Egypt. J. Chem. **Vol. 66**, No. 7 pp. 119 - 129 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Development and Evaluation of Simple Spectrophotometric Methods for the Simultaneous Determination of Alogliptin Benzoate Metformin Hydrochloride and Melamine

Nehal F. Farid [a], Nouruddin W. Ali [a], Ahmed A. Taha [b] Ahmed M. Elgebaly[c] and Maimana A. Magdy[a]

[a]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt*

[b]*Chemistry Department, Faculty for Girls, Ain Shams University, Cairo, Egypt*

[c]*Analytical Chemistry Department, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt*

Abstract

For an analyst, quantitative determination of a ternary combination with strongly overlapped spectra is a difficult task. The resolution and quantification of a ternary combination consisting of alogliptin benzoate, metformin HCl, and metformin impurity; melamin were achieved without the requirement for any preliminary separation processes by manipulating spectral data of this overlap using simple spectrophotometric techniques. For the determination of the three components, two straightforward, sensitive, and reproducible procedures were developed. Combining many spectrophotometric techniques, method (A) identifies the three components under investigation; Zero order spectrophotometric analysis was used to identify alogliptin benzoate (0D) at 276 nm, metformin HCl and melamin were determined by ratio difference spectrophotometric method; using 5 µg mL-1 of alogliptin benzoate as a divisor and measuring the difference in absorbance between 226.6, 240nm and 207.6, 255.8nm for determination of metformin HCl and melamin respectively. In method (B), the ratio spectra were centred using the mean approach, with the mean amplitudes at279.2, 236.2, and 232.2 nm were used for the estimation of alogliptin benzoate, metformin HCl and melamin, respectively. The methods' accuracy, precision, and selectivity have all been validated in accordance with ICH recommendations. The techniques have also been applied to Westirizide® tablets that include both Alogliptin benzoate and Metformin HCl, and the results showed that there was no interference from additives. When these approaches were contrasted with the described one, no significant difference was discovered.

Keywords: Alogliptin Benzoate, Metformin Hydrochloride and Melamin, spectrophotometry, mean centering of ratio spectra

1. Introduction

Alogliptin benzoate (AGT) "2-({6-[(3R)-3-aminopiperidin-1- yl]-3-methyl-2, 4-dioxo-1, 2, 3, 4 tetrahydropyrimidin-1-yl} methyl) benzonitrile benzoate"(Figure1) belongs to a novel class of anti-diabetic medications called dipeptidyl peptidase-4 inhibitors that works by boosting glucose-dependent insulin release [1]. Therapeutically, type 2 diabetes is treated with dipeptidyl peptidase-4 inhibitors alone or in conjunction with other medications that make insulin more sensitive at the target site [2-4].

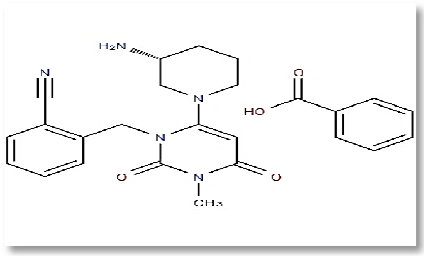


Figure 1. Alogliptin benzoate

Pharmacopoeia does not list analogliptin benzoate as an official medication. Numerous methods for

*Corresponding author e-mail: ahmad.moustafa@nub.edu.eg; (Ahmed M. Elgebaly).

EJCHEM use only: Received date 17 July 2022; revised date 17 September 2022; accepted date 14 October 2022

DOI: 10.21608/EJCHEM.2022.150683.6527

estimating AGT in drug products have been published in the literature employing chromatographic techniques [5-7], spectrophotometric techniques [8], or capillary electrophoresis, either alone or in combination with other medications. [9].

Metformin hydrochloride (MET), chemically known as "1, 1- dimethylbiguanide hydrochloride and it belongs to biguanide class of oral anti-diabetic drugs" appearance By reducing hepatic glucose production, gluconeogenesis, and boosting peripheral glucose uptake, MET acts as an anti-hyperglycemic and lowers blood glucose levels [10]. Indian Pharmacopeia, USP, and BP all consider metformin hydrochloride as a legitimate medication [11–13]. Numerous techniques for estimating the presence of metformin hydrochloride in medicinal products, either alone or in combination with other medications have been published in the literature [14–17].

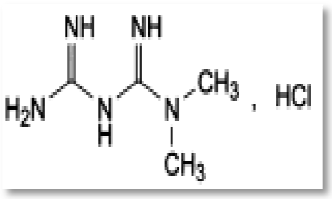


Figure 1. Metformin HCl

Numerous specialized techniques for MET determination in plasma, serum, and urine, as well as its interaction with other medications, were also documented in the literature [18-21].Metformin HCl may include an impurity called Melamin (MLN) "1,3,5-triazine-2,4,6- triamine ". According to the World Health Organization, animal studies have demonstrated that high dose exposure to MLN alone can result in kidney stone formation. [22].

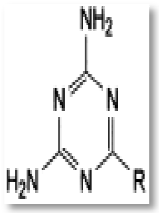


Figure 1. Melamin

Different chromatographic techniques [23–30], as well as UV-Spectrophotometric techniques [31 - 33], have all been used to examine the binary mixture of AGT and MET concurrently in both pure form and pharmaceutical formulation. Only one method has been reported for the determination of AGT and MET in presence of MLN [34]

Recently, analysis of mixtures of many drugs or drugs together with their impurities were developed and validated by analysts using different spectrophotometric methods [35-39].

This work is concerned with development and validation of economic, simple, accurate and precise spectrophotometric methods for determination of the suggested drugs in their raw materials, in combined dosage form and in presence of MET impurity (Melamin). So, it's the first spectrophotometric method which determine AGT and MET in presence of Metformine impurity (MLN).

2. Experimental

2.1. Instrument

The UV-PC personal software version 3.7 was used with a double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm. The wavelength-scanning speed was 2800 nm/min, and the spectral bandwidth was 2 nm. MATLAB® version 6.5 and PLS-Toolbox 2.0 were used for all data analysis. [40]. UV lamp with short wavelength 254nm UV Lamp (Viber Lourmat, Marine LA VALLEE Cedex 1, France).

2.2. Materials

2.2.1. Pure samples

Western Pharmaceutical Industries Co. provided the metformin HCl and AGT, with certified purities of 99.84 and 100.07 percent for MET and AGT, respectively. A certified pure standard MLN with a 99.56 percent purity was purchased from Sigma-Aldrich Co. Chemie GmbH in Germany.

2.2.2. Pharmaceutical formulation

Westirizide® pills (Batch No. W15812) are made by Western Pharmaceutical Industries, Cairo, Egypt, and are labelled to contain 500 mg MET and 10 mg AGT.

2.2.3. Chemicals and reagents

Methanol was of analytical grade (El-Nasr Pharmaceutical Chemicals Co., Abu- Zabaal, Cairo, Egypt).

2.3. Solutions

*AGT, MET, and MLN stock standards (1 mg mL-1) for the spectrophotometric technique were produced in methanol by weighing 0.025 g of each in three separate 25 mL volumetric flasks, and the volume was completed with methanol.

**Working standard solution of* MET, AGT, and MLN (0.1 mg.mL^{-1}):

Working standard solutions were created by transferring 2.5 mL of AGT, MET, and MLN from their respective stock standard solutions (1 mg.mL-1 each) into three distinct 25 mL measuring flasks, and then individually adjusting the volume of each solution with methanol.

**Pharmaceutical formulation solution:*

The weight and fine grinding of ten Westerizide® tablets was performed. Weighed material equal to 12.5 mg MET and 0.25 mg AGT has been added to a 25 mL volumetric flask. Methanol was used to separate the active components from the excipients. 15 minutes of sonication and 0.45 m membrane filtering were used to filter the solution. The volume was then adjusted after taking 0.5 mL of the prepared solution in 10 mL measuring flask and completing the volume with methanol.

3. Procedure

3.1. Instrumental conditions

For spectral characteristics and wavelengths selection: The absorption spectra of 6 µg.mL-1 ALG, 20 µg.mL-1 MET and 6 µg.mL-1 MLN were measured using methanol as a blank spanning the wavelength range of 200-400 nm. The spectra were examined to choose appropriate wavelengths for spectrophotometric analysis.

3.2. Construction of the calibration curves

3.2.1. Zero order and ratio difference spectrophotometric methods

Different aliquots equivalent to 5-60 µg of AGT, 30-500 µg of MET and 10-100 µg MLN were separately transferred from their respective working standard solutions (0.1 mg mL-1 for AGT, MET and MLN) into three separate series of 10-ml volumetric flasks and the volume was completed using methanol to obtain final concentrations of 0.5-6 µg mL-1 for AGT, 3-50 µg.mL-1 for MET and 1-10 µg for MLN.

For measuring AGT, the prepared solutions were scanned in the range of 200 – 400 nm and the absorbance values at 276 nm for AGT were measured with no interference from MET or MLN. The regression equation was derived using the calibration curve that connected the absorbance of AGT at the chosen wavelength to the appropriate drug concentration.

The absorption spectra of MET in the range of (3–50 µg.mL^{-1}) were split by the reference spectrum of 5 µg.mL^{-1}AGT, and the ratio between 226.6 and 240 nm was determined to determine the amount of MET. By charting the ratio difference between the chosen wavelengths against their corresponding concentrations, the calibration curve for MET was created, and the regression equation was then calculated.

For testing MLN, the standard spectrum of 5 µg.mL^{-1}AGT was divided by the MLN absorption spectrum in the range of 1 to 10 µg.mL^{-1}and the ratio difference between 207.6 and 255.8 nm was measured. Plotting the ratio difference between the chosen wavelengths against their corresponding concentrations and computing the regression equation allowed for the construction of the MLT calibration curve.

3.2.2. Mean centering of ratio spectra (MCR) method

Three separate series of 10-mL volumetric flasks were used to transfer different aliquots equivalent to 5-60 µg for AGT, 10-90 µg for MLN, and 30-500 µg for MET from their respective working standard solutions (0.1 mg.mL-1 for AGT, MET, and MLN). The volume was then completed with methanol to obtain final concentrations of 0.5-6 µg.mL-1 for AGT, 1-9 µg.mL-1 for ML. The first ratio spectra, which were later mean-centered, were created by dividing the recorded spectra of various concentrations of AGT in the range of 0.5 to 6 µg.mL^{-1} by the standard spectrum of 5 µg.mL-1 of MET. The mean centering of the second ratio spectra was then derived by dividing these vectors by the mean centered ratio of α MLN/αMET. Similar to this, the MET spectra in the range of 3- 50 g mL-1 were divided by the MLN spectra of 4 µg.mL^{-1}, and the resulting ratio spectra were mean centered. These vectors (mean centered ratio spectra) were then divided by the mean centered ratio of AGT/MLN to obtain the second ratio spectra, which were then mean centered. For MLN, the ratio spectra were mean-centered by dividing the scanned spectra of its produced solutions in the range of 1 to 9 µg.mL^{-1} by the reference spectrum of 3 µg.mL-1 of AGT. The

second ratio spectra were then mean-centered after these vectors were divided by the mean-centered ratio of (αMET/αAGT). By comparing the amplitude values of the mean-centered ratio spectra at 279.2, 236.2, and 232.2 nm for AGT, MET, and MLN, respectively, against their corresponding concentrations, calibration curves were created. A regression equation was then calculated for each component.

3.3. Statistical analysis

The new method and the reported HPLC [34] were statistically compared using Student's t-test and F-test.

4. Results and discussion

Alogliptin benzoate and Metformin hydrochloride is a combination medicine that is used together with healthy diet and exercise for improvement of blood sugar control in adults with type 2 diabetes mellitus. On the other hand, the literature showed only TLC-densitometric and reversed phase HPLC methods [34] for the simultaneous determination of Alogliptin benzoate and Metformin hydrochloride and its hazardous impurity (Melamin). Due to the wide application of the studied combination and due to the draw backs of the previous published spectrophotometric methods for resolving the studied ternary mixture, we aimed in this work to develop and validate accurate, precise, sensitive and selective spectrophotometric methods for measuring the studied drugs in their combined formulation without preparing or treating the sample.

4.1. Spectrophotometric methods

As demonstrated in Figure 2,

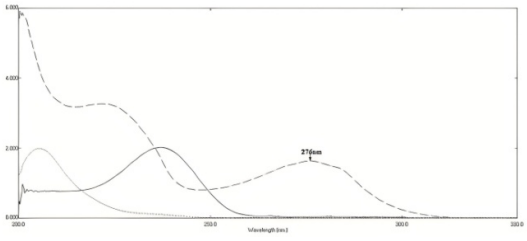


Figure 2. Zero order absorption spectra of 6 µg mL^{-1} of Alogliptin benzoate (_ _ _),20 µg mL^{-1} of Metformin HCl (____) and 6 µg mL^{-1} of Melamin (….), using methanol as a blank

Zero order spectrophotometric measurement (0D) at 276 nm, where there was no interference from MET or MLN, allowed for the direct determination of AGT in the range of of (0.5-6 µg.mL^{-1}). The ratio between 226.6 and 240 nm was used to evaluate MET by dividing the absorption spectra of MET in the range of (3-50 µg.mL-1) by the standard spectrum of 5 µg.mL^{-1} AGT (Figure 3)

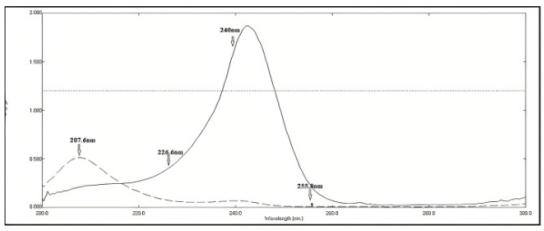


Figure 3. Division spectra of 20 µg mL-1 of Metformin HCl (___), 6µg mL-1 of Melamin (_ _ _) and 6 µg mL-1 of Alogliptin benzoate (….) using 5 µg mL-1 of Alogliptin benzoate as a divisor and methanol as a solvent

The absorption spectra of MET in the (1–10) µg.mL-1 range were split by the reference spectrum of 5 µg.mL-1 AGT, and the ratio between 207.6 and 255.8 nm was then determined (Figure 3).

Linear correlation was obtained and the regression equation were calculated and found to be :

AAGT = 0.327 CAGT-0.003 rAGT=0.9999 Accuracy=100.13±1.049

AMET = 0.046 CMET + 0.002 rMET = 0.9999 Accuracy=99.87±0.436

AMLN = 0.084 CMLN - 0.008 rMLN = 0.9999 Accuracy=99.84±1.033

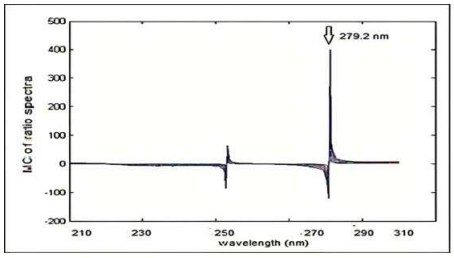
where AAGT, AMET and AMLN are the absorbance values of AGT,MET and MLN respectively, CAGT, CMET and CMLN are the concentrations of AGT,MET and MLN in µgml-1respectively and rAGT, rMET and rMLN are the correlation coefficients of AGT,MET and MLN respectively.

4.2. Mean centering of ratio spectra (MCR) spectrophotometric method.

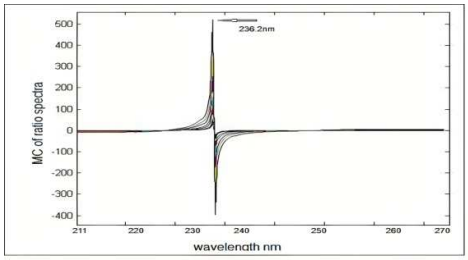
The mean centering of ratio spectra serves as the foundation for the presented approach. Afkhami and Bahram [41] created the technique and provided examples for it. By using this technique, we may avoid the steps involved in spectrum derivatization, which increases the method's sensitivity. Different parameters were examined in order to optimize the MCR method that was established. Different wavelength ranges were tried, and it was discovered that the chosen wavelength range had a significant

impact on the ratio spectra's MCR. The wavelength range from (211.2-300.8 nm) for AGT (Figure 4a), (211.2-270 nm) for MET (Figure 4b), and (211.2-250 nm) for MLN (Figure 4c) produced the greatest results. AGT, MET, and MLN were tested at various concentrations in order to determine the impact of divisor concentration on the method's selectivity. Using 3, 4 and 5 µg.mL-1 of AGT, MLN, and MET, respectively, as divisors, produced the greatest results in terms of sensitivity and selectivity.

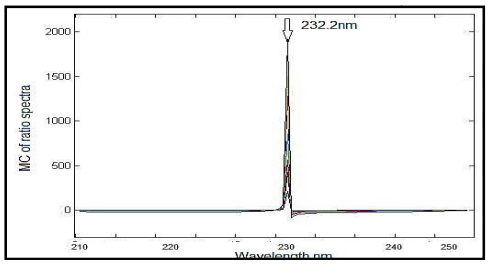
Regression parameters were determined and reported in Table 1 to show that Beer's Lambert rule was followed in the range of 0.5-6 µg.mL^{-1} at 279.2 nm for AGT, 3-50 µg.mL^{-1} at 236.2 nm for MET, and 1-9 µg.mL^{-1} at 232.2 nm for MLN.



(a)



(b)



(c)

Figure 4. Mean centered ratio spectra of (a) Alogliptin benzoate in the range of 0.5-6 µg mL^{-1}, (b) Metformin HCl in the range of 3-50 µg mL^{-1} and (c) Melamin in the range of 1-9 µg mL^{-1} using methanol as a blank

Linear correlation was obtained and the regression equation were calculated and found to be :

PAGT=7.016CAGT+21.48 rAGT=0.9999 Accuracy=100.32±1.085

PMET = 3.45CMET + 0.192 rMET = 0.9999 Accuracy=100.11±0.596

PMLN = 216.1CMLN +0.133 rMLN = 0.9998 Accuracy=100.55±0.909

where PAGT, PMET and PMLN are the peak amplitude at the selected wavelengths of AGT,MET and MLN respectively, CAGT, CMET and CMLN are the concentrations of AGT,MET and MLN in µgml-1respectively and rAGT, rMET and rMLN are the correlation coefficients of AGT,MET and MLN respectively.

4.3. Analysis of laboratory prepared mixtures

Different laboratory-made mixes comprising various ratios of AGT, MET, and MLN were created, and the processes under calibration curve development were followed, to assess the specificity of the spectrophotometric and MCR techniques. When there is no interference from MET or MLN, the zero order (0D) spectrophotometric technique at 276 nm can be used to detect AGT directly (Figure 2). In order to calculate MET, the absorption spectra of laboratory-prepared mixes were multiplied by the standard spectrum of 5 µg.mL-1 AGT, and the ratio between 226.6 and 240 nm was then measured, as shown in (Figure 3).

The ratio difference between 207.6 and 255.8 nm was measured after dividing the absorption spectra of laboratory-prepared mixes by the reference spectrum of 5 µg.mL-1 AGT, as shown in (Figure 3). From the estimated regression equations, concentrations of AGT, MET, and MLN in the prepared samples were determined. The directions under the building of calibration curves were adhered to in order to determine the concentration of each component in the laboratory-prepared mixture using the mean centering of ratio spectra approach.

4.4. Application of the developed methods on pharmaceutical formulation

The newly discovered approach was used to analyze Westerizide® tablets in order to evaluate their viability. The process used for building calibration curves was followed to analyze the previously prepared sample solutions. The AGT and MET content of Westerizid® tablets have been

calculated using the previously computed regression equations, and good results were obtained as indicated in Table 2.

Additionally, using the conventional addition method, three different levels of pure AGT and MET were added. The concentrations of the pure added AGT and MET were then calculated as percent Recovery. Results reveal that without the intervention of tablet excipients, the established methods can accurately assess both MET and AGT in Westerizide® tablets.

5. Method validation

Each developed approach has its validity examined in accordance with ICH recommendations [42].

5.1. Linearity and range

The feasible range required to provide accurate, precise, and linear results was taken into consideration when establishing the calibration ranges for AGT, MET, and MLN. MET, AGT, and MLN linearity ranges are displayed in (Table 1).

5.2. Accuracy

The % recoveries of pure samples of the investigated components were used to calculate the proposed methods' accuracy. The results of the concentration calculations using the related regression equations are displayed in Table 1. By using the conventional addition technique on Westerizide® tablets, accuracy was further evaluated. Good percentage recoveries were obtained, demonstrating no excipient interference (Table 2).

5.3. Precision

Repeatability

The proposed methods were applied to three intra-daily analyses of three concentrations (3, 15 and 50 µg.mL-1 of MET and 1, 3 and 6 $\mu g.mL^{-1}$ for both AGT and MLN). Table 1 shows that positive outcomes and manageable relative standard deviations (RSD) were attained.

Intermediate precision

For the analysis of the selected concentrations under repeatability, the suggested procedures were put to the test everyday on three different days. Table 1 shows that positive findings and reasonable RSD values were attained.

5.4. Selectivity and specificity

By analyzing several synthetic laboratory-prepared mixtures with various ratios of (AGT, MET, and MLN) within respective linearity ranges, the suggested spectrophotometric techniques' specificity was evaluated. Results that are satisfactory are displayed in Tables 1 and 3.

5.5. LOD and LOQ

Detection and quantitation limits were determined for MLN in order to verify the method's sensitivity, and low values were obtained (see Table 1), indicating the high sensitivity of the established approaches.

Results from the proposed methods and the reported HPLC method [24] for determining the proposed pharmaceuticals in their pure forms were statistically evaluated, and as indicated in Table 4, no significant differences were found between them. The test determines whether the proposed methods are comparable to one another and equally exact and accurate to the published HPLC method [33]. A comparison between proposed, published chromatographic methods [22-29], published spectrophotometric methods [30-32] and reference method [33] indicated in Table 5

6. Conclusion

In this article, spectrophotomeric techniques for quantifying AGT, MET, and its hazardous impurity are presented (MLN). The developed methods have a number of benefits, and spectrophotometric procedures are thought to be straightforward, quick, and economical. The described techniques have been used to measure AGT and MET in their purest forms, in tablet dosage form, and in the presence of toxic MET impurities with success (MLN). The techniques can be used in quality control laboratories while analyzing drugs.

7. Conflicts of interest

"There are no conflicts to declare".

8. References

[1] Doupis, J.; Veves, A. DPP4 Inhibitors: A New Approach in Diabetes Treatment. Adv. Ther. 25 (7), 627–643.(2008).

[2] Seshadri, K. G.; Kirubha, M. H. B. Gliptins: A New Class of Oral Antidiabetic Agents. Indian J. Pharm. Sci., 71 (6), 608(2009).

[3] Ruscica, M.; Baldessin, L.; Boccia, D.; Racagni, G.; Mitro, N. Non-Insulin Anti-Diabetic Drugs: An Update on Pharmacological Interactions. Pharmacol. Res., 115, 14–24(2017).

[4] Andukuri, R.; Drincic, A.; Rendell, M. Alogliptin: A New Addition to the Class of DPP-4 Inhibitors. Diabetes, Metab. Syndr. Obes. targets Ther., 2, 117(2009)

[5] Mokhtar, M. Development and Validation of a Reversed Phase HPLC Method for Simultaneous Determination of Antidiabetic Drugs Alogliptin Benzoate and Pioglitazone HCl. Der Pharm. Sin. (2016).

[6] El-Bagary, R. I.; Elkady, E. F.; Ayoub, B. M. Liquid Chromatographic Determination of Alogliptin in Bulk and in Its Pharmaceutical Preparation. Int. J. Biomed. Sci. IJBS, 8 (3), 215(2012).

[7] Deshpande, P. B.; Butle, S. R. Stability Indicating High Performance Thin Layer Chromatographic Determination of Alogliptin Benzoate as Bulk Drug and in Tablet Dosage Form. Eurasian J. Anal. Chem., 12 (4), 325–335(2017)

[8] Mabrouk, M. M.; Hammad, S. F.; Mansour, F. R.; Amer, M. M. Three Spectrophotometric Methods for Determination of Alogliptin Benzoate and Pioglitazone HCl in Combined Tablet Dosage Form. Anal. Chem. Lett., 8 (3), 368–378(2018)

[9] Fejős, I.; Urbancsok, Z.; Zhou, W.; Sohajda, T.; Hu, W.; Szente, L.; Béni, S. Separation of Alogliptin Enantiomers in Cyclodextrin-modified Capillary Electrophoresis: A Validated Method. Electrophoresis, 35 (19), 2885–2891(2014)

[10] Klip, A.; Leiter, L. A. Cellular Mechanism of Action of Metformin. Diabetes Care, 13 (6), 696–704(1990)

[11] Pharmacopoeia, B.; Commission, B. P. The Stationery Office. London, UK 2009, 1 (123,128).

[12] Pharmacopoeia, I.; Commission, I. P. Ghaziabad. Govt. India Minist. Heal. Fam. Welf. 2010, 1, 192–193.

[13] Convention, G. M.; Pharmacopoeia, G. C. for the F. of the A.; Convention, N. M.; Pharmacopoeia, N. C. for R. the; Convention, U. S. P. Pharmacopoeia of the United States of America: The United States Pharmacopoeia; United States Pharmacopoeial Convention, Incorporated, 1893.

[14] Thomas, A. B.; Patil, S. D.; Nanda, R. K.; Kothapalli, L. P.; Bhosle, S. S.; Deshpande, A. D. Stability-Indicating HPTLC Method for Simultaneous Determination of Nateglinide and Metformin Hydrochloride in Pharmaceutical Dosage Form. Saudi Pharm. J. 19 (4), 221–231(2011).

[15] Habib, I. H. I.; Kamel, M. S. Near Infra-Red Reflectance Spectroscopic Determination of Metformin in Tablets. Talanta, 60 (1), 185–190(2003).

[16] Al-Rimawi, F. Development and Validation of an Analytical Method for Metformin Hydrochloride and Its Related Compound (1-Cyanoguanidine) in Tablet Formulations by HPLC-UV. Talanta, 79 (5), 1368–1371(2009)

[17] Sebaiy, M. M., El-Adl, S. M., Baraka, M. M., & Hassan, A. A. (2019). Rapid RP-HPLC Method for Simultaneous Estimation of Some Antidiabetics; Metformin, Gliclazide and Glimepiride in Tablets.. Egyptian Journal of Chemistry, 62(3), 429-440. doi: 10.21608/ejchem.2018.4394.1388

[18] Zarghi, A.; Foroutan, S. M.; Shafaati, A.; Khoddam, A. Rapid Determination of Metformin in Human Plasma Using Ion-Pair HPLC. J. Pharm. Biomed. Anal. 31 (1), 197–200(2003).

[19] Skrzypek, S.; Mirčeski, V.; Ciesielski, W.; Sokołowski, A.; Zakrzewski, R. Direct Determination of Metformin in Urine by Adsorptive Catalytic Square-Wave Voltammetry. J. Pharm. Biomed. Anal., 45 (2), 275–281(2007).

[20] Marques, M. A. S.; de Souza Soares, A.; Pinto, O. W.; Barroso, P. T. W.; Pinto, D. P.; Ferreira-Filho, M.; Werneck-Barroso, E. Simple and Rapid Method Determination for Metformin in Human Plasma Using High Performance Liquid Chromatography Tandem Mass Spectrometry: Application to Pharmacokinetic Studies. J. Chromatogr. B, 852 (1–2), 308–316(2007).

[21] Chen, X.; Gu, Q.; Qiu, F.; Zhong, D. Rapid Determination of Metformin in Human Plasma by Liquid Chromatography– Tandem Mass Spectrometry Method. J. Chromatogr. B, 802 (2), 377–381(2004).

[22] WHO, C. O. F. World Health Organization. Responding to Community Spread COVID-19. Ref. WHO/COVID-19/Community_Transmission/2020.1 2020.

[23] Thangabalan, B.; Parvathareddy, S. Method Development and Validation for Metformin HCl and Alogliptin in Bulk and Pharmaceutical Formulation by RP-HPLC Method.". Indian polit Sci rev, 2, 1451–1464(2014).

[24] Sultana, N.; Rizwana, I. Development and Validation of Stabilty Indicating RP-HPLC Method for Simultaneous Estimation of Metformin and Alogliptin in Bulk and Tablet Dosage Form. Pharma Innov. 7, 319–324(2018)

[25] Runja, C.; Ravikumar, P.; Avanapu, S. R. Stability Indicating RP-HPLC Method for

Simultaneous Estimation of Alogliptin Benzoate and Metformin Hydrochloride in Tablet Dosage Form. Int. J. Pharm. Pharm. Sci, 8, 116–120(2016).

[26] Mowaka, S.; Elkady, E. F.; Elmazar, M. M.; Ayoub, B. M. Enhanced LC-MS/MS Determination of Alogliptin and Metformin in Plasma: Application to a Pharmacokinetic Study. Microchem. J. 130, 360–365(2017).

[27] Malathi, S.; Vijayalakshmi, M. Development and Validation of HPTLC Method for Simultaneous Determination of Alogliptin and Metformin in Fixed Dose Combination Tablets. Am. J. PharmTech Res, 7, 2249–3387(2017).

[28] Marianne A.M, Nesrine T.L. Experimental design methodology for optimization and robustness determination in ion pair RP-HPLC method development: Application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate and repaglinide in tablets. Microchemical Journal, 147, 691-706(2019).

[29] Patel. H. P, Christian, J. R, Shah, P. A, Patel, K. G and Gandhi, T. R. Box-Behnken Design Assisted Protein Precipitation Optimization for Simultaneous Determination of Metformin Hydrochloride and Alogliptin Benzoate in Plasma along with Pharmacokinetic Application.Indian J Pharm Sci;83(4):785-793(2021)

[30] Sen, D.D.B. Simultaneous Assessment of Alogliptin Benzoate and Metformin Hydrochloride in Tablet Dosage Form by Reversed-phase High-performance Liquid Chromatography. Asian Journal of Pharmaceutics (AJP). 16(2) 2022 .

[31] Sen, D. B.; Sen, A. K.; Zanwar, A.; Balaraman, R.; Seth, A. Determination of Alogliptin Benzoate and Metformin Hydrochloride in Tablet Dosage Form by Simultaneous Equation and Absorption Ratio Method. International Journal of Pharmacy and Pharmaceutical Sciences 7(8), 2015.

[32] Chirag, P. A. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Metformin Hydrochloride and Alogliptin Benzoate in Bulk Drugs and Combined Dosage Forms. Pharma Chem, 6, 303–311(2014).

[33] Zaghary,w; Hassan,M.A; Mowaka,S; Ayoub,B.M. Comparative study between different simple methods manipulating ratio spectra for the analysis of alogliptin and metformin co-formulated with highly different concentrations. Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy 186 (2017).

[34] Magdy, M. A.; Ali, N. W.; Taha, A. A.; Elgebaly, A. M.; Farid, N. F. Different Chromatographic Methods for Determination of Alogliptin Benzoate, Metformin Hydrochloride, and Metformin Impurity in Bulk and Pharmaceutical Dosage Form. J. Sep. Sci., 44 (4), 833–842(2021)

[35] Meselhy, E. M.; Kheir, A. A. A.; El Henawee, M. M.; Elmasry, M. S. Simultaneous Determination o fNebivolol Hydrochloride and Valsartan in Their Binary Mixture Using Different Validated Spectrophotometric Methods.Spectrochim. Acta Part A Mol. Biomol. Spectrosc., 230, 118083(2020)

[36] Farid, N. F.; Abdelwahab, N. S. Two Different Spectrophotometric Determinations of Potential Anticancer Drug and Its Toxic Metabolite. Spectrochim. Acta Part A Mol. Biomol. Spectrosc., 145, 360–367(2015).

[37] Amin, K. F. M.; Abdullah, A. M.; Fakhre, N. A. Mean Centering-Triple Divisor and Ratio Derivative-Zero Crossing for Simultaneous Determination of Some Diabetes Drugs in Their Quaternary Mixture with Severely Overlapping Spectra. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 222, 117261(2019).

[38] Abdelrahman, M. M.; Naguib, I. A.; El Ghobashy, M. R.; Ali, N. A. Quantitative Determination of Zopiclone and Its Impurity by Four Different Spectrophotometric Methods. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 137, 617–624(2015).

[39] Abdel-Ghany, M. F.; Abdel-Aziz, O.; Ayad, M. F.; Tadros, M. M. Validation of Different Spectrophotometric Methods for Determination of Vildagliptin and Metformin in Binary Mixture. Spectrochim. Acta Part A Mol. Biomol. Spectrosc., 125, 175–182(2014).

[40] Wise, B. M.; Gallagher, N. B.; Bro, R.; Shaver, J.; Windig, W.; Koch, R. S. PLS Toolbox 4.0. 2007.

[41] Afkhami, A.; Bahram, M. Mean Centering of Ratio Spectra as a New Spectrophotometric Method for the Analysis of Binary and Ternary Mixtures. Talanta, 66 (3), 712–720(2005).

[42] Healy, E. M.; Kaitin, K. I. The European Agency for the Evaluation of Medicinal Products' Centralized Procedure for Product Approval: Current Status. Drug Inf. J. 33 (4), 969–978(1999).

Table 1.

Regression and analytical parameters of the proposed Spectrophotometric and (MCR) methods for determination of Alogliptin Benzoate, Metformin Hydrochloride and Melamin

Parameters	Spectrophotometric methods			(MCR)		
	AGT	MET	MLN	AGT	MET	MLN
Range (μgmL^{-1})	0.5-6	3-50	1-10	0.5-6	3-50	1-9
Slope	0.327	0.046	0.084	7.016	3.45	216.1
Intercept	-0.003	0.002	-0.008	21.48	0.192	0.133
Correlation coefficient	0.9999	0.9999	0.9999	0.9999	0.9999	0.9998
Accuracy[a] (%)	100.13	99.87	99.84	100.55	99.86	100.32
Specificity±SD	99.33± 1.527	99.73± 1.006	98.68± 0.577	99.80± 1.058	99.93± 0.642	99.33± 1.527
Precision						
Repeatability RSD %	1.169	0.729	0.609	0.921	0.630	1.12
Intermediate precision [cc] RSD %	1.214	0.811	0.708	0.993	0.754	1.231
LOD[d] (μgmL^{-1})	-	-	0.334	-	-	0.432
LOQ[d] (μgmL^{-1})	-	-	0.668	-	-	0.874

a Accuracy was expressed as mean percentage recovery and it was performed on 7 different samples (in triplicates) each of pure MET, AGT and MLN.

b Standard deviation of 3 concentrations of each drug (3, 15 and 50µg/mL of MET and 1, 3 and 6 µg/mL of both AGT and MLN) for spectrophotometric and MCR methods analyzed in triplicates on the same day.

c Standard deviation of 3 concentrations of each drug (3, 15and 50µg/mL of MET and 1, 3 and 6 µg/mL of both AGT and MLN) for spectrophotometric, and MCR methods analyzed in triplicates on three successive days.

d LOD =3.3×S.D./Slope ; while LOQ =10×S.D./Slope, were calculated using the lower part of the calibration graphs.

Table 2.

Quantitative determination of Alogliptin Benzoate, Metformin Hydrochloride in Westerizide® tablets by the Spectrophtometric and MCR methods and application of standard addition technique

Pharmaceutical Formulation	Method		Taken ($\mu g\ mL^{-1}$)	%Recovery[a]	Standard addition technique	
					Pure added ($\mu g\ mL^{-1}$)	%Recovery[b]
Westerizide® tablets Batch No. W15812 Labeled to contain 500 mg MET &10 mg AGT/Tablet	Spectrophotometric method	**AGT**	0.5	100.46±1.432	0.50	102.00
					0.80	101.25
					1.00	101.00
					Mean ± SD	**101.42±0.520**
		MET	25.00	100.65±1.305	10.00	100.66
					20.00	101.00
					25.00	100.66
					Mean ± SD	**100.77±0.196**
	MCR method	**AGT**	0.5	100.94±1.129	0.50	98.00
					0.80	98.75
					1.00	99.00
					Mean ± SD	**98.58±0.520**
		MET	25.00	101.01±1.221	10.00	100.26
					20.00	100.80
					25.00	101.33
					Mean ± SD	**100.80±0.535**

a average of 6 determinations

b average of 3 determinations

Table 3
Determination of Alogliptin Benzoate, Metformin Hydrochloride and Melamin in laboratory prepared mixtures by the proposed spectrophotometric and MCR methods

Concentration (µg/mL)			**spectrophotometric method Recovery %***			**MCR Recovery %***		
AGT	**MET**	**MLN**	**AGT**	**MET**	**MLN**	**AGT**	**MET**	**MLN**
1.00	50.00	1.00	98.00	98.80	99.00	101.00	100.40	98.00
1.00	25.00	1.00	101.00	100.80	98.00	99.00	99.20	101.00
5.00	50.00	1.00	99.00	99.60	99.00	99.40	100.20	99.00
2.00	50.00	1.00	99.50	99.00	101.00	98.50	99.50	101.00
Mean ± SD			99.38± 1.25	99.55± 0.90	99.25± 1.25	99.48± 1.08	99.83± 0.56	99.75± 1.50

* Average of 3 determinations

Table 4
Statistical comparison of the proposed spectrophotometric methods and the reported methods for determination of Alogliptin Benzoate, Metformin Hydrochloride in their dosage form

Parameter	**Spectrophotometric methods**		**(MCR)**		**Reported HPLC Method[34]***	
	AGT	**MET**	**AGT**	**MET**	**AGT**	**MET**
Mean %	100.46	100.65	100.94	101.01	102.43	101.52
SD	1.432	1.305	1.129	1.221	1.911	0.795
Variance	2.05	1.703	1.275	1.491	3.653	0.632
n	6	6	6	6	6	6
Student *t*- test (2.26)	2.027	1.389	1.651	0.868	___	____
***F*- value (5.05)**	1.780	2.695	2.866	2.359	_____	_____

* Reported HPLC method using a mobile phase consisted of sodium lauryl sulfate buffer 0.1% w/v, pH 3: methanol in the ratio 70:30, v/v and measurement was done at 220 nm

Table 5
comparison of the proposed spectrophotometric methods and the published methods for determination of Alogliptin Benzoate, Metformin Hydrochloride

	proposed spectrophotometric methods	**Published chromatographic methods[22–29]**	**Published spectrophotometric methods[30-32]**	**Reference chromatographic method[33]**
Methods used	-zero order , ratio difference - mean centering of ratio spectra	-RP-HPLC - LC-MS/MS -HPTLC	- simultaneous equation , absorption ratio - Absorbance ratio (Qanalysis method), simultaneous equation method (Vierodt's Method) - g derivative ratio, ratio subtraction coupled with extended ratio subtraction and spectrum subtraction coupled with constant multiplication	-RP-HPLC
Determination of metformin hazardous impurity	yes	no	no	yes
Best accuracy %				
AGT	100.13	99.98	100.16	100.51
MET	99.87	100.22	100.03	100.41
MLN	99.84	_____-	_____	99.95
Best sensitivity				
AGT	0.5-6 μgmL^{-1}	5-400 ng mL^{-1}	2.5-25 μgmL^{-1}	0.2-10 μgmL^{-1}
MET	3-50 μgmL^{-1}	25-2000ngmL^{-1}	2.5-15 μgmL^{-1}	1-15 μgmL^{-1}
MLN	1-10 μgmL^{-1}	_____	_____	0.2-10 μgmL^{-1}
Low cost	Low cost	Higher cost	Low cost	Higher cost
simplicity	More simple	Less simple	More simple	Less simple
Availability of instrument	More available	less available	More available	less available