



## Research article

## Induced mathematical filtration as an innovative strategy for discrimination and estimation of glycemic control drugs in fixed dose combination

Hayam M. Lotfy<sup>a,\*</sup>, Ekram H. Mohamed<sup>b,c</sup><sup>a</sup> Pharmaceutical Chemistry Department, Faculty of Pharmaceutical Science & Pharmaceutical Industries, Future University in Egypt, 12311, Cairo, Egypt<sup>b</sup> Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, The British University in Egypt, 11837, El-Sherouk City, Cairo, Egypt<sup>c</sup> The Center of Drug Research and Development, Faculty of Pharmacy, The British University in Egypt, 11837, El-Sherouk City, Cairo, Egypt

## ARTICLE INFO

## Keywords:

Pharmaceutical chemistry  
Induced mathematical filtration  
Induced dual wavelength  
Induced concentration subtraction  
Induced amplitude modulation

## ABSTRACT

An innovative strategy was developed for the estimation of a fixed dose combination containing Alogliptin (ALO) and pioglitazone (PIO) using induced concept for resolving the overlapped spectra, lacking isoabsorptive point. This strategy is based on coupling factors as numerical values or ratios as spectrum form with the recorded signals leading to induced mathematical filtration of the drug of interest and complete elimination of the interfering one in the combination without prior physical separation. The calculated factors were factor of equality in induced dual wavelength (IDW) or absorptivity factor in induced concentration subtraction method (ICS) while absorptivity ratio spectrum for induced amplitude modulation method (IAM). The calibration curves displayed linearity within 1.0–16.0 µg/mL for ALO and 2.0–22.0 µg/mL for PIO with good correlation coefficients. The induced methods specificity was also assured through the assaying different synthetic mixtures prepared to contain the two drugs in ratios approaching the ratio actually found in the marketed dosage form. The methods were applicable and suitable for estimating ALO and PIO in both bulk form and their fixed dose combination. Induced methods have been extensively validated in accordance with ICH guidelines and results demonstrated the accuracy and reproducibility in comparison to the reported method.

## 1. Introduction

Compliance is crucial in the management of chronic diseases, where the higher the daily intakes number, the worst the adherence to therapy and compliance. The case applies to type II diabetes mellitus that usually necessitate more than one drug with different mechanism of actions but with synergetic effect to be taken simultaneously. Unfortunately, there is no single best combination for all patients and choosing the optimum diabetes therapy is as much an art as a science. The only way to find the best therapeutic regimen for each patient is through trial and error.

Although the combination therapy was found to be fully beneficial to the patients, it created a challenge to the analysts in quality control labs to find easy affordable and at the same time economic and accurate methods for the analysis of the new drug combinations that are launched in the market every day.

Recently new analytical methods based on spectrophotometric technique have been implemented and validated for the concurrent assaying and resolution of multicomponent mixtures with overlapped spectra. The methods depend on simple mathematical manipulation; combine

different smart approaches through facile software already built in modern spectrophotometers and other available tools as excel and don't require any sophisticated instruments or programs. These methods proved to be applicable and reliable and could be adopted for simultaneous estimation of different components in dosage form without specific requirement such as isoabsorptive point.

Alogliptin (ALO) is considered to be an incretin-based drug. It selectively binds to and inhibits dipeptidyl peptidase-4 slowing the incretin hormones deactivation and thus keeping their level in blood high which in turn reduce concentrations of glucose in a glucose-dependent manner [1, 2]. In the past few years, various analytical methods were applied and reported for determination of ALO in single or combined pharmaceuticals and in different biological fluids. The developed techniques included spectrophotometry [3, 4, 5, 6, 7, 8, 9], chromatography including TLC [10, 11, 12], LC [13, 14, 15] and electrophoresis [16].

Pioglitazone (PIO) acts as a selective agonist at nuclear receptor peroxisome proliferator-activated receptor gamma in liver, fat tissues and skeletal muscles. Activation of these receptors control glucose utilization, production and transport. Thus, PIO insulin tissue sensitivity is

\* Corresponding author.

E-mail addresses: [hayamlotfyhm@yahoo.com](mailto:hayamlotfyhm@yahoo.com), [hayam.mahmoud@fue.edu.eg](mailto:hayam.mahmoud@fue.edu.eg) (H.M. Lotfy).

<https://doi.org/10.1016/j.heliyon.2020.e05289>

Received 4 July 2020; Received in revised form 14 July 2020; Accepted 14 October 2020

2405-8440/© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

enhanced and reduces liver gluconeogenesis is reduced with no increase in pancreatic  $\beta$  cells secretion of insulin [17, 18]. PIO is widely used either alone with a proper diet and exercise program or in combination with other medications. Analysis of PIO was achieved using different methods and reported in the literature [19]. The recently developed techniques included spectrophotometry [20, 21, 22], chromatography including TLC [23] and HPLC [24, 25].

The pharmaceutical combination of ALO and PIO was approved by FDA for management of type II diabetes and this new drug combination was chosen as a model to apply the newly proposed induced technique. The structures of ALO and PIO were displayed in Figure 1.

In this combination product, ALO targets the dysfunction in the pancreatic islets that causes increased glucose levels in the blood diabetic patients, while PIO ameliorates insulin resistance and improves insulin sensitivity and both drugs were found to have protective effects on  $\beta$ -cell mass [26].

Few methods were previously developed for estimating ALO and PIO simultaneously including spectrophotometry using derivative, dual wavelength, ratio subtraction coupled with extended ratio subtraction and constant multiplication coupled with spectrum subtraction methods [27, 28], TLC [23, 29] and LC [30, 31, 32, 33].

The privilege of spectrophotometric methods over other analytical methods is their cheap cost, need of less labor, low chemical consumption and wastes thus eco-friendly and time saving with excellent precision. The applications of innovative UV spectrophotometry for determination of different components in pharmaceutical dosage form have lately increased to eliminate restrictions or specific requirement which can arise upon using conventional methods. The innovative spectrophotometric methods exhibited simplicity without complicated mathematical principles found in chemo metric methods.

In present investigation the authors propose a simple, sensitive and reproducible spectrophotometric methods for resolution and estimation of ALO and PIO in both their bulk powder and synthetic prepared mixtures including any possible additive or excipients. These methods are based on smart original mathematical manipulations to calculate simple factors or ratios whether in their zero order or ratio spectra for resolving the binary mixture of ALO and PIO. The proposed induced spectrophotometric methods via coupling the recorded signals with calculated factors or ratios provided the new proposed methods the power and the ability to resolve the cited drugs with optimum sensitivity and accuracy without restriction.

## 2. Theoretical background

Induced filtration technique in order to quantify each drug in the combination by coupling the recorded signal by mathematical calculated factor(s) or ratio. Thus, the signal can be used to determine the drug of interest.

### 2.1. Induced dual wavelength (IDW)

The IDW [34] was recently applied to resolve and estimate two components or X and Y with complete overlapping spectra in a binary mixture. To apply IDW, any two wavelengths may be selected ( $\lambda_1$  and  $\lambda_2$ ) without any restrictions. For determination the concentration of the drug of interest, an equality factor (Eq.F) at the selected wavelengths for the pure interfering substance should be calculated to cancel its contribution upon subtracting the absorbances of the mixture at  $\lambda_1$  and  $\lambda_2$ .

### 2.2. Induced concentration subtraction (ICS)

Induced Concentration Subtraction is considered to be a novel approach that could be used for determination of two overlapped components X and Y, where Y is displaying an extension over X. For successful application of ICS, Unified Regression Equation (URE) constructed at  $\lambda_{max}$  of Y which representing as  $\lambda_F$  where the two absorptivity factors [35] are needed to be computed.

The first absorptivity factor ( $F_{\lambda_F}$ ) representing the ratio between the absorptivity of X and that of Y at ( $\lambda_F$ ). It could be calculated by division the absorbance of same concentration of X and Y at ( $\lambda_F$ ). Thus,  $F_{\lambda_F} = a_X / a_Y$  where  $b_X = b_Y = 1$  cm and  $C_X = C_Y$ .

While the second absorptivity factor ( $F_Y$ ) is computed through obtaining the average ratio between the two absorbance values  $\{a_Y(\lambda_F) / a_Y(\lambda_1)\}$  of different concentration of component Y in its pure form to get  $\{a_Y(\lambda_F) / a_Y(\lambda_1)\}$

The Unified Regression Equation (URE) is computed after constructing a linear correlation between pure Y absorbance at the  $\lambda_F$  (Which is the maxima of Y) versus its corresponding concentrations.

For analysis of scanned mixture, the absorbance is recorded at two wavelengths  $\lambda_F$  and  $\lambda_1$ .

Thus, the  $F_{\lambda_F} C_X + C_Y$  of the mixture could be calculated via substitution of the recorded absorbance ( $A_m$ ) at  $\lambda_F$  in the constructed URE at  $\lambda_F$ .

The absorbance of component Y alone could be obtained using its previously calculated absorptivity factor ( $F_Y$ ) as briefly discussed below:

Y absorbance at  $\lambda_F$  in mixture =  $[a_Y(\lambda_F) / a_Y(\lambda_1)] \cdot \text{Abs (mixture)}_{\lambda_1}$

$a_Y(\lambda_F) b C_Y = [a_Y(\lambda_F) / a_Y(\lambda_1)] \cdot a_Y(\lambda_1) b C_Y$  (mixture) $_{\lambda_1}$

where  $a_Y(\lambda_F) / a_Y(\lambda_1)$  is absorptivity factor ( $F_Y$ ) of pure Y at  $\lambda_F$  and  $\lambda_1$ , while  $[\text{abs (mixture)}_{\lambda_1}]$  is total mixture absorbance at  $\lambda_1$  where X has no contribution at ( $\lambda_1$ ).

Thus, the Y concentration can be calculated via substitution of the calculated absorbance of Y alone at  $\lambda_F$  in the constructed URE at  $\lambda_F$ .

Finally, upon subtracting the concentration of Y from the mixture total concentration, the result will represent ( $F_{\lambda_F} C_X$ ), which consequently will be multiplied by  $1/F_{\lambda_F}$  to finally obtain X concentration.

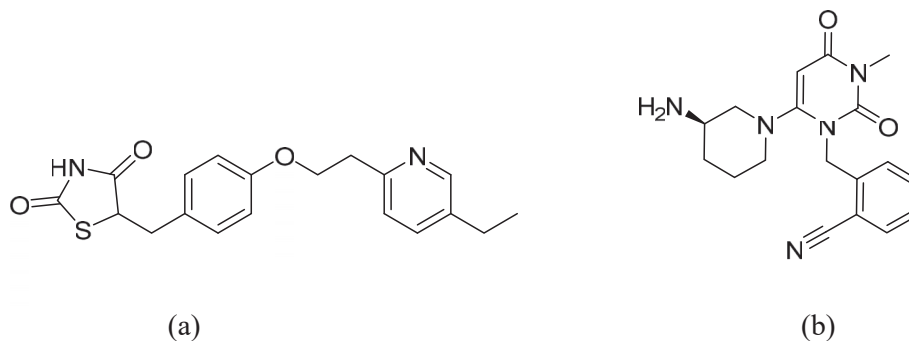


Figure 1. (a) Pioglitazone and (b) Alogliptin.

The ICS method offered the advantage of determination of X and Y with a regression equation unified for both components at  $\lambda_F$  unlike absorbance subtraction [36] or advanced absorbance subtraction methods [37] which were limited for drugs having isoabsorptive point. It also showed superiority over the absorptivity factor method [35] that determined the sum of the concentrations of both drugs collectively but one drug should be estimated using another complementary method to obtain its concentration separately to subtract it from the total concentration of both drugs and get that of the second drug.

### 2.3. Induced amplitude modulation method (IAM)

The recently developed IAM extended the idea of amplitude modulation [36, 38] with an effective modification allowing the assay of a severely overlapped components X and Y lacking is absorptive point [34]. For successful application of IAM, an absorbance ratio spectrum ( $a_r$ ) representing the ratio between the normalized spectrum of Y and X [ $a_Y/a_X$ ] is needed to be obtained by dividing Y normalized spectrum by X normalized spectrum using spectrophotometer software.

The method commences by dividing total spectrum of mixture by Y normalized spectra ( $a_Y$ ) and recording the mixture ( $P_m$ ) amplitude at a selected wavelength ( $\lambda_p$ ). The amplitude difference a step in constant center method [39, 40] will be applied to calculate X postulated amplitude ( $P_x$ ) in the binary mixture at ( $\lambda_p$ ) by construct a regression equation representing difference between recorded amplitude of the ratio spectra of different concentration of component X using normalized Y as a divisor at two selected wavelengths ( $\lambda_p, \lambda$ ) versus amplitude value at  $\lambda_p$

Subtracting the X postulated amplitude from the mixture total recorded amplitude at ( $\lambda_p$ ) will result in a constant representing ( $a_Y C_Y / a_Y$ ) which modulated to concentration of Y would be obtained since the used divisor is the normalized spectra of Y.

The ratio spectrum of X ( $a_X C_X / a_Y$ ) will be obtained via constant subtraction ( $a_Y C_Y / a_Y$ ) from the recorded spectrum of the total mixture ( $a_Y C_Y / a_Y + a_X C_X / a_Y$ ) followed by its multiplication with the ( $a_r$ ) which represents  $a_Y / a_X$  all over the wavelengths to get a constant representing the concentration of X directly ( $C_X$ ).

X and Y concentrations are obtained after substitution in the corresponding regression equations.

## 3. Experimental

### 3.1. Instrument

All measurements were performed using double beam UV/Vis spectrophotometer model S/N C367961148, JASCO V-630 with a built in software (Spectra Manager II). The absorption spectra were scanned within 200 nm to nm wavelength range in quartz cells of 1.00 cm.

## 4. Material and solvents

Alogliptin (ALO) and Pioglitazone (PIO) were kindly supplied by Takeda Pharmaceutical Company, Japan with purity  $100.34 \pm 0.27$  and  $99.41 \pm 0.28$  in accordance with the reported method [28]. Oseni® tablets, labeled to contain 25.0 mg (ALO)/30.0 mg (PIO) was obtained from the local market.

Solvents- Spectroscopic analytical grade methanol (Sigma- Germany).

(d) Standard solutions -Stock standard solutions 1000.0  $\mu\text{g/mL}$  of both ALO and PIO were separately prepared using methanol as solvent, working standard solutions- 20.0  $\mu\text{g/mL}$  of ALO and 40.0  $\mu\text{g/mL}$  PIO were obtained by appropriate dilution from the previously prepared stock solutions using the same solvent.

## 5. Procedures

### 5.1. Spectral characteristic

Serial concentrations (1.0–16.0  $\mu\text{g/mL}$ ) of ALO and (2.0–22.0  $\mu\text{g/mL}$ ) of PIO were accurately prepared in methanol. Zero-order spectra ( $D^0$ ) of all the solutions were recorded and stored separately using Spectra Manager II.

### 5.2. Calculating the resolving numerical factors and absorptivity ratio spectrum

#### 5.2.1. For IDW method

The equality factor (Eq.  $F_{ALO}$ ) of ALO was calculated using the absorbance values at 224 nm and 276 nm ( $F = [A_{224}/A_{276}]$ ) and an absorptivity factor of ALO ( $F_{ALO}$ ) was calculated using those at 224 nm and 297 nm. While for PIO, the equality factor (Eq.  $F_{PIO}$ ) was the ratio of the absorbance values at 224 and 276 nm of pure PIO ( $F = [A_{224}/A_{276}]$ ).

#### 5.2.2. For ICS method

An absorptivity factor of ALO ( $F_{ALO}$ ) was calculated which represent the average value of absorbance readings for different ALO concentrations at 224 and 297 nm.

#### 5.2.3. For IAM method

Absorptivity ratio spectrum ( $a_r$ ) obtained by dividing ALO normalized spectrum by PIO normalized spectrum using the spectrophotometer software.

### 5.3. Construction of calibration graphs

#### 5.3.1. Calibration graphs for IDW method

For ALO, Construction of calibration graph was achieved using the stored  $D^0$  of ALO to represent the correlation between the absorbance difference ( $\Delta A$ ) ( $A_{ALO224} - \text{Eq. } F_{PIO} [A_{ALO276}]$ ) and the corresponding concentration of pure ALO. For PIO, a single calibration graph was constructed to represent the correlation between  $\Delta A$  ( $A_{PIO224} - \text{Eq. } F_{ALO} [A_{PIO276}]$ ) and the corresponding concentration of pure PIO.

#### 5.3.2. Calibration graphs for ICS method

A Calibration graph was constructed between the absorbance values of  $D^0$  spectra of ALO at 224 nm against the corresponding concentrations. An absorptivity factor of ALO ( $F_{ALO}$ ) was calculated which represent the absorbance values average for different ALO concentrations at 224 and 297 nm.

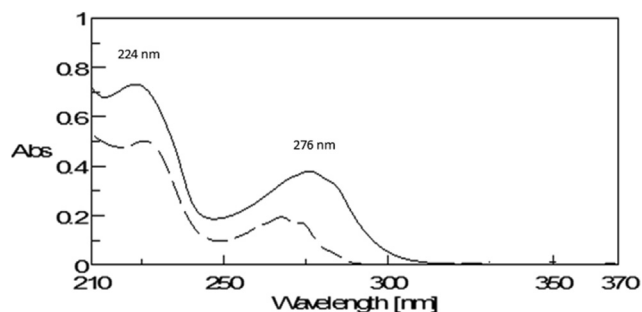
#### 5.3.3. Calibration graphs for IAM method

The  $D^0$  spectra of different ALO and PIO concentrations were separately divided by the normalized spectrum of ALO and PIO. Two calibration graphs were constructed for each drug representing the relation between their recorded amplitude values at 224 nm and the corresponding concentrations of ALO and PIO, respectively. Another regression equation was constructed representing relation between the amplitude difference at 224 nm and 267 nm for different ratio spectra of PIO versus the amplitude values at 224 nm.

The corresponding regression equations were then computed and used to calculate the unknown concentrations of ALO and PIO.

### 5.4. Application to synthetic mixtures

In a set of 10 mL calibrated volumetric flasks, different predetermined portions of ALO and PIO working solutions were accurately transferred



**Figure 2.** Zero order absorption curve of 10.0 µg/mL Alogliptin (—) and 10.0 µg/mL Pioglitazone (---).

and to methanol was used to complete the volume. Seven synthetic mixtures with different ratios of ALO and PIO were prepared, scanned and stored as shown in Figure 2.

Each drug in the binary mixture can be estimated by three methods using different approaches as follows:

#### 5.4.1. IDW method

The absorbance of each mixture was recalled at 224 nm and 276 nm for ALO and at 224 nm and 297 nm for PIO then multiply each by its appropriate factor as in construction of calibration graph.

#### 5.4.2. ICS method

The mixture absorbance values was recalled at 224 nm and 297 nm, the later was then multiplied by ALO absorbance factor, previously calculated, to get the postulated value of ALO at 224 nm. This calculated absorbance value and the recorded value were used to calculate the ALO and PIO concentrations.

#### 5.4.3. IAM method

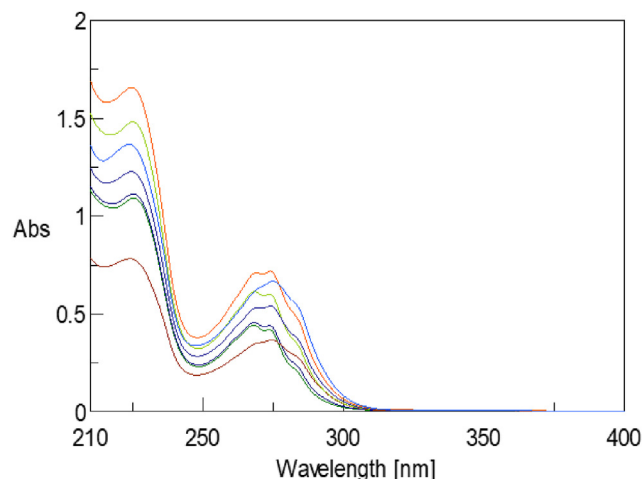
Ratio spectra of each synthetic mixture was obtained using ALO normalized spectrum as a divisor then the  $\Delta P$  between 224 nm and 267 nm was calculated and substituted in the constructed equation to get the amplitude of PIO at 224 nm then subtracted from recorded one at 224 nm to get the amplitude of ALO ( $P_{ALO}$ ). The  $P_{ALO}$  was then subtracted its corresponding mixture, to resolve ratio spectrum of PIO, followed by multiplication with absorptivity ratio spectra to finally obtain a constant representing PIO concentration.

#### 5.5. Application to fixed dose combination

An accurately weighed amount of the Oseni® tablets powder equivalent to ALO 5.0 mg and PIO 6.0 mg was dissolved using methanol with the aid of ultrasonic bath for half an hour. The obtained solution was filtered and transferred to a measuring flask 100-mL, and the final volume was completed with the same solvent, then 0.5 mL of the extracted tablet solution were transferred into a volumetric flask (10-mL) and completed to volume with methanol to get solution claimed to contain 2.5 µg/mL of ALO and 3.0 µg/mL of PIO. The steps of IDW, ICS and IDW methods were applied for the analysis of the pharmaceutical preparations solutions and the concentration of both ALO and PIO were calculated using the corresponding regression equations for each method.

## 6. Results and discussion

The scanned  $D^0$  of ALO and PIO showed highly overlapped bands in their  $\lambda_{max}$  region which hindered their direct determination using conventional methods. In addition, there were no isosbestic points but fortunately ALO exhibited slight extension over PIO in the region over 295 nm (Figure 2) which was useful in calculating a factor to solve their superimposed spectra.



**Figure 3.** Zero order spectra of seven synthetic mixtures containing Alogliptin and Pioglitazone in different ratios using methanol as blank.

The induced methods were used for selective determination of the two drugs under investigation in the synthetic mixtures (Figure 3).

#### 6.1. IDW method

Both ALO and PIO were determined progressively adopting IDW using two wavelengths (224 nm and 276 nm) where both drugs showed prominent peaks and thus significant absorbance difference values (Figure 2). The equality factors were calculated to be 1.93 and 3.49 for PIO and ALO, respectively. Thus the calibration graphs were constructed representing  $\Delta A (A_{224} - 1.93 A_{276})$  for ALO ( $A_{224} - 3.49 A_{276}$ ) for PIO against their corresponding concentrations. To estimate ALO and PIO in the mixtures, the absorbance values were recorded at the chosen wavelengths and multiplied by factors of equality as mentioned in the constructed calibration graphs.

The main limitation of ordinary dual wavelength method (DWM) was the obligation to select specific wavelengths where the values of absorbance of the interfering drug must be the same and difference in the absorbance ( $\Delta A$ ) would be equal zero. This critical choice may have its reverse effects on the sensitivity and robustness of the method. IDW overcame this limitation where it could be applied using two wavelengths where  $\Delta A$  of the interfering drug is not zero while its contribution is eliminated through calculating an equality factor that equalizes its absorbances at the selected wavelengths and thus  $\Delta A$  is induced to be zero. The selection of the two wavelengths isn't restricted to certain wavelengths as in case dual wavelength method. Thus, the selected wavelengths show high absorbance value so minimum error and consequently good correlation was obtained between the difference in the absorbance  $\Delta A (A_{224} - 1.93 A_{276})$  for ALO ( $A_{224} - 3.49 A_{276}$ ) for PIO versus the corresponding concentration.

#### 6.2. ICS method

The novel ICS is smart accurate method developed to determine both drugs in interest using a unified regression equation at 224 nm. Although ALO was more extended over PIO but it ALO show very poor absorbance in the extended region over 350 nm which make the analysis of ALO at the extension region is impossible since it is neither sensitive nor robust. This newly proposed method exploits this extended region through calculating two factors the first one is absorptivity factor ( $F_{\lambda F}$ ) (ratio between the absorbance of the same concentration of pure ALO and PIO at the absorptivity point ( $\lambda_F$ ) and it was calculated to be 1.5. While, the second one is (F) for pure ALO representing the relation between the absorbance at 224 nm ( $\lambda_{max}$  of ALO) and that at 297 nm (extended region with no contribution of PIO) and it was found to be 9.11. This novel

method based on a progressive technique on the scanned ( $D^0$ ) of the mixture with no requirement for a divisor or any further manipulation.

For analysis of synthetic mixtures, the values of absorbance were recorded at 224 nm and 297 nm (Figure 3). The sole absorbance value of ALO at 224 nm was obtained by multiplying the recorded absorbance of each mixture at 297 nm by 9.11. The calculated absorbance value of ALO at 224 nm and the recorded absorbance value at 224 nm were substituted separately in a previously constructed unified regression equation representing the absorbance at  $\lambda_F$  224 nm versus corresponding concentration of pure ALO, to get the concentration of ALO and (ALO+1.5 PIO) in their mixture, respectively. Finally, the concentration of ALO was subtracted from the cumulative concentration (ALO+1.5 PIO) to get 1.5 [PIO] and actual concentration of PIO in each mixture was obtained through multiply by  $1/F_{AF}$  (1/1.5).

This method could be applied for the determination of the two drugs in the mixture by using two factors calculated using the spectra of the pure drugs and only one regression equation only.

### 6.3. IAM method

The novel method IAM [34] was based on the smart amplitude modulation method [38] that modulated amplitude to concentration by using normalized spectra as divisors but didn't require the existence of isobestic point or spectrum extension. Although,  $D^0$  of ALO displays extension over PIO but the original amplitude modulation method which necessitated the extended region of one of the drugs failed to give satisfactory results. In contrary, IAM was successfully applied for simultaneous determination of ALO and PIO. Two regression equation of ratio spectra at 224 nm of different concentrations of ALO and PIO using normalized spectrum of ALO' and PIO' as a divisor were constructed against the corresponding concentration of ALO and PIO. For analysis of synthetic mixtures, each mixture was divided by the normalized spectra of ALO ( $a_{ALO}$ ). Use the amplitude difference at (224 nm–267 nm) for each mixture and substitute in the regression equation representing a relationship between the differences of ratio amplitudes at (224 nm–267 nm) of different concentrations of pure PIO versus the corresponding ratio amplitude 224 nm as presented in (Figure 4a). The obtained amplitude value of PIO for each mixture at 224 nm was then subtracted from the recorded amplitude of its corresponding mixture at the same wavelength to obtain a constant value of ALO/ALO' then the concentration of ALO in each mixture was calculated via its corresponding regression equation representing amplitude values of ALO/ALO' versus corresponding concentration of ALO'.

$$P_{\text{Recorded}} = 0.9978 C + 0.0380$$

where  $P_{\text{Recorded}}$  represented the recorded amplitude of ALO and C represented the corresponding actual concentration of ALO.

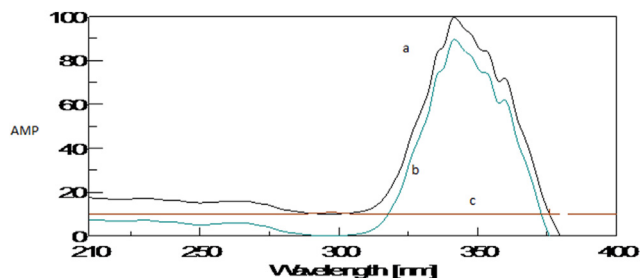


Figure 4. (a) Ratio spectrum of the binary mixture of (10 µg/mL of ALO: 10 µg/mL of PIO) using the normalized spectrum of ALO as a divisor. (b) Resolved ratio spectrum of 10 µg/mL of PIO/ALO' after subtraction of ALO/ALO' constant from the ratio spectrum of the binary mixture using the normalized spectrum of ALO as a divisor. (c) Modulated amplitude equivalent to concentration of PIO/PIO' in mixture after multiplication by the absorptivity ratio spectra of ALO/PIO'.

The resolved spectrum of PIO/ALO' (Figure 4b) in each mixture was obtained by subtraction the constant value of ALO/ALO' from the ratio spectrum of its corresponding synthetic mixture via spectrophotometer software. The constant represent PIO/PIO (Figure 4c) was obtained after multiplying PIO/ALO' by absorbance ratio spectrum representing ALO'/PIO'. PIO concentration in the mixture was calculated via its corresponding regression equation representing amplitude values of PIO/PIO' versus corresponding concentration of PIO'.

$$P_{\text{Recorded}} = 1.0032 C + 0.0236$$

where  $P_{\text{Recorded}}$  represented the recorded amplitude of PIO and C represented the corresponding actual concentration of PIO.

The induced methods was developed and successfully applied for quantifying ALO and PIO within 1.0–16.0 µg/mL and 2.0–22.0 µg/mL, respectively as presented in Table 1.

The methods also proved to be sensitive upon the analysis of different synthetic mixtures and the results were presented in Table 2.

The induced methods were also adopted for the assay of ALO and PIO in tablet dosage form and satisfactory results were obtained and presented in Table 3.

## 7. Method validation

The induced methods were accomplished and validated in agreement with the ICH guidelines [41] as follows:

### 7.1. Linearity and range

Induced methods were found to be linear within the concentration ranges of 1.0–16.0 µg/mL and 2.0–22.0 µg/mL for ALO and PIO, respectively. The characteristic parameters of the computed regression equations are expressed in Table 1.

### 7.2. Accuracy

The accuracy of the methods was checked by calculating the recovery of ALO and PIO standard solutions at three concentration levels within the specified linearity range (5.0, 9.0, 15.0 µg/mL) of pure ALO and (5.0, 9.0, 15.0 µg/mL) of pure PIO, i.e., multilevel recovery study. Good mean of recovery% ± SD of standard drugs analyzed by methods indicating that the proposed method was accurate. Results of recovery study are shown in Table.

### 7.3. Precision

repeatability was investigated through estimating three concentrations of pure drugs, 4.0, 10.0, 16.0 µg/mL for ALO and 4.0, 10.0, 20.0 µg/mL for PIO in triplicates on the same day while the intermediate precision was checked through repeating the assay of the same concentrations for nine times on four successive days. The results were satisfactory and did not exceed 2% assuring the precision of induced methods (Table 1), proving the high reproducibility of the results and the precision of the method.

### 7.4. Specificity

The term specificity refer to 100% selectivity where no interference was obtained from the component expected or unexpected to be present [42]. For analytical methods, specificity of methods for analyzing drugs in combination could be achieved by estimation and discrimination the analytes of interest among mixtures (synthetic mixtures) and this is applied to check whether the co-formulated drug (s) is influence the results of the proposed methods. It is assessed via the preparation of seven mixtures containing both ALO and PIO prepared in different ratios around the actual ratio expected in the marketed tablets.

**Table 1.** validation and assay parameters of the induced spectrophotometric methods for of ALO and PIO determination.

Parameters	ALO			PIO			
	IDW	ICS	IAM	IDW	ICS	IAM	
Linearity ( $\mu\text{g/mL}$ )	1.0–16.0			2.0–22.0			
Slope	0.059	0.073	1.0008	0.0222	0.073	0.6798	
Intercept	0.0016	0.0001	0.0001	-0.0002	0.0001	-0.005	
Correlation Coefficient(r)	0.9999	1.0000	1.0000	1.0000	1.0000	0.9999	
Accuracy							
Mean $\pm$ SD	100.03 $\pm$ 0.30	100.04 $\pm$ 0.14	100.01 $\pm$ 0.24	100.01 $\pm$ 0.35	99.82 $\pm$ 0.28	100.08 $\pm$ 0.53	
Precision	RSD% <sup>a</sup>	0.537	0.441	0.075	0.570	0.523	0.091
	RSD% <sup>b</sup>	0.852	0.833	0.168	0.794	0.777	0.215

RSD%<sup>a</sup>: the intra-day precision, RSD%<sup>b</sup>: inter-day precision, (n = 3) relative standard deviation of concentrations ALO (4.0, 10.0, 16.0  $\mu\text{g/mL}$ ).and PIO (4.0, 10.0, 20.0  $\mu\text{g/mL}$ ).

**Table 2.** Determination of ALO and PIO in synthetic mixtures by the proposed spectrophotometric methods.

No.	Concentration ( $\mu\text{g/mL}$ )		Recovery% <sup>a</sup>					
			ALO			PIO		
	ALO	PIO	IDW	ICS	IAM	IDW	ICS	IAM
1	10.0	10.0	100.74	99.25	100.45	99.49	100.52	100.31
2	5.0	15.0	100.99	100.06	99.67	99.86	99.97	100.16
3 <sup>b</sup>	12.5	15.0	100.68	100.01	99.75	99.56	100.03	100.13
4	8.0	18.0	100.79	99.79	99.88	99.78	100.15	100.17
5	8.0	4.0	100.81	99.16	100.16	99.03	100.97	100.52
6	16.0	4.0	100.64	99.79	100.28	100.04	100.59	100.25
7	4.0	16.0	101.11	100.11	99.77	99.9	99.95	100.31
Mean			100.82	99.73	99.99	99.67	100.31	100.26
$\pm$ SD			0.169	0.385	0.301	0.339	0.388	0.133

<sup>a</sup> The average of three measurements.

<sup>b</sup> The ratio of the lab mixture in Oseni® tablets.

**Table 3.** assay of ALO and PIO in fixed dose combination applying the three induced spectrophotometric methods and standard addition technique results.

Fixed dosage combination		Found % <sup>a</sup>					
		ALO			PIO		
		IDW	ICS	IAM	IDW	ICS	IAM
Oseni®tablet 25.0 (ALO)/30.0 (PIO)	Mean	100.76	100.11	100.02	99.61	100.06	99.82
	$\pm$ SD	$\pm$ 0.32	$\pm$ 0.21	$\pm$ 0.18	$\pm$ 0.59	$\pm$ 0.25	$\pm$ 0.18
Standard addition (Recovery% $\pm$ SD)	Mean	100.45	100.25	99.68	100.66	100.33	100.75
	$\pm$ SD	$\pm$ 0.26	$\pm$ 0.29	$\pm$ 0.53	$\pm$ 0.45	$\pm$ 0.51	$\pm$ 0.32

<sup>a</sup> The average of five determinations.

**Table 4.** Results of statistical comparison between the induced spectrophotometric methods and the reported method [28] for determination of ALO and PIO.

Values	ALO			Reported Method [28] <sup>a</sup>	PIO			
	Proposed methods				Proposed methods			
	IDW	ICS	IAM		IDW	ICS	IAM	
Mean	100.76	100.11	100.02	100.34	99.61	99.06	99.72	99.41
SD	0.32	0.21	0.18	0.27	0.59	0.25	0.18	0.28
RSD%	0.318	0.209	0.179	0.269	0.592	0.253	0.180	0.281
n	5	5	5	5	5	5	5	5
Variance	0.1024	0.0441	0.0324	0.0729	0.3481	0.0625	0.0324	0.0784
Student's t-test <sup>b(2.306)</sup>	2.243	1.504	2.205		0.684	2.085	2.082	
F-value <sup>b(6.38)</sup>	1.41	1.65	2.25		4.44	1.25	2.42	

<sup>a</sup> Spectrum subtraction coupled with constant multiplication.

<sup>b</sup> The values in the parenthesis are the corresponding t and F theoretical values at p = 0.05.

Satisfactory percentage recoveries and standard deviations were obtained and results were displayed in Table 2.

### 7.5. Estimation of fixed dose combination

The matrix interference effect was investigated to check whether the matrix including the inert excipients and formulation additives have any influence or impact on the resulting data either through quenching or enhancing effects. This was achieved via preparation of synthetic solutions of tablets containing concentration of ALO and PIO equivalent to their actual concentrations in the marketed tablets. The spectra obtained after analyzing the solutions of synthetic and marketed tablets were found to be identical assuring that excipients have no influence or interfering effect on the accurate estimation of ALO and PIO upon applying induced spectrophotometric methods, thus considered to be specific. The results are presented in Table 3 where good percentage recoveries and SD not exceeding 2 were obtained. The standard addition technique was also applied at different fortification levels and satisfactory results of recovery percentages of the added standard reveal to accurate estimation of the ALO and PIO in Oseni® tablets by the proposed methods as abridged in Table 3.

### 7.6. Statistical analysis

Statistical comparison [43] was performed between the results obtained from the three induced methods and those obtained from the reported one [28], Table 4. The difference was found to be insignificant with the perspective of accuracy and precision where the calculated t and F values was lower than theoretical values as demonstrated in Table 4.

The results obtained by the proposed methods were statistically [43] compared to those obtained by the reported method [28] as demonstrated in Table 4. No significant difference regarding the accuracy and precision of the proposed and reported methods was observed as indicated from the lower values of the calculated t and F tests than that of the theoretical ones as demonstrated in Table 4.

### 7.7. Advantages of the proposed methods over reported spectrophotometric methods [27, 28]

The induced mathematical filtration strategy was considered to be the most sensitive compared to other reported spectrophotometric methods [27, 28] where they permitted the determination of lower concentrations limits up to 1.0 µg/mL for ALO and 2.0 µg/mL for PIO. While the concentration limit for all the previously reported methods was 5.0 µg/mL for both drugs. The three induced methods among this innovative technique could be applied for ALO and PIO in their synthetic mixtures containing different ratios of them to overcome the restrictions of these reported methods such as:

- 1 The proposed methods could be applied at wavelengths leading to maximum sensitivity, thus they overcome restriction on the selection of certain wavelengths which showing equal absorbance for the interfering component as in case of dual wavelength method
- 2 The proposed methods applied on zero order and ratio spectra with good signal to noise ration, thus they overcome restriction on using certain derivatized mode where the interfering drug showing zero crossing or zero contribution leading to poor signal to noise ratio.
- 3 The induced methods are applicable on completely overlapped spectra thus, they overcome the restriction of the analysis of the two drugs in definite concentration where one of the proposed drugs should extended over the other one to get extended regular constant as in case of ratio subtraction coupled with extended ratio subtraction and spectrum subtraction coupled with constant multiplication.

## 8. Conclusion

The present paper described innovative strategy via induced mathematical filtration technique to accurately and specifically estimate the concentration of ALO and PIO in their fixed dose tablets. Three methods (IDW, ICS and AAM) among this strategy based on coupling factors and ratio as smart approach for enhancement specification and resolving strong overlapped spectra without any restriction in wavelengths selection and showing good signal to noise ratio. The novel ICS has main privilege over IDW and AAM that it needs minimum manipulation steps at zero order spectra of the mixtures and only one regression equation is used to estimate both drugs in tablets. The procedures presented here do not need necessitate any expensive apparatus; and can be used advantageously as a routine method for the assaying ALO and PIO in quality control laboratories, especially those found in poor or developing countries lacking expensive facilities and instrumentation.

## Declarations

### Author contribution statement

Hayam M. Lotfy, Ekram Hany: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

## References

- [1] J.E. Campbell, D.J. Drucker, Pharmacology, physiology, and mechanisms of incretin hormone action, *Cell Metabol.* 17 (2013) 819–837.
- [2] J.A. Lovshin, D.J. Drucker, Incretin-based therapies for type 2 diabetes mellitus, *Nat. Rev. Endocrinol.* 5 (5) (2009) 262–269.
- [3] A. Nikalje, M.S. Baig, M.I. Anees, A. Qureshi, Simultaneous estimation of Alogliptin and Metformin from its tablet dosage form by area under curve and multicomponent UV spectrophotometric method, *World J. Pharm. Pharm. Sci.* 4 (2015) 1329–1339.
- [4] B.P. Patel, R.C. Mashru, Sensitive and selective approaches for real time estimation of alogliptin benzoate and metformin hydrochloride in synthetic mixture, *Int. Bull. Drug Res.* 4 (2014) 148–159.
- [5] R. Anjali, P. Pares, Spectrophotometric estimation of Glibenclamide and Alogliptin in synthetic mixture by area under curve method, *Int. J. Pharm. Pharmaceut. Res.* 6 (2016) 228–236.
- [6] D.B. Sen, A.K. Sen, A. Zanzwar, R. Balaraman, A.K. Seth, Determination of Alogliptin benzoate and Metformin hydrochloride in tablet dosage form by simultaneous equation and absorption ratio method, *Int. J. Pharm. Pharmaceut. Sci.* 7 (2015) 380–383.
- [7] A.S. Kumara, T.V. Reddy, C.B. Sekharan, Utility of picric acid and 2,4 dinitrophenol as chromogenic reagents for visible spectrophotometric quantification of Alogliptin, *Bull. Fac. Pharm. Cairo Univ.* 5 (2017) 177–184.
- [8] A.S. Kumara, T.V. Reddy, C.B. Sekharan, Spectrophotometric determination of alogliptin in bulk and tablet dosage form using bromate–bromide mixture as brominating agent, *Karbala Int. J. Mod. Sci.* 3 (2017) 8–17.
- [9] P.J. Yadav, V.N. Kadam, S.K. Mohite, Development and validation of UV spectrophotometric method for alogliptin benzoate in bulk drug and tablet formulation, *Curr. Pharma Res.* 4 (2014) 1286–1290.
- [10] K.B. Bodiwala, S. Shah, J. Thakor, Degradation kinetics study of Alogliptin benzoate in alkaline medium by validated stability-indicating HPTLC Method, *J. AOAC Int.* 99 (2016) 1505–1512.
- [11] P.B. Deshpande, S.R. Butle, Stability indicating high performance thin layer chromatographic determination of Alogliptin benzoate as bulk drug and in tablet dosage form, *Eurasian J. Anal. Chem.* 12 (2017) 325–335.

- [12] K. Sharma, A. Parle, Development and validation of HPTLC method for Estimation of alogliptin benzoate in bulk drugs and tablet dosage forms, *Int. Bull. Drug Res.* 5 (2015) 81–89.
- [13] C.D. Bertol, M.T. Friedrich, G. Carlos, Analytical stability-indicating methods for Alogliptin in tablets by LC-CAD and LC-UV, *J. AOAC Int.* 100 (2017) 400–405.
- [14] S. Mowaka, B.M. Ayoub, Comparative study between UHPLC-UV and UPLC-MS/MS methods for determination of alogliptin and metformin in their pharmaceutical combination, *Die Pharmazie* 72 (2017) 67–72.
- [15] S. Mowaka, E.F. Elkady, M.M. Elmazar, B.M. Ayoub, Enhanced LC-MS/MS determination of alogliptin and metformin in plasma: application to a pharmacokinetic study, *Microchem. J.* 130 (2017) 360–365.
- [16] I. Fejős, Z. Urbancsok, W. Zhou, T. Sohajda, W. Hu, L. Szente, S. Béni, Separation of alogliptin enantiomers in cyclodextrin-modified capillary electrophoresis: A validated method *Electrophoresis* 35 (2014) 2885–2891.
- [17] T. Dehmer, M.T. Heneka, M. Sastre, J. Dichgans, J.B. Schulz, Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I $\kappa$ B $\alpha$  induction and block of NF $\kappa$ B and iNOS activation, *J. Neurochem.* 88 (2004) 494–501.
- [18] U. Smith, Pioglitazone, Mechanism of action, *Int. J. Clin. pract. Suppl.* 121 (2001) 13–18.
- [19] N. Satheeshkumar, S. Shantikumar, R. Srinivas, Pioglitazone, A review of analytical methods, *J. Pharm. Anal.* 4 (2014) 295–302.
- [20] S. Naveed, Z. Ashraf, T. Mukhtar, Assay of Pioglitazone HCL by using UV visible spectrophotometer, *Br. J. Res.* 2 (2015) 24–29.
- [21] M.T. Jafari, M. Kamfirozi, E. Jazan, S.M. Ghoreishi, Selective extraction and analysis of pioglitazone in cow plasma using a molecularly imprinted polymer combined with ESI ion mobility spectrometry, *J. Sep. Sci.* 37 (2014) 573–579.
- [22] A.A. Abdelmonem, G.H. Ragab, H. Hashem, E.A. Bahgat, Simple atomic absorption spectroscopic and spectrophotometric methods for determination of pioglitazone hydrochloride and carvedilol in pharmaceutical dosage forms, *Int. J. Spectrosc.* (2014) 1–17.
- [23] K. Sharma, A. Parle, Development and validation of HPTLC method for simultaneous estimation of alogliptin benzoate and pioglitazone hydrochloride in bulk drugs and combined dosage forms, *Int. J. Pharm. Rev. Res.* 4 (2015) 35–42.
- [24] P.S. Prasad, S.S. Imam, M. Aqil, M. Rizwan, Y. Sultana, A. Ali, Validated reversed phase HPLC method for determination of pioglitazone hydrochloride in bulk drug and tablet formulations, *J. Anal. Chem.* 70 (2015) 744–746.
- [25] A.M. Mohamed, F.A. Mohamed, S. Ahmed, Y.A. Mohamed, An efficient hydrophilic interaction liquid chromatographic method for the simultaneous determination of metformin and pioglitazone using high-purity silica column, *J. Chromatog. B.* 997 (2015) 16–22.
- [26] S. Kawashimaa S, T. Matsukoaa, H. Kanetoo, Y. Tochinoa, K. Katoa, K. Yamamotoa, T. Yamamotoa, M. Matsuhisab, I. Shimomuraa, Effect of alogliptin, pioglitazone and glargine on pancreatic  $\beta$ -cells in diabetic db/db mice, *Biochem. Biophys. Res. Comm.* 404 (2011) 534–540.
- [27] R. Kashyap, U. Srinivasa, S. Dadhaniya, First order derivative and dual wavelength spectrophotometry methods development and validation for simultaneous estimation of alogliptin and pioglitazone in bulk and dosage form, *Int. J. Pharm. Chem.* 4 (2014) 21–31.
- [28] M.F. Abdel-Ghany, O. Abdel-Aziz, M.F. Ayad, M.M. Tadros, Comparative study between multivariate and univariate analysis of two antidiabetic combinations, *J. AOAC Int.* 100 (2017) 1379–1391.
- [29] D.A. Shah, U. Gajjar, F.A. Mehta, V.B. Patel, U.K. Chhalotiya, Development of HPTLC method for the estimation of pioglitazone and alogliptin in synthetic mixture, *Indian Drugs* 54 (2017) 44–52.
- [30] B.M. Ayoub, O. Abdel-Aziz, A guide for using experimental design in chromatographic method development: applied to the analysis of selected anti-diabetic pharmaceutical combinations, *Die Pharmazie* 71 (2016) 683–690.
- [31] M.F. Abdel-Ghany, O. Abdel-Aziz, M.F. Ayad, M.M. Tadros, New LC–UV methods for pharmaceutical analysis of novel anti-diabetic combinations, *Acta Chromatogr.* 29 (2017) 448–452.
- [32] B.H. Babu, P.R. Veni, P.B. Krishna, K.L. Prameela, RP-HPLC estimation of alogliptin and pioglitazone simultaneously in combined tablet dosage forms, *Marmara Pharm. J.* 21 (2017) 345–354.
- [33] A.A. El-Zaher, E.F. Elkady, H.M. Elwy, M.A. Saleh, Synchronized determination of four antidiabetic and antihyperlipidemic drugs by a validated LC method, *Chromatographia* 80 (2017) 87–97.
- [34] H.M. Lotfy, S.S. Saleh, N.Y. Hassan, H. Salem, Novel two wavelength spectrophotometric methods for simultaneous determination of binary mixtures with severely overlapping spectra, *Spectrochim. Acta A.* 136 (2015) 1786–1796.
- [35] A. Samir, H. Salem, M. Abdelkawy, New developed spectrophotometric method for simultaneous determination of salmeterol xinafoate and fluticasone propionate in bulk powder and Seritide® diskus inhalation, *Bull. Fac. Pharm. Cairo Univ.* 50 (2012) 121–126.
- [36] H.M. Lotfy, M.A. Hegazy, S. Mowaka, E.H. Mohamed, Novel spectrophotometric methods for simultaneous determination of amlodipine, valsartan and hydrochlorothiazide in their ternary mixture, *Spectrochim. Acta A.* 140 (2015) 495–508.
- [37] E.H. Mohamed, H.M. Lotfy, M.A. Hegazy, S. Mowaka, Different applications of isosbestic points, normalized spectra and dual wavelength as powerful tools for resolution of multicomponent mixtures with severely overlapping spectra, *Chem. Cent. J.* 11 (2017) 1–15.
- [38] H.M. Lotfy, Absorbance subtraction and amplitude modulation as novel spectrophotometric methods for the analysis of binary mixtures, *Int. J. Pharm. Pharm. Sci.* 6 (2014) 735–741.
- [39] H.M. Lotfy, Determination of simvastatin and ezetimibe in combined tablet dosage forms by constant center spectrophotometric method, *Int. J. Pharm. Pharm. Sci.* 4 (2012) 673–679.
- [40] H.M. Lotfy, M.H. Hegazy, S. Mowaka, E.M. Mohamed, Validated spectrophotometric methods for simultaneous determination of Omeprazole, Tinidazole and Doxycycline in their ternary mixture, *Spectrochim. Acta A* 153 (2016) 321–332.
- [41] S. Walfish, Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods, *Bio Pharm. Inter.* 19 (2006) 1–6.
- [42] J. Vessman, R.I. Stefan, J.F. Van staden, K. Danzer, W. Lindner, D.T. Burns, A. Fajgelj, H. Müller, Selectivity in analytical Chemistry (IUPAC recommendations 2001), *Pure Appl. Chem.* 73 (2001) 1381–1386.
- [43] J.N. Miller, J.C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, fifth ed., Pearson Education Limited, England; Harlow, 2005.