IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2018 Vol.:11, Issue:3

Spectrofluorimetric and First Derivative Spectrophotometric Determination of the New Bronchodilator Drug; Olodaterol in Presence of its Oxidative Degradation Product

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ISSN 2349-7203
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Submission: 24 January 2018
Accepted: 29 January 2018
Published: 28 February 2018

Keywords: Olodaterol, olodaterol degradation, spectrofluorimetry, first derivative, Striverdi

ABSTRACT

Olodaterol is a novel, long-acting β-2-adrenergic agonist used for the treatment of chronic obstructive pulmonary disease. The present study has been considered to be the first spectroscopic analytical methods for the quantitative and selective determination of olodaterol in the presence of its oxidative degradation product. Derivative mathematical manipulation (first derivative) has been introducing to resolve the overlapped UV absorption spectra by measuring the amplitude at 280 nm without interference from the degradation product. Alternatively, a spectrofluorometric method has been developed for the selective quantitative determination of olodaterol in presence of its oxidative degradation product by measuring the fluorescence of olodaterol in ethanol at 409 nm after excitation at 250 nm without interference from its degradation product. The proposed methods were validated according to ICH guidelines and show high sensitivity, accuracy, and precision. Also, these methods were successfully applied to the analysis of olodaterol in the pharmaceutical dosage form and the results were statistically compared to a reported method showing no significant difference.

INTRODUCTION

Olodaterol is a novel, long-acting β-2-adrenergic agonist that exerts its pharmacological effect by binding and activating β-2-adrenergic receptors located primarily in the lungs and used for the treatment of chronic obstructive pulmonary disease. Olodaterol; **Figure. 1** is 6-hydroxy-8-[(1R)-1-hydroxy-2-{[1-(4-methoxyphenyl)-2-methylpropan-2yl]amino}ethyl]-3,4-dihydro-2H-1,4-benzoxazin-3-one with molecular formula $C_{21}H_{26}N_2O_5$ and molecular weight 386.44. It is a white fine powder slightly soluble in water, freely soluble in ethanol [1].

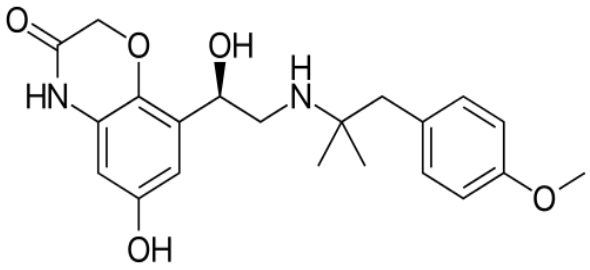

Figure (1): Structural formula of olodaterol hydrochloride

Due to this novel bronchodilator is newly formulated there is only reported HPLC method for its analysis in a combination form [2].

To the best of our knowledge, no spectroscopic methods have been reported for determination of olodaterol. Hence, the aim of this work was to develop a new spectrophotometric and spectroflurimetric method with a superior simple operation, time-saving for analysis of olodaterol in its pure form and pharmaceutical formulation.

MATERIALS AND METHODS

Instruments

- Shimadzu UV-Visible 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells.

- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 W Xenon lamp, holographic gratings excitation and emission monochromators for all measurements. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were carried out at medium sensitivity.

- pH meter 3510 (Jenway, U.S.A.).
- UV lamp with short wavelength 254nm (Viber Lourmat, VL-4LC, France).
- FT-IR, Nicolet IR 200 (Thermo electron corporation, USA).
- GCMS-QP-1000 EX mass spectrometer at 70 EV (Shimadzu, Tokyo, Japan).

Materials and reagents

- Pure olodaterol hydrochloride was kindly supplied by Boehringer Ingelheim Company (Ingelheim, Germany).
- Striverdi® Respimate: containing 60 metered inhalation (2.5 µg olodaterol / actuation) (B.No.L5583A, was supplied by Boehringer Ingelheim Pharmaceutical Company).
- Acetonitrile, chloroform, ethanol, and methanol all of HPLC grades (Sigma-Aldrich, USA).
- Hydrogen peroxide 30% (El-Nasr Company, Egypt).
- Sodium hydroxide and hydrochloric acid; analytical grade, (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Monobasic potassium phosphate, potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate; analytical grade, (El-Nasr Company, Egypt).
- Deionized double distilled water.
- Buffers of different pH values prepared as prescribed in US pharmacopeia [3]:

1. Acetate buffer pH 4 to 6.
2. Phosphate buffer pH range from 6 to 8.
3. Borate buffer pH range from 8 to 10.

Standard solutions

Standard solution of olodaterol

A stock standard solution of olodaterol (100 µg/mL) was prepared by dissolving 10 mg of the drug powder in 50 the volume was completed to 100 mL with ethanol. Working standard solution (10 µg/mL) was prepared by further dilution of the stock solution with ethanol.

Standard solution of olodaterol oxidative degradation product

100 mg of pure olodaterol hydrochloride powder was dissolved in 45 mL of ethanol and transferred to a 100-mL round-bottomed flask to which 5 mL of 30% H_2O_2 was added. The solution was heated under reflux for 4 hours and evaporated to dryness under vacuum. The obtained residue was extracted with ethanol (2×10 mL), filtered into a 100-ml volumetric flask and diluted to volume with ethanol to obtain a stock solution labeled to contain degradation product derived from 1 mg/ml of olodaterol hydrochloride. Working solution of degradate (10µg/ml) was obtained by further dilution of the stock solution with ethanol.

Procedures

Construction of calibration graphs

First derivative spectrophotometric method (^{1}D)

Different aliquots of olodaterol standard solution (10 µg/mL) ranging from (20–80µg) were transferred to 10-mL to volume with ethanol. The absorption spectra (from 200 to 400 nm) solutions were recorded using ethanol as a blank. The first derivative of the recorded absorption spectra was obtained, using $\Delta\lambda$ = 2 nm and scaling factor 40. The amplitude values were measured at 280 nm. The measured amplitude values versus olodaterol concentrations in µg/ mL were plotted equation was derived. **Spectrofluorimetric method**

Aliquots from olodaterol working standard solution (10µg/mL) ranging from (5–25 µg) were transferred to a series of 10-mL volumetric flasks containing 1 mL of acetate buffer pH 5 and the volume was completed to the mark with ethanol. The emission of these solutions was measured at 409 nm after excitation at 250 nm and then plotted versus the final olodaterol concentrations in µg/mL to get the calibration graph. Alternatively, the

regression equation was derived.

Assay of olodaterol in synthetic mixture with its oxidative degradation product

Aliquots of olodaterol and its degradation product were mixed in different ratios covering the working concentration range. The concentrations of olodaterol were determined using the mentioned general procedure for each method.

Application to pharmaceutical preparation

Contents of four cartridges of Striverdi® Respimate device were transferred to 50 mL volumetric flask containing 25 ml of ethanol and washed several times using ethanol until obtaining an amount equivalent to 600µg olodaterol then the volume was adjusted with ethanol to obtain a solution labeled to contain (12µg/mL of olodaterol hydrochloride). By using aliquots covering the working concentration ranges. The content of the Respimate was calculated using the mentioned general procedure for each method.

RESULTS AND DISCUSSION

In the present study, a simple and sensitive spectrofluorimetric and spectrophotometric methods were suggested for determination of olodaterol in presence of its oxidative degradation product.

Oxidative degradation study of olodaterol:

For investigation of olodaterol stability, the drug was subjected to the oxidative stress where complete degradation was attained when refluxed with 30% H_2O_2 for 4 hours. Degradation of olodaterol was checked by TLC using chloroform– acetonitrile (60:40, v/v) as a developing system and UV detection at 254 nm. Degradation product was confirmed through IR spectral analysis, **Figures (2, 3).**

IR spectrum for the intact olodaterol show medium intense band at 1457.93 of amide group; While IR spectrum of the olodaterol degradation product showed the highly intense band at 1724.38 cm-1 of C=O, which indicate the formation of COOH group of the carboxylic acid in the oxidative degradate.

The molecular ion peak of olodaterol degradate m/z=404.20 in the formed oxidative degradation product. This finding suggests that oxidation of olodaterol with hydrogen

peroxide leads to the formation of 2-(2-amino-4-hydroxy-6-(1-hydroxy-2-(1-(4-methoxyphenyl)-2-methylpropane-2-ylamino)ethyl)phenoxy)acetic acid. The suggested degradation pathway is shown in **Figure (4).**

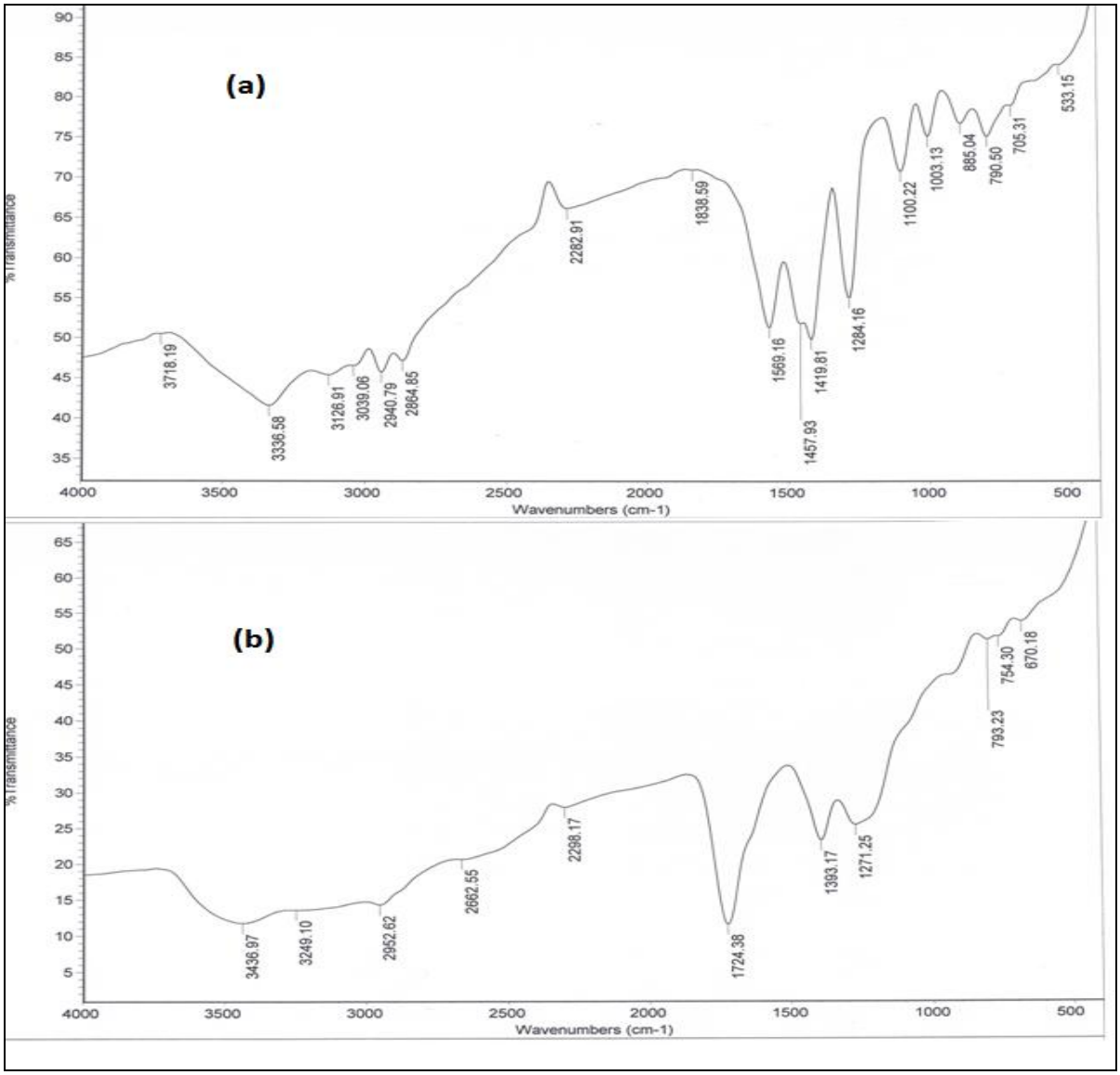

Figure (2): IR spectrum of (a) olodaterol and (b) olodaterol degradation product

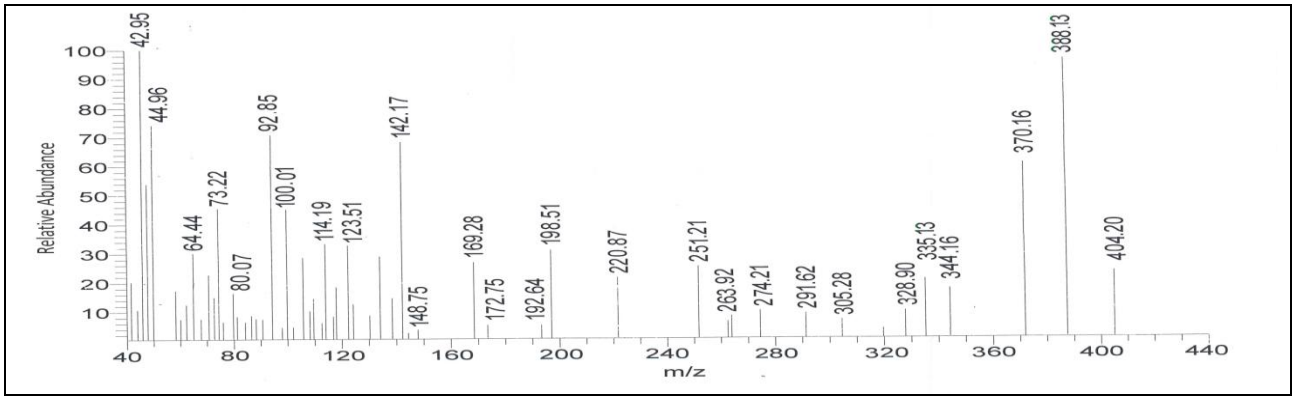

Figure (3): Mass spectrum of olodaterol degradation product

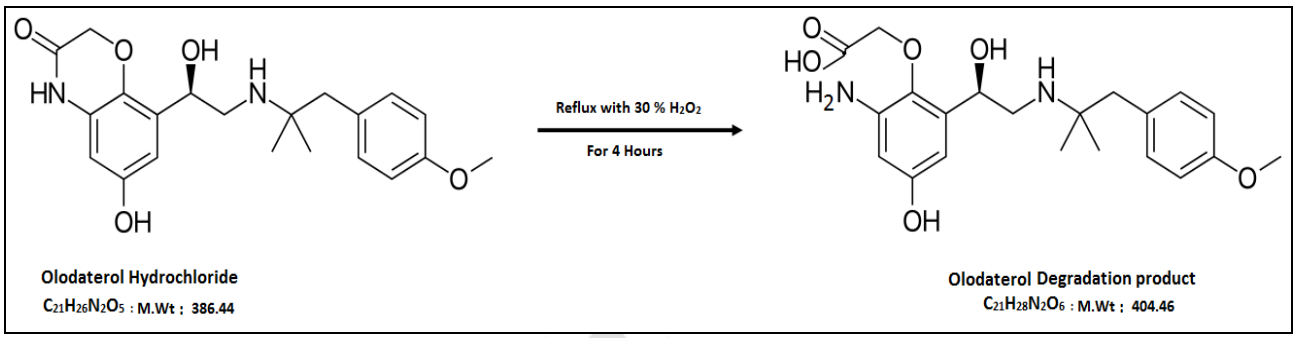

Figure (4): Suggested degradation pathway of olodaterol

First derivative spectrophotometric method

UV-absorption spectra of olodaterol and its degradation product, **Figure (5)**, display severe overlap that made direct quantitative very difficult. The first derivative could be applied to the overlapped UV absorption spectra. The first derivative spectra enabled the estimation of olodaterol by measuring the peak amplitude at 280 nm where no contribution of its degradation product as shown in **Figure (6)**. Calculating the derivative spectra at $\Delta\lambda=2$ minimum noise.

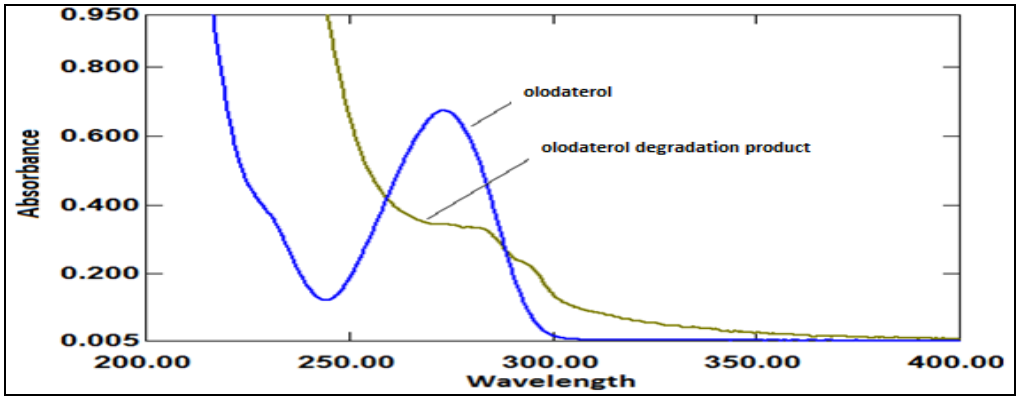

Figure (5): Zero-order absorption spectra of olodaterol (7 µg/mL) and olodaterol degradation product (7 µg/mL).

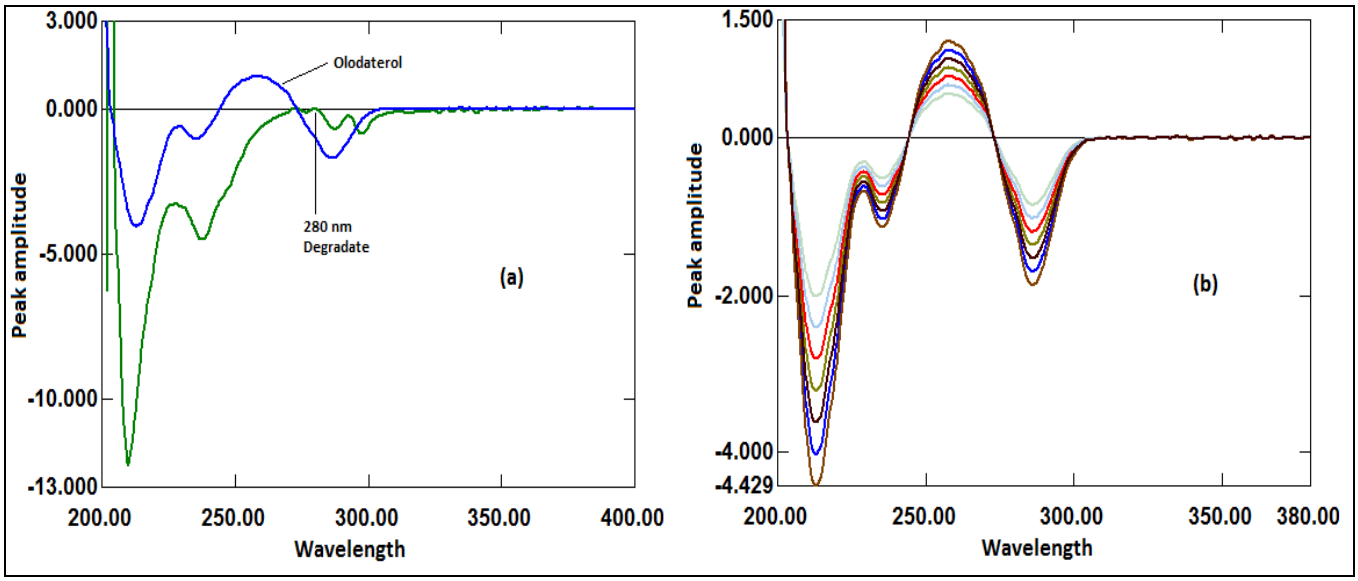

Figure (6): (a) First derivative of the UV spectra of olodaterol and olodaterol degradation product (7 µg/mL) of each and (b) First derivative of the UV spectra of olodaterol (2-8 µg/mL).

Spectrofluorimetric method

Method development

Olodaterol exhibits a native fluorescence in ethanol and its emission can be measured at 409 nm (λem) after excitation at 250 nm (λex), without interference from its oxidative degradation product. The emission and excitation spectra of olodaterol in ethanol are shown in **Figure 7**, and emission spectra of olodaterol and its degradation product are shown in **Figure 8.**

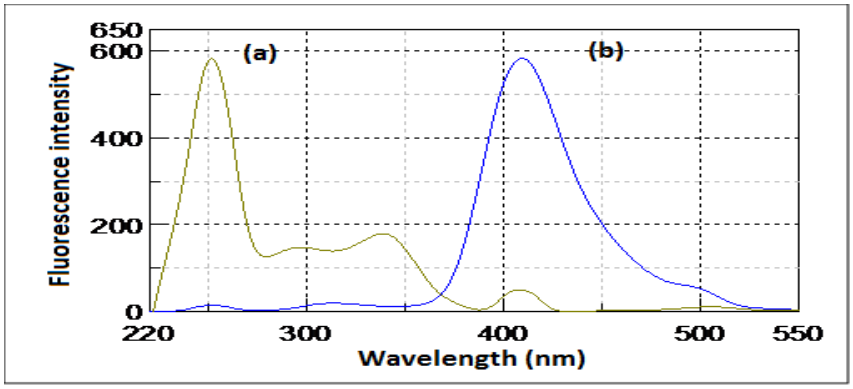

Figure (7): Excitation (a) and emission (b) spectra of olodaterol (1.5 µg/mL) in ethanol.

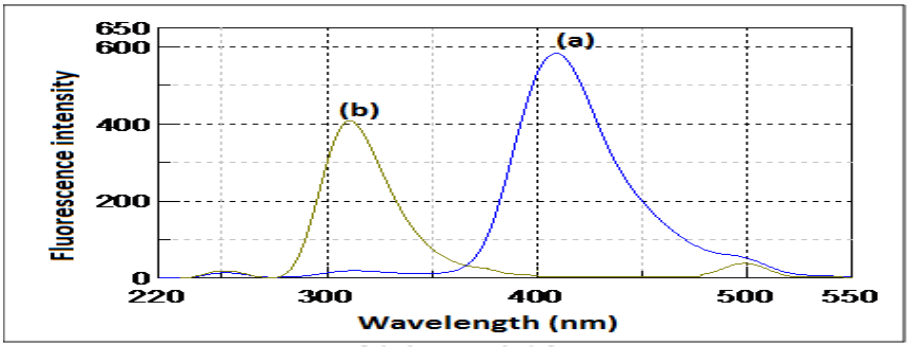

Figure (8): Emission spectra of (a) olodaterol (1.5 µg/mL) and (b) olodaterol degradation product (1.5 µg/mL), in ethanol

Method Optimization

Affecting the fluorescence intensity of olodaterol was the selective determination of olodaterol.

Effect of diluting solvents

The general procedure for the method was repeated using a fixed amount of olodaterol (1.5 µg) and different diluting solvents, the results as shown in **Figure 9**. prove that; ethanol is the best diluting solvent.

Effect of pH and buffer

The general procedure for the method was repeated using a fixed amount of olodaterol (1.5 µg) and different buffers with different pH values, the results as shown in **Figure 10,** prove that; acetate buffer pH 5 gives the best results.

Effect of buffer volume

The general procedure for the method was repeated using a fixed amount of olodaterol (1.5 µg) and different volumes of acetate buffer pH 5, the results as shown in **Figure 11,** prove that; 1 mL of acetate buffer pH 5 gives the best results.

Effect of time

The general procedure for the method was repeated using a fixed amount of olodaterol (1.5 µg) at different time intervals, the results as shown in **Figure 12** prove that; the fluorescence intensities were stable from zero time up to 60 min.

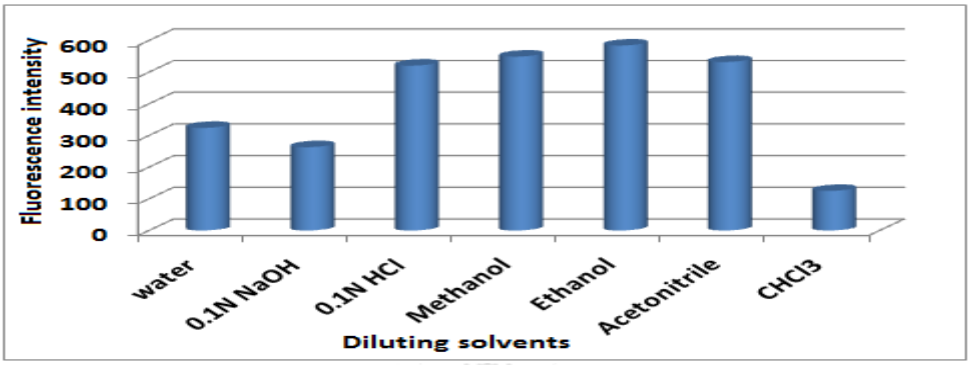

Figure (9): Effect of different diluting solvents on the fluorescence intensity of olodaterol (1.5 µg/mL).

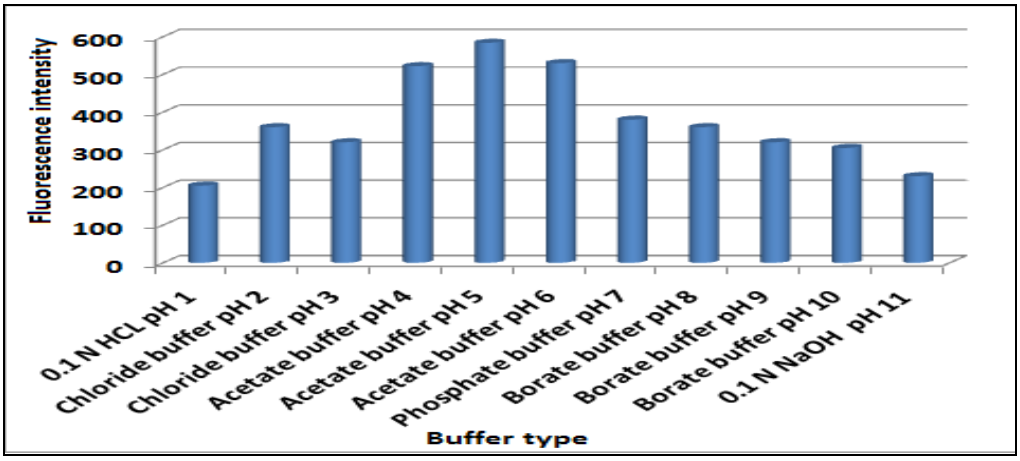

Figure (10): Effect of pH on fluorescence intensity of olodaterol (1.5 µg/mL).

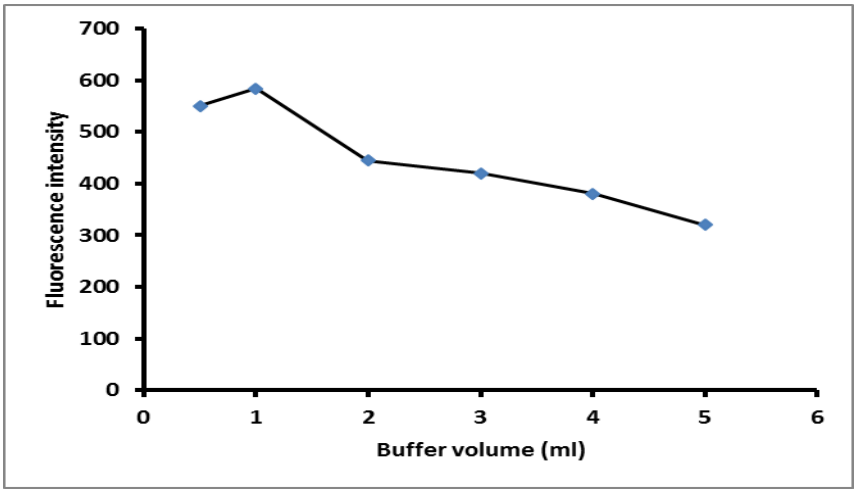

Figure (11): Effect of buffer volume on the fluorescence intensity of olodaterol (1.5 µg/mL).

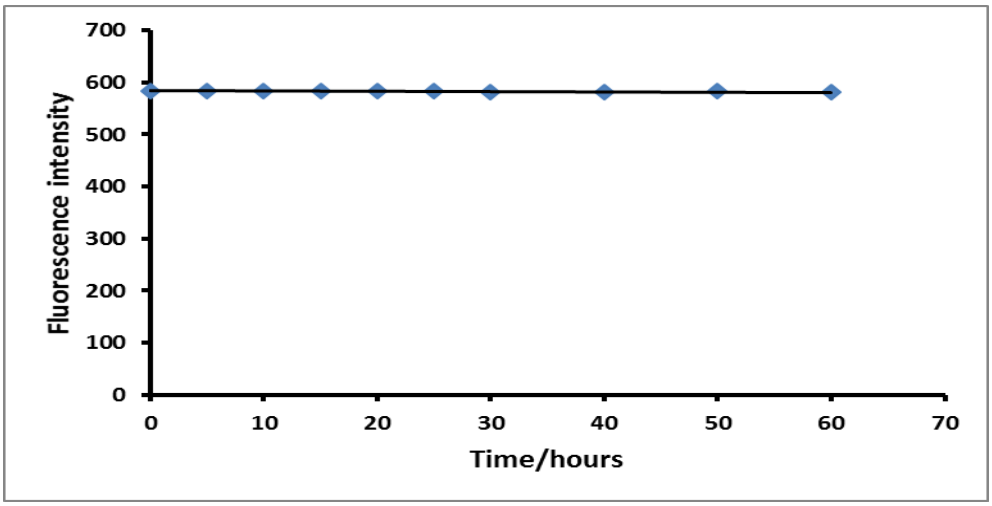

Figure (12): Effect of time on fluorescence intensity of olodaterol (1.5 µg/mL).

Method Validation

The proposed methods were validated according to ICH guidelines [4]**.** Linearity range, limits of detection and quantification, accuracy, precision, and specificity were investigated.

Linearity and range

Proposed methods were constructed by plotting amplitude values of the UV spectra versus in µg/ mL for first derivative and spectrofluorimetric method respectively. The regression analysis data for the described methods were presented in **Table (1).** The regression plots were found to be linear over the ranges of 0.5-2.5 µg/mL and 2-8 µg/mL for

spectrofluorimetric and first derivative spectrophotometric methods respectively.

Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the following equations:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where **σ** is the residual standard deviation of the regression line and **S** is the slope. The results in **Table (1)** indicated that spectrofluorimetric method is more sensitive than first derivative spectrophotometric method.

Accuracy

The accuracy by calculating the mean percent recovery (% R), three concentration levels covering the linearity range (3, 5 and 7 µg/mL) and (0.5, 1 and 2 µg/mL) for first derivative spectrophotometric and spectrofluorimetric methods respectively. The results in **Table (1)** indicated the good accuracy of the proposed methods.

Precision

Different concentrations of olodaterol (3, 5 and 7µg/mL) and (0.5, 1 and 2 µg/mL) for first derivative spectrophotometric and spectrofluorimetric was checked. This days for intermediates precision. The % RSD was calculated and found to be satisfactory as shown in **Table (1).**

Table (1): Regression and validation parameters for determination of olodaterol by the proposed methods:

Parameters		First derivative spectrophotometric method	Spectrofluorimetric method
Wavelength (nm)		280	$\lambda_{emission}$ (409 nm) $\lambda_{excitation}$ (250 nm)
Linearity range (µg/ml)		2-8	0.5-2.5
- Regression Equation - Slope (b) - Intercept (a)		*y= b x+ a* 0.0957 0.2970	*y= b x+ a* 389.7912 4.4024
Coefficient of determination (r^2)		0.9996	0.9997
Accuracy (%R)*		99.13	99.93
Precision (%RSD)*	Repeatability	0.762	0.557
	Intermediate precision	0.896	0.783
LOD (µg/mL)		0.135	0.044
LOQ (µg/mL)		0.408	0.133

*** Values for 3 determinations of 3 different concentrations**

Specificity

Ratios of olodaterol and its degradation product were mixed well. Then, the mixtures were analyzed using the previously described procedure for each method and the percent recovery of the studied drug in the presence of its degradation product was calculated. Good satisfactory results were obtained and listed in **Table (2).**

Table (2): Determination of olodaterol in laboratory prepared mixtures with its degradation product by the proposed methods.

Olodaterol (µg/ml)		Degradation product (µg/ml)		% Degradate		% Recovery of olodaterol	
^{1}D	FLUORO	^{1}D	FLUORO	^{1}D	FLUORO	^{1}D	FLUORO
5	2	3	0.5	37.5	20	100.12	99.56
4	1.5	4	1	50	40	99.82	99.84
3	1	5	1.5	62.5	60	99.87	99.34
2	0.5	6	2	75	80	98.6	98.6
Mean ± %RSD						99.60 ± 0.684	99.34 ± 0.535

Application to pharmaceutical preparation

The proposed procedures were applied for the determination of olodaterol in Striverdi®Respimate. Satisfactory results were obtained in good agreement with the label claim. The obtained results were statistically compared to those obtained by the reported method [2]. No significant differences were found by applying t-test and F-test at 95% confidence level [5], indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **Table 3.**

Furthermore, to check the specificity of the described methods. It was done by adding known quantities of olodaterol the percent recovery of the pure added concentrations were calculated. The data listed in **Table (4)** proved that the proposed method could selectively analyze the studied drug without any interference from the additives or the excipients.

Table (3): Determination of olodaterol in Striverdi® Respimate by the proposed and reported methods:

Parameters	Proposed method		Reported method[(2)]
	^{1}D	FLUORO	
n*	5	5	5
Average (% Recovery)	100.02	99.84	99.79
%RSD	1.121	0.943	1.177
t** (2.31)	0.32	0.08	——
F** (6.39)	1.10	1.56	——

*Number of samples.

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

[(2)] HPLC using C_{18} column, mobile phase was phosphate buffer: methanol [55:45 v/v], pH 4.4) at a flow rate (1mL/min) and UV detection at 236 nm.

Table (4): Recovery study of olodaterol by adopting standard addition technique via the proposed methods.

Pharmaceutical taken (µg/ml)		Pharmaceutical found (µg/ml)		Pure added (µg/ml)		%Recovery of olodaterol	
^{1}D	FLUORO	^{1}D	FLUORO	^{1}D	FLUORO	^{1}D	FLUORO
2.4	1.2	2.390	1.194	2	0.5	99.98	99.46
				3	0.8	99.31	98.59
				4	1.5	100.59	98.25
Mean ± %RSD						99.96 ± 0.640	98.77 ± 0.632

CONCLUSION

This work has introduced the first spectroscopic quantitative analytical methods for the selective determination of olodaterol. First derivative spectrophotometric and native spectrofluorimetric methods have been applied to the estimation of olodaterol either in its pure powder form or in the pharmaceutical dosage form in the presence of its oxidative degradation product. The suggested methods have been validated according to the ICH

guidelines where accuracy, precision, and specificity have been found to be within the acceptable limit.

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