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Original Article

ANALYSIS OF THREE CARDIOVASCULAR DRUGS IN THEIR TERNARY MIXTURE USING GREEN ANALYTICAL METHODOLOGY OF SMART SPECTROPHOTOMETRIC METHODS AND RP-HPLC METHOD

MARWA K. EL JAMAL*, AZZA A. GAZY

Department of Pharmaceutical Technology, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon
Email: marwa.jamal@bau.edu.lb

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ABSTRACT

Objective: Development and validation of two new, simple and specific analytical techniques for the simultaneous determination of a ternary mixture of co-administered cardiovascular drugs ticagrelor (TICA), irbesartan (IRB) and hydrochlorothiazide (HCT).

Methods: Chemometric assisted UV spectrophotometric methods (considered as green analytical chemistry) and RP-HPLC method were developed and validated. The different applied chemometric methods are based on Fourier function convolution (FF) and Double divisor ratio spectra Fourier function (FFR). As for the HPLC method, it was developed on a C_{18} Sunfire© waters column with a mobile phase composed of Acetonitrile: sodium dihydrogen phosphate (adjusted to pH 3.6 using orthophosphoric acid) in the ratio of 60:40 v/v at a flow rate of 1 ml/min. Quantification was based on measuring peak areas at 230 nm.

Results: By applying the chemometric assisted methods, good percentage recoveries were obtained in quantifying each of the drugs in their triple mixture. The RP-HPLC method, peaks were well resolved with retention times 4.6, 8.0 and 10.0 min, for HCT, TICA and IRB, respectively. The established method was also applied in samples spiked with plasma; the peaks were well separated from each other and from plasma protein peaks, proving the ability of the HPLC method to be applied in biological samples. The methods (RP-HPLC and chemometric assisted methods) were validated in terms of linearity, LOD, LOQ, precision and accuracy. The results obtained from the analysis of the co-administered mixture by the proposed chemometric assisted methods were statistically compared to those obtained by the applied RP-HPLC method.

Conclusion: The applied RP-HPLC methods were proved to be more sensitive when compared to the applied chemometric methods, thus could be used for determination of drug concentration in human plasma. However, the applied chemometric methods, considered as green analytical chemistry, is a simple, time-saving method that requires minimal use of a hazardous solvent.

Keywords: Fourier Function, Double divisor Fourier function ratio spectra, Spectrophotometry, RP-HPLC, Spiked plasma

INTRODUCTION

Combination therapy (or polytherapy) is a broad term for the use of multiple medications, in order to fight a particular condition; It is a therapeutic intervention in which more than one pill (each containing separate drug) are administered to the patient.

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. A dose combination therapy for the treatment of blood pressure, diuretic and antiplatelet treatments has been proposed as one strategy to reduce the global burden of CVD by remarkable percentage. So, developing a method for the estimation of a mixture of cardiovascular co-administered drugs would be of great importance [1].

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 the environment. Therefore, chemometrics addresses 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 software on the primary data generated in the lab work. After some recent years, chemometrics has become one of the mathematical and statistical techniques for the resolution of overlapping spectra of multi-component mixtures, which is difficult to separate using the traditional spectroscopic method as well as it has a wide application in different disciplines [1].

Irbesartan (IRB), chemically described as 2-butyl-3-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]-methyl1-3-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 [2].

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 the distal convoluted tubule. Because of their synergistic antihypertensive action, irbesartan and hydro-chlorothiazide are available in the market as a combined dosage form [2].

Ticagrelor (TICA), chemically described as (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3*H*-[1,2,3]-tria zolo [4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxycyclo pentane-1,2-diol2, (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 cardiovascular thrombotic events in patients with the 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 [2].

Several analytical methods have been reported for the determination of irbesartan in pure drug, pharmaceutical dosage forms using spectrophotometry [3], RP-HPLC [4]. Also, irbesartan has been determined in human plasma by LC [5]. In the presence of hydrochlorothiazide, irbesartan has been determined by UV-Spectroscopy [6], RP-HPLC [7, 8], HPLC by the aid of chemometry [9]. Hydrochlorothiazide and irbesartan are simultaneously determined by LC-MS in human plasma [10], or in spiked human plasma using microemulsion-LC [11]. Irbesartan, in combination with other drugs, has been determined by RP-HPLC [12], HPTLC [13], LC-MS [13].

Hydrochlorothiazide has been determined individually by spectrophotometry [14], HPLC [15]. In combination with many other drugs, hydrochlorothiazide has been determined by HPLC [8, 16, 13], spectrophotometric chemometric analysis [17, 18], HPTLC with UV-absorption densiometry [19].

Ticagrelor has been assayed by spectrophotometry [20], HPLC [21]. In plasma sample, ticagrelor has been determined by LC-MS [22], LC-MS-MS [23] and UPLC-MS [24].

To the best of our knowledge, neither of the already published papers has been related to any chemometric nor RP-HPLC method for simultaneous determination of HCT, TICA and IRB. So the present study emphasizes on the determination of HCT, TICA and IRB using different chemometric methods that will be compared to a RP-HPLC method. The developed methods will be validated according to ICH guidelines [25].

MATERIALS AND METHODS

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 absorption spectra were measured using 1-cm quartz cells. For DDRD, FF and FFR, the absorption spectra were recorded using 1 cm quartz cells.

HPLC system (waters 1525) is composed of binary HPLC pump, connected to water 2487 dual wavelength absorbance detector. The liquid chromatographic system is equipped with waters 717 plus autosampler. Liquid separations are performed on RPC Sunfire © C18 analytical column (250 x 4.6 mm x 5µm) at ambient temperature. The mobile phase used was degassed and filtered by passing through 0.5 µm pore size membrane filter at a pumping speed of 30L/min (Glassco diaphragm Vacuuum pump). The samples were also filtered using PTFE 0.2 µm Minisart SRP 15 (Sartorius Stedim) disposable filters.

Chromatographic conditions

The mobile phase was prepared by mixing potassium dihydrogen phosphate buffer (adjusted to pH 3.6 with orthophosphoric acid) and acetonitrile in a ratio (40:60) by volume.

Materials and reagents

Ticagrelor (from supplied by Omnipharma, Astrazenica, Lebanon), irbesartan and hydrochlorothiazide (from Algorithm, Lebanon) were used as working standards. Acetonitrile (SIGMA-ALDRICH CHROMASOLV®FOR HPLC>99.9%), KH_2PO_4 (Merck KGaA), Na_2HPO_4 (BDH), human plasma was supplied by Dar Al Ajaza, Lebanon.

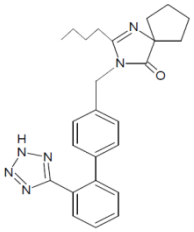

Fig. 1: Irbesartan

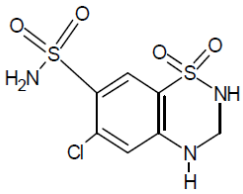

Fig. 2: Hydrochlorothiazide

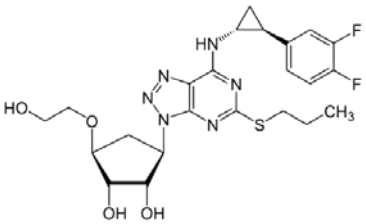

Fig. 3: Ticagrelor

Chemometric assisted methods

Standard stock solutions

Standard solutions of TICA (1000 µg/ml, IRB (100 µg/ml) and HCT (400 µg/ml), were prepared separately in methanol.

Working standard solutions

Portions of the above stock solutions were separately diluted with methanol to attain the concentration of 100 µg/ml TICA, 10 µg/ml IRB and 40 µg/ml HCT.

Calibration graphs

Accurate volumes of the working standard solutions of TICA, IRB or HCT into separate sets of 10-mL calibrated flasks were transferred and completed to volume with methanol to obtain the concentration ranges stated in table 2. Absorbance values were recorded at λ range of 200-300 nm for each drug, at 1 nm interval, against methanol as blank.

Synthetic mixtures

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 4. The absorbance values for each solution at 1-nm intervals in the wavelength range 200-300 nm, were recorded.

HPLC method

Standard stock solutions

Standard solutions of TICA (100 µg/ml), IRB (100 µg/ml) and HCT (100 µg/ml), were prepared in HPLC-grade methanol.

Working standard solutions

Portions of the above stock solutions were separately diluted with methanol to attain the concentration range of 10 µg/ml TICA, 1 µg/ml IRB and 10 µg/ml HCT.

Calibration graph

Pure drugs

Into series of 10-mL measured flasks, volumes from working solutions of TICA, IRB and HCT were transferred and diluted with methanol to give the final concentrations stated in table 3. The above solutions were filtered using 0.2 µm disposable filters. 20µL portions of the working solutions of TICA, IRB and HCT were injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak area values of each drug were plotted against the corresponding concentrations to obtain the calibration graph for each drug. The concentrations of TICA, IRB and HCT from the corresponding calibration graphs were computed.

Spiked plasma

To calculated volumes of the working solutions of TICA, IRB and HCT, 100 µL of human plasma and 100 µL of acetonitrile were added (to deproteinize biological sample), to produce a final concentration in the range stated in table 3. After vortex mixing and centrifugation (6000 rpm/min), the supernatant solutions were filtered using 0.2 µm disposable filters transferred to separate Eppendorf tubes and 20 uL of each were injected into the HPLC column. The peak areas for each drug were measured and the corresponding concentrations in the mixtures were derived referring to calibration graph.

Synthetic mixtures

Pure drugs

Accurate volumes of working standard solutions of TICA, IRB and HCT were transferred into a four separate 10-mL calibrated flasks and diluted to the mark with methanol to give synthetic mixtures containing IRB, HCT and TICA in the ratios stated in table 5. The solutions were filtered using 0.2 µm disposable filters. Volumes of 20 µL portions of the mixture solutions were injected in triplicates and chromatographed under the chromatographic conditions

mentioned above. The peak areas for each drug were measured and the corresponding concentrations in the mixtures were derived referring to calibration graph.

Spiked plasma

Accurate volumes (from the supernatant layer obtained from centrifugation) of each drug were pipetted into Eppendorf tubes to give synthetic mixtures containing TICA, IRB and HCT in the ratios stated in table 5. Volumes of 20 µL portions of the mixture solutions were injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak areas for each drug were measured and the corresponding concentrations in the mixtures were derived referring to calibration graph.

RESULTS

Chemometric assisted methods

Spectral characteristics

Fig. 4 represents the absorption spectra of TICA, IRB and HCT and their mixtures, recorded over the range of 200-300 nm against methanol.

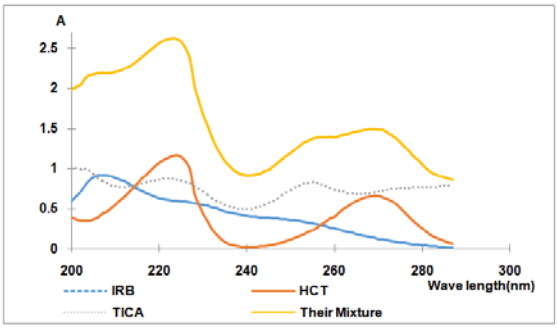

Fig. 4: Absorption curves of 1 µg/ml IRB in methanol, 8 µg/ml of HCT, 25 µg/ml TICA in methanol and their mixture

Two chemometric methods, FF (Trigonometric Fourier function) and hybrid double divisor ratio spectra **(**FFR) were applied and validated.

TICA was determined in presence of IRB and HCT by applying FF and FFR. The following equations were applied for FF (eq. 1) and FFR (eq. 2), respectively:

$$t'_j = [(1.707)\ A_{0+}(0.707)\ A_1+(-0.707)\ A_2+(-1.707)\ A_3+(-1.707)\ A_4+(-0.707)\ A_5+(0.707)\ A_{6+}(1.707)\ A_7]/4 \ldots\ldots (1)$$

Where A_0–A_7 stand for eight absorbance values at 4-nm intervals for 20 µg/ml TICA, 2 µg/ml IRB+6 µg/ml HCT (that is the double divisor) and their mixtures within the range (240-268 nm). The number in brackets are the selected combined Fourier functions and T′= [cos x_i+cos (x_i+45 °)] (fig. 5).

$$t'_j = [(-0.866)\ FFR_{0+}(0)\ FFR_1+(0.866)\ FFR_2+(0.866)\ FFR_3+(0)\ FFR_4-(0.866)FFR_5]/3 \ldots. (2)$$

Where R_0–R_5 stand for six ratio values at 4-nm intervals for 20 µg/ml TICA, 2 µg/ml IRB+6 µg/ml HCT (that is the double divisor) and their mixtures within the range (244-264 nm). The number in brackets are the selected combined Fourier functions and T′= [sin x_i-sin (x_i+60 °)] (fig. 6).

IRB was also determined in the presence of TICA and HCT by applying FF and FFR. The following equations were applied for FF and FFR, (eq. 3, 4) respectively:

$$t'_j = [(-0.866)\ A_{0+}(1.732)\ A_1-(00866)\ A_2-(0.866)\ A_3+(1.732)\ A_4-(0.866)\ A_5]/4 \ldots\ldots\ldots (3).$$

Where A_0–A_5 stand for six absorbance values at 4-nm intervals for 3 µg/ml IRB, 8 µg/mlTICA+20 µg/ml HCT and their mixtures within the range (204-224 nm). The number in brackets are the selected combined Fourier functions and T′= [sinx_i-sin (2x_i+60 °)] (fig. 7).

$$t'_j = [(-0.866)\ FFR_{0+}(0)\ FFR_1+(0.866)\ FFR_2+(0.866)\ FFR_3+(0)\ FFR_4-(0.866)\ FFR_5]/3 \ldots\ldots (4)$$

Where R_0–R_5 stand for six ratio values at 4-nm intervals for 3 µg/ml IRB, 8 µg/ml TICA+20 µg/ml HCT (that is the double divisor) and their mixtures within the range (240-260 nm). The number in brackets are the selected combined Fourier functions and T′= [sin x_i-sin (x_i+60 °)] (fig. 8).

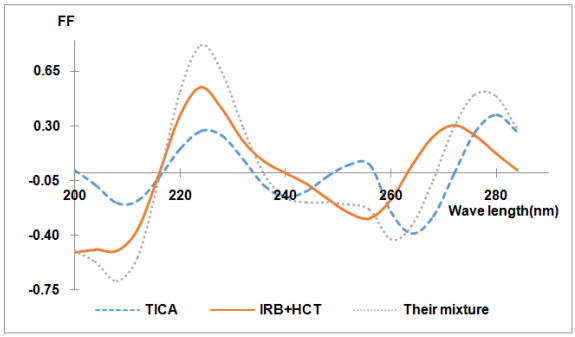

Fig. 5: Fourier functions convoluted curves for 25 µg/ml TICA in methanol, 2 µg/ml IRB+6 µg/ml HCT in methanol and their mixture

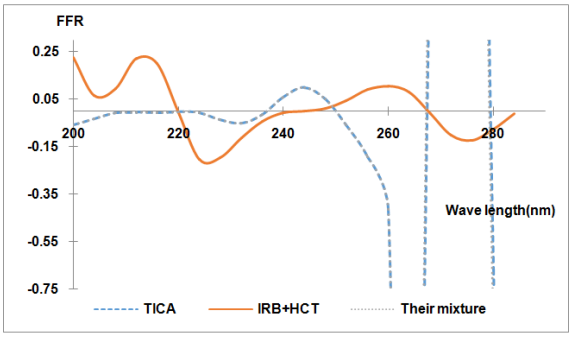

Fig. 6: Fourier functions convolution of ratio for 25 µg/ml TICA in methanol, 2 µg/ml IRB+6 µg/ml HCT in methanol and their mixture

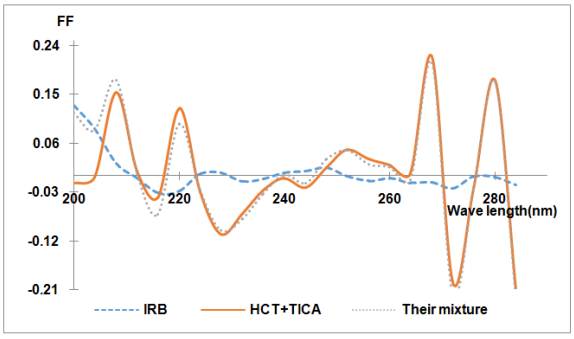

Fig. 7: Fourier functions convoluted curves for 3 µg/ml IRB in methanol, 8 µg/ml HCT+20 µg/ml TICA in methanol and their mixture

HCT was finally determined in presence of TICA and IRB by applying FF and FFR. The following equations were applied for FF and FFR, (eq. 5, 6) respectively:

$$t'_j = [(1.5)\ A_{0+}(0)\ A_1+(-1.5)\ A_2+(-1.5)\ A_3\ (0)\ A_4+(1.5)\ A_5]/3 \ldots\ldots\ldots\ldots (5)$$

Where A_0–A_5 stand for six Absorbance values at 4-nm intervals for 10 µg/ml HCT, 2 µg/ml IRB+20 µg/ml TICA (that is the double divisor) and their mixtures within the range (212-232 nm). The

number in brackets are the selected combined Fourier functions and T′= [sin x_i-sin (x_i+60 °)] (fig. 9).

t'_j = [(-0.866) FFR_{0+}(0) FFR_1+(0.866) FFR_2+(0.866) FFR_3+(0) FFR_4-(0.866) FFR_5]/3 ... (6)

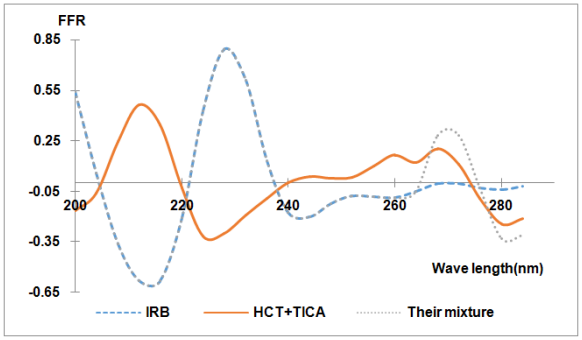

Fig. 8: Fourier functions convoluted curves of ratio for 3 µg/ml IRB in methanol, 8 µg/ml HCT+20 µg/mlTICA in methanol and their mixture

Where R_0–R_5 stand for six ratio values at 4-nm intervals for 10 µg/ml HCT, 2 µg/ml IRB+20 µg/ml TICA (that is the double divisor) and their mixtures within the range (240-260 nm). The number in brackets are the selected combined Fourier functions and T′= [sin x_i-sin (x_i+60 °)] (fig. 10).

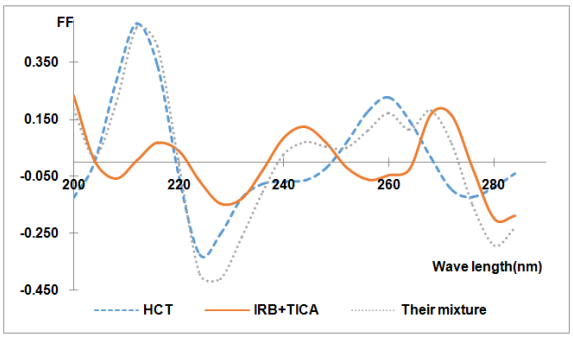

Fig. 9: Fourier functions convoluted curves for 10 µg/ml HCT, 2 µg/ml IRB+20 µg/ml TICA in methanol and their mixture

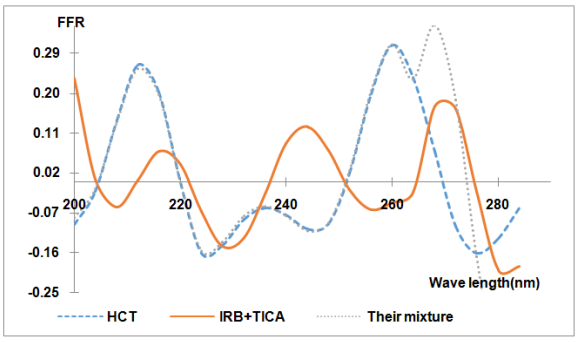

Fig. 10: Fourier functions convoluted curves of ratio for 10 µg/ml HCT, 2 µg/ml IRB+20 µg/ml TIC in methanol and their mixture

DISCUSSION

Chemometric assisted methods

As fig. 4 shows, there is clear spectral overlapping, preventing their resolution by direct traditional spectrophotometric measurements. Thus, the applied a chemometric assisted methods showed their ability to quantify each of the drugs in their ternary mixture.

The FF method depends on the convolution of absorption spectra of TICA, IRB and HCT using combined trigonometric Fourier function (FF). Following the general rules for the use of Fourier function in the processing of the absorption spectral data [26, 27], the associated different parameters were optimized. FFR method that is considered as a hybrid double divisor ratio spectra method depends on the convolution of the double divisor ratio spectra (to cancel the interfering effect of other drugs in the mixture, and obtain better resolution) using trigonometric Fourier functions, wherein this method, Fourier functions are applied to double divisor ratio spectra.

The optimum parameters were selected for each drug in order to give precise and accurate results for the analyte in the presence of the other interfering drugs. These include the selection of function order, number of points and wavelength intervals corresponding to spectral characteristics. Both methods gave good % recoveries. However, the application of the hybrid method (FFR) proved its excellence in resolving this triple mixture (table 4).

HPLC method

Optimization of chromatographic conditions

To establish and validate an accurate method for the analysis of these drugs in pharmaceutical formulations and plasma, C_{18} column was used. The effect of mobile phase composition, flow rate, and pH were studied. The best resolution with reasonable retention time was obtained with a mobile phase composed of acetonitrile: phosphate buffer in the ratio of (60:40).

Upon studying the effect of pH of the aqueous mobile phase, strongly acidic pH values gave peaks of short retention time that interfered with plasma protein peak. However, alkaline pH gave very long retention time. The best separation in terms of system suitability and the resolution was achieved by using the value of pH of 3.6. The optimum wavelength for detection was 230 nm at which much better detector response for HCT, TICA and IRB was obtained.

According to FDA 1994 [28], system suitability tests are an integral part of any liquid chromatographic method. System suitability uses to verify capacity factor, number of theoretical plates, asymmetry factor, selectivity and resolution (table 1) and they were found to be satisfactory and within the reported acceptance criteria listed in the reference

The optimum chromatographic conditions mentioned previously were applied for all measurements. Fig. 11, shows the separation of HCT at 4.8 min, TICA at 7.9 min and IRB at 9.8 min, at the optimized chromatographic conditions. Fig. 12, shows the separation of HCT at 4.4 min, TICA at 8.0 min and IRB at 9.9 min, in spiked plasma at the optimized chromatographic conditions.

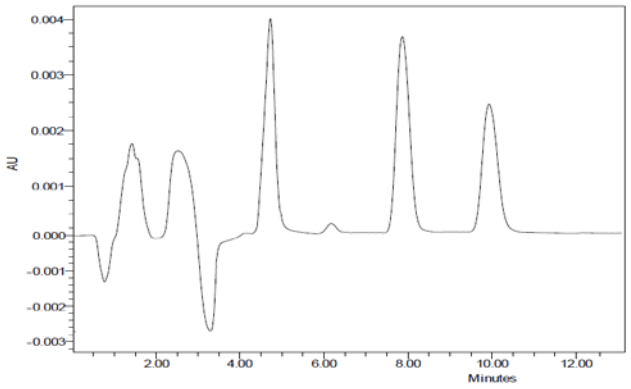

Fig. 11: HPLC chromatogram of a 20 µl injection of a standard mixture of 0.3 µg/ml TICA, 0.05 µg/ml IRB, and 0.5 µg/ml HCT using the optimized chromatographic conditions

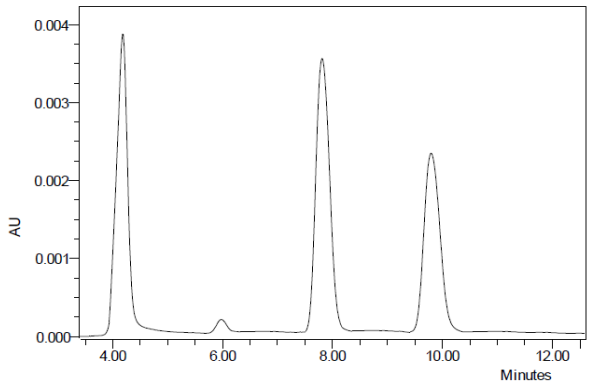

Fig. 12: HPLC chromatogram of a 20 µl injection of a mixture of 0.3µg/ml TICA, 0.05 µg/ml IRB, and 0.5 µg/ml HCT in spiked plasma using the optimized chromatographic conditions

Method validation and statistical analysis [25, 29]

The proposed methods were validated according to ICH guidelines [25]. The validation parameters included: linearity, limits of detection and quantification, accuracy, precision and specificity. The applied analytical methods are able to separate and quantify the studied drugs in their triple mixture.

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 2 and 3. Linearity data and statistical parameters for the proposed methods were calculated, including linear regression equation parameters (intercepts, slopes, correlation coefficients, the standard deviation of intercept and standard deviation of the slope (table 2 and 3). 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) or peak areas (HPLC method) and concentrations in significance 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. The 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 scattering 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).

Table 1: HPLC system suitability parameters for the determination of HCT, TICA and IRB using the proposed method

Analyte	Retention time (t_r)	Capacity Factor(k´)	N° of theoretical plates (N)	Asymmetry factor (A_F)	Selectivity (α)	Resolution (R_s)
HCT	4.8	0.66	536	1.66		3.44
TICA	7.9	1.72	1198	1.16	2.61	2.20
IRB	9.8	2.38	1433	1.00	1.24	

Limit of detection (LOD) and limit of qualification (LOQ)

Limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines [25]. 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 table 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 [30].

Table 2: Assay parameters for the determination of TICA, IRB and HCT using the applied chemometric assisted methods

	TICA		IRB		HCT	
Conc. Range(µg/ml)	10-30		1-3		4-10	
Parameters	FF	FF	FF	FFR	FFR	FFR
λ or $\lambda_{range(nm)}$	240-268	204-224	212-232	212-232	240-260	244-264
$\Delta\lambda_{(nm)}$	4	4	4	4	4	4
r	0.9997	0.9993	0.9993	0.9992	0.9992	0.9998
$S_{y/x}$	7.00×10^{-7}	2.75×10^{-6}	2.77 x 10-[34]	8.44 x 10-[34]	3.09×10^{-5}	1.89×10^{-7}
F	65×10^{3}	2299	1.02 x 10[33]	9.82 x 10[31]	2044	97×10^{3}
Significance F	1.31×10^{-7}	2.00×10^{-5}	6.75 x 10-[50]	2.27 x 10-[48]	2.38×10^{-5}	7.24×10^{-8}
a (intercept)	-0.03×10^{-2}	0.28×10^{-3}	0.2 x 10-[16]	5.55 x 10-[17]	0.65×10^{-2}	-0.17×10^{-3}
b (slope)	-0.78×10^{-1}	0.29×10^{-1}	0.48×10^{-1}	0.26×10^{-1}	0.159	0.49×10^{-1}
S_a	0.68×10^{-3}	0.13×10^{-2}	1.01 x 10-[17]	1.76 x 10-[17]	0.74×10^{-2}	0.33×10^{-3}
S_b	0.31×10^{-3}	0.61×10^{-3}	1.52 x 10-[18]	2.65 x 10-[18]	0.35×10^{-2}	0.159×10^{-3}
LOD (µg/ml)	-0.24×10^{-1}	-0.24×10^{-1}	6.22 x 10-[16]	2.01 x 10-[15]	0.14	0.20×10^{-1}
LOQ (µg/ml)	-0.82×10^{-1}	0.44	2.07 x 10-[15]	6.69 x 10-[15]	0.46	0.67×10^{-1}
a/S_a	-0.46	0.22	1.98	3.15	0.88	-0.49
$(S_b)^2$	9.33×10^{-8}	3.66×10^{-7}	2.31 x 10-[36]	7.03 x 10-[36]	1.24×10^{-5}	2.52×10^{-8}
S_b %	9.33×10^{-6}	3.66×10^{-5}	1.52 x 10-[16]	2.65 x 10-[16]	1.24×10^{-3}	2.52×10^{-8}

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 on the same day and on different days. Good accuracy expressed as relative percentage error (Er %), and high precision, expressed as percentage RSD, were obtained. The results, summarized in tables 4, 5 and 6 show that the (Er %) and RSD % values do not exceed the accepted limits, which demonstrate the accuracy an repeatability of the developed methods.

Table 3: Assay parameters for the determination of HCT, TICA and IRB using the proposed HPLC method

Parameters	HCT	IRB	TICA	HCT	IRB	TICA
	In pure drugs			In spiked plasma		
Conc. Range (µg/ml)	0.1-0.9	0.01-0.05	0.1-0.5	0.1-0.9	0.01-0.05	0.1-0.5
$\Delta\lambda_{(nm)}$	230	230	230	230	230	230
r	0.9999	0.9993	0.9996	0.9999	0.9858	0.9996
$S_{y/x}$	25.51×10^4	74.04×10^6	48.35×10^2	76.73×10^4	78.62×10^6	11.04×10^3
F	74.53×10^3	23.34×10^2	40.40×10^3	24.40×10^3	103.69	16.29×10^3
Significance F	1.08×10^{-7}	1.95×10^{-5}	2.71×10^{-7}	5.78×10^{-7}	2.02×10^{-3}	1.06×10^{-6}
a (intercept)	-17.20	23.33×10^2	-29.40	255.75	-16.36×10^3	-87.3
b (slope)	12.58×10^4	24.00×10^4	25.52×10^4	12.49×10^4	28.55×10^4	24.48×10^4
S_a	264.84	16.47×10^2	42.11	459.34	92.99×10^2	63.61
S_b	461.04	49.67×10^2	12.69×10^2	799.62	28.04×10^3	19.18×10^2
LOD (µg/ml)	0.631×10^{-2}	0.205×10^{-1}	0.495×10^{-3}	0.11×10^{-3}	9.77×10^{-2}	7.79×10^{-4}
LOQ (µg/ml)	0.21×10^{-1}	0.686×10^{-1}	0.165×10^{-2}	3.67×10^{-2}	0.33	2.59×10^{-3}
a/S_a	-0.64×10^{-1}	1.41	-0.698	0.556	-1.76	-1.37
S_b^2	21.25×10^4	24.67×10^6	16.11×10^5	63.93×10^4	7.86×10^8	36.78×10^5
S_b %	46.10×10^3	13.94×10^4	12.69×10^4	79.96×10^3	28.04×10^5	19.18×10^4

Table 4: Accuracy for the simultaneous determination of TICA, HCT o IRB in laboratory-made mixtures using the proposed chemometric assisted methods

TICA: IRB: HCT µg/ml	Mean recovery±SD[a], RSD %[b], Er %[c]	
	FF	FFR
20:1:4	100.38±1.59	100.00±1.25
	1.58	1.25
	0.38	0.00
20:2:4	101.38±1.35	100.79±1.45
	1.33	1.44
	1.38	0.79
10:3:4	98.66±1.56	100.00±0.73
	1.58	0.73
	-1.34	0.00
20:3:10	97.99±0.92	100.65±0.99
	0.94	0.98
	-2.01	0.65
3:30:10	101.24±0.92	100.00±0.99
	0.90	0.99
	1.24	0.00
15:2:10	97.00±0.89	100.48±0.54
	0.91	0.54
	-3.0	0.48

[a]mean±SD for the three determinations, [b]% Relative standard deviation, [c]% Relative error

Table 5: Accuracy for the simultaneous determination of IRB, HCT and TICA in laboratory-made mixtures and spiked plasma using the proposed HPLC method

HCT: IRB: TICAµg/ml	Mean recovery±SD[a], RSD%[b], Er%[c]					
	HCT	IRB	TICA	HCT	IRB	TICA
	In laboratory prepared mixtures			In spiked plasma		
0.1:0.01: 0.1	98.75±0.86	99.38±0.87	101.64±1.73	99.39±0.99	100.90±1.02	100.16±1.11
	0.87	0.87	1.70	0.99	1.01	1.11
	0.39	-0.62	1.64	-0.61	1.02	0.16
0.3:0.02:0.2	98.92±0.98	98.20±1.52	100.09±1.25	99.44±0.89	101.47±0.85	101.46±0.86
	0.99	1.55	1.25	0.89	0.84	0.851.46
	0.58	-1.8	0.09	-0.26	1.47	
0.5:0.03:0.3	99.47±1.18	99.61±1.34	101.10±0.68	99.68±1.48	101.56±0.61	100.10±0.68
	1.18	1.35	0.67	1.48	0.60	0.68
	-0.53	-0.39	1.10	-0.32	1.56	0.10
0.7:0.04:0.4	99.6±1.09	99.82±0.88	100.50±1.32	98.88±1.57	100.04±0.76	101.20±0.32
	1.09	0.88	1.31	1.59	0.76	0.32
	-0.40	-0.18	0.48	-1.70	3.44	1.20
0.9:0.05:0.5	98.98±1.09	99.26±1.09	100.56±1.32	99.34±0.87	101.04±0.98	100.05±0.87
	1.10	1.09	1.31	0.88	0.97	0.87
	-1.02	-0.74	0.56	-0.66	1.01	0.05

[a]mean±SD for the five 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 chemometric and HPLC methods

HCT: IRB: TICA µg/ml	Analytical method	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
10: 3: 30	FF	100.5±0.42	99.3±0.99	100±0.63	100.2±0.54	100.0±0.73	100±0.89
		0.42	0.99	0.63	0.54	0.73	0.89
		0.50	-0.70	0.00	0.20	0.00	0.00
10: 3: 30	FFR	100.22±1.32	101.90±1.34	101.13±1.45	101.64±1.73	101.11±1.34	101.53±1.01
		1.32	1.32	1.43	1.70	1.33	1.51
		0.22	1.90	1.13	1.64	1.11	1.54
0.9: 0.05:0.5	HPLC	99.0±0.61	99.94±1.52	98.73±1.68	100.00±0.75	100.22±1.32	100.46±0.68
		0.61	1.52	1.70	0.75	1.32	0.67
		-1.00	-0.06	-1.27	0.00	0.22	0.46

[a]mean±SD for the five determinations, [b]% Relative standard deviation, [c]% Relative error

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

RatioTICA+IRB+HCTµg/ml9:1.5:0.5	Mean recovery±SD[a]RSD %[b]Er %[c]		
	HPLC	FF	FFR
TICA			
Mean Recovery±SD[a]	98.76±0.45	98.87±0.40	99.19±1.09
RSD %[b]	0.45	0.40	1.09
Er %[c]	-1.01	-1.13	-0.81
**t-test	––	0.43	0.82
**F-test	––	0.79	5.86
IRB			
Mean Recovery±SD[a]	98.99±0.87	99.35±0.71	99.82±0.70
RSD %[b]	0.87	0.71	0.70
Er %[c]	-1.01	-0.65	-0.18
**t-test	––	0.71	1.67
**F-test	––	0.69	0.65
HCTMean Recovery±SD[a]RSD %[b]Er %[c]**t-test**F-test	99.08±0.610.61-0.92––––	99.23±0.840.84-0.770.381.89	99.63±0.770.77-0.371.241.59

[a]mean±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.

Laboratory made mixture

Table 7 present a statistical comparison between the assay of TICA, IRB and HCT in the laboratory made a mixture by the proposed methods 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 applied methods for determination of the two drugs in commercial tablets.

CONCLUSION

For analytical purposes, it is always important to establish methods capable of analyzing co-administered drugs in a short period of time with acceptable accuracy and precision. The chemometric assisted methods are simple, inexpensive and require easy treatment of the samples. The use of ratio spectra in the chemometric methods can magnify the prediction ability of the usual Spectrophotometric techniques. A comparative study of the use of RP-HPLC and chemometric assisted methods for resolution of ticagrelor, irbesartan, hydrochlorothiazide has been accomplished. Showing that chemometric assisted methods provide, with adequate software support, a clear example of the high resolving power of this technique. Although the RP-HPLC method is more sensitive, it needs expensive equipment and materials. The chemometric assisted methods are less expensive and do not require sophisticated instruments or prior separation steps. The proposed RP-HPLC were found to be suitable for the assay of this ternary mixture in plasma and can be used for further bioequivalence studies.

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CONFLICT OF INTERESTS

Declared none

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