





UV-spectrophotometry-assisted chemometric methods for simultaneous determination of ambroxol hydrochloride and doxofylline in pharmaceutical formulation

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Abstract: Two methods, ratio spectra derivative method and partial least squares regression (PLS) were developed for the simultaneous estimation of ambroxol hydrochloride and doxofylline in combined dosage form. In the first method, ratio spectra derivative method, analytical signals were measured at the wavelengths corresponding to either maximums or minimums for both drugs in the first derivative spectra of the ratio spectra obtained by dividing the standard spectrum of one of two drugs in water. Partial least squares regression (PLS) was used for data analysis of and the parameters of the chemometry procedures were optimized. In this study, the simultaneous determination of ambroxol hydrochloride and doxofylline in pharmaceuticals by chemometric approaches using UV spectrophotometry has been reported. Spectra of ABH and DOX were recorded at several concentrations within their linear ranges between 2-12 µg/mL and 15-40 µg/mL respectively and applied to pharmaceutical formulation, tablet, with no interference with excipients as indicated by the results of the recovery study. The proposed methods are simple, rapid and can be easily used in the quality control of drugs as alternative analytical tools.

Keywords: Ambroxol hydrochloride; doxofylline; partial least squares; UV-visible spectrophotometry; ratio derivative spectroscopy; Validation. © 2020 ACG Publications. All rights reserved.

1. Introduction

Ambroxol hydrochloride (ABH), chemically trans-4-[(2-Amino-3, 5-dibrombenzyl) amino]-cyclohexane, has been shown to inhibit the nitric oxide dependent activation of soluble guanylate cyclase, thereby suppressing excessive mucus secretion, lowers the phlegm viscosity and thereby improves the mucociliary transport of bronchial secretions [1,2]. Doxofylline (DOX), chemically is 7-(1, 3-dioxolan-2-ylmethyl)-1, 3-dimethylpurine-2,6 dione [3,4], and a phosphodiesterase IV inhibitor is used as a mucolytic agent (Figure S1, see supporting information). The combination of ABH and DOX is used for the treatment of patients suffering with asthma and chronic obstructive pulmonary

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disease [5]. Literature reports, analysis of ABH and DOX by HPLC, HPTLC and UV spectrophotometric methods [6-12].

Chromatographic methods like Liquid chromatography [13] and Gas chromatography [14] finds major applications in pharmaceutical analysis and hence are most commonly used for the analysis of binary and ternary mixtures. Additionally, chromatographic methods have high sensitivity and selectivity, however these techniques are tedious in terms of time-consuming methodology of sample preparation, also require expensive higher-grade solvents and sophisticated instruments. UV-Visible spectrophotometry compared to chromatographic technique is much simpler and faster resulting in a cost-effective technique. But the critical challenge for the estimation of binary and ternary mixtures is occurrence of overlapping spectra and matrix interference [15]. Recently, there is a prodigious growth in the determination of mixture by chemometric approach [16-19]. The interference of other analytes in mixture, extensive spectral overlap, less resolution and using limited spectral data has resulted in failure of traditional spectrophotometric methods. Hence application of chemometric methods can overcome these challenges and problems [20]. Chemometrics has enabled the application of spectroscopic methods for the analysis of complex mixtures without the need for a prior separation and faster in analysis. Hence, chemometrics coupled to spectroscopic characterization, is an essential and useful tool for pharmaceutical analysis [21, 22].

Chemometric methods viz classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS), reported in the literature are finding increasing use for determination of complex mixtures with the assistance of modern instrumentation and computers for acquiring and computing spectral data offering a remarkable alternative to chromatographic techniques. PLS regression hence is a multivariate method that can be used for the quantitative analysis of mixtures of drugs even if spectral differences are insignificant [23]. The advantage of PLS is the transformation of the numerous original variables into a small number of latent vectors, which are a linear combination of the original variables. PLS regression and Multivariate calibration model can be used effectively by mathematical resolution of the overlapped spectra and the results are similar to those obtained by chromatographic techniques [24]. In context to this, two methods, ratio first order derivative and PLS method have been applied for the determination of ABH and DOX. To the best of our knowledge, this is the first study for simultaneous determination of ABH and DOX in tablet formulation.

2. Experimental

2.1. Instrument, Reagents and Software

Double beam UV-Visible Spectrophotometer (Shimadzu Corporation, UV1800) having two matched quartz cells with 1 cm light path length. Shimadzu electronic analytical balance (AUW-220D), Sonicator (Ultrasonic cleaner USC 100) was used for the study. Additionally, all instrument and glassware were calibrated. ABH and DOX was obtained as a gift sample from Ami Life Sciences Pvt. Ltd., Mumbai, India. The formulation used was tablet dosage form containing labelled claim of 30 mg of ABH and 400 mg of DOX per tablet from Ami Life Sciences Pvt. Ltd., Mumbai, India. Methanol AR grade was procured from S.D. Fine Chemical Ltd, Mumbai. The design expert 8.0 software and PLS solo and XL stat were used for preparation of calibration and validation set of binary mixture and the statistical treatment of the data with generation of model. In this method, the spectrum mode is used with medium scan speed and scaling factor 1. Initial base line correction was carried out by using Double distilled water.

2.2. Preparation of Standard Solution for Ratio First Order Derivative Method

Accurately weighed 10 mg of ABH and DOX was transferred into 100 ml volumetric flask and dissolved in small volume of methanol. The volume was adjusted to the mark with methanol to obtain final concentration of ABH and DOX (100 µg/mL). From standard stock solution, 1 ml was transferred in 10 ml volumetric flask and volume was adjusted to the mark with methanol, to prepare a final concentration of 10 µg/mL.

2.3. Preparation of Standard Solution for PLS Method

Accurately weighed 10 mg of ABH and DOX was transferred into 100 ml volumetric flask and dissolved in small volume of distilled water. The volume was adjusted to the mark with distilled water to obtain final concentration of ABH (100 µg/mL) and DOX (100 µg/mL).

2.4. Selection of Analytical Wavelength

Zero order spectra of standard solution of ABH and DOX were recorded and further, spectra were divided by 12 µg/mL of ABH and 10 µg/mL of DOX respectively and these ratio spectra of ABH and DOX were converted into first derivative for selection of wavelength for both the drugs. Similarly, for PLS method wavelength range was selected from zero order spectra.

2.5. Ratio first Order Derivative Spectrophotometric Method

Ratio spectra involves critical selection of divisor where different concentrations of both drugs were tried. 12 µg/mL of ABH and 10 µg/mL of DOX was selected as divisor as it resulted in minimum noise in ratio spectra, best linearity and maximum sensitivity. Ratio spectra of ABH were obtained by dividing the zero order spectra of mixture by the spectrum of DOX (10 µg/mL). The obtained ratio spectra of ABH were converted into the first order derivative (D_1) spectra. Likewise, ratio first order derivative spectra of DOX were recorded using ABH (12 µg/mL) as divisor using the interval of $\Delta\lambda$ value of 8 nm and scaling factor 20. The concentrations of both drugs were quantified by measuring the amplitude maxima from their first order derivative (D_1) spectrum at wavelength of 239.07 nm and 272.78 nm for ABH and DOX respectively. Calibration curve was plotted with amplitude vs. concentration for both drugs and evaluated by ordinary linear regression analysis.

Developed Ratio first order derivative Spectrophotometric method has been validated according to ICH guideline by determination of various analytical method validation parameters like linearity, sensitivity, precision and accuracy. The linearity was evaluated through a linear regression analysis. The linearity for ABH (2-12 µg/mL) at 239.07 nm and DOX (15-40 µg/mL) at 272.78 nm was determined in terms of correlation coefficient. Precision was evaluated by performing repeatability and intermediate precision. The concentration for precision study of ABH and DOX selected within linearity range were 2, 6, 10 µg/mL and 20, 30, 40 µg/mL respectively. Three replicates for each concentration for ABH and DOX were analyzed for repeatability on the same day, and three replicates were analyzed on different days to ascertain intermediate precision. Over all mean and RSD % were calculated. To demonstrate the accuracy of the proposed method, recovery studies were carried out by spiking with 50%, 100% and 150% concentration of standard for ABH (1.5, 3 and 4.5 µg/mL) and for DOX (6, 12 and 18 µg/mL) respectively to formulation containing 3 µg/mL of ABH and 12 µg/mL of DOX. % recovery was then calculated by using regression equation.

2.6. PLS Method

Study design for validation of the PLS method as shown in Table S1 and S2 (see supporting information). Before finalizing the calibration data, to avoid over fitting, the optimum number of latent variables or factors were selected by applying the cross-validation method, leaving one sample at a time [25].

The statistical parameters representing the quality of fit of data are the root mean square difference (RMSECV), square of the correlation coefficient (R^2) and relative error of prediction (REP %) [26].

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both precision and accuracy of predictions. Appropriate selection of the number of factors to be used to construct the model is the key to achieving correct quantitation in PLS calibrations. The usual procedure involves choosing the number of factors that result in the minimum RMSECV.

Different figures of merit have been reported in the literature to quantify the quality of a given multivariate model including selectivity, sensitivity, analytical sensitivity and limit of detection and further used for method comparison to study the quality of a given analytical technique.

2.7. Analysis of Marketed Formulation

Twenty tablets (label claim of 30 mg ABH and 400 mg DOX) were weighed and finely powdered. Powder equivalent to 30 mg and 400 mg ABH and DOX was accurately weighed and transferred to 100 ml volumetric flask followed by addition of methanol and sonicated for 15 min. The volume was made up to mark with methanol. The solution was filtered through whatman filter paper (0.45μ). From this solution, 1 mL was transferred to 100 mL volumetric flask and diluted up to the mark to give a solution containing $3\ \mu\text{g/mL}$ ABH and $40\ \mu\text{g/mL}$ DOX. This solution was used for the estimation of ABH and DOX, by scanning in the range of 220-330 nm. Estimation was carried out by both ratio first order derivative and PLS method.

3. Results and Discussion

3.1. Ratio First Order Derivative Method

The zero order absorption spectra were transformed into ratio spectra by choosing optimum concentration of divisor. The chosen divisor concentration for ABH ($12\ \mu\text{g/mL}$) and DOX ($10\ \mu\text{g/mL}$) gave good results for slope, intercept and correlation coefficient, at wavelength of 239.07 nm for ABH and 272.78 nm for DOX as shown in Figure 1.

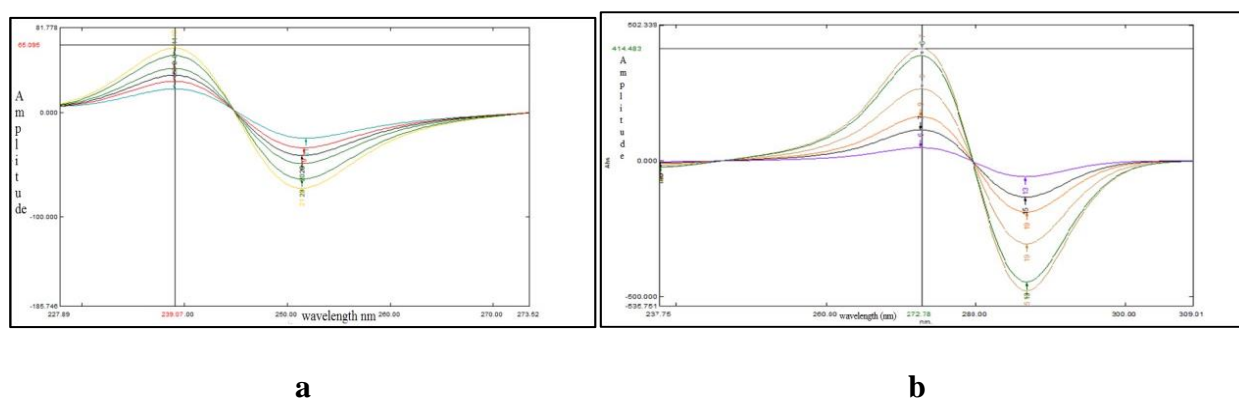


Figure 1. Ratio first derivative absorption spectra of (A) ABH (2-12 $\mu\text{g/mL}$) and (B) DOX (15-40 $\mu\text{g/mL}$)

Calibration curve constructed showed good correlation coefficient of 0.9937 and 0.9934 respectively for ABH and DOX in the given concentration range of 2-12 $\mu\text{g/mL}$ for ABH ($y=3.7852x+17.622$) and 15-40 $\mu\text{g/mL}$ for DOX ($y=13.95x+155.88$). LOD and LOQ for ABH were 0.04514 and 0.13679 $\mu\text{g/mL}$ while for DOX were 0.02906 and 0.08807 $\mu\text{g/mL}$ respectively indicating high sensitivity of the method. Results of intraday precision resulted in RSD % below 0.17 for ABH and 0.10 for DOX respectively. Similarly, interday precision results reveals RSD % below 0.17 and 0.07 for ABH and DOX respectively. RSD % values less than 2%, demonstrated good repeatability and

reproducibility of the method. The proposed method afforded recovery of 98.42- 101.93 % after spiking the standard drug at 3 concentration levels of 50, 100 and 150 %. The RSD % was found to be less than 2% indicating that the proposed method was accurate.

3.2. Development of PLS Method

PLS method as a chemometric model is widely applied for the simultaneous estimation of multi-component formulations and especially for the binary and ternary mixture of drugs with extensive overlapping in their absorption spectrum. This chemometric model involves the determination of the concentration of the drugs present in mixture by measuring their absorbances at many numbers of wavelengths available in the spectral data. This type of measurements increases the accuracy of spectral analysis and one of the important advantages is selection of the most informative data, and removing the unnecessary ones, which further makes the models meaningful. Hence, chemometric assisted spectrophotometric methods was found to be more preferable and advantageous being economical, simple, rapid and sensitive when compared to conventional time consuming analytical techniques. Zero order absorption spectra revealed considerable overlap between 220 to 330 nm. The zero order spectra of ABH and DOX are as shown in Figure S2 (see supporting information). Hence, the overlap observed between the absorption spectra, indicates that univariate methods may not be directly applied to the resolution of the ABH and DOX, and physical prior separation processes could be necessary for the simultaneous determination of the drugs. In contrast, multivariate methods may resolve band overlapping, without physical separations.

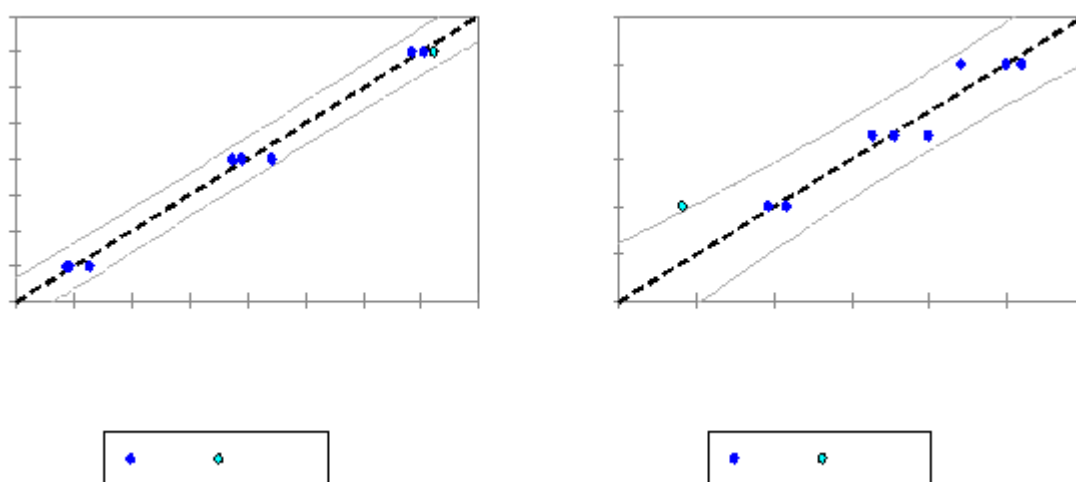


Figure 2. Plot of actual versus predicted value of ABH and DOX

From a practical point of view, experimental setups that do not require data pretreatment, such as PLS-1 should be preferred due to their time saving characteristics which makes them comparatively more efficient. The statistical parameters obtained after applying PLS to the spectrophotometric data of cross validation and validation are shown in Table 1. From the obtained results, it is evident that the present method is accurate and precise suggested by the low RMSE and REP values. Moreover, the actual and predicted values are also in agreement with each other (Figure 2).

Analytical figure of merits are very important to quantify the quality of a given methodology or for method comparison in multivariate calibration. Several figure of merits, sensitivity, analytical sensitivity, limit of detection (LOD) are as summarized in Table 1.

Table 1. Statistical parameters for the optimized PLS model

Statistical parameters and Figure of merit		
Parameters	ABH	DOX
Factor	2	2
RMSEC	0.283	0.312
R ²	0.997	0.998
Intercept	0.473	-0.898
Slope	1.019	1.0005
Press	5.42	0.02
REP%	3.45	6.07
Bias	0.104	0.32
RMSEP	0.242	1.67
R²	0.990	0.925
Intercept	-17.74	0.69
Slope	1.00	0.002
Press	0.0001	0.00
Bias	-0.001	0.56
Figure of merits		
Parameters	ABH	DOX
LOD($\mu\text{g/mL}$)	1.28	2.22
Sensitivity($\text{mL}/\mu\text{g}$)	1.019	1.0005
Analytical sensitivity($\mu\text{g/mL}$)	2.56	1.48

*The figures in bold indicates Parameters of the validation

3.3. Analysis of Tablet Formulation

The developed methods were applied for analyzing the ABH and DOX in marketed formulations and the study was repeated three times. The results obtained were complying with the label claim (Table 2). The developed methods can serve as a good alternative to the available methods. The methods are beneficial in terms of ease of performing and reduced cost of the analysis for routine analysis, quality control of mixture and tablet formulation containing these two drugs.

Table 2. Assay of marketed formulation by proposed methods

Formulation	Label claim	% Mean drug found^a	% Mean drug found^a
		PLS	Ratio first derivative Spectrophotometric method
DOXOLLIN –AX	ABH 30 mg	99.76	100.2
	DOX 400 mg	100.91	99.17

4. Conclusion

Analytical methods with attributes of good accuracy and precision and capable of performing analysis of binary or ternary mixtures that are multicomponent formulations within a very less span of time are always attracting the interest of analysts. This can be achieved when analytical methods are coupled with chemometric tools. The assistance of chemometrics to the spectroscopic methods makes them powerful and efficient in the simultaneous determination of drugs in multi-component formulations. Ratio derivative UV Spectrophotometric method and PLS chemometric method were applied successfully for the simultaneous estimation of ABH and DOX. The developed methods can serve as a good alternative to the available methods. The methods are beneficial in terms of ease of performing and reduced cost of the analysis for routine analysis, quality control of mixture and commercial preparation containing these two drugs. Thus, spectrophotometric technique can generate large amounts of data within a short period of analysis; however, when coupled with chemometric tools, the quality of the spectral information can be markedly increased, converting this combined technique

into a powerful and highly convenient analytical tool. PLS is a simple but powerful approach for the analysis of data of complicated problems. In the present study, two UV-Spectrophotometric methods were developed and validated for the estimation of ABH and DOX in combination and useful for the quality control of marketed formulation.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/journal-of-chemical-metrology>

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