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Research

Development and validation of rp-hplc method for the simultaneous estimation of serdexmethylphenidate and dexmethylphenidate in bulk and pharmaceutical dosage form

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Abstract

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Serdexmethylphenidate and Dexmethylphenidate in bulk and combined pharmaceutical tablet dosage forms. Serdexmethylphenidate and Dexmethylphenidate were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size; Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Serdexmethylphenidate and Dexmethylphenidate was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Serdexmethylphenidate and Dexmethylphenidate in the range of 20-100µg/ml for Serdexmethylphenidate and 60-140µg/ml for Dexmethylphenidate with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Serdexmethylphenidate and 100.21% for Dexmethylphenidate respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form.

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

INTRODUCTION

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the

analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatography

Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.
Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.
Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.
Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.
Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.
Mobile phase: Methanol, Acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.
Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.
Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.
Mobile phase: Methanol, Acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.
Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase
Stationary phase:

	Strong	Weak
Cation exchanger	SO_3^-	COO^-
Anion exchanger	NR_3^+	NHR_2^+

Mobile phase: Aqueous buffer systems.
Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

MATERIALS AND METHOD

Serdexmethylphenidate (Pure)-Sura labs, Dexmethylphenidate (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Telma-LN 40-Glenmark

HPLC method development
Trails
Preparation of standard solution: Accurately weigh and transfer 10 mg of Serdexmethylphenidate and Dexmethylphenidate working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.6ml of Serdexmethylphenidate and 1ml of Dexmethylphenidate from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: 0.1% Orthophosphoric acid in proportion 64:36 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	38ºC
Column	:	Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Mobile phase	:	Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Flow rate	:	1ml/min
Wavelength	:	224nm
Injection volume	:	20µl
Run time	:	7.0minutes

Method validation
Preparation of mobile phase
Preparation of mobile phase: Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Trial: (Optimized Condition)

Mobile phase	:	Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Column	:	Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Flow rate	:	1 ml/min
Wavelength	:	224 nm
Column temp	:	38ºC
Sample Temp	:	Ambient
Injection Volume	:	20 µl
Run time	:	7 minutes

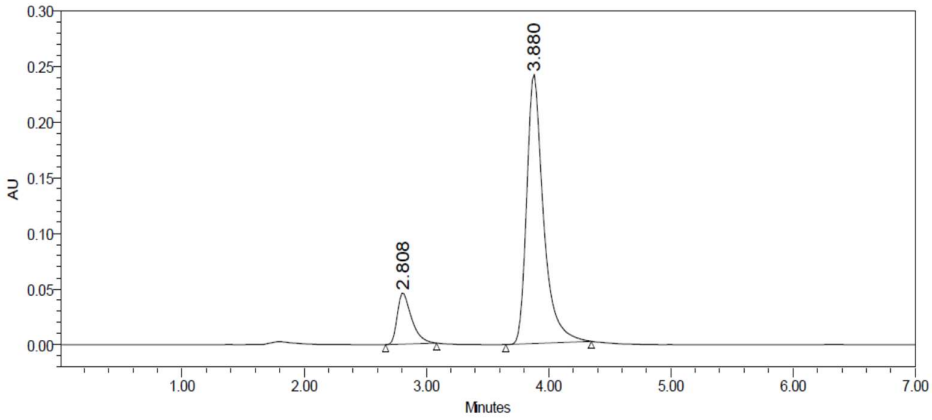

Fig 1: Chromatogram for Trail 5

Table 1: Peak Results for Trail 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Serdexmethylphenidate	2.808	65258	4326		1.08	5685.4
2	Dexmethylphenidate	3.880	8659854	659823	5.68	1.42	6895.7

From the above chromatogram it was observed that the Serdexmethylphenidate and Dexmethylphenidate peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial. Retention time of Serdexmethylphenidate –2.808min; Retention time of Dexmethylphenidate – 3.880 min

System Suitability

Table 2: Results of system suitability parameters for Serdexmethylphenidate and Dexmethylphenidate

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Serdexmethylphenidate	2.816	65358	4536		1.08	5689.6
2	Dexmethylphenidate	3.893	8658746	658985	5.69	1.42	6892.4

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Showing assay standard Results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Serdexmethylphenidate	2.813	65684	4365		1.08	5632.4	1
2	Dexmethylphenidate	3.886	8659824	659824	5.69	1.42	6859.2	1
3	Serdexmethylphenidate	2.813	65985	4329		1.09	5682.3	2
4	Dexmethylphenidate	3.886	8645872	658266	5.68	1.43	6824.1	2
5	Serdexmethylphenidate	2.813	65784	4426		1.08	5692.8	3
6	Dexmethylphenidate	3.886	8657847	6589412	5.69	1.43	6895.4	3

Assay (sample)

Table 4: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Serdexmethylphenidate	2.799	66859	4458		1.09	5785.4	1
2	Dexmethylphenidate	3.863	8756854	669585	5.69	1.43	6956.7	1
3	Serdexmethylphenidate	2.799	66258	4462		1.10	5789.5	2
4	Dexmethylphenidate	3.861	8769582	663598	5.68	1.44	6945.2	2
5	Serdexmethylphenidate	2.799	66435	4438		1.09	5784.1	3
6	Dexmethylphenidate	3.863	8754985	668548	5.69	1.44	6927.7	3

Table 5: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Serdexmethylphenidate	10mg	59.84	99.68%
2	Dexmethylphenidate	40mg	499.63	99.46%

The retention time of Serdexmethylphenidate and Dexmethylphenidate was found to be 2.808mins and 3.880mins respectively. The % purity of Serdexmethylphenidate and Dexmethylphenidate in pharmaceutical dosage form was found to be 99.68% and 99.46% respectively.

Precision

Table 6: Results of method precision for Serdexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Serdexmethylphenidate	2.808	65898	4365	5682.2	1.08
2	Serdexmethylphenidate	2.808	65487	4375	5628.6	1.09
3	Serdexmethylphenidate	2.808	65324	4395	5649.7	1.08
4	Serdexmethylphenidate	2.808	65982	4328	5638.4	1.09
5	Serdexmethylphenidate	2.808	65248	4371	5698.3	1.08
6	Serdexmethylphenidate	2.808	65734	4391	5682.7	1.09
Mean			65612.17			
Std. Dev			304.8425			
% RSD			0.464613			

Table 7: Results of method precision for Dexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dexmethylphenidate	3.880	8659824	658784	6859.4	1.42	5.68
2	Dexmethylphenidate	3.880	8658547	657489	6824.6	1.43	5.69
3	Dexmethylphenidate	3.880	8659824	652368	6829.3	1.42	5.68
4	Dexmethylphenidate	3.880	8659875	658745	6892.7	1.43	5.69
5	Dexmethylphenidate	3.880	8658745	658213	6875.2	1.42	5.68
6	Dexmethylphenidate	3.880	8659862	652354	6859.8	1.42	5.69
Mean			8659446				
Std. Dev			623.2924				
% RSD			0.007198				

- *%RSD for sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate Precision/Ruggedness

DAY 1

Table 8: Results of Intermediate precision for Serdexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Serdexmethylphenidate	2.808	66895	4468	5784.2	1.09

2	Serdexmethylphenidate	2.808	66986	4523	5835.1	1.09
3	Serdexmethylphenidate	2.808	66258	4475	5864.4	1.10
4	Serdexmethylphenidate	2.808	66457	4514	5864.6	1.09
5	Serdexmethylphenidate	2.808	66539	4489	5784.9	1.10
6	Serdexmethylphenidate	2.808	66298	4565	5748.5	1.10
Mean			66572.17			
Std. Dev			304.536			
% RSD			0.457452			

Table 9: Results of Intermediate precision for Dexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dexmethylphenidate	3.882	8758568	669583	6982.4	1.43	
2	Dexmethylphenidate	3.882	8756982	665984	6935.3	1.44	5.69
3	Dexmethylphenidate	3.882	8746925	665345	6984.7	1.44	
4	Dexmethylphenidate	3.882	8723654	665325	6952.8	1.43	5.70
5	Dexmethylphenidate	3.882	8754982	669852	6898.9	1.44	
6	Dexmethylphenidate	3.882	8754698	665874	6976.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

%RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

DAY 2

Table 10: Results of Intermediate precision for Serdexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Serdexmethylphenidate	2.799	66510	4310	5711.6	1.01
2	Serdexmethylphenidate	2.813	66216	4219	5826.2	1.03
3	Serdexmethylphenidate	2.808	66501	4316	5715.1	1.05
4	Serdexmethylphenidate	2.816	66129	4501	5756.0	1.06
5	Serdexmethylphenidate	2.860	66016	4468	5891.6	1.09
6	Serdexmethylphenidate	2.824	66519	4419	5892.8	1.08
Mean			66315			
Std. Dev			222.72			
% RSD			0.3358			

Table 11: Results of Intermediate precision for Dexmethylphenidate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dexmethylphenidate	3.861	8761210	668200	6952.1	1.44	
2	Dexmethylphenidate	3.886	8721601	666111	6971.5	1.43	5.70
3	Dexmethylphenidate	3.880	8739120	664626	6990.4	1.43	
4	Dexmethylphenidate	3.893	8742810	664462	6960.1	1.44	5.71
5	Dexmethylphenidate	3.949	8784519	665511	6941.2	1.44	
6	Dexmethylphenidate	3.914	8712915	668440	6950.9	1.44	5.70
Mean			8743695				
Std. Dev			26194.05				
% RSD			0.299				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 12: Accuracy (recovery) data for Serdexmethylphenidate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	35921.67	30	30.134	100.446%	100.30%
100%	70894.33	60	60.205	100.341%	
150%	105654.7	90	90.093	100.103%	

- *The % Recovery for each level should be between 98.0 to 102.0%.*

Table 13: Accuracy (recovery) data for Dexmethylphenidate

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4276302	50	50.208	100.416%	100.21%
100%	8484717	100	100.148	100.148%	
150%	10160609	150	150.091	100.060%	

- *The percentage recovery was found to be within the limit (97-103%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Linearity

Chromatographic data for linearity study of serdexmethylphenidate

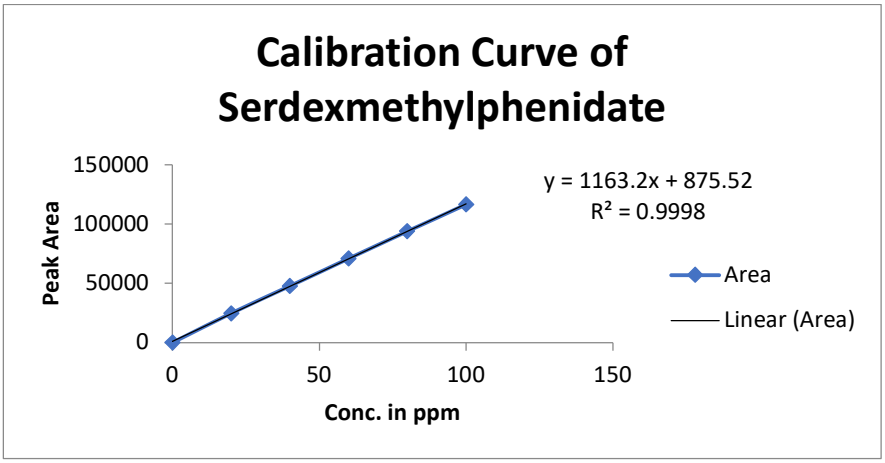

Fig 2: Calibration graph for Serdexmethylphenidate

Table 14: Linearity Results: (for Serdexmethylphenidate)

S.No	Linearity Level	Concentration (ppm)	Area
1	I	20	24759
2	II	40	47859
3	III	60	70898
4	IV	80	93985
5	V	100	116698
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999.

Linearity Results: (for Dexmethylphenidate)

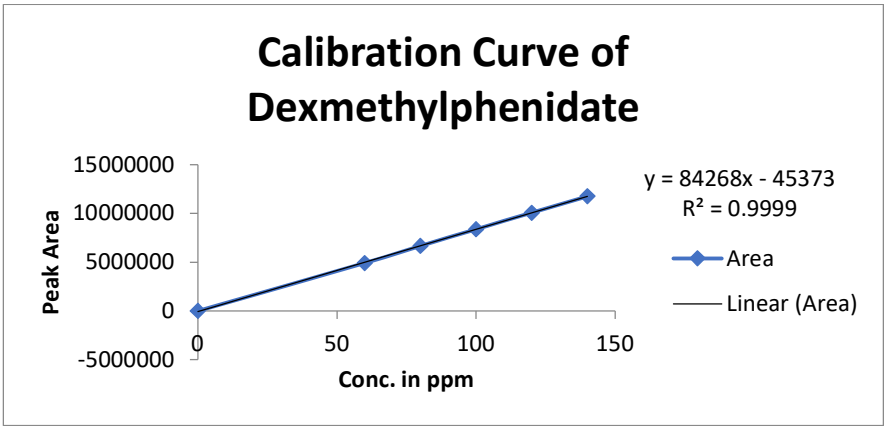

Fig 3: Calibration graph for Dexmethylphenidate

Table 15: Linearity Results (for Dexmethylphenidate)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	60	4928578
2	II	80	6687842
3	III	100	8389878
4	IV	120	10085847
5	V	140	11769854
Correlation Coefficient			0.999

- *Correlation coefficient should be not less than 0.99.*

Robustness

Table 16: System suitability results for Serdexmethylphenidate

S.No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	0.9	5784.6	1.06	3.091
2	1.0	5685.4	1.08	2.813
3	1.1	5869.5	1.09	2.553

** Results for actual flow (1.0 ml/min) have been considered from Assay standard.*

Table 17: System suitability results for Dexmethylphenidate

S.No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	0.9	6698.3	1.46	4.274
2	1.0	6895.7	1.42	3.886
3	1.1	6983.6	1.49	3.538

** Results for actual flow (1.0ml/min) have been considered from Assay standard.*

Table 18: System suitability results for Serdexmethylphenidate

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	10% less	5895.3	1.12	3.301
2	*Actual	5685.4	1.08	2.813
3	10% more	5964.2	1.16	2.469

Table 19: System suitability results for Dexmethylphenidate

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	10% less	6785.2	1.46	4.344
2	*Actual	6895.7	1.42	3.886
3	10% more	6982.4	1.49	3.508

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Serdexmethylphenidate and Dexmethylphenidate in bulk and tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Serdexmethylphenidate and Dexmethylphenidate.

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