Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 4, October - December 2014, p. 1359 - 1372

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF DIPHENHYDRAMINE, BENZONATATE, GUAIFENESIN AND PHENYLEPHRINE IN THEIR QUATERNARY MIXTURE USING PARTIAL LEAST SQUARES WITH AND WITHOUT GENETIC ALGORITHM AS A POWERFUL VARIABLE SELECTION PROCEDURE

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Diphenhydramine HCl, benzonatate, guaifenesin and phenylephrine HCl are co-formulated together in Bronchofree ™ capsule in the ratio of 2.5:10:10:1 respectively. Literature review showed only one reported HPLC method for this mixture. Simultaneous chemometric- assisted spectrophotometric analysis of the multi-component dosage form has been carried out using two chemometric methods. These methods includes partial least squares (PLS-1) and PLS-1 proceeded by genetic algorithm (GA-PLS). Results demonstrated the efficiency of the two methods as quantitative tool of analysis of the four components without any interference of the excipient added, that eliminates the need for preliminary extraction of analytes from the pharmaceutical formulation. The four analytes were determined precisely using the afore-mentioned methods in an independent data set as well as in dosage form after optimization of the experimental conditions.Both methods are robust,accurate and precise in addition to their remarkable simplicity in comparison to other sophisticated techniques such as HPLC.

(Received May 28, 2014; Accepted October 15, 2014)

Keywords: Diphenhydramine HCl, Benzonatate, Guaifenesin, Phenylephrine HCl, Spectrophotometry, PLS, Genetic algorithm

1. Introduction

Diphenhydramine hydrochloride (DP) [2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride] [1] is an effective antihistaminic, and has been used for the treatment of motion sickness and extrapyramidal symptoms, as well as an antitussive and night-time sleep-aid. Recently, its use has been reported, in combination with other drugs, as antiemetic for the prevention of cisplatin-induced emesis in chemotherapy treatment. It has also been used as sedative in dentistry for children and in local anaesthesia [2]. It is a common ingredient of cough and cold preparations and is also used as a hypnotic [3]. Benzonatate (BN)[4-(Butylamino)benzoic acid 3,6,9,12,15,18,21,24,27-nanooxaoctacos-1-yl ester)] is an effective antitussive [1] . Guaifenesin (GU) [3-(2-Methoxyphenoxy)-1, 2-propanediol] is reported to reduce the viscosity of sputum and used as an effective expectorant [3] . Phenyl ephrine hydrochloride (PH) [3-(2-Methoxyphenoxy)-1, 2-propanediol] is an alpha-adrenergic sympathomimetic agent which stimulates alpha-adrenergic receptors, producing pronounced vasoconstriction [2]. The combination of the four drugs is used for treating bronchial spasm and as antitussive. The UV absorption spectra of DP, BN, GU and PH display considerable overlap that the application of the conventional spectrophotometry and its direct derivative and derivative ratio technique failed to resolve the overlapping of their spectra.

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Only one recent reported HPLC method [4] (just accepted) is available for the simultaneous analysis of this quaternary mixture. Under computer-controlled instrumentation, multivariate calibrations methods are playing a very important role in the multi-component analysis of mixtures by UV–Vis molecular absorption spectrophotometry. The approach is useful in the resolution of band overlapping in quantitative analysis. The multivariate calibration has been found to be the method of choice for complexed mixtures. The advantage of multi-component analysis using multivariate calibration is the speed of the determination of the components in a mixture, avoiding a preliminary separation step [5]. Control analyses on pharmaceutical preparations using multivariate calibration method, has been proved to be a valid alternative to HPLC[6]. The application of quantitative chemometric methods needs a calibration step where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum. In PLS calibration the information from the concentration values is introduced into the calculation of the so-called latent variables; thus, it is may be that the eliminating wavelengths after variable selection, change the structure and/or the order of the latent variables of the PLS model. Consequently, in practice, spectral wavelength selection continues to be the process of interest because, a selection procedure which optimizes the prediction capacity will lead to those wavelengths for which the analyte of interest absorbs and where its absorbance is different from other analytes. In other words, this procedures lead to the rejection of wavelengths not related to the analyte of interest. Partial least squares (PLS-1) perform the optimization of the number of latent variables for only one component at a time [7]. Genetic algorithms (GA)[8-10] have been used to solve difficult problems with objective functions that do not possess 'nice' properties such as continuity, differentiability, etc. [11]. These algorithms maintain and manipulate a family, or population, of solutions and implement a 'survival of fittest' strategy in their search for better solutions. GA searches the solution space of a function through the use of simulated evolution, i.e. the survival of the fittest strategy. GA have been shown to solve the optimization problem by exploring all regions of the potential solutions and exponentially exploiting promising area through mutation, crossover and selection operation applied to individuals in the populations. A complete discussion of genetic algorithms can be found in [11-13]. This work presents PLS-1 and GA-PLS methods for simultaneous spectrophotometric determination of DP, BN, GU and PH in dosage form, whereas the UV absorption spectra of the four components show severe overlap which hinders their assay by traditional methods.

2. Experimental

Instrumentation

Schimadzu UV- Visible Spectrophotometer 1601 PC equipped with 1 cm quartz cells.The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The wavelength scanning speed was 2800 nm min^{-1}. PLS-1 analysis was carried out by using PLS-Toolbox software version 2.0–PC for use with MATLAB (Version 7, Math Work, Inc.).

Materials and reagents

Pharmaceutical grade of DP, BN, GU and PH were used and certified to contain 99.90%, 99.87%, 99.15% and 99.30% respectively. Potassium hydroxide and methanol used were analytical grade. Bronchofree capsules, batch number 811062 (Adwia Pharmaceuticals and Chemical Industries Co., Cairo, Egypt) were used. Each capsule was labeled to contain 25 mg DP, 100 mg BN, 100 mg GU and 10 mg PH.

Standard and working solutions

Stock standard solutions of DP, BN, GU and PH were prepared separately by dissolving 125 mg of DP, 250 mg BN, 250 mg GU and 125 mg PH in 100 ml distilled water. DP was dissolved in least amount of methanol and complete to volume with water. Corresponding working solutions were prepared by transferring accurately 12.5 ml, 25 ml, 25 ml and 6 ml from DP, BN,

GU and PH stock standard solutions separately in 250-ml measuring flasks and volume was completed with distilled water.

Procedures

A- Calibration

1- Constuction of the training set

Multilevel multifactor design [14] was used for the construction of the calibation set. A calibration set of 16 samples was prepared for calibration. A four-level, two –factor calibration design was used in which 4, 4.5, 5.5 and 6 ml aliquots of the four working solutions were combined and 1 ml of 0.05 N KOH was added then volume completed to 25 ml with distilled water. The UV absorption spectra were recorded over the range 200–350 nm against solvent blank contains 30μg ml^{-1} GU treated excactly as the samples. The data points of the spectra were collected at every 1 nm. Final concentration ranges were 10-15 μg ml^{-1} for DP, 40–60 μg ml^{-1} for BN and GU , 4.8-7.2 μg ml^{-1} for PH. (table 1)

2-Pre-procxessing the data

reject the regions from 200- 215 nm and above 340 nm. Mean centered of the data was performed.

3-Selection of the optimum numbers of latent variables to build the PLS-1 model

The RMSECV values were calculated using a cross-validation method leaving out one sample at a time [15].

B- Validation

Validastion set of 6 samples was prepared to check the performance of the PLS-1 and GA-PLS models. The composition of 6 mixtures is shown in table 2

C- Application to pharmaceutical preparation

Weigh accurately liquid content of 10 capsules and stirred for 5 min. with 20 ml methanol. Complete to 250 ml with distilled water. Transfer accurately 5 ml of this solution to 100-ml measuring flask and complete to volume with distilled water . The final concentratons were 50 μg ml^{-1} for DP, 200 μg ml^{-1} for both BN and GU, 20 μg ml^{-1} for PH. Take 5, 6, 7 and 7.5 from this solution and and add 1 ml of 0.05 N KOH then complete volume to 25 ml with distilled water. The general procedures described under calibration were followed and the concentration of each compound was calculated.

3. Results and discussion

Optimization of spectral measurements

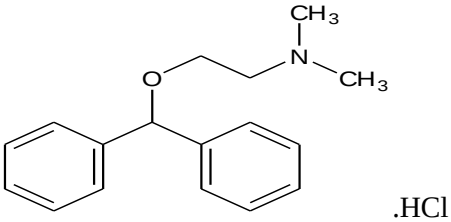
The chemical structures of DP, BN, GU and PH are shown in Fig. 1. Fig. 2 shows the UV absorption spectra of DP, BN, GU and PH at their nominal concentrations in capsules. First-order derivatives spectra for the drugs are shown in Fig. 3. As these figures show there is a clear overlapping between them especially DP, GU and PH; the spectral overlapping of the drugs prevents resolution of the mixtures by direct spectrophotometric measurements. Thus, the univariate analysis cannot be applied to resolve their mixtures. The optimum conditions for quantitative estimation of considered compound were established via a number of preliminary experiments. The medium is rendered alkaline to produce hyperchromic shift for PH [16] by addition of 1 ml of 0.05 N KOH just before measurements to prevent hydrolysis of BN that may occurred in alkaline medium [17]. Mixtures were measured against solvent blank contain 30 μg ml^{-1} of GU to decrease absorbance of overall mixture sample.

Table 1: Concentrations and percent recoveries of four components used in the training set

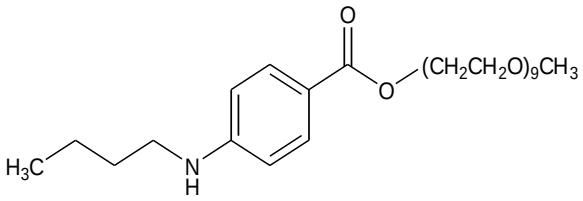
Mix. no.	Mix. composition(µg.ml $^{-1}$)				R% PLS-1 method				R% GA-PLS method		
	DP	BN	GU	PH	DP	BN	GU	PH	BN	GU	PH
1	10	40	40	4.8	99.40	100.63	99.50	100.68	99.55	100.26	99.59
2	10	45	45	7.2	97.11	100.45	99.53	98.71	99.65	99.12	98.97
3	11.25	45	60	5.4	101.35	100.26	99.95	100.13	99.85	99.26	100.58
4	11.25	60	45	4.8	98.32	99.43	99.43	99.58	100.35	100.68	100.58
5	15	45	40	6.6	102.90	101.01	99.98	101.17	100.83	100.35	101.07
6	11.25	40	55	6.6	97.47	98.89	99.41	99.18	99.53	100.39	99.28
7	10	55	55	5.4	101.61	100.32	100.28	100.96	99.22	98.45	100.87
8	13.75	55	45	6.6	99.22	99.12	99.44	99.99	97.90	96.72	97.06
9	13.75	45	55	4.8	100.40	101.08	100.94	101.56	101.04	99.97	100.62
10	11.25	55	40	7.2	102.65	100.98	100.29	100.38	100.85	102.24	101.14
11	13.75	40	60	7.2	99.58	98.75	100.38	100.03	100.11	101.32	100.62
12	10	60	60	6.6	102.45	100.05	100.26	100.03	99.02	99.70	99.49
13	15	60	55	7.2	97.45	100.34	99.73	100.38	100.93	100.78	100.62
14	15	55	60	4.8	100.01	100.34	99.38	98.82	100.80	100.21	98.81
15	13.75	60	40	5.4	98.25	98.97	101.11	99.14	100.03	101.14	100.54
16	15	40	45	5.4	100.60	99.45	100.69	99.65	100.83	99.35	100.69
Mean					99.92	99.96	100.02	100.02	100.03	100.12	100.15
S.D					1.907	0.792	0.570	0.827	0.870	1.005	0.824

Table 2: Concentrations and percent recoveries of four components used in the validation set

Mix. no.	Mix. composition (µg.ml $^{-1}$)				R% PLS-1 method				R% GA-PLS method		
	DP	BN	GU	PH	DP	BN	GU	PH	BN	GU	PH
1	15	60	60	6	100.87	98.34	99.55	98.70	98.73	100.05	101.03
2	12.5	40	40	7.2	98.64	101.81	99.74	98.07	101.31	100.11	98.28
3	12.5	40	45	6.6	101.36	100.40	99.82	101.01	100.70	100.44	100.86
4	15	60	45	7.2	102.67	101.41	101.97	98.41	101.62	100.83	98.98
5	15	40	40	6.6	97.87	97.67	102.39	99.73	97.92	101.93	98.80
6	12.5	50	50	6	102.90	99.83	102.53	97.73	99.19	102.53	98.37
Mean					100.72	99.91	101.00	98.84	99.91	100.98	99.39
S.D					2.07	1.648	1.435	1.222	1.508	1.023	1.234

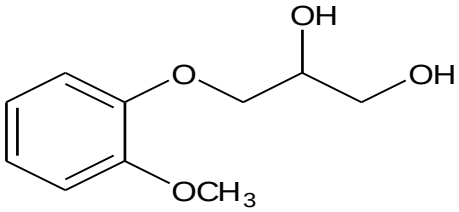


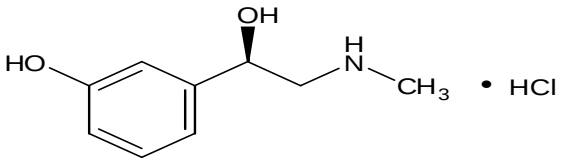
.HCl



Diphenhydramine HCl

Benzonatate





Guaifenesin

Phenyl ephrine HCl

Fig. 1. Chemical structures of four components of Bronchofree capsule

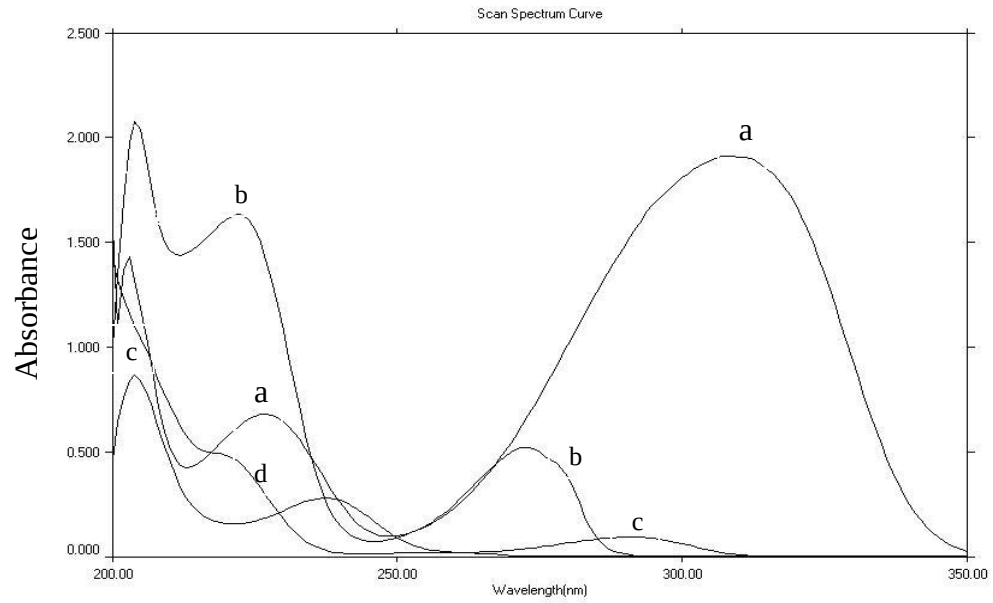


Fig. 2. UV absorption spectra of 60 μg ml^{-1} of BN (a), 60 μg ml^{-1} of GU (b), 6μg ml^{-1} of PH (c) and 15 μg ml^{-1} of DP (d)

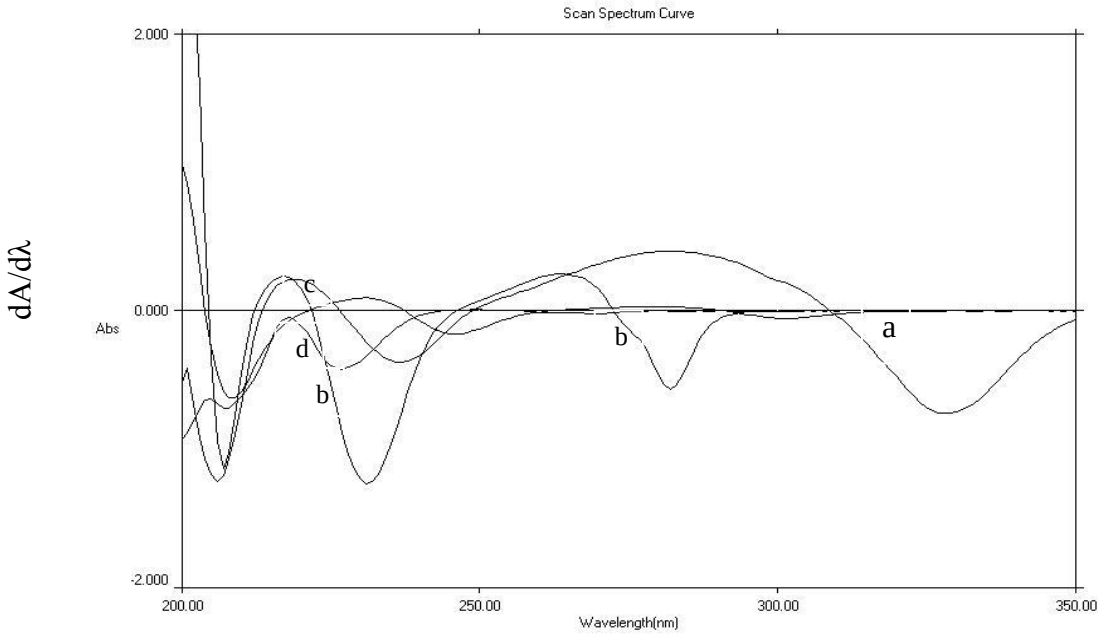


Fig. 3. First derivative absorption spectra of 60 μg ml−1 of BN (a), 60 μg ml−1 of GU (b), 6μg ml−1 of PH (c) and 15 μg ml−1 of DP (d)

PLS-1 method

The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used [18]. PLS procedures are designated to be full spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened, by discarding particularly noisy wavelengths. The wavelengths used were in range 215–340 nm in all cases. Wavelengths less than 215 nm, were rejected due to the noisy content. Wavelengths more than 280 nm for DP, 300 nm for GU and PH and 340 nm for BN were not used because the corresponding components do not absorb in these regions(table 3). The first step in simultaneous determination of DP, BN, GU and PH in mixtures by multivariate methods involved constructing the calibration matrix. In this work, we performed the calibration with the absorption spectra and the first-order derivative spectra. The derivative of each spectrum was calculated with MATLAB. The wavelength interval ($\Delta\lambda$) used for calculation of derivative spectra was optimized and $\Delta\lambda$ = 5 nm was considered to be optimum which gives the best signal-to-noise ratio for all drugs. The multivariate calibration requires a careful experimental design of the standard composition of calibration set for providing the best predictions. Multilevel multifactor design [14] was used for the construction of the calibration set. A calibration set consisting of 16 samples was used. The concentration of DP, BN, GU and PH was varied over the range 10–15 μg ml^{-1} for DP, 40-60 μg ml^{-1} for BN and GU and 4.8–7.2 μg ml^{-1} for PH in the calibration set. Table 1 shows the composition of the calibration set. PLS-1 method was run on the calibration data of absorption (zero-order) UV spectra and first-order derivative spectra. Percent variance captured by PLS-1 using zero order spectra (D^0) and first order derivative spectra (D^1) was shown in table 4.

To select the number of factors in the PLS-1 algorithm, a cross-validation method leaving out one sample at a time [15] was employed using calibration set of 16 calibration spectra. PLS-1 calibration on 15 calibration spectra was performed and, using this calibration, the concentration of the sample left out during the calibration process was predicted. This process was repeated 16 times until each training sample had been left out once. The predicted concentrations of the components in each sample were compared with the actual concentrations in this calibration samples and the root mean squares error of cross-validation (RMSECV) was calculated for each method as follows:

$$\mathrm{RMSECV} = \sqrt{\frac{1}{\mathrm{I}}\sum_{\mathrm{i}=1}^{\mathrm{I}}\left(c_i - \hat{c}^{A}_{i\_cv}\right)^2}$$

where I is the number of objects in the calibration set, c_i is the known concentration for sample i and $\hat{c}^{A}_{i\_cv}$ is the predicted concentration of sample i using A components. Mean centering was performed on the training set each time successive samples were left out. The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both of the precision and accuracy of predictions. It was recalculated upon addition of each new factor to the PLS-1 model.

Appropriate selection of the number of factors to be used to construct the model is a key to achieving correct quantitation in PLS-1 calibration. The most usual procedure for this purpose involves choosing the number of factors that result in the minimum RMSECV. However, this criterion is subjected to some constraints since, occasionally; the RMSECV does not reach a sharp minimum, but decreases gradually above a given number of factors. For these reasons, the method developed by Haaland and Thomas [19] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that results in an insignificant difference between the corresponding RMSECV and the minimum RMSECV. Number of factors used for each drug is shown in table 5. As the difference between the minimum RMSECV and other RMSECV values become smaller, the probability that each additional factor is significant becomes smaller [20]. In order to validate proposed PLS-1 method, a validation set composed of 6 synthetic mixtures of DP, BN, GU and PH were analyzed with the proposed PLS-1 method. The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E.) of the prediction concentration [21].

$$R.S.E\ (\%) = 100 \times \left(\frac{\sum_{j=1}^{N} (\hat{C}_j - C_j)^2}{\sum_{j=1}^{N} (C_j)^2} \right)^{1/2}$$

where N is the number of samples; C_j, the concentration of the component in the jth mixture and $\hat{C}_j$ is the estimated concentration.

Table 3: statistical parameter values for simultaneous determination of DP, BN, GU and PH using optimized PLS-1 and GA-PLS methods

Parameter of interest	PLS-1 method				GA-PLS method		
	DP	BN	GU	PH	BN	GU	PH
Spectral range (nm)	215-280	240-340	240-300	240-300	261-264, 273-276, 329-332, 337-340	240, 261-264, 269-272, 277-280, 289-292	240-256, 277-280, 297-300
Conc. range (µg ml $^{-1}$)	10-15	40-60	40-60	4.8-7.2	40-60	40-60	4.8-7.2
No. of factors	5	2	2	3	2	2	3
RMSECV	0.280	0.723	0.752	0.102	0.675	0.629	0.086
Intercept	0.0274	0.12	0.0408	0.0264	0.2374	0.2306	0.0303
slope	0.9969	0.9977	0.9993	0.9957	0.9954	0.9951	0.9951
Correlation coefficient (r)	0.9935	0.9989	0.9994	0.9988	0.9984	0.9973	0.9974
Order of calibration data	Zero order	First order	First order	First order	First order	First order	First order

Table 4: percent variance captured by PLS-1 using zero order spectra (D0) and first order derivative spectra (D1).

Drug		% variance captured by PLS-1 using (D^0)				% variance captured by PLS-1 using (D^1)			
	No. of LV.	X-Block		Y-Block		X-Block		Y-Block	
		This LV	Total	This LV	Total	This LV	Total	This LV	Total
DP	1	83.66	83.66	2.85	2.85	72.43	72.43	2.91	2.91
	2	12.24	95.90	8.31	11.16	8.34	80.77	30.76	33.67
	3	0.63	96.53	50.64	61.80	12.27	93.04	22.49	56.16
	4	3.43	99.95	2.71	64.51	4.37	97.41	31.76	87.92
	5	0.01	99.96	33.59	98.09	0.82	98.24	7.52	95.45
	6	-----	-----	-----	-----	0.26	98.50	3.6	99.05
BN	1	93.30	93.30	99.03	99.03	68.59	68.59	97.03	97.03
	2	6.13	99.44	0.46	9949	24.78	93.37	2.50	99.54
	3	0.47	99.91	0.03	99.51	1.07	94.44	0.33	99.87
	4	-----	-----	-----	-----	2.06	96.50	0.08	99.95
	5	-----	-----	-----	-----	0.56	97.06	0.04	99.99
GU	1	13.41	13.41	97.42	97.42	61.62	61.62	89.63	89.63
	2	85.28	98.69	0.83	98.25	31.22	92.84	9.23	98.85
	3	1.28	99.97	0.35	98.60	2.74	95.57	0.43	99.29
	4	-----	-----	-----	-----	2.92	98.49	0.14	99.43
PH	1	85.24	85.24	8.07	8.07	26.79	26.79	41.62	41.62
	2	8.43	93.67	48.85	56.92	34.97	61.75	34.89	76.51
	3	6.29	99.97	39.33	96.25	35.13	96.88	21.52	98.04
	4	0.01	99.98	3.16	99.41	0.33	98.94	1.30	99.34

Table 5: Statistical comparison between application of PLS-1 model on absorption spectrum and first-order derivative spectra derivative of quaternary mixtures of DP, BN, GU and PH.

Drug	Method	RMSECV	Number of factors	Mean Recovery (%)	R.S.E.(%)
DP	1	0.280	5	100.72	2.022
	2	1.417	5	93.44	10.263
BN	1	0.799	2	100.16	1.421
	2	0.723	2	99.96	1.293
GU	1	1.612	2	102.74	3.418
	2	0.752	2	101.00	1.593
PH	1	0.211	3	99.76	3.180
	2	0.102	3	98.84	1.533

(1) PLS on absorption UV spectra, (2) PLS on first-order derivative spectra

The method was evaluated using statistical comparison between different applied methods on quaternary mixtures of DP, BN, GU and PH by models optimized; the results are in table 5. By considering values of RMSECV and relative standard error, it can be seen that application of PLS-1 method on first-order derivative spectra of BN, GU and PH; zero order spectra for DP represents better results for their determination in quaternary mixtures. the application of PLS-1 to first-order derivative spectra improved the performance of PLS-1 modeling for GU and PH and has bad or little significant effect on the prediction of DP or BN respectively.PLS-1 method was run on the calibration data using optimal number of latent variables. The concentrations of four components in calibration set were calculated as shown in table 1. By plotting predicted concentrations of each component versus actual concentrations, a straight line is obtained. The data of the straight line for

each component including slope, intercept and correlation coefficient is collected in table 3. PLS-1 method was run on the validation set using optimal number of factors and predicted concentrations of DP, BN, GU and PH are given in table 2.

GA-PLS method

Constructing the PLS model after selecting the optimal variables (wavelengths) improves the prediction capacity of the model [12, 13]. GA can be used successfully for wavelength selection in PLS calibration. GA for wavelength selection consists of five steps: A-Initiation: different combinations of wavelengths are generated randomly, each combination represents a possible solution. Each wavelength in the spectrum is assigned randomly a value of 1 or 0, where 1 indicates selection and 0 indicates omitting. The different possible solutions are called population and every single solution is a chromosome, where the wavelengths are the genes. B-Evaluation: each different chromosome is used to construct a PLS model and cross validation is used to evaluate the prediction error of each chromosome. C-Exploitation: selection of good chromosomes. D- Exploration: recombination of good genes. E- Mutation: changing chromosomes locally to hopefully form better chromosomes. The new chromosomes produced are tested again for performance and the algorithm continues until a certain number of generations are produced. A critical issue of successful GA performance is the adjustment of GA parameters. The parameters allowed for adjustment in PLS-Toolbox are: the maximum number of generations, the number of wavelengths in a window, percent genes included at initiation, the mutation rate, breeding cross over rule and percent of population the same at convergence. Other parameters to be chosen by the user are : maximum number of latent variables for the PLS, cross validation type random or contiguous blocks, number of subsets to divide data into for cross validation, number of iterations for cross validation at each generation. The configuration of GA parameters is shown in table 6. The fitness values were used as response variables. Mutation rate was 0.005 in all cases except in BN was 0.001 as when it increased above these values, no convergence occurred between average fitness and best fitness values and model stop at generation number 7. Mean centering was used as preprocessing data process as auto scaling lead to poor recovery % for application and standard addition. Each solution (chromosome) is evaluated using the PRESS value reached in the calibration.

Table 6: Parameters of the genetic algorithms

Parameter	value
Population size	20
Maximum generations	50
Mutation rate	0.005 (except in BN=0.001)
the number of variables in a window (window width)	4
per cent of population the same at convergence	100
% wavelengths used at initiation	50
Crossover type	Single
Maximum number of latent variables	4
number of subsets to divide data into for cross validation	4
number of iterations for cross validation at each generation	2

The genetic algorithm searches for the minimum RMSECV in the space of all the possible chromosomes without establishing, a priori, the latent structure of the calibration. The GA was run for 66 variables (in the range 215–280 nm) for DP, 101 variables (in the range 240–340 nm) for

BN and 61 variables (in the range 240–300 nm) for GU and PH using a PLS regression method with maximum number of factors allowed is the optimal number of components determined by cross-validation on the model containing all the variables, and the selected variables were used for running of PLS. For DP, the GA model was very poor in predicting DP concentration in calibration and validation mixtures this is may be due to lack of characteristic features in DP spectrum so all the wavelengths have to be included in the model while the GA –PLS model predicted other three analytes with high efficiency. Fig 4, 5 and 6 show the frequency with which each variable was preselected for the three analytes. For obtaining the optimum set of wavelength for determination of each drug, the GA procedure was repeated 10 times. Finally a wavelength was selected if the percent of selection for that variable exceeds a critical value. The thresholds of 80% were obtained for the three drugs, according to minimum error of prediction for each analyte. The selected wavelengths are shown in table 3.GA did not reduce the optimal number of factors for three analytes as shown in table 3 but RMSECV was slightly decreased indicating an increase in power of prediction of GA-PLS model than PLS model. The predictive ability of the method was further checked on validation set, the results are shown in table1and table 2. The comparison of GA-PLS results with PLS-1 shows, the GA-PLS is more suitable for simultaneous determination of these three analytes.

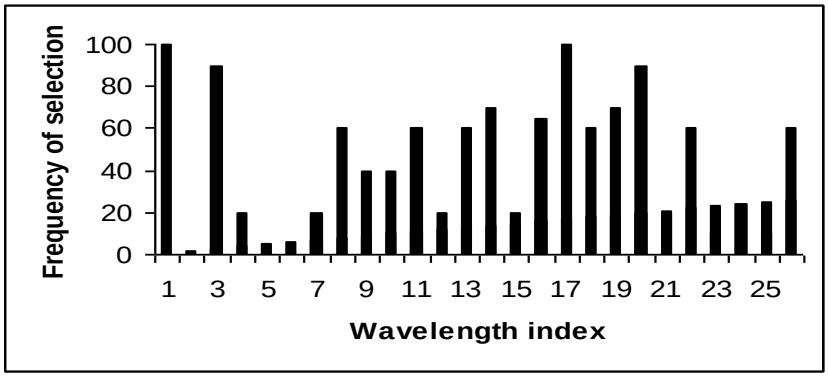


Fig. 4. Frequency of selection of wavelength for BN.

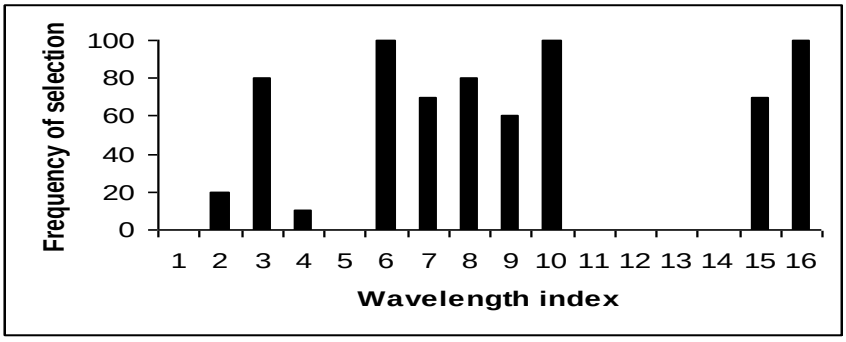


Fig. 5. Frequency of selection of wavelength for GU.

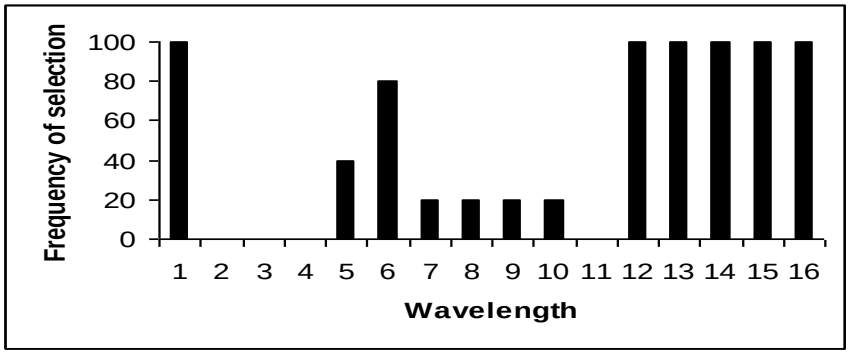


Fig. 6. Frequency of selection of wavelength for PH.

Application to pharmaceutical preparation

The produced models were used for the analysis of pharmaceutical preparation containing the mixture. The results are shown in table 7. Each value indicated is the mean of 3 determination of the same commercial batch. The results obtained from the analysis of pure powders of the analytes in presence of pharmaceutical excipients added by the manufacturer (polypropylene glycol and polyethylene glycol) are indicated in Table 8. The results obtained were compared for the mean and the standard deviation using the t-test and F-test, respectively. There were no significant differences. In addition, the results found were in good agreement with the data indicated in the formulation given by the manufacturer.

Table 7: Results of analysis of Bronchofree Capsules by proposed methods

	PLS-1				**GA-PLS**		
Sample no.	DP R%	BN R%	GU R%	PH R%	BN R%	GU R%	PH R%
1	98.15	101.00	100.09	98.15	100.15	99.06	98.50
2	97.67	98.70	100.50	100.88	99.02	99.02	99.38
3	97.14	99.07	97.10	101.32	99.89	99.82	98.57
4	98.47	100.01	99.37	100.78	101.53	98.27	100.17
Mean	97.86	99.70	99.27	100.28	100.15	99.04	99.15
S.D.	0.580	1.030	1.517	1.441	1.041	0.635	0.783

Table 8: Statistical comparison between analysis results of four components in presence and absence of pharmaceutical excipients by proposed methods

	PLS-1 method								GA-PLS					
	DP R%		BN R%		GU R%		PH R%		BN R%		GU R%		PH R%	
Sample no.	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence
1	99.62	98.81	100.62	100.97	99.62	100.76	100.08	100.63	100.46	101.15	98.52	99.98	101.00	101.50
2	100.38	100.50	100.16	100.52	100.05	99.50	101.03	101.05	100.34	101.25	99.07	99.30	99.17	100.63
3	101.50	100.54	98.81	98.82	100.04	100.49	100.42	99.33	100.65	100.50	100.27	101.38	100.67	100.17
Mean	100.50	99.95	99.86	100.10	99.90	100.25	100.51	100.34	100.48	100.97	99.29	100.22	100.28	100.76
S.D.	0.946	0.987	0.941	1.134	0.245	0.663	0.481	0.896	0.156	0.407	0.893	1.062	0.977	0.677
Variance	0.895	0.974	0.885	1.286	0.060	0.440	0.231	0.803	0.024	0.166	0.797	1.128	0.955	0.458
Degree of freedom	4		4		4		4		4		4		4	
t-test(2.776)*	0.524		0.792		0.468		0.787		0.166		0.311		0.521	
F-teat(19)*	1.088		1.453		7.33		3.476		6.92		1.415		2.085	

* tabulated value at p= 0.05

4. Conclusion

The two proposed chemometric methods were simple, rapid, sensitive and precise and could be easily applied in quality-control laboratories for the simultaneous spectrophotometric determination of DP, BN, GU and PH in pure powder and in Bronchofree capsules**.** The first method is PLS-1 using full spectrum and it was suitable for determination of 4 components. The second method was GA-PLS in which GA function present in PLS_ Toolbox was used as a mean of wavelength selection. GA-PLS had an advantage of reducing variables and so increasing predictive ability of PLS model but it was not suitable for determination of DP may be due to lack of characteristic features in its spectrum, so all wavelengths should be included in the model. The fundamental advantages of the investigated methods are the simultaneously analysis of the mixture components without any chemical pre-treatment and during a short period of time, as well as no complex instruments are required. Moreover, the proposed methods are the first publication for the simultaneous determination of DP, BN, GU and PH in pharmaceutical preparationCC and HCT in pure bulk powders. Moreover, they could be applied for dosage form analysis as well as in pure powder form without any preliminary separation step.

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-VPP-322

References

[1] S. Budavari, M. O'Neal, A. Smith, P. Heckelman, and J. Kinneary, "The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 12th edn. Whitehouse Station, NJ: Merck & Co," ed: Inc, 1996.
[2] A. Goodman-Hillman, T. Rall, A. Nier, and P. Taylor, Eds., The Pharmacological Basis of Therapeutics. New York: McGraw-Hill, 1996.
[3] S. C. Sweetman, Martindale-The Complete Drug Reference, 36 ed. London, UK: The Pharmaceutical Press, 2009.
[4] S. M. Kishk, I. Salama, S. Mostafa, and M. El-Sadek, Journal of Liquid Chromatography & Related Technologies, 2013.
[5] T. Pham-Gia and Q. Duong, Mathematical and Computer Modelling, **12**, 1613 (1989).
[6] R. G. Brereton, Analyst, **125**, 2125 (2000).
[7] J. Nevado, J. R. Flores, and G. C. Penalvo, Analytica chimica acta, **340**, 257 (1997).
[8] C. B. Lucasius, G. Kateman, Chemometrics and Intelligent Laboratory Systems, **19**, 1 (1993).
[9] C. B. Lucasius and G. Kateman, Chemometrics and Intelligent Laboratory Systems, **25**, 99 (1994).
[10] D. B. Hibbert, Chemometrics and Intelligent Laboratory Systems, **19**, 277 (1993).
[11] Z. Michalewicz, Genetic algorithms+ data structures: Springer, 1996.
[12] R. Leardi, Journal of Chemometrics, **14**, 643 (2000).
[13] R. Leardi, M. B. Seasholtz, R. J. Pell, Analytica Chimica Acta, **461**, 189 (2002).
[14] R. G. Brereton, Analyst, **122**, 1521(1997).
[15] A. Espinosa-Mansilla, F. Salinas, I. De Orbe Paya, Analytica Chimica Acta, **313**, 103 (1995).
[16] A. C. Moffat, D. M. Osselton, B. Widdop, Clarke's analysis of drugs and poisons: in pharmaceuticals, body fluids, and postmortem material, 3 ed.: Pharmaceutical press, 2004.
[17] W.-J. Lin, Y.-Y. Chen, and R. R.-L. Chen, International journal of pharmaceutics, **176**, 179 (1999).

[18] M. Blanco, J. Coello, F. Gonzalez, H. Iturriaga, and S. Maspoch, Journal of pharmaceutical sciences, **82**, 834 (1993).
[19] D. M. Haaland and E. V. Thomas, Analytical Chemistry, **60**, 1193 (1988).
[20] N. A. El-Ragehy, S. S. Abbas, S. Z. El-Khateeb, Analytica chimica acta, **461**, 155 (2002).
[21] H. Martens, T. Naes, Multivariate calibration: John Wiley & Sons, 1991.