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Chemometric-assisted UV Spectrophotometric and RP-HPLC Methods for the Simultaneous Determination of Caffeine and Sodium Benzoate in Synthetic Mixture

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Abstract

In present work, chemometric-assisted UV spectrophotometric and RP-HPLC methods weredeveloped for the simultaneous estimation of caffeine (CAF) and Sodium benzoate (SB) in their combined pharmaceutical dosage form. The two chemometric methods i.e. principle component regression (PCR) and partial least square regression (PLS) were successfully applied to quantify each drug in mixture using UV absorption spectra in range of 220 – 280 nm at $\Delta\lambda$ of 1 nm. Chemometric model development was done using 30 binary solutions and 10 solutions were used for validation of models. The chemometricmethod does not require any prior separation step. In addition RP-HPLC method was developed using HiQSil C18 column with a mobile phase consisting of methanol: acetate buffer (50:50 % v/v), pH adjusted to 4.4 with UV detection at 224 nm and flow rate of 1 ml/min. The methods were successfully applied for the simultaneous determination of these drugs in synthetic mixture.The results obtained for analysis by PCR and PLS methods were compared with RP-HPLC method and a good agreement was found.

Keywords: Chemometric, PCR, PLS, HPLC, Caffeine, Sodium benzoate

Introduction

Caffeine (CAF) is chemically1, 3, 7-trimethylpurine-2; 6-dione(Figure 1a)and is a well-known CNS stimulant.CAFstimulates medullary, vagal, vasomotor and respiratory centers, promoting bradycardia, vasoconstriction and increased respiratory rate by antagonists at adenosine-receptors within the plasma membrane of virtually every cell (1). Sodium benzoate (SB) is added to increase the solubility of CAF and its structure is depicted in Figure 1b. The USP specifies gravimetric assay for CAF and titrimetric method for SB (2).

Chemometric was introduced in 1972 by SvanteWold (3). Chemometric is the science of extracting information from chemical systems. Multivariate calibration method (e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data are the important chemometric approach for determination of mixtures including drugs combination (4).

Literature survey reveals that there are reported methods on estimation of CAF and SB in combination by UV (5, 6) and HPLC(7) methods. The reported UV spectrophotometric methods are based on multicomponent analytical

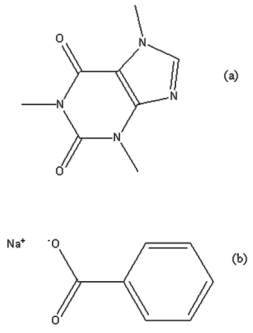

Fig. 1. Chemical structure of (a) caffeine and (b) sodium benzoate

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method butin the present work, the chemometricmultivariate calibration methods are applied for the multicomponent analysis of drug substances which provides more powerful analysis tool. HPLC method was also developed and validated for the simultaneous determination of CAF and SB. The injection sample (blend)was assayed with the optimized chemometric-assisted spectrophotometric method and HPLC method for comparison.

Material and Methods

Instrumentation: The apparatus used for chemometric analysis was double beam UV-Vis spectrophotometer (Jasco V-550, Japan) with matching pair of 1 cm quartz cells. The absorbance spectra of calibration set and validation set were recorded in range of 220-280 nm with $\Delta\lambda$ of 1 nm. PCR and PLS calculations were performed on Unscrambler X 10.3 (trial version) and MS-Excel 2007.

The RP-HPLC was carried on JASCO HPLC (PU 2080 Plus, Japan) equipped withJasco PDA detector (PU 2010 Plus, Japan). Samples were injected through Rheodyne sample injection port (20 µl). HiQSilC18 Column(250 x 4.5mm, i.d. 5 ìm) was used. Data acquisition and integration was performed usingBorwin software (version 1.5).

Material and Reagent: Pure drug samples of Caffeine and Sodium benzoate were kindly gifted by S. D. Fine Chemicals ltd. (Mumbai, MH, India). The standards were used without further purification. HPLC grade water was obtained from ELGA LABWATERpurification system (PURELAB UHQ-11, United Kingdom). Methanol used for HPLC was of HPLC grade and that used for spectrophotometry was of AR grade (LOBA Chemie, Mumbai, MH, India).

Chromatographic Conditions: The mobile phase was prepared by mixing methanol and 10mMAcetate buffer (pH adjusted to 4.4 using glacial acetic acid) in ratio (50:50 % v/v). The flow rate was 1 ml/min. Quantitation based on peak area was achieved using PDAdetector at 224 nm. All determinations were performed at ambient temperature.

Standard stock solutions: Stock solution of CAF and SB were prepared by dissolving accurately weighed 10 mg of standard drugs in 10 ml of double distilled water, separately (1000 ìg/ml). Gentle warming was carried out to dissolve caffeine properly in water. From above solution further 5 ml was pipetted and diluted to 50 ml (100 ìg/ml) of CAF and SB, respectively.

Working solutions: Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution to obtain final concentration of 2.5-15 µg/ml for HPLC (dilution with mobile phase) and 5-25 µg/ml for chemometric analysis (dilution with double distilled water) for both the drugs.

Construction of calibration and validation set for chemometric approach: A total set of 40 mixtures were prepared by combining working standard of CAF and SB in their linear concentration range of 5-25 µg/ml for both drugs (Table 1). From these 30 mixtures were used for calibration set and 10 mixtures were used for validation set. The validation set was randomly selected. The absorbance spectra were recorded in range of 220- 280 nm with 1 nm interval.The absorbance data of the calibration set were then processed throughUnscramblersoftware for development of PCR and PLS models. For validation generated models, the concentrations of drugs in validation set were predicted by using the proposed PCR and PLS models.

Single Component Calibration: To find linear concentration range of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 5.0-30.0 µg/ml for both drugs. Figure 2 represents overlain spectra CAF and SB.

Calibration curves for the HPLC method: Working solutions in the concentration range of 2.5-15.0 µg/ml were injected in triplicateand chromatogram was obtained under the specified chromatographic conditions described previously. The calibration graph was constructed by plotting peak area versus concentration of each drug and the regression equation was calculated.

Analysis of Drug in synthetic mixture: The sample solution of injection of caffeine and sodium benzoate was prepared in the laboratory as it was not easily available in the market. The injection was prepared by dissolving caffeine in water for injection with sodium benzoate to increase the solubility of caffeine. The volume was made up to make a solution containing dose of 100 ìg/ml each. The sample solution of injection was then used for assay performance and % recovery studies by preparing dilutions in double distilled water to get standard concentration of 10µg/ml each, procedure was repeated for six times

Validation by HPLC method: For validation of the developed method, the ICH Q2 (R1) guidelines were followed. The requirement for drug assay follows these topics: linearity, precision, accuracy, specificity, robustness, LOD and LOQ.

Linearity*:* Working standard solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 2.5-15 µg/ml of CAF and SB, respectively. Six sets of such solutions

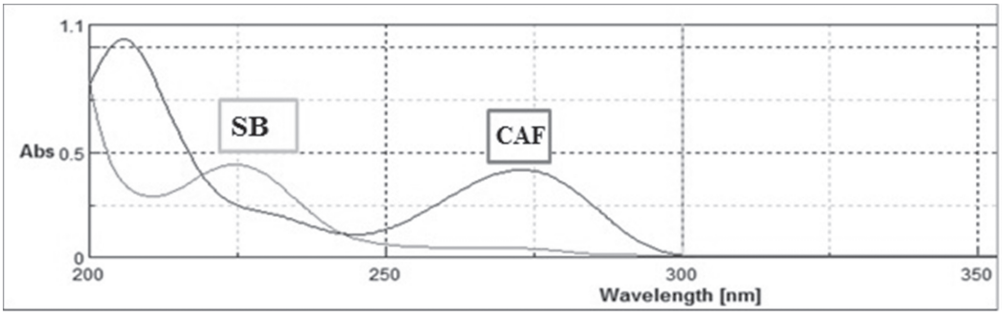

Fig. 2. Overlaid spectra of caffeine and sodium benzoate(10 µg/ml each)

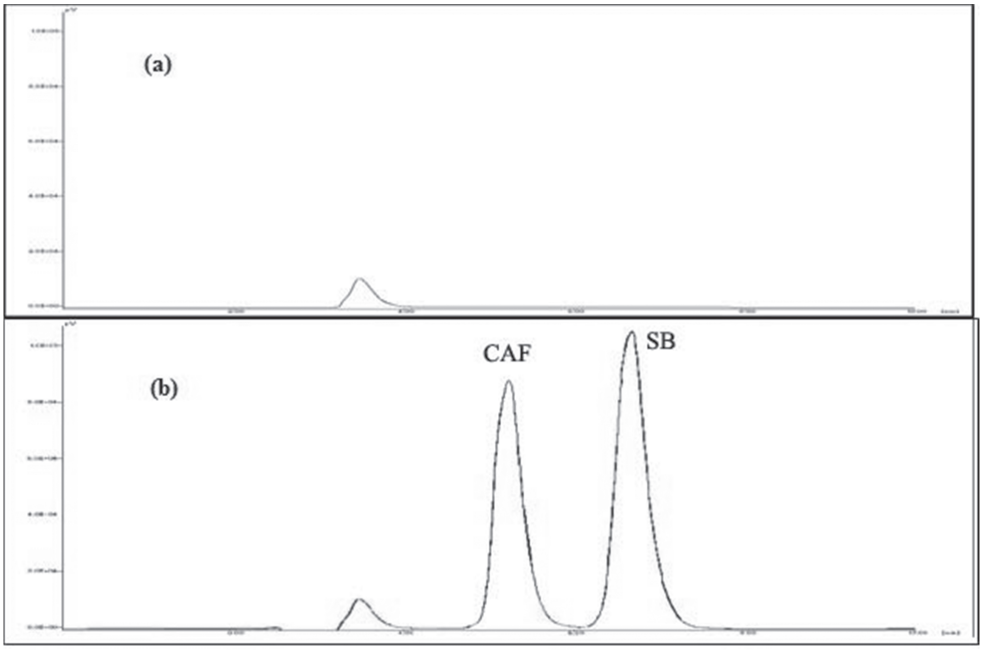

Fig. 3. (a) Blank (b) Chromatogram of caffeine and sodium benzoate

were prepared. Each set was analyzed to plot a calibration curve. Standard deviation (SD), slope, intercept and correlation coefficient (R^2) of the calibration curves were calculated to ascertain the linearity of the method.

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies, 6 replicates of CAF (10 µg/ml) and SB (10µg/ml) were analyzed in a day and % RSD was calculated. For the inter day variation studies, 3 different concentrations (5, 7.5 and 10 µg/ml) were analyzed on 3 consecutive daysfor both the drugs and % RSD were calculated.

Accuracy*:* To check accuracy of the method, recovery studies were carried by spiking the standard drug to the sample injection solution, at three different levels of 50, 100 and 150 %.The % recovery and % RSD were calculated.

Specificity*:* The specificity of the method was ascertained by injecting placebo preparations without main drugs in the mixture and further confirmed by peak purity profiling studies. The peak purity values were found to be more than 991, indicating no interference of excipients at analytes Rt.

Robustness*:* Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate were altered and the effect on the area were noted.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) : The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at a signal-to-noise ratio (S/N) of 3 and 10. TheLOD and LOQ were theoretically verified by the equations. LOD = 3.3 σ/Sand LOQ = 10σ/S, where, σ is the standard deviation of the intercept and S is the slope of calibration curve.

Result and Discussion

Chemometric method: The first step in multivariate methods involved constructing the calibration matrix. The wavelength range used was 220 to 280 nm. Sixty one spectral points at 1 nm intervals were selected within this range. The compositions of the calibration mixtures were randomly designed in order to collect maximum information from the spectra of these mixtures.The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used. The PCR and PLS models were developed by the Unscrambler® program. Model development was performed by using 30 mixtures as calibration standards. Leave-one-out cross-validation (LOO-CV) was used to validate the PCR and PLS models in model development and obtain optimum latent variables (number of factors) of the model. The predicted concentrations of the components in each sample were compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated for each method. The RMSECV was used as a diagnostic test for examining the error in the predicted concentrations. The resulting models were also validated by prediction of the concentration of analytes in a separate validation set shown in Table 2. Statistical parameters of the chemometric methods are represented in Table 3.

HPLC method: The validity of the analytical procedure as well as the resolution between the peaks of interest is ensured by the system suitability test (Table 4). All critical parameters tested met the acceptance criteria. As shown in the chromatogram (Figure 3), the two analytes are eluted by forming symmetrical single peaks well-separated from each other. CAF and SB showed a good correlation coefficient in the concentration range of 2.5-15 µg/ml. The linear regression analysis obtained by plotting the peak areas of analytes vs. concentration showed excellent correlation coefficients (correlation coefficients greater than 0.990). The proposed method afforded high recoveries of almost 100 % for CAF and SBin synthetic formulation, indicating that this assay procedure can be used for the routine quality control analysis of the pharmaceutical dosage form.

The system precision is a measure of the method variability that can be expected for a given

Table 1. Calibration set and Validation set

Mix. No	SB	CAF	Mix. No	SB	CAF
1	5	5	21	25	5
2	5	10	22	25	10
3	5	15	23	25	15
4	5	20	24	25	20
5	5	25	25	25	25
6	10	5	26	5	30
7	10	10	27	10	30
8	10	15	28	15	30
9	10	20	29	20	30
10	10	25	30	25	30
11	15	5	31	10	5
12	15	10	32	15	5
13	15	15	33	20	10
14	15	20	34	25	10
15	15	25	35	5	15
16	20	5	36	10	15
17	20	10	37	10	20
18	20	15	38	30	20
19	20	20	39	15	25
20	20	25	40	30	25

1-30: Calibration set; 31-40: Validation set

Table 2. Percentage recovery results of caffeine and sodium benzoate in validation set by the proposed PCR and PLS chemometric methods.

METHOD		PCR				PLS			
SB	CAF	SB		CAF		SB		CAF	
Actual (ìg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
10	5	9.928	99.2	5.041	100.8	9.963	99.6	5.144	102.8
15	5	15.517	103.4	5.123	102.4	15.125	100.8	5.138	102.7
20	10	20.371	101.8	10.085	100.8	20.312	101.5	10.051	100.5
25	10	24.830	99.3	9.988	99.8	24.891	99.5	9.987	99.8
5	15	4.960	99.2	14.915	99.4	4.971	99.4	14.892	99.2
10	15	10.126	101.2	14.948	99.6	10.029	100.2	14.970	99.8
10	20	10.013	100.1	20.161	100.8	10.113	101.1	19.866	99.3
30	20	29.816	99.3	20.132	100.6	29.768	99.2	20.193	100.9
15	25	15.017	100.1	24.941	99.7	15.087	100.5	24.913	99.6
30	25	29.774	99.2	25.024	100.0	29.757	99.1	25.128	100.5

%R*: percent recovery

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Table 3. Statistical parameters of caffeine and sodium benzoate using the proposed PCR and PLSchemometric methods

Statistical Parameters	SB		CAF	
	PCR	PLS	PCR	PLS
Range (ig/ml)	5.0-30.0		5.0-30.0	
Offset	0.039	0.039	0.029	0.029
Regression Coefficient (R^2)	0.995	0.995	0.997	0.997
RMSE	0.714	0.714	0.616	0.616
PC/Factors	2		2	

Table 4. System suitability parameters for CAF and SB

Drug	Concentration (ig/ml)	RT (Min)	Area	Plates	Asymmetry	Resolution
CAF	10	5.30 ± 0.05	772011	4546	1.04	2.73
SB	10	6.90 ± 0.09	2171667	3897	0.98	3.12

Table 5. Summary of validation parameters by proposed HPLC method

Sr. No.	Validation Parameter	CAF	SB
1.	Regression Equation	y = 93883x + 17560r^2 = 0.998	y = 19060x + 27869 r^2 = 0.992
2.	Range	2.5-15 µg/ml	2.5-15 µg/ml
3.	Intraday precision (% RSD)	0.505	1.337
4.	Interday precision (% RSD)	1.427	1.584
5.	LOD (µg/ml)	0.419	0.464
6.	LOQ (µg/ml)	1.270	1.407
7.	Accuracy (% recovery)	100.150	100.500
8.	Robustness (% RSD)	<2	<2
9.	Specificity	Specific	Specific

Table 6. Assay results of CAF and SB by the proposed PCR, PLS chemometric methods and HPLC method

DRUG Actual amount	**CAF** 5µg/ml			**SB** 5 µg/ml		
Method	HPLC	PCR	PLS	HPLC	PCR	PLS
*Amount found	4.984	4.998	4.979	5.029	5.007	5.009
% Amount found	99.68	99.97	99.59	100.58	100.15	100.18
P value		0.7983			0.7148	
F value		0.2287			0.3435	

*Average of 6 determinations for HPLC, PCR and PLS methods.

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analyst performing the analysis and was determined by performing six repeats. The %RSD for CAF and SB response was found to be less than 2.0 (values in limit). The intermediate precision was assessed by analyzing three different concentrations from the calibration linearity on three different days and intraday precision was assessed by analyzing three different concentrations from the calibration curve on the same day. % RSD for precision was in limit. The summary of HPLC results are depicted in Table 5.

Comparison of the Chemometric method with the HPLC Method: In order to compare the results of the proposed PCR and PLS models for the determination of CAF and SB in synthetic mixture, the HPLC method was also employed. The same samples solutions used for the PCR and PLS models were analyzed by the HPLC method. The determination results of PCR, PLS, and HPLC methods are presented in Table 6. The data were expressed in terms of percent labeled amount. The results showed that the average percent labeled amount obtained from the PCR and PLS models were not significantly different from those obtained from the HPLC method with the confidence limit of 95%.

Conclusion

RP-HPLC techniques are generally used for separation and determination of components in pharmaceutical formulations and are considered superior with regard to identification and specificity. However, the chemometric methods are less expensive by comparison and do not require sophisticated instrumentation nor any prior separation step. The proposed chemometric-assisted spectrophotometric methods are applicable and specific for the simultaneous determination of CAF and SB in their synthetic mixtures. The results obtained were compared with the proposed RP-HPLC method and good coincidence in the means of recovery was observed as there was no significant difference between the methods compared. The three proposed methods were accurate, precise with good reproducibility and sensitivity; hence can be used for the routine analysis of these in their combined pharmaceuticals.

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