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Spectrophotometric and chemometric determination of hydrochlorothiazide and spironolactone in binary mixture in the presence of their impurities and degradants

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Hydrochlorothiazide (HCT) and spironolactone (SPR) are mostly co-formulated in antihypertensive formulations. Several methods have been developed and validated for their determination; these methods include spectrophotometric and chemometric-assisted spectrophotometric methods. The developed spectrophotometric methods were isosbestic point (ISO) and ratio subtraction (RS) methods. The absorbance values at 232.4 (λ_{iso1}) and 257.6 nm (λ_{iso2}) were used for determination of the total mixture concentration, while HCT could be directly determined at 317.2 nm (λ_{max}) and by subtraction SPR concentration could be obtained. Also SPR concentration could be calculated by RS method using the absorbance at 243.8 nm (λ_{max}). A wavelength selection method based on genetic algorithm (GAs) was developed and compared to the conventional partial least squares method (PLS). In this method, several parameters were adjusted and the optimum parameter settings were determined using experimental design. The developed chemometric methods were successfully applied for the determination of the HCT and SPR, as well as for determination of their impurities and degradation products. The proposed methods were successfully applied for determination of HCT and SPR in commercial tablets and they were statistically compared to each other and to the reported method. No significant difference was found, providing their accuracy and precision.

Keywords: hydrochlorothiazide; spironolactone; chlorothiazide; salamide; partial least squares; genetic algorithm; isosbestic point; ISO; ratio subtraction; RS*

Introduction

Hydrochlorothiazide (HCT; Figure 1A), is chemically designated as (6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide).[1] It is one of the benzothiadiazines diuretics that are widely used in antihypertensive formulations either alone or in combination with other drugs. It decreases active sodium absorption and reduces peripheral vascular resistance.[2] Spironolactone (SPR; Figure 1B), is chemically designated according to IUPA nomenclature as 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone. It is a synthetic steroid and an aldosterone competitive antagonist which has been used as an effective diuretic, especially in patients with heart failure or liver cirrhosis.[3] The combination of both HCT and SPR is used to improve urine output and lung function in infants with bronchopulmonary dysplasia[4] and so, it is important to develop and validate several methods for their determination.

The literature comprised several analytical methods for determination of the binary mixture in pharmaceutical formulations or in biological fluids; these methods are either official[5] or unofficial[6–15] methods. Gas chromatography-mass spectrometry (GC-MS) method was described for determination of a number of drugs including HCT, SPR and chlorothiazide (HCT process impurity).[16] Lahuerta-Zamora *et al*.[17] studied the chemiluminescent behaviours of several diuretics, including HCT and SPR.

Several spectrophotometric methods were reported for determination of the binary mixture, including ratio spectra,[18] chemometrics[19,20] and derivative spectrophotometric methods.[21] Also indirect spectrophotometric[22] and colorimetric[23] methods were described for determination of HCT and SPR. The literature survey revealed that up to the present time, no analytical method has been published for the analysis of HCT and SPR in the presence of hydrochlorothiazide impurities (chlorothiazide (CT) and salamide (DSA)) and/or in the presence of spironolactone degradation products (SPR Deg).

CT and DSA are considered process impurities of HCT according to BP.[24] On the other hand, HCT undergoes hydrolytic degradation yielding DSA and formaldehyde.[25] CT was found to have lower pharmacological activity than the parent drug (HCT).[26,27] Besides, it is incompletely and variably absorbed compared with HCT.[28] According to ICH guidelines[29] SPR was subjected to acid

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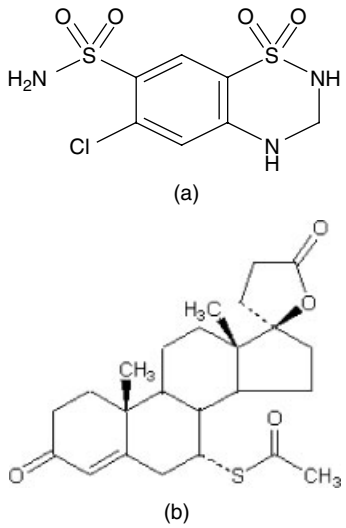

Figure 1. Chemical Structure of a- HCT and b- SPR.

and alkaline hydrolysis, oxidation, and photodegradation. The drug degraded only under acidic and basic conditions producing the same degradation product.

This work focuses on the development of accurate, simple, selective and appropriate spectrophotometric and chemometric methods for analysis of the suggested drugs in pure forms and in pharmaceutical preparations, as well as the study of their stability over time in the presence of reported impurities or hydrolytic degradation products. The suggested spectrophotometric methods include ratio subtraction (RS)[30] and isosbestic point (ISO).[31–37]

Experimental

Instruments

Double beam UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan), model UV-1601 PC with 1 cm quartz cells, connected IBM compatible computer. Matlab® version 6.5[38] was used for the proposed chemometric methods, the PLS and GA methods were performed with PLS_Toolbox[39] for use with Matlab® 6.5.

Samples

Authentic samples

HCT was kindly supplied by Amriya Pharmaceutical Industries (Alexandria, Egypt). It was certified to contain 98.5% according to the manufacturer method. SPR was kindly supplied by El Kahira Pharm. & Chem. Ind. Co. (Cairo, Egypt). It was certified to contain 99% according to the manufacturer method. CT and DSA were supplied by Sigma-Aldrich Chemie GmbH, Taufkirchen (Germany), their purity was labelled to be 99.5% and 99.25%, respectively.

Commercial products

Aldactazide® tablets (Batch No. 0 611 108) was manufactured by El Kahira Pharm. & Chem. Ind. Co. (Cairo, Egypt) under license from Searle Pharmaceuticals, a division of G. D. Searle & Co. Ltd (UK), while *Spirozide® tablets*(Batch No. 0 207 104) were manufactured by Sedico Pharmaceutical Co. (6 October City, Egypt). Each was labelled to contain 25 mg of HCT and SPR.

Degraded samples

Preparation of spironolactone degradate (SPR Deg). 0.5 gm of pure SPR was accurately weighed and refluxed with 75 mL of 0.1 N NaOH for 2 h. The degradation process was followed using TLC densitometric technique ($CHCl_3$-EtOAc-HCOOH-TEA (5 : 5 : 0.1 : 0.15, by volume) was used as a developing system).

The obtained solution was cooled and extracted with $CHCl_3$ to remove any traces of SPR that might have been present and the pH was adjusted to pH 6 using 0.1 N HCl. A yellow precipitate of SPR Deg was then obtained, filtered, washed three times with 10 mL of water, and left to dry at 60 °C for 5 h.

Chemicals and solvents

All chemicals and solvents used throughout were of analytical grade and were purchased from El-Nasr Pharmaceutical Chemicals Co. (Abu- Zabaal, Cairo, Egypt).

Solutions

Stock solutions of HCT, SPR, CT, DSA and SPR Deg were prepared in methanol in the concentration of 1 mg/mL. Working solutions of HCT, SPR, CT, SA and SPR Deg were prepared in methanol in the concentration of 0.1 mg/mL.

Procedure

Spectral Characteristics

The absorption spectra of 10 µg/mL of each of HCT, SPR, CT, DSA and SPR Deg were recorded over the range of 200–400 nm using 0.1N HCl as a blank. Also the absorption spectrum of a binary mixture of the two suggested drugs in the ratio of (1 : 1), containing 5 µg/mL of each was recorded using the same solvent (Figure 2).

Linearity

Isosbestic (ISO) and RS methods

Aliquots of HCT and SPR were separately transferred from their stock (1 mg/mL) and working (0.1 mg/mL) standard solutions, respectively, into two separate sets of 10-ml volumetric flasks. The volume was then made up to the mark with 0.1N HCl to prepare samples having concentrations in the range of 15–90 and 3–50 µg/mL for HCT and SPR, respectively. Zero order absorption spectra (^{0}D) of the prepared samples were recorded, the absorbance values of HCT at 317.2 nm (λ_{max}) and of SPR at 232.4 nm (λ_{iso1}), 257.6 nm (λ_{iso2}) were recorded.

Chemometric Methods

Multivariate calibrations are useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of a single wavelength greatly improves the precision and predictive ability.[40] Zero order absorption spectra of the two drugs, their impurities and degradates (Figure 2), showed severe spectral overlapping that seriously hindered the resolution of the binary mixture in the presence of the other components by conventional spectrophotometry. PLS is considered a standard multivariate calibration method. Several studies proved that the performance of PLS can be improved through wavelength selection; in most

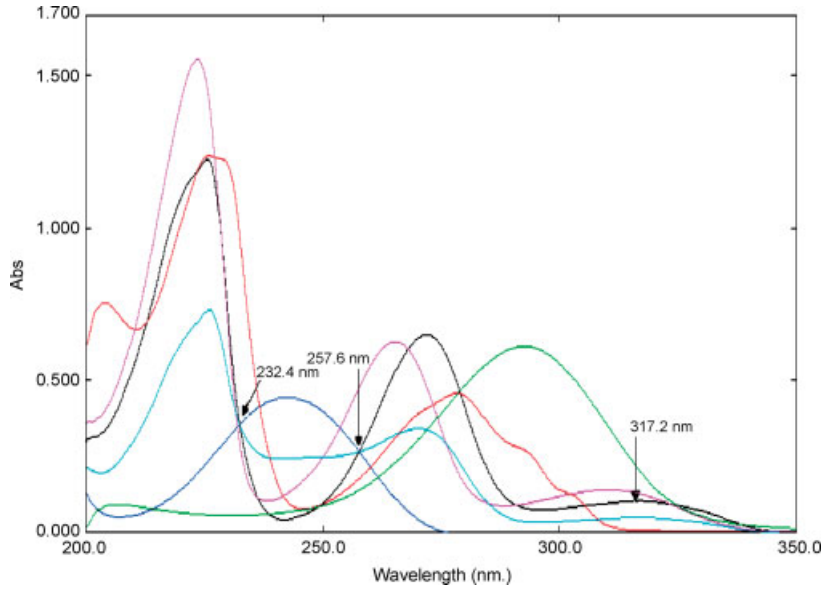

Figure 2. Zero order absorption spectra of 10 µg/mL of each of HCT (——), SPR (——), CT (——), DSA (——), SPR Deg (——) and a mixture contains 5 µg/mL of each of HCT and SPR (——) using 0.1N HCl as a solvent.

cases either the predication ability of multivariate calibration methods is improved or the developed models are more simple to interpret.[41–43] GAS were introduced by John Holland[44] and were successfully used as a feature selection technique after suitable modifications.[41,45–51] The developed GA model was applied to find the optimum set of wavelengths that best predicts the concentrations of the five compounds.

Multilevel multifactor experimental design was used for the construction of the calibration and validation sets.[52] A five-level, five-factor calibration design was used and 30 mixtures of the five proposed components were prepared. Eighteen samples were used for calibration and the other twelve samples were used as an external validation set. The concentration ranges and the composition of the calibration and validation samples are given in Table 1. The absorbance of these solutions in the range of 216–300 nm was recorded and the spectra were exported to Matlab® 6.5 for subsequent data manipulation. The spectra were digitized at 0.4 nm intervals, thus the produced spectral data matrix has 30 rows representing different samples and 211 columns representing wavelengths (30 × 211).

1. PLS method
2. In order to determine the correct number of LVs to be used for modelling the auto-scaled data, cross-validation with random subsets selection was performed to calculate the cumulative PRESS (prediction residual error sum of squares). Haaland and Thomas's criterion[44] was used to calculate the optimum number of LVs (latent variables) to be used in the models.

GA_PLS method

To improve this method, several parameters were adjusted: population size, maximum number of generations, mutation rate, percentage at convergence, percentage at initiation, the type of crossover, and window width. The number of subsets, maximum number of latent variables, and the number of iterations were kept at constant values. Plackett-Burman's[53] design for the seven assigned variables was applied in which all parameters were changed together in order to determine the optimal parameter settings for the GAs model. The average fitness value and number of LVs were used as response variables in the study.

Analysis of Laboratory-prepared Mixtures

ISO and RS methods

The absorption spectra of different laboratory-prepared mixtures containing different ratios of HCT and SPR were recorded and the absorbance values at 317.2 nm were measured, from which HCT concentrations were calculated. The absorbance values for each mixture at 232.4 and 257.6 nm were used to obtain the total mixture concentration and by subtraction, the concentration of SPR in the mixture was then calculated.

For measuring SPR content in the same mixtures using RS method, the spectra of the laboratory-prepared mixtures were divided by the standard spectrum 50 µg/mL of HCT. The value of the absorbance in the plateau region (at λ from 331–335 nm) was subtracted and the obtained curves were multiplied by the standard spectrum of 50 µg/mL HCT. The obtained curves were used for determination of SPR using the regression equation previously calculated at 243.8 nm.

Correct choice of the divisor concentration is playing an important role in the RS method, regarding selectivity and sensitivity. If the concentration of the divisor is increased or decreased, the resulting constant value will be proportionally decreased or increased. Standard spectrum of 50 µg/mL HCT gave the best results.

Application to Commercial Tablets

Twenty tablets each of Aldactazide® and Spirozide® were separately weighed, powdered and mixed well. An accurately weighted portion equivalent to 100 mg of each of HCT and SPR were transferred into 100-ml calibrated measuring flask and then 75 ml methanol was added. The prepared solutions were sonicated for 45 min, the volume was completed with the same solvent to get 1 mg/mL of each drug, and the solution was then filtered. Appropriate dilutions of the prepared solutions were made to prepare their working solutions (0.1 mg/mL).

Table 1. Concentrations of HCT, SPR, CT, DSA, and SPR Deg in the calibration and validation sets

Sample No.	HCT (μg/mL)	SPR (μg/mL)	CT (μg/mL)	DSA (μg/mL)	SPR Deg (μg/mL)
1	5.00	3.00	2.00	5.00	2.00
2	5.00	7.00	3.00	2.00	4.00
3	2.00	7.00	5.00	4.00	5.00
4	4.00	4.00	3.00	3.00	1.00
5	6.00	7.00	4.00	5.00	3.00
6	5.00	6.00	1.00	4.00	3.00
7	5.00	3.00	4.00	3.00	5.00
8	5.00	5.00	5.00	1.00	1.00
9	4.00	7.00	1.00	1.00	2.00
10	6.00	3.00	1.00	2.00	1.00
11	3.00	3.00	←	Zero	→
12	4.00	4.00	←	Zero	→
13	2.00	3.00	2.00	1.00	3.00
14	3.00	3.00	4.00	4.00	2.00
15	2.00	5.00	4.00	2.00	2.00
16	4.00	6.00	2.00	2.00	5.00
17	6.00	6.00	←	Zero	→
18	3.00	4.00	5.00	2.00	3.00
19	3.00	6.00	4.00	1.00	3.00
20	3.00	7.00	2.00	3.00	1.00
21	6.00	4.00	3.00	1.00	5.00
22	3.00	5.00	1.00	5.00	5.00
23	2.00	4.00	1.00	3.00	4.00
24	6.00	5.00	2.00	4.00	4.00
25	4.00	3.00	5.00	5.00	4.00
26	6.00	6.00	5.00	3.00	2.00
27	2.00	7.00	3.00	2.00	1.00
28	2.00	6.00	3.00	5.00	1.00
29	4.00	5.00	3.00	3.00	3.00
30	4.00	4.00	1.00	2.00	2.00

* The shaded samples are those used for model validation.

Results and Discussion

After preparation, complete separation and purification of SPR Deg, the drug, and the degradate were subjected to IR and mass analyses. The assignment of IR spectrum of SPR Deg was based on comparison to that of the intact drug. It showed the disappearance of the thiol ester group band at 1690 cm^{-1} which was present in the parent compound and the appearance of a new band of SH group at 2607 cm^{-1} indicating the hydrolysis of the thiol ester group. While the bands of carbonyl groups at 1679.2 and 1767.9 cm^{-1} in the parent drug shifted to 1654.5 and 1723.2 cm^{-1}, a new broad band at 3289.9 cm^{-1} corresponding to OH group indicated the hydrolysis of the cyclic ester to its acid derivative. The obtained mass spectra (MS) for SPR ($C_{24}H_{32}O_4S$) and SPR Deg ($C_{22}H_{31}O_4S$) showed molecular ion peaks at m/z 416.85 and m/z 392 (m + 1), respectively, where the cyclic ester was opened (H_2O molecule was added) and the thiol ester was hydrolyzed to SH group.

D^0, ISO and RS methods

Zero order absorption spectra (^0D) of the two drugs showed spectral overlap from 200 to 275 nm (Figure 2), while from 275 to 350 nm SPR does not interfere with the direct determination of HCT. So the absorbance at 317.2 nm (λ_{max}) was used for direct determination of HCT, while the absorbance values at 232.4 (λ_{iso1}) and (λ_{iso2}) (Figure 2) were used for determination of the total mixture concentration and by subtraction SPR concentration could be calculated. Also, SPR content could be obtained by RS method using the absorbance at 243.8 nm (λ_{max}) as shown in Figure 3.

Calibration curves were constructed for the above linear relations relating the absorbance values to the corresponding concentrations. The linear relationships were obtained in the ranges of 15–90 μg/mL for HCT at 317.2 nm and 5–50, 3–50 and 5–45 μg/mL for SPR at 232.4, 257.6 and 243.8 nm, respectively. The regression equations were computed and given in Table 2. The other regression equation parameters in Table 2 show good linear relationship for the suggested methods as revealed by the correlation coefficients. Method validation including linearity and range, accuracy, precision, specificity, and limits of detection and quantitation, was performed according to ICH guidelines.[29]

In order to test the validity and the applicability of the proposed methods, laboratory-prepared mixtures containing different ratios of the two drugs were analyzed; good results were obtained and are shown in Table 3.

The suggested methods were successfully applied for determination of both drugs in Aldactazide® and Spirozide® tablets. The results presented in Table 3 show satisfactory results and good agreement with the labelled amounts. By applying the standard addition technique, no interference from the excipients was observed (Table 3).

Table 2. Results of regression, assay validation parameters and statistical analysis of the proposed methods for determination of HCT and SPR

Parameters	HCT (D^0) at 317.2 nm	SPR: ISO method 232.4 nm	SPR: ISO method 257.6 nm	SPR: RS method 243.8 nm	Chemometric methods: HCT PLS	Chemometric methods: HCT GAPLS	Chemometric methods: SPR PLS	Chemometric methods: SPR GAPLS	Reported method**[18] HCT	Reported method**[18] SPR
Linearity										
Range	15–90 µg/mL	5–50 µg/mL	3–50 µg/mL	5–45 µg/mL	2–6 µg/mL		3–7 µg/mL			
Slope	0.0108	0.0374	0.0284	0.0450	0.9916	0.9990	1.0182	1.0105		
Intercept	−0.0006	−0.0197	−0.0191	−0.0096	0.0170	0.0179	−0.0192	−0.0556		
(r)	0.9999	0.9999	0.9999	0.9999	0.9994	0.9994	0.9987	0.9987		
Accuracy	100.0 ± 0.59	100.0 ± 1.08	100.1 ± 0.99	99.8 ± 1.07					100.1 ± 1.05	99.8 ± 1.13
Precision										
Repeatability	0.64	0.80	1.46	1.03						
Intermediate precision	0.77	1.29	1.41	1.05						
LOD	4.28 µg/mL	1.50 µg/mL	0.81 µg/mL	1.54 µg/mL						
LOQ	12.96 µg/mL	4.55 µg/mL	2.46 µg/mL	4.67 µg/mL						
N	12	10	9	9	12	12	12	12	6	8
Student's	0.34	0.17	0.68	0.02	0.76	0.62	0.56	0.20		
t-test	**(2.12)***	**(2.12)***	**(2.13)***	**(2.13)***	**(2.12)***	**(2.10)***	**(2.12)***	**(2.10)***		

r: the correlation coefficient. ** First derivative of ratio spectra spectrophotometric determination of HCT at 270.7 nm and SPR at 237 nm using (0.1N HCl/MeOH) as a solvent. * Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.

Table 3. Determination of the studied drugs in the laboratory-prepared mixtures (L.P.) and in tablets by the proposed methods

		SPR			Chemometric methods									
		ISO method		RS method 243.8 nm	PLS					GA_PLS				
Sample	HCT ^{0}D (317.2 nm)	232.4 nm	257.6 nm		HCT	SPR	CT	DSA	SPR Deg	HCT	SPR	CT	DSA	SPR Deg
L.P. Mixtures*	101.7 ± 0.91	100.7 ± 1.37	100.1 ± 1.37	101.1 ± 1.56	99.6 ± 1.27	100.1 ± 1.48	102.6 ± 2.32	102.1 ± 3.00	100.3 ± 1.16	100.4 ± 1.14	99.9 ± 1.47	102.5 ± 2.36	101.1 ± 2.42	99.8 ± 1.27
RMSEP					0.053	0.076	0.070	0.090	0.461	0.049	0.070	0.070	0.084	0.048
Aldactazide® ** (B.N. 0611108)	100.5 ± 0.91	100.5 ± 1.66	99.7 ± 1.51	98.50 ± 1.06	99.00 ± 1.76	98.50 ± 1.91	–	–	–	99.00 ± 1.47	98.85 ± 1.72	–	–	–
Standard addition*	101.5 ± 1.91	100.9 ± 0.98	100.5 ± 1.40	99.6 ± 1.62	101.1 ± 0.75	101.4 ± 0.94	–	–	–	101.00 ± 0.83	101.4 ± 0.89	–	–	–
Spirozide® ** (B.N. 0207104)	97.2 ± 1.01	96.7 ± 0.80	96.6 ± 0.90	95.00 ± 1.17	95.2 ± 0.48	95.3 ± 1.38				95.4 ± 0.67	95.4 ± 1.08			
Standard addition*	97.7 ± 1.12	98.4 ± 0.74	98.6 ± 0.81	1005 ± 1.01	99.4 ± 0.71	102.24 ± 0.068				101.07 ± 0.910	102.11 ± 0.294			

* Average of 3 determinations. ** Average of 6 determinations. RMSEP: root mean square error of prediction.

Table 4. Plackett-Burman Design for GAs parameter settings

	Assignedvariables(A-G)					Unassignedvariables(H-K)					
Trial	A	B	C	D	E	F	G	H	I	J	K	Percent improvement
1	–	–	–	–	–	–	–	–	–	–	–	–74.95
2	+	–	+	+	+	–	–	–	+	–	+	–322.96
3	–	+	+	+	–	–	–	+	–	+	+	–83.30
4	+	+	+	–	–	–	+	–	+	+	–	6.45
5	+	+	–	–	–	+	–	+	+	–	+	–41.37
6	+	–	–	–	+	–	+	+	–	+	+	–84.82
7	–	–	–	+	–	+	+	–	+	+	+	–21.44
8	–	–	+	–	+	+	–	+	+	+	–	–3.98
9	–	+	–	+	+	–	+	+	+	–	–	–18.98
10	+	–	+	+	–	+	+	+	–	–	–	–63.57
11	–	+	+	–	+	+	+	–	–	–	+	–90.89
12	+	+	–	+	+	+	–	–	–	+	–	–1.20

Note: A-population size, B-% wavelengths at initiation, C-maximum generations, D-% at convergence, E- mutation rate, F-crossover type and G- window width. Percent improvement- percentage improvement of RMSEP of GA-PLS in comparison with that of PLS.

Table 5. Levels of GAs parameters setting

Parameters	Low (–)	High (+)	Chosen level
Population size	16	40	16
% Wavelength used at initiation	20	50	20
Maximum generations	25	75	75
% at convergence	10	50	10
Window width	3	10	3
Mutation rate	0.003	0.01	0.003
Crossover type	single	double	double
Number of subsets	–	–	5
Maximum no. of latent variables	–	–	2
Number of iterations	–	–	2

Table 6. Percentage improvement upon using the five components in GAs optimization

	improvement **%**				
Compound used for optimization	HCT	SPR	CT	DSA	SPR Deg
HCT	6.5	7.4	–0.4	6.1	–4.8
SPR	–19.2	11.7	–12.3	–9.1	5.2
CT	–28.5	–9.1	–25.5	–45.2	–1.7
DSA	–42.3	11.7	–2.4	–7.9	–14.3
SPR Deg	–10.8	5.5	–7.6	38.9	–7.6

Chemometric Methods

After optimization of the developed PLS model (as previously mentioned), it was found that seven LVs were suitable for constructing the model. Plackett-Burman's design[53] was applied in order to obtain the optimum parameters setting for GAs model and the GA configuration that gave the best fitness value with minimum number of LVs was selected. The percent improvement in RMSEP (root mean square error of prediction) of GA_PLS relative to PLS for each parameters setting was calculated[54] and is given in Table 4. The change in each parameter greatly affected the process of wavelength selection; for example: population size caused an obvious increase in the computation time. Decreasing the mutation rate and percentage at convergence and increasing the maximum number of generations gave better fitness values. Both window width and percentage at initiation were found to have little effect on the fitness values. The optimum configuration used is given in Table 5.

RMSEP values compared to PLS without wavelength selection were calculated when GA_PLS was used for optimization of each of the five proposed components. Results in Table 6 show the percentage improvement in each case. It was observed that when GAs was used for optimization of HCT, the RMSEP of HCT, SPR, and DSA improved, while nearly no effect was noticed on CT, and so the selected wavelengths can be used for adequate calibrations of the five components.

In order to asses the predictive ability of the developed models, an external validation set was used. Satisfactory results were obtained and given in Table 3. Regression parameters of the developed models are shown in Table 2.

The suggested chemometric methods are applicable in determination of both HCT and SPR in their pharmaceutical formulation. Application of standard addition technique assessed the validity of these methods and satisfactory results were obtained and are presented in Table 3. When the PLS model was preceded by GA wavelengths selection, an improved prediction of both HCT and SPR was observed as described by decrease in RMSEP values relative to their PLS models.

When results obtained by applying the proposed methods for analysis of pure HCT and SPR were compared to those obtained by applying the reported spectrophotometric method,[18] they showed no significance difference regarding both accuracy and precision (Table 2).

Conclusion

In this work, simple, selective, rapid and accurate methods were described for determination of HCT and SPR in their bulk powder and pharmaceutical preparations, either in their binary mixture or in the presence of their impurities and degradation products. The suggested methods were validated according to (ICH) guidelines,[29] and they were statistically compared to each other and to the reported method.

The isosbestic and RS methods are rapid, simple, and economic methods for simultaneous determination of the binary mixture. But these methods cannot determine HCT and SPR in the presence of their impurities and degradation products.

Chemometric methods can be used for determination of the intact drugs and all their impurities and degradation products and so they are selective. stability-indicating methods. Applying the reported spectrophotometric method[18] for determination of HCT and SPR in the presence of other related components (CT, DSA, and SPR Deg) gave bad results while good results were obtained by applying the proposed chemometric methods for detemination of the five components in the validation set (Table 3), confirming its higher selectivity.

The developed chemometric methods have higher selectivity than other published methods reported for analysis of the binary mixture, so they can be useful for the stability investigation of the

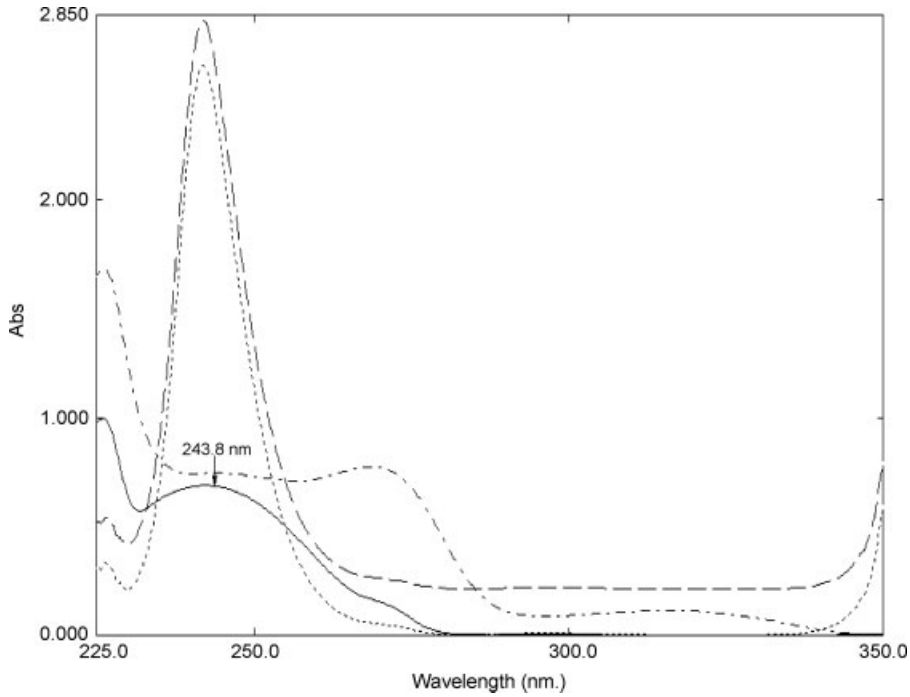

Figure 3. Ratio spectra of the laboratory prepared mixture of HCT and SPR (-.-.-.-), (using 50 µg/mL of HCT as a divisor and 0.1N HCl as a solvent.

two cited drugs and for checking the extent of degradation in pharmaceutical formulations.

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