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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DEXMETHYLPHENIDATE AND SERDEXMETHYLPHENIDATE BY RP-HPLC

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantitative analysis of Dexmethylphenidate and Serdexmethylphenidate in pharmaceutical dosage form. Chromatographic separation of Dexmethylphenidate and Serdexmethylphenidate was achieved on Waters Alliancee2695, by using X-Bridge Phenyl, 250x4.6mm, 5µm column and the mobile phase containing acetonitrile and hexane sulphonic acid in the ratio of 60:40% v/v. The flow rate was 1.0 mL/min; detection was carried out by absorption at 236nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Dexmethylphenidate and Serdexmethylphenidate were NLT 2000 and should not more than 2 respectively. % Relatives tandard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, precise, accurate and robust method for quantitative analysis of Dexmethylphenidate and Serdexmethylphenidate and study of its stability.

Keywords: Dexmethylphenidate, Serdexmethylphenidate, RP-HPLC and Validation.

INTRODUCTION

Dexmethylphenidate (Fig. 1) is a norepinephrine-dopamine reuptake inhibitor (NDRI), a psychostimulant and central nervous system stimulant used to treat attention deficit hyperactivity disorder (ADHD) in those over the age of five years1.Chemically it is Methyl (2R)-2-phenyl-2-[(2R)-piperidin-2-yl]acetate.

Dexmethylphenidate inhibits dopamine and norepinephrine reuptake transporters in synapses, especially in the thalamus and striatum. It blocks the reuptake of norepinephrine and dopamine into the presynaptic neuron and increase the release of these monoamines into the extraneuronal space[2].

Serdexmethylphenidate (Fig. 2) is a prodrug of the CNS stimulant Dexmethylphenidate used as a first-line treatment for ADHD in children, adolescents and adults[3]. Chemically it is (2*S*)-3-hydroxy -2-[[1-[[(2*R*)-2-[(1*R*)-2-methoxy-2-oxo-1-phenylethyl]piperidine-1-carbonyl] oxymethyl] pyridin-1- ium-3-carbonyl] amino] propanoate. Serdexmethylphenidate increases extracellular levels of dopamine and norepinephrine in the CNS, leading to altered neurotransmission. Serdexmethylphenidate is combined with Dexmethylphenidate to provide extended plasma concentrations and therapeutic benefit with once-daily dosing[4].

Literature survey reveals that few HPLC methods were reported for simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate in combined dosage form[5-9]. The aim of the present study is to develop a simple, rapid and specific RP-HPLC method for the simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate in bulk and combined

pharmaceutical dosage form in accordance with ICH guidelines[10-11].

MATERIALS AND METHODS

Instrumentation

To develop a high performance liquid chromatographic method for simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate using Waters Alliance e 2695 HPLC system on X-Bridge Phenyl (250 x 4.6 mm I.D., 5 µm particle size) column was used. The instrument is equipped with an auto sampler and PDA detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software. A Eutech pH meter was used for pH measurements.

CHEMICALS AND SOLVENTS

The working standards of Dexmethylphenidate and Serdexmethylphenidate were obtained as gift samples from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. The marketed formulation of Dexmethylphenidate and Serdexmethylphenidate tablets (Azstarys contains 9 mg of Dexmethylphenidate and 42 mg of Serdexmethylphenidate) were procured from local market. Acetonitrile, hexane sulphonic acid, ortho phosphoric acidand HPLC grade water were obtained from Rankem Chemicals Ltd., Mumbai, India.

Chromatographic conditions

Acetonitrile and hexane sulphonic acid in the ratio of 60:40% v/v was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 10 µL and the column was maintained at a temperature of 25^0C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 236 nm. The run time was set as 6 minutes.

Preparation of hexane sulphonic acid (HSA) buffer

2.5 mg of hexane sulphonic acid was weighed accurately, transferred into a 1000 mL volumetric flask and dissolved in 500 mL of HPLC grade water. The solution was sonicated for 30 minutes, degassed and then made to total volume with water. The resulting solution was filtered through 0.45 µm nylon filter.

Preparation of mobile phase and diluent

The mobile phase was prepared by mixing 600 mL of acetonitrile with 400 mL of hexane sulphonic acid. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The mobile phase solution was used as diluent.

Preparation of standard solution

Accurately weighed and transferred 9 mg of Dexmethylphenidate and 42 mg of Serdexmethylphenidate working standards into 100 mL volumetric flasks and was dissolved in 3/4th volume of diluent. Sonicated the solution for few minutes and dissolved the drugs completely and make up to the final volume with diluent. Then it was filtered through 0.45 µm filter. Further pipette 1mL of the above stock solutions into a 10mL volumetric flask and dilute upto the mark with diluent (9 µg/mL of Dexmethylphenidate and 42 µg/mL of Serdexmethylphenidate).

Preparation of sample preparation

Twenty commercial tablets were weighed, powdered and weighed accurately the tablet powder equivalent to 9 mg of Dexmethylphenidate and 42 mg of Serdexmethylphenidatetransferred into 100 mL volumetric flask and was dissolved in 70 mL of the diluent. Sonicated the solution for few minutes and dissolved the drugs completely and make up to the final volume with diluent. Then it was filtered through 0.45 µm filter. Furtherpipette1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent(9 µg/mL of Dexmethylphenidate and 42 µg/mL of Serdexmethylphenidate).

Procedure

The column was maintained at a temperature of 25^0C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 10 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

METHOD VALIDATION

Linearity

Several aliquots of standard solutions of Dexmethylphenidate and Serdexmethylphenidate were taken in six

different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 2.25-13.50 µg/mL for Dexmethylphenidate and 10.50-63.00 µg/mL for Serdexmethylphenidate. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 236 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drug in tablet dosage form.

Precision

Precision for Dexmethylphenidate and Serdexmethylphenidate was determined in terms of system precision, repeatability and intermediate precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy

The accuracy of the method was assessed by recovery studies of Dexmethylphenidate and Serdexmethylphenidate at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Dexmethylphenidate and Serdexmethylphenidate. Each level was repeated three times. The % recovery of Dexmethylphenidate and Serdexmethylphenidate were calculated.

System suitability

The system suitability parameters like retention time, theoretical plate count, tailing factor and resolution were evaluated by six replicate analysis of Dexmethylphenidate and Serdexmethylphenidateand compared with standard values. The acceptance criteria for theoretical plates number (N) at least 3000 per each peak, tailing factors not more than 2.0 and% RSD of peak areas not more than 2% for Dexmethylphenidate and Serdexmethylphenidate.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Dexmethylphenidate and Serdexmethylphenidate using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio.

Robustness

The robustness of the method was determined by making small deliberate changes in method like variation of flow rate, mobile phase ratio and temperature.

Assay

Standard preparations are made from the bulk drug and sample preparations are made from formulation. Both standard and sample solutions were injected in six homogeneous samples. 10 µL of sample solution was injected into the chromatographic system and measure the peak areas of Dexmethylphenidate and Serdexmethylphenidate and calculate the % assay by using the formula. The results were compared with the label claim of Dexmethylphenidate and Serdexmethylphenidate in tablet dosage form.

DEGRADATION STUDIES

Acid degradation

To 1 mLof stock solution of Dexmethylphenidate and Serdexmehylphenidate,1 mLof 2N hydrochloric acid was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 9 µg/mL & 42 µg/mL solution and 10 µL solutions into the system and the chromatograms were recorded to assess the stability of sample

Alkali degradation

To 1 mL of stock solution of Dexmethylphenidate and Serdexmehylphenidate, 1 mLof 2N sodium hydroxide was added and refluxed for 30 min at 60^{0}C. The resultant solution was diluted to obtain 9 µg/mL & 42 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Peroxide degradation

To 1 mLof stock solution of Dexmethylphenidate and Serdexmehylphenidate, 1 mL of 20% hydrogen peroxide was added separately. The solutions were kept for 30 min at 60^{0}C. For HPLC study, there resultant solution was diluted to obtain 9 µg/mL & 42 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation

The standard drug solution was placed in oven at 105°C for 1 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 9 µg/mL & 42 µg/mL

solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photolytic degradation

The photochemical stability of the drug was also studied by exposing the sample solutions to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 9 µg/mL & 42 µg/mL solutions and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Hydrolytic degradation

Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hr at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 9 µg/mL & 42 µg/mL solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop a novel, accurate, precise and reproducible method for simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate in tablet dosage form using X-Bridge Phenyl (250 x 4.6 mm I.D., 5 µm particle size) column in isocratic mode with mobile phase composition of acetonitrile and hexane sulphonic acid in the ratio of 60:40% v/v resulted in peak with maximum separation, good shape and resolution. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time. Total run time was 6 minutes. The drug components were measured with UV detector at 236 nm. The results of optimized chromatographic conditions were shown in Table 1.

Linearity was obtained in the range of 2.25-13.50 µg/mL for Dexmethylphenidate and 10.50-63.00 µg/mL for Serdexmethylphenidate. The correlation coefficient (r^2) was found to be 0.999 for both Dexmethylphenidate and Serdexmethylphenidate respectively. The regression equation of the linearity plot of concentration of Dexmethylphenidate over its peak area was found to be y=443300x+30330, where x is the concentration of Dexmethylphenidate (µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Serdexmethylphenidate over its peak area was found to be y=77745x+56896, where x is the concentration of Serdexmethylphenidate (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results was shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

The % RSD for system precision, repeatability and intermediate precision for Dexmethylphenidate were found to be 0.13%, 0.30% and 0.36% respectively (limit % RSD<2.0%). The % RSD for system precision, repeatability and intermediate precision for Serdexmethylphenidate were found to be 0.54%, 0.91% and 0.62% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Dexmethylphenidate and Serdexmethylphenidate were furnished in Table 4 and Table 5.

The mean % recovery of the drugs Dexmethylphenidate and Serdexmethylphenidate were found to be 99.74% and 99.69% respectively and the high percentage of recovery of Dexmethylphenidate and Serdexmethylphenidate indicates that the proposed method is highly accurate. The results of accuracy studies of Dexmethylphenidate and Serdexmethylphenidate were shown in Table 6 and Table 7.

The retention times for the drugs Dexmethylphenidate and Serdexmethylphenidate was 3.112 minutes and 4.399 minutes respectively. The number of theoretical plates calculated for Dexmethylphenidate and Serdexmethylphenidate was 3634 and 7825 respectively. The tailing factor for Dexmethylphenidate and Serdexmethylphenidate was 1.10 and 1.05 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Dexmethylphenidate were found to be 0.27 µg/mL and 0.81 µg/mL; 1.26 µg/mL and 4.20 µg/mL for Serdexmethylphenidate respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Dexmethylphenidate and Serdexmethylphenidate were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate in commercial tablet

dosage forms. The % Assay of Dexmethylphenidate and Serdexmethylphenidate were found to be 99.88% and 100.02% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Dexmethylphenidate and Serdexmethylphenidate by the proposed HPLC method. The assay results are shown in Table 9.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Dexmethylphenidate and Serdexmethylphenidate standard were shown in Fig. 5. All the degradation products formed during forced degradation studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The results of the degradation studies are presented in Table 10.

CONCLUSION

The proposed HPLC method is simple, rapid, sensitive, precise and accurate for the simultaneous estimation of Dexmethylphenidate and Serdexmethylphenidate and can be reliably adopted for routine quality control analysis of Dexmethylphenidate and Serdexmethylphenidate in bulk and its tablet dosage form.

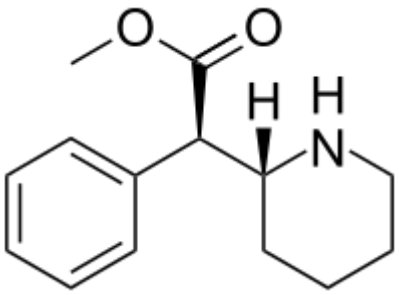

Fig. 1: Chemical structure of Dexmethylphenidate

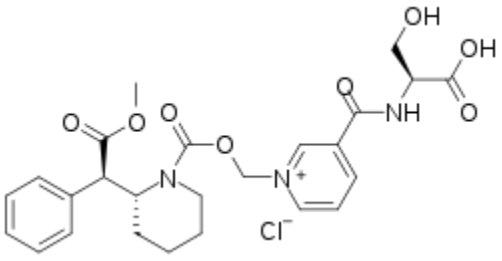

Fig. 2: Chemical structure of Serdexmethylphenidate

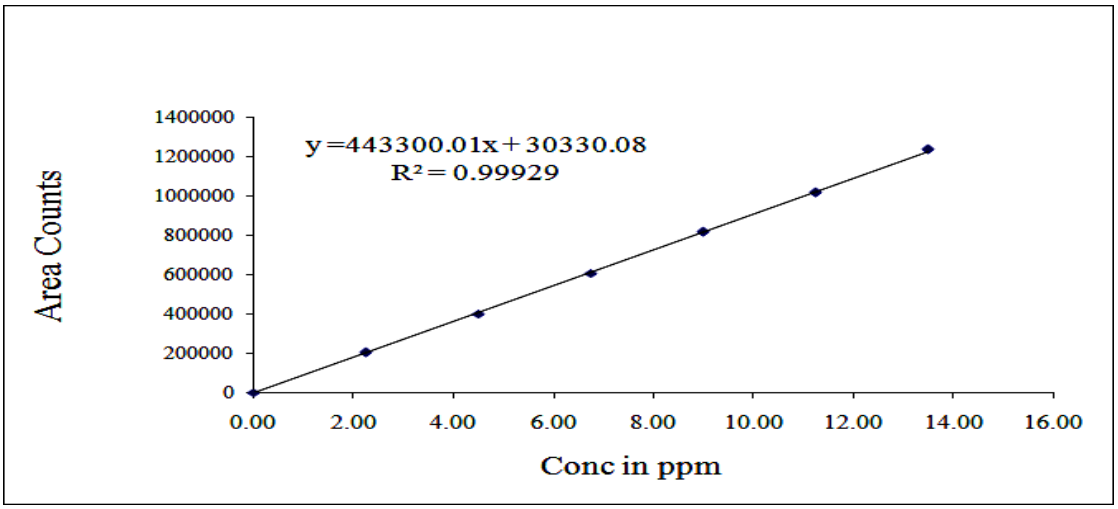

Fig. 3: Calibration curve for Dexmethylphenidate

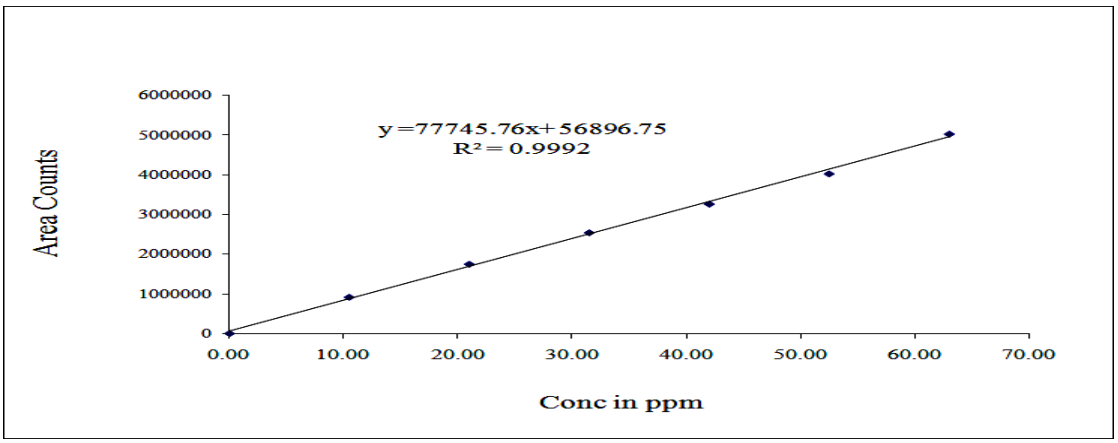

Fig. 4: Calibration curve for Serdexmethylphenidate

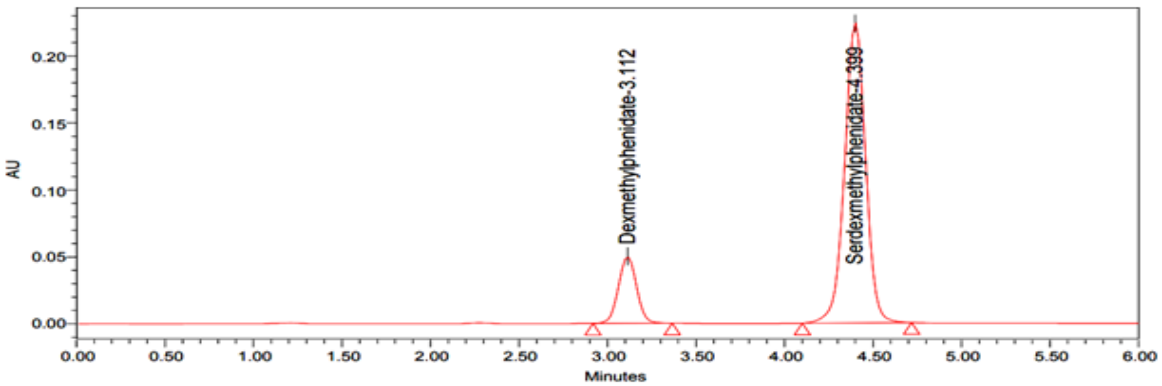

Fig. 5: Chromatogram of Dexmethylphenidate and Serdexmethylphenidate

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase	Acetonitrile:Hexane sulphonic acid (60:40% v/v)
Diluent	Mobile phase
Column	X-Bridge Phenyl (250 mm x 4.6 mm;5µm)
Column temperature	25^0C
Wave length	236 nm
Injection volume	20 µL
Flow rate	1.0 mL/min.
Run time	6 min.

Table 2: Linearity results of Dexmethylphenidate and Serdexmethylphenidate

S. No.	Concentration of Dexmethylphenidate (µg/mL)	Peak area	Concentration of Serdexmethylphenidate (µg/mL)	Peak area
1	2.25	204785	10.50	915181
2	4.50	400156	21.00	1748692
3	6.75	606425	31.50	2544693
4	9.00	814715	42.00	3265524
5	11.25	1014593	52.50	4030598
6	13.50	1235144	63.00	5036529

Table 3: System precision data of Dexmethylphenidate and Serdexmethylphenidate

S. No.	Peak area of Dexmethylphenidate	Peak area of Serdexmethylphenidate
Injection-1	815476	3265914
Injection-2	814896	3274718
Injection-3	812659	3225043
Injection-4	814478	3265698
Injection-5	815685	3256849
Injection-6	814798	3264583
Mean	814665	3258801
SD	1079.65	17483.47
% RSD	0.13	0.54

Table 4: Repeatability data of Dexmethylphenidate and Serdexmethylphenidate

S. No.	Peak area of Dexmethylphenidate	Peak area of Serdexmethylphenidate
Injection-1	816259	3256478
Injection-2	814987	3214562
Injection-3	818502	3232588
Injection-4	812471	3275964
Injection-5	815985	3246124
Injection-6	819374	3296983
Mean	816263	3253783
SD	2480.975	29704.124
% RSD	0.30	0.91

Table 5: Intermediate precision data of Dexmethylphenidate and Serdexmethylphenidate

S. No.	Peak area of Dexmethylphenidate	Peak area of Serdexmethylphenidate
Injection-1	817896	3214154
Injection-2	813063	3225987
Injection-3	819674	3231647
Injection-4	814897	3231496
Injection-5	811876	3267487
Injection-6	815462	3256201
Mean	815478	3237828
SD	2918.821	19980.941
% RSD	0.36	0.62

Table 6: Accuracy results of Dexmethylphenidate

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	% Mean recovery
50 %	4.5	4.46	99.11	99.74%
100%	9.0	9.01	100.11	
150%	13.5	13.5	100.00	

Table 7: Accuracy results of Serdexmethylphenidate

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	% Mean recovery
50 %	21	20.85	99.28	99.69%
100%	42	41.7	99.28	
150%	63	63.33	100.52	

Table 8: System suitability parameters of Dexmethylphenidate and Serdexmethylphenidate

S. No.	Parameters	Dexmethylphenidate	Serdexmethylphenidate
1	Linearity (µg/mL)	2.25-13.50	10.50-63.00
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	3.112	4.399
4	Resolution	--	5.26
5	Tailing factor	1.10	1.05
6	Theoretical plates (N)	3634	7825
7	LOD (µg/mL)	0.27	1.26
8	LOQ (µg/mL)	0.81	4.20

Table 9: Assay results of Dexmethylphenidate and Serdexmethylphenidate

Formulation		Label claim	Amount found	% Assay
AZSTARYS	Dexmethylphenidate	9 mg	8.99 mg	99.88%
	Serdexmethylphenidate	42 mg	42.01 mg	100.02%

Table 10: Degradation results for Dexmethylphenidate and Serdexmethylphenidate

S. No.	Degradation condition	Dexmethylphenidate		Serdexmethylphenidate	
		Peak area	% Degradation	Peak area	% Degradation
1	Acid	697234	14.4	2746875	15.7
2	Alkali	705124	13.4	2834102	13.1
3	Peroxide	680156	16.5	2732257	16.1
4	Thermal	728389	10.6	2889631	11.3
5	Photolytic	804577	1.2	3235247	0.7
6	Hydrolytic	814498	1.6	3245896	0.4

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