World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Development and validation of UV spectroscopic Q-absorbance ratio method for zonisamide and aripirazole in synthetic mixture

Naimuddin Riyazuddin Saiyed [1,*], Vineet C Jain [2], Zarana Dedania [3], Harshad K Patel [1] and Rajendrakumar C. Patel [1]

[1] *Research Scholar, Department of Pharmaceutical Science, Bhagwan Mahavir University, BMEF Campus, Nr. Aakash E-Space, Bharthana, Vesu, Surat-395017.*
[2] *Principal and Professor, Bhagwan Mahavir University, BMEF Campus, Nr. Aakash E-Space, Bharthana, Vesu, Surat-395017.*
[3] *Professor and Head, Pharmaceutical Quality Assurance of Bhagwan Mahavir University, BMEF Campus, Nr. Aakash E-Space, Bharthana, Vesu, Surat-395017.*

World Journal of Biology Pharmacy and Health Sciences, 2023, 13(02), 266–276

Publication history: Received on 06 December 2022; revised on 26 January 2023; accepted on 28 January 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.13.2.0043

Abstract

A simple, specific, accurate and precise Q-Absorbance ration spectrophotometric method was developed and validated for estimation of Zonisamide and Aripirazole in Synthetic Mixture. Zonisamide and Aripirazole showed and iso-absorptive point at 230.50nm in Distilled water. The second wavelength used was 240.60nm which is λmax of Aripirazole in distilled water. The concentration of the drugs was determined by using ratio of absorbance at iso-absorptive point (λ1 =240.60 nm) and at the λmax of Aripirazole (λ2 =230.50 nm). This method is linear for both drugs; in range 10-30 µg/ml at λ1 R2 = 0.996 at λ2 (R2 = 0.997) for Aripirazole, and in the range of 10–30 µg/mL for Zonisamide found at λ1 (R2 = 0.990) and at λ2 (R2 = 0.996). The % Recovery was 101.12 % of Aripirazole and 101.89 % of Zonisamide by standard addition method. The LOD was found to be 0.084 µg/mL and 0.110 µg/mL for both drugs at λ1 and λ2 respectively. The LOQ was found to be 0.257 µg/mL and 0.330 µg/mL for both drugs at λ1 and λ2 respectively. The method was found to be precise as % RSD was less than 2.00 in Repeatability, Interday and Intraday precision for Zonisamide and Aripirazole. The % assay of analyte drugs in synthetic mixture was found to be 100.48 % of Zonisamide which showed good applicability of the developed method.

Keywords: Zonisamide; Aripirazole; Q-Absorbance Ratio method; UV Spectroscopy

1 Introduction

One of the most common Neurodegenerative disorders amongst the elderly, leading to Dementia. The term 'Dementia' refers to several illnesses, which affect the functioning of the brain, leading to disruptions in memory, reasoning and emotional stability. In India, dementia is commonly associated with cerebrovascular disease. Earlier for the loss of memory drugs that improve blood flow like amphetamine, Pentoxyphylline were prescribed. Evidence of decrease in cholinergic mechanisms in Alzheimer's disease led to use of cholinergic drugs.

Zonisamide with Aripirazole on ECT- and Benzodiazepine-Resistant periodic catatonia, J Neuropsychiatry Clin Neurosci 24:3, 2012. Hence, there is a scope to develop analytical methods for Zonisamide and Aripirazole in combination.

Literature review reveals that, various analytical methods have been reported for the estimation of Zonisamide and Aripirazole in biological fluids, pharmaceutical formulation and bulk drug include UV spectrophotometric, High-

* Corresponding author: Naimuddin Riyazuddin Saiyed

performance liquid chromatography method (HPLC), Stability indicating RP-HPLC method, HPTLC method, TLC method, LC/MS/MS method and UPLC method in individual and/or in combination of other drug.

Literature review shows that, there is no reported method available for Q-absorbance estimation of both the drugs in combination. Therefore it is thought of interest to developed simple, accurate, precise and rapid methods for Q-absorbance estimation of Zonisamide and Aripirazole in combination.

2 Material and methods

2.1 Instrument and apparatus

Table 1 Lists of Instrument and Apparatus

Component	Model/Software	Manufacturer
Double Beam UV-Visible Spectrophotometer	Shimadzu-2450, UV Probe 2.34	Shimadzu
Analytical Balance	Sartorius (CD 2250)	Wensar
Volumetric Flask	-	Borosil
Pipettes	-	Borosil
Beaker	-	Borosil

2.2 Reagents and material

All the Reagents and Solvents used were of AR or HPLC grades.

Table 2 Working Standard API

Standard	Purpose	Source
Zonisamide	Analysis	Sun Pharmaceuticals Industries Ltd.
Aripirazole	Analysis	Sun Pharmaceuticals Industries Ltd.

2.3 Preparation of solutions

2.3.1 Standard Stock Solution of Zonisamide (ZON)

Accurately weighed quantity of ZON 10 mg was transferred to 100ml volumetric flask, add 5 ml of Methanol and 20 ml of water, sonicate it for 15min and dilute it up to the mark with Water to make 100µg/ml solution of ZON

2.3.2 Standard Stock Solution of Aripirazole (APZ)

Accurately weighed quantity of APZ 10 mg was transferred to 100ml volumetric flask, add 5 ml of Methanol and 20 ml of water, sonicate it for 15min and dilute it up to the mark with Water to make 100µg/ml solution of APZ.

2.3.3 Preparation of Standard Mixture Solution (ZON + APZ):

Take 10mg ZON and 10 mg APZ in 100ml volumetric flask. Add 5 ml of Methanol and 20 ml of water, sonicate it for 15min and dilute it up to the mark with Water to make 100µg/ml solution of ZON and 100µg/ml solution of APZ.

2.4 Q-absorbance method

2.4.1 Spectrophotometric condition

Table 3 Spectrophotometric conditions for Spectroscopic Method

Mode	Spectrum
Scan Speed	Medium
Wavelength Range	400-200 nm
Initial base line correction	Distilled Water

2.5 Preparation of calibration curve

2.5.1 Calibration Curve for Zonisamide

This series consisted of five concentrations of standard ZON solution ranging from 10-30µg/ml. The solutions were prepared by pipetting out Standard ZON stock solution (1ml, 1.5ml, 2ml, 2.5ml, 3ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with water. A zero-order derivative spectrum of the resulting solution was recorded and, measured the absorbance at 231.50 nm against a reagent blank solution (Water). Calibration curve was prepared by plotting absorbance versus respective concentration of ZON.

2.5.2 Calibration Curve for Cilostazol

This series consisted of five concentrations of standard APZ solution ranging from 10-30µg/ml. The solutions were prepared by pipetting out Standard APZ stock solution (1ml, 1.5ml, 2ml, 2.5ml, 3ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with Water. A zero-order derivative spectrum of the resulting solution was recorded and, measure the absorbance at 240.60 nm against a reagent blank solution (Water). Calibration curve was prepared by plotting absorbance versus respective concentration of APZ.

2.6 Validation of proposed method

2.6.1 Linearity and range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10-30µg/ml and 10-30µg/ml for Zonisamide and Aripirazole respectively (n=5).

2.7 Precision

2.7.1 Intraday Precision

The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three Standard solutions containing concentrations 26,28,30 µg/ml for ZON and 26,28,30 µg/ml for APZ and three replicate (n=3) each on same day. Q-Absorbance Ratio was measured at 231.50 nm for ZON and 240.60nm for APZ. The % RSD value of the results corresponding to the absorbance was expressed for intra-day precision

2.7.2 Interday Precision

The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three Standard solutions containing concentrations 26,28,30 µg/ml for ZON and 26,28,30 µg/ml for APZ and three replicate (n=3) each on different day. Q-Absorbance Ratio was measured at 231.50 nm for ZON and 240.60nm for APZ. The % RSD value of the results corresponding to the absorbance was expressed for inter-day precision.

2.8 Accuracy

It was determined by calculating the recovery of ZON and APZ by standard addition method. Accuracy was done by adding both API standard solution and test solution.

Each solution was taken and diluted with Distilled Water up to 10ml volumetric flask and scanned between 200nm to 400nm against Distilled Water as a blank. The amount of ZON and APZ was calculated at each level and % recoveries were computed.

Table 4 Solutions for Accuracy Study

Concentration of Formulation (µg/ml)		Concentration of API in spiking solution (µg/ml)		Total concentration of (µg/ml)	
ZON	**APZ**	**ZON**	**APZ**	**ZON**	**APZ**
10	00	-	-	10	-
10	00	08	08	18	08
10	00	10	10	20	10
10	00	12	12	22	12

2.9 LOD and LOQ

The Limit of detection and Limit of Quantification of the developed method was assessed by analyzing ten replicates of standard solutions containing concentrations 10 µg/ml for ZON and 10 µg/ml for APZ.

The LOD and LOQ may be calculated as

$$LOD = 3.3 \times \frac{SD}{Slope}$$

$$LOQ = 10 \times \frac{SD}{Slope}$$

Where,

- SD = ten replicates of absorbance
- Slope = the mean slope of the 6 calibration curves

2.10 Robustness & ruggedness

- Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:
- Change in Analyst-1 and Analyst-2.
- Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450),
- % RSD was calculated.

2.11 Assay by UV spectrophotometric method

- Synthetic mixture was taken in water. Take 10mg ZON and 10 mg APZ in 100ml volumetric flask. conc of Zonisamide and Aripirazole was 100 µg/mL. From which 2.5 ml transferred in 10 ml volumetric flask and made up to the mark with the water. Final formulation contained 25µg/mL ZON and 25µg/mL APZ.
- Label claim = 25 mg Zonisamide
- Excipients = q.s.
- Dissolve 25 mg ZON in 250 ml distilled water.
- Take 2.5 ml in 10 ml volumetric flask and dilute up to the mark with distilled water.
- So Final formulation contained 25µg/mL ZON.

3 Result and discussion

The methods were validated with respect ICH Q_2R_1 guidelines.

The standard solution of ZON and APZ were scanned separately between 200-400nm, and zero-order spectra were showed overlapping peaks. (figure: 1)

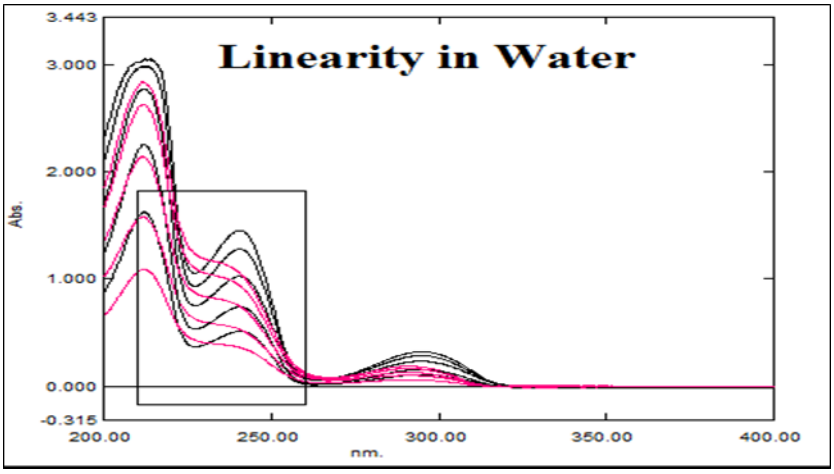

Figure 1 Overlain zero order spectra of ZON and APZ (1:1) ratios, respectively

Thus, obtained spectra were then processed to obtain Q-Absorbance Ratio Spectrophotometric Method Iso absorptive point is 231.50 nm. λmax of Zonisamide 289.60 nm and λmax of Aripirazole is 240.60 nm.

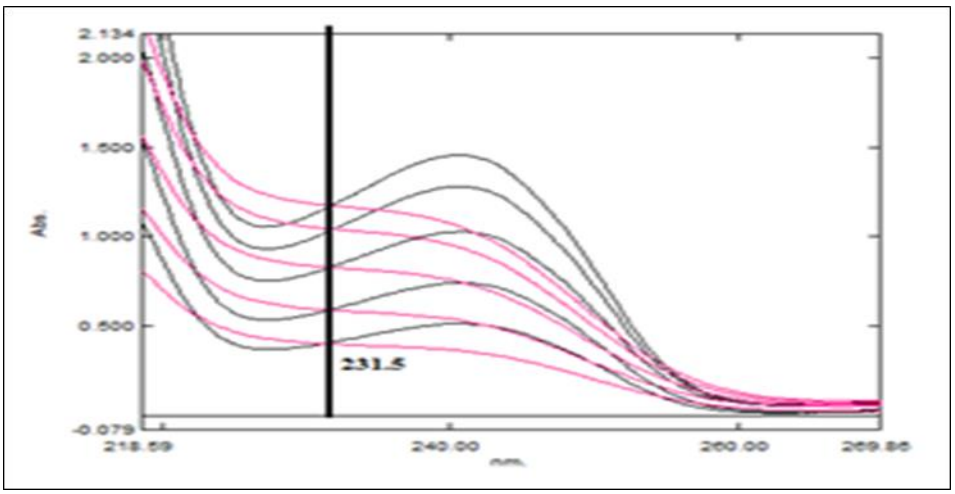

Figure 2 Overlain first order spectra of ZON and APZ in 1:1 ratio, Respectively Showing Iso absorptive point

3.1 Validation of proposed spectrophotometric method for q-absorbance method

3.1.1 Linearity and range

The Q-Absorbance Ratio Spectrophotometric Method (fig.6.2.1) showed linear absorbance at 231.50 nm (Iso absorptive point) for ZON (10-30µg/ml) and 240.60 nm for APZ (10-30µg/ml) with correlation coefficient (r2) of 0.9961 and 0.9973 for ZON and APZ, respectively.

This method obeyed beer's law in the concentration range 10-30 µg/ml and 10-30µg/ml for ZON and APZ, respectively.

Correlation coefficient (r2) form calibration curve of ZON and APZ was found to be 0.9961 and 0.9973, respectively

The regression line equation for ZON and APZ are as following,

$$y = -0.055x - 0.0527 \text{ for ZON} \quad (1)$$

$$y = -0.0631x - 0.02 \text{ for APZ} \quad (2)$$

Table 5 Calibration data for ZON and APZ at231.50nm and 240.60nm, respectively. *(n=6)

Conc. (µg/ml)	ZON Mean Abs. ± SD (231.50nm)	ZON Mean Abs. ±SD (240.60nm)	APZ Mean Abs. ± SD (231.50nm)	APZ Mean Abs. ± SD (240.60nm)
10	0.577±0.0012	0.563±0.0004	0.581±0.0007	0.640±0.0008
15	0.897±0.0008	0.874±0.0005	0.895±0.0029	0.965±0.0005
20	1.184±0.0010	1.152±0.0023	1.187±0.0010	1.281±0.0007
25	1.397±0.0009	1.340±0.0010	1.399±0.0005	1.637±0.0007
30	1.701±0.0004	1.561±0.0018	1.710±0.0008	1.881±0.0008

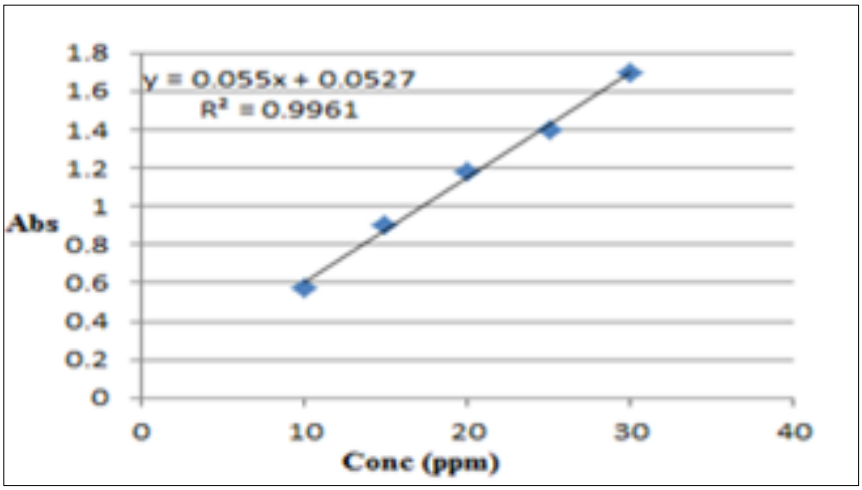

Figure 3 Calibration curve for ZONISAMIDE at 231.50nm

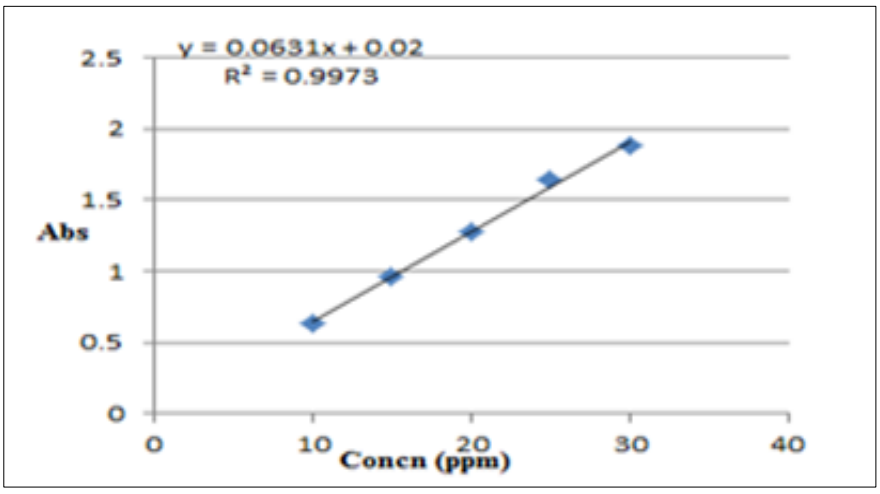

Figure 4 Calibration curve for APZ at 240.60nm

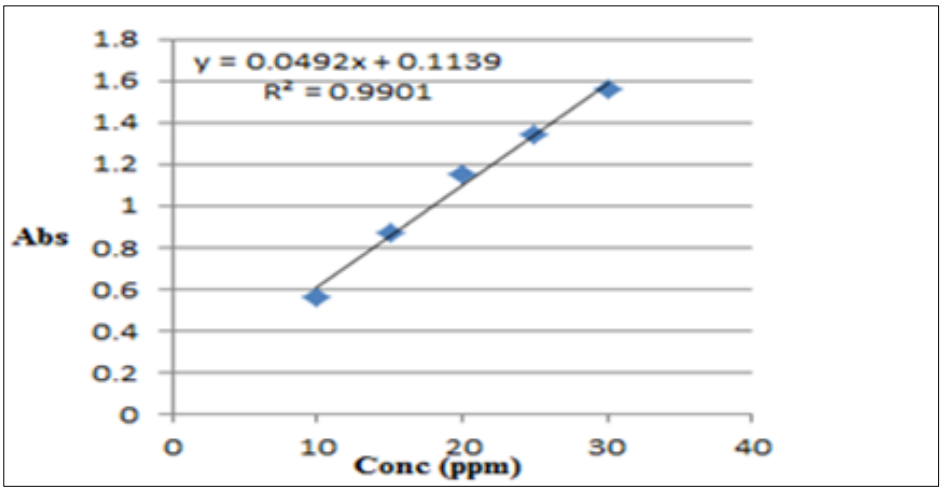

Figure 5 Calibration curve for ZONISAMIDE at 240.60nm

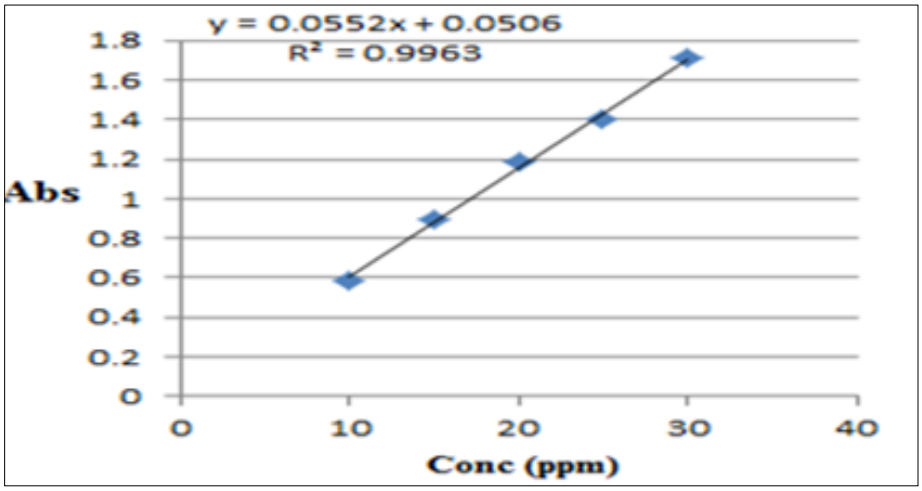

Figure 6 Calibration curve for APZ at 231.50nm

3.2 Precision

3.2.1 Intraday precision

- The % R.S.D was found to be 0.062-0.120 % for ZON and 0.038-0.082% for APZ.

Table 6 Intraday precision data for estimation of ZON and APZ *(n=3)

Conc. (µg/ml)		Abs.* (ZON) Avg. ± SD(231.50nm)	% RSD	Abs. (APZ)* Avg.± SD(240.60nm)	% RSD
ZON	APZ				
26	26	2.453 ±0.001	0.062	2.628 ±0.001	0.038
28	28	2.642 ±0.002	0.078	2.833 ±0.001	0.061
30	30	2.831 ±0.003	0.120	3.032 ±0.002	0.082

3.2.2 Interday precision

- The % R.S.D was found to be 0.084-0.140 % for ZON and 0.070-0.079 % for APZ.

Table 7 Interday precision data for estimation of ZON and APZ *(n=3)

Conc. (µg/ml)		Abs.* (ZON) Avg. ± SD(231.50nm)	% RSD	Abs. (APZ)* Avg.± SD(240.60nm)	% RSD
ZON	APZ				
26	26	2.456 ±0.002	0.084	2.633 ±0.002	0.079
28	28	2.645 ±0.002	0.095	2.836 ±0.002	0.070
30	30	2.833 ±0.004	0.140	3.037 ±0.002	0.076

3.3 Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition. The % recovery values are tabulated in Table 5 and 6. Percentage recovery for ZON and APZ by this method was found in the range of 99.95 to 100.12% and 99.57 to 100.25%, respectively. The value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 8 Recovery data of ZON *(n=3)

Conc. of ZON from formulation (µg/ml)	Amount of Std. ZON added (µg/ml)	Total amount of ZON(µg/ml)	Total amount of ZON found (µg/ml) Mean* ± SD	% Recovery* (n=3)	% RSD ZON
10	00	10	09.98±0.002	99.80	0.200
10	08	18	18.32± 0.001	101.80	0.098
10	10	20	20.42± 0.002	102.10	0.195
10	12	22	21.88±0.0003	99.45	0.030

Table 9 Recovery data of APZ*(n=3)

Conc. of APZ from formulation (µg/ml)	Amount of Std. APZ added (µg/ml)	Total amount of APZ (µg/ml)	Total amount of CIT found (µg/ml) Mean* ± SD	% Recovery* (n=3)	% RSD APZ
00	00	00	-	-	-
10	08	18	7.95±0.002	99.38	0.020
10	10	20	10.48±0.002	104.80	0.252
10	12	22	12.18±0.003	101.50	0.189

3.4 LOD and LOQ

Table 10 LOD and LOQ data of ZON and APZ *(n=10)

Conc. (µg/ml)		Zonisamide			APZ		
ZON	APZ	Avg±SD (n=3)	%RSD	Slop	Avg±SD (n=3)	%RSD	Slop
10	10	0.577±0.0014	0.245	0.055	0.641±0.0021	0.328	0.0631
LOD (µg/ml)		0.084			0.110		
LOQ (µg/ml)		0.257			0.334		

3.5 Robustness & ruggedness

Table 11 Robustness and Ruggedness data of ZON and APZ *(n=3)

Condition	Concentration (µg/ml)	Different Analyst			
		Analyst 1 Abs. ± SD	Analyst 2 Abs. ± SD	Analyst 1 %RSD	Analyst 2 %RSD
ZON (231.50 nm)	26	2.451±0.002	2.454±0.002	0.081	0.100
	28	2.640±0.001	2.643±0.002	0.057	0.078
	30	2.831±0.003	2.834±0.002	0.100	0.073
APZ (240.60 nm)	26	2.630±0.001	2.633±0.002	0.058	0.075
	28	2.833±0.002	2.834±0.001	0.073	0.053
	30	3.033±0.002	3.035±0.001	0.068	0.032

Table 12 Robustness and Ruggedness data of ZON and APZ *(n=3)

Condition	Concentration (µg/ml)	Different Instrument			
		UV 2450 Abs. ± SD	UV 1800 Abs. ± SD	UV 2450 %RSD	UV 1800 %RSD
LEV (231.50 nm)	26	2.452±0.001	2.454±0.001	0.040	0.062
	28	2.643±0.002	2.645±0.002	0.075	0.095
	30	2.832±0.002	2.835±0.002	0.081	0.093
APZ (240.60 nm)	26	2.628±0.0005	2.631±0.0010	0.021	0.038
	28	2.831±0.0011	2.834±0.0020	0.040	0.070
	30	3.031±0.0020	3.035±0.0005	0.068	0.019

3.6 Application of the proposed method for analysis of ZON and APZ in synthetic mixture

Table 13 Analysis data of ZON and APZ in Synthetic Mixture *(n=3)

Drug	Conc taken(µg/ml)	Avg±SD (n=3)	Conc found(µg/ml)	% found	Limit	%RSD
ZON	25	2.276±0.002	25.025	100.10%	98.5 -101 %	0.11
APZ	25	2.241±0.003	24.7	98.80%	-	0.13

- Label claim = 25 mg Zonisamide
- Excipients = q.s.
- Dissolve 25 mg ZON in 250 ml distilled water.
- Take 2.5 ml in 10 ml volumetric flask and dilute up to the mark with distilled water.
- So Final formulation contained 25µg/mL ZON.

Table 14 Analysis data of ZON and APZ in commercial formulation *(n=3)

Drug	Conc taken(µg/ml)	Avg±SD (n=3)	Conc found(µg/ml)	% found	Limit	%RSD
ZON	25	1.205±0.001	25.12	100.48%	98.5 -101 %	0.12
APZ	-	-	-	-	-	-

Table 15 Summary of validation parameters

Sr no.	Parameters	Q absorbance Ratio Method	
		ZON	**APZ**
1.	λmax (nm)	231.50 nm	240.60nm
2.	Linearity range (µg/mL)	10 - 30	10 – 30
3.	Regression equation	Y =0.055x +0.0527	Y =0.0631x+0.02
4.	Correlation coefficient (r^2)	0.9961	0.9973
5.	Precision		
	Intraday % RSD (n = 3)	0.062 – 0.120	0.038 – 0.082
	Inter day % RSD (n = 3)	0.084 – 0.140	0.070 – 0.079
6.	Accuracy % Recovery (n = 3)	101.12 %	101.89%
7.	Limit of detection (µg/mL) (n = 10)	0.084	0.110
8.	Limit of quantification(µg/mL) (n = 10)	0.257	0.330
9.	Assay %	100.48%	–

4 Conclusion

All the parameters for two substances met the criteria of the ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Ruggedness and Robustness of method was confirmed as no significant were observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in fair agreement. The method is validated as per ICH Q_2R_1Guidelines.

Compliance with ethical standards

Acknowledgments

The authors are thankful to Dr. Vineet C. Jain, Director and Principal of Bhagwan Mahavir College of Pharmacy for providing all facilities for my research work and Sun Pharmaceuticals Industries Ltd. for providing drug samples Zonisamide and Aripirazole to carry out this work.

Disclosure of conflict of interest

No conflict of interest.

References

[1] R.Valarmathi, C. S. Dhivya Dhharshini, R. Senthamarai and S. Farisha Banu "Simple UV Spectrophotometric method development and Validation for determination of Lacosamide in bulk and its Tablet Dosage Form" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 373-376.

[2] C Kestelyn , M Lastelle, N Higuet, S Dell'Aiera, L Staelens, P Boulanger, H Boekens and S Smith, "A simple HPLC-UV method for the determination of lacosamide in human plasma", Bioscience, 2011 Nov;3(22):2515-22. doi: 10.4155/bio.11.261.

[3] Seenuvasan, R E, "Development and Validation of New Analytical Methods for the Estimation of Lacosamide by UV Spectroscopy, RP-HPLC and HPTLC Method. Masters thesis, Adhiparasakthi College of Pharmacy, Melmaruvathur, 2012.

[4] Rajeshri D. Chaudhari, vishruti H. Choksi, Tanvi Divan, Bhavna A. Patel and Shraddha J. Parmar, "Development and validation of first order derivative spectrophotometric method for estimation of lacosamide in bulk and tablet dosage form", Asian J Pharm Clin Res, Vol 6, Supply 3, 2013, 162-164.

[5] V.Kalyan Chakravarthy and D.Gowri Sankar, "Development And Validation Of Rp-hplc Method For Estimation Of Lacosamide In Bulk And Its Pharmaceutical Formulation", Rasayan J. Chem. Vol.4, No.3 (2011), 666-672.

[6] R. Valarmathi*, S. Farisha Banu, S. Akilandeswari, R. Senthamarai and CS. Dhivya Dhharshini, "A Review on New Antiepileptic Drug – Lacosamide and its Analytical Methods", IJPCS, 2013, Vol.2 (1), Jan-Mar, 181-184.

[7] Shaik Nazma*, P. Yasaswini, M. Sai Supraja, M. Vijayalakshmi and Buchi N Nallur, "Development And Validation Of RP-HPLC Method For The Estimation Of Lacosamide In Bulk And Parenteral Dosage Form ", IJRPC 2015, 5(2), 355-360.

[8] Ganji Ramanaiah, D. Ramachandran, G.Srinivas, Srilakshmi and Purnachanda Rao, "Development and validation of UV spectroscopy method for estimation of lacosamide in bulk and formulations", IJPBS, 2012, Vol.03(01).

[9] M.S.Kondawar and S.R.Shinde, "Development and Validation of Bioanalytical Method for Lacosamide from Rat Brain Tissues", IJPRIF 2012, Vol.4, No.4, 1417-1421.

[10] Usmangani K. Chhalotiya, Kashyap K. Bhatt, Dimal A. Shah, Sunil l. Baldania, and Jigar R. Patel, "Stability-indicating Liquid Chromatographic Method For Quantification Of New Anti-epileptic Drug Lacosamide In Bulk And Pharmaceutical Formulation", Chemical Industry & Chemical Engineering Quarterly, 2012, 18 (1), 35–42.

[11] Kishore Sonkusare and Hemant V. Barkate, "Lacosamide as monotherapy in focal seizure: Literature review", IJJEP, 2017.

[12] Mahesh H. R. K. and Sudhakar K Babu , "Quantitative estimation of related compounds of lacosamide in oral solution by using reverse phase HPLC", Schloars Research Library, 2015, 7 (1):285-291.

[13] Jarogniew J Luszczki , Mirosław Zagaja, Barbara Miziak, Maria W Kondrat-Wrobel, Katarzyna Zaluska, Paula Wroblewska-Luczka, Piotr Adamczuk, Stanislaw J Czuczwar, Magdalena Florek-Luszczki, "Beneficial Combination of Lacosamide with Retigabine in Experimental Animals: An Isobolographic Analysis", Pharmacology, 2018;101(1-2):22-28.

[14] Natalia Casado, Zhengjin Jiang, María Ángeles García and María Luisa Marina, "Enantiomeric Separation of Colchicine and Lacosamide by Nano-LC. Quantitative Analysis in Pharmaceutical Formulations", Separation, 2020; 7 (55), 10-12.

[15] EP2992891A1; Pharmaceutical Composition Comprising Brivaracetam and Lacosamide with Synergistic Anticonvulsant Effect, Dressen, Frank, UCB Pharma GmbH 40789 Monheim,2016.

[16] Masanori Nakagawa, Satoshi Yamamura, Eishi Motomura, Takashi Shiroyama, Hisashi Tanii, and Motohiro Okada; "Combination Therapy of Zonisamide With Aripiprazole on ECT- and Benzodiazepine-Resistant Periodic Catatonia", J Neuropsychiatry Clin Neurosci 24:3, 2012.

[17] ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005