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STABILITY-INDICATING SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF RISPERIDONE IN PURE FORM AND PHARMACEUTICAL PREPARATION.

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ABSTRACT

Three simple, rapid, sensitive, accurate and precise methods were developed for the determination of risperidone in bulk powder, in pharmaceutical preparation and in presence of its degradate.Method (A) ratio difference method; is based on measuring the difference in the amplitude of intact risperidone in presence of its degradation product at two different wavelengths, this is done at 240 nm and 287 nm in the range of 5 – 35 µg ml^{-1} with LOD of 0.0053 µg ml^{-1} and LOQ of 0.018 µg ml^{-1}.Method (B) mean centering method; the method was applied for analysis of risperidone in presence of its degradation product this is done at 285.6 nm in the range of 5 – 35 µg ml^{-1} with LOD of 0.161 µg ml^{-1} and LOQ of 0.535 µg ml^{-1}. Method (C) Derivative ratio method(1DD); is used for the determination of intact risperidone in presence of its degradation product at 231 nm in the range of 5 – 35 µg ml^{-1} with LOD of 0.087 µg ml^{-1} and LOQ of 0.289 µg ml^{-1}. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

KEYWORDS: Risperidone, Ratio difference, Mean centering, Derivative ratio.

INTRODUCTION

Risperidone (Fig.1) is 4-[2-[4-(6-fluorobenzo [d] isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one,belongs to the chemical class of benzisoxazole derivatives. It is an atypical antipsychotic agent and acts through selective antagonism of

serotonin $5HT_2$, dopamine D_2 receptors[1]. Clinically, it is used in the treatment of schizophrenia and other psychoses [2]. The therapeutic importance of the drug has promoted the development of several analytical methods for its quantitative determination. The British Pharmacopeia adopts a non-aqueous titrimetric method for the determination of risperidone[3]. Other analytical techniques include several spectrophotometric[4-10], polarographic[11] and chromatographic[12-18] methods for determination of risperidone in pure form, pharmaceutical preparations and/or biological fluids have been reported.

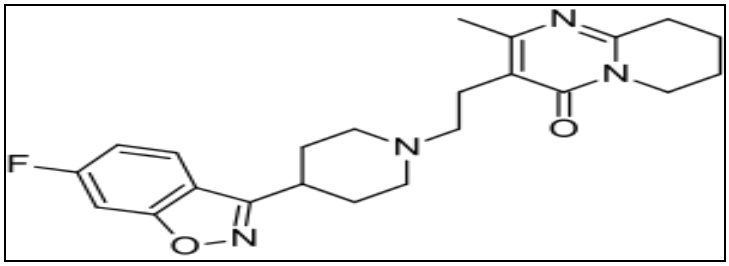

Figure 1: Structural formula of Risperidone

Under computer-controlled instrumentation, ratio difference, mean centering and derivative ratio methods are playing a very important role in the analysis of risperidone in presence of its degradation product without previous separation by UV–VIS spectrophotometry[19-23].

MATERIALS AND METHODS

Apparatus

- Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).

Materials and reagents

Pure sample

Risperidone; was kindly supplied by multi-apex company, Egypt, B. No.(RN0030612).

Pharmaceutical preparation

Sigmadone ® tablets: product of Sigma company, Egypt, Batch No.(40708), labeled to contain 3 mg of Risperidone per tablet purchased from local pharmacies.

Reagents and solvents

All chemicals and reagents used throughout the work were of analytical grade.

- Water used throughout the procedures was freshly double distilled.
- Methanol (Sigma–Aldrich, USA).
- 3% Hydrogen peroxide.

Standard solution

- Stock solution of risperidone (0.1 mg ml^{-1}) was prepared by dissolving 10 mg of risperidone in 100 ml methanol and this is the working standard solution.

Degraded sample[18]

100 mg of risperidone sample was taken in 100 mL round bottom flask, 10 mL of 3% hydrogen peroxide solution was added, and contents were mixed well at room temperature. After 4 hr, 1 mL of this solution was diluted to 100 mL with methanol. The obtained solution was claimed to contain (0.1 m g ml^{-1}).

Procedures

Construction of the calibration curve (General procedure)

a. Ratio difference method: Aliquots equivalent to (0.05 – 0.35 mg) of risperidone working standard solution were accurately transferred into a series of 10 - ml volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer.The absorption spectra of risperidone are divided by the spectrum of (20 µg ml^{-1}) of the oxidative degradate to obtain the ratio spectra. The amplitude difference at 240 and 287 nm ($\Delta P_{287-240}$) was plotted against the corresponding risperidone concentration in µg ml^{-1} and the regression equation was computed**.**

b. Mean centering method: The same as in ratio difference method but the ratios here are mean centered using MATLAB. The amplitude of the mean centered peak of (intact / degradate) is measured at 285.6 nm. A calibration graph relating the peak amplitude to the corresponding concentrations in µg ml^{-1} of risperidone was constructed.

c. Derivative ratio method: The same as in ratio difference method. The stored spectra of risperidone are divided by the spectrum of 20 µg ml^{-1} degradate, smoothed with $\Delta\lambda = 16$ nm, then the first derivative of the ratio spectra (1DD) with $\Delta\lambda = 4$ nm is obtained. The amplitude of the first derivative peak of (intact risperidone / degradate) is measured at 231 nm.A calibration graph relating the peak amplitude at 231 nm to the corresponding concentrations in µg $ml^{-1}$of risperidone is constructed Alternatively, the regression equation was derived.

Analysis of pharmaceutical preparation

Ten **Sigmadone ® 3 mg** tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of risperidone was shaken three times with 25 ml methanol 10 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with methanol to obtain a concentration of (0.1 mg ml^{-1}). Proceed as described under "General Procedure" for each method.

RESULTS AND DISCUSSION

Spectral characteristics and optimization of the methods

Ratio difference method

The zero-order absorption spectra of risperidone (Fig.2) show an overlaping, so we develop a spectrophotometric method which allow the determination of the drug in presence of its degradate without previous separation.

In this method, the absorption spectra of the drug were divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra. Different concentrations of divisor are used (30,25,20,15 and10 µg ml^{-1}) of risperidone degradate and the divisor concentration 20 µg $ml^{-1}$of risperidone degradate is found the best regarding average recovery percent. The difference in peak amplitudes between two selected wavelengths in the ratio spectra is proportional to the concentration of the drug without interference from its divisor (Fig.3).The method comprises two critical steps, the first is the choice of the divisor. The selected divisor should compromise between minimal noise and maximum sensitivity. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and good linearity is present at each wavelength individually. The selected wavelengths are 240 and 287 nm ($\Delta P_{287\text{-}240\ nm}$) which gave the best results.

Mean centering method

In this method, the absorption spectra of the drug were divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra(Fig.3).The best divisor concentration was 20 µg/ml of risperidone degradate. The obtained ratio spectra were mean centered using MATLAB and the concentration of risperidone was determined by measuring the amplitude at 285.6 nm (Fig. 4).

Derivative ratio method

Salinas et al.[24] designed a spectrophotometric method, which is based on the derivation of the ratio-spectra for resolving binary mixtures. The main advantage of the ratio-spectra derivative spectrophotometry is the chance of doing easy measurements in correspondence of peaks so it permits the use of the wavelength of highest value of analytical signals (a maximum or a minimum)[25-27]. The main parameters that affect the shape of the ratio spectra are wavelength, scanning speed, the concentration of the standard solution used as a divisor, the wavelength increment over which the derivative is obtained (Δλ) and the smoothing function are carefully tested. The ratio spectra presented in (Fig.3) and the first derivative of the ratio spectra presented in (Fig.4) may provide a good proof for this understanding.

The peak amplitude at 231 and 267 nm of the first derivative of ratio spectra are then recorded respectively. Good linearity was observed but the recovery percent at 231 nm was better, which may be attributed to its higher signal to noise ratio.

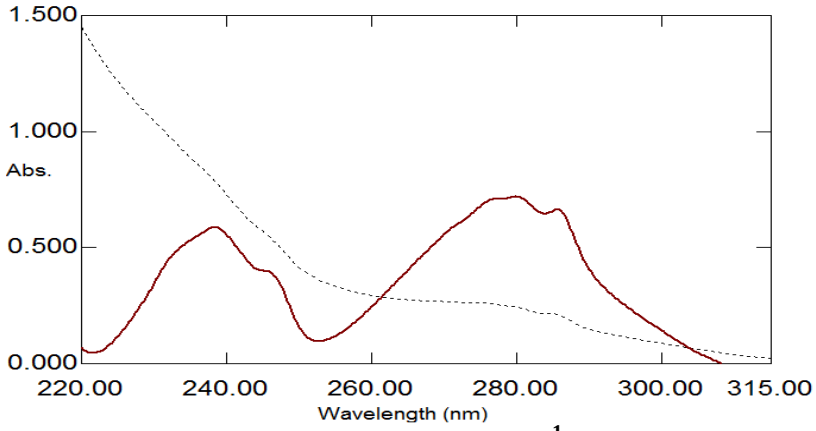

Figure (2): UV- Spectra of Intact risperidone (25µg ml^{-1})(—), Degradate Risperidone 25 µg ml^{-1}) (…..).

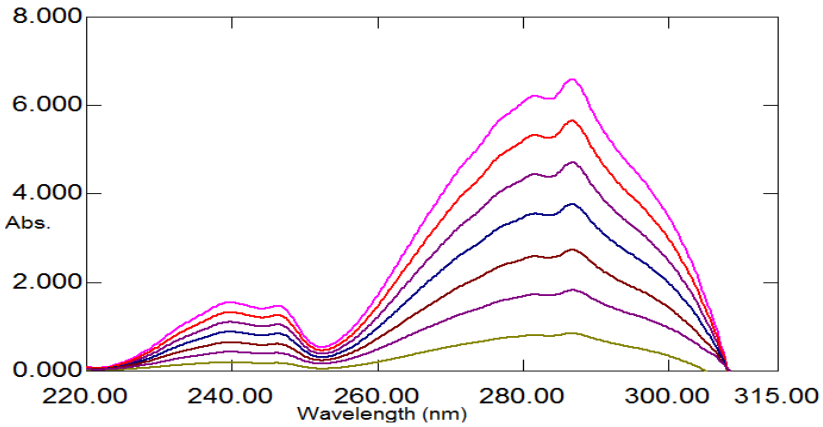

Figure (3): Ratio Spectra of Risperidone (5 – 35 µg ml^{-1}) using(20 µg ml^{-1}) of Degradate as a Divisor and Methanol as a Blank.

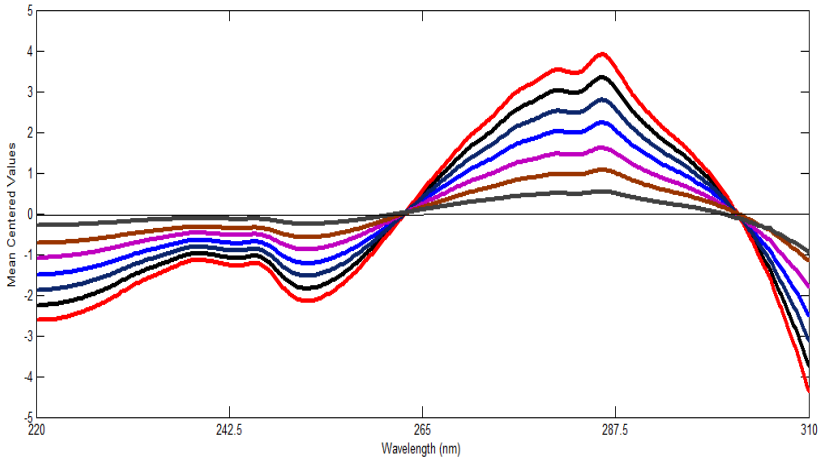

Figure (4): Mean Centered Ratio Spectra of Risperidone (5 – 35 µg ml^{-1}) Using (20 µg ml^{-1}) of its Degradate as a Divisor and Methanol as a Blank.

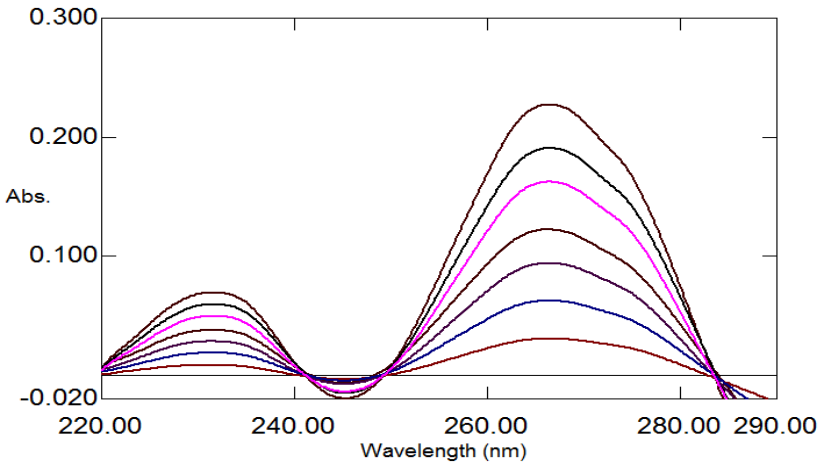

Figure (5): First Derivative of Smoothed Ratio Spectra of Risperidone (5– 35 µg ml^{-1}) Using (20 µgml^{-1}) Risperidone Degradate as a Divisor and Methanol as a Blank.

Validation of the methods

Linearity and range

For ratio difference method: Linear correlation was obtained between the differences in amplitudes at 240 and 287 nm, against the corresponding concentration of risperidone. Good linearity is obtained in the concentration range of 5 - 35 µg ml^{-1}.The corresponding regression equation was computed to be:

$\Delta P_{287-240} = 0.1433\ C - 0.0534$ ($r^2 = 0.9997$)

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient as shown in table 1.

For mean centering method: Linear correlation was obtained between the mean centered values at 285.6 nm,against the corresponding concentration of risperidone. Good linearity is obtained in the concentration range of (5 - 35 µg ml^{-1}). The corresponding regression equation was computed to be:

$MCN_{285.6}$ = 0.1129 C - 0.0500 (r^2 = 0.9997)

Where MCN is the peak amplitude of the mean centered ratio spectrum curve, C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient, as shown in table 1.

For derivative ratio method: Under the described experimental conditions, the calibration graph for the method was constructed by plotting peak amplitude at 231 nm versus concentration in µg/ml. The regression plot was found to be linear over the range of 5-35 µg/ml. The linear regression equation for the graph is:

P_{231} nm= 0.002 C - 0.0022 (r^2 = 0.9998)

Where C is the concentration of risperidone in µg ml^{-1}, P is the peak amplitude of the first derivative of the ratio spectrum curve at 231 nm and r^2 is the correlation coefficient, as shown in table 1.

Limits of detection and quantitation: The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines[28] from the following equations

LOD = 3.3 S_a / slope

LOQ = 10 S_a / slope

Where S_a is the standard deviation of y-intercepts of regression lines.

LOD and LOQ values of lornoxicam for each method were listed in table 1.The small values of LOD and LOQ indicate good sensitivity.

Accuracy and precision

According to the ICH guidelines[28], three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method.Accuracy as recovery percent (R%) and precision as percentage relative standard

deviation (RSD%) were calculated and results are listed in table 2.The small values of RSD% indicates high precision of the methods.Morever,the good R% confirms excellent accuracy.

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drug and its degradate.The results are listed in table 3.

Pharmaceutical Applications

The proposed methods were applied to the determination of the studied drug in **Sigmadone ® 3 tablets**. The results were validated by comparison to a previously reported method.[10] No significant differences were found by applying t-test and F-test at 95% confidence level,[29] indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form(table 4).

Table (1): Spectral data for determination of the studied drug by the proposed methods

Parameters	Ratio difference	Mean centering	Derivative ratio
Wavelength (nm)	240&287	285.6	231
Linearity range (µgml^{-1})	5-35	5 — 35	5 — 35
LOD (µgml^{-1})	0.0053	0.161	0.087
LOQ (µgml^{-1})	0.0176	0.535	0.289
Regression equation*			
Slope (*b*) Intercept (*a*)	0.1433 0.0534	0.1129 0.050	0.0020 0.0022
Correlation coefficient (r^2)	0.9997	0.9997	0.9998

* $y = a + bx$ where y is the response and x is the concentration.

Table (2): Intraday and interday accuracy and precision for the determination of the risperidone by the proposed methods

Method	Conc µg.ml^{-1}	Intraday			Interday		
		Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
Ratio difference	15	14.91±0.0002	99.39	0.009	14.90±0.0016	99.34	0.078
	20	19.92±0.00058	99.61	0.021	19.91±0.0012	99.57	0.041
	25	25.47±0.006	101.89	0.169	25.42±0.019	101.69	0.531
Mean centering	15	14.74±0.014	98.27	0.845	14.73±0.011	98.19	0.702
	20	20.18±0.015	100.92	0.687	20.04±0.014	100.18	0.641
	25	25.09±0.0023	100.36	0.084	25.09±0.0026	100.37	0.095
Derivative ratio	15	15.07 ± 0.00012	100.44	0.413	15.22 ± 0.00025	101.44	0.891
	20	19.93 ± 0.00029	99.67	0.766	20.18 ± 0.00029	100.92	0.756
	25	25.43± 0.00029	101.73	0.593	25.33 ± 0.00046	101.33	0.953

Table (3): Determination of risperidone and its degradate in their laboratory mixtures by the proposed methods

	Intact in (µg ml^{-1})	Degradate in (µg ml^{-1})	Percent of degradate	Intact found in (µ ml^{-1})	Recovery % of intact
Ratio difference	30	5	14.29	30.45	101.50
	25	10	28.57	25.42	101.70
	20	15	42.86	20.40	102
	15	20	57.14	15.17	101.11
	10	25	71.43	10.07	100.73
	Mean ± SD%				101.41±0.500
Mean centering	30	5	14.29	29.67	98.91
	25	10	28.57	25.24	100.97
	20	15	42.86	19.96	99.82
	15	20	57.14	15.00	99.97
	10	25	71.43	10.10	100.97
	Mean ± SD%				100.13 ±0.872
Derivative ratio	30	5	14.29	29.60	98.67
	25	10	28.57	24.60	98.40
	20	15	42.86	20.10	100.50
	15	20	57.14	15.10	100.67
	10	25	71.43	10.10	101
	Mean ± SD%				99.85±1.216

Table (4): Determination of risperidone in Sigmadone ® tablets by the proposed and reported methods

	Ratio difference	Mean centering	Derivative ratio	Reported method[10]
N*	7	7	7	6
$\bar{X}$	100.00	100.53	100.47	100.16
SD	0.633	1.256	0.647	0.631
RSD%	0.633	1.265	0.644	0.631
t**	0.455 (1.7959)	0.685 (1.7959)	0.873 (1.7959)	———
F**	0.994 (4.95)	3.96 (4.95)	0.951 (4.95)	———

** No. of experimental.*

*** The values in the parenthesis are tabulated values of t and F at (p= 0.05).*

CONCLUSION

The proposed methods are simple, rapid, accurate and precise and can be used for the determination of risperidone in pure form and in pharmaceutical dosage form as well as in presence of its degradation product.

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REFERENCES

1. Hardman G, Limbid IE, Gilman AG. The Pharmaceutical Basis of Therapeutics, 10th ed, McGraw Hill, 2001; 279.
2. Physicians' Desk Reference, 59th ed, Thomson PDR, New Jersey, 2005; 1742.
3. British Pharmacopeia, 2008, 2: 1898.
4. Narayana B and Shetty DN. Development and Validation of Spectrophotomtric Method for the Determination of Risperidone. Der Pharmacia Lettre, 2011; 3 (5): 104-109.
5. Akachukwu I, Nwodo NJ and Mbah CJ. Spectrophotometric Method for Determination of Risperidone In Phrmacutical Bulk and Dosage Forms. Int J Pharm, 2013; 3(4): 710-715.
6. Kutty SV, Babu YH, Greeshma , Vidhya PM. Difference Spectrophotometric Method for the Determination of Risperidone in Bulk and Tablet Dosage Form. The Pharma Innovation – Journal, 2013; 2(2): 44-49.
7. Wafaa El-Sayed Hassan. Extractive colorimetric method for the determination of dothiepin hydrochloride and risperidone in pure and in dosage forms. Chemical & Pharmacutical Bulletin, Zagazig University, 2008; 56(8): 1092-6.
8. Archana S, Prasanna YN, Pavitra P, Krupa DS, Hema MS. Development and Validation of Spectrophotometric Method for Determination of Risperidone by MBTH. IJPSR, 2013; 4(3): 1116-1119.
9. Kishore VNV, Krishna KB, Ramana GV. Development of Stability indicating spectrophotometric method for determination and validation of Risperidone in formulation and bulk drug. Afro Asian J SciTech, 2014; 1(2): 098-108.
10. Kumar MS, Smith AA, Vasagam GA, Muthu AK, Manavalan R. Development of analytical method for Risperidone by UV Spectrophotometry using methanol as a solvent. Der Pharma Chemica, 2010; 2(3): 309-315.
11. Jeyaseelan C, Jugade R, Joshia AP. Differential Pulse Polarographic Studies of Risperidone in Pharmaceutical Formulations. Croatica Chemica Acta, 2006; 79 (4): 541-544.

12. Ashour S, Kattan N. Sensitive Method for the Quantitative Determination of Risperidone in Tablet Dosage Form by High-Performance Liquid Chromatography Using Chlordiazepoxide as Internal Standard. International journal of Biomedical science, 2013; 9(2).
13. Anthony GK, Ramya SS. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Risperidone and Haloperidol in Tablet Dosage Forms. Int.J.Pharm Drug Anal, 2012; 2(9): 666-671.
14. Mohammad Y, Kumar BP. Development and Validated New RP-HPLC Method for the Determination of Risperidone in Bulk and Tablet Dosage Forms. Research Journal of Pharmaceutical, Biological and Chemical Sciences.2010; 1(2): 165-171.
15. Svirskis D, Travas-Sejdic J, Garg S. A Stability Indicating HPLC Method for the Determination of Electrochemically Controlled Release of Risperidone. Journal of Chromatographic Science, 2011; 49.
16. Huang M, Shentu J, Chen J, Liu J, Zhou H. Determination of risperidone in human plasma by HPLC-MS/MS and its application to a pharmacokinetic study in Chinese volunteers. J Zhejiang Univ Sci B, 2008; 9(2): 114-120.
17. Suthar AP, Dubey SA, Patel SR, Shah AM. Determination of Risperidone and forced degradation behavior by HPLC in tablet dosage form. International Journal of PharmTech Research, 2009; 1(3): 568-574.
18. Dedania ZR, Dedania RR, Sheth NR, Patel J, Patel B. Stability Indicating HPLC Determination of Risperidone in Bulk Drug and Pharmaceutical Formulations. International Journal of Analytical Chemistry, 2011.
19. Elzanfaly ES, Saad AS, Abd-Elaleem AE. A smart simple spectrophotometric method for simultaneous determination of binary mixtures. J. Pharm. Anal., 2012; 2(5): 382 – 385.
20. Darwish HW, Hassan SA,Salem MY, El-Zeiny BA.Three different methods for determination of binary mixture of Amlodipine and Atorvastatin using dual wavelength spectrophotometry. Spectrochimica Acta, 2013; 104: 70 – 76.
21. Darwish HW,Hassan SA, Salem MY, El-Zeiny BA. Three different spectrophotometric methods manipulating ratio spectra for determination of binary mixture of amlodipine and atorvastatin. Spectrochimica Acta Part A, 2011; 83(1): 140– 148.
22. Abou-Seada HHM, Attia KAS, Nassar MW, Emara MS. Spectrophotometric and Stability-Indicating Spectrophotometric Methods for Determination of Lornoxicam in Pure Form and Pharmaceutical Preparation. European Journal of Biomedical and Pharmaceutical Sciences, 2015; 2(1): 59-79.

23. Attia KA, Nassar MW, Abou-Seada HM and Emara MS. Stability-Indicating Spectrophotometric Methods for Determination of Cefdinir in Pure Form and Pharmaceutical Preparation. IJPSR, 2014; 5(6): 2230-2237.
24. Salinas F, Nevado J, Mansilla A. A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of salicylic and salicyluric acids. Talanta, 1990; 37(3): 347 – 351.
25. El-Gindy A, Ashour A, Abdel-Fattah L, Shabana M. Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorothiazide in binary mixtures. Journal of Pharmaceutical and Biomedical Analalysis, 2001; 24 (4): 527 – 534.
26. Lemus J, Arroyo P. Spectrophotometric resolution of ternary mixtures of Dexamethasone, Polymyxin B and Trimethoprim in synthetic and pharmaceutical formulations. Journal of Analytica Chimica Acta, 2001; 437(2): 247- 257.
27. Tena RC, Delgado MA, Sanchez MJ, Montelongo FG.Comparative-Study of the Ratio Spectra Derivative and Partial Least-Squares Methods Applied to the Simaltaneous Determination of Atrazine and Ametryn In-Ground Waters. Talanta, 1997; 44(4): 673-683.
28. ICH Q2 (R1): Validation of analytical procedure. Text and methodology.Geneva: International conference on Harmonization, 2005.
29. Armitage P, Berry G. Statistical Methods in Medical Research. 3rd ed. Oxford, UK; Blackwell, 1994.