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Research Article

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Serdexmethylphenidate and Dexmethylphenidate in Bulk and Formulation

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Abstract

The objective of this study was to develop and validate the stability-indicating method for the simultaneous estimation of the Serdexmethylphenidate and Dexmethylphenidate in capsule dosage form. Chromatogram was gone through Std Discovery C8 (150 x 4.6 mm, 5µ) Mobile phase prepared with Buffer 0.01N KH_2PO_4:Acetonitrile taken in the proportion 60:40 was siphoed through column with flow of 1ml/min. Buffer utilized in this strategy was potassium dihydrogen ortho phosphate buffer. Temperature was kept up with 30°C. Optimized wavelength chose was 260 nm. Retention time of Serdexmethylphenidate and Dexmethylphenidate were viewed as 2.555 min and 3.207 min. %RSD of the Serdexmethylphenidate and Dexmethylphenidate were and found to be 1.3 and 1.3 respectively. Serdexmethylphenidate and Dexmethylphenidate got the 99.93% and 99.93% recovery. From the regression equations the LOD, LOQ values calculated, for Serdexmethylphenidate 0.25 µg/ml, 0.76 µg/ml and Dexmethylphenidate 0.04 µg/ml, 0.11 µg/ml respectively. Regression equation of Serdexmethylphenidate is y = 47119x + 4918, and y = 67584x + 1883 of Dexmethylphenidate. As the Retention time and run time decreased which shows the developed method was simple and economic.

Keywords: RP-HPLC; Serdexmethylphenidate; Dexmethylphenidate

Introduction

Serdexmethylphenidate (SDX) is a prodrug of dexmethylphenidate marketed by KemPharm Company. As a part of the treatment for Attention Deficit Hyperactivity Disorder (ADHD) in children, adolescents, and adults Azstarys was a FDA approved drug in March 2021 [1-3]. As compared to the dexmethylpheidate which is a parent compound, the prodrug SDX has a delayed onset of action and a prolonged duration of effects. IUPAC of Serdexmethylphenidate: (1-((((R)-2-((R)-2-methoxy-2-oxo-1-phenylethyl)piperidine-1-carbonyl)oxy)methyl)pyridin-1-ium-3-carbonyl)-L-serinate chloride.

Dexmethylphenidate is a strong central nervous system stimulant used to treat attention deficit hyperactivity disorder (ADHD) in above five years [4].

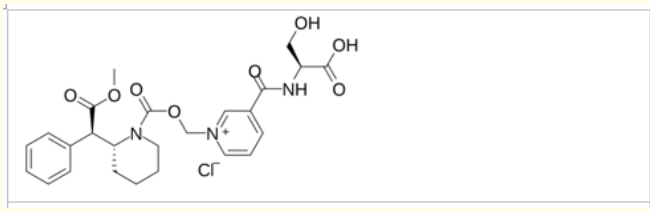

Figure 1: Chemical structure of sedexmethylphenidate.

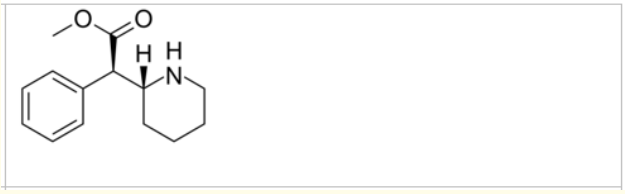

Figure 2: Chemical structure of dexmethylphenidate.

The combined dose of serdexmethylphenidate and dexmethylphenidate is used to treat attention deficit hyperactivity disorder (ADHD). The above combinations are taking as central nervous system stimulants and also habit forming agents. They act by changing the amounts of natural substances in the cerebrum [5-8].

A far reaching survey of the literature on serdexmethylphenidate and dexmethylphenidate revealed only a few techniques like HPLC [9-12] for determining these compounds in pharmaceutical formulations and bulk substances. 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. The developed method was sensitive, stability indicating to the previous works.

Materials and Methods

Materials

Serdexmethylphenidate and Dexmethylphenidate pure drugs Azstarys, Combination Serdexmethylphenidate and Dexmethylphenidate, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid.

Instrumentation and chromatographic condition

A HPLC system of younglin gradient system pump SP930D with UV 730 detect was used working via chemastation 10.1 software. The separation was carried on Agilent column with C18 packaging and 4.6×100 mm dimensions, 2.5 µm particle size. The mobile phase consists acetonitrile: 0.1% OPApH6.2 with 0.1% Triethylamine. In the ratio of 80:20 with flow rate of 1 mL/min. Wavelength selected for the determination serdexmethylphenidate and dexmethylphenidate was 260.0 nm according to observation. Instruments such as Electronics balance denver, pH meter BVK enterptises, waters HPLC system series with Binary pumps, photo diode array detector and manual sampler integrated with empower software. Lab India UV double beam spectrometer with UV win 5 software was used for meaning absorbances of serdexmethylphenidate and dexmethylphenidate solutions.

Methodology

Based up on the solubility of the drugs, diluent was chosen, Acetonitrile and Water taken in the proportion of 50:50.

Preparation of Standard stock solution

Transfer the precisely weighed 26.1mg of Serdexmethylphenidate and5.2mg of Dexmethylphenidate into100ml volumetric flask. Furthermore, 70 ml of diluents was added to these flask and sonicated for 20 minutes. Flask were made up with diluents and named as Standard stock solution (261 µg/ml of Serdexmethylphenidate and 52 µg/ml of Dexmethylphenidate).

Preparation of Standard working solutions (100% solution)

From the above stock solution 1ml was pipetted out and taken into a 10 ml volumetric flask and made up with diluent (26.1 µg/ml Serdexmethylphenidate of and 5.2 µg/ml of Dexmethylphenidate).

Preparation of Sample stock solutions

20 capsules were weighed and equivalent to 1 tablet is transferred to 100 ml volumetric flask. Latter 5 ml of acetonitrile added and sonicated for 20 minutes. After dissolving the remaining volume is make up with diluents and filtered through 0.45 µm or finer porosity membrane filter (theoretical concentration is 261 µg/ml of Serdexmethylphenidate and 52 µg/ml of Dexmethylphenidate).

Preparation of Sample working solutions (100% solution)

1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (theoretical concentration is 26.1 µg/ml of Serdexmethylphenidate and 5.2 µg/ml of Dexmethylphenidate).

Preparation of buffer: (0.01N Potassium dihydrogen ortho phosphate buffer)

Weighed 1.36 gm of Potassium dihydrogen ortho phosphate in a 1000 ml of Volumetric flask add about 900 ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 ml of Triethylamine then PH adjusted to 4.0 with dil. Orthophosphoric acid solution.

Validation of sedexdimethylphenidate and dexmethylphenidate

The RP-HPLC method for sedexmethylphenidate and dexmethylpheniate assay was validated in term of accuracy, linearity, specificity, LOD, LOQ and robustness according to ICH Harmonized Guidelines [13-15].

System specificity

The system suitability parameters were determined by preparing standard solutions of Serdexmethylphenidate (26.1 ppm) and Dexmethylphenidate (5.2 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. Interference of the substances is not absorbed. By this we can say the method was specific. As per ICH guidelines %RSD should not be more than 2%.

Linearity and range

Transfer the precision weighed 26.1mg of Serdexmethylphenidate and 5.2 mg of Dexmethylphenidate and transferred to 100ml volumetric flask. Further 70 ml of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution (261 µg/ml of Serdexmethylphenidate and 52 µg/ml of Dexmethylphenidate).

The area of the linearity peak versus different concentrations has been evaluated for dexmethylphenidate as 25, 50, 75, 100, 125, 150 percent dilutions, respectively. Linearity was performed in the range of 14-84 µg/ml of serdexmethylphenidate and 3-18 µg/ml of dexmethylphenidate. The correction coefficient achieved greater than 0.999 for all.

Accuracy

Preparation of standard stock solutions

Accurately weighed 26.1mg of Serdexmethylphenidate and 5.2 mg of Dexmethylphenidate and transferred to 100 ml volumetric flask. And 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (261 µg/ml of Serdexmethylphenidate and 52 µg/ml of Dexmethylphenidate).

Precision

In this method of precision study prepare six different sample solutions with similar concentrations of Serdexmethylphenidate (56 µg/ml) and Dexmethylphenidate (12 µg/ml) were injected into HPLC system. The % assay results were found to be in the range of 98% to102%.

Limit of detection and limit of quantification

Transfer 0.25 ml each standard stock solutions into two 10 ml volumetric flasks and made up with diluents. Pipette out 0.1 ml each of Serdexmethylphenidate and Dexmethylphenidate solutions from the above and were transferred to 10 ml volumetric flasks and made up with the same diluents.

Robustness

Robustness conditions like variation in Flow (0.9 ml/min to 1.1 ml/min), decrease and increase in mobile phase ratio temperature varies (25°C to 35°C) and samples were injected multiply.

Forced degradation study

Oxidation

Add 1 ml of 20% hydrogen peroxide to the 1 ml of stock solution of Serdexmethylphenidate and Dexmethylphenidate and maintained at 60°C over a period of 30 minutes.

Acid degradation studies

Add 1 ml of 2N Hydrochloric acid to 1 ml of stocks solution Serdexmethylphenidate and Dexmethylphenidate, and by maintain at 60°C reflux for 30 mins.

Alkali degradation studies

To 1 ml of stock solution Serdexmethylphenidate and Dexmethylphenidate, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60°C.

Dry heat degradation studies

For dry heat studies, drug solution was set in oven at 105°C for 1h.

Photo Stability studies

By exposing the 261 µg/ml and 52 µg/ml sample under UV Chamber for 24 hours to perform photo stability studies.

Neutral Degradation Studies:

By refluxing the drug in water over a period of 1 hour at 60°C for neutral degraded studies.

The solutions from the degradation conditions were diluted to 26.1 µg/ml and 5.2 µg/ml solution and 10 µl injected into the HPLC. As per ICH guidelines degraded studies were performed.

Results and Discussion

Method development

Optimized Chromatographic conditions:	
Mobile phase	0.01N KH_2PO_4 60%:40% Acetonitrile
Flow rate	1 ml/min
Column	Discovery C8 (4.6 x 150 mm, 5 µm)
Detector wave length	260 nm
Column temperature	30°C
Injection volume	10 µL
Run time	6 min

Table a

Figure 3: Optimized Chromatogram for Serdexmethylphenidate and Dexmethylphenidate.

Observation

Serdexmethylphenidate and Dexmethylphenidate were eluted at 2.555 min and 3.207 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

As per ICH regulations the SST parameters satisfied with optimized method.

S. No	Serdexmethylphenidate			Dexmethylphenidate			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.535	6881	1.03	3.221	6902	1.11	4.3
2	2.543	6609	1.03	3.223	6810	1.11	4.3
3	2.544	6620	1.03	3.224	6907	1.13	4.7
4	2.548	6826	1.05	3.224	6975	1.10	4.7
5	2.584	6830	1.04	3.233	6947	1.10	4.9
6	2.589	6484	1.05	3.244	6850	1.11	4.8

Table 1: System suitability parameters for Serdexmethylphenidate and Dexmethylphenidate.

Linearity

Serdexmethylphenidate		Dexmethylphenidate	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
6.525	323040	1.3	86994
13.05	631099	2.6	177518
19.575	910762	3.9	273591
26.1	1248563	5.2	352510
32.625	1529687	6.5	443823
39.15	1854322	7.8	523808

Table 2: Linearity table for Serdexmethylphenidate and Dexmethylphenidate.

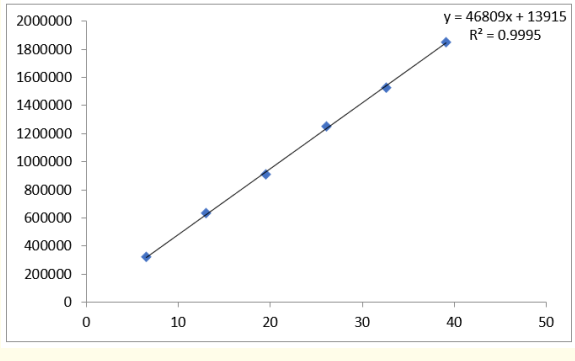

Figure 4: Calibration curve of Serdexmethylphenidate.

Figure 5: Calibration curve of Dexmethylphenidate.

Discussion

Six linear concentrations of Serdexmethylphenidate (6.525-39.15 µg/ml) and Dexmethylphenidate (1.3-7.8 µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Serdexmethylphenidate was y = 47037x + 7454 and of Dexmethylphenidate was y = 67584x + 1883 Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System precision

Six multiple injections were taken from the working standard solution and injected to HPLC. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 1.3% and 1.3% respectively for Serdexmethylphenidate and Dexmethylphenidate.

Intermediate precision (Day Day Precision)

The working sample solutions with same concentrations were prepared and injected onto the HPLC after 24 hours from the preparation of the sample with different analyst. The % RSD for two drugs are 1.0% and 0.7% respectively for Serdexmethylphenidate and Dexmethylphenidate which are within limit as per ICH guidelines.

Accuracy

Triple concentrations and three injections were given at each concentration and mean %Recovery was obtained as 99.93% and 99.88% for Serdexmethylphenidate and Dexmethylphenidate respectively.

Sensitivity

Molecule	LOD	LOQ
Serdexmethylphenidate	0.25	0.76
Dexmethylphenidate	0.04	0.11

Table 3: Sensitivity table of Serdexmethylphenidate and Dexmethylphenidate.

Figure 6: LOD Chromatogram of Standard.

Figure 7: LOQ Chromatogram of Standard.

Robustness

Robustness conditions like flow (0.9 ml/min) and (1.1 ml/min), mobile phase (65B:35A), mobile phase (55B:45A), temperature (25°C) and (35°C) was maintained and sampling done in duplicate manner. As per ICH regulations SST parameters were not deviated and %RSD was within the limit.

Analysis of a marketed formulation

Azstarys, bearing the label claim containing Serdexmethylphenidate 26.1 mg + Dexmethylphenidate 5.2 mg. Assay was performed with the above formulation. Average % Assay for Serdexmethylphenidate and Dexmethylphenidate obtained was 99.60% and 99.61% respectively.

S. No.	Standard Area	Sample area	% Assay
1	1228220	1228503	99.42
2	1243948	1234001	99.86
3	1210969	1217132	98.50
4	1224754	1226232	99.23
5	1244415	1231708	99.68
6	1254448	1247089	100.92
Avg	1234459	1230778	99.60
St. dev	15960.0	9893.0	0.80
%RSD	1.3	0.8	0.8

Table 4: Assay data of Serdexmethylphenidate.

S. No.	Standard Area	Sample area	% Assay
1	359812	347049	98.42
2	352344	352413	99.94
3	346964	350097	99.28
4	349020	352874	100.07
5	353644	352019	99.82
6	351917	353012	100.11
Avg	352284	351244	99.61
St. dev	4418.5	2309.3	0.65
%RSD	1.3	0.7	0.66

Table 5: Assay data of Dexmethylphenidate.

Force degradation of sedexmethylphendate and dexmethylphenidate

Type of degradation	Serdexmethylphenidate		Dexmethylphenidate	
	%Recovered	% Degraded (100- % Recovered)	%Recovered	% Degraded (100-% Recovered)
Acid	94.26	5.74	94.73	5.27
Base	95.16	4.84	94.91	5.09
Peroxide	96.01	3.99	95.54	4.46
Thermal	97.63	2.37	97.25	2.75
UV	98.47	1.53	98.81	1.19
Water	99.39	0.61	99.42	0.58

Table 6: Degradation studies.

Parameters	Serdexmethylphenidate	Dexmethylphenidate	LIMIT
Linearity Range (µg/ml)	6.525-39.15 µg/ml	1.3-7.8 µg/ml	R< 1
Regression coefficient	0.999	0.999	
Slope (m)	47037	67584	
Intercept (c)	7454.3	1883.8	
Regression equation (Y = mx+c)	y = 47037x + 7454.3.	y = 67584x + 1883.8	
Assay (% mean assay)	99.60%	99.61%	90-110%
Specificity	Specific	Specific	No interference of any peak

System precision %RSD		1.3	1.3	NMT 2.0%
Method precision %RSD		0.8	0.7	NMT 2.0%
Accuracy (%recovery)		99.93%	99.88%	98-102%
LOD		0.25	0.04	NMT 3
LOQ		0.76	0.11	NMT 10
Robustness	FM	0.7	1.4	%RSD NMT 2.0
	FP	0.8	0.3	
	MM	0.2	0.7	
	MP	0.7	1.1	
	TM	0.9	1.2	
	TP	0.4	0.4	

Table 7: Validation Summary Table for Serdexmethylphenidate and Dexmethylphenidate.

Conclusion

The work concludes that the developed method was simple, precise, accurate and sensitive for the simultaneous assessment of the Serdexmethylphenidate and Dexmethylphenidatein drug product. Retention time of Serdexmethylphenidate and Dexmethylphenidate were found to be 2.555 and 3.207 minutes. %RSD were found to be 1.3 and 1.3 respectively. Three levels of Accuracy samples (50%, 100% 150%) were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.93% and 99.88% for Serdexmethylphenidate and Dexmethylphenidate respectively. LOD, LOQ values for Serdexmethylphenidate and Dexmethylphenidate were 0.25, 0.76 µg/ml and 0.04, 0.11 µg/ml respectively. Regression equation of Serdexmethylphenidate is y = 47119x + 4918, and y = 67584x + 1883 of Dexmethylphenidate.

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