

Simultaneous estimation of beclomethasone dipropionate and salbutamol sulphate in capsules by chemometric assisted UV-spectrophotometric method

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Abstract

A convenient new method for simultaneous estimation of Beclomethasone dipropionate and Salbutamol sulphate, with minimum sample pretreatment is described in this paper. The procedure, based on the multivariate analysis of spectral data in the 220–260 nm region by the partial least squares method (based on SIMPLS algorithm) has been evaluated. The evaluation of calibration model is relied on the coefficient of determination (R^2) and root mean square error of calibration (RMSEC). The coefficient of determination (R^2) for the relationship between actual values and predicted values of both drugs was higher than 0.9930, indicating good accuracy of the developed method while RMSEC values (0.8637) obtained were relatively low which indicate acceptable precision of analytical method. The experimental calibration and validation matrixes were constructed with 55 and 24 samples, respectively. The concentration range considered was 10.0–60.0 $\mu\text{g/ml}$ for both the drugs. The method was successfully applied for estimation of drugs in pharmaceutical formulation, with no interference with excipients as indicated by the recovery studies. The proposed method is simple, rapid and can be easily used in the quality control of drugs as alternative analysis tools.

Key Words: Chemometric, Partial least square, Beclomethasone dipropionate, Salbutamol sulphate

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Introduction

Beclomethasone dipropionate (BEC) chemically is 9 α -chloro-11 β -hydroxy-16 β -methyl-3, 20-dioxopregna-1, 4-diene-17, 21-diyl dipropionate (Fig. 1 A) and Salbutamol sulphate (SAL) chemically is (RS)-1-(4-hydroxy-3-hydroxy-methylphenyl)-2-(tert-butylamino) ethanol sulphate (Fig. 1-B). Beclomethasone dipropionate is a steroidal drug used in asthma while Salbutamol Sulphate is a β_2 agonist. There are many drugs in individual form and in combination forms available in the market for the management of bronchial asthma but Salbutamol sulphate in combination with Beclomethasone dipropionate is highly prescribed¹.

Literature survey reveals, simple UV², RP-HPLC^{3,4}, stability indicating HPLC⁵ and UPLC⁶ methods for estimation of Beclomethasone dipropionate and Salbutamol sulphate in combination.

Under computer controlled instrumentation, chemometric methods (multivariate calibration) are playing a very prominent role in the multicomponent analysis of mixtures by UV-VIS spectrophotometry⁷. The approach is useful in the resolution of band overlapping in quantitative analysis. The chemometric

methods have been found to be the method of choice for complexed mixtures. The advantage of multicomponent analysis using multivariate calibration is the speed of the determination of the components in a mixture, avoiding a preliminary separation step⁸. Control analysis on pharmaceutical preparations using the chemometric method has been proved to be a valid alternative to HPLC.

To the best of our knowledge no chemometric-assisted UV spectrophotometric method has been reported for simultaneous estimation of Beclomethasone dipropionate and Salbutamol sulphate hence, the work was undertaken.

Materials and Methods

Instrumentation: Double beam UV-Vis spectrophotometer (Jasco V-550, Japan) with matched pair of 1 cm quartz cells were used to record spectra of all solutions. Spectra were acquired between 220 - 260 nm (data pitch 1 nm) i.e. from 41 different wavelengths. The spectra were recorded at scanning speed 400 nm/min. PLS toolbox (version 4) with MATLAB 7.0 and Microsoft Excel 2007 were used for application of chemometric and mathematical calculations.

Reagents and chemicals: Authentic sample of Beclomethasone dipropionate and Salbutamol sulphate were obtained from Cipla Pharmaceuticals Ltd (Mumbai, MH, India) as gift samples. The formulation Aerocort Rotacaps[®] (Cipla Pharmaceuticals Ltd, Mumbai, MH, India) labeled to contain Beclomethasone dipropionate (IP) 100 mg and Levosalbutamol sulphate (IP) equivalent to salbutamol 100 mg was procured from local market. Methanol AR

grade was obtained from S.D. Fine Chem. Ltd (Mumbai, MH, India).

Preparation of standard stock and working solutions: Standard solutions of each drug BEC and SAL were prepared by separately dissolving 10 mg of each drug in 10 ml methanol (1000 µg/ml). From this solution, further 1 ml was pipetted and diluted to 10 ml (100 µg/ml). Working solutions were prepared from standard stock solution of (100 µg/ml) by appropriate dilution to obtain final concentration of 10.0-60.0 µg/ml for both the drugs.

Construction of calibration and validation set: The calibration and validation mixture sets were prepared by mixing the BEC and SAL solutions in different ratios varying within their individual linearity range viz. 10.0 – 60.0 µg/ml. A total of 79 mixtures were prepared out of which 55 were used for calibration set whereas, the rest 24 served as validation set (Table 1 and Table 2). The validation set was randomly selected from linearity range. All the mixtures were scanned at 220 - 260 nm range. The PLS model was developed utilizing absorption data using MATLAB software.

Preparation of sample solution for assay: Twenty capsules (Aerocort Rotacaps®) were emptied and weighed. Powder equivalent to 10 mg of BEC and 12.04 mg of SAL (equivalent to 10 mg of salbutamol) was transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 minutes and volume was made to 10 ml. Solution was filtered and further dilutions were made with methanol to get final concentration of 10 µg/ml of BEC and 12.04 µg/ml of SAL. Procedure was repeated for six times. % recovery was determined by applying developed PLS model (Table 3).

Accuracy: To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the Aerocort Rotacaps® sample solution (assay solution) at three different levels of 50, 100 and 150%. Basic concentration of sample solution chosen was 10 µg/ml and 12.04 µg/ml for BEC and SAL, respectively. Results are depicted in Table 4 as % recovery.

Results and Discussion

The overlay of absorption UV spectra of pure BEC and SAL in methanol in wavelength range of 220-260 nm is shown in Fig. 2, which exhibited excessive overlapping of drugs making the difficulty of analysis of these drugs using UV spectrophotometry without any treatment, hence PLS was applied.

Quantitative analysis of BEC and SAL was performed with the aid of PLS calibration (multivariate method). The first step in multivariate methods involves constructing the calibration matrix. To confirm good predictability of generated model the 2D scores plot for the first two latent variables (LVs) of the whole calibration matrix was obtained to confirm the well position of the mixtures in space, orthogonality, symmetry and rotatability as indicated in Fig. 3. Mean centering of the data was done for getting the optimum results. Leave one out (LOO) cross validation is used in our study for optimizing the number of PLS components and is calculated using below formula,

$$RMSECV = \sqrt{\frac{\sum (C_{act} - C_{pre})^2}{Ic}}$$

Where,

RMSECV = Root mean square error of cross validation

C_{act} = actual concentration of calibration set

C_{pre} = predicted concentration of validation set

Ic = Total number of samples in calibration set

The generated model showed RMSECV value of 0.9088. The selection of the optimum number of LVs was a very important preconstruction step, if the number of factors retained was more than required, more noise would be added to the data; if the number retained was too small, meaningful data that could be necessary for the calibration might be lost. After the PLS model has been constructed, it was found that the optimum number of LVs described by the developed models was two factors as shown in Fig. 4.

After optimization of parameters and calibration step, model was applied successfully for analysis of BEC and SAL in validation set consisting of 24 samples (Table 2). The plot of the predicted concentration versus actual values is shown in Fig. 5. The results suggested that the present method is accurate in concern to the validation samples, as suggested by the low RMSE (root mean square error) and REP (relative error of prediction) value for validation set.

In order to test the applicability of the proposed method, the produced model was used to predict the concentrations of the BEC and SAL in pharmaceutical formulation. The mean assay results obtained were 100.04% and 99.99% for BEC and SAL respectively (Table 3). Accuracy results obtained also showed almost 100 % recovery for both the drugs with % RSD of less than 2 (Table 4).

Table 1: Concentration data of the different mixtures of BEC and SAL used in the calibration set

Mix. No.	BEC (µg/ml)	SAL (µg/ml)	Mix. No.	BEC (µg/ml)	SAL (µg/ml)
1	10	10	29	30	10
2	10	15	30	30	15
3	10	20	31	30	20
4	10	25	32	30	25
5	10	30	33	30	30

6	10	35	34	30	35
7	10	40	35	30	40
8	15	10	36	35	10
9	15	15	37	35	15
10	15	20	38	35	20
11	15	25	39	35	25
12	15	30	40	35	30
13	15	35	41	35	35
14	15	40	42	35	40
15	20	10	43	40	10
16	20	15	44	40	15
17	20	20	45	40	20
18	20	25	46	40	25
19	20	30	47	40	30
20	20	35	48	40	35
21	20	40	49	40	40
22	25	10	50	50	10
23	25	15	51	50	20
24	25	20	52	10	50
25	25	25	53	15	60
26	25	30	54	10	60
27	25	35	55	60	15
28	25	40	-	-	-

Table 2: Concentration data of the different mixtures of BEC and SAL used in the validation set along with its prediction data and % recovery

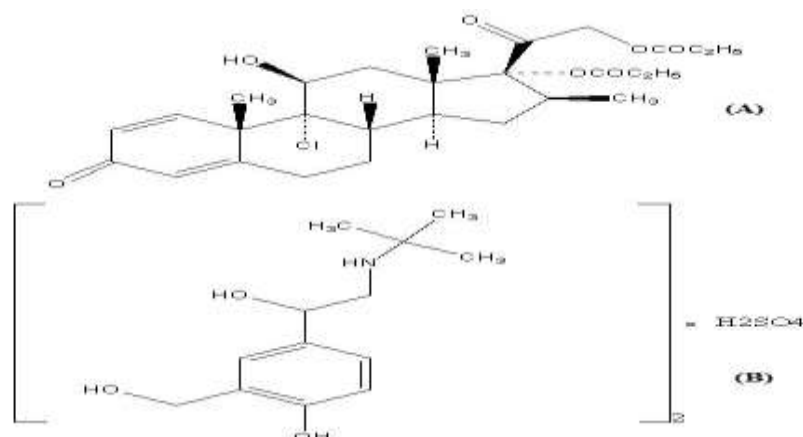
Mix. No.	BEC Actual (µg/ml)	SAL Actual (µg/ml)	BEC Predicted (µg/ml)	BEC % recovery	SAL Predicted (µg/ml)	SAL % recovery
1	10	15	10.024	100.241	14.882	99.213
2	10	25	10.018	100.184	25.101	100.406
3	10	35	10.051	100.510	35.073	100.208
4	15	10	14.931	99.541	9.991	99.907
5	15	20	14.880	99.197	19.888	99.439
6	15	30	14.885	99.232	29.963	99.877
7	15	40	14.898	99.318	40.090	100.225
8	20	15	19.901	99.503	14.879	99.191
9	20	25	20.371	101.854	25.013	100.052
10	20	35	20.206	101.031	34.874	99.640
11	25	10	25.313	101.253	10.119	101.186
12	25	20	24.894	99.576	19.997	99.984
13	25	30	25.258	101.032	30.044	100.146
14	25	40	25.190	100.761	40.422	101.055
15	30	15	30.292	100.972	15.203	101.353
16	30	25	30.261	100.870	25.278	101.111
17	30	35	30.015	100.052	35.112	100.319
18	35	10	34.887	99.678	10.106	101.058
19	35	20	34.859	99.598	19.808	99.040
20	35	30	35.055	100.157	30.186	100.619
21	35	40	34.960	99.885	39.875	99.688
22	40	15	40.112	100.281	14.982	99.877
23	40	25	40.134	100.335	25.188	100.751
24	40	35	39.890	99.726	34.802	99.435
Mean % recovery			-	100.199	-	100.157

Table 3: Result for BEC and SAL obtained from commercial formulations (assay)

Mix. No	BEC			SAL		
	Actual Conc. (µg/ml)	Predicted Conc. (µg/ml)	% Purity	Actual Conc. (µg/ml)	Predicted Conc. (µg/ml)	% Purity
1	10	9.8983	98.983	12.04	12.0116	99.6896
2	10	9.9708	99.708	12.04	12.2321	101.52
3	10	10.1038	101.038	12.04	12.1136	100.536
4	10	10.0212	100.212	12.04	12.1001	100.424
5	10	9.9191	99.191	12.04	11.9212	98.9393
6	10	10.1104	101.104	12.04	11.9111	98.8555
Mean	-	10.0039	100.039	-	12.0483	99.9941
SD	-	0.09062	0.9062	-	0.12414	1.0303
%RSD	-	0.90584	0.90584	-	1.03036	8.55143

Table 4: Accuracy results for BEC and SAL by proposed PLS method

Level	Sample (µg/ml)		Standard (µg/ml)		Predicted concentration (µg/ml)		% Recovery	
	BEC	SAL	BEC	SAL	BEC	SAL	BEC	SAL
50%	10	12.04	5	5	15.036	16.949	100.24	99.413
					15.113	17.135	100.753	100.504
					15.104	16.972	100.693	99.548
	Mean						99.399	100.156
	SD						0.280	0.594
% RSD						0.278	0.595	
100%	10	12.04	10	10	19.892	22.023	99.46	99.882
					20.112	21.974	100.56	99.659
					20.072	22.136	100.36	100.394
	Mean						100.182	100.057
	SD						0.585	0.376
% RSD						0.585	0.376	
150%	10	12.04	15	15	25.036	27.028	100.144	100.103
					24.985	26.887	99.94	99.581
					25.024	27.249	100.096	100.922
	Mean						101.980	101.524
	SD						0.106	0.675
% RSD						0.106	0.674	

**Fig. 1: Chemical structures of (A) Beclomethasone dipropionate and (B) Salbutamol sulphate**

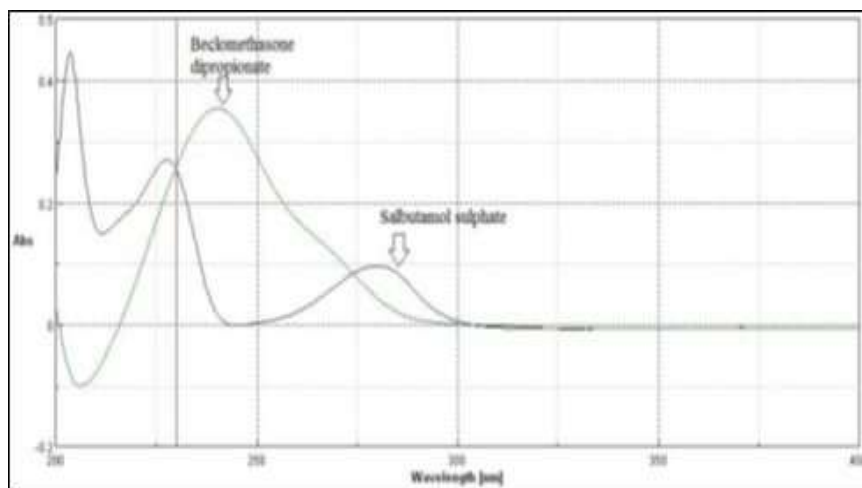


Fig. 2: Overlaid spectra of Beclomethasone dipropionate and Salbutamol sulphate (10 µg/ml each)

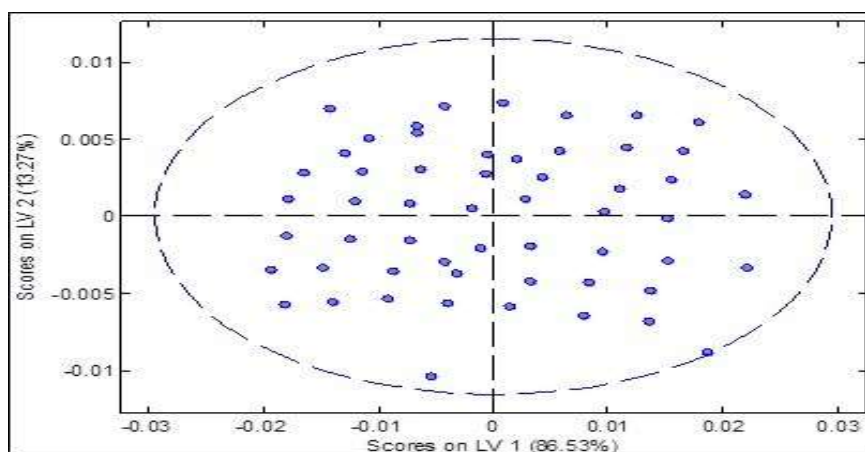


Fig. 3: 2D scores plot for the first two latent variables (LV 1 Versus LV 2) generated by using MATLAB software

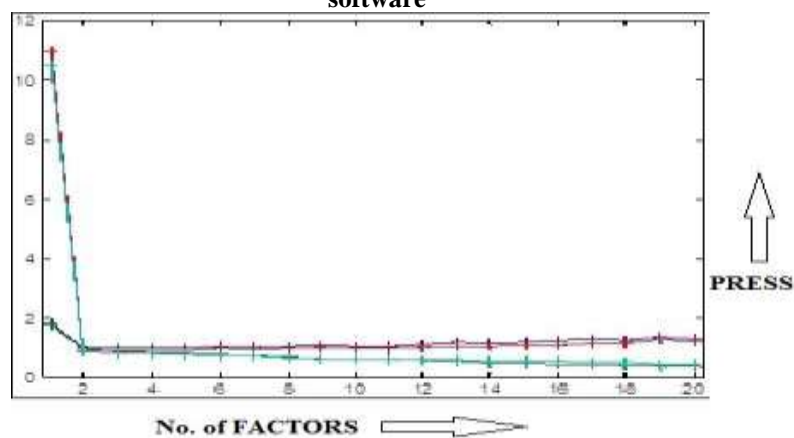


Fig. 4: Plot of PRESS versus number of factors by PLS method

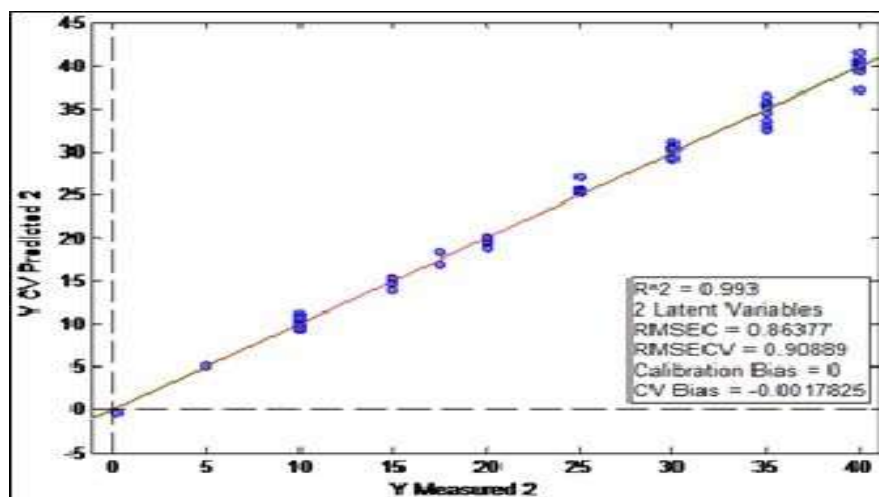


Fig. 5: Plots of predicted concentration versus actual concentration for BEC and SAL by PLS method

Conclusion

Partial least-square regression model was successfully developed and validated for determination of Beclomethasone dipropionate and salbutamol sulphate. The resulting model was proved for its efficiency by determination of these drugs in test set samples. The proposed methods do not need prior separation of drugs before analysis. The model was also successfully used to evaluate the content of these drugs in Aerocort Rotacaps® capsules. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well as for in-process quality control.

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