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SELECTIVE H-POINT STANDARD ADDITION AND DOUBLE DIVISOR RATIO DERIVATIVE CHEMOMETRIC METHODS FOR DETERMINATION OF TERNARY MIXTURE OF CARDIOVASCULAR DRUGS

Marwa El Jamal

Assistant Professor, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon,
marwa.jamal@bau.edu.lb

Azza Gazy

Professor, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon, azza.ghazi@bau.edu.lb

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SELECTIVE H-POINT STANDARD ADDITION AND DOUBLE DIVISOR RATIO DERIVATIVE CHEMOMETRIC METHODS FOR DETERMINATION OF TERNARY MIXTURE OF CARDIOVASCULAR DRUGS

Abstract

Green analytical chemistry is concerned with the development of analytical procedures that minimize consumption of hazardous reagents and solvents, and maximize safety for operators and environment. Chemometrics, considered as green analytical chemistry, have become one of the important mathematical and statistical techniques for the resolution of overlapping spectra of multi component mixtures. This work relates simple, accurate and specific analytical chemometric techniques for the simultaneous determination of a ternary mixture of co-administered cardiovascular drugs (Ticagrelor (TICA), Irbesartan (IRB) and Hydrochlorothazide (HCT)). The different applied Chemometric methods are based on H-point standard addition method (HPSAM) and Double divisor ratio spectra Derivative method (DDRD). The applied methods were compared to Derivative spectrophotometry (First derivative (D1) and second derivative (D2)), and shows their superiority in resolving the ternary mixture. TICA, IRB and HCT were determined simultaneously at concentration ratios varying from 0.5: 4: 12.5 $\mu\text{g.mL}^{-1}$ to 1: 8: 25 or from 10:1:10 to 20:3:8 $\mu\text{g.mL}^{-1}$, by applying HPSAM or DDRD respectively in a mixed sample. The methods were validated in terms of linearity, LOD, LOQ, precision and accuracy and the results were statistically compared to an established RP-HPLC method.

Keywords

Double divisor Ratio Spectra derivative; H-point; RP-HPLC

1. INTRODUCTION

Cardiovascular diseases accompanied with high blood pressure are the largest risk factor for premature death. Some patients with hypertension require two or more antihypertensive and anti-platelet drugs with complementary mechanisms of action to control their cardiovascular condition. The angiotensin II type 1-receptor antagonist Irbesartan, the diuretic Hydrochlorothiazide and the anti-platelet Ticagrelor have recognised clinical efficacy and protective effect on the cardiovascular system. So, the combination of the three drugs been found to be more effective than either drug alone in the treatment of cardiovascular diseases not adequately controlled by monotherapy (Umesh R. Desai, Ph.D ,2004).

In recent years, there have been significant developments in methodological and technological tools to prevent and reduce the deleterious effects of analytical procedures. In modern analytical chemistry, it is essential to reduce the chemicals used, and the analysis time. Therefore, Chemometrics address the constraints that happen due to reducing the solvents used and the duration of analysis by doing most of the works on the front desk using microcomputers with appropriate softwares on the primary data generated in the lab work.

Irbesartan (IRB), chemically described as 2-butyl-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]-methyl-1,3-diazaspiro[4,4]-non-1-en-4-one, (Fig. 1), is an angiotensin II blocker, which acts mainly by selective blockade of AT1 receptors and reduces the effects of angiotensin II. IRB may be used alone or in combination with other antihypertensive or diuretic agents (Charles, L. ,2005).

Hydrochlorothiazide (HCT), chemically described as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide, (Fig. 2), is a thiazide diuretic. It increases sodium and chloride excretion in distal convoluted tubule. Because of their synergistic anti-hypertensive action, Irbesartan and Hydrochlorothiazide are available in the market as a combined dosage form (O'Neil MJ,2001).

Ticagrelor (TICA), chemically described as (1S,2S,3R,5S)-3-[7-[[[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-(propylthio)-3H-[1,2,3]-triazolo [4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol, (Fig. 3), is an orally active antiplatelet agent, act as an inhibitor for platelet activation and aggregation mediated by the P2Y₁₂ ADP-receptor1. Ticagrelor is indicated to reduce the rate of thrombotic cardiovascular events in patients with acute coronary syndrome. Ticagrelor and its major metabolite reversibly interact with the platelet P2Y₁₂ ADP-receptor to prevent signal transduction and platelet activation, which inhibits platelet aggregation and thrombus formation in atherosclerotic disease (Siragy H.,1999).

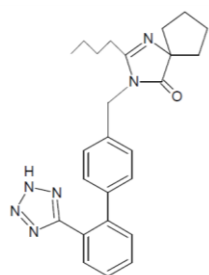


Fig.1: Irbesartan

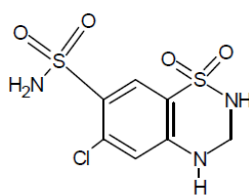


Fig.2: Hydrochlorobiazide

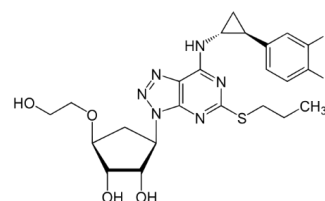


Fig.3: Ticagrelor

Several analytical methods have been reported for the determination of Irbesartan in pure drug and pharmaceutical dosage forms using spectrophotometry (Ramakrishna VS,2012) ,(Rani GT, S,2012), Extractive and non-extractive spectrophotometry (Anupama B,2012),(El-sutohy MM, 2013), spectrofluorometry (M.Farouk O ab.,2011,). In presence of hydrochlorothiazide, Irbesartan has been determined by direct UV-Spectroscopy using absorbance ratio method and simultaneous equation (Patel K.R.,2011) or using multicomponent mode of analysis(D. S, A. T, V. R, S. S, B. PK.,2010). Hydrochlorothiazide has been determined individually by spectrophotometry (Hapse SA,2012). In combination with many other drugs, Hydrochlorothiazide has been determined by spectrophotometric Chemometric analysis using Partial least Squares (PLS), Principal component Regression (PCR) (Sivasubramanian L,2015), absorbance subtraction (AS), amplitude modulation (AM) method and extended ratio subtraction (ERS) method (Khadiga M. K.,2015), Sequential Spectrophotometry (Darwish HW,2013). Hydrochlorothiazide was assayed in combination with

valsartan (Lakshmi KS,2011) form one side and Irbesartan and Telmisartan from the other side by the use of H-point standard addition(Sivasubramanian L,2014). Ticagrelor has been assayed by spectrophotometry (Pandya D,2016).

In this work, green analytical spectrophotometric chemometric assisted methods, are developed for the analysis of IRB, TICA and HCT in synthetic mixture. The suggested Chemometric assisted methods are able to resolve the overlapped spectra. The chemometric assisted methods were validated according to ICH guide lines (ICH,1996) . The results obtained were compared to a reference RP-HPLC method.

2. MATERIALS AND METHODS

2.1. Apparatus

The spectrophotometric measurements were carried out on a Jasco V-530 double beam UV-Vis Spectrophotometer connected to a computer loaded with Jasco UVPC software and HP Deskjet 5652 printer. The spectrophotometer is supported with Jasco Spectra Manager software for GULLIVER Ver. 1.53. The absorption spectra were recorded using 1 cm quartz cells.

2.2. Materials and Reagents

Ticagrelor (supplied by Omnipharma, Astrazenica, Lebanon), Irbesartan and Hydrochlorothiazide (from Algorithm, Lebanon) were used as working standards. Methanol (SIGMA-ALDRICH CHROMASOLV® FOR HPLC>99.9%).

2.3. Standard Stock Solutions

Standard solutions of TICA (1000 $\mu\text{g.mL}^{-1}$), IRB (100 $\mu\text{g.mL}^{-1}$) and HCT (400 $\mu\text{g.mL}^{-1}$), were prepared separately in methanol.

2.4. Working Standard Solutions

The standard stock solutions were separately diluted with methanol to prepare working standards having the concentration of 100 $\mu\text{g.mL}^{-1}$ TICA, 10 $\mu\text{g.mL}^{-1}$ IRB and 40 $\mu\text{g.mL}^{-1}$ HCT.

2.5. Calibration Graphs:

Into series of 10-mL measured flasks, volumes from working standard solutions of TICA, IRB or HCT were separately transferred and diluted with methanol to give the final concentrations stated in table 1.

2.6. Synthetic Mixtures for D1, D2, or DDRD:

Six validation synthetic mixtures were prepared by mixing appropriate volumes of the working standard solutions of TICA, IRB and HCT and diluting to volume with methanol. The combination of TICA, IRB and HCT are illustrated in table 5.

The absorbance values for each solution of the calibration graph and the prepared synthetic mixtures at 1-nm intervals in the wavelength range 200-300 nm, were recorded.

2.7. Synthetic Mixtures for HPSAM

Accurate volumes each of TICA, IRB and HCT working standard solutions were transferred into 10-ml volumetric flasks and diluted to the volume with methanol to prepare a synthetic mixture containing 25 $\mu\text{g.mL}^{-1}$ TICA, 8 $\mu\text{g.mL}^{-1}$ HCT and 1 $\mu\text{g.mL}^{-1}$ IRB. A portion of the solution was transferred into a quartz cell to measure its absorbance at appropriate wavelengths.

For IRB determination

To the above-prepared mixture, standard addition of 0-2 $\mu\text{g.mL}^{-1}$ IRB (at 0.5 $\mu\text{g.mL}^{-1}$ interval) is performed. For each of the prepared mixture the absorbance values were recorded at 214 and 228 nm.

For TICA determination

To the above-prepared mixture, standard addition of 0-20 $\mu\text{g.mL}^{-1}$ TICA (at 5 $\mu\text{g.mL}^{-1}$ interval) is performed. For each of the prepared mixture the absorbance values were recorded at 215 and 257 nm.

For HCT determination

To the above-prepared mixture, standard addition of 0-8 $\mu\text{g.mL}^{-1}$ HCT (at $2\mu\text{g.mL}^{-1}$ interval) is performed. For each of the prepared mixture the absorbance values were recorded at 205 and 215 nm.

Regression equations of the signals obtained (whether Absorbance values or the ratios calculated) versus the standard concentration of analyte added, are derived to quantify each of the studied drugs in their ternary mixture.

3. RESULTS AND DISCUSSION

Spectral characteristics: Quantitative spectrophotometry has been greatly improved by the use of novel chemometric methods since one of the main drawbacks of spectrophotometric methods for simultaneous determination of drug mixtures is the high degree of spectral overlapping. Fig.4. shows the extensive overlapping between spectra of TICA, IRB and HCT. The developed Chemometric assisted methods have the ability to quantify each of the drug in their ternary mixture.

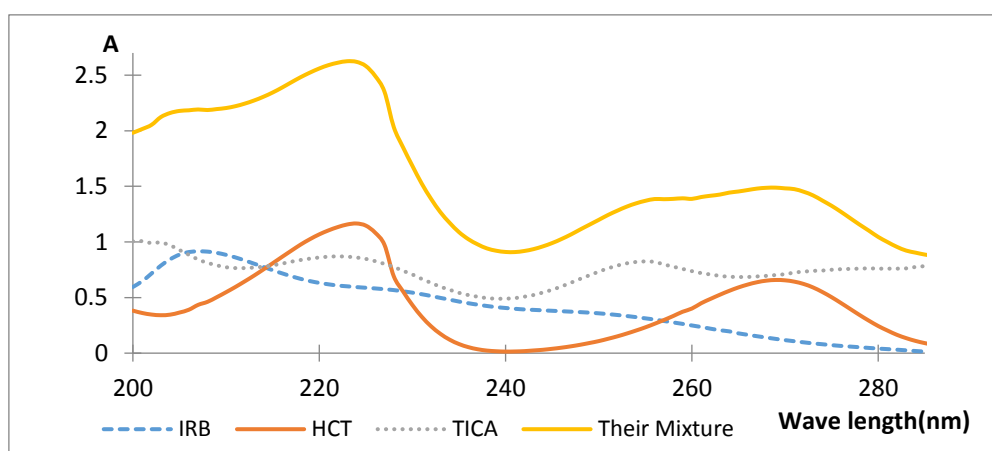


Fig.4: Absorption curves of 1 $\mu\text{g.mL}^{-1}$ IRB in methanol, 8 $\mu\text{g.mL}^{-1}$ of HCT, 25 $\mu\text{g.mL}^{-1}$ TICA in methanol and their mixture.

Different Chemometric methods including: H-point Standard Addition Method (HPSAM), Double Divisor Ratio Derivative (DDRD), First Derivative (D_1) and Second Derivative (D_2) were applied and validated.

3.1. First-Derivative Spectrophotometric Method (D1-method)

The D_1 spectra of TICA, IRB or HCT in presence of each other were derived at 4-nm intervals, over the wavelength range 200-300 nm. The D_1 value of TICA at 244 nm, IRB at 268 nm and HCT at 228 nm could be applied to determine the concentration of each drug in presence of the two other interfering drugs, where at the stated wavelength values, D_1 spectrum of interfering drugs exhibit a zero crossing.

3.2. Second-Derivative Spectrophotometric Method (D2-method)

The D_2 spectra of TICA, IRB or HCT in presence of each other were derived at 4-nm intervals, over the wavelength range 200-300 nm. The D_2 value of TICA at 248 nm, that of IRB at 212 nm and finally that of HCT at 228 nm could be applied to determine the concentration of each drug in presence of the two other interfering drugs, where at the stated wavelength values, D_2 spectrum of interfering drugs exhibit a zero crossing. Both D_1 and D_2 gave high percentage recoveries indicating their inability to resolve the studied mixture and to quantify each of TICA, IRB or HCT in presence of each other.

3.3. Double Divisor Ratio Derivative Method (DDRDM) (Rachana V.,2014)

This method depends on using signals of the double divisor ratio spectra and deriving their first derivative. So to calculate the signals of double divisor ratios: spectra of 25 $\mu\text{g.mL}^{-1}$ TICA were divided by that of a mixture of 2 $\mu\text{g.mL}^{-1}$ IRB + 6 $\mu\text{g.mL}^{-1}$ HCT, spectra of 3 $\mu\text{g.mL}^{-1}$ IRB were

divided by $8 \mu\text{g.mL}^{-1}$ HCT + $20 \mu\text{g.mL}^{-1}$. Spectra of $10 \mu\text{g.mL}^{-1}$ HCT were divided by $2 \mu\text{g.mL}^{-1}$ IRB + $20 \mu\text{g.mL}^{-1}$. The obtained signals were used to derive the DDRD spectra for TICA, IRB and HCT (Fig. 5, 6 and 7, respectively). The influence of different parameters was studied to optimize the signal of the derivative ratio spectra, i.e. to give good selectivity and high sensitivity in the determination. The influence of $\Delta\lambda$ was tested to obtain the optimum wavelength intervals. The $\Delta\lambda$ value affects both, shape and position of peaks of the analyzed compounds as well as the position of the zero-crossing points of interfering compounds in the synthetic mixtures. Divisor concentration is mainly reflected to sensitivity, while the effect of $\Delta\lambda$ is affecting the resolution and thereby increasing selectivity. Other $\Delta\lambda$ -values give poor resolution. The DDRD spectra were calculated at $\Delta\lambda = 4 \text{ nm}$. The concentration of TICA, IRB were determined by measuring the DDRD values at 244 nm for TICA (Fig. 5), at 268 nm for IRB (Fig. 6), whereas HCT concentration was determined at 248 nm (Fig. 7). Compared to D_1 and D_2 methods, better percentage recoveries were obtained indicating the ability of the DDRD method to correct the interferences and determining each of TICA, IRB and HCT in their ternary mixture.

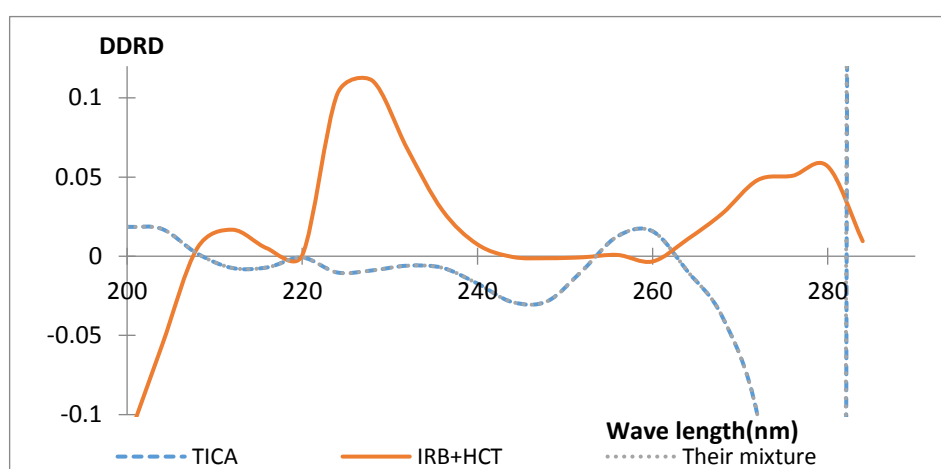


Fig.5: DDRD spectra of $25 \mu\text{g.mL}^{-1}$ TICA in methanol, $2 \mu\text{g.mL}^{-1}$ IRB + $6 \mu\text{g.mL}^{-1}$ HCT in methanol and their mixture.

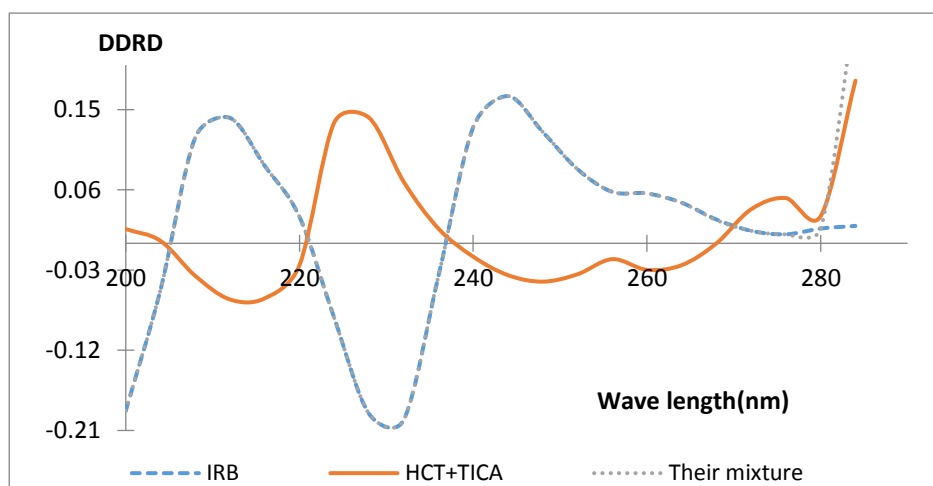


Fig.6: DDRD spectra of $3 \mu\text{g.mL}^{-1}$ IRB in methanol, $8 \mu\text{g.mL}^{-1}$ HCT + $20 \mu\text{g.mL}^{-1}$ TICA in methanol and their mixture.

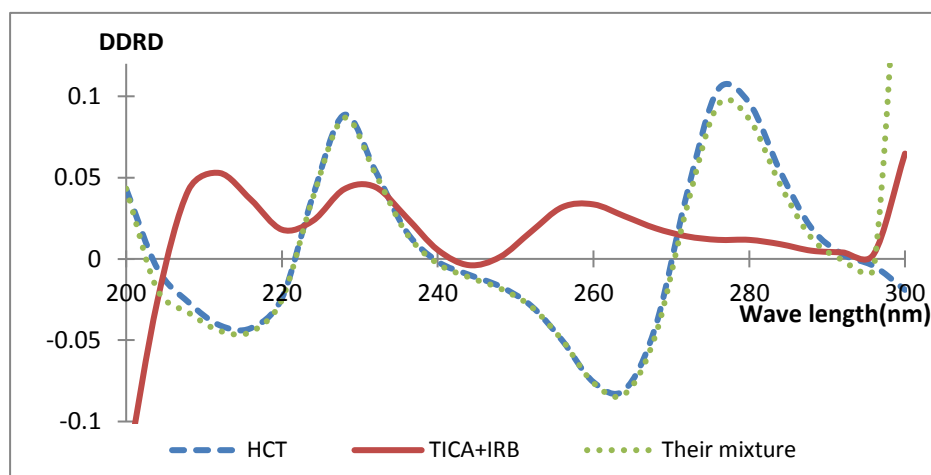


Fig.7: DDRD spectra of $10 \mu\text{g.mL}^{-1}$ HCT, $2 \mu\text{g.mL}^{-1}$ IRB + $20 \mu\text{g.mL}^{-1}$ TICA in methanol and their mixture.

3.4. H-point Standard Addition Method (HPSAM)

H-point standard addition method (HPSAM) is a simple two variable Chemometric technique. It permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. HPSAM allows the determination of the concentration of analyte in the presence of a direct interference and even the concentration of interference can be determined. The requirements for the application of HPSAM is the choice of two wavelengths (λ_1 and λ_2), where at λ_1 both analytes have the same analytical signal (Absorbance or any other related signal), however at λ_2 the value of the analytical signal, of the analyte, that will be determined differs significantly. By plotting the analytical signals versus added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H, S_H$). Where $-C_H$ is the unknown analyte concentration and S_H is the analytical signal due to the interferent species, from S_H can conclude the concentration of the other drug in the mixture. To demonstrate the analytical applicability HPSAM for the assay of the studied ternary mixture, the absorption spectra of pure solutions of $1 \mu\text{g.mL}^{-1}$ IRB, $8 \mu\text{g.mL}^{-1}$ HCT and $25 \mu\text{g.mL}^{-1}$ TICA were recorded separately between 200 and 300 nm. The spectral overlapping in Fig. 4, allows the selection of λ_1 and λ_2 that are necessary for the application of HPSAM. As shown in Fig. 4; the same absorbance value is exhibited by all the three analyte at 214 nm, and by HCT and TICA at 228 nm. The A value of IRB at 228 nm differs, allowing its determination using HPSAM in presence of TICA and HCT.

IRB determination: IRB is considered as analyte, HCT and TICA are considered as interferences. The A values of the ternary mixture ($1 \mu\text{g.mL}^{-1}$ IRB + $8 \mu\text{g.mL}^{-1}$ HCT + $25 \mu\text{g.mL}^{-1}$ TICA) after standard addition of IRB (0, 0.5, 1, 1.5 $\mu\text{g.mL}^{-1}$) are recorded at 214 and 228 nm. The obtained A values are treated mathematically and graphically to extract the concentration of IRB from the mixture.

By applying the mathematical method, regression equations (equations 7, 8 and 9) of absorbance values of the prepared mixtures (after standard addition of IRB) versus concentration of IRB added, were derived at 214 and 228 nm, and concentration of IRB could be calculated.

$$\text{At } 214 \text{ nm: } A_1 = a_1 + b_1C \dots\dots\dots (7)$$

$$\text{At } 228 \text{ nm: } A_2 = a_2 + b_2C \dots\dots\dots (8)$$

$$-C_{\text{IRB}} = (A_2 - A_1) / (b_1 - b_2); \dots\dots\dots (9); \quad C_{\text{IRB}} = 0.982 \mu\text{g.mL}^{-1}$$

$$\% \text{ RSD} = 98.2\%$$

Where,

$-C_{\text{IRB}}$ is the concentration of IRB in the original mixture

A_2, A_1 are the absorbance of the mixture at λ_1 and λ_2 before any addition.

b_1, b_2 are the slopes of the regression equations at 214 and 228 nm, respectively.

In Fig. 6, **the graphical method** is shown (H-point standard addition plot). By plotting the analytical signals (A values of the prepared mixtures after standard addition) versus the added analyte concentrations, at 214 and 228 nm, two straight lines are obtained that have a common point with coordinates H ($-C_H, S_H$). The coordination of the H-point at X-axis is equal to the concentration of IRB ($-C_H$) in the original ternary mixture before any addition, however the coordination of the H-

point on the Y-axis (S_H) is equal the absorbance of the interfering substances (TICA and HCT) in the ternary mixture. S_H (that refers to the summation of A values of TICA and HCT) could not be used to quantify TICA or HCT, individually, since the obtained signal is the summation of the absorbance values of both interfering substances.

To be able to quantify TICA or HCT, in their triple mixture, using HPSAM, the interfering effect of IRB should be cancelled, so that the obtained treated ratios will be equivalent to the concentration of TICA and HCT in the mixture. By dividing the absorption spectra of TICA, HCT and the studied mixture by the absorption spectra of IRB (Fig. 7), the interfering effect of IRB will be removed. HPSAM will then be applied on the ratio spectra and the concentration of the two other analyte (TICA and HCT) could be quantified. TICA and HCT were determined using two pairs of wavelength, where at each pair one drug is considered as the analyte and the other is considered as the interfering substance, and vice versa.

Determination of TICA: The spectra of the ternary mixtures ($1 \mu\text{g.mL}^{-1}$ IRB, $8 \mu\text{g.mL}^{-1}$ HCT and $25 \mu\text{g.mL}^{-1}$ TICA) after standard addition of 0, 5, 10, 15, 20 $\mu\text{g.mL}^{-1}$ of TICA are divided by the spectra $1 \mu\text{g.mL}^{-1}$ IRB. TICA (as analyte) is determined in presence of HCT at 215 and 257 nm where at those λ pair, both analyte exhibit the same ratio signal value, however at 257 nm the signal ratio value related to TICA differs significantly allowing its determination. The regression equations of the ratio signals of the mixtures (after standard addition of TICA) versus concentrations of TICA added at the two different λ s were calculated, and the regression lines were plotted. Concentration of TICA is then determined by two methods, mathematical (equations 10, 11 and 12) and graphical (Fig. 8), respectively.

$$\text{At 215 nm: } R_1 = 3.142 + 0.0424 C \dots\dots\dots (10)$$

$$\text{At 257 nm: } R_2 = 4.790 + 0.110 C \dots\dots\dots (11)$$

$$-C_{\text{TICA}} = (A_2 - A_1) / (b_1 - b_2); \dots\dots\dots (12); \quad C_{\text{TICA}} = 24.26 \mu\text{g.mL}^{-1}$$

$$\% \text{ RSD} = 97.44\%$$

Where, $-C_{\text{TICA}}$ is the concentration of TICA in the original mixture

R_1, R_2 are the ratio signals of the mixture at λ_1 and λ_2 before any addition.

b_1, b_2 are the slopes of the regression equations at 215 and 257 nm, respectively.

The ratio signal of HCT (considered as interference) could be calculated using equations 10 and 11, but S_H value refers to the ratio spectra signal of both (HCT+IRB).

So, $S_H = (A_{\text{HCT}}/A_{\text{IRB}}) + (A_{\text{IRB}}/A_{\text{IRB}})$; then $S_H = (A_{\text{HCT}}/A_{\text{IRB}}) + \text{cst}$; thus $(A_{\text{HCT}}/A_{\text{IRB}}) = S_H - \text{cst}$

$$\text{At 215 nm } \% \text{ RSD}_{\text{HCT}} = 97.6\%$$

$$\text{At 257 nm } \% \text{ RSD}_{\text{HCT}} = 97.82\%$$

Determination of HCT: The spectra of the ternary mixtures ($1 \mu\text{g.mL}^{-1}$ IRB, $8 \mu\text{g.mL}^{-1}$ HCT and $25 \mu\text{g.mL}^{-1}$ TICA) after standard addition of 0, 2, 4, 6, 8 $\mu\text{g.mL}^{-1}$ of HCT are divided by the spectra $1 \mu\text{g.mL}^{-1}$ IRB. HCT (as analyte) is determined in the presence of TICA at 205 and 215 nm (Fig. 7), where at 215 nm both analyte exhibit the same ratio signal value, however at 205 nm the signal ratio value related to HCT differs significantly allowing its determination. The regression equations of the ratio signals of the mixtures (after standard addition of HCT) versus concentrations of HCT added at the two different λ s were calculated, and the regression lines were plotted. The regression equations of the ratio signals of the mixtures (after standard addition of HCT) versus the concentrations of HCT added at the two different λ s were calculated, and the regression lines were plotted enabling the determination of the concentration of HCT by two methods, mathematical (equations 11 and 12) and graphical (Fig. 9), respectively.

$$\text{At 205 nm: } R_1 = 2.45 + 0.050C \dots\dots\dots (11)$$

$$\text{At 215 nm: } R_2 = 3.14 + 0.135C \dots\dots\dots (12)$$

$$-C_{\text{HCT}} = (A_2 - A_1) / (b_1 - b_2); \quad C_{\text{TICA}} = 8.14 \mu\text{g.mL}^{-1}$$

$$\% \text{ RSD} = 101.76\%$$

Where, $-C_{\text{HCT}}$ is the concentration of HCT in the original mixture

R_1, R_2 are the ratio signals of the mixture at λ_1 and λ_2 before any addition.

b_1, b_2 are the slopes of the regression equations at 205 and 215 nm, respectively.

The ratio signal of TICA (considered as interference) could be calculated using equations 11 and 12, but S_H value refers to the ratio spectra signal of both (TICA + IRB).

So, $S_H = (A_{\text{TICA}}/A_{\text{IRB}}) + (A_{\text{IRB}}/A_{\text{IRB}})$; then $S_H = (A_{\text{TICA}}/A_{\text{IRB}}) + \text{cst}$; thus $(A_{\text{TICA}}/A_{\text{IRB}}) = S_H - \text{cst}$

$$\text{At 205 nm } \% \text{ RSD}_{\text{TICA}} = 100.43 \%$$

$$\text{At 215 nm } \% \text{ RSD}_{\text{TICA}} = 99.43\%$$

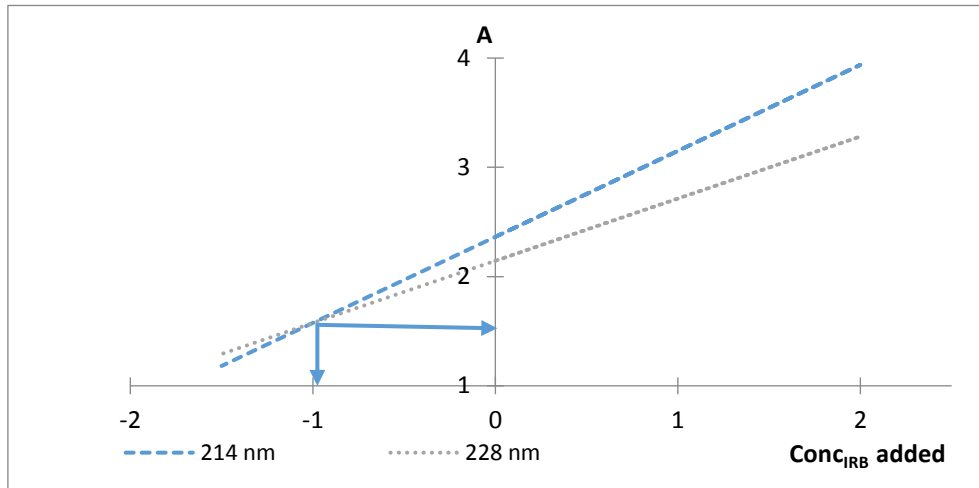


Fig. 8: H-point standard addition plot constructed at wavelength pair of 214 and 228 nm, for $1 \mu\text{g}\cdot\text{mL}^{-1}$ IRB, $8 \mu\text{g}\cdot\text{mL}^{-1}$ HCT and $25 \mu\text{g}\cdot\text{mL}^{-1}$ TICA after standard addition of 0, 0.5, 1, 1.5 $\mu\text{g}\cdot\text{mL}^{-1}$ of IRB.

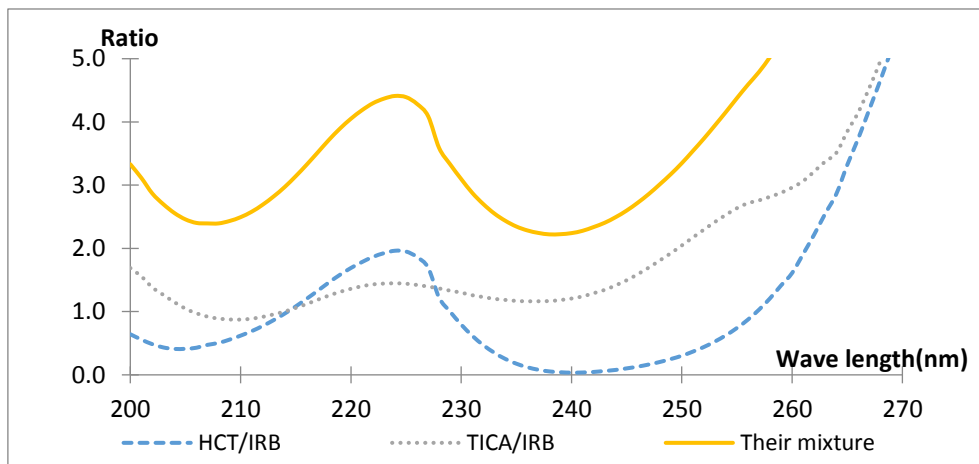


Fig. 7: The spectra of $8 \mu\text{g}\cdot\text{mL}^{-1}$ HCT, $25 \mu\text{g}\cdot\text{mL}^{-1}$ TICA and their mixture, divided by the spectra of $1 \mu\text{g}\cdot\text{mL}^{-1}$ IRB.

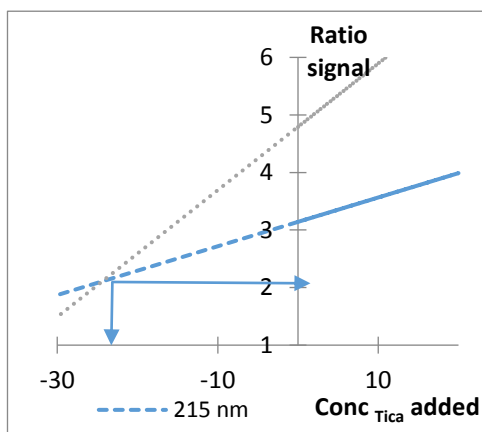


Fig.8: H-point standard addition plot constructed at wavelength pair of 215 and 257 nm, for $8 \mu\text{g}\cdot\text{mL}^{-1}$ HCT and $25 \mu\text{g}\cdot\text{mL}^{-1}$ TICA after standard addition of 0, 0.5, 1, 1.5 $\mu\text{g}\cdot\text{mL}^{-1}$ of TICA, over $1 \mu\text{g}\cdot\text{mL}^{-1}$ IRB.

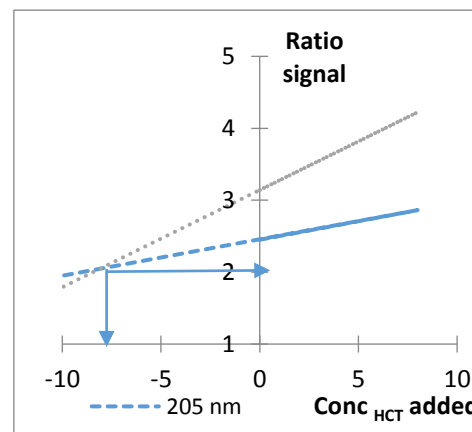


Fig. 9: H-point standard addition plot constructed at wavelength pair of 205 and 215 nm, For $8 \mu\text{g}\cdot\text{mL}^{-1}$ HCT and $25 \mu\text{g}\cdot\text{mL}^{-1}$ TICA after standard addition of 0, 2, 4, 6 and 8 $\mu\text{g}\cdot\text{mL}^{-1}$ of HCT, over $1 \mu\text{g}\cdot\text{mL}^{-1}$ IRB.

4. METHOD VALIDATION AND STATISTICAL ANALYSIS (Mileer JCM.,2005)

The proposed methods were validated according to ICH guidelines. The validation parameters included: linearity, limits of detection and quantification, accuracy, precision and specificity. The applied analytical methods are able to quantify the studied drugs in their triple mixture.

4.1. Linearity and Concentration Ranges

Under the described experimental conditions, the graphs obtained by plotting the signals of the proposed methods versus concentration for TICA, IRB and HCT gave linear relationships over the concentration ranges stated in table 1. Linearity data and statistical parameters for the proposed methods were calculated, including linear regression equation parameters (intercepts, slopes, correlation coefficients, standard deviation of intercept and standard deviation of the slope (Table 1. Regression analysis confirmed good linearity as shown from correlation coefficient value ($r > 0.999$). The high F-value proved that the linear correlation between calculated signals (Chemometric methods) and concentrations is significant to a high level of confidence. The high values of the correlation coefficient (r) with negligible intercepts indicate the good linearity of the calibration graphs. Standard deviation of residuals ($S_{y/x}$), of intercept (S_a) and of slope (S_b) are presented for each drug. ($S_{y/x}$) is a measure of the extent of deviation of the found (measured) y -values from the calculated ones. Also, the small degree of scatter of the experimental data point around the line of regressions was confirmed by the small values of the variances around the slopes (S_b^2). The variance test for the regression lines revealed that, for equal degrees of freedom, the increase in the variance ratio (F-values) means an increase in the mean squares due to regression and a decrease in the mean squares due to residuals, (i.e. the less is the scatter of experimental points around the regression line). Consequently, regression lines with high F-values (low significance F) are much better than those with lower ones. Good regression lines show high values for both r and F statistical parameters (P. and Berry G., 2001).

4.2. Limit of Detection (LOD) and Limit of Qualification (LOQ)

Limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines. LOD was defined as $10 S_a/b$, where S_a is the standard deviation of the intercept and b is the slope of the calibration curve. The sensitivity of the proposed methods can be confirmed by the low LOD and LOQ values obtained in tables 1.

Table 1: statistical analysis of the regression equations of the applied Chemometric assisted method

Parameters	TICA		IRB		HCT	
	DDRD	HPSA	DDRD	HPSAM	DDRD	HPSAM
Conc. Range ($\mu\text{g.mL}^{-1}$)	10-30	25 ^a	1-3	1 ^b	4-10	8 ^c
λ or λ range (nm)	244	257	268	214	252	215
$\Delta\lambda$ (nm)	4	—	4	—	4	—
r	0.9994	0.9995	0.9999	0.9999	0.9996	0.9994
$S_{y/x}$	3.00×10^{-9}	1.73×10^{-7}	5.24×10^{-7}	1.60×10^{-6}	1.53×10^{-35}	1.17×10^{-15}
F	528×10^3	52×10^6	43096	2904768	2.66×10^{32}	1.88×10^5
Significance F	5.74×10^{-9}	5.74×10^{-12}	2.46×10^{-7}	4.45×10^{-10}	5.07×10^{-49}	2.71×10^{-12}
a (intercept)	0.14×10^{-3}	4.79	0.61×10^{-3}	2.36	-6.9×10^{-18}	3.14
b (slope)	-0.14×10^{-2}	0.11	0.54×10^{-1}	0.78	5.82×10^{-2}	0.13
S_a	0.64×10^{-3}	0.18×10^{-3}	0.561×10^{-3}	0.56×10^{-3}	2.37×10^{-18}	1.53×10^{-11}
S_b	2.00×10^{-5}	1.52×10^{-5}	0.26×10^{-3}	0.46×10^{-3}	3.57×10^{-19}	3.12×10^{-10}
LOD ($\mu\text{g/mL}$)	-0.87×10^{-2}	0.51×10^{-2}	0.31×10^{-1}	0.21×10^{-2}	1.22×10^{-15}	3.39×10^{-5}
LOQ ($\mu\text{g/mL}$)	-0.29×10^{-1}	0.16×10^{-1}	0.10	0.72×10^{-2}	4.06×10^{-15}	1.13×10^{-2}
a/S_a	3.29	25761	1.08	4177	-2.93	2.05×10^1
$(S_b)^2$	4.00×10^{-10}	2.31×10^{-10}	6.99×10^{-8}	2.13×10^{-7}	1.27×10^{-37}	9.76×10^{-4}
S_b %	4.00×10^{-8}	1.25×10^{-3}	6.99×10^{-6}	0.46×10^{-1}	3.57×10^{-17}	3.12×10^{-8}

^a25 $\mu\text{g.mL}^{-1}$ TICA with standard addition of 0 - 20 $\mu\text{g.mL}^{-1}$ TICA

^b1 $\mu\text{g.mL}^{-1}$ IRB with standard addition of 0 - 2 $\mu\text{g.mL}^{-1}$ IRB

^c8 $\mu\text{g.mL}^{-1}$ HCT with standard addition of 0 - 8 $\mu\text{g.mL}^{-1}$ HCT

4.3. Accuracy and Precision

The applicability of the developed methods was tested by the analysis of TICA, IRB and HCT in several synthetic mixtures of different proportions. Good accuracy, expressed as percentage recovery and high precision, expressed as percentage RSD were obtained. The results, summarized in tables 2, 3, 4, 5 and 6 show that the % recovery values do not exceed the accepted limits, which demonstrate the accuracy and repeatability of the developed methods.

Table 2: Accuracy for the simultaneous determination of IRB in presence of HCT and TICA in laboratory-made mixtures using the proposed HPSA method.

IRB added $\mu\text{g.mL}^{-1}$	IRB:HCT:TICA $\mu\text{g.mL}^{-1}$	IRB found $\mu\text{g.mL}^{-1}$	IRB % Recovery at 214 & 228 nm
0, 0.5, 0.6, 0.7, 0.8	0.5: 4: 12.5	0.5	100.00
0, 0.4, 0.5, 0.6, 0.7	0.6: 4.8: 15	0.59	98.41
0, 0.6, 0.7, 0.8, 0.9	0.7: 5.6: 17.5	0.713	101.87
0, 0.6, 0.7, 0.8, 0.9	0.9: 7.2: 22.5	0.905	100.66
0, 0.5, 1, 1.5, 2	1: 8: 25	0.986	98.62

Table 3: Accuracy for the simultaneous determination of HCT or TICA in laboratory-made mixtures using the proposed HPSA method.

HCT added $\mu\text{g.mL}^{-1}$	IRB:HCT:TIC A $\mu\text{g.mL}^{-1}$	HCT found $\mu\text{g.mL}^{-1}$	HCT % Recovery at 205 & 215 nm	TICA % Recovery at 205 nm	TICA % Recovery at 215 nm
0, 1, 2, 3, 4	0.5: 4: 12.5	0.5	100.00	99.02	97.88
0, 2, 3, 4, 5	0.6: 4.8: 15	0.59	98.41	99.33	79.85
0, 3, 4, 5, 6	0.9: 7.2: 22.5	0.713	101.87	99.04	97.97
0, 4, 5, 6, 7	0.9: 7.2: 22.5	0.905	100.66	99.11	97.91
0, 2, 4, 6, 8	1: 8: 25	8.14	101.75	99.85	98.31

Table 4: Accuracy for the simultaneous determination of TICA or HCT in laboratory-made mixtures using the proposed HPSA method.

TICA added $\mu\text{g.mL}^{-1}$	IRB:HCT:TICA $\mu\text{g.mL}^{-1}$	TICA found $\mu\text{g.mL}^{-1}$	TICA % Recovery at 215 & 257 nm	HCT % Recovery at 215 nm	HCT % Recovery at 257 nm
0, 4, 8, 12, 16	0.5: 4: 12.5	12.36	98.91	103.28	106.56
0, 8, 12, 16, 20	0.6: 4.8: 15	14.85	98.99	100.97	102.57
0, 12, 16, 18, 22	0.9: 7.2: 22.5	17.40	99.55	106.03	102.03
0, 18, 20, 22, 24	0.9: 7.2: 22.5	22.5	100.06	102.08	104.90
0, 5, 10, 15, 20	1: 8: 25	20	96.94	102.77	109.00

Table 5: Assay results for the determination of TICA in presence of IRB and HCT in synthetic mixtures using the **D₁**, **D₂** and **DDRD** methods.

TICA: IRB:HCT µg.mL ⁻¹	Mean Recovery ± SD ^a RSD % ^b Er % ^c		
	D₁	D₂	DDRD
10:1:10	93.00 ± 0.92 0.98 -7.00	90.53 ± 1.59 1.75 -9.47	100.00 ± 0.86 0.86 0.00
15:3:6	92.36 ± 1.25 1.35 -7.64	95.83 ± 0.56 0.58 -4.17	100.79 ± 0.34 0.33 0.79
20:1:4	106.98 ± 1.02 0.95 6.98	94.44 ± 1.37 1.45 5.60	100.91 ± 1.78 1.76 0.91
20:3:8	94.18 ± 1.78 1.88 -5.82	96.38 ± 0.89 0.92 -3.62	96.18 ± 0.69 0.71 -3.82
20:2.5:6	105.37 ± 0.78 0.74 5.37	97.00 ± 0.54 0.55 -3.00	96.62 ± 0.73 0.75 -3.38
30:3:10	106.10 ± 0.97 0.91 6.10	103.05 ± 0.86 0.83 3.05	101.24 ± 0.99 0.97 1.24

^a Mean ± SD for the three determinations^b % Relative standard deviation^c % Relative error

Table 6: Intra-day and inter-day precision for the simultaneous determination of TICA, IRB and HCT in laboratory-made mixtures using the proposed chemo-metric assisted methods.

Analytical Method	HCT:IR B:TICA µg.mL ⁻¹	Intra-day precision Mean Recovery ± SD ^a RSD % ^b Er % ^c			Inter-day precision Mean Recovery ± SD ^a RSD % ^b Er % ^c		
		TICA	IRB	HCT	TICA	IRB	HCT
DDRD	10: 3: 30	100.26 ± 0.15 0.15 0.26	100.59 ± 0.22 0.22 0.59	100.82 ± 0.47 0.47 0.82	99.6 ± 0.42 0.42 -0.40	100.32 ± 0.24 0.24 0.32	99.59 ± 0.37 0.37 -0.41
HPSAM	1: 8: 25	100.26 ± 0.15 0.15 0.26	100.59 ± 0.22 0.22 0.59	99.6 ± 0.42 0.42 -0.40	100.82 ± 0.47 0.47 0.82	99.59 ± 0.37 0.37 -0.41	99.59 ± 0.37 0.37 -0.41

^a Mean ± SD for the five determinations^b % Relative standard deviation^c % Relative error

4.4. Laboratory made mixture

Table 7 present a statistical comparison between the proposed methods and a reference RP-HPLC(Jamal MK El,2016) method for the assay of TICA, IRB and HCT in laboratory made mixture by using

the student's t-test and the variance ratio F-test. Since the calculated t- and F- values for each drug did not exceed the theoretical ones, this indicated that there was no significant difference between the reference HPLC and each of the proposed chemometric methods for the analysis of the investigated mixture in laboratory-made mixtures.

Table 7: Assay results for TICA, IRB and HCT in their laboratory made mixture using the proposed chemometric assisted methods

Ratio TICA+IRB+HCT $\mu\text{g}\cdot\text{mL}^{-1}$ 9:1.5:0.5	Mean Recovery \pm SD ^a RSD % ^b Er % ^c		
	HPLC(23)	DDDR	HPSAM
TICA	98.76 \pm 0.45	99.13 \pm 1.05	98.99 \pm 0.87
Mean Recovery \pm SD ^a	0.45	1.05	0.87
RSD % ^b	-1.01	-0.85	-1.01
Er % ^c			
**t-test	—	0.72	0.54
**F-test	—	5.44	3.73
IRB	98.99 \pm 0.87	99.42 \pm 0.40	98.76 \pm 0.87
Mean Recovery \pm SD ^a	0.87	0.41	0.88
RSD % ^b	-1.01	-0.58	-1.24
Er % ^c			
**t-test	—	1.01	0.74
**F-test	—	0.21	1.00
HCT	99.08 \pm 0.61	99.18 \pm 0.75	99.71 \pm 0.67
Mean Recovery \pm SD ^a	0.61	0.75	0.67
RSD % ^b	-0.92	-0.82	-0.29
Er % ^c			
**t-test	—	0.24	1.54
**F-test	—	1.51	1.21

^a Mean \pm SD for the five determinations

^b% Relative standard deviation

^c% Relative error

**Theoretical values of t- and F- at P = 0.05 are 2.13 and 6.93, respectively.

5. CONCLUSION

For analytical purposes, it is always important to establish methods capable of analysing co administered drugs in short period of time with acceptable accuracy and precision. The Chemometric assisted methods are simple, inexpensive and require easy treatment of the samples. This work demonstrates the ability and advantages of HPSAM and DDRD, chemometric assisted methods, to resolve the ternary co-administered mixture and individually quantify each of TICA, IRB and HCT. HPSAM and DDRD could be well adopted since it gave satisfactory results in synthetic mixtures, and proved to be superior when compared to first and second derivative methods. The methods offers good selectivity, accuracy and precision that can be applied for different concentration ratios.

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REFERENCES

- Anupama, B., Ramakrishna, V.(2012) Assay of Irbesartan by extractive spectrophotometry. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 2(4),529-531.
- Armitage, P., Berry, G., & Matthews, J. N. S. (2008). *Statistical methods in medical research*. John Wiley & Sons.
- Charles, L., Lora, A., Morton, G., and Leonard, L.(2005). *Drug Information Handbook*. Lexi-Comp Inc.
- Deka, S., Patel, Meghna., P., Shah, N., Vivek, S., & Saralai, M. G. (2012). Review on Simultaneous Determination of Antihypertensive Analytes in Pharmaceutical Dosage Form by Different Analytical Methods. *International Journal*, 2(2),876-879.
- Darwish, H. W., Hassan, S. A., Salem, M. Y., & El-Zeany, B. A. (2013). Sequential spectrophotometric method for the simultaneous determination of amlodipine, valsartan, and hydrochlorothiazide in coformulated tablets. *International journal of spectroscopy*, 1,1-8.
- El-Sutohy, M. M., El-Shaboury, S. R., Hussein, S. A., & Mohamed, N. A. (2013). Validated Non-Extractive Spectrophotometric Methods for Determination of Some Angiotensin II Receptor Antagonists. *Asian Journal of Pharmaceutical Analysis*, 3(1), 3-8.
- El Jamal, MK., Gazy, AA.(2016) Analysis Of Three Cardiovascular Drugs in Their Ternary Mixture Using Green Analytical Methodology of Smart Spectrophotometric Methods and RP-HPLC Method. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(8),243-250
- Farouk, M., EL-Aziz, O. A., Hemdanb, A., & Shehata, M. (2011). Simple novel spectrophotometric and spectrofluorimetric methods for determination of some anti-hypertensive drugs. *Journal of American Science*, 7(1), 300-312.
- Hapse, S. A., Wagh, V. S., Kadaskar, P. T., Dokhe, M. D., & Shirsath, A. S. (2012). Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. *Der Pharma Chemica*, 4(1), 10-14.
- ICH Harmonized Tripartite guideline.(1996). Validation of Analytical Procedures text and Methodology.
- Khadiga M Kelani, Abdallah A Shalaby, Magda Y Elmaamly MKH.(2015) Spectrophotometric and Chemometric Methods for Simultaneous Determination of Two Anti-Hypertensive Drugs in their Combined Dosage Form. *Pharmaceutica Analytica Acta*.6(1),2153–435.
- Lakshmi, K., & Lakshmi, S. (2011). Simultaneous spectrophotometric determination of valsartan and hydrochlorothiazide by H-point standard addition method and partial least squares regression. *Acta Pharmaceutica*, 61(1), 37-50.
- Miller, J.N.,(2005) *Statistics and Chemometrics for Analytical Chemistry*. 5th ed. London, Pearson Prentice Hall.
- O'Neil, M. J., Smith, A., Heckelman, P. E., & Budavari, S. (2001). The Merck Index-An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: *Merck and Co. Inc*, 767, 4342.
- Patel, K.R., Patel, S.A., Darji, V.C, SRN.(2011). Simultaneous Spectrophotometric estimation of Irbesartan and Hydrochlorothiazide in tablets. *International Research Journal of Pharmacy*, 2(3),202–207.
- Pandya, D., Patel, M., Ghediya, R., Shah, A., & Khunt, R. (2016). UV-Vis spectrophotometric assay determination of oral antiplatelet ticagrelor drug in pharmaceutical formulation: Application to content uniformity. *Journal of Chemical and Pharmaceutical Research*, 8(1), 316-321.
- Ramakrishna, S., SudhaLakshmi, P. B., & Rambabu, C. (2012). Visible Spectrophotometric Methods for The Determination Of Irbesartan In Pharmaceutical Formulations. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3), 86-87.
- Rani, GT., Shankar, DG., Satyanarayana, B.(2012) *Academi c*.4(1),1–5.
- Rachana, V., Gohel, Shraddha J., Parmar, B.A.P.(2014). Development and Validation of

Double Divisor-Ratio Spectra Derivative Spectrophotometric Method for Simultaneous estimation of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorothiazide in Tablet Dosage Form. *IntJResAyurveda Pharm.*5(4),523–530.

- Siragy, H. (1999). Angiotensin II receptor blockers: review of the binding characteristics. *The American journal of cardiology*, 84(10), 3-8.
- Sivasubramanian, L., & Ks, L. (2015). Spectrophotometric multicomponent analysis of telmisartan, hydrochlorothiazide and ramipril in pharmaceutical formulations by chemometric techniques. *World journal of pharmacy and pharmaceutical sciences*, 4(3), 536-550.
- Sivasubramanian, L., & Lakshmi, K. S. (2014). H-point standard addition method for simultaneous spectrophotometric determination of irbesartan, hydrochlorothiazide and telmisartan in tablets. *International journal of research in pharmacy and chemistry*, 4(2), 373-380.
- Umesh R. Desai, Ph.D (2004) Cardiovascular drugs, VCU, Richmond.