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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DEXMETHYLPHENIDATE AND SERDEXMETHYLPHENIDATE BY USING RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the simultaneous measurement of active pharmaceutical ingredients of Dexmethylphenidate and Serdexmethylphenidate.

Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the simultaneous determination of Dexmethylphenidate and Serdexmethylphenidate. The chromatographic strategy utilized inertsil ODS column of dimensions 250x4.6 mm, 5 µ, using isocratic elution with a mobile phase of acetonitrile and 0.1% orthophosphoric acid (70:30). A flow rate of 1 ml/min and a detector wavelength of 262 nm utilizing the PDA detector were given in the instrumental settings. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the active ingredient were established with respect to test concentration. The calibration chart plotted was linear with a regression coefficient of R^2>0.999, which means the linearity was within the limit.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Dexmethylphenidate, Serdexmethylphenidate, RP-HPLC, Development, Validation

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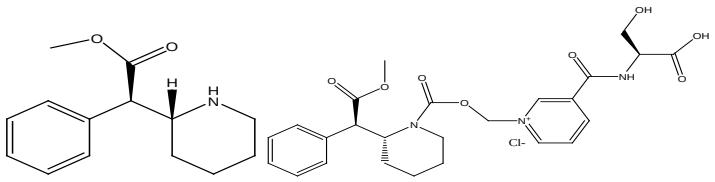
INTRODUCTION

Dexmethylphenidate, sold under the brand name Focalin among others, is a medication [1, 2] used to treat attention deficit hyperactivity disorder (ADHD) [3, 4] in those over the age of five years. If no benefit is seen after four weeks, it is reasonable to discontinue its use. It is taken by mouth [5]. The immediate-release formulation lasts up to five hours, while the extended-release formulation lasts up to twelve hours. Common side effects include abdominal pain [6, 7], loss of appetite [8], and fever [9, 10]. Serious side effects may include abuse [11], psychosis [12], sudden cardiac death [13, 14], mania [15], anaphylaxis [16], seizures [17], and dangerously prolonged erection. Safety during pregnancy and breastfeeding is unclear. Dexmethylphenidate is a central nervous system (CNS) stimulant [18]. How it works in ADHD is unclear. It is the more active enantiomer of methylphenidate.

Serdexmethylphenidate (SDX) is a prodrug of dexmethylphenidate created by the pharmaceutical company KemPharm. The compound was first approved by the FDA as one of the active ingredients in Azstarys for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children, adolescents, and adults in March 2021. Co-formulation of SDX with dexmethylphenidate allows for a more rapid onset of action while still retaining up to 13 h of therapeutic efficacy. Due to the delayed onset and prolonged duration of effects following oral administration of SDX, several dosage forms containing SDX are currently under investigation for use as long-acting psychostimulant [19, 20] in the treatment of various CNS disorders [21], substance use disorder (SUD) [22, 23], and sleep disorders [24]. Under the developmental codename KP484, SDX is being investigated as part of a potential "super-extended duration" psychostimulant, with therapeutic efficacy lasting up to 16 h following oral administration. The aim of the study is to estimate the pharma ingredients Dexmethylphenidate and Serdexmethylphenidate by using RP-HPLC.



A B

Fig. 1: Structure of (A) Dexmethylphenidate and (B) Serdexmethylphenidate

Till today there is only one HPLC method [25] was reported in the literature. Hence we developed a method for the quantification of Dexmethylphenidate and serdexmethylphenidate. The developed HPLC method was utilized for the estimation of the drug by *in vitro* method.

MATERIALS AND METHODS

Chemicals

Acetonitrile (HPLC-grade), orthophosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of

Dexmethylphenidate and Serdexmethylphenidate standard was procured from Glenmark, Mumbai.

The instrumentation

Waters alliance liquid chromatography (model 2695) was monitored with empower 2.0 data handling system and a detector of photodiode array (model 2998) was used for this study [25, 26].

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention time. Finally, 0.1% ortho phosphoric acid buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} and amino, phenyl columns were tested. From these trials the peak shape was relatively good with inertsil ODS column of 250 x 4.6 mm, 5 µ with a PDA detector. The mobile phase flow rate has been done at 262 nm in order to obtain enough sensitivity. By using above conditions we get retention time of Dexmethylphenidate was about 4.535 min with a tailing factor of 1.24. The number of theoretical plates for Dexmethylphenidate was 7328 and Serdexmethylphenidate retention time was 2.936 min with a tailing of 1.24, plate count 3319 which indicate the column's successful output the % RSD for six replicate injections were 0.25% for dexmethylphenidate and 0.25% for serdexmethylphenidate, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [27-29].

Preparation of buffer

1 ml of orthophosphoric acid (OPA) is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Chromatographic conditions

The HPLC analysis was performed on a reverse-phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% OPA and inertsil ODS (250x4.6 mm, 5 µ) column with a flow rate of 1 ml/min.

Diluent: Mobile phase was used as a diluent.

Preparation of the standard solution

For standard solution preparation, add 70 ml of diluents to 12 mg of Dexmethylphenidate and 56 mg of Serdexmethylphenidate taken in a 100 ml volumetric flask and sonicate for 10 min to fully dissolve the contents and then makeup to the mark with diluents (stock solution). Further, 5 ml of solution was drawn from the above normal stock solution into a 50 ml volumetric flask and diluted up to the level.

Preparation of sample solution

For sample solution preparation, add 70 ml of diluents to 118 mg of Dexmethylphenidate and Serdexmethylphenidate sample taken in a 100 ml volumetric flask and sonicate for 20 min to fully dissolve the contents and then makeup to the mark with diluents (stock solution). Further, 5 ml of solution was drawn from the above normal stock solution into a 50 ml volumetric flask and diluted up to the level.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized.

System suitability

In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1 and the standard chromatogram was shown in fig. 2 [30].

Table 1: Results of system suitability

System suitability parameter	Serdexmethylphenidate			Dexmethylphenidate		
	Mean	Std Dev	% RSD	Mean	Std Dev	% RSD
USP Plate Count	3319	43.614	1.31	7238	48.726	0.67
USP Tailing	1.15	0.008	0.66	1.03	0.008	0.79
USP Resolution	-	-	-	7.53	0.036	0.48
Retention time	2.937	0.002	0.08	4.540	0.005	0.10

(n=6)

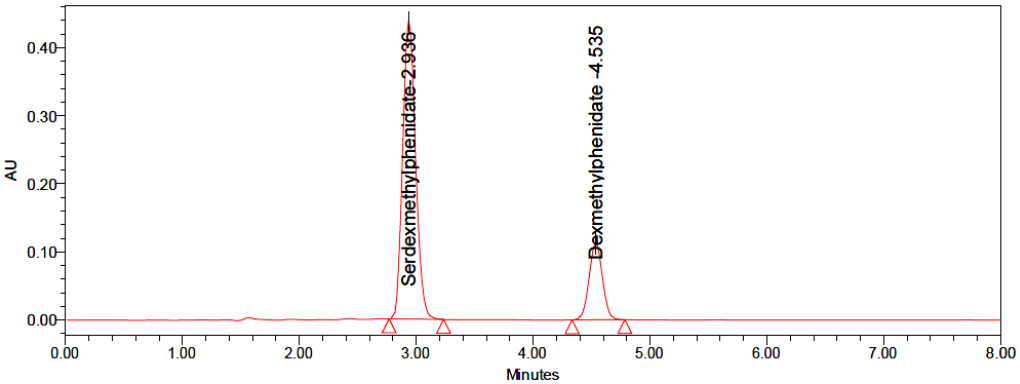


Fig. 2: Chromatogram of standard

Specificity

In this test method, placebo and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific [30].

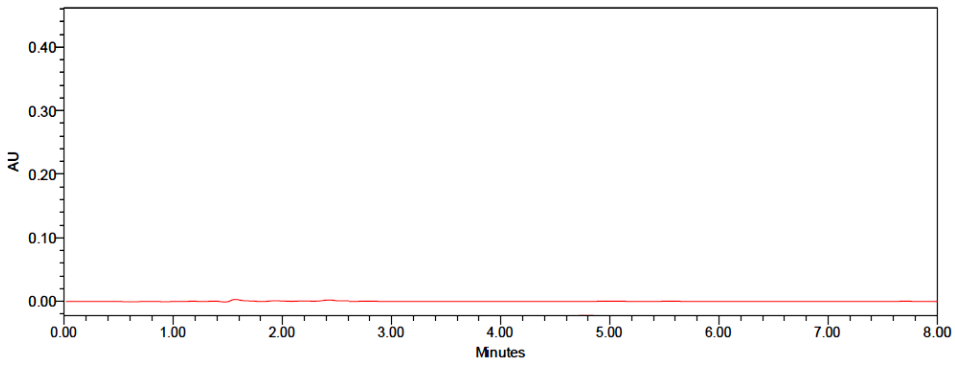


Fig. 3: Chromatogram of blank

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Serdexmethylphenidate as 25, 50, 75, 100, 125, 150 percent dilutions [30], respectively. Linearity was performed in the range of 14-84 µg/ml of Serdexmethylphenidate and 3-18 µg/ml of Dexmethylphenidate. The correlation coefficient achieved greater than 0.999 for all.

Accuracy

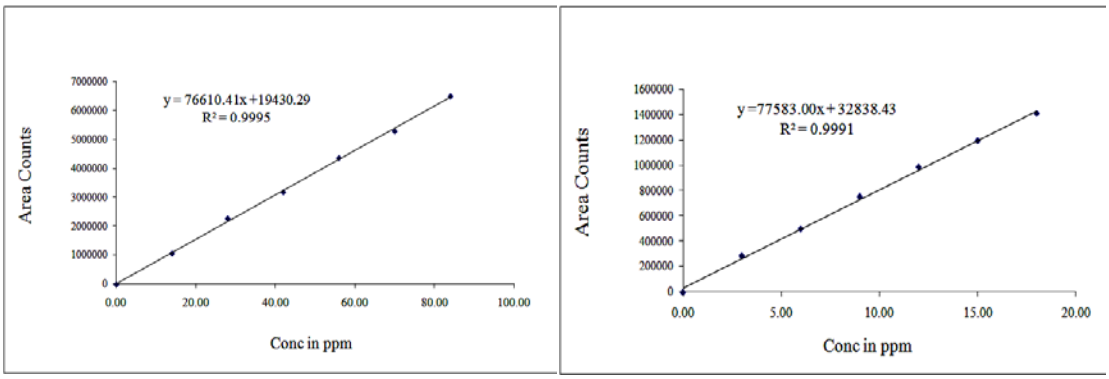
Three kinds of concentration levels of 50, 100, and 150 percent at a specified limit were used in this process to assess the accuracy of this particular method. The developed method was found to be highly accurate and reliable. The recovery percentages were given in table 3 [31].

Precision

In method, precision study prepare six different sample solutions in the concentration 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% to 102%. Peak areas were calculated, which were used to calculate mean, SD and % RSD values. These results are given below table 4 [32].

Table 2: Linearity results

S. No.	Serdexmethylphenidate		Dexmethylphenidate	
	Conc. µg/ml	Serdexmethylphenidate area count	Conc. µg/ml	Dexmethylphenidate area count
1	14.00	1058753	3.00	285651
2	28.00	2271560	6.00	498128
3	42.00	3182175	9.00	751473
4	56.00	4366503	12.00	985033
5	70.00	5287600	15.00	1190570
6	84.00	6492881	18.00	1406743
Correl coef		0.99954		0.99905
Slope		76610.41		77583.00
intercept		19430.29		32838.43



A **B**

Fig. 4: Calibration plot of (A) Serdexmethylphenidate and (B) Dexmethylphenidate

Table 3: Results of accuracy

S. No.	% Level	Serdexmethylphenidate		Dexmethylphenidate	
		Mean % recovery	Std dev	Mean % recovery	Std dev
1	50	99.6	0.392	99.7	0.611
2	100	100.2	0.474	100.1	0.427
3	150	98.8	0.306	98.8	0.483

n=3

Table 4: Intraday precision results

S. No.	Serdexmethylphenidate			Dexmethylphenidate		
	Conc. (µg/ml)	Area counts	% Assay as is	Conc. (µg/ml)	Area counts	% Assay as is
1	56	4362891	100.3	12	994314	100.2
2		4353789	100.1		990341	99.8
3		4335810	99.7		989739	99.7
4		4354608	100.2		996352	100.4
5		4359396	100.3		991343	99.9
6		4341312	99.8		990561	99.8
% RSD	0.26			0.27		
mean	100.1			100.0		
SD	0.258			0.273		

n=6

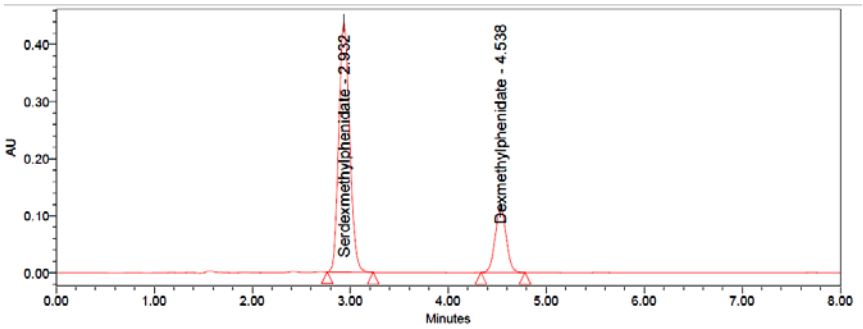


Fig. 5: Chromatogram of method precision

Intermediate precision

Separate instruments were used on different days, in different locations, for independent investigations into six different replicates of the sample solution. Mean RSD values have been calculated and determined from the peak regions. The following table shows the results. Serdexmethylphenidate (56 µg/ml) and Dexmethylphenidate (12 µg/ml) were analysed on 6 different days with 6 different samples. Mean, standard deviation and percent related standard deviation values were calculated from peak areas. Thus, it has been found that the current method yields very accurate results, with RSD values less than 2 percent and percent assay values near 100 percent. In table 5 [33] we can see the results.

LOD and LOQ

The LOD concentration of serdexmethylphenidate was 1.68 µg/ml and s/n values is 5 and Dexmethylphenidate was 0.36 µg/ml and s/n values is 3. The LOQ concentration for Serdexmethylphenidate was 5.6 µg/ml and their s/n values are 26, and Dexmethylphenidate was 1.2 µg/ml and s/n values is 22. The method is validated as per the ICH guidelines [34, 35].

Robustness

The design of the experiment was intentionally altered in order to test the robustness of the system. Examples of such changes include changing the flow rate, organic to inorganic ratio, and so on. The results were robust and tabulated in table 7 [36].

Degradation studies

Partial degradation of the drug was accomplished using various forced degradation conditions on the Serdexmethylphenidate and Dexmethylphenidate sample. Research has been carried out to see if the method works for degrading products [37, 38]. Additionally, the studies describe the conditions under which the drug is unstable, providing further information so that appropriate precautions are taken during the process of formulation in order to avoid possible instabilities [39, 40].

Table 5: Inter-day outcomes of the accuracy of dexmethylphenidate

S. No.	Serdexmethylphenidate			Dexmethylphenidate		
	Conc.(µg/ml)	Area counts	% assay as is	Conc. (µg/ml)	Area counts	% assay as is
1	56	4352889	100.1	12	994320	100.2
2		4353794	100.1		991346	99.9
3		4335815	99.7		991747	99.9
4		4354611	100.1		995359	100.3
5		4339397	99.8		990540	99.8
6		4341324	99.8		994556	100.2
% RSD	0.19			0.21		
mean	99.9			100.1		
SD	0.186			0.207		

n=6

Table 6: LOD and LOQ for dexmethylphenidate

Serdexmethylphenidate				Dexmethylphenidate			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	Concentration	s/n	Concentration	s/n
1.68µg/ml	5	5.6µg/ml	26	0.36µg/ml	3	1.2µg/ml	22

Table 7: Robustness data of dexmethylphenidate

Parameter name	Serdexmethylphenidate			Dexmethylphenidate		
	Mean	SD	%RSD	Mean	SD	%RSD
Flow minus (0.8 ml/min)	99.7	0.252	0.25	99.7	0.205	0.21
Flow plus (1.2 ml/min)	99.5	0.265	0.27	99.9	0.379	0.38
Organic minus (63:37)	100.2	0.101	0.10	100.1	0.208	0.21
Organic plus (77:23)	100.1	0.265	0.26	99.9	0.265	0.27

RSD-Relative standard deviation; n=3

Acid degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 1N HCl and left it for 15 min. After 15 min add 1 ml of 1N NaOH and makeup to the diluent mark. Filter the solution using a syringe filter and injected it into HPLC system.

Alkali degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 1N NaOH and left it for 15 min. After 15 min add 1 ml of 1N HCl and make up to the mark. Filter the solution using syringe filter and injected into HPLC system.

Peroxide degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected it into HPLC system.

Reduction degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml and add 1 ml of 30% sodium bi sulphate solution and makeup to the mark with diluents. Filter the solution using a syringe filter and injected into HPLC system.

Thermal degradation

The sample solution was set in an oven at 105 °C for 6 h. The resultant solution was injected into HPLC system.

Hydrolysis degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml and add 1 ml of HPLC grade water and makeup to the mark with diluents. Filter the solution using syringe filter and injected it into HPLC system.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of dexmethylphenidate

Degradation condition	Serdexmethylphenidate		Dexmethylphenidate	
	% assay*	% degradation*	% assay*	% degradation*
Acid degradation	87.8	12.2	89.0	11.0
Alkali degradation	87.9	12.8	88.6	11.4
Peroxide degradation	86.4	13.6	87.5	12.5
Reduction degradation	88.6	11.4	80.6	9.4
Thermal degradation	97.9	2.1	98.1	1.9
Hydrolysis degradation	98.3	1.7	98.9	1.1

*Data expressed as mean; n=3

CONCLUSION

The developed method was accurate, precise and reliable for the simultaneous analysis of Serdexmethylphenidate and Dexmethylphenidate in pharmaceutical formulations. This method was validated for linearity, accuracy, precision, robustness, forced degradation of Serdexmethylphenidate and Dexmethylphenidate. The RSD values for all parameters were found to be less 2, which indicates the validity of the method and results obtained by this method are in fair agreement. Finally, this method can be used for better analysis of Serdexmethylphenidate and Dexmethylphenidate.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Kesselheim AS, Avorn J, Sarpatwari A. The high cost of prescription drugs in the United States: Origins and prospects for reform. JAMA. 2016;316(8):858-71. doi: 10.1001/jama.2016.11237, PMID 27552619.
2. Qato DM, Wilder J, Schumm LP, Gillet V, Alexander GC. Changes in prescription and over-the-counter medication and dietary supplement use among older adults in the united states, 2005 vs 2011. JAMA Intern Med. 2016;176(4):473-82. doi: 10.1001/jamainternmed.2015.8581, PMID 26998708.
3. de Cock M, Maas YG, van de Bor M. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? review. Acta Paediatr. 2012;101(8):811-8. doi: 10.1111/j.1651-2227.2012.02693.x, PMID 22458970.
4. Thapar A, Cooper M, Jefferies R, Stergiakouli E. What causes attention deficit hyperactivity disorder? Arch Dis Child. 2012;97(3):260-5. doi: 10.1136/arch dis child-2011-300482, PMID 21903599.
5. Paton DC, Collins BG. Bills and tongues of nectar-feeding birds: A review of morphology, function and performance, with intercontinental comparisons. Austral Ecol. 1989;14(4):473-506. doi: 10.1111/j.1442-9993.1989.tb01457.x.
6. Viniol A, Keunecke C, Biroga T, Stadje R, Dornieden K, Bosner S, Donner Banzhoff N, Haasenritter J, Becker A. Studies of the symptom abdominal pain--a systematic review and meta-analysis. Fam Pract. 2014;31(5):517-29. doi: 10.1093/fampra/cmu036, PMID 24987023.
7. Cartwright SL, Knudson MP. Evaluation of acute abdominal pain in adults. Am Fam Physician. 2008;77(7):971-8. PMID 18441863.
8. Langhans W. Anorexia of infection: current prospects. Nutrition. 2000;16(10):996-1005. doi: 10.1016/s0899-9007(00)00421-4, PMID 11054606.

9. Axelrod YK, Diringer MN. Temperature management in acute neurologic disorders. Neurol Clin. 2008;26(2):585-603, xi, xi. doi: 10.1016/j.ncl.2008.02.005, PMID 18514828.
10. Ludwig J, McWhinnie H. Antipyretic drugs in patients with fever and infection: a literature review. Br J Nurs. 2019;28(10):610-8. doi: 10.12968/bjon.2019.28.10.610, PMID 31116598.
11. McArdle P. Alcohol abuse in adolescents. Arch Dis Child. 2008;93(6):524-7. doi: 10.1136/adc.2007.115840, PMID 18305075.
12. Grant KM, LeVan TD, Wells SM, Li M, Stoltenberg SF, Gendelman HE, Carlo G, Bevins RA. Methamphetamine-associated psychosis. J Neuroimmune Pharmacol. 2012;7(1):113-39. doi: 10.1007/s11481-011-9288-1, PMID 21728034.
13. Rubart M, Zipes DP. Mechanisms of sudden cardiac death. J Clin Invest. 2005;115(9):2305-15. doi: 10.1172/JCI26381, PMID 16138184.
14. Sandroni C, D'Arrigo S, Nolan JP. Prognostication after cardiac arrest. Crit Care. 2018;22(1):150. doi: 10.1186/s13054-018-2060-7, PMID 29871657.
15. Berrios GE. Of mania: introduction (Classic text no. 57). Hist Psychiatry. 2004;15(57 Pt 1):105-24. doi: 10.1177/0957154X04041829, PMID 15104084.
16. Khan BQ, Kemp SF. Pathophysiology of anaphylaxis. Curr Opin Allergy Clin Immunol. 2011;11(4):319-25. doi: 10.1097/ACI.0b013e3283481ab6, PMID 21659865.
17. Gunn BG, Baram TZ. Stress and seizures: space, time and hippocampal circuits. Trends Neurosci. 2017;40(11):667-79. doi: 10.1016/j.tins.2017.08.004, PMID 28916130.
18. Moen MD, Keam SJ. Dexmethylphenidate extended-release: a review of its use in the treatment of attention-deficit hyperactivity disorder. CNS Drugs. 2009;23(12):1057-83. doi: 10.2165/11201140-000000000-00000, PMID 19958043.
19. Avois L, Robinson N, Saudan C, Baume N, Mangin P, Saugy M. Central nervous system stimulants and sport practice. Br J Sports Med. 2006;40;Suppl 1:i16-20. doi: 10.1136/bjsm.2006.027557, PMID 16799095.
20. Docherty JR. Pharmacology of stimulants is prohibited by the world anti-doping agency (WADA). Br J Pharmacol. 2008;154(3):606-22. doi: 10.1038/bjp.2008.124, PMID 18500382.
21. Cacabelos R, Torrellas C, Fernandez Novoa L, Lopez Munoz F. Histamine and immune biomarkers in CNS disorders. Mediators Inflamm. 2016;2016:1924603. doi: 10.1155/2016/1924603.
22. Haber PS, Day CA. Overview of substance use and treatment from Australia. Subst Abus. 2014;35(3):304-8. doi: 10.1080/08897077.2014.924466, PMID 24853496.
23. Guha M. Diagnostic and statistical manual of mental disorders: DSM-5. 5th ed. Reference Reviews. 2014;28:36-7.
24. Wang CF, Sun YL, Zang HX. Music therapy improves sleep quality in acute and chronic sleep disorders: a meta-analysis of 10 randomized studies. Int J Nurs Stud. 2014;51(1):51-62. doi: 10.1016/j.ijnurstu.2013.03.008.
25. Bhavani P, Prasada Rao K, Mohan S. Novel validated reversed-phase high-performance liquid chromatography method for determination of glucosamine, diacerein, and methyl sulfonyl methane in micro-sample rat plasma and its application to pharmacokinetic and dissolution studies. Asian J Pharm Clin Res. 2020;13:50-63. doi: 10.22159/ajpcr.2020.v13i12.36547.
26. Supriya T, Naresh D, Vijaya Kumar G, Haneer MA. Stability indicating RP-HPLC method development and validation for simultaneous estimation of escitalopram and flupentixol pure and marketed formulation. Asian J Pharm Res. 2018;8:4-10.
27. Naykode MD, Bhagwat DA, Jadhav SD, More HN. Analytical and bioanalytical method for quantification of pure azilsartan, not its salts by RP-HPLC. Res J Pharm Technol. 2017;10(3):708-14. doi: 10.5958/0974-360X.2017.00133.0.
28. Singh M, Charde M, Shukla R, Rita MC. Determination of calcipotriene in calcipotriene cream 0.05% w/w by RP-HPLC method development and validation. Res J Pharm Technol. 2011;4:1219-23.
29. Malathi S, Arunadevi N. Development and validation of stability-indicating simultaneous estimation of metformin and alogliptin in tablets by high-performance thin-layer chromatography. Int J Pharm Pharm Sci. 2020;12:68-73.
30. Manoranjani M. A study of method development, validation and forced degradation for simultaneous quantification of cisplatin and fluorouracil in bulk and pharmaceutical dosage form by RP-HPLC. J Pharm Sci Res. 2021;13:155-61.
31. Vijayakumari M, reddy Ch B. Stability indicating validated hplc method for the determination of zanubrutinib in the bulk and pharmaceutical dosage form. Asian J Pharm Clin Res. 2020;13:159-62.
32. Shivani CP, Maheshwari DG. Development and validation of UV spectrometric and HPLC method for estimation of escitalopram oxalate and flupentixol dihydrochloride in combined dosage form. AJPTI. 2016;4:59-70.
33. Shanmugasundaram P, Kamarapu SK. RP-HPLC method for the simultaneous estimation and validation of amlodipine besylate and atenolol in bulk and tablet dosage form in biorelevant dissolution medium (Fassif). Res J Pharm Technol. 2017;10(10):3379-85. doi: 10.5958/0974-360X.2017.00601.1.
34. International conference on harmonization. ICH harmonized tripartite guideline. Validation of analytical procedures: text and methodology. Vol. Q2(R1); 2005.
35. Raziq A Syed Umer Jan. Relative comparison of stability and degradation of methylcobalamin tablets of different brands at different storage settings. Int J Appl Pharm. 2021;13:171-5.
36. Syed Rafi, Kantipudi Rambabu. Stability indicating validated HPLC method for the determination of aceclofenac and misoprostol in bulk and pharmaceutical formulation. Int J Res Pharm Sci. 2020;11:7848-53.
37. Swati K, Abhishek P, Sushank S, Bothiraja C, Atmaram P. High-performance liquid chromatography for the simultaneous estimation of cefoperazone and sulbactam in rat plasma and its importance in therapeutic drug monitoring. Int J Pharm Pharm Sci. 2020;12:92-7.
38. Rajakumari R, Sreenivasa Rao S. Stress degradation studies and development of a validated RP-HPLC method for determination of tiagabine in the presence of its degradation products. Int J Pharm Pharm Sci. 2016;8:230-6.
39. Charu Pandya P, Sadhana Rajput J. Development and validation of stability-indicating method RP-HPLC method of acotiamide. Int J Pharm Pharm Sci. 2018;10:1-8.
40. Athavia BA, Dedania ZR, Dedania RR, Swamy SMV, Prajapati CB. Stability indicating HPLC method for determination of vilazodone hydrochloride. Int J Curr Pharm Sci 2017;9(4). doi: 10.22159/ijcpr.2017v9i4.20975.