INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RELATED SUBSTANCES OF LISDEXAMPHETAMINE DIMESYLATE BY RP-HPLC

Khushboo Pasbola *[1], Meenu Chaudhary [2], Praveen Chaudhary [2]

[1]Student, Shri Guru Ram Rai Institute of Technology and Sciences, Division of Pharmaceutical Science, Patel Nagar Dehradun, Uttarakhand, India

[2]Assistant Professor, Shri Guru Ram Rai Institute of Technology and Sciences, Division of Pharmaceutical Science, Patel Nagar Dehradun, Uttarakhand, India

*Corresponding Author Email: priyanka22pasbola@gmail.com

Article Received on: 18/05/17 Approved for publication: 19/06/17

DOI: 10.7897/2230-8407.08697

ABSTRACT

Reversed phase high performance liquid chromatography method, for estimation of related substances or chromatographic impurities of lisdexamphetamine dimesylate was developed and validated. A selected wavelength maximum for lisdexamphetamine dimesylate was 210nm. All peaks were well separated under selected chromatographic conditions. YMC Pack C_{18} (150 x 4.6), 5µm column was used for separation of lisdexamphetamine dimesylate and its related impurities. 1.0 mL/min flow rate and 55^{0}C column compartment temperature was the chromatographic conditions. Validation of Developed method was performed according to ICH (Q2R1). Linearity plot for lisdexamphetamine and its related impurities showed good concentration ranges 0.6ppm-2.4ppm for lisdexamphetamine and 0.6ppm- 3.6ppm for lisdexamphetamine dimesylate showed linear relation between concentration and peak areas, correlation coefficient for all components were within acceptance limit as >0.99. The precision of method was determined by method, system and intermediate precision which show % RSD under limits (<10%) for all impurities and lisdexamphetamine dimesylate. LOD and LOQ for all impurities and lisdexamphetamine dimesylate was 0.01% and 0.03% respectively with respect to test concentration. Accuracy was calculated as percentage recovery which was under acceptance criteria (90-110%) for all related impurities of lisdexamphetamine dimesylate. The robustness study was performed for flow rate variations (±0.1 mL/min) and column temperature variations (±5.0^{o}C), changes in retention time and resolution was negligible. The proposed method is simple as selected chromatographic conditions are not so difficult to apply in routine analysis for testing the chromatographic impurity of lisdexamphetamine dimesylate.

Keywords: RP-HPLC, Chromatographic Impurity, Related substances, Validation

INTRODUCTION

Lisdexamphetamine dimesylate (LIS) is a drug of choice for ADHD (Attention Deficit Hyperactivity Disorder) [1] and binge eating disorder.[2] It is inactive in nature because it is a prodrug which shows therapeutic effect after metabolism. The drug is converted into dextroamphetamine after metabolism which also shows central nervous system (CNS) stimulant action. Chemically in its structure it contains dextroamphetamine joint with L-lysine (Essential amino acid). IUPAC name for lisdexamphetamine dimesylate is (2s)-2, 6-diamino-N-[(2S)-1-phenylpropan-2-yl]hexanamide, (Figure 1). Pure drug is hydrophilic in nature so it is freely soluble in water.[1] Enzyme hydrolysis takes place after oral administration. Lisdexamphetamine breaks into L-lysine, a naturally occurring essential amino acid and active d-amphetamine which is the component responsible for the drug's activity.[3-4] GI pH does not change this conversion and the attachment of the L-lysine slows down the amount of d-amphetamine available to the blood stream and therefore to the CNS.[5] The pharmacological effects for lisdexamphetamine are show because of its converting nature to d-amphetamine which then actually acts with moderate potency to inhibit dopamine and norepinephrine transporter, the vesicular monoamine transporter. As a result, d-amphetamine increase catecholamines in synaptic space through transporter inhibition and reverse transport of catecholamines out of nerve terminal.[6]

For quality purposes, there are some important parameters like purity and efficacy of any pharmaceutical product for customer satisfaction should come under consideration during manufacturing a product. To check these important parameters of any pharmaceutical drug component a method for their analysis should be available and if doesn't available in official monographs, it can be developed and validated by own[7-8]. Chromatographic technique like HPLC is now a day mostly utilize for the qualitative and quantitative analysis of pure drug and combination of drugs.[4]

MATERIAL AND METHOD

Reagent & Solvents

1-Octane sulfonic acid (AR grade, Merck)
Acetonitrile (HPLC grade, Merck)
Orthophosphoric acid (AR grade, Spectrochem)
Water (HPLC grade, Merck)

Instruments

Uv-visible (Shimadzu, UC 1700)
HPLC (WATERS, Alliance 2695 seperation Module with 2996 PDA Detector)

Analytical balance (Precisa, EP225SM-DR)
Sonicator (Power sonic, 410)

Chromatographic Conditions: YMC Pack ODS (150 x 4.6), 5µm column was selected for the separation of all chromatographic impurities of lisdexamphetamine dimesylate. Mobile phase was octance-1-sulfonic acid sodium salt buffer and acetonitrile in gradient program of 55 minutes. Diluent was buffer and ACN in ratio 70:30. Flow rate 1 mL/min, column compartment temperature 55^0C, injection volume 15µL and wavelength maxima 210nm was selected for separation of all impurities and lisdexamphetamine dimesylate.

Sample Preparation: Accurately weigh and transfer 50mg test sample in 25ml volumetric flask. Add about 10ml of diluent to dissolve and make up the volume upto the mark with diluent. Sonicate the resultant solution and filled in a HPLC vial then injected for analysis.

Table 1: Retention time and Resolution of different components of lisdexamphetamine dimesylate and its related substances in trial VI

Name of component	Retention time	Resolution
Lisdexamphetamine	8.90	9.10
Impurity 1	4.72	0.00
Impurity 2	11.89	5.08
Impurity 3	28.91	45.06
Impurity 4	32.335	11.95
Impurity 5	34.434	7.84

Table 2: Percent relative standard deviation for all impurities

Day	Percent relative standard deviation				
	Impurity-1	Impurity-2	Impurity-3	Impurity-4	Impurity-5
1	5.1	3.2	NA	7.6	NA
2	4.1	4.2	NA	5.7	NA
Over all % RSD	5.4	4.2	NA	6.8	NA

Table 3: Selected LOD level and Signal to noise ratio

Compound	Concentration w.r.t. to Test preparation (%)	Signal to noise ratio
Impurity-1	0.01	5
Impurity-2	0.01	6
Impurity-3	0.01	5
Impurity-4	0.01	5
Impurity-5	0.01	6
Lisdexamphetamine	0.01	7

Table 4: Selected LOQ level and Signal to noise ratio

Compound	Conc. w.r.t. to test (%)	S/N ratio
Impurity-1	0.03	18
Impurity-2	0.03	20
Impurity-3	0.03	16
Impurity-4	0.03	19
Impurity-5	0.03	15
Lisdexamphetamine	0.03	18

Table 5: Intercept, Slope, Correlation coefficient and linear equation for Lisdexamphetamine dimesylate

Concentration (%)	Concentration w.r.t test preparation (%)	Concentration Spiked w.r.t test preparation (ppm)	Areas
LOQ%	0.03	0.6	8384
50%	0.075	1	28147
80%	0.12	1.6	49939
100%	0.15	2	68782
120%	0.18	2.4	89793
Intercept	**0.14**		
Slope	**0.46**		
Correlation coefficient	**0.994**		
Equation	**y = 0.46x-0.14**		

Table 6: Percentage recovery for individual Impurities

% Recovery				
Impurity-1	**Impurity-2**	**Impurity-3**	**Impurity-4**	**Impurity-5**
98%	96%	100%	95%	98%

Table 7(a): Resolution between all peaks by variation in flow rate

Flow Rate (mL/min)	Resolution					
	Imp-1	LIS	Imp-2	Imp-3	Imp-4	Imp-5
0.9	0.0	9.10	5.08	45.06	11.95	7.84
1.0	0.0	9.10	5.08	45.06	11.95	7.84
1.1	0.0	9.10	5.08	45.06	11.95	7.84

Table 7(b): Resolution between peaks by variation in Column temperature

Column Temperature (ºC)	Resolution					
	Imp-1	LIS	Imp-2	Imp-3	Imp-4	Imp-5
50	0.0	9.12	5.06	45.03	11.97	7.80
55	0.0	9.10	5.08	45.06	11.95	7.84
60	0.0	9.13	5.10	45.10	11.99	7.86

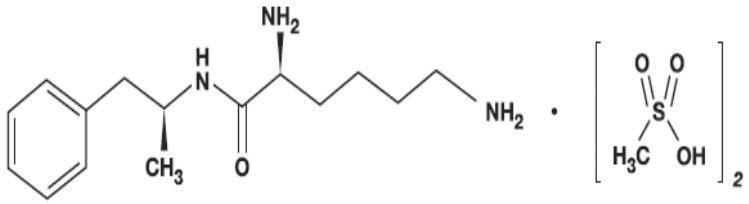

Figure 1: Structure of Lisdexamphetamine dimesylate

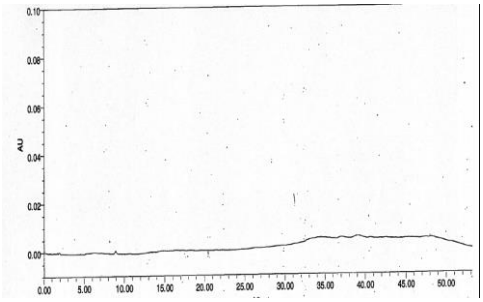

Figure 2(a): Chromatogram for blank solution

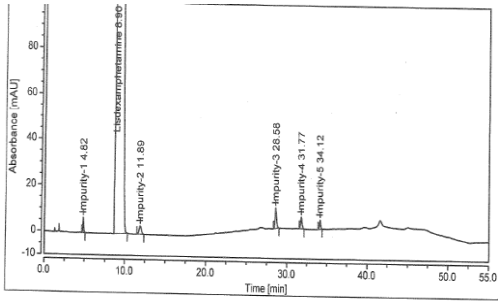

Figure 2(b): Chromatogram for test solution

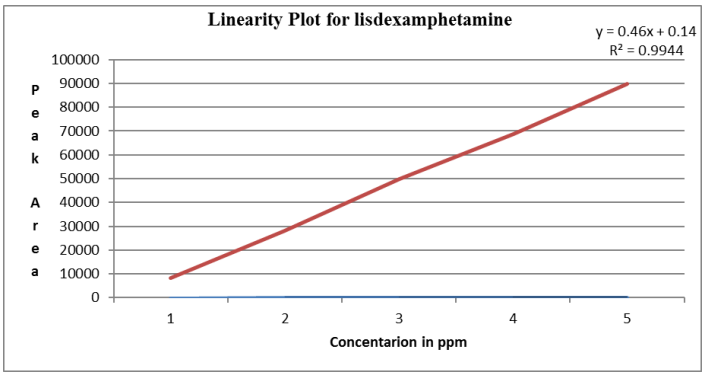

Figure 3: Linearity Plot for lisdexamphetamine dimesylate

RESULTS AND DISCUSSION

Method Development and Optimization

Solvent was selected on the basis of its solubility as the drug lisdexamphetamine dimesylate was freely soluble in water. Pure API scanned by Photo Diode Array (PDA) detector using Uv-vis spectroscopy under 200-400nm wavelength range. At 210nm maximum absorbance was observed. Using different chromatographic conditions, different trial was conducted. In trial I-II there weak separation between lisdexamphetamine and Imp-II as both peaks was not resolved properly, other impurities was not separated. In trial III-IV, by gradient program and change in column chemistry, other impurities like Imp-3, Imp-4, Imp-5 were separated but the resolution and other system suitability parameters was not complying. As BDS Hypersil C18 column show better resolution between Imp-3 and Imp-4 but Lisdexamphetamine and Imp-1 was not resolved. In trial V-VI, YMC columns show with given conditions show better peak separation. All peaks were well resolved and accurate retention time was found. So, the method VI was selected for chromatographic purity of lisdexamphetamine dimesylate. Retention time and resolution between peaks illustrated in Table 1. Chromatograms for blank and test were shown in Figure-2(a&b). Developed method was optimized for checking the

stability of lisdexamphetamine dimesylate peak and all related peaks, in the same chromatographic conditions. All peaks were in good shape as retention time and resolution for all peaks were same as previously detect.

Method Validation

Specificity: There was no interference in any peaks. There was no change in retention time, resolution and relative retention time between individual preparation and composite preparation of all components.

Precision: System Precision, Method Precision and Intermediate Precision were performed. All results was under acceptance criteria as obtained percentage relative standard deviation for areas of lisdexamphetamine dimesylate and all related impurities, were less than 10%. Results obtained are listed in Table 2.

Limit of Detection (LOD): The LOD was determined for lisdexamphetamine dimesylate and its related impurities. Calculated LOD values for all components was under acceptance limits of signal to noise ratio (>3). Results are shown in Table 3.

Limit of Quantification (LOQ): The LOQ was determined for lisdexamphetamine dimesylate and its related impurities. The LOQ level for all components was under limits of signal to noise ratio (>10). Results are shown in Table 4.

Linearity: Linearity was calculated for lisdexamphetamine and its related impurities in five different concentrations ranging from LOQ to 120% with respect to specification limits for drug and its related impurities. Correlation coefficient for linear equation was under limit (Not less than 0.99). Obtained results are shown for lisdexamphetamine dimesylate in Table 5. Calibration curve was plotted for lisdexamphetamine dimesylate and mentioned as Figure 3.

Accuracy: Accuracy was calculated as percentage recovery which was under acceptance criteria (90-110%) for all related impurities of lisdexamphetamine dimesylate. Results are tabulated in Table 6.

Robustness: The robustness study was performed for effect of flow rate and effect of column temperature. The flow rate of mobile phase was varied by $\pm$0.1 mL/min and the effect of same on system suitability were studied. There was no change in resolution between peaks. The results obtained are tabulated in Table 7(a). The column temperature was varied by$\pm$5.0ºC and the effect of sample of same on system suitability were studied. There was negligible variation in Resolution between peaks. The results obtained are recorded in Table 7(b).

CONCLUSION

A Simple, accurate and precise method for estimation of chromatographic impurities of lisdexamphetamine dimesylate, was developed and validated. All peaks were well resolved from each other and separate with an appropriate retention time. Selected chromatographic conditions for separation of all impurities were same so it is easy to estimate all impurities in a single time from a test sample of lisdexamphetamine dimesylate.

REFERENCES

1. Eloisa Comiran, Fabiano Barreto, Leonardo Z. Meneghini, Graciela Carlos, Pedro E. Froehlich, Renata Pereira Limberger. Method validation and determination of lisdexamphetamine and amphetamine in oral fluid, plasma and urine by LC–MS/MS. Biomedical Chromatography 2017; 31(3):1523-1525.
2. Fda.gov. FDA News Release: FDA expands uses of Vyvanse to treat binge-eating disorder [updated 2015 January 30; cited 2017 May 5]. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncement/ucm432543.htm.
3. James E. Frampton. Lisdexamphetamine: A Review in ADHD in Adults. Springer International Publishing [Online ISSN 1179-1934]. 2016 April [cited 2017 May 6]; 30(4): 343. Available from: https://link.springer.com/article/10.1007/s40263-016-0327-6.
4. Graciela Carlos, Eloisa Comiran, Marcella Herbstrith de Oliveira, Renata Pereira Limberger, Ana Maria Bergold, Pedro Eduardo Froehlich. Development, validation and comparison of two stability-indicating RP-LC methods using charged aerosol and UV detectors for analysis of lisdexamphetamine dimesylate in capsules. Arabian Journal of Chemistry 2015; 9:1905-1914.
5. WHO; Expert Committee on Drug Dependence. 2014; [cited on 2017 May 5]. Available from: http://www.who.int/medicines/areas/quality_safety/5_1_Preview.pdf.
6. Ann C Childress and Floyd R Sallee. The use of lisdexamphetamine dimesylate for the treatment of ADHD. Journal Expert of Neurotherapeutics 2012; 12(1): 13-26.
7. USP United States Pharmacopoeia, 31st edition NF 26. United States Pharmacopoeia Convention, Asian edition, Rockville 2008; 683-687.
8. ICH, Validation of Analytical Procedures: Text and Methodology Q2(R1); International Conference on Harmonization, Geneva. 1996; [cited on 2017 May 5]. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf.

Cite this article as:

Khushboo Pasbola *et al*. Development and validation for estimation of related substances of Lisdexamphetamine dimesylate by RP-HPLC. Int. Res. J. Pharm. 2017;8(6):59-62 http://dx.doi.org/10.7897/2230-8407.08697

Source of support: Nil, Conflict of interest: None Declared