.50	100.0	
	1292741	4.5	4.49	99.8	
The Accuracy results for Serdexmethylphenidate					
%Concentration	**Response**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	1926625	7.0	7.02	100.3	100.0
	1913642	7.0	6.97	99.6	
	1924509	7.0	7.01	100.1	
100%	3833250	14.0	13.96	99.7	100.3
	3854831	14.0	14.04	100.3	
	3873564	14.0	14.11	100.8	
150%	5793987	21.0	21.10	100.5	100.4
	5789765	21.0	21.09	100.4	
	5785420	21.0	21.07	100.3	

Table 4. Sensitivity parameters (LOD & LOQ)

Medication Name	LOD(µg/ml)	s/n	LOQ(µg/ml)	s/n
Dexmethylphenidate	0.09	3	0.42	10
Serdexmethylphenidate	0.30	3	1.40	10

Table 5. Robustness results of Dexmethylphenidate

Parameter	Dexmethylphenidate						
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow(0.45ml)	1.178	849461	-	0.91	9523	0.15
	Actual(0.50ml)	1.024	864123	-	0.87	9654	0.21
	More flow(0.55ml)	0.942	885120	-	0.83	9788	0.25
Organic Phase change	Less Org (45:55)	1.351	826315	-	0.89	9412	0.55
	Actual (50:50)	1.021	860740	-	0.82	9686	0.21
	More Org (55:45)	0.818	895123	-	0.77	9837	0.21
Robustness results of Serdexmethylphenidate							
Parameter	**Serdexmethylphenidate**						
	Condition	**RT (min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**	**%RSD**
Flow rate Change (mL/min)	Less flow(0.45ml)	1.824	3665241	5.72	1.12	11613	0.25
	Actual(0.50ml)	1.673	3851620	5.78	1.08	11735	0.17
	More flow(0.55ml)	1.477	3931402	4.96	1.03	11850	0.50
Organic Phase change	Less Org (45:55)	2.013	3545621	5.89	1.14	11568	0.46
	Actual (50:50)	1.676	3847832	5.74	1.09	11760	0.17
	More Org (55:45)	1.332	4056412	4.77	1.05	11979	0.57

Table 6. Forced Degradation results for Dexmethylphenidate and Serdexmethylphenidate

Results: % Degradation results	Dexmethylphenidate					Serdexmethylphenidate				
	Area	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	863021	100	0	6.325	9.959	3841562	100	0	5.128	16.295
Acid	772889	89.5	10.5	6.318	9.947	3416178	88.9	11.1	5.163	16.241
Alkali	765526	88.7	11.3	6.359	9.921	3355291	87.3	12.7	5.154	16.255
Peroxide	747846	86.6	13.4	6.336	9.963	3240325	84.3	15.7	5.102	16.239
Reduction	825567	95.6	4.4	6.344	9.989	3765840	98.0	2.0	5.187	16.203
Thermal	775348	89.8	10.2	6.302	9.914	3431868	89.3	10.7	5.149	16.257
Photolytic	834126	96.6	3.4	6.369	9.932	3801243	98.9	1.1	5.183	16.298
Hydrolysis	839788	97.3	2.7	6.387	9.991	3776982	98.3	1.7	5.106	16.243

DISCUSSION

Serdexmethylphenidate and dexmethylphenidate had Rt of 1.024 and 1.673 minutes, consecutively. The % RSD of, (d-MPH) and (SDX) considerably, was found to be 0.17 and 0.21 %. For serdexmethylphenidate and dexmethylphenidate, % recoveries were 100.2 % and 100.2 %, respectively. The LOD and LOQ values for serdexmethylphenidate and dexmethylphenidate expressed from regression equations were 0.30 µg/ml, 1.40 µg/ml, and 0.09 µg/ml, 0.42 µg/ml, consecutively. Regression equation of serdexmethylphenidate is y = 26905.45x + 38928.57 and y =28635.39x + 571.43 of dexmethylphenidate. Rt of Dexmethylphenidate and Serdexmethylphenidate were 1.024 min and 1.673 min considerably. The ICH criteria were followed throughout the method validation process. The ICH criteria were followed throughout the method validation process. % RSD obtained as 0.21% and 0.17% respectively for Dexmethylphenidate and Serdexmethylphenidate. As the Precision limit was below "2" the system precision was precise in this method. The conventional addition procedure was used to create three levels of Accuracy samples. Dexmethylphenidate had a mean % Recovery of 100.2 % and Serdexmethylphenidate of 100.2 % after three doses were administered for each accuracy level. The drugs were observed to be degraded in the acidic, alkaline, peroxide and thermal conditions in the stress studies [14-20].

CONCLUSION

A precise, rapid, accurate and sensitive approach was established for the synchronous quantification of serdexmethylphenidate and dexmethylphenidate in marketed tablets. The peaks were pure in all degradation parameters, evaluating the results of the stress studies. Hence the Rt and run times were decreased, the approach established was sensitive and affordable, making it suitable for use in QC labs for quantitative assessment of pharmaceuticals in both individual and marketed tablet forms.

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ABBREVIATIONS

ADHD: Attention-deficit/hyperactivity disorder; RP-UPLC: Reverse Phase Ultra Performance Liquid Chromatography; RSD: Relative Standard Deviation; LOD: Limit of Detection; LOQ: Limit of Quantitation; mL: Milliliter; ppm: Parts per million; W: Watts; V: Volts; L/min: Liter/minute; mg: milligram; min: minutes; Hrs: Hours; °C: degree Celsius; µL: microliter; Sec: seconds; mm: millimeter; Std: Standard. VF: Volumetric Flask; (d-MPH): Dexmethylphenidate; (SDX): Serdexmethylphenidate; ICH: International Council for Harmonization;

Ethics Committee Approval:
Not applicable.

Informed Consent:
Not applicable.

Competing interests:
Authors have declared that no competing interests exist.

Financial Disclosure: The authors declared that this study received no financial support.

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