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Research Article

Different Spectrophotometric Methods Manipulating Ratio Spectra Applied for the Analysis of Aclidinium in Duaklir® Genuair® Inhalation Powder

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Two simple, accurate, and selective spectrophotometric methods were developed to determine aclidinium in the presence of formoterol as interferent compound in Duaklir Genuair inhalation powder. The methods under study are ratio derivative and ratio subtraction spectrophotometric methods. These methods are based on different mathematical processing of the obtained ratio spectra. The methods are linear over the concentration range of 5–50 μg/mL for aclidinium and validated according to the ICH guidelines. The accuracy and precision are found to be within the acceptable limits and assessed by applying the standard addition technique. The specificity of the proposed methods was tested using laboratory-prepared mixtures. Furthermore, the methods were statistically compared to the reported RP-HPLC method, and good results were obtained.

1. Introduction

Duaklir Genuair is a recently approved two-component inhalation mixture of aclidinium and formoterol used to relieve symptoms in adult patients with chronic obstructive pulmonary disease [1]. Aclidinium (Figure 1(a)) acts through its binding to M3 receptors located on the bronchial smooth muscle and the vascular endothelium in the lung thus resulting in bronchodilation [2, 3]. Formoterol (Figure 1(b)) exhibits its bronchodilator activity through its long and selective sympathomimetic action on the bronchial smooth muscles [4].

The literature survey revealed that several methods were developed for the determination of formoterol either alone [5–8] or in many other combinations [9–13], and only one liquid chromatographic method was developed for the assay of aclidinium and formoterol in their combined dosage form [14].

Time saving, cost effectiveness, and the ability for resolving overlap of binary and multicomponents without pretreatment of the sample are well-characterized advantages of using different spectrophotometric techniques in pharmaceutical analysis over other techniques such as HPLC and electrochemical techniques [15–17]. Hence, the aim of the present work is to develop two accurate and precise spectrophotometric methods manipulating ratio spectra, namely, ratio derivative [18] and ratio subtraction [19, 20]. The methods were applied for the determination of aclidinium in the presence of formoterol as the interferent compound in Duaklir Genuair and in their laboratory-prepared mixtures.

2. Experimental

2.1. Materials and Reagents. Aclidinium bromide (99.84%) and formoterol fumarate dihydrate (99.94) were kindly supplied by the National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Duaklir Genuair inhalation powder (batch number 208K) was kindly supplied by NODCAR, Giza, Egypt. Each dose was claimed to contain 396 micrograms of aclidinium bromide and 11.8 micrograms of formoterol fumarate dihydrate. Methanol, HPLC grades (Sigma-Aldrich, Germany).

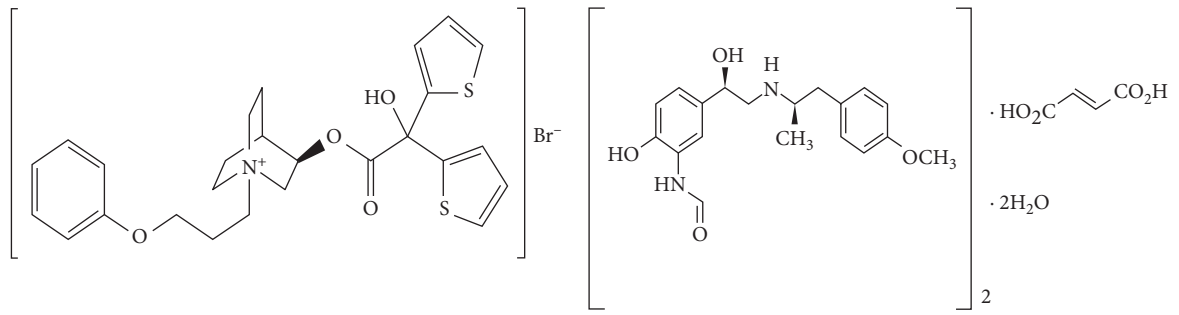

Figure 1: Chemical structure of (a) aclidinium bromide and (b) formoterol fumarate dihydrate.

2.2. Instruments. Shimadzu dual beam UV-visible spectrophotometer (Kyoto, Japan) model UV-1800 PC connected to a compatible IBM and an HP 1020 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.43 (Shimadzu), was used.

2.3. Software. The bundled software, UV-Probe personal spectroscopy software version 2.43 (Shimadzu), was used. Ratio derivative and ratio subtraction methods were implemented in Matlab 8.2.0.701 (R2013b) using our own written scripts. The *t*-test, *F*-test, and one-way ANOVA were performed using Microsoft Excel.

2.4. Standard Solutions. Stock standard solutions of aclidinium bromide and formoterol fumarate dihydrate (100 μg/mL) were prepared by dissolving 10 mg of each drug in 75 mL methanol, and the volume was completed to 100 mL with the same solvent.

2.5. Procedure

2.5.1. General Procedures. In a series of 10 mL volumetric flasks, aliquots of aclidinium (100 μg/mL) equivalent to 50–500 μg were transferred and diluted to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank and then divided by the spectrum of formoterol solution (8 μg/mL).

(i) Ratio derivative method: The first derivative corresponding to each ratio spectrum was recorded, using $\Delta\lambda = 8$ nm and scaling factor = 5. The amplitude values were measured at 229 nm.

(ii) Ratio subtraction method: The amplitude values at 290 nm in the ratio spectra were subtracted from each ratio spectrum, followed by multiplication of the obtained spectra by the divisor (8 μg/mL formoterol). The amplitude values were measured at 238 nm.

2.5.2. Application to Laboratory-Prepared Mixtures. Aliquots of aclidinium bromide and formoterol fumarate dihydrate were mixed in different ratios. The absorption spectra of the prepared mixtures were recorded, and the concentrations of aclidinium bromide were calculated using the procedure described under the mentioned methods.

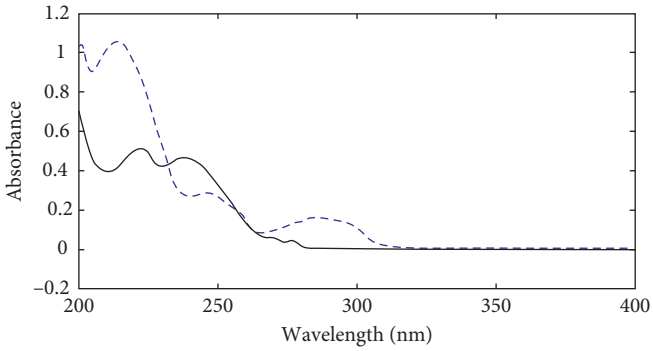

Figure 2: Zero-order absorption spectra of (20 μg/mL) aclidinium bromide (_____) and (10 μg/mL) formoterol fumarate dehydrate (--------).

2.5.3. Application to Pharmaceutical Preparation. A quantity equivalent to 10 mg of aclidinium bromide was accurately weighed and transferred, then the volume was made up to 75 mL with methanol. The solution was shaken vigorously for 15 min, then sonicated for 30 min and completed to 100 mL with the same solvent to obtain a solution labeled to contain 100 μg/mL of aclidinium bromide. The solution was filtered. Further dilutions with the same solvent were performed to obtain working solutions to be assessed by referring to the procedure described under the mentioned methods.

3. Results and Discussion

Aclidinium bromide and formoterol fumarate dihydrate are the active ingredients of Duaklir Genuair inhalation powder. The zero-order absorption spectra of these two drugs show severe overlap as shown in Figure 2, which creates difficulty in their analysis. It is noteworthy to mention that several methods were devoted for the analysis of formoterol either alone or in many other combinations. Moreover, the authors believe that the application of sample enrichment technique to determine formoterol as a minor component will produce false-positive results, and it will be difficult to determine formoterol in the pharmaceutical dosage form accurately.

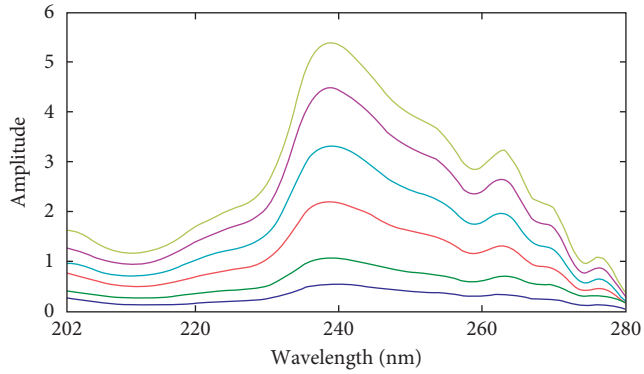

Figure 3: Ratio spectra of aclidinium at various concentrations (5–50 μg/mL) using 8 μg/mL of formoterol as a divisor.

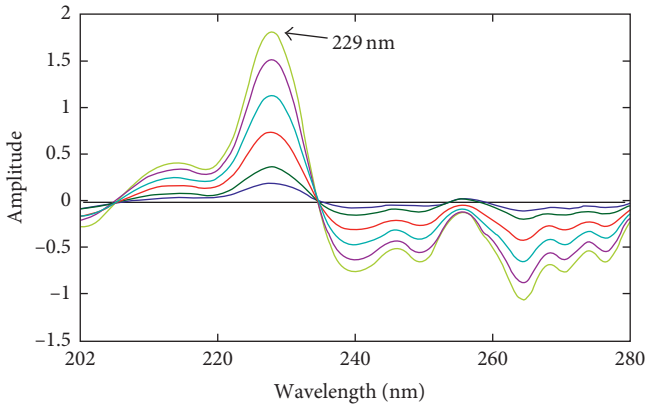

Figure 4: First derivative of the ratio spectra of aclidinium at various concentrations (5–50 μg/mL) using 8 μg/mL of formoterol as a divisor.

Hence, the main theme of this work is to determine aclidinium bromide in the presence of formoterol fumarate dihydrate as the interferent using two methods manipulate ratio spectra, namely, ratio derivative and ratio subtraction. The ratio spectra were obtained by dividing aclidinium spectra by a definite concentration of formoterol. Different concentrations of the divisor were tried (6, 8, and 10 μg/mL), and the divisor concentration (8 μg/mL) was found to be the best choice regarding signal-to-noise ratio, repeatability, sensitivity, and average recovery percent when used for the prediction of aclidinium by the proposed methods is shown in Figure 3.

In the ratio derivative method, the amplitudes of the first derivative of the ratio spectra at 229 nm were proportional to the concentrations of aclidinium without interference from formoterol (divisor) is shown in Figure 4.

In the ratio subtraction method, the laboratory-prepared mixtures were divided by the spectrum of formoterol to get the ratio spectra is shown in Figure 5, then subtraction of the absorbance values in plateau region at 290 nm (the constant) is shown in Figure 6, followed by multiplication of the obtained spectra by the spectrum of the divisor is shown in Figure 7. The peak amplitude at 238 nm in the final spectra is proportional to the concentrations of aclidinium without interference from formoterol.

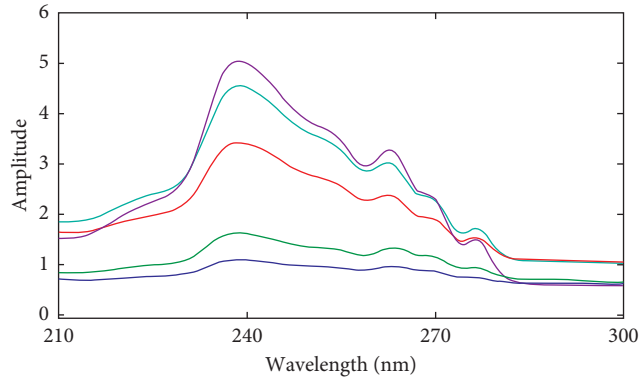

Figure 5: Ratio spectra of laboratory-prepared mixtures of aclidinium (5–40 μg/mL) and formoterol (5–10 μg/mL) using 8 μg/mL of formoterol as a divisor.

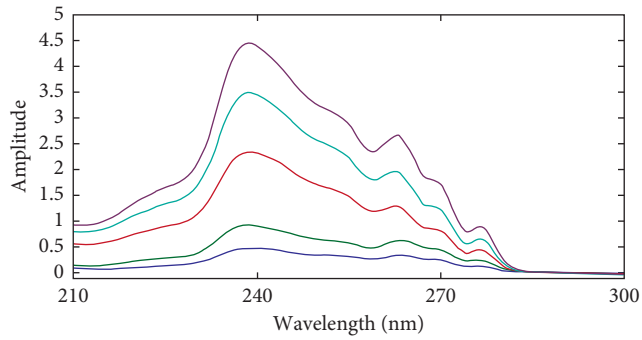

Figure 6: Ratio spectra of laboratory-prepared mixtures of aclidinium (5–40 μg/mL) and formoterol (5–10 μg/mL) using 8 μg/mL of formoterol as a divisor after subtraction of the constant.

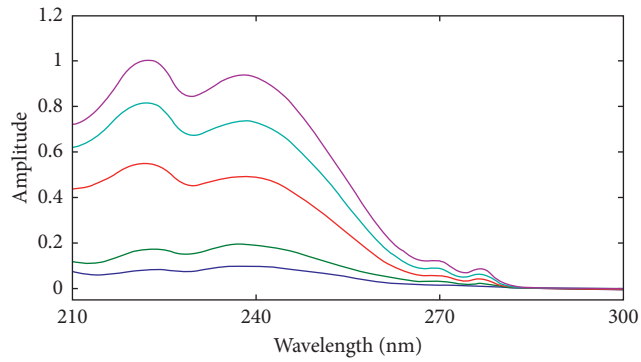

Figure 7: The zero-order absorption spectra of aclidinium (5–40 μg/mL) obtained by the proposed ratio subtraction method for the analysis of laboratory-prepared mixtures with formoterol (5–10 μg/mL) after multiplication by the divisor.

3.1. Method Validation. Validation of the proposed methods was done according to the ICH guidelines [21]. The linearity of all the methods was determined over the concentration range of 5–50 μg/mL for aclidinium bromide. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to the ICH guidelines. The lower values of the LODs and LOQs indicate the sensitivity of the developed methods.

Table 1: Regression and validation of data for determination of aclidinium by the proposed ratio derivative and ratio subtraction methods.

Parameters		Ratio derivative	Ratio subtraction
Wavelength (nm)		229	238
Linearity range (μg/mL)		5–50	
(i) Regression equation		$y^a = bx^b + a$	$y^a = bx^b + a$
(ii) Slope (b)		0.0380	0.0230
(iii) Intercept (a)		−0.0007	0.0032
Coefficient of determination (r^2)		0.9997	0.9996
LOD (μg/mL)		0.9681	1.1428
LOQ (μg/mL)		2.9336	3.4630
Accuracy (%R)[c]		101.55	100.82
Precision (% RSD)[c]	Repeatability	1.237	1.149
	Intermediate precision	1.707	1.520

y^a is the response of each method; x^b is the concentration of aclidinium in μg/mL in each method; [c]values for 3 determinations of 3 different concentrations.

Table 2: Determination of aclidinium in mixtures with formoterol by the proposed ratio derivative and ratio subtraction methods.

Aclidinium (μg/mL)	Formoterol (μg/mL)	% recovery	
		Ratio derivative	Ratio subtraction
5	5	97.77	98.12
10	5	100.08	99.81
20	10	100.28	101.10
30	10	101.76	101.32
40	5	102.48	98.73
Mean ± % RSD		100.47 ± 1.806	99.82 ± 1.415

Table 3: Recovery study of aclidinium by adopting standard addition technique via the proposed ratio derivative and ratio subtraction methods.

Pharmaceutical conc. (μg/mL)	Pharmaceutical found (μg/mL)	Pure added (μg/mL)	% recovery	
			Ratio derivative	Ratio subtraction
35	35.073[a]	6	101.26	100.29
	9.955[b]	8	100.23	101.28
		10	98.25	99.67
Mean ± % RSD			99.91 ± 1.531	100.41 ± 0.809

[a]Average of five determinations by the ratio derivative method; [b]average of five determinations by the ratio subtraction method.

The accuracy of the methods was determined by calculating the mean percent recovery of three determination of three different concentration of pure aclidinium bromide (20, 30, and 40 μg/mL). Good percent recoveries were obtained as indicated in Table 1. The precision of the developed methods was checked by measuring percent relative standard deviation (% RSD) of three concentrations for aclidinium bromide (20, 30, and 40 μg/mL) repeated three times in the same day (repeatability) and in three days (intermediate precision). The obtained values of % RSD were <2%, confirming the high precision of the developed method. The summary of validation and regression parameters for the method is shown in Table 1.

The specificity of the developed methods was determined by analysis of laboratory-prepared mixtures of both aclidinium bromide and formoterol fumarate dihydrate, where good recoveries of aclidinium bromide were obtained confirming the specificity of the developed methods as shown in Table 2. Moreover, the standard addition technique was applied, where pure aclidinium bromide was added to already analyzed pharmaceutical preparation. Good recoveries of the pure standard drug were obtained confirming that the proposed methods could be adopted for the determination of aclidinium bromide without any possible interference from formoterol fumarate dihydrate, excipients, or additives. The obtained results are shown in Table 3.

3.2. Application of the Developed Method. The developed methods were applied for the assay of aclidinium bromide in its commercial pharmaceutical formulation (Duaklir Genuair inhalation powder). The obtained results of the developed methods compared with the reported HPLC method [14] are shown in Table 4. The calculated t and

Table 4: Determination of aclidinium in Duaklir Genuair inhalation powder by the proposed ratio derivative, ratio subtraction, and the reported methods.

Parameters	Ratio derivative	Ratio subtraction	Reported method [14]
n[a]	5	5	5
Average (% recovery)	99.23	100.22	99.65
SD	1.462	1.599	1.717
% RSD	1.474	1.595	1.724
T (2.306)[b]	0.949	0.030	—
F (6.388)[b]	1.400	1.171	—

[a]Number of measurements; [b]The values in parenthesis are tabulated values of "t" and "F" at ($P = 0.05$).

Table 5: One-way ANOVA testing for the different proposed methods used for the determination of aclidinium bromide in Duaklir Genuair inhalation powder.

	Source	DF	Sum of squares	Mean square	F value
Aclidinium	Between experiments	2	3.186	1.593	0.622 (3.885)
	Within experiments	12	30.744	2.562	

The values between parentheses are the theoretical F values; the population means are not significantly different.

F values are less than the tabulated ones indicating that there are no significant differences between the two methods and the reported method.

Another statistical comparison of the obtained results by the proposed methods and the reported method was performed for the determination of aclidinium bromide in its pharmaceutical product using one-way ANOVA test is shown in Table 5. The results obtained by applying these methods show no significant differences between all of them.

4. Conclusion

Two spectrophotometric methods manipulating ratio spectra were applied for the selective determination of aclidinium bromide in the presence of formoterol fumarate dihydrate as the interferent without prior separation or pretreatment. The proposed methods are simple, sensitive, accurate, and precise manipulating ratio spectra. It does not need any sophisticated apparatus and could be easily applied in quality control laboratories. Moreover, the proposed method does not require multiple steps or complicated handling associated with chromatographic methods. There is no derivatization step in the ratio subtraction method which enhance the signal-to-noise ratio. However, special software such as Matlab is needed for doing the data analysis of this method. The developed methods have been statistically compared to the reported HPLC method by analyzing their mean and variance using different methods. The results indicate that no significance difference was found regarding the developed spectrophotometric methods and the reported HPLC method.

Data Availability

The scripts used for calculating the ratio derivative and ratio subtraction methods along with data in Matlab format are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] *Duaklir® Genuair® Approved in the European Union for Chronic Obstructive Pulmonary Disease*, 2017, https://www.astrazeneca.com/media-centre/press-releases/2014/duaklir-genuiar-approved-european-union-chronic-obstructive-pulmonary-disease-24112014.html#!.

[2] *Aclidinium Bromide*, 2017, https://pubchem.ncbi.nlm.nih.gov/compound/Aclidinium_bromide.

[3] M. Pisano and N. Mazzola, "Aclidinium bromide inhalation powder (Tudorza): a long-acting anticholinergic for the management of chronic obstructive pulmonary disease," *Pharmacy and Therapeutics*, vol. 38, no. 7, pp. 393–396, 2013.

[4] Formoterol, https://pubchem.ncbi.nlm.nih.gov/compound/formoterol, 2017.

[5] B. Demircigil, S. Özkan, Ö. Coruh, and S. Yılmaz, "Electrochemical behavior of formoterol fumarate and its determination in capsules for inhalation and human serum using differential-pulse and square-wave voltammetry," *Electroanalysis*, vol. 14, no. 2, pp. 122–127, 2002.

[6] H. Kamimura, H. Sasaki, S. Higuchi, and Y. Shiobara, "Quantitative determination of the β-adrenoceptor stimulant formoterol in urine by gas chromatography mass spectrometry," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 229, no. 2, pp. 337–345, 1982.

[7] M. Gousuddin, S. A. Raju, and M. S. Sultanuddin, "Development and validation of spectrophotometric methods for estimation of formoterol bulk drug and its pharmaceutical dosage forms," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, pp. 300–309, 2011.

[8] S. T. Hassib, A. A. El-Zaher, and M. A. Fouad, "Validated stability-indicating derivative and derivative ratio methods for the determination of some drugs used to alleviate respiratory tract disorders and their degradation products," *Drug Testing and Analysis*, vol. 3, no. 5, pp. 306–318, 2011.

[9] R. I. El-Bagary, M. A. Fouad, A. Manal, and E. H. Tolba, "Forced degradation of mometasone furoate and development of two RP-HPLC methods for its determination with formoterol fumarate or salicylic acid," *Arabian Journal of Chemistry*, vol. 9, no. 3, pp. 493–505, 2016.

[10] K. H. Assi, W. Tarsin, and H. Chrystyn, "High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in Symbicort Turbuhaler," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 1, pp. 325–328, 2006.

[11] V. K. Parmar, H. N. Patel, and B. K. Patel, "Sensitive, and robust methods, for simultaneous determination of beclomethasone dipropionate and formoterol fumarate dihydrate in rotacaps," *Journal of Chromatographic Science*, vol. 52, no. 10, pp. 1255–1266, 2014.

[12] M. Rizk, M. Sultan, N. Talaat, and N. Youssef, "A validated TLC—densitometric method for the simultaneous determination of formoterol fumarate and budesonide in

pressurized metered-dose inhaler," *JPC-Journal of Planar Chromatography-Modern TLC*, vol. 30, no. 1, pp. 63–67, 2017.

[13] H. A. Merey, S. S. El-Mosallamy, N. Y. Hassan, and B. A. El-Zeany, "Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form," *Bulletin of Faculty of Pharmacy, Cairo University*, vol. 54, no. 1, pp. 99–106, 2016.

[14] R. Gowda, "Simultaneous RP-HPLC method for determination of impurities in formoterol fumarate and aclidinium bromide in pharmaceutical dosage forms," *International Journal of Chemical and Pharmaceutical Analysis*, vol. 3, no. 3, p. 1005, 2016.

[15] K. A. Attia, N. M. El-Abasawi, A. El-Olemy, and A. Serag, "Different spectrophotometric methods applied for the analysis of simeprevir in the presence of its oxidative degradation product: a comparative study," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 190, pp. 1–9, 2018.

[16] K. A. Attia, M. W. Nassar, M. B. El-Zeiny, and A. Serag, "Stability indicating methods for the analysis of cefprozil in the presence of its alkaline induced degradation product," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 159, pp. 1–6, 2016.

[17] K. A. Attia, M. W. Nassar, M. B. El-Zeiny, and A. Serag, "Firefly algorithm versus genetic algorithm as powerful variable selection tools and their effect on different multivariate calibration models in spectroscopy: a comparative study," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 170, pp. 117–123, 2017.

[18] K. A. Attia, M. W. Nassar, M. B. El-Zeiny, and A. Serag, "Stability-indicating methods for the analysis of ciprofloxacin in the presence of its acid induced degradation product: a comparative study," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 159, pp. 219–222, 2016.

[19] H. W. Darwish, S. A. Hassan, M. Y. Salem, and B. A. El-Zeany, "Sequential spectrophotometric method for the simultaneous determination of amlodipine, valsartan, and hydrochlorothiazide in coformulated tablets," *International Journal of Spectroscopy*, vol. 2013, Article ID 273102, 8 pages, 2013.

[20] K. A. Attia, M. W. Nassar, M. B. El-Zeiny, and A. Serag, "Different spectrophotometric methods applied for the analysis of binary mixture of flucloxacillin and amoxicillin: a comparative study," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 161, pp. 64–68, 2016.

[21] ICH Harmonised Tripartite Guidelines, *Validation of Analytical Procedures: Text and Methodology Q2(R1)*, 2016, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.