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Research Article

A validated stability indicating Rp-Hplc method development and validation for simultaneous estimation of serdexmethylphenidate and dexmethylphenidate in pharmaceutical dosage form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Serdexmethylphenidate and Dexmethylphenidate in Tablet dosage form. Chromatogram was run through Std Ascentis C18 (150 x 4.6 mm, 2.4m) Mobile phase containing Buffer 0.01N NAH2PO4: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.9 ml/min. Buffer used in this method was 0.01N NAH2PO4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 228 nm. Retention time of Serdexmethylphenidate and Dexmethylphenidate were found to be 2.133 min and 2.925 min. %RSD of the Serdexmethylphenidate and Dexmethylphenidate were and found to be 0.6 and 0.4 respectively. %Recovery was obtained as 99.98% and 100.50% for Serdexmethylphenidate and Dexmethylphenidate respectively. LOD, LOQ values obtained from regression equations of Serdexmethylphenidate and Dexmethylphenidate were 0.24, 0.73 µg/ml and 0.04, 0.12µg/ml respectively. Regression equation of Serdexmethylphenidate is y = 71326x + 10084, and y = 85514x + 10852.of Dexmethylphenidate. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Serdexmethylphenidate, Dexmethylphenidate, RP-HPLC

INTRODUCTION

Azstarys is a central nervous system Stimulant approved by FDA in March2021 to treat ADHD. It is unique because first combined medication of serdexmethylphenidate and Dexmethylphenidate. Serdexmethylphenidate is a prodrug of the CNS stimulant dexmethylphenidate used as a first-line treatment for Attention Deficit Hyperactivity Disorder (ADHD).Serdexmethylphenidate is a prodrug of dexmethylphenidate that is indicated in combination with dexmethylphenidate for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in patients aged six years and older Serdexmethylphenidate is a prodrug of the CNS stimulant dexmethylphenidate, which increases extracellular levels of dopamine and

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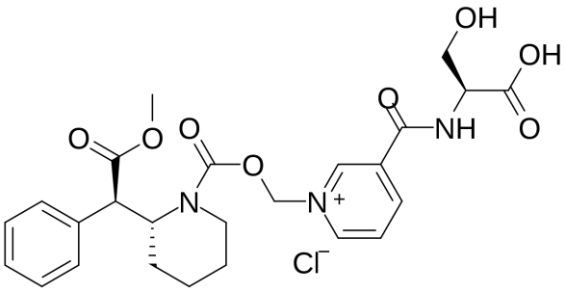
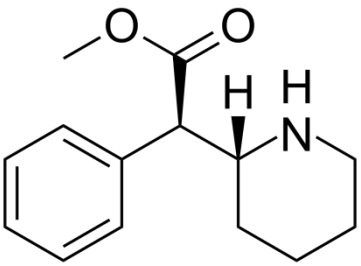
norepinephrine in the CNS, leading to altered neurotransmission.3 As a CNS stimulant, serdexmethylphenidate carries a risk of abuse, misuse, and dependence, which should be monitored. Also, CNS stimulants are associated with increased blood pressure, heart rate, and risk of serious cardiovascular reactions, including stroke, myocardial infarction, and sudden death; patients should be assessed before starting therapy and monitored for cardiovascular abnormalities. Dexmethylphenidate compound belongs to the class of organic compounds known as aralkylamines. These are alkylamines in which the alkyl group is substituted at one carbon atom by an aromatic hydrocarbyl group. Dexmethylphenidate is the d-enantiomer of methylphenidate. This enantiomer is more pharmacologically active than the racemic mixture and may block norepinephrine and dopamine reuptake in synapses. Literature survey revealed that there are some methods reported for the simultaneous estimation of these drugs, some methods for estimation of individual drugs or with other drugs. UV-Spectrophotometry methods [12-16] RP-HPLC[17-22]. On basis of the review of literature, no official method for the stability-indicating simultaneous estimation of serdexmethylphenidate and dexmethylphenidate by RP-HPLC in pharmaceutical dosage form. The main of this study is to develop a simple, accurate relatively sensitive and rapid RP-HPLC technique for Estimation of serdexmethylphenidateand dexmethylphenidate in bulk and pharmaceutical dosage. A validated method also applied for serdexmethylphenidate and dexmethylphenidate estimation as per the ICH guidelines.



Structure of serdexmethylphenidate



Structure of Dexmethylphenidate

MATERIALS AND REAGENTS

Spectrum Pharma research solution, Hyderabad, has provided the serdexmethylphenidate and dexmethylphenidate pure drugs. The combination tablet serdexmethylphenidate and dexmethylphenidate (AZSTARYS) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this Method.

Instrumentation and Chromatographic: HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector. Discovery (C18 150 mm x 4.6mm, 5µm) column was used for the separation. 0.01N Sodium hydrogen phosphate is used as mobile phase A and Acetonitrile is used as mobile phase B (60:40 Ratio). Analysis was carried out in isocratic mode with flow rate of 1.0 mL/min and injection volume was 10 µL. The column temperature was 30°C; the run time was 6 min. The data was acquired at 215 nm. The output signal was monitored and integrated using Empower 2 software.

Preparation of solutions:
Buffer: 0.01N Sodium hydrogen phosphateAccurately weighed 1.42gm of Sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.5 with dil. Orthophosphoric acid solution.

Preparation of Standard stock solutions:
Accurately weighed 26.1mg of Serdexmethylphenidate and 5.2mg of Dexmethylphenidate and transferred to 50ml volumetric flask. And ¾ th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (522µg/ml of Serdexmethylphenidate and 104µg/ml of Dexmethylphenidate)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (52.2µg/ml Serdexmethylphenidate of and 10.4µg/ml of Dexmethylphenidate)

Preparation of Sample stock solutions:
5 tablets were weighed and equivalent to 1 tablet is weighed and transferred to 50 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 50ml with diluents and filtered through 0.45 µm or finer

porosity membrane filter (522µg/ml of Serdexmethylphenidate and 104µg/ml of Dexmethylphenidate).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (52.2µg/ml of Serdexmethylphenidate and 10.4µg/ml of Dexmethylphenidate)

METHOD VALIDATION

The validation of HPLC method was carried out for the simultaneous estimation of serdexmethylphenidate and dexmethylphenidate drug substance as per the ICH guidelines to demonstrate that the method is proposed for the routine analysis.

System suitability: The system suitability was performed for each validation parameters by injecting standard solution containing serdexmethylphenidate 52.2µg/ml, dexmethylphenidate10.4µg/ml. System suitability chromatogram was shown in figure 2 and values are mentioned in the table 1.

Specificity (Selectivity): Checking of the interference in the optimized method. We didn't found interfering peaks in blank and placebo at retention times of these drugs in this method. So this **method** was said to be specific.

Table1:Systemsuitability parameters for Serdexmethylphenidate and Dexmethylphenidate

s.no	Serdexmethylphenidate			Dexmethylphenidate			
Inj	RT(min)	USP plate Count	Tailing	RT (min)	USP plate count	Tailing	Resolution
1	2.123	7229	1.23	2.897	8487	1.37	6.5
2	2.128	7337	1.27	2.915	8475	1.31	6.6
3	2.131	7279	1.29	2.915	8459	1.29	6.7
4	2.132	7261	1.24	2.917	8469	1.3	6.4
5	2.133	7221	1.22	2.918	8490	1.3	6.7
6	2.136	7283	1.26	2.923	8439	1.31	6.7

Table 2: Specificity data

Sample name	Retention time (min)	Area
Serdexmethylphenidate	2.133	3693282
Dexmethylphenidate	2.925	1025164

Specificity:

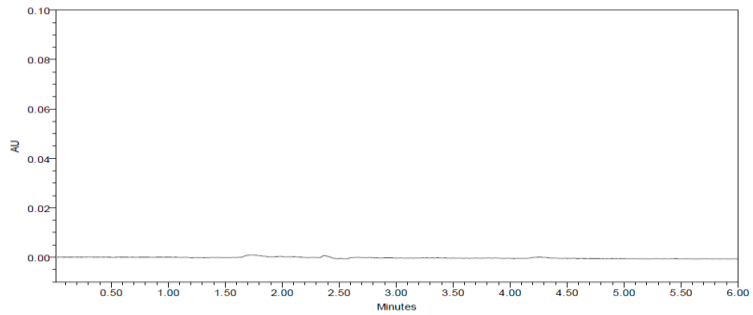


Figure2: Chromatogram of blank.

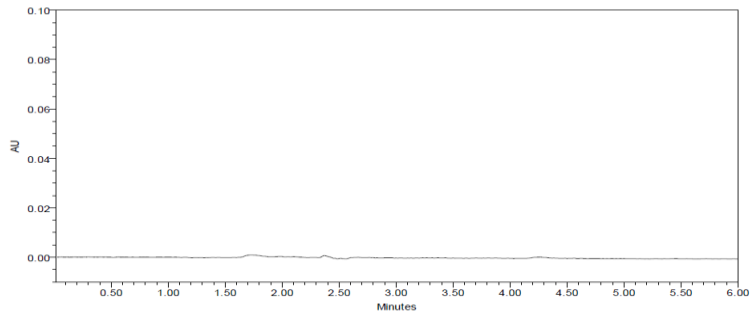


Figure 3: Specificity Chromatograms of serdexmethylphenidate and dexmethylphenidate

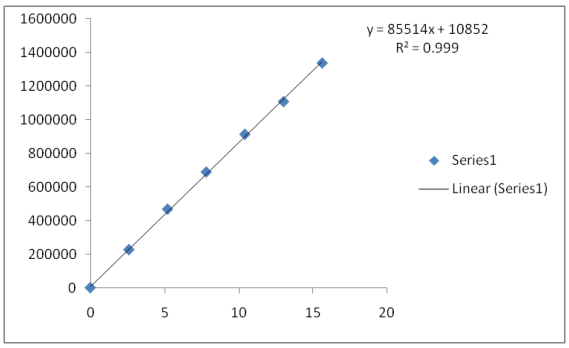


Figure 4: Calibration curve of dexmethylphenidate

Table2: Linearity of Serdexmethylphenidate and Dexmethylphenidate

Serdexmethylphenidate		**Dexmethylphenidate**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
13.5	979296	2.6	226777
26.1	1846157	5.2	467949
39.15	2837845	7.8	689164
52.2	3759166	10.4	914045
62.25	4428700	13	1108430
78.3	5584497	15.6	1338681

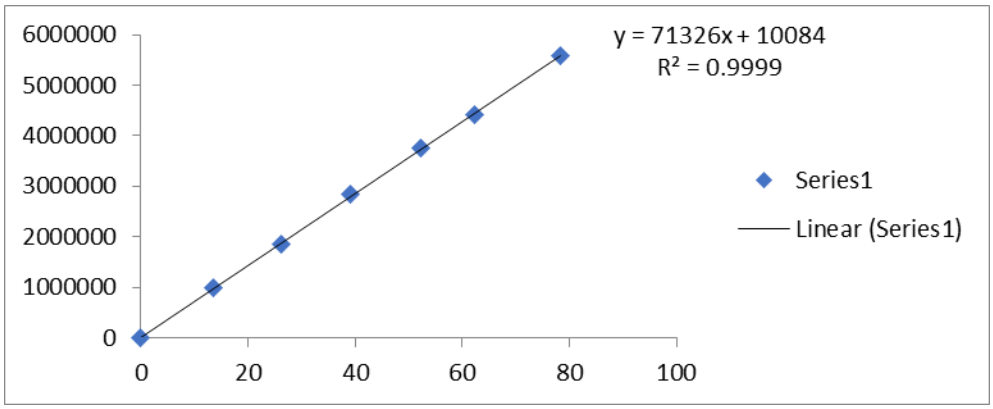


Figure 5:Calibration curve of serdexmethylphenidate

Table 3: Accuracy table of Serdexmethylphenidate

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	26.1	26.2	100.3	99.98%
	26.1	25.9	99.2	
	26.1	26.2	100.3	
100%	52.2	52.2	100.0	
	52.2	52.6	100.7	
	52.2	52.0	99.7	
150%	78.3	77.9	99.5	
	78.3	78.4	100.1	
	78.3	78.3	100.0	

Table4:Accuracy table of dexmethylphenidate

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5.2	5.25	100.95	100.50%
	5.2	5.21	100.14	
	5.2	5.20	99.91	
100%	10.4	10.47	100.69	
	10.4	10.50	100.94	
	10.4	10.49	100.88	
150%	15.6	15.67	100.44	
	15.6	15.65	100.32	
	15.6	15.64	100.28	

Table 5:System Precision

S. No	Area of Serdexmethylphenidate	Area of Dexmethylphenidate
1.	3734505	913065
2.	3777698	911696
3.	3759038	912305
4.	3793036	910446
5.	3787298	915329
6.	3779822	919818
Mean	3771900	913777
S.D	21651.9	3376.5
%RSD	0.6	0.4

Table 6:Method Precision

S no	Serdexmethylphenidate	Dexmethylphenidate
1	3749825	918374
2	3766859	911160
3	3779822	915329
4	3771990	915555
5	3760469	9198146
6	3724718	913176
Avg	**3758947**	**915290**
Std dev	**19624**	**2802.3**
%RSD	**0.5**	**0.3**

From the above results, the % RSD of method precision study was within the limit for serdexmethylphenidate and dexmethylphenidate is (<2%).

Table 7: Robustness

S.no	Condition	%RSD of Serdexmethylphenidate	%RSD of Dexmethylphenidate
1	Flow rate (-) 0.8ml/min	0.3	0.3
2	Flow rate (+) 1.0ml/min	0.3	0.8
3	Mobile phase (-) 55B:45A	0.3	0.8
4	Mobile phase (+) 65B:35A	0.9	0.4
5	Temperature (-) 25°C	0.5	0.3
6	Temperature (+) 35°C	0.2	0.7

Table 8: Forced degradation for serdexmethylphenidate and dexmethylphenidate

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table 9: Degradation results of serdexmethylphenidate and dexmethylphenidate

Degradation condition	Serdexmethylphenidate % Degraded	dexmethylphenidate % Degraded
Acid	6.22	6.44
Base	4.77	4.99
Oxidation	4.72	4.98
Thermal	2.14	2.65
Photolytic	1.98	1.61
Hydrolytic	0.42	0.82

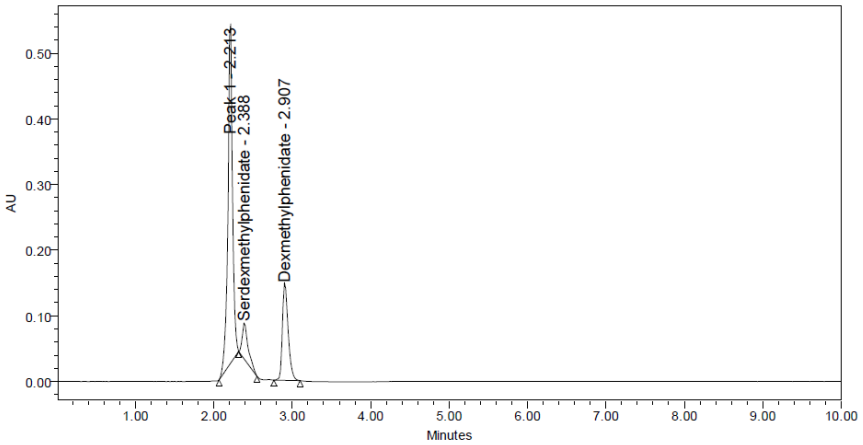


Figure 6:Acid degradation Chromatogram

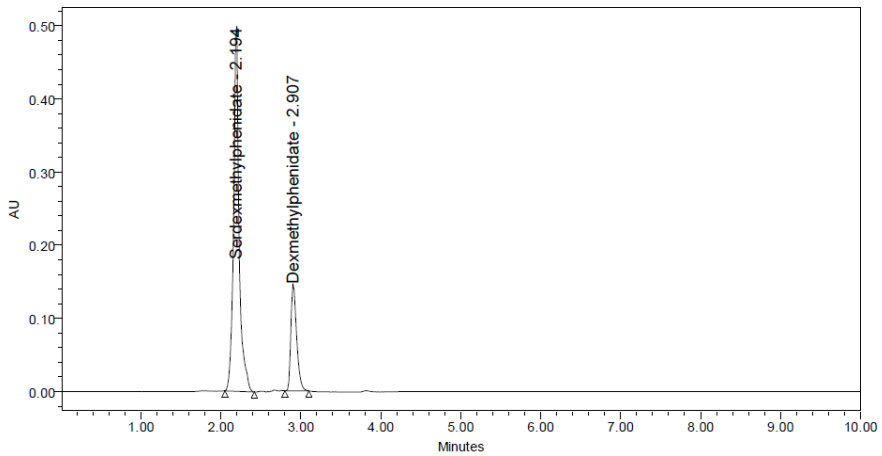


Figure 7: Base degradation Chromatogram

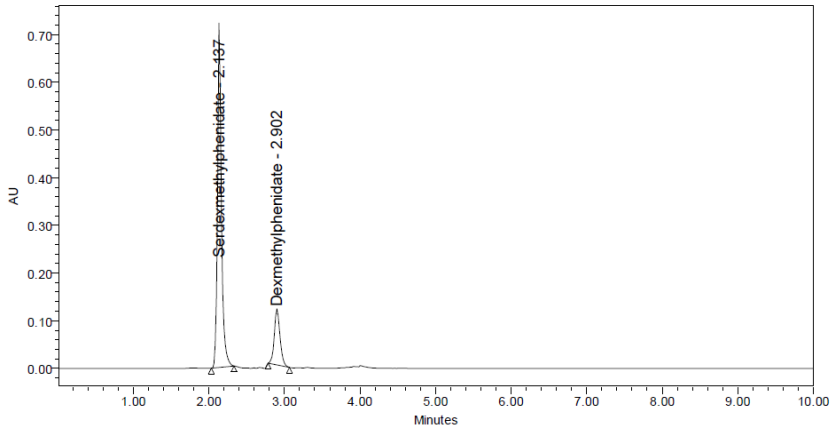


Figure 8: Oxidation degradation Chromatogram

From the results, degradation was observed when the samples were exposed to acid, base, Peroxide reaction. No degradation was observed for thermal, light and hydrolysis reaction. According to the stress study, none of the degradants were co-eluted with the active drug peaks formed

Table 10: Assay of serdexmethylphenidate

S.no	Standard Area	Sample area	% Assay
1	3734505	3749825	99.32
2	3777698	3766859	99.77
3	3759038	3779822	100.11
4	3793036	3771990	99.90
5	3787298	3760469	99.60
6	3779822	3724718	98.65
Avg	3771900	3758947	99.56
Stdev	21651.9	19624.7	0.52
%RSD	0.6	0.5	0.5

Table11 :Assay of Dexmethylphenidate

S.no	Standard Area	Sample area	% Assay
1	913065	918374	100.40
2	911696	911160	99.61
3	912305	915329	100.07
4	910446	915555	100.09
5	915329	918146	100.38
6	919818	913176	99.83
Avg	913777	915290	100.07
Stdev	3376.5	2802.3	0.31
%RSD	0.4	0.3	0.3

Conclusion:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Serdexmethylphenidate and Dexmethylphenidate in injection dosage form. Retention time of Serdexmethylphenidate and Dexmethylphenidate were found to be 2.133 min and 2.925 min. %RSD of the Serdexmethylphenidate and Dexmethylphenidate were and found to be 0.6 and 0.4 respectively. %Recovery was obtained as 99.98% and 100.50% for Serdexmethylphenidate and Dexmethylphenidate respectively. LOD, LOQ values obtained from regression equations of Serdexmethylphenidate and Dexmethylphenidate were 0.24, 0.73 µg/ml and 0.04, 0.12µg/ml respectively. Regression equation of Serdexmethylphenidate isy = 71326x + 10084, and y = 85514x + 10852.of Dexmethylphenidate. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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