6-1-2020

Sample Enrichment of Canagliflozin Prior to Its Spectrophotometric Determination in Presence of Metformin: Application to Recently Approved Binary Dosage Form

A. Zaghary Wafaa
Helwan University

Shereen Mowaka
The British University in Egypt, shereen.hassib@bue.edu.eg

Moataz S. Hendy
Pharmaceutical Chemistry Department, Faculty of Pharmacy, The British University in Egypt - Center of Drug Research and Development, moataz.sobhy@bue.edu.eg

ISSN 1061-9348, Journal of Analytical Chemistry, 2020, Vol. 75, No. 6, pp. 742–753. © Pleiades Publishing, Ltd., 2020.

ARTICLES

Sample Enrichment of Canagliflozin Prior to Its Spectrophotometric Determination in Presence of Metformin: Application to Recently Approved Binary Dosage Form

Wafaa A. Zaghary[a], Shereen Mowaka[b, c, d], and Moataz S. Hendy[b, d, *]

[a] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo, 11795 Egypt*

[b] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, The British University in Egypt, El-Sherouk city, Cairo, 11837 Egypt*

[c] *Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, Ain Helwan, Cairo, 11795 Egypt*

[d] *The Center for Drug Research and Development (CDRD), Faculty of Pharmacy, The British University in Egypt, El-Sherouk city, Cairo, 11837 Egypt*

**e-mail: Moataz.sobhy@bue.edu.eg*

Received November 29, 2017; revised June 5, 2018; accepted December 25, 2019

Abstract—This work illustrates four different spectrophotometric methods for metformin (**MET**) and canagliflozin (**CANA**) quantification in their recently approved pharmaceutical with trade name Invokamet®. The proposed methods are simple, definitive and accurate. Certain ensuing steps implementing zero spectra and/or ratio and/or derivative spectra were applied. Via implementing spectrum subtraction method accompanied with constant multiplication, zero order spectra were obtained for these drugs followed by their determination at their correlated λ_{max} at 237 and 290 nm for MET and CANA, respectively. Meanwhile, through employing method of derivative subtraction the two drugs were obtained in their first derivative spectra and quantified at 242.7 and 319.0 nm for MET and CANA, respectively. The suggested methods were inspected via laboratory prepared (diverse ratios) mixtures and they were excellently applied for analysis of Invokamet® tablets. Moreover, sample enrichment via spiking technique was elected for a pharmaceutical formulation analysis containing CANA as a minor component. Accuracy, precision and specificity were between the valid limits. Validation steps were done in accordance with the ICH guidelines. Also, statistical compression was carried out between the obtained and reported results and no crucial divergence appeared.

Keywords: canagliflozin, metformin, SGLT2 inhibitors, Invokamet

DOI: 10.1134/S1061934820060180

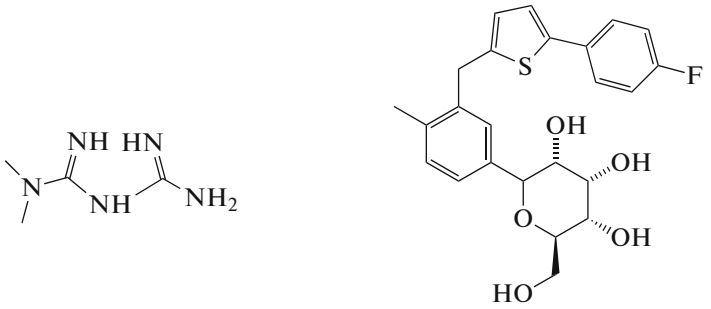
Diabetes mellitus Type 2 (**DMT2**) is a big global health issue, accounting for about 90% of the diagnosed cases of diabetes. The pervasiveness of DMT2 is increasing globally at an alarming rate with an estimated increment to 552 million by 2030. Low- and middle-income countries challenge the greatest threat of diabetes with nearly 80% of all diabetes-related fatality occurring in these countries [1].

In DMT2, combination of drugs not only reduces the pill burden, which leads to better patient compliance, but also provides broader control of disease and its complication. Invokamet® [2], a relatively new drug combination, includes 1000/500 mg of metformin hydrochloride and 50/150 mg of canagliflozin.

Metformin (N,N-dimethylimidodicarbonimidic diamide, Scheme 1), a biguanide anti-hyperglycemic drug, controls blood glucose levels by boost insulin sensitivity and augmenting glucose uptake in the liver [3]. It is widely adopted in the therapy of diabetes mellitus type 2.

Canagliflozin [4] ((2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)-oxane-3,4,5-triol, Scheme 1) is a sodium glucose co-transporter (**SGLT2**) suppressor approved by the Food and Drug Administration for the treatment of DMT2 in March 2013. SGLT2 inhibitors reduce blood sugar by decreasing the reabsorption of filtered glucose in the kidneys resulting in raising glucose excretion in urine.

Because CANA is considered to be a new drug, it has no legitimate official method in any pharmacopoeia yet in contrary to MET which has an official method of determination in both United States Pharmacopeia [5] and British Pharmacopeia [6].



Metformin Canagliflozin

Scheme 1. Structures of metformin and canagliflozin.

Referring to the literature review, sparse chromatographic techniques were applied [7, 8] for simultaneous quantitation of CANA and MET in pharmaceutical preparations or in laboratory prepared mixtures. Other few methods were found for determination of CANA alone, either in dosage form [9–11] or in plasma [12, 13]. On the contrary, various methodologies were developed for MET investigation using both spectrophotometry and chromatographic techniques [14–36].

Analysis of binary or ternary mixtures occasionally requires an earlier separation before spectrophotometric analysis. So far, there have been methods used to resolve those mixtures showing interfered spectra like spectrophotometry methods with derivative manipulations. Derivative techniques in spectrophotometry are considered to be effective for resolving the interfered spectra and wipe out matrix interventions [37–39].

The present study aims to provide easy, sensitive, valid and precise analytical spectrophotometric methods for concurrent determination and quantification of CANA as a minor component in presence of MET in bulk and tablet forms with credible values of validation parameters.

THEORETICAL DESCRIPTION OF THE METHODS

Spectrum subtraction accompanied with the constant multiplication method (SS–CM). This method has been recently evolved for continuous resolution of components in binary mixtures [40]. It is implemented by constant multiplication method and eradication of the retrieved zero order spectrum [41–43].

In case of X and Y mixture, Y is found to be more extended than X so we can use spectrum subtraction to erase Y followed by estimation of X. An advantage of this technique is its suitability when sample enrichment is needed either with spiking or spectrum addition in case of small concentrations of Y or low absorptivity of one of the components in the extended part.

It can be expressed mathematically via dividing mixture spectrum by a divisor (certain concentration of the component which is more extended), new spectrum will be delivered as follows:

$$(X + Y)/Y' = X/Y' + Y/Y', \quad (1)$$

$$(X + Y)/Y' = X/Y' + \text{Constant}, \quad (2)$$

to estimate the constant value from the $(X + Y)/Y'$ spectrum which is a straightforward parallel line to the wavelength axis.

The first component (Y) can be concluded in constant multiplication method by multiplication of Y' divisor by the already estimated constant Y/Y', then we get the D^0Y curve as follows:

$$Y = Y/Y'Y'. \quad (3)$$

By subtraction Y spectrum from zero order spectrum of the binary mixture ($X + Y$), X spectrum alone is obtained. Then we get X concentration by referring to the regression equation illustrating linearity between each absorbance against concentration of X at its λ_{max} in zero order spectrum. The same is done to get Y concentration using its corresponding regression equation.

Derivative subtraction accompanied with the constant multiplication method (DS–CM). This is a recently established technique for resolution of components in mixture analysis. It is applied for binary mixtures like X and Y, where Y spectrum is found to be more extended than X, so by using derivative subtraction we can eliminate the spectrum of Y when no effect from the interfering one is observed. Another component which is less extended will be eliminated in its first derivative [40–44].

This is used for components quantification in the binary mixture by utilizing the constant multiplication method for removing the obtained first order spectrum of the more extended drug. In case of the binary mixture (X and Y), if Y is found to be more extended it will be eliminated by first order spectrum subtraction leading to determination of X. Via dividing the first

derivative mixture spectrum obtained by the first derivative of more extended Y' (divisor), new spectrum is obtained as illustrated:

$$D/D\lambda(X+Y)/D\lambda(Y') = D/D\lambda(X/Y') + D/D\lambda(Y/Y'), \quad (4)$$

$$D/D\lambda(X+Y)/Y' = D/D\lambda(X/Y') + \text{Constant}, \quad (5)$$

to estimate the constant value from the $D/D\lambda(X + Y)/Y'$ spectrum which is a straightforward parallel line to the wavelength axis.

The Y component is then calculated using constant multiplication method by multiplying Y' divisor with the already estimated constant of $D/D\lambda(Y/Y')$, then the first derivative curves of Y can be obtained using the following equation:

$$D/D\lambda(Y) = D/D\lambda(Y/Y')\, D/D\lambda(Y'). \quad (6)$$

The X component is then determined by subtracting $D/D\lambda(Y)$ spectrum from the first derivative curve of the mixture, which results in first derivative curve of X. X concentration is then computed using regression equation of the linear relationship between the first derivative amplitudes at their λ_{max} against the correlated concentrations.

First derivative spectrophotometry (D^1). Derivative spectrophotometry is broadly applied in the investigation of multicomponent mixtures with interfering spectra. This eradicates overlapping from matrix via applying the zero-crossing techniques [44]. Simultaneous determination of CANA (Y) and MET (X) depending on the first derivative mode technique was carried out. The first derivative spectra of these drugs permitted individual and simultaneous determination of CANA and MET in different concentration intervals by measuring the amplitude of one drug at zero crossing point of the other. The concentrations of X and Y were then established using linear regression equations between the first derivative amplitudes at λ_{max} against the correlated concentrations of X and Y, respectively.

Amplitude modulation method (AM). Application of amplitude modulation method was done on the binary mixture using an isoabsorptive point [45, 46] in zero order spectrum. This in turn retained as an isoabsorptive wavelength in the ratio spectrum [47–49]. A normalized spectrum was equal to the divisor absorptivity along the spectrum range. At these wavelengths the resulted absorbances of the ratio spectrum were adjusted to concentration values expressing each component concentration.In case of X and Y mixture, zero order spectra showed that Y was found to be more extended than X. Meanwhile X and Y spectra came together at an isoabsorptive point (λ_{iso}).When it comes to computing the modulated amplitude correlated to Y concentration, it would be quantified from the spectrum of $(X + Y)/Y'$ that gave a straightforward line parallel to wavelength axis in the extended part of Y spectrum. The divisor used was normalized Y so it gave Y concentration after division of the binary mixture. The amplitudes retrieved from the ratio spectrum at the isoabsorptive point gave concentrations of both Y and X, so by subtracting the estimated value of the constant from the ratio spectrum, amplitude referring to component X at λ_{iso} was steadily computed. To eradicate any inaccuracy, the obtained X and Y concentrations may be interchanged to the true concentrations via utilizing merged equation of regression at

$$\lambda_{iso} : c_{Rec} = \text{Slope}c \pm \text{Intercept}, \quad (7)$$

where c_{Rec} is the adjusted amplitude at λ_{iso} and c corresponds to X or Y true concentration, the slope approaches one and the intercept approaches zero.

EXPERIMENTAL

Device. BIO Double-beam UV-Vis spectrophotometer JASCO V-630 (S/N: C367961148) with 1.00 cm quartz cells was used in the scanning range of 200–400 nm and 0.1 nm intervals. Data manipulation was done via Spectra Manager II software.

Chemicals and reagents. Metformin was graciously obtained from Chemical Industries Development (Cairo, Egypt), canagliflozin was purchased from BaoJi Guokang Bio-technology co. (China). The purities were certified to be 99.90 and 99.88% as reported in certificates from suppliers for MET and CANA, respectively. Invokamet® tablet labelled to contain 1000 and 50 mg of MET and CANA, respectively, was manufactured by Ortho-McNeil-Janssen Pharmaceuticals (USA). This dosage form was bought from USA market. HPLC grade methanol was from Fisher Scientific (Loughborough, Leicestershire, UK).

Standard solutions. Stock standard solutions of MET and CANA were prepared at 1 mg/mL in methanol. 100 μg/mL working standards were prepared for both analytes by further dilution with methanol.

Procedures. 10 μg/mL MET and CANA solutions were used to measure zero order absorption against blank (methanol) in the range of 200–400 nm.

Construction of calibration graphs. Aliquots guaranteed to have 2–15 and 5–20 μg/mL of MET and CANA, respectively, were solely prepared in methanol. These samples were used to measure zero order and first derivative spectra (smoothing factor ($\Delta\lambda$) of 10 and scaling factor of 10). Results obtained were stored in computer. Then, by relating the absorbance in zero order spectra of MET and CANA at their λ_{max} of 237 and 290 nm, respectively, against the equivalent concentrations calibration graphs were constructed. Regression equations were then obtained separately for each analyte.

For the first derivative spectra, calibration graphs were constructed by relating the peak amplitudes of MET and CANA at 242.7 and 319 nm, respectively, vs.

the analogous concentrations of each of them. Regression equations were subsequently obtained.

Prepared mixtures. A series of MET and CANA accurate aliquots were pipetted from working standards and added into 10 mL volumetric flasks. Six mixtures with accurate diverse ratios of the studied drugs were prepared. After completion to the volume with methanol, spectra of each drug were measured and recorded in range of 200–400 nm.

While applying amplitude modulation method, cited drugs concentrations (MET and CANA) were continuously computed using the recorded amplitudes at 252.9 and 295.5 nm, respectively, on the same spectrum. A unified regression equation at λ_{iso} was constructed. This enabled to determine concentration of each drug separately, while concentration determination in other methods could be achieved via substitution in the analogous regression equation of the method.

Application to tablets. Ten Invokamet® tablets were precisely weighed and efficiently powdered. A fixed amount of this powder guarantying to have 100 mg of MET was weighed and dissolved in methanol using ultrasonic bath sonicator for 25 min. Then filtration was done in another 100 mL volumetric flask followed by completion to the mark with methanol. Aliquots of MET and CANA were transferred accurately to a 10 mL volumetric flask to get a final mixture containing 10 and 0.5 μg/mL of MET and CANA, respectively. The concentration of CANA in a tablet mixture was then enriched with 5 and 7 μg via spiking technique using pure standard of CANA prior employing each proposed method.

RESULTS AND DISCUSSION

In order to resolve interfered spectra of more than one compound in the mixture without preliminary separation of the analytes, certain spectrophotometric methods must be employed. Lately, these methods have been greatly evolved and utilized. Mathematical spectrophotometric analytical methods are quite expedient over other analytical techniques or instrumentations like gas chromatography–mass spectrometry, liquid chromatography–nuclear magnetic resonance, liquid chromatography–mass-spectrometry, etc. Those techniques are more complicated, demanding continuous changes in conditions and precise optimization of such parameters as temperature, flow rate, pH, etc. On the other hand, spectrophotometric techniques are easy to employ and operate, fast, sensitive and surprisingly economical. While developing these analytical methods, chemists focus chiefly on how to implement new mathematical techniques. The most essential advantage in spectrophotometric techniques is their diversity and flexibility according to conditions of each mixture, where the analysts have the opportunity to apply the most convenient method for analysis and resolution.

The ratio of MET and CANA in the studied tablets was 20 : 1, zero order spectra was found to be very hard to determine the concentration of each compound simultaneously, since low CANA concentrations were out of the linearity range to attain this dosage content form the ratio. Spectral properties of each drug, its absorptivity and amount in the mixture restricted the choice of the ideal concentration range.

In Fig. 1 CANA spectrum was found to be more extended than MET spectrum. Canagliflozin showed a peak at 290 nm while Metformin showed the highest amplitude at 237 nm. Both CANA and MET showed two isoabsorbative points at 223.9 and 252.9 nm, respectively. The contribution of MET in all prepared mixtures was higher than that of CANA mimicking the provided pharmaceutical dosage form Invokamet® (1000 and 50 mg of MET CANA, respectively). CANA in the extended region exhibited slight absorbance at low concentrations, which in turn prevented its direct determination in the combined dosage form.

Sample enrichment was chosen to be carried out to maintain precise CANA quantification in the spectrum extended region that in turn showed much lower absorptivity than other peaks. It was performed by adding accurate known amounts of the minor drug pure standards in the binary mixtures leading to its precise determination simultaneously with MET.

Spectrum subtraction accompanied with multiplication of constant (SS–CM) or derivative subtraction accompanied with multiplication of constant (DS–CM) as well were successfully utilized for investigation of CANA and MET binary mixtures. Generally, constant multiplication is considered to be a relatively new tactic [47–49].

SS–CM mainly depends on employing the intelligent mathematical techniques that uses the constants appearing in spectra of the mixture, and then by further developing the genuine spectrum of the overlapped component is obtained. In order to evaluate the other drug content in combination, spectrum subtraction of the resolved component is carried out from the initially used mixture. This method is used for both quantification and resolution of two investigated drugs in their binary prepared mixture. It differs from the formerly used ratio subtraction method which is applied only for resolution of the investigated drugs [50].

This new spectrophotometric method has the advantage of acquiring spectra of the two investigated compounds after employing these resolution techniques. This leads to further determination of the drugs at their λ_{max} of 237 and 289 nm for MET and CANA, respectively, in zero order spectra. Maximum ranges of linearity and sensitivity were achieved, the applied methods could be used in concentration

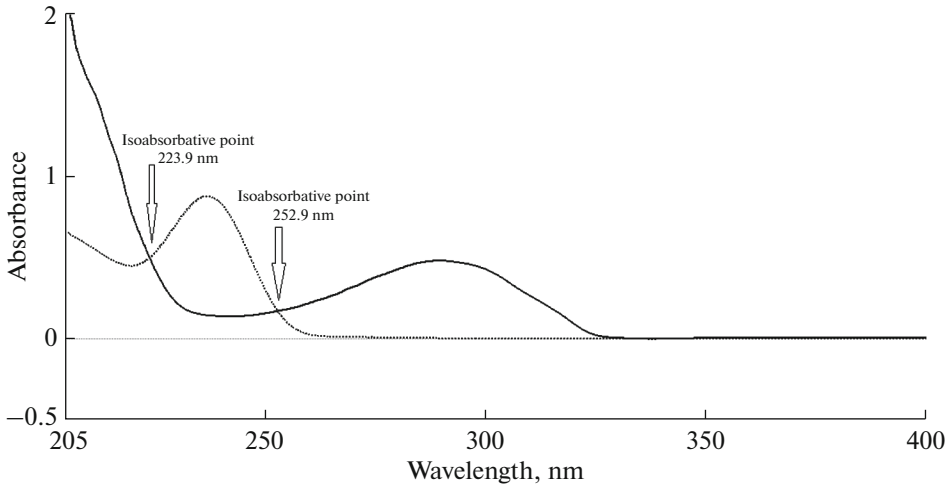


Fig. 1. Zero order absorption spectra of 10 μg/mL canagliflozin (—) and metformin (- -) using methanol as blank.

ranges of 2 and 2.5 μg/mL for CANA and MET, respectively.

Consequently, upon tablet analysis CANA only was enriched with the pure standard enabling its simultaneous determination where there was no need for MET enrichment. On the contrary, other applied methods like amplitude modulation allow to quantify each compound in mixture by choosing a standard solution to act as divisor and a unified equation of regression at the isoabsorptive point for accurate determination of the two drugs in the laboratory prepared mixtures and tablets.

The presented methods were found to be very easy, fast, simple and precise. The compounds under investigation can be determined and quantified either in prepared mixtures or in the tablet dosage form. They differ from other sophisticated techniques such as chemometric methods which need a particular software (Matlab), diverse preparation of standard solutions and various laboratory prepared mixtures with diverse amounts of analytes. Not to mention HPLC methods which also require more complicated optimization of conditions.

Spectrum subtraction accompanied with constant multiplication. CANA was found to be more extended than MET in zero order absorption spectrum as shown in Fig. 1.

To apply this method, first step is dividing laboratory prepared mixture spectrum (Fig. 2a) with a selected concentration of CANA as the divisor spectrum that represents pure CANA standard (5 μg/mL). A straightforward line parallel to the wavelength axis in the region extended in spectrum (CANA/CANA') (270–320 nm) is obtained (Fig. 2b). Then, upon multiplying the value of this constant by the initially used divisor spectrum of CANA original CANA curve in the zero order is obtained. Corresponding absorbance is measured at λ_{max} of 289 nm (Fig. 2c).

To evaluate CANA concentration, substitution of the absorbance value in the corresponding linear regression equation between the absorbance and CANA concentration was performed.

Finally, by subtracting the obtained zero order CANA spectrum from the corresponding laboratory mixture the D^0 spectrum of MET was obtained (Fig. 2d). Subsequently, determination of MET concentration via substitution in its corresponding regression equation constructed between concentration and its correlated absorbance at λ_{max} of 237 nm was achieved.

By employing the spectrum subtraction method for components resolution, we obtained the genuine spectrum of each component with no interferences from the other one. Therefore, using this method is beneficial in determining the two drugs at their λ_{max}. In addition, the regenerated spectra can be considered as a spectral profile.

Derivative subtraction accompanied with constant multiplication. While analysis of the prepared mixtures possessing high ratio of MET by implementing spectrum subtraction accompanied with constant multiplication technique two changes were observed. Firstly, contribution of MET was raised, secondly, the extended part changed to very small. This resulted in a boisterous constant and accordingly the constant determination in the plateau area turned out to be tougher resulting in imprecise constant values and afterwards imprecise recoveries of the unscathed CANA. Derivative subtraction accompanied by constant multiplication offers a solution for this error.

To efficiently employ the first derivative method, initially various scaling and smoothing factors were tried. Smoothing factor of 10 and scaling factor of 10 gave the best ratio between signal and noise with good spectra resolution. Figure 3 showed the overlaid D^1 spectra of the two components.

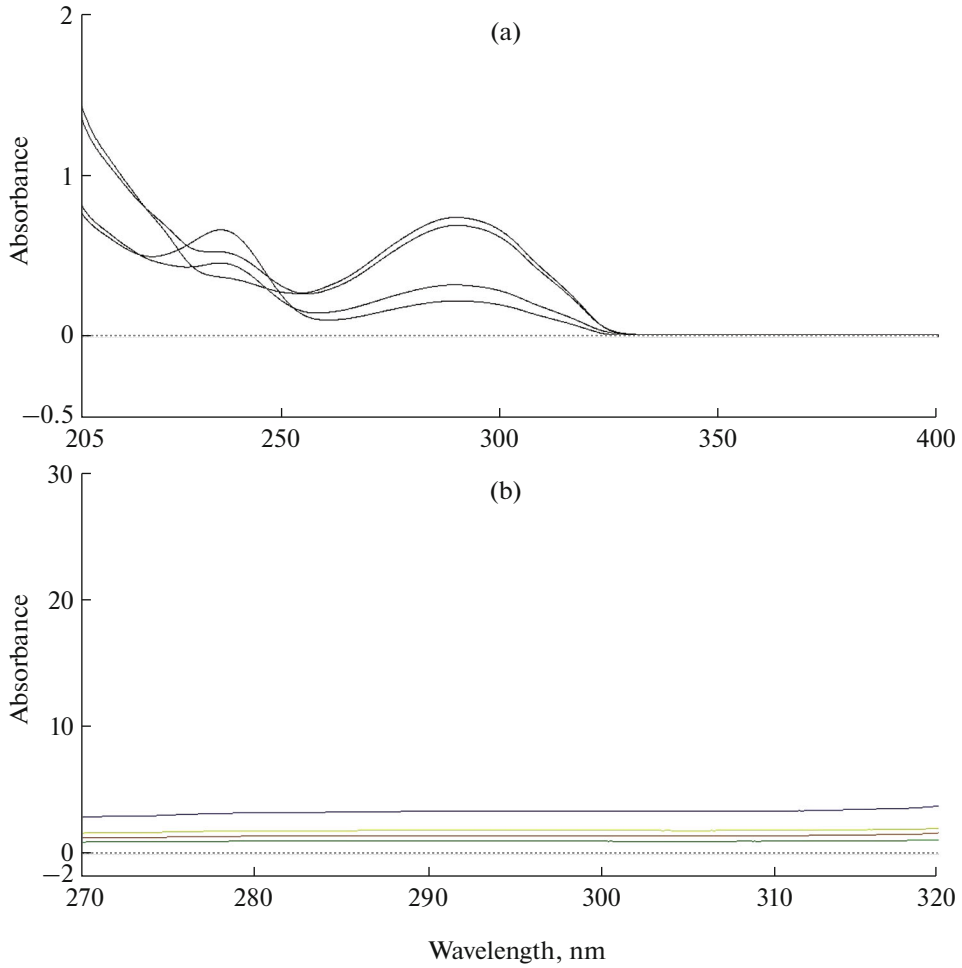


Fig. 2. Zero order absorption spectra of laboratory prepared mixtures with different canagliflozin (CANA) and metformin (MET) concentrations (a); CANA (5 μg/mL) as a divisor showing constants in the plateau region (b); different CANA (5–20 μg/mL) concentrations after constants multiplication by a divisor of CANA (5 μg/mL) (c); different MET (2–15 μg/mL) concentrations after subtraction of CANA zero order absorption spectra (d).

To enhance the sensitivity, derivative subtraction accompanied with constant multiplication method was employed to get the D^1 spectrum of one component with no interference from the other one in the tablet dosage form or prepared mixture. In this work the amplitudes were measured at zero crossing points of 319 and 242.7 for CANA and MET, respectively. The concentrations of CANA and MET were then computed via regression equations of the linear relationship between the peak amplitudes against the correlated concentrations of CANA and MET.

The divisor concentration of 5 μg/mL was chosen for CANA that offered perfect results concerning the average percentage recovery and it was compromised regarding noise and sensitivity.

The constant value was obtained by dividing the first derivative spectrum of the laboratory prepared mixtures (Fig. 4a) by a divisor spectrum (first derivative spectrum of 5 μg/mL standard CANA solution). The constant was obtained from the straight line which was parallel to wavelength axis in the spectrum extended part (292–328 nm) as shown in Fig. 4b. By multiplication with the divisor spectrum, we got the spectrum that represented the first derivative curve of CANA (Fig. 4c). Subtracting the first derivative spectrum of CANA from the first derivative spectrum of the mixture resulted in obtaining the D^1 spectrum of MET (Fig. 4d). The concentrations of CANA and MET were then evaluated via their corresponding equations with linear relationships between the peak amplitudes and the correlated concentrations.

Derivative subtraction coupling with constant multiplication and ordinary derivative subtraction are only utilized in case of the noisy constant when measuring zero order spectra. A noisy constant prevents accurate application of the method and unsatisfactory recoveries are further obtained. In addition, like other methods that utilize the derivative technique, DS–CM has the advantage of enhanced peaks leading to a precise

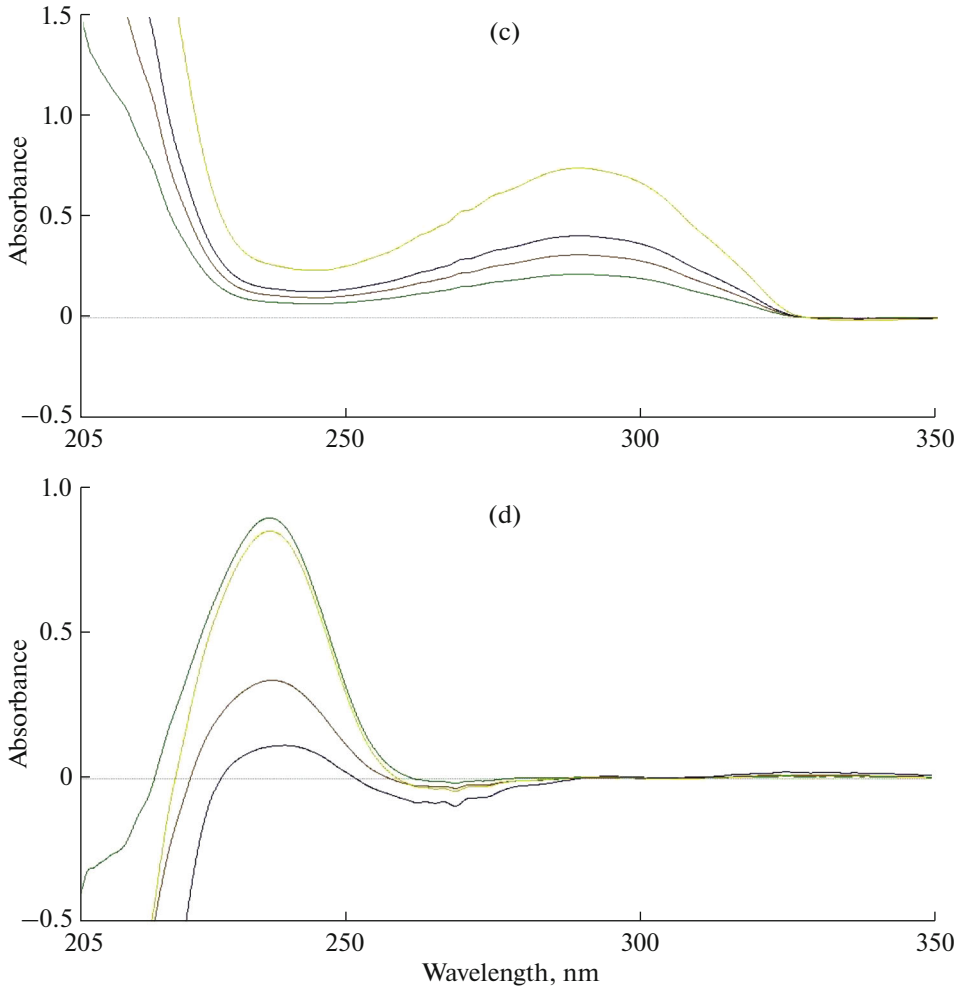


Fig. 2. (Contd.)

determination and quantification of the constants resulting in good final recoveries.

Amplitude modulation method. This technique relies on the presence of an isoabsorptive point in the overlaid spectra of both components. This point will be preserved with no change in spite of the division by a certain chosen divisor. The results obtained were markedly influenced by the divisor change. Therefore, to get rid of the consequences of choosing wrong divisor, normalized CANA spectra that exhibited the absorptivity of CANA at various wavelengths were used.

Firstly, the binary mixture spectrum was divided by the normalized CANA spectrum as a divisor. A ratio spectra was obtained showing a constant in the plateau area (275–316 nm) that in turn was equivalent to CANA constant amplitude over any region in the spectrum. The amplitude of the ratio spectra at this isoabsorpative point (252.9 nm) was equivalent to the summation of CANA and MET responses. Ratio mathematical handling via normalized divisor resulting in the changeover of CANA curve to a constant showed as a straightforward line parallel to axis of wavelength with no change in the amplitude value corresponding to CANA concentration. By subtracting the already concluded constant from the mixture amplitude at λ_{iso} of 252.9 nm, MET amplitude was found:

$$P_{mix} \text{ at } 252.9 \text{ nm} = P_{MET} \text{ at } 252.9 \text{ nm} + \text{Constant}. \quad (8)$$

P_{CANA} at 252.9 nm is the estimated constant in the plateau area between 275–316 nm and it is considered to be the concentration of CANA.

$$P_{MET} \text{ at } 252.9 \text{ nm} = P_{mix} \text{ at } 252.9 \text{ nm} - P_{CANA} \text{ at } 252.9 \text{ nm}. \quad (9)$$

P_{MET} at 252.9 nm is the estimated amplitude at 252.9 nm considered to be the recorded concentration of MET.

The substantive values represented CANA and MET concentrations which were further computed by using a corresponding unified regression equation at

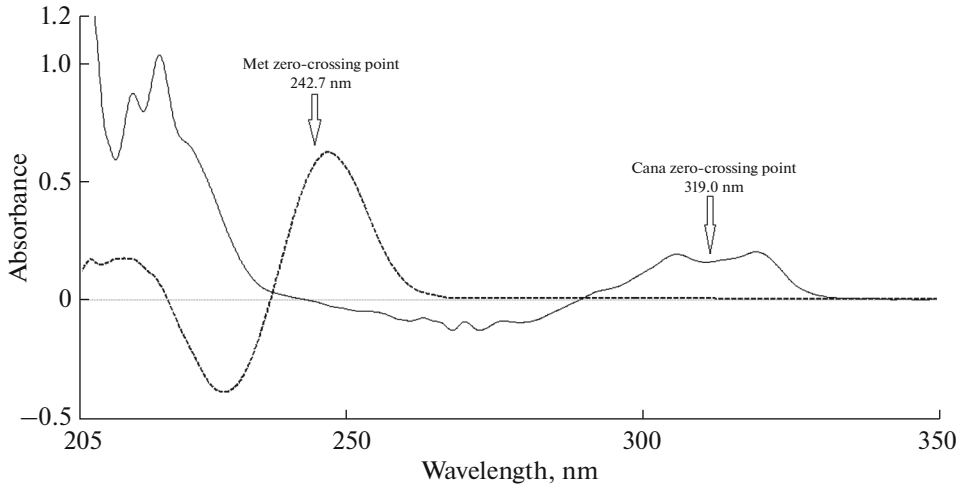


Fig. 3. Overlaid first derivative spectra of 10 μg/mL canagliflozin (—) and metformin (- - -) using methanol as blank.

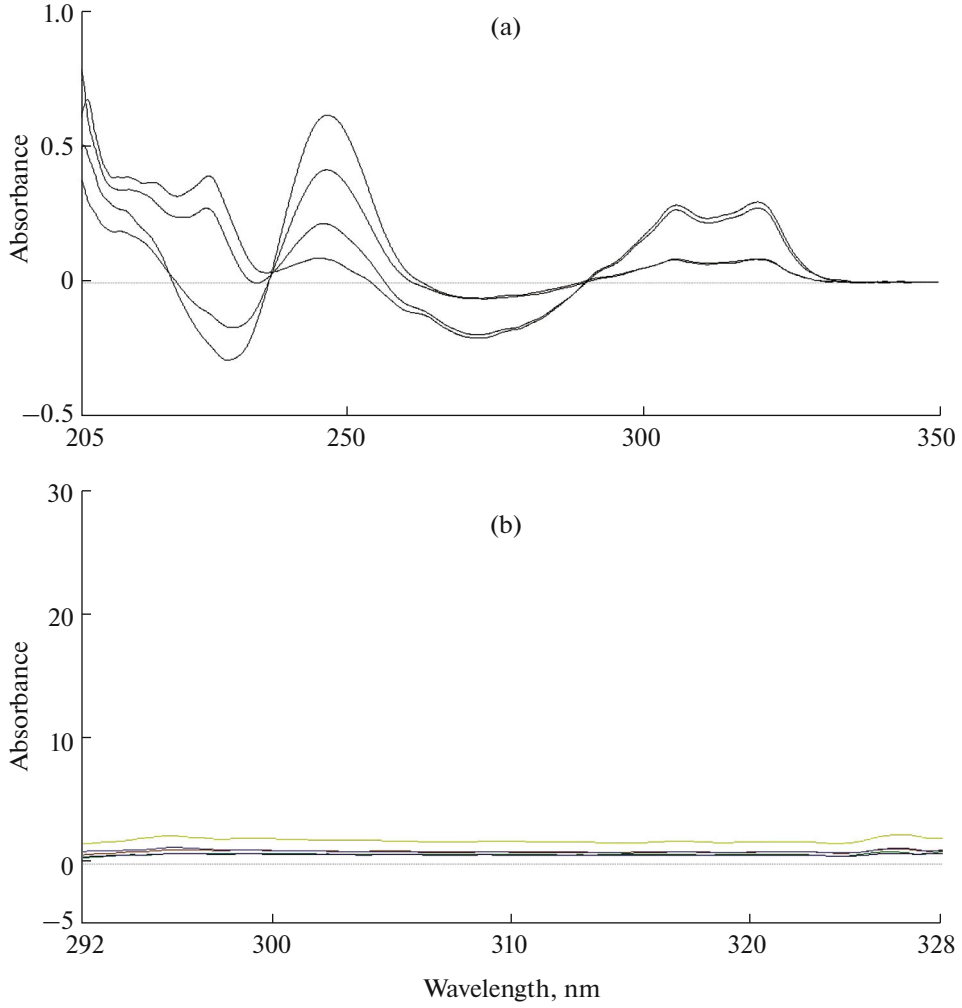


Fig. 4. First derivative absorption spectra of lab prepared mixtures with different canagliflozin (CANA) and metformin (MET) concentrations (a); CANA (5 μg/mL) as a divisor showing constants in the plateau region (292–382 nm) (b); different CANA concentrations (5–20 μg/mL) after constants multiplication by a divisor of CANA (5 μg/mL) (c); different MET concentrations (2–15 μg/mL) after subtraction of CANA first derivative absorption spectra (d).

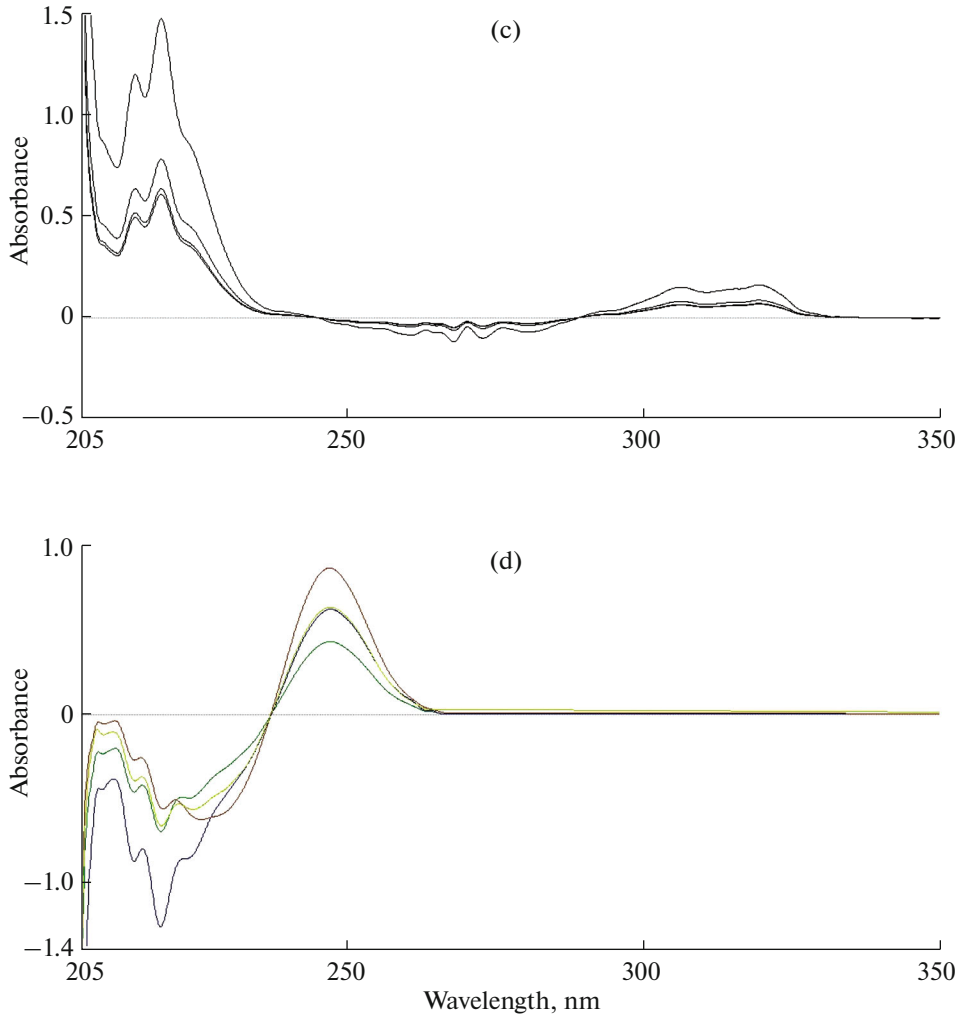


Fig. 4. (Contd.)

252.9 nm. This was performed to get rid of any flows related to signal to noise:

$$c_{rec} = 1.002c + 0.002. \quad (10)$$

Method validation. These applied spectrophotometric resolution and quantification methods were fully validated and certified in consonance with the ICH guidelines [51] as represented in Table 1. The analysis of laboratory prepared mixtures containing differing ratios and amounts of the two drugs gives adequate and accurate values regarding each method specificity and calibration range as represented in Table 2. Further investigation was done via employing those methods to the quantification of the two cited drugs in Invokamet® tablets. Rationality, viability and sustainability of reported techniques were further estimated via the standard addition application (Table 2).

Results of statistical comparison and validation. Statistical comparison and validation of the obtained results were done via comparison with the literature methods [9, 24]. Calculated *t* and *F* values were tested. They were found to be lower than the corresponding theoretical values. This proved that no notable differences were between the investigated and the reported methods regarding accuracy and precision (Table 3).

CONCLUSIONS

In this work new, simple and smart spectrophotometric methods were utilized and fully presented for concurrent determination and quantification of CANA and MET in their mixtures. To investigate binary mixtures with the lowest number of steps, spectrum subtraction and derivative subtraction methods have been applied. These two techniques have the main advantage of their applicability in mixture analysis when one overlapped component has a noted contribution in the extended area in the spectrum while measuring the constant value. Using the derivative technique leads to the complete elimination of this contribution. When derivative resolution accompanied with constant multiplication is employed, the

Table 1. Assay parameters and method validation by applying the proposed spectrophotometric methods for determination of canagliflozin and metformin

Parameter	CANA				MET			
	SS–CM	DS–CM	D[1]	AM	SS–CM	DS–CM	D[1]	AM
Linearity, μg/mL	5–20	5–20	5–20	5–20	2–15	2–15	2–15	2–15
Slope	0.050	0.0214	0.0216	0.0507	0.0791	0.0422	0.0427	0.0776
Accuracy, %	99 ± 1	99.6 ± 0.9	100 ± 1	100 ± 1	100.7 ± 0.8	99 ± 1	99 ± 2	100 ± 2
Intercept	0.030	0.014	0.015	0.034	0.012	0.002	0.006	0.007
Correlation coefficient	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999
RSD[a], %	1.31	1.10	1.72	1.26	2.57	2.19	1.25	1.95
RSD[b], %	1.46	0.81	0.42	1.51	1.91	1.38	1.11	1.42

[a] Intra-day relative standard deviation of CANA (5, 10, 15 μg/mL) and MET (3, 6, 9 μg/mL) ($n = 3$), [b] inter-day relative standard deviation of CANA (5, 10, 15 μg/mL) and MET (3, 6, 9 μg/mL) ($n = 3$).

Table 2. Determination of canagliflozin and metformin in laboratory prepared mixtures and the pharmaceutical dosage form by the proposed methods and results obtained by the standard addition techniques

Concentration, μg/mL		Recovery[a], %							
		SS–CM		DS–CM		D[1]		AM	
CANA	MET	CANA	MET	CANA	MET	CANA	MET	CANA	MET
Lab prepared mix									
5	10	98.3	99.8	98.7	101.5	98.9	100.1	100.9	97.8
10	2	97.9	99.9	98.7	100.2	98.0	100.2	101.9	101.2
20	5	97.5	101.4	100.5	99.3	101.2	97.8	98.4	98.9
10	15	100.8	101.8	99.2	98.5	101.1	99.0	98.3	98.0
6	8	99.6	100.5	100.5	99.6	101.3	94.9	99.3	102.0
5	15	101.0	100.6	100.9	99.8	99.9	100.4	98.7	101.4
Mean, %		99.2	100.7	99.7	99.8	100.1	98.7	99.5	99.9
SD		1.49	0.82	0.99	1.01	1.39	2.12	1.46	1.85
Invokamet tablet (1000 mg of MET/50 mg of CANA)									
10/0.5 + 5 pure added[b]		97.3	100.7	97.1	100.8	102.9	98.5	101.9	99.4
Mean ± SD		97.29 ± 0.03	97.6 ± 0.7	101.4 ± 0.8	97.29 ± 0.03	99.1 ± 0.8	102 ± 2	99 ± 1	101.4 ± 0.8
10/0.5 + 7 pure added[b]		97.7	100.9	98.1	101.2	100.6	99.6	100.8	97.8
Mean ± SD		98.0 ± 0.3	99 ± 1	98.4 ± 0.5	100 ± 2	100.2 ± 0.3	99.6 ± 0.4	100.3 ± 0.5	99 ± 1

[a] Average of three determinations, [b] the ratios present in the pharmaceutical formulations spiked with known pure amounts of canagliflozin.

resulting spectrum obtained is the first derivative of any component equal to that of pure compound with no other components overlapping in the mixture. Derivative spectra of any of the two cited drugs would be obtained directly at a fixed particular zero crossing wavelength. Another method using the isoabsorpative point in ratio spectra is the amplitude modulation method employed for both resolution and quantification. This method offers the advantages of minimum mathematical intervention and no need for complementary methods. Sample enrichment via sample spiking was adopted to reveal accurate results. The ease, accuracy, rationality and validity of those represented methods not needing any particular program for data manipulation make them possible to be used in routine determination of cited drugs in their original pharmaceutical dosage forms in quality control laboratories.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 3. Statistical comparison of the proposed methods and the reference method for canagliflozin [9] and metformin [24] determination

Value	Proposed method								Reported method	
	SS–CM		D[1]		DS–CM		AM		CANA	MET
	CANA	MET	CANA	MET	CANA	MET	CANA	MET		
Mean	99.2	100.7	100.1	98.7	99.6	99.5	99.5	99.9	99.5	100.6
SD	1.49	0.82	1.39	2.11	0.87	1.24	1.46	1.85	0.76	0.81
RSD, %	1.49	0.82	1.39	2.11	0.87	1.24	1.46	1.85	0.76	0.81
n	5	5	5	5	5	5	5	5	5	6
Variance	2.22	0.67	1.93	4.45	0.76	1.54	2.13	3.42	0.58	0.66
Student's *t*-test[a] (2.571)	0.468	0.155	0.762	2.007	0.155	1.706	0.014	0.786		
F-value[a] (6.388)	3.844	1.025	3.345	6.786	1.310	2.344	3.690	5.216		

[a] The values in the parenthesis are the corresponding theoretical values of t and F at $P = 0.05$.

REFERENCES

1. Whiting, D.R., Guariguata, L., Weil, C., and Shaw, J., *Diabetes Res. Clin. Pract.*, 2011, vol. 94, p. 311.
2. Madaan, T. and Akhtar, M., *Eur. J. Pharm. Sci.*, 2016, vol. 93, p. 244.
3. Sengupta, P., Bhaumik, U., Ghosh, A., Sarkar, A.K., Chatterjee, B., and Bose, A., *Chromatographia*, 2009, vol. 69, p. 1243.
4. Bailey, R.A., Schwab, P., Xu, Y., Pasquale, M., and Renda, A., *Clin. Ther.*, 2016, vol. 38, p. 2046.
5. *The United States Pharmacopoeia 30, the National Formulary 25*, Rockville, MD: US Pharmacopeial Convention, 2007.
6. *The British Pharmacopoeia 2009*, London: The Stationery Office, 2009.
7. Panigrahy, U. and Reddy, A., *Orient. J. Chem.*, 2015, vol. 31 p, p. 1489.
8. Gaware, D., Patil, R., and Harole, M., *World J. Pharm. Pharm. Sci.*, 2015, vol. 4, p. 631.
9. Patel, N., Shah, D., and Maheshwari, D., *Int. J. Pharm. Technol.*, 2015, vol. 7, p. 9779.
10. Kaur, I., Wakode, S., and Singh, H., *Pharm. Methods*, 2015, vol. 6, p. 82.
11. Suneetha, A. and Sharmila, D., *Res. J. Pharm. Biol. Chem. Sci.*, 2015, vol. 6 p, p. 1186.
12. Iqbal, M., Ezzeldin, E., Al-Rashood, K., Asiri, Y., and Rezk, N., *Talanta*, 2015, vol. 132, p. 29.
13. Iqbal, M., Khalil, N., Alanazi, A., and Al-Rashood, K., *Anal. Methods*, 2015, vol. 7, p. 3028.
14. Bhushan, R., Gupta, D., and Jain, A., *J. Planar Chromatogr.—Mod. TLC*, 2006, vol. 19 p, p. 288.
15. Sengupta, P., Bhaumik, U., Ghosh, A., and Sarkar, A., *Chromatographia*, 2009, vol. 69, p. 1243.
16. Elbagary, R., Elkady, E., and Ayoub, B., *Int. J. Biomed. Sci.*, 2011, vol. 7, p. 201.
17. Wang, M. and Miksa, I., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, vol. 856, p. 318.
18. Georgita, C., Albu, F., David, V., and Medvedovici, A., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, vol. 854, p. 211.
19. Ali, M., Rafiuddin, S., Ghori, M., and Khatri, A., *Chromatographia*, 2008, vol. 67, p. 517.
20. Lai, E. and Feng, S., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2006, vol. 843, p. 94.
21. Elbagary, R., Elkady, E., and Ayoub, B., *Talanta*, 2011, vol. 85, p. 673.
22. Zhang, L., Tian, Y., Zhang, Z., and Chen, Y., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, vol. 854, p. 91.
23. Ali, A., Duraidi, I., Saket, M., and Abu-Nameh, E., *J. AOAC Int.*, 2009, vol. 92, p. 119.
24. Elbagary, R., Elkady, E., and Ayoub, B., *Eur. J. Chem.*, 2013, vol. 4, p. 360.
25. Ghassempour, A., Ahmadi, M., Ebrahimi, S., and Aboul-Enein, H., *Chromatographia*, 2006, vol. 64, p. 101.
26. Elbagary, R., Elkady, E., and Ayoub, B., *Int. J. Biomed. Sci.*, 2011, vol. 7, p. 62.
27. Tahara, K., Yonemoto, A., Yoshiyama, Y., Nakamura, T., Aizawa, M., Fujita, Y., and Nishikawa, T., *Biomed. Chromatogr.*, 2006, vol. 20, p. 1200.
28. Pawar, S., Meshram, G., and Phadke, M., *Chromatographia*, 2008, vol. 68, p. 1063.
29. Elbagary, R., Elkady, E., and Ayoub, B., *Eur. J. Chem.*, 2013, vol. 4, p. 444.
30. Mowaka, S. and Ayoub, B., *Pharmazie*, 2017, vol. 72, p. 67.
31. Mowaka, S., Elkady, E., Elmazar, M., and Ayoub, B., *Microchem. J.*, 2017, vol. 130, p. 360.
32. Ayoub, B. and Abdel-Aziz, O., *Pharmazie*, 2016, vol. 71, p. 683.
33. Ayoub, B., *RSC Adv.,* 2015, vol. 5, p. 95703.
34. Mowaka, S. and Mohamed, D., *RSC Adv.*, 2015, vol. 5, p. 60 467.
35. Khan, G., Sahu, D., Agrawal, Y.P., Sabarwal, N., Jain, A., and Gupta, A.K., *Asian J. Biochem. Pharm. Res.*, 2011, vol. 1, p. 352.
36. Peraman, R., Gowra, C.S., Reddy, Y.P., and Peruru, K.K., *Chromatographia*, 2013, vol. 76, p. 1153.

37. Wahbi, A.M., Abounassif, M.A., and Al-Kahtani, H.G., *Analyst*, 1986, vol. 111, p. 777.
38. Erk, N., *J. Pharm. Biomed. Anal.*, 2000, vol. 23, p. 1023.
39. Rajic, K., Novovic, D., Marinkovic, V., and Agbaba, D., *J. Pharm. Biomed. Anal.*, 2003, vol. 32, p. 1019.
40. El-Ghobashy, M.R. and Abo-Talib, N.F., *J. Adv. Res.* (Cairo Univ.), 2010, vol. 1, p. 323.
41. Lotfy, H.M., Saleh, S.S., Hassan, N.Y., and Elgizawy, S.M., *Anal. Chem. Lett.*, 2013, vol. 3, p. 70.
42. Lotfy, H.M., *Int. J. Pharm. Pharm. Sci.*, 2012, vol. 4, p. 673.
43. Lotfy, H.M. and Hegazy, M.A., *Spectrochim. Acta, Part A*, 2013, vol. 113, p. 107.
44. Donmez, O.A., Bozdogan, A., Kunt, G., and Div, Y., *J. Anal. Chem.*, 2010, vol. 65, p. 30.
45. Lotfy, H.M., Hassan, N.Y., and Salem, H., *Spectrochim. Acta, Part A*, 2014, vol. 132, p. 239.
46. Samir, A., Salem, H., and Abdelkawy, H., *Bull. Fac. Pharm. (Cairo Univ.)*, 2012, vol. 50, p. 121.
47. Lotfy, H.M., *Int. J. Pharm. Pharm. Sci.*, 2014, vol. 6, p. 735.
48. Lotfy, H.M., Hagazy, M.A., Rezk, M.R., and Omran, Y.R., *Spectrochim. Acta, Part A*, 2014, vol. 126, p. 197.
49. Lotfy, H.M., Saleh, S.S., Hassan, N.Y., and Salem, H., *Spectrochim. Acta, Part A*, 2014, vol. 126, p. 112.
50. Lotfy, H.M. and Hagazy, M.A., *Spectrochim. Acta, Part A*, 2012, vol. 96, p. 259.
51. *International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology 62*, US FDA, Federal Register, 1997.