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A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Serdexmethylphenidate and Dex-Methylphenidate in Pharmaceutical Dosage Form

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HUMAN

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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 Discovery C18 (150 x 4.6 mm, 3.5µm) Mobile phase containing Buffer 0.01N Na_2HPO_4: 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 Na_2HPO_4 & 0.1% OPA is added to adjust pH of buffer(pH-3.4) buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 228 nm. Retention time of Serdexmethylphenidate and Dexmethylphenidate were found to be 2.435 min and 2.791 min. % RSD of the Serdexmethylphenidate and Dexmethylphenidate were and found to be 0.8 and 0.6 respectively. % Recovery was obtained as 100.13% and 100.16% for Serdexmethylphenidate and Dexmethylphenidate respectively. LOD, LOQ values obtained from regression equations of Serdexmethylphenidate and Dexmethylphenidate were 0.51, 1.54µg/ml and 0.14, 0.44µg/ml respectively. Regression equation of Serdexmethylphenidate is y =70879x + 11533, and y = 168658x + 5402.1 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.

INTRODUCTION:

Attention deficit hyperactivity disorder (ADHD)[1] is a neurodevelopmental disorder characterized by excessive amounts of inattention, hyperactivity, and impulsivity that are pervasive, impairing in multiple contexts, and otherwise age-inappropriate[2,3]**.** ADHD symptoms arise from executive dysfunction[4], and emotional dysregulation is often considered a core symptom. In children, problems paying attention may result in poor school performance. ADHD is associated with other neurodevelopmental and mental disorders as well as some non-psychiatric disorders, which can cause additional impairment, especially in modern society.

Serdexmethylphenidate[5] is a prodrug of the CNS stimulant dexmethylphenidate, which increases extracellular levels of dopamine and norepinephrine in the CNS, leading to altered neurotransmission. As a CNS stimulant, Serdexmethylphenidate carries a risk of abuse, misuse, and dependence, which should be monitored.

Methylphenidate[6] (MPH) non-competitively blocks the reuptake of dopamine and noradrenaline into the terminal by blocking dopamine transporter (DAT) and noradrenaline transporter (NAT), increasing levels of dopamine and noradrenaline in the synaptic cleft.

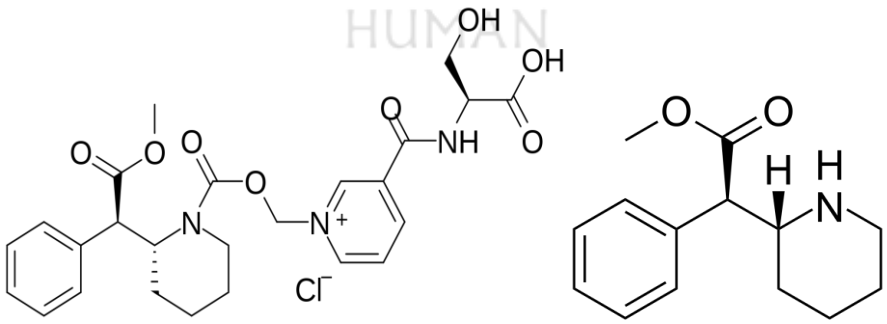

Figure No. 1: Structures of Serdexmethylphenidate, Dexmethylphenidate.

High Performance Liquid chromatography is one of the effective separation analytical tools to determine drug combination. There are some RP-HPLC [7-10] methods were described in the literature for estimation of Serdexmethylphenidate, Dexmethylphenidate. There are some RP-HPLC methods of single and double described in the literature for estimation of Serdexmethylphenidate, Dexmethylphenidate. On basis of the review of literature, no official method for the stability-indicating simultaneous estimation of Serdexmethylphenidate, Dexmethylphenidate by RP-HPLC in pharmaceutical dosage form was proposed as of now.

Hence, there is an effort should be applied for research that has been made to develop and validate a novel and simple analytical method for the stability-indicating simultaneous estimation of Serdexmethylphenidate, Dexmethylphenidate in the pharmaceutical dosage form. The proposed new method is able to separate all the active analyte present in the pharmaceutical dosage form and validated as per the guidelines of ICH (Q2 specification).

Optimization Experiments:

In the process of developing drug separation by HPLC method, different parameters were studied which influence the separation such as using various columns, different mobile phases and column temperatures. HPLC columns used for development of method were Ascentis, Discovery with various combinations of mobile phases. In this development separation between two combination drugs was barely achieved. Hence, we tried with Discovery column, 0.01N Disodium Hydrogen phosphate buffer in various combination of mobile phase with organic solvents acetonitrile. Finally specific method was optimized in this column. Method was finalized with satisfactory resolution among all the drugs with Mobile phase A is 0.01N Disodium Hydrogen phosphate and mobile phase B is Acetonitrile with isocratic elution.

MATERIALS AND REAGENTS

Serdexmethylphenidate and Dexmethylphenidate pure drugs were procured from Spectrum Pharma Labs. The HPLC grade methanol and acetonitrile procured from Rankem chemical division, India. Sodium hydrogen phosphate procured from Rankem, India and Pure milli-Q water is used with the help of 0.45µ Millipore filters (Rankem, india).

Instrumentation and Chromatographic Conditions

WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector. Discovery 150 mm x 4.6mm, 3.5µm column was used for the separation. 0.01N Disodium Hydrogen phosphate buffer is used as mobile phase A and Acetonitrile is used as mobile phase B (60:40 Ratios). Analysis was carried out in isocratic mode with flow rate of 0.9 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 228 nm. The output signal was monitored and integrated using Empower 2 software.

Preparation of Solutions

Diluent: Mixed Water and Acetonitrile in the ratio of 50:50v/v.

Preparation of buffer:

Buffer (0.01N Disodium Hydrogen Phosphate) Accurately weighed 1.42gm of Disodium 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 3.4 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 3/4 th of diluents was added to these flasks 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 working standard:

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 solution: 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: 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).

System suitability: The system suitability parameters were determined by preparing standard solutions of Serdexmethylphenidate (52.2ppm) and Dexmethylphenidate (10.4ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Table No. 1: System suitability table

Serdexmethylphenidate			Dexmethylphenidate			
RT(min)	**TP**	**Tailing**	**RT(min)**	**TP**	**Tailing**	**RS**
2.437	4504	1.52	2.972	4593	1.35	3.3
2.439	4608	1.51	2.972	4667	1.35	3.3
2.439	4718	1.50	2.974	4496	1.35	3.2
2.439	4673	1.50	2.975	4659	1.35	3.3
2.440	4729	1.50	2.975	4749	1.36	3.3
2.453	4456	1.51	2.987	4634	1.34	3.3

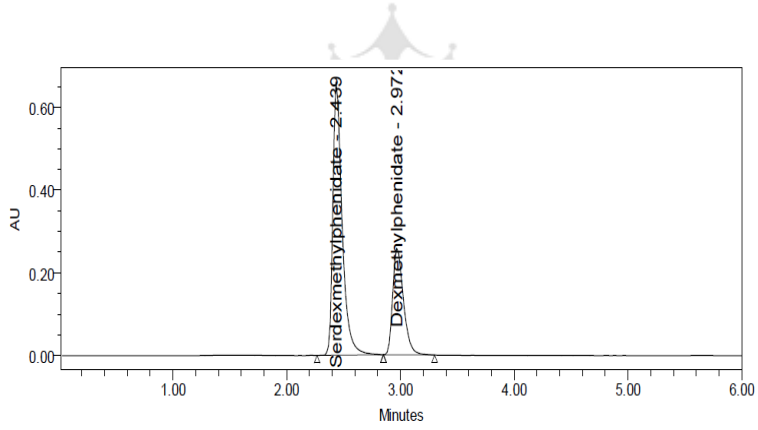

Figure No. 2: System suitability Chromatogram of Serdexmethylphenidate and dexmethylphenidate

Table No. 2: Specificity data

Sample name	**Retention time(mins)**
Serdexmethylphenidate	2.437
Dexmethylphenidate	2.972

There was no interference observed in blank at the retention times of Serdexmethylphenidate and dexmethylphenidate. Both compounds are separated with good resolution.

In order to assess the stability indicating nature of the HPLC method, Serdexmethylphenidate and dexmethylphenidate samples were stressed by acid, base, oxidation, heat, light and humidity. The degraded samples were analyzed by using a photodiode-array detector. The peak purity of Serdexmethylphenidate and dexmethylphenidate were passed. The forced degradation conditions are mentioned in Table 3 and the results are mentioned in Table 4.

Table No. 3: Forced degradation conditions 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
Oxidation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	1 hour
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	1 hour

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, light, humidity and heat. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table No. 4: Degradation profile results

Degradation condition	Serdexmethylphenidate % Undegraded	Dexmethylphenidate % Undegraded
Acid	96.02	96.54
Base	93.18	92.63
Oxidation	93.17	93.66
Thermal	97.24	95.82
Photolytic	97.39	98.94
Hydrolytic	99.24	98.94

Limit of detection (LOD) and Limit of quantitation (LOQ): The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated. The detection limit was determined as the lowest

concentration for which the response is approximately three times greater than the baseline noise. The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The LOD values obtained for Serdexmethylphenidate and dexmethylphenidate are listed in Table 5 and corresponding representative chromatogram is shown in Figure 3.

Table No. 5: Summary of limit of detection& limit of Quantification

Sample	LOD		LOQ	
	Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
Serdexmethylphenidate	0.14	36375	0.51	109839
Dexmethylphenidate	0.44	23739	1.54	74607

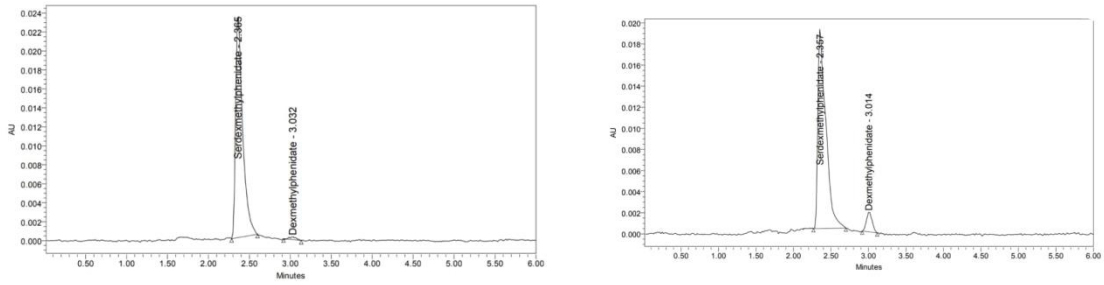

Figure No. 3: LOD & LOQ Chromatograms of Serdexmethylphenidate and dexmethylphenidate

Linearity: The linearity of the method was demonstrated for Serdexmethylphenidate and dexmethylphenidate by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 6). The correlation coefficient for Serdexmethylphenidate and dexmethylphenidate was 0.999. This indicates good linearity (Figures 4-5).

Table No. 6: Linearity data

% Level	Serdexmethylphenidate		Dexmethylphenidate	
	Area	Conc(µg/ml)	Area	Conc(µg/ml)
25%	961632	13.5	441950	2.6
50%	1873814	26.1	882487	5.2
75%	2791728	39.15	1334470	7.8
100%	3702245	52.2	1748038	10.4
125%	4469087	62.25	2222166	13
150%	5525765	78.3	2617447	15.6

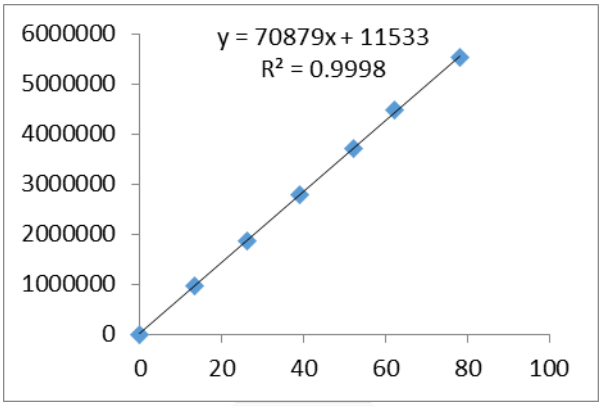

Figure No. 4: Linearity plot of Serdexmethylphenidate

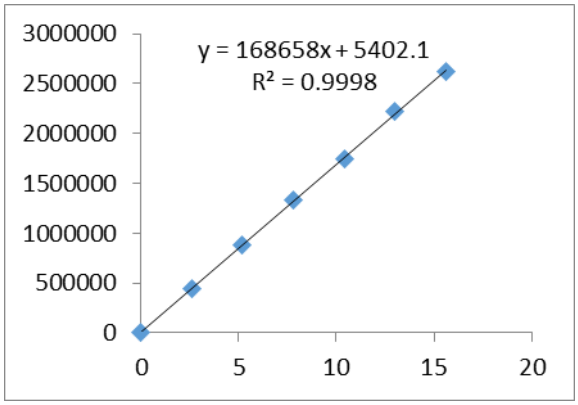

Figure No. 5: Linearity plot of Dexmethylphenidate

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Serdexmethylphenidate and dexmethylphenidate at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analyzed. Percentage recovery results obtained for each impurity was listed in Table 7.

Table No. 7: % Recovery data

% Level	% Recovery	
	Serdexmethylphenidate	Dexmethylphenidate
50%	99.6	100.75
	100.6	100.43
	100.2	99.63
100%	100.2	99.97
	100.6	100.63
	100.1	99.49
150%	100.5	99.61
	99.5	99.98
	99.9	100.97

The percentage recovery values obtained at level 50%, 100% and 150% were within the acceptable limit.

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Serdexmethylphenidate and dexmethylphenidate. Results of peak area are summarized in Table 8.

Table No. 8: System precision data

Injection	Serdexmethylphenidate	Dexmethylphenidate
1	3736193	1784968
2	3722542	1755905
3	3704393	1762320
4	3773105	1765406
5	3698536	1757644
6	3703855	1754346
Avg	3723104	1763432
Std dev	28269.9	11328.2
% RSD	0.8	0.6

The % RSD for the peak areas of Serdexmethylphenidate and dexmethylphenidate obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Serdexmethylphenidate and dexmethylphenidate (Six individual sample preparations). Data obtained is summarized in Table 9.

Table No. 9: Method precision data

Injection	Serdexmethylphenidate	Dexmethylphenidate
1	3761966	1765296
2	3723628	1772410
3	3742880	1752951
4	3720494	1769294
5	3756100	1759166
6	3755565	1775228
Avg	3743439	1765724
Std dev	17719.2	8415.6
%RSD	0.5	0.5

From the above results, the % RSD of method precision study was within the limit for Serdexmethylphenidate and dexmethylphenidate.

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase and system suitability also complied as per methodology. The robustness results are summarized in Table 10.

Table No. 10: Robustness results

Condition	% RSD	
	Serdexmethylphenidate	Dexmethylphenidate
Flow (-)	0.6	0.5
Flow (+)	0.2	0.4
Temp (-)	0.4	0.6
Temp (+)	0.8	0.7
Mobile phase (-)	0.5	0.3
Mobile phase (+)	0.8	0.7

From the robustness study, system suitability criteria comply with the results.

CONCLUSION:

From the above experimental results, it was concluded that, the newly developed method for the simultaneous estimation of Serdexmethylphenidate and dexmethylphenidate was found to be simple, precise and accurate with high resolution, shorter retention time and with separated degradants in forced degradation studies. The present proposed methodology makes it cost effective which can be implemented for routine analyses in pharmaceutical industry.

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