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VALIDATED STABILITY – INDICATING SPECTROSCOPIC METHODS FOR DETERMINATION OF AGOMELATINE

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ABSTRACT

Three simple, sensitive and accurate stability- indicating UV-spectroscopic methods were developed and validated for the determination of agomelatine in the presence of its alkaline degradation product. The first method was a bivariate calibration algorithm involving the use of data from four linear regression calibration equations, two calibrations for each component at two selected wavelengths 230 nm and 235 nm. The second method was a derivative ratio spectrophotometry in which the drug was determined through first derivative responses at 243 nm using 0.5 µg mL^{-1} degradation product used as a divisor. The third one is dual wavelength method in which two wavelengths 222 nm and 235 nm were selected for the estimation of intact agomelatine in presence of its degradation product as the alkaline degradate shows the same absorbance at these wavelengths. Calibration curves showed linearity in the concentration range 0.5 - 4 µg mL^{-1} for the bivariate and 1- 4 µg mL^{-1} for the derivative ratio or dual wavelength methods with mean percentage recoveries of 99.8 ± 0.76 and 99.4 ± 1.34 or 100.4 ± 1.12; respectively. The bivariate method retains its accuracy in the presence of up to 90% degradation product, while the other two methods retain up to 70% degradation product. The three proposed methods were successfully applied to analyse the drug in its preparation; the results obtained were statistically analysed and found to be in accordance with those given by the compendial method.

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INTRODUCTION

Agomelatine; chemically N-[2-(7-methoxy napthalen-1-yl) ethyl] acetamide is a sleep modulating antidepressant, approved by the European Medicines Agency for the treatment of major depressive disorder (MDD) in 2009[1]. It acts as a melatonergic receptor (MT1 /MT2) agonist and serotonergic receptor (5- HT2C) antagonist. Because of its action upon the melatonine receptors, agomelatine shows a marked improvement on sleep. It has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders [2]. Literature survey of agomelatine revealed few stability – indicating methods based on HPLC [3,4] spectrophotometry [4] and TLC[5]. The main task of the present work simple is to establish simple, accurate and precise stability – indicating methods for the determination of agomelatine in the presence of its degradation product in raw material and pharmaceutical preparations.

2. EXPERIMENTAL

2.1. Instrumentation

-Shimadzu UV-Vis 1601 spectrophotometer (Tokyo, Japan) connected with PC program and two matched 1 cm path-length quartz cell.
-UV lamp with short wavelength (254 nm).
-Raypa ultrasonic sonicator.
-Sartorius analytical balance (Germany).
-Jenway pH meter.

2.2. Samples

2.2.1. Pure samples

Agomelatine was kindly supplied by Mash Company for Pharmaceutical Industry, Badr City, Egypt. Its purity was found to be 99.8% as stated by the supplier.

2.2.2. Market sample

Agovald® tablets; batch number M170415, each tablet labeled to contain 25 mg agomelatine, product of Mash Company for Pharmaceutical Industry, Badr City, Egypt.

2.2.3. Degraded sample

100 mg of agomelatine was dissolved in 10 mL methanol and heated under reflux for 12 hours with 50 mL 2.5 N NaOH, followed by cooling, neutralization with 2.5 N HCl and evaporation till dryness under vacuum. The resulted residue was then extracted three times with methanol each with 25 mL, filtered and diluted to 100 mL with the same solvent to obtain a solution labeled to contain degradate derived from 1mg mL^{-1} agomelatine [4].

2.3. Chemicals and reagents

All reagents used were of analytical grade and solvents were of spectroscopic grade. Distilled water was used throughout the work.
-Methanol (Fischer Chemicals, UK; Lab Scan, Ireland; El-Nasr Chemicals, Egypt).
-Hydrochloric acid (Sigma-Aldrich, Germany).
-Sodium Hydroxide (Qualikems fine chemical Pvt. Ltd, India).

2.4. Standard solutions

Standard stock solution of agomelatine was prepared by dissolving 25 mg in 25 mL methanol (1 mg mL^{-1}). This solution was stable for 24 hour either kept in refrigerator or at room temperature [4]. Working solution of agomelatine (0.1 mg mL^{-1}) was prepared by transferring 5 mL of the standard stock solution into 50 mL volumetric flask and completing volume with methanol.

2.5. Procedures

2.5.1. Construction of calibration curves

2.5.1.1. Bivariate method

Aliquot volumes of working standard agomelatine solution or its alkaline degradate equivalent to 0.005-0.04 mg were transferred separately into two sets of 10 mL volumetric flasks then diluted to volume with methanol. Calibration curves at different wavelengths 220, 225, 230, 235, 240 and 245 nm were constructed and the regression equation at each wavelength was calculated. From both sets of regression equations, the sensitivity matrices K was calculated, the optimum pair of wavelengths were chosen (230 and 235 nm) to carry out the determination and the regression equations used in the bivariate algorithm were deduced, Table (1).

2.5.1.2. Derivative ratio method

Aliquot volumes of equivalent to 0.01-0.04 mg agomelatine or its degradate were accurately introduced into two separate series of 10 mL volumetric flasks then completed to the volume with methanol. The UV absorption spectra of the prepared solutions were scanned from 200 – 400 nm against methanol and stored in the computer. The spectra of intact agomelatine were divided by the spectrum of 0.5 µg mL^{-1} degradate, then first derivative were calculated for the obtained ratio spectra with $\Delta\lambda$= 4 and scaling factor = 5. The amplitudes at 243 nm of the first derivative ratio spectra were recorded. Calibration curves relating the amplitudes at 243

nm to drug concentration in µg mL^{-1} were constructed.

2.5.1.3. Dual wavelength method

Into two separate sets of 10 mL volumetric flasks, aliquots containing 0.01-0.04 mg of standard drug solution or its degradate were transferred, then both sets were diluted to the volume with methanol. Absorption difference of intact agomelatine was measured at 222 and 235nm. Calibration curves were constructed by plotting the absorption difference versus drug concentration in µg mL^{-1}.

2.5.2. Assay of laboratory prepared mixtures

Different volumes of pure agomelatine solution (0.1 mg mL^{-1}) containing 0.004-0.036 mg of intact drug were transferred into a series of 10 mL volumetric flasks containing 0.036-0.004 mg of agomelatine degradation product and diluted to the volume with methanol. The concentration of each drug was calculated by substitution in the corresponding regression equation after applying the corresponding manipulating steps for each method.

2.6. Application to pharmaceutical preparation

Ten Agovald® tablets were accurately weighed, powdered and mixed well, a portion of the powder equivalent to 100 mg agomeltine was introduced into a 100 mL volumetric flask, 15 mL methanol were added and the flask was sonicated for 10 minutes, cooled, then completed to volume with methanol and filtered. The clear filtrate claimed to contain 1 mg mL^{-1} of agomeltine was further diluted and analyzed by the proposed bivariate, first derivative ratio and dual wavelength methods. The drug concentrations were calculated from the appropriate regression parameters.

3. Results and Discussion

Three different spectrophotometric methods were developed for the selective determination of agomelatine in the presence of its degradation product.

Degradation of agomelatine

Accelerated degradation of agomelatine was carried out by heating under reflux with 0.5-3 N NaOH for 1-13 hours. Complete degradation was achieved upon refluxing with 2.5 N NaOH for 12 hours; this was confirmed by the absence of the spot of the intact drug in the region of the degradation product. However, reflux of agomelatine with 2.5 N HCl for 12 hours causes incomplete degradation of the drug [4]. IR spectrum of the intact agomelatine showed sharp band at 1634.38 cm^{-1} which characterizes the ester carbonyl moiety, while the IR spectrum of degraded agomelatine showed forked band at 3408.57 and 3228.25 cm^{-1} which characterizes the primary amino group.

The suggested degradation pathway [4] was shown in figure (1).

3.1. Bivariate method

Bivariate calibration spectrophotometric method is a direct method proposed for the selective determination of agomelatine in the presence of its degradation product, as the zero-order spectra of the intact and degraded agomelatine showed severe overlapping; Figure (2). The principle of the bivariate method is the measurement of the two components (A and B) at two selected wavelengths (λ_1 and λ_2) to obtain two equations [6,7].

$$A_{AB1} = m_{A1} C_A C_B + e_{AB1}$$

$$A_{AB2} = m_{A2} C_A C_B + e_{AB2}$$

The resolution of such equations sets allows evaluation of C_A and C_B values:

$$C_A = (A_{AB1} - e_{AB1} - m_{B1} C_B) / m_{A1}$$

$$C_B = [m_{A2} (A_{AB1} - e_{AB1}) + m_{A1} (e_{AB2} - A_{AB2})] / m_{A2} m_{B1} - m_{A1} m_{B2}$$

Where C_A , C_B are the concentration of component A (agomelatine), component B (degradation product); m_{A1}, m_{A2} are the slope values of intact agomelatine at λ_1, λ_2; m_{B1}, m_{B2} are the slope values of agomelatine degradation product at λ_1, λ_2; A_{AB1}, A_{AB2} are the absorbance of their mixture at λ_1, λ_2; e_{AB1}, e_{AB2} are the sum of the intercepts of intact agomelatine and its degradation product at λ_1 and λ_2, respectively. This simple mathematic algorithm allows the resolution of the two components by measuring the absorbance of their mixture at the two selected wavelengths and using the parameters of the linear regression functions evaluated individually for each component at the same wavelengths. In order to apply the bivariate method for the determination of agomelatine in the presence of its degradation product, the absorbance of the two components at several different selected wavelengths was recorded into in the region of overlap from 220-245 nm at 5 nm interval. The calibration curve equations and their respective linear regression coefficients were determined in order to ensure that there was a linear relationship between the absorbance and

the corresponding concentration. According to Kaiser method [7], the slope values of the linear regression equations for agomelatine and its degradation product at the selected wavelengths were used to calculate the sensitivity matrices K in order to obtain the optimum pair of wavelengths for the mixture to be resolved.

$$K = \begin{bmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{bmatrix}$$

The optimum pair of wavelengths were found to be 230 and 235 nm, which gave maximum value for K; Table (1). The linear regression formulas used for the bivariate algorithm were presented in table (2).

3.2. Derivative ratio method

Derivative ratio spectroscopic method is a useful tool in quantification of drugs. The principle of this method depends on measuring the absorption spectra of intact agomelatine having concentration range of (1-4 µg mL^{-1}) in the region of 200-400 nm then divided by the absorption spectra of degraded agomelatine having concentration range of (0.5 – 4 µg ml^{-1}) in order to select the best divisor; Figure (3). It was found that 0.5 µg mL^{-1} degraded agomelatine is the best divisor. Then first derivative of these ratio spectra were recorded using $\Delta\lambda = 4$ and scaling factor = 5. The peak amplitude was measured at 243nm; Figure (4).

3.3. Dual wavelength spectrophotometric method

The third method eliminating the interference in spectra caused by presence of degradation product is dual wavelength spectrophotometric method. In this method the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of intact drug independent of the interfering degradation product [8,9]. The difference in absorbance at 222 and 235 nm was zero for degraded agomelatine so they were selected for the determination of intact drug; Figure (2).

3.4. Methods Validation

3.4.1. Linearity

Linear relationship between the absorbance and the corresponding drug concentration in the bivariate method was found in the range of (0.5-4 µg mL^{-1}) at the two wavelengths 230, 235 nm while a linear relationship between the peak amplitude at 243 nm and the drug concentration was found in the range of (1-4 µg mL^{-1}) for the derivative ratio method. Also linearity between the absorbance difference between the two wavelengths 222, 235 nm and the drug concentration was found in the range of (1-4 µg mL^{-1}) for the dual wavelength method; Table (3).

3.4.2. LOD and LOQ

The experimental limit of detection (LOD) and limit of quantitation (LOQ) were determined according to ICH [10] using the standard deviation of multiple blank samples and the slope of the calibration curve; Table (3).

3.4.3. Accuracy and precision

The accuracy and precision of the proposed methods were determined using three different concentrations of pure samples of drug covering the linearity range, each in triplicate, within one day for intraday and three different days for interday; Table (4).

3.4.4. Specificity

The specificity of the proposed methods was assessed by applying the proposed methods to laboratory prepared mixtures of the intact drug together with its degradation product. Table (5) revealed that intact agomelatine can be determined selectively in the presence of up to 90% of its degradation product using the proposed bivariate method and up to 70% of its degradation product using derivative ratio and dual wavelength methods.

3.4.5. Robustness

The robustness of the proposed bivariate, derivative ratio and dual wavelength methods was assessed by using different sources of methanol. It was found that, using methanol of Fischer, Lab-scan and El-Nasr gave RSD did not exceed 0.6%.

3.5. Application to pharmaceutical dosage form

The proposed methods were applied successfully for the determination of agomelatine in pharmaceutical dosage form in the presence of excipients and additives without interference. The standard addition technique was used to determine the recovery of the proposed methods; Table (6). Statistical analysis of the results obtained by the proposed methods compared with a reported method of agomelatine [11] revealed no significant difference between them confirming accuracy and precision at 95% confidence limit[12]; Table (7)

Table (1): Values of the sensitivity matrix determinates calculated according to Kaiser's method (K × 10^{-6}) for the mixture of agomelatine and its degradation product by the proposed bivariate method.

λ / λ	220	225	230	235	240	245
220	0					
225	1700	0				
230	2700	963	0			
235	16.3	-2200	**-3600**	0		
240	-520	-1530	-2000	-700	0	
245	-480	-1140	-1550	-650	87	0

Table (2): Linear regression calibration formula used for the bivariate algorithm.

Component	Calibration equations	
	at λ_{230}	at λ_{235}
Agomelatine	A = 0.26701 C + 0.018 r^2 = 0.9998	A = 0.2073 C + 0.0168 r^2 = 0.9996
Degradation product	A = 1.7142 C + 0.004 r^2 = 0.9996	A = 1.2175 C + 0.0092 r^2 = 0.9994

Table (3): Spectral data of calibration curves for the determination of agomelatine by the proposed bivariate, derivative ratio and dual wavelength methods.

Parameter	Bivariate method	Derivative ratio method	Dual wavelength method
λ_{max}	230, 235 nm	243 nm	222, 235 nm
Linearity range (µg mL^{-1})	0.5-4	1-4	1-4
Regression parameters:			
Slope	0.26701	1.5276	0.0194
Intercept	0.0180	-0.2746	-0.0008
Correlation coefficient (r^2)	0.9998	0.9993	0.9998
LOD	0.06	0.11	0.052
LOQ	0.18	0.34	0.16

Table (4): Intraday and interday accuracy and precision for the determination of agomelatine by the proposed bivariate, derivative ratio and dual wavelength methods.

	Taken (µg mL^{-1})	Intraday			Interday		
		Found (µg mL^{-1}) ± SD	Accuracy R%	Precision RSD%	Found (µg mL^{-1}) ± SD	Accuracy R%	Precision RSD%
Bivariate method	2	2.01 ± 0.015	100.5	0.75	2.01 ± 0.015	100.5	0.75
	3	2.98 ± 0.033	99.3	1.1	2.99 ± 0.028	99.7	0.94
	4	3.99 ± 0.023	99.8	0.58	4.01 ± 0.025	100.3	0.61
Derivative ratio method	2	1.98 ± 0.004	99	0.96	2.01 ± 0.017	100.5	0.85
	2.5	2.47 ± 0.001	98.8	0.03	2.47 ± 0.005	98.8	0.20
	4	3.98 ± 0.001	99.5	0.04	3.97 ± 0.005	99.3	0.13
Dual wavelength method	1	1.02 ± 0.004	102.0	0.39	1.02 ± 0.001	102.0	0.13
	2.5	2.49 ± 0.0396	99.6	1.59	2.48 ± 0.029	99.2	1.17
	4	4.01 ± 0.003	100.3	0.75	4.02 ± 0.016	100.5	0.39

Table (5): Determination of agomelatine in laboratory prepared mixtures by the proposed bivariate, derivative ratio and dual wavelength methods.

Intact (µg mL^{-1})	Degraded (µg mL^{-1})	R %		
		Bivariate method	Derivative ratio method	Dual wavelength method
3.6	0.4	101.8	100.6	100.4
3.2	0.8	100.1	99.4	99.6
2.8	1.2	98.6	98.6	101.4
2.4	1.6	99.9	100.4	99.2
2	2	99.5	99.0	101.3
1.6	2.4	99.8	101.3	101.5
1.2	2.8	100.8	100.3	100.9
0.8	3.2	98.8	108.5*	108.1*
0.4	3.6	98.0	107.3*	108.6*
Mean ± SD		99.7 ± 1.16	99.9 ±0.97	100.6 ± 0.92

* Rejected

Table (6): Recovery of the proposed bivariate, derivative ratio and dual wavelength methods for the determination of agomelatine in its pharmaceutical preparation.

Bivariate method				Derivative ratio method				Dual wavelength method			
Recovery ± SD%	Standard addition			Recovery ± SD%	Standard addition			Recovery ± SD%	Standard addition		
	Claimed taken (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery % of pure added		Claimed taken (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery % of pure added		Claimed taken (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery % of pure added
99.8 ± 0.76	0.5	0.5	99.5	99.4 ± 1.34	1	1	100.9	100.4 ± 1.12	1	1	102.1
	0.5	1	99.9		1	1.5	101.8		1	1.5	100.0
	0.5	1.5	99.5		1	2	99.9		1	3	100.8
	0.5	3	99.6		1	2.5	100.1		1	2.5	99.2
	0.5	3.5	99.1		1	3	99.3		1	3	102.0
Mean ± SD	99.5 ± 0.29			100.4 ± 0.96				100.8 ± 1.26			

Table (7): Results obtained by the proposed methods compared with the reported method [11] for the analysis of agomelatine in its pharmaceutical dosage form.

	Agovald® Tablets			
Parameters	Bivariate method	Derivative ratio method	Dual wavelength method	Reported method
Linearity range (µg mL^{-1})	0.5 – 4	1-4	1-4	70 – 130
N	8	7	7	5
Mean %	99.8	99.4	100.4	100.8
SD	0.76	1.34	1.12	0.89
Variance	0.58	1.80	1.25	0.79
T	2.166 (2.201)	2.025 (2.228)	0.697 (2.228)	-
F	1.36 (4.12)	2.28 (4.53)	1.58 (4.53)	-

- Figures in parenthesis are theoretical t and F values at p = 0.05.
- The reported method determines agomelatine by HPLC using Chromolith C_{18} column, mobile phase consisting of phosphate buffer pH 2.5: acetonitrile: methanol (70:12:18 v/v/v) with flow rate - 6 ml/min and UV detection at 230 nm.

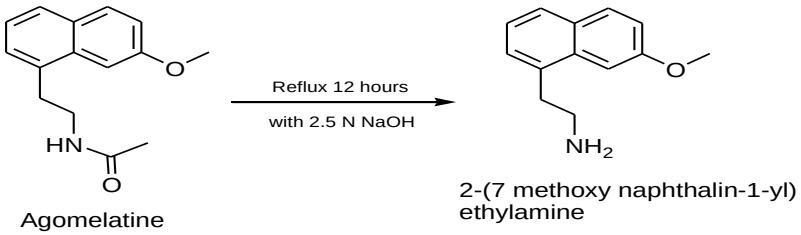

Figure (1): Proposed degradation pathway of agomelatine.

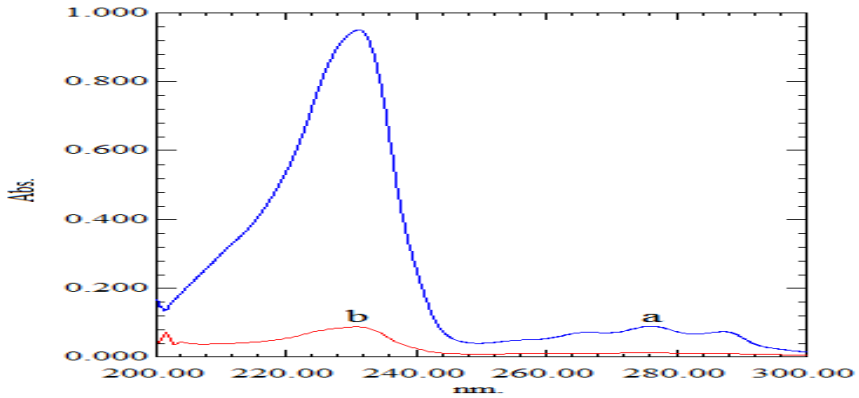

Figure (2): Zero-order spectra 3.5 µg mL^{-1} intact agomelatine (a) and 3.5 µg mL^{-1} degraded agomelatine (b) in methanol.

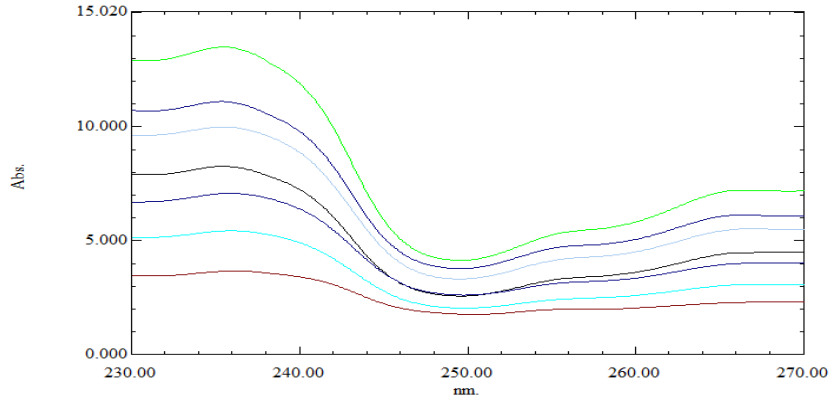

Figure (3): Ratio spectra of agomelatine (1-4 µg mL^{-1}) in methanol.

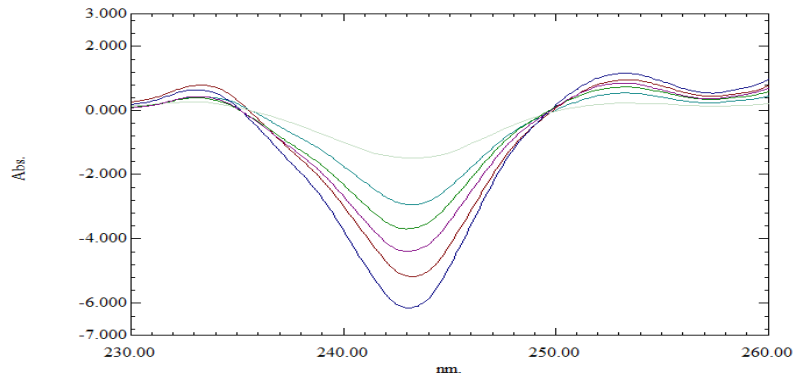

Figure (4): First derivative ratio spectra of agomelatine (1-4 µg mL^{-1}) in methanol.

CONCLUSION

The proposed bivariate, derivative ratio and dual wavelength spectrophotometric methods are simple, accurate, precise and selective as they enable the quantitation of agomelatine in the presence of its degradation product with good accuracy and precision either in laboratory prepared mixtures or in pharmaceutical dosage forms. All validation parameters were found to be highly satisfactory. Therefore, they can be applied efficiently for the determination, stability studies and quality control analysis of agomelatine.

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