Development and Validation of New Analytical Method for the Simultaneous Estimation of Serdexmethylphenidate and Dexmethylphenidate in Pharmaceutical Dosage Forms

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Abstract:

The simultaneous estimate of serdexmethylphenidate and dexmethylphenidate in bulk and pharmaceutical dose form in an isocratic mode has been accomplished using a new uncomplicated, rapid, sensitive, accurate, precise, and reproducible RP-HPLC method utilizing Standard discovery C18 (150X4.6mm, 5µm) column. These drugs were used to treat attention deficit hyperactivity disorder. Serdexmethylphenidate is a prodrug of dexmethylphenidate. Acetonitrile and 0.01N orthophosphoric acid (55:45v/v) served as the mobile phase for smooth retention and good resolution. At 260 nm, the detection was performed with good resolution of 4.3. For serdexmethylphenidate and dexmethylphenidate, the technique was linear over the concentration range of 13.1-78.3µg/ml and 2.6-15.6 µg/ml, respectively. Serdexmethylphenidate and dexmethylphenidate were found to be accurate with percent recoveries of 100.32% and 99.2%, respectively. The approach was validated according to ICH criteria. The stability-indicating HPLC approach described here was effectively used to analyse a medicinal formulation.

Key-Words: Serdexmethylphenidate, Dexmethylphenidate, RP-HPLC, Method Development, stability–indicating.

Introduction:

Dexmethylphenidate is a drug used to treat attention deficit hyperactivity disorder (ADHD) in people older than five years old. It is marketed under the trade name Focalin. It is sensible to stop using it if there is no improvement after four weeks. It is consumed orally. While the extended-release formulation can be taken for up to twelve hours, the immediate-release formulation can be taken for up to five hours. Abdominal pain, a decrease in appetite, and fever are typical adverse effects. Abuse, psychosis, sudden cardiac death, mania, allergy, convulsions, and dangerously extended erections are examples of serious adverse effects. Uncertainty surrounds safety during pregnancy and breast feeding. It's unclear how it functions in ADHD. It is methylphenidate's more energetic enantiomer.

Serdexmethylphenidate, the pharmaceutical company KemPharm developed the prodrug serdexmethylphenidate (SDX) as a form of dexmethylphenidate. Of March 2021, the FDA granted its initial approval for the substance as one of the active components in Azstarys, a medication used to treat adults, adolescents, and children with attention deficit hyperactivity disorder (ADHD). The co-formulation of SDX with dexmethylphenidate enables a quicker start of action while maintaining therapeutic efficacy for up to 13 hours. In order to treat a variety of CNS disorders substance use disorder[1-3], and sleep disorders , several dosage forms containing SDX are currently being studied for their potential use as long-acting psychostimulants [4-6]. This is because the effects of oral administration of SDX take a little while to start and last for a long time. SDX is being developed under the identifier KP484.

Metabolism:

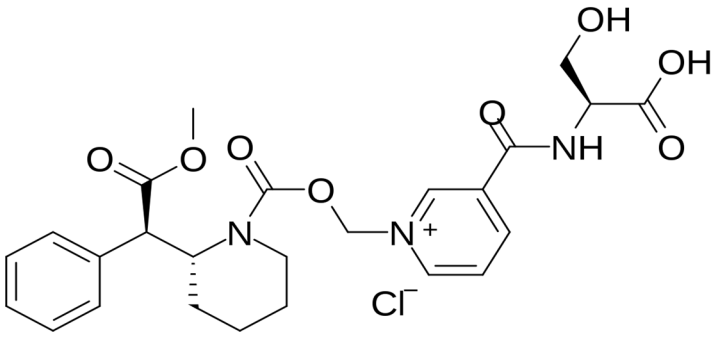


Figure 1: Structure of serdexmethylphenidate

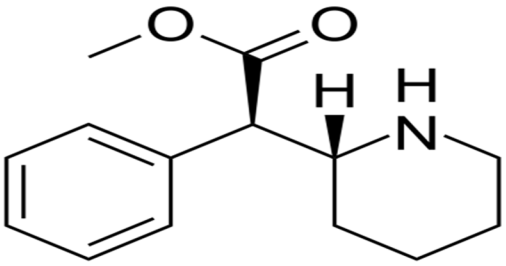


Figure 2: Structure of dexmethylphenidate

Experimental:

Apparatus:

The HPLC WATERS, software: Empower 2, 2695 separation module, UV detector, Electronic weighing balance (Denver), pH Meter (BVK enterprises, India).

Reagents and Chemicals:

All the chemicals and reagents in this experiment were of analytical grade. Water was double distilled and filtered with a membrane filter.Acetonitrile and ortho phosphoric acid (Rankem) were used to prepare mobile phase. Pharmaceutical grade standard drugs viz.,

Methodology: [7-9]

Preparation of (0.1%OPA Buffer): 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water. (pH-3.0)

Preparation of mobile phase:

Accurately measured 550ml (55%) of buffer solution, 450ml (45%) of HPLC grade acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µm filter under vacuum filtration.

Diluent Preparation:

Based up on the solubility of the drugs, Diluent was selected, Acetonitrile and Water taken in the ratio of 50:50v/v.

Standard Solution Preparation:

Accurately weighed and transferred 26.1mg ofserdexmethylphenidate5.2mg of dexmethylphenidate into a 100ml clean dry volumetric flask and made volume up to the mark with the diluent and sonicated to dissolve it completely (Stock solution A).

From the stock solution A, serial dilutions were made to obtain the concentrations of 13.1-78.3µg/mL for serdexmethylphenidateand 2.6-15.6µg/mL for dexmethylphenidate.

Method Development: [10-14]

The method was developed with different buffers and organic solvents but the composition of potassium dihydrogen ortho phosphate and acetonitrile showed symmetrical peaks, with good resolution- high theoretical plates, and low retention times of both serdexmethylphenidate and dexmethylphenidate.

S.No	Parameters	Description
1	Stationary phase	C18(150*,4.6mm,5µ)
2	Mobile phase	0.1%OPA:Acetonitrile
3	Flow rate	1ml/min
4	Detection wave length	230nm
5	Detector	Photo diode array
6	Injection	Auto sampler
7	Injection volume	10µl
8	Column temperature	30◦c
9	run time	6min

Method Validation:

The proposed RP-HPLC method was validated as per ICH guidelines.

System Suitability Constraints:

The system suitability parameters were showed good theoretical plates 6328 and 7457forserdexmethylphenidate and dexmethylphenidate. The tailing factor was found to be less than 2 for both drugs and good resolution exists between the peaks.

S.No	Name	RT (min)	Area(µVsec)	USP resolution	USP Tailing	USP Plate count
1	Serdexmethylphenidate	2.762	5146286	-	1.08	6328
2	Dexmethylphenidate	3.186	625173	4.5	1.12	7457

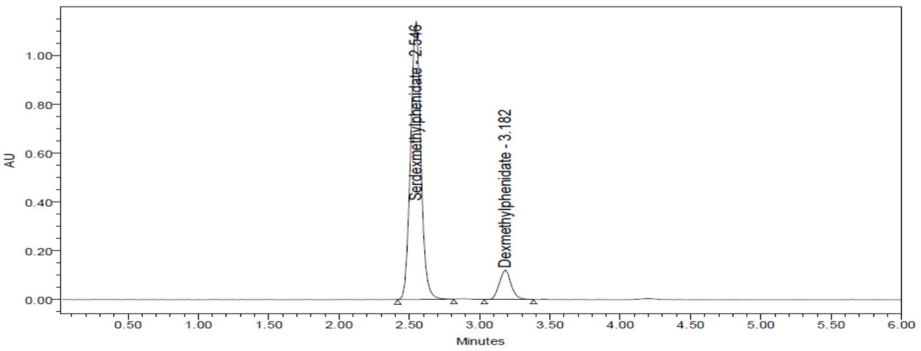
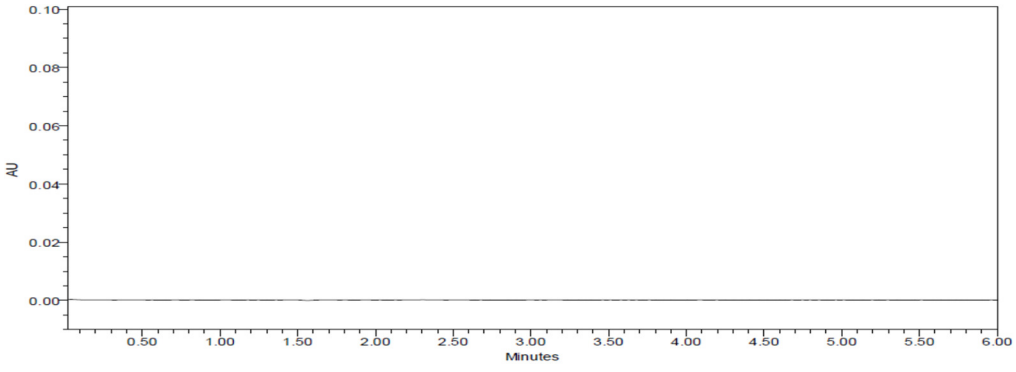


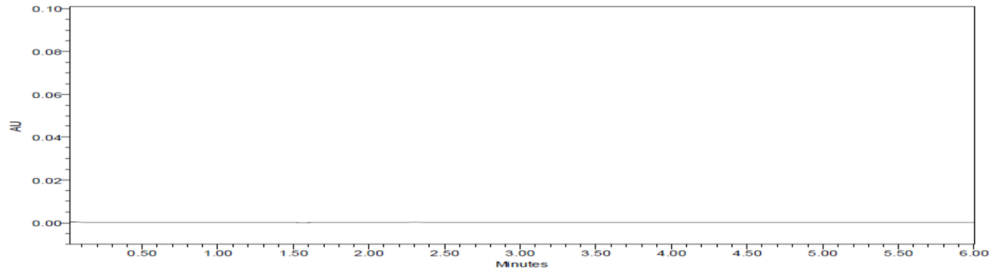
Figure 3: Chromatogram for system suitability

Specificity:

The stress degradation studies implies the specificity of the method. Different parameters were evaluated depending upon the separation between degradants and active moiety, as well as method ability to analyze analyte in the presence of other products as interfering peaks were found in blank at retention times of the drugs. Hence the method was specific.



Chromatogram For Blank



Chromatogram For Placebo

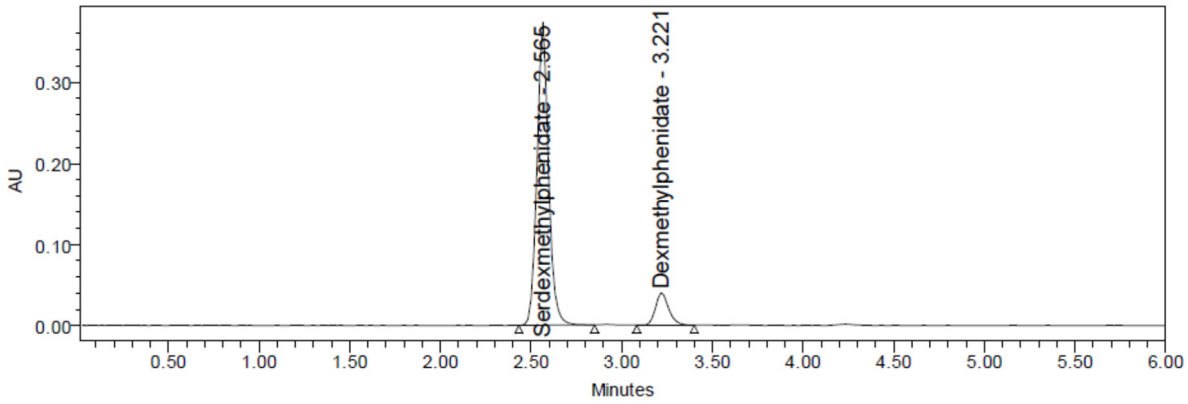
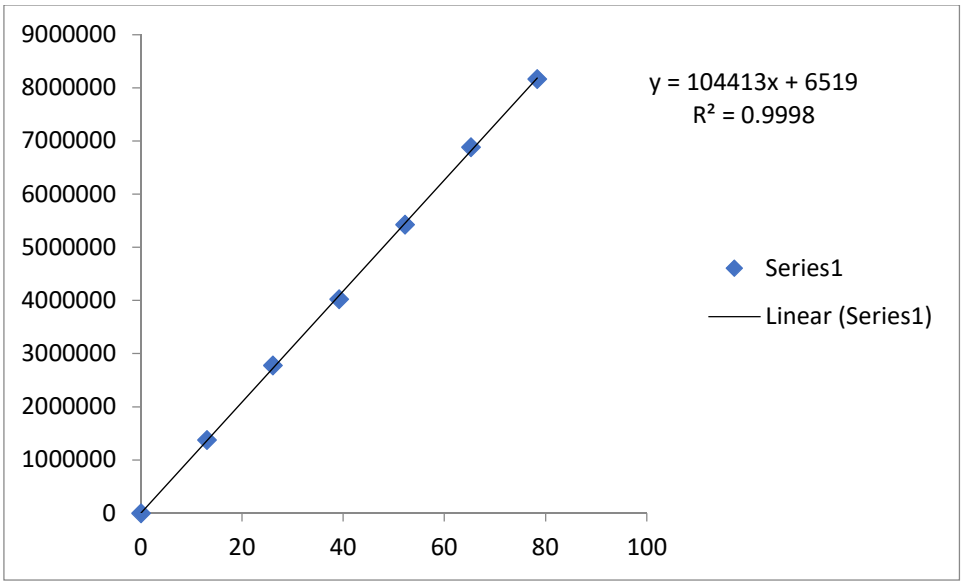


Figure 4: Standard Chromatogram of serdexmethylphenidate and dexmethylphenidate.

Linearity:

The calibration curve was linear over concentration range of 13.1-78.3µg/mL for serdexmethylphenidate and 2.6-15.6µg/mL for dexmethylphenidate and R2 values were found to be 0.999 for both drugs.



Calibration Graph for Serdexmethylphenidate.

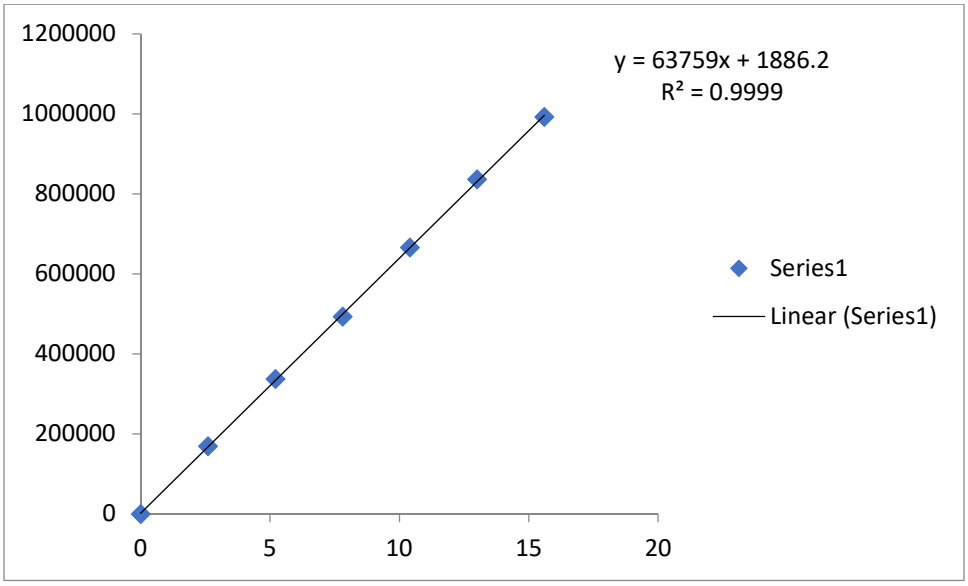


Figure 5: Calibration Graph for Dexmethylphenidate.

System Precision:

The precision was assessed through system precision and method precision. The method precision was estimated through assay. The optimized concentrations of standard and sample solutions were injected into chromatographic system six times and the % RSD of peak areas were found to be less than 0.5. There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Accuracy:

The accuracy of the method was performed by standard addition process at three different levels in triplicate. The concentration of 50% solution showed % mean recovery 100.1 and99.25 forserdexmethylphenidate and dexmethylphenidate, respectively. The concentration of 100% solution showed % mean recovery 101.5 and 98.56 for serdexmethylphenidate and dexmethylphenidate, respectively. The concentration of 150% solution showed % mean recovery 100.3 and 99.33 forserdexmethylphenidate and dexmethylphenidate, respectively.

Accuracy data for Serdexmethylphenidate.

Spiked level	Amount added(µg/ml)	Amount recovered(µg/ml)	%Recovery	Mean recovery
50%	26.1	26.1	100.1	100.32%
100%	52.2	53.0	101.5	
150%	78.3	78.5	100.3	

Accuracy data for Dexmethylphenidate.

Spiked level	Amount added(µg/ml)	Amount recovered(µg/ml)	%Recovery	Mean recovery
50%	5.2	5.16	99.25	99.28%
100%	10.4	10.25	98.56	
150%	15.6	15.50	99.33	

Limit of detection and Limit of quantification:

The limit of detection and quantification limits performed based on the slope and standard deviation. The method showed ability to detect serdexmethylphenidate and dexmethylphenidate at low level of concentrations. The LOD and LOQ were found to be0.18 µg/ml–0.55µg/ml for serdexmethylphenidate and 0.02µg/ml-0.06µg/ml dexmethylphenidate.

Robustness:

The robustness of the method was performed with deliberate change in flow rate, temperature and mobile phase composition. There was no change in the results which indicates that the method was more robust.

Force Degradation Studies:

The stability studies were implemented on the serdexmethylphenidate and dexmethylphenidate. The method showed, there was no interference of degradants and blank. The developed RP-HPLC method verifies the proficiency of stability indicating method for the analysis ofserdexmethylphenidate and dexmethylphenidate. Different stress indicating studies were conducted with 2 N HCl, refluxed for 30min at 60ºC, Base (2 N NaOH refluxed for 30min at 60ºC), H2O2(20% H2O2 Stored at room temperature for 30min 60ºC), hydrolytic for 1H at 105oC and UV light (near UV 200 Watt forone hour).

Sample name	**serdexmethylphenidate Area**	**%degraded**	**dexmethylphenidate Area**	**% degraded**
Standard	5449250	-	666153	-
Acid	5146286	5.90	625173	6.05
Base	5221846	4.70	637179	4.25
Peroxide	5226310	4.44	637876	4.14
Thermal	5342925	2.31	651582	2.08
Photo	5374829	1.72	658769	1.00

Assay: AZSTARYS, bearing the label claim containing Serdexmethylphenidate 26.1mg + Dexmethylphenidate5.2mgAssay wasperformed with the above formulation. Average % Assay for Serdexmethylphenidate and Dexmethylphenidate obtained was 99.99% and 99.81% respectively.

Conclusion: The developed and validated RP-HPLC method for the simultaneous estimation of serdexmethylphenidate and dexmethylphenidateshowed low tailing factor and high theoretical plates, good precision, accuracy and robustness, met the all values within the limits according to ICH guidelines. Hence it can be used for quality control analysis.

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