## SPECTROPHOTOMETRIC ASSESSMENT OF A SPECTRALLY OVERLAPPING MIXTURE OF CINCHOCAINE HYDROCHLORIDE AND BETAMETHASONE VALERATE IN THE PRESENCE OF THEIR DEGRADATION PRODUCTS[**]

**A. S. Fayed, M. R. Rezk, H. M. Marzouk[*], S. S. Abbas**

*Cairo University, Analytical Chemistry Department, Faculty of Pharmacy, Kasr El-Aini Str., ET-11562 Cairo, Egypt; e-mail: h.marzouk@yahoo.com; hodaallah.marzouk@pharma.cu.edu.eg*

*This work represents a contribution to the analytical spectrophotometric methods applied to resolve the spectral overlapping of cinchocaine hydrochloride (CIN) and betamethasone valerate (BMV), for the sake of their simultaneous determination in the presence of their hydrolytic degradation products and in the pharmaceutical formulation, with no need for preliminary separation. Conventionally assisted mathematical univariate techniques, including the use of the isoabsorptive point, dual wavelength, ratio subtraction, first derivative of the ratio spectra and ratio difference, are applied; these methods are able to determine the two drugs simultaneously in binary mixtures but not in the presence of their degradation products. For the determination of CIN, a direct measurement of its zero order absorption spectra at 327.0 nm is performed, where BMV showed zero absorbance. Betamethasone valerate is determined by the developed univariate methods after resolving the CIN spectral overlapping. For the determination of both CIN and BMV in the presence of their degradation products, two multivariate calibration methods are proposed using principal component regression and partial least squares. The developed methods are validated as per the ICH guidelines and can be applied for routine quality control analysis.*

***Keywords:*** *betamethasone valerate, cinchocaine hydrochloride, hydrolytic degradation products, univariate spectrophotometry, multivariate calibrations.*

## СПЕКТРОФОТОМЕТРИЧЕСКАЯ ОЦЕНКА СПЕКТРАЛЬНО ПЕРЕКРЫВАЮЩЕЙСЯ СМЕСИ ЦИНХОКАИНА ГИДРОХЛОРИДА И БЕТАМЕТАЗОНА ВАЛЕРАТА В ПРИСУТСТВИИ ПРОДУКТОВ ИХ ДЕГРАДАЦИИ

**A. S. Fayed, M. R. Rezk, H. M. Marzouk[*], S. S. Abbas**

*УДК 543.243*

*Каирский университет, ET-11562 Каир, Египет;*
*e-mail: h.marzouk@yahoo.com; hodaallah.marzouk@pharma.cu.edu.eg*

*(Поступила 3 ноября 2017)*

*Предложено развитие аналитических спектрофотометрических методов, которые могут быть применены для одновременного определения спектрально перекрывающихся цинхокаина гидрохлорида (CIN) и бетаметазона валерата (BMV) в присутствии их продуктов гидролитической деградации и в фармацевтической композиции без их предварительного разделения. Обычные математические одномерные методы используют изоабсорбционную точку, две длины волны, первую производную спектров и разностные спектры. Эти методы способны одновременно определять два препарата в бинарных смесях, но не в присутствии их продуктов разложения. Для определения CIN проводилось прямое измерение спектров поглощения нулевого порядка на длине волны 327.0 нм, где BMV показывал нулевое поглощение. Бетаметазона валерат определяли с помощью разработанных одномерных методов после разрешения спектрального перекрытия CIN. Для определения как CIN, так и BMV в присутствии их продуктов разложения предложены два метода многомерной калиб-*

[**] Full text is published in JAS V. 86, No. 1 (http://springer.com/10812) and in electronic version of ZhPS V. 86, No. 1 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

*ровки с использованием регрессии главных компонент и частных наименьших квадратов. Разработанные методы проверены в соответствии с рекомендациями ICH и могут применяться для контроля качества.*

***Ключевые слова:*** *бетаметазона валерат, цинхокаина гидрохлорид, продукты гидролитической деградации, одномерная спектрофотометрия, многовариантные калибровки.*

**Introduction.** Cinchocaine hydrochloride (CIN) is co-formulated with betamethasone valerate (BMV) in ointment preparation. CIN acts pharmacologically as a local anesthetic, while BMV is an anti-inflammatory drug of the glucocorticoid group. This combination is found to be useful for the local treatment of hemorrhoids. Drug stability studies are essential for ensuring the efficacy of drugs. The stability of CIN was previously studied by Morch [1]. The study showed that the drug was liable to acid degradation, but it was relatively stable towards oxidative, alkali, and thermal stress conditions. Derivative spectrophotometry [2, 3] and chromatographic methods, including HPLC [2] and TLC-densitometry [3], were reported as stability-indicating methods for the determination of CIN in the presence of its acid-induced degradation products. On the other hand, BMV undergoes degradation by alkali, acid, and thermal stress conditions, but it is relatively stable when subjected to oxidative stress conditions [4–7]. In the recent literature only one HPLC and HPTLC-densitometric method is reported for the simultaneous determination of both CIN and BMV in the presence of their hydrolytic degradation products [8]. There is no reported spectrophotometric method for the determination of the studied drugs either in their binary mixture or in the presence of their degradation products.

UV spectrophotometry is one of the analytical techniques used in pharmaceutical analysis. It is characterized as simple, rapid, and cheap, which promotes its wide application for the analysis of mixtures containing two or more components in pure form and in the pharmaceutical formulation without chemical pretreatment or using sophisticated equipment. Accordingly, the goal of the present work is to develop and validate simple, accurate, and precise spectrophotometric methods for the determination of CIN and BMV in pure form as well as in the presence of their potential degradation products and in their pharmaceutical formulations. The advantages of the mathematical manipulation of data to resolve the spectral overlapping of active compounds and their degradation products, when found in mixtures, are introduced.

The application of all the proposed methods for quality control can be easily performed for the analysis of CIN and BMV in pharmaceutical preparations. ICH guidelines [9] are followed to ensure their suitability for the intended use.

**Experimental.** Spectrophotometric measurements are performed on a Shimadzu UV-Visible spectrophotometer dual beam (Kyoto/Japan), model UV-1650 PC, with two matched 1-cm quartz cells. UV-Probe personal spectroscopy software version 2.21 (Shimadzu) is used. Multivariate computations are performed with the support of Matlab® 7.0 (Mathwork Inc., USA). For the extraction of drugs from pharmaceutical formulations, a Soniclean 160T sonicator (Soniclean, Thebarton, Australia) is used.

Standard CIN, with 100.05 ± 1.32% assessed purity according to the BP official method (1), is kindly supplied by Alexandria Co. Pharmaceuticals, Alexandria, Egypt. BMV standard is kindly supplied by GlaxoSmithkline S.A.E., Cairo, Egypt. Its purity is checked and found to be 100.03 ± 1.17%, according to the BP official method [10].

Sodium hydroxide, hydrochloric acid, glacial acetic acid, toluene, and ethanol are obtained from El-Nasr Pharmaceutical Chemical Co., Egypt; chloroform, acetonitrile, and n-hexane are from Sigma-Aldrich, Steinheim, Germany. The water used is double distilled.

Supraproct-S® ointment (Batch No. 0014) is manufactured by Julphar, Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE. Each gram is labeled to contain 5 mg of CIN and 1 mg of BMV.

*Preparation of CIN acid-induced degradation products.* A weighed amount of 25.0 mg of CIN is transferred into a 100-mL flask; 25 mL of 2 M hydrochloric acid is then added and the whole refluxed for 2 h. After the reflux, the solution is cooled. A pre-calculated volume of 2 M sodium hydroxide is then added to adjust the pH to 7.0. Complete degradation is confirmed by HPTLC using toluene-chloroform-ethanol-glacial acetic acid (4.5:4.5:1:1, by volume) [8].

*Preparation of BMV alkali-induced degradation products.* Twenty five milligrams of BMV is accurately weighed, transferred into a small beaker, and dissolved in 25 mL of acetonitrile. Then one milliliter of 2 M sodium hydroxide solution is added. The solution is mixed and allowed to stand for 90 min at room temperature. The pH is then adjusted to 7.0 with a pre-calculated amount of 2 M hydrochloric acid. Complete degradation is checked by the HPTLC method previously reported [8].

*Standard stock solutions of CIN and BMV (each, 1.0 mg/mL).* Standard stock solutions either of CIN or BMV are prepared by dissolving 100.0 mg of the drug pure powder in 100 mL of the solvent mixture of acetonitrile-water (50:50, v/v).

*Stock solution of CIN acid-induced degradation products (200.0 μg/mL).* The procedure mentioned for the preparation of CIN acid-induced degradation products is followed, but using 20.0 mg of CIN and the volume being then made up to 100 mL with the solvent mixture of acetonitrile-water (50:50, v/v).

*Working solution of CIN acid-induced degradation products (20.0 μg/mL).* Five milliliters of the respective stock solution (200.0 μg/mL) is accurately transferred into a 50 mL volumetric flask, and the volume is made up to the mark with the solvent mixture of acetonitrile: water (50:50, v/v).

*Stock solution of BMV alkali-induced degradation products (200.0 μg/mL).* The procedure mentioned for the preparation of BMV alkali induced degradation products is followed, but using 20.0 mg of BMV and the volume being then made up to 100 mL of the solvent mixture of acetonitrile-water (50:50, v/v).

*Working solution of BMV alkali-induced degradation products (10.0 μg/mL).* A volume of 2.5 mL of the respective stock solution (200.0 μg/mL) is accurately transferred into a 50 mL volumetric flask, and the volume is made up to the mark with the solvent mixture of acetonitrile-water (50:50, v/v).

All the stock solutions are freshly prepared on the day of the analysis and stored in the refrigerator to be used within 24 h and protected from light.

*Construction of calibration curves for the CIN and BMV univariate spectrophotometric methods.* Aliquots are accurately transferred from CIN and BMV standard working solutions (200.0 μg/mL) into two separate series of 10 mL volumetric flasks and then made up to the volume with acetonitrile:water (50:50, v/v). The absorption spectra of the prepared standard solutions are scanned from 200.0 to 400.0 nm and stored on the computer. For the determination of CIN in the presence of BMV, a calibration curve is constructed, relating the values of absorbance of the zero-order ($^0D$) absorption spectra of CIN at 327.0 nm to its corresponding concentration (10.0–120.0 μg/mL), and the regression equation is then computed. For the determination of BMV in the presence of CIN, the subsequent manipulations are carried out:

*Construction of calibration curves for the isoabsorptive point method (ISO).* Calibration curves are constructed, relating the absorbance of the zero-order absorption spectra of BMV at the isoabsorptive point 248.1 nm ($\lambda_{iso}$) to its corresponding concentrations (5.0–50.0 μg/mL), and the regression equations are computed.

*Construction of calibration curves for the dual wavelength method (DW).* A calibration graph is plotted, relating the difference in absorbance at 264.5 and 271.5 nm of the zero-order spectrum ($\Delta A$ = 264.5 and 271.5) to the corresponding concentration of BMV (5.0–90.0 μg/mL). The regression equation is computed.

*Construction of calibration curves for the ratio subtraction method (RS).* For the determination of BMV in the presence of CIN by RS, the stored scanned spectra of BMV are divided by a standard spectrum of 30.0 μg/mL CIN, and the ratio spectra are obtained, the constant at the extended part of the curve from 310.0–320.0 nm is subtracted, then curves are multiplied by the $^0D$ of CIN. A calibration curve representing the relation between the absorbance of BMV at $\lambda_{max}$ = 240.0 nm and the concentrations (5.0–50.0 μg/mL) is constructed, and the regression equation is computed.

*Construction of calibration curves for the first derivative of the ratio spectra method ($^1DD$).* For the determination of BMV in the presence of CIN by $^1DD$, the stored scanned spectra of BMV are divided by a standard spectrum of 30.0 μg/mL CIN, and the ratio spectra are obtained. Then the first derivatives of the ratio spectra ($^1DD$) are computed, using $\Delta\lambda$ = 4 nm and the scaling factor 10. The peak-to-peak amplitudes of the first derivative peak of (BMV/CIN) are measured at 253.0 and 271.0 nm. A calibration graph relating the peak-to-peak amplitudes to the concentrations (5.0–60.0 μg/mL) of BMV is constructed, and the regression equations are computed.

*Construction of calibration curves for the ratio difference spectrophotometric method (RD).* BMV is determined from the previously calculated ratio spectra, where the difference in amplitudes at 259.0 and 240.0 nm ($\Delta P$ = 259.0–240.0 nm) are plotted against the concentration in the range 5.0–50.0 μg/mL. The regression equation is then computed from the obtained calibration graph.

*Construction of calibration curves for the multivariate calibration methods (PLS and PCR).* Multivariate models using the PLS and PCR methods are required for the calibration of different concentrations of the four components (CIN, BMV, and CIN acid degradation products and BMV alkali degradation products). The solution preparation is based on the five levels–four factors design [11]. The calibration set consists of fifteen mixtures, randomly chosen. The concentration range of the solutions is 10.0–30.0, 5.0–45.0, 0.5–2.5, and 0.25–1.25 μg/mL, respectively, being prepared by mixing different aliquots of CIN, BMV, and CIN acid degradation products and BMV alkali degradation products working solutions in a series of 10 mL volumet-

ric flasks. The UV absorption spectra of the prepared solutions are recorded over the range 220.0–370.0 nm. Data analysis and the construction of multivariate calibration models are performed by transferring the data points to Matlab® for subsequent processing. The mean centered pre-processing step is carried out for all the spectral data before building the PLS and PCR models.

*Analysis of laboratory-prepared mixtures containing different ratios of CIN and BMV using the suggested methods for the conventional spectrophotometric methods (ISO, DW, RS, $^1$DD, and RD).* For testing the specificity of the proposed methods, aliquots of intact CIN and BMV are mixed to prepare mixtures containing different ratios of CIN and BMV. The absorbance of the resulting solutions is measured at 327.0 nm, which corresponds to the concentration of CIN alone, so its concentration in each mixture is determined with the help of its regression equation. For the determination of BMV, the general procedure previously mentioned for each method is followed to calculate its concentrations.

*Analysis of laboratory-prepared mixtures containing different ratios of CIN and BMV using the suggested methods for the multivariate calibration methods (PLS and PCR).* An external validation set is used and randomly chosen. This set includes up to 10 mixtures with different ratios of CIN and BMV and their degradation products. Aliquots of CIN, BMV, and CIN acid degradation products and BMV alkali degradation products are mixed in 10 mL volumetric flasks, and then the volumes are completed with acetonitrile: water (50:50, v/v). The spectra of these solutions are recorded from 220.0–370.0 nm and used for the determination of the concentration of CIN and BMV in each mixture.

*Application to the pharmaceutical formulation.* Two grams of the ointment is accurately weighed and transferred into a 100 mL beaker, dispersed into a 20 mL *n*-hexane, and then subjected to sonication for 15 min. Extraction is carried out by quantitatively transferring the solution into a 250-mL funnel, then shaking well till complete dispersion of the ointment occurs. Threefold extraction is performed with each 15 mL of the solvent mixture (acetonitrile-water, 50:50, v/v). The lower layer, containing the active ingredients, is collected into a 50 mL volumetric flask. The volume is made up to the mark with the same solvent mixture and filtered through filter paper. Appropriate dilutions are made used the same solvent mixture. The procedures previously described for each method are then followed for the calculation of the CIN and BMV concentrations.

**Results and discussion.** The aim of this work is to develop simple and accurate methods for the simultaneous determination of CIN and BMV in their bulk powders and pharmaceutical formulations in addition to the analysis of the studied drugs in the presence of their potential hydrolytic degradates. The spectrophotometric analysis of the CIN and BMV binary mixture without prior separation suffered from two problems: the overlapped spectra of the two drugs along with the pronounced difference in their absorptivities (Fig. 1). For their effective pharmacological action they are co-formulated in a ratio of CIN:BMV (5:1), which can prevent the determination of BMV accurately. CIN could be analyzed “in the extended region” in the presence of BMV at 327.0 nm, but a direct analysis of BMV in the presence of CIN is not possible. Accordingly, this work is directed at the use of simple univariate spectrophotometric mathematically manipulated methods (ISO, DW, RS, $^1$DD, and RD) for the determination of BMV in the presence of CIN in their binary mixture without any interference due to their spectral overlapping. On the other hand, in the presence of a complex mixture of CIN, BMV, and their degradation products, the methods are unsuccessful in the resolution of overlapping and fail to determine any of the two drugs (Fig. 1).

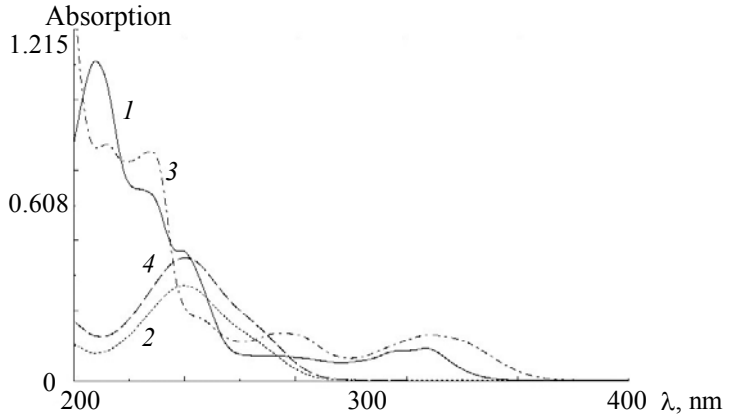


Fig. 1. Zero-order absorption spectra of 10.0 μg/mL of each of CIN (1), BMV (2), CIN acid degradation products (3) and BMV alkali degradation products (4), using acetonitrile and water (50:50) as a blank.

Thus, the multivariate calibration spectrophotometric methods are tried and shown to give excellent results for the determination of CIN and BMV in spite of the presence of their hydrolytic degradation products without pre-separation techniques. The proposed methods have advantages over the traditional chromatographic methods. The success of the methods is due to the development of the models that relate the multiple spectral intensities from many calibration samples to the known analyte concentrations of the samples. This verifies the resolution and determination of CIN and BMV in this multi-component mixture and permits their use as stability-indicating methods.

*For the univariate spectrophotometric methods (ISO, DW, RS, $^{1}$DD and RD). Isoabsorptive point method (ISO).* Erram, et al. [12] previously described a simple isoabsorptive point method for the determination of a binary mixture. The point is used for the determination of the total mixture concentration. In the present work, the analysis of the absorption spectra of the solutions of CIN and BMV separately (20.0 μg/mL) and a mixture of CIN and BMV with the same concentration (10.0 μg/mL) shows isoabsorptive points at 248.1 and 273.1 nm. The later wavelength is not used, as it suffers low sensitivity for the two drugs. The total content of CIN and BMV in the mixture could be calculated by measuring the absorbance at the chosen isoabsorptive point in the zero-order absorption spectrum, while the concentration of CIN alone could be calculated using the zero-order spectrum without any interference from BMV. Thus, the BMV concentration is calculated by subtraction.

*Dual wavelength method (DW).* In order to successfully apply DW, one needs to properly choose two wavelengths at which one of the two compounds has exactly equal absorptivity (in our study, CIN) for eliminating its interference and thus permitting selective determination of the other (BMV). Meanwhile, the absorbance difference between the two chosen wavelengths (264.5 and 271.5 nm) for the determination of BMV is directly proportional to its concentration, and the absorbance difference of CIN at these wavelengths is zero (Fig. 1). A careful selection of wavelengths with constant absorbance of the interfering substance is key to the success of the method. Compliance with Beer's law for the difference of the amplitude with a high curve slope greatly affects the sensitivity of the method [13].

*Ratio subtraction method (RS).* For the determination of BMV, the ratio subtraction method [14] is applied to cancel the CIN spectrum. The method depends on the fact that, in a mixture of CIN (Y) and BMV (X) where the spectrum of (Y) is more extended (Fig. 1), the determination of BMV (X) can be done by scanning the zero-order absorption spectra of the laboratory-prepared mixtures (CIN and BMV), dividing them by a carefully chosen concentration (30.0 μg/mL) of standard CIN (Y′= divisor), and producing a new ratio spectrum that represents X/Y + constant (Fig. 2a). This is followed by the subtraction of the absorbance values of these constants (Y/Y′) in the plateau region (Fig. 2b). Finally, the original spectrum of BMV (X) can be restored by the multiplication of the obtained spectra by (Y') the divisor, as shown in Fig. 2c. These spectra are used for the direct determination of BMV at 240.0 nm and the calculation of the concentration from the corresponding regression equation.

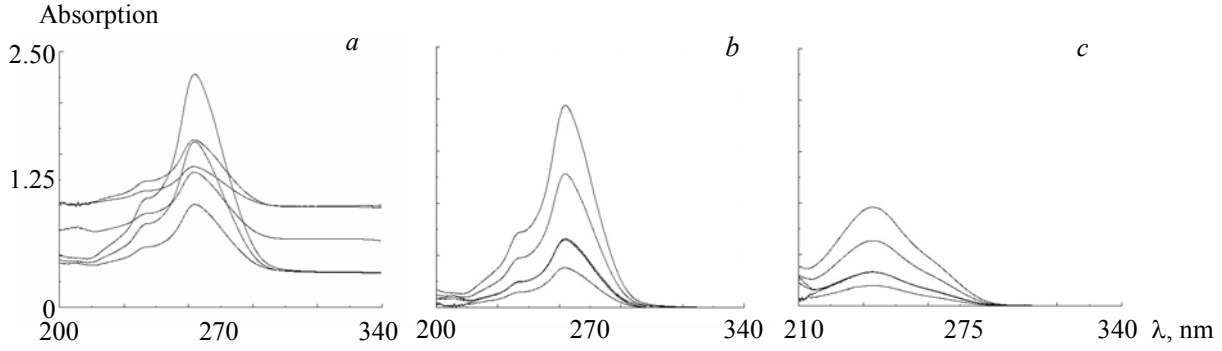


Fig. 2. Ratio spectra of laboratory-prepared mixtures of CIN and BMV using 30.0 μg/mL of CIN as a divisor (a); ratio spectra after the constant subtraction (b); zero-order spectra of BMV obtained by the proposed ratio subtraction method for the analysis of laboratory-prepared mixtures after multiplication by the divisor (c).

*First derivative of the ratio spectra spectrophotometric method* ($^{1}$*DD*). In this method, the absorption spectrum of the two-component mixture is divided by the absorption spectrum of a standard solution of one

fixed component (divisor), and the first derivative of the ratio spectrum is obtained, which is independent of that component (divisor), and the other component can be determined with no interference [15].

Different concentrations of the divisor are tested (10.0, 20.0, 30.0 μg/mL), but the concentration of 30.0 μg/mL is found to be the best in terms of minimum noise in the ratio spectra and maximum sensitivity. The wavelength increment over which the derivative is obtained (Δλ) is carefully tested; Δλ = 4 is chosen to minimize the noise. The ratio spectra for BMV (5.0–60.0 μg/mL) are obtained (Fig. 3a), and then the first derivative of the ratio spectra is calculated, as shown in Fig. 3b. Several amplitude peaks are observed with good linearity, but 253.0 and 271.0 nm show minimum noise and the best percentage recovery in the laboratory-prepared mixtures. Measurements of the peak-to-peak amplitude between 253.0 and 271.0 nm show a significant improvement of the sensitivity and recovery percentage. The concentrations are calculated using the regression equation representing the linear relationship between the $^1$DD at 253.0 and 271.0 nm versus the concentrations of BMV.

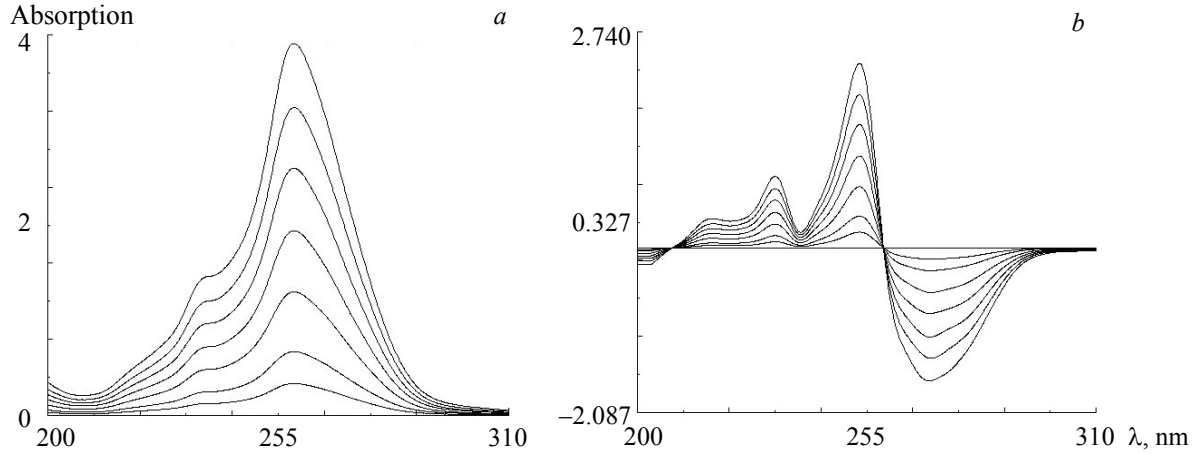


Fig. 3. Ratio spectra (a) and the first derivative of ratio spectra $^1$DD (b) of 5.0–60.0 μg/mL of BMV, using CIN (30.0 μg/mL) as a divisor.

*Ratio difference spectrophotometric method (RD).* A simple, high selectivity, and innovative method (RD) is developed for the determination of BMV in a binary mixture with CIN [16]. After recording the ratio spectra using the previously selected divisor, wavelengths for the determination of BMV are carefully selected. The linearity at each wavelength is separately checked. The linearity of the amplitude difference to the corresponding concentration of the drug is also checked. When $\Delta P$ = 259.0–240.0 nm is selected for the determination of BMV, the ratio spectrum of CIN shows the same amplitudes (constant), but the ratio spectrum of BMV shows a significant difference from the concentration in these two amplitude values at these two selected wavelengths, as shown in Fig. 4. The selection is based on the best sensitivity.

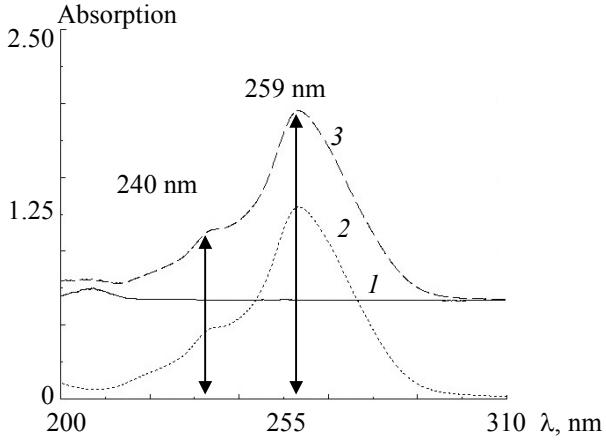


Fig. 4. Ratio spectra of 20.0 μg/mL CIN (1), 20.0 μg/mL BMV (2) and a laboratory-prepared mixture, containing 20.0 μg/mL of both (3) using CIN (30.0 μg/mL) as a divisor.

Analysis of laboratory mixtures of CIN and BMV prepared in different ratios proves that the proposed methods are applicable and valid and can be used for the assay of commercial ointment.

Recovery studies are performed from the obtained results (Table 1).

TABLE 1. Determination of Cinchocaine Hydrochloride and Betamethasone Valerate in Laboratory-prepared Mixtures by the Proposed Univariate Spectrophotometric Methods

| CIN:BMV | | %Recovery ± SD of the claimed amount [a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | CIN | BMV | | | | |
| Ratio | *C*, (μg/mL) | $^0$D at 327.0 nm | ISO at 248.1 nm | DW Δ*A*, 264.5 and 271.5 nm | RS at 240.0 nm | $^1$DD, nm, at 253.0–271.0 nm | RD $\Delta P_{259.0\text{-}240.0\ nm}$ |
| 1:1 | 10:10 | 100.75 | 100.90 | 98.96 | 100.16 | 100.02 | 100.02 |
| 1:2 | 10:20 | 99.35 | 99.70 | 99.48 | 98.82 | 98.95 | 101.26 |
| 2:1 | 20:10 | 99.91 | 100.80 | 101.00 | 100.47 | 99.90 | 100.02 |
| 3:1 | 30:10 | 100.25 | 100.20 | 101.04 | 98.58 | 101.20 | 99.03 |
| 1:3 | 10:30 | 99.81 | 99.40 | 101.03 | 99.84 | 100.30 | 99.10 |
| 5:1[b] | 30:6 | 99.63 | 100.17 | 99.00 | 98.79 | 99.50 | 99.96 |
| Mean ± SD | | 99.95 ± 0.49 | 100.20±0.59 | 100.09 ± 1.04 | 99.44 ± 0.81 | 100.01 ± 0.77 | 99.90 ± 0.81 |

[a] Average of three separate determinations.
[b] Dosage form ratio.

*For the multivariate calibration methods (PLS and PCR).* The models obtained are applied for the analysis of CIN and BMV in the presence of their degradation products. Fifteen samples containing different concentrations of both drugs and their degradation products are prepared and used as a calibration set (Table 2). In order to achieve accurate determination, the concentration ratios of the four components in this set are designed to give symmetric and orthogonal distribution. Optimization of the data handling reveals that the best results are obtained when spectra are digitized at 0.1 nm in the range 220.0–370.0 nm, where 1501 experimental points are used in the calculation. Before constructing the model for the developed PLS and PCR, the selection of the optimum number of latent variables is a crucial step.

TABLE 2. Concentrations of Cinchocaine Hydrochloride, Betamethasone Valerate, Cinchocaine Hydrochloride Acid-induced Degradation Products, and Betamethasone Valerate Alkali-induced Degradation Products in the Calibration and Validation Sets in μg/mL

| Sample No. | CIN | BMV | CIN acid degradates | BMV alkali degradates |
|---|---|---|---|---|
| 1 | 20.00 | 25.00 | 1.50 | 0.75 |
| 2 | 20.00 | 5.00 | 0.50 | 1.25 |
| 3 | 10.00 | 5.00 | 2.50 | 0.50 |
| 4 | 10.00 | 45.00 | 1.00 | 1.25 |
| 5 | 30.00 | 15.00 | 2.50 | 0.75 |
| 6 | 15.00 | 45.00 | 1.50 | 0.50 |
| 7 | 30.00 | 25.00 | 1.00 | 0.50 |
| 8 | 20.00 | 15.00 | 1.00 | 1.00 |
| 9 | 15.00 | 15.00 | 2.00 | 1.25 |
| 10 | 15.00 | 35.00 | 2.50 | 1.00 |
| 11 | 25.00 | 45.00 | 2.00 | 0.75 |
| 12 | 30.00 | 35.00 | 1.50 | 1.25 |
| 13 | 25.00 | 25.00 | 2.50 | 1.25 |
| 14 | 20.00 | 45.00 | 2.50 | 0.25 |
| 15 | 30.00 | 45.00 | 0.50 | 1.00 |
| 16 | 30.00 | 5.00 | 2.00 | 0.25 |
| 17 | 10.00 | 35.00 | 0.50 | 0.75 |
| 18 | 25.00 | 5.00 | 1.50 | 1.00 |
| 19 | 10.00 | 25.00 | 2.00 | 1.00 |

*Continue Table 2*

| Sample No. | CIN | BMV | CIN acid degradates | BMV alkali degradates |
|---|---|---|---|---|
| 20 | 20.00 | 35.00 | 2.00 | 0.50 |
| 21 | 25.00 | 35.00 | 1.00 | 0.25 |
| 22 | 25.00 | 15.00 | 0.50 | 0.50 |
| 23 | 15.00 | 5.00 | 1.00 | 0.75 |
| 24 | 10.00 | 15.00 | 1.50 | 0.25 |
| 25 | 15.00 | 25.00 | 0.50 | 0.25 |

* The shaded samples are those used for the model validation.

The leave-one-out cross-validation method is used in this study to determine the optimum number of variables. The RMSECV values (root mean square of cross validation) of different models are compared. The selected model is that of the smallest number of variables, which is found to be four for the mean centered data.

To test the ability of the developed models (PLS and PCR) to predict concentration data, the spectra of ten samples (other than the training set) as a validation set are subjected to analysis using the developed models (PLS and PCR) (Table 2). The percentage recoveries of the validation set are shown in Table 3. For examining the errors in the predicted concentrations, the root mean square error of prediction (RMSEP) is calculated as a diagnostic tool; it indicates both accuracy and precision (Table 3).

TABLE 3. Recovery Percentage of Cinchocaine Hydrochloride and Betamethasone Valerate in the Validation Set by the Proposed PLS and PCR Models

| Mixture No. | %Recovery* | | | |
|---|---|---|---|---|
| | CIN | | BMV | |
| | PLS | PCR | PLS | PCR |
| 1 | 100.35 | 100.38 | 100.08 | 100.01 |
| 2 | 99.36 | 99.30 | 101.17 | 101.16 |
| 3 | 100.28 | 100.29 | 102.30 | 102.27 |
| 4 | 100.50 | 100.54 | 99.68 | 99.68 |
| 5 | 100.40 | 100.39 | 102.32 | 102.32 |
| 6 | 99.53 | 99.56 | 99.93 | 99.95 |
| 7 | 99.66 | 99.59 | 100.06 | 100.07 |
| 8 | 98.92 | 98.86 | 101.31 | 101.35 |
| 9 | 99.25 | 99.01 | 99.74 | 99.65 |
| 10 | 101.23 | 100.98 | 98.55 | 98.57 |
| Mean ± SD | 99.95±0.71 | 99.89±0.72 | 100.51±1.22 | 100.50±1.22 |
| RMSEP** | 0.1032 | 0.1031 | 0.3150 | 0.3146 |

* Average of three determinations.
** Root Mean Square of Error of Prediction.

Analysis of CIN and BMV in their pharmaceutical formulation can be achieved by the proposed spectrophotometric methods. The standard addition technique is used to assess the accuracy of the methods. Accurate results are obtained, indicating that no interference is observed from the excipients (Tables 4 and 5).

Both PLS and PCR regression models are used for the determination of CIN and BMV in Supraproct-S® ointment. Good recovery with high accuracy is obtained. Further testing of the accuracy by applying the standard addition technique reveals applicability of the methods with high accuracy, as shown in Table 6.

For the univariate methods, all the proposed methods show a linear correlation between the concentration and the selected wavelength absorbance, each according to the applied method within the stated range for the method. For the PLS and PCR multivariate methods, linear correlations are obtained between the predicted and the original concentrations of both drugs. The concentration ranges and regression parameters are shown in Table 7.

TABLE 4. Determination of Cinchocaine Hydrochloride and Betamethasone Valerate in the Pharmaceutical Formulation Supraproct-S® Ointment* by the Proposed Univariate Spectrophotometric Methods

| %Found of the claimed amount ± SD * | | | | | |
|---|---|---|---|---|---|
| CIN | BMV | | | | |
| $^0D_{327.0}$ | $ISO_{248.1}$ | DW $\Delta A$ (264.5 and 271.5 nm) | $RS_{240.0}$ | $^1DD_{253.0–271.0}$ | RD $\Delta P_{240.0–259.0}$ |
| 99.52 ± 0.48 | 99.68 ± 0.13 | 99.72 ± 0.86 | 99.95 ± 0.55 | 99.72 ± 1.07 | 99.67 ± 0.60 |

* Each gram was labeled to contain 5.0 mg CIN and 1.0 mg BMV, Batch No. 0014. Average of three determinations.

TABLE 5. Application of the Standard Addition Technique for the Determination of Cinchocaine Hydrochloride and Betamethasone Valerate by the Proposed Univariate Spectrophotometric Methods in Supraproct-S® *

| Taken (μg/mL) | | Added (μg/mL) | | %Recovery of the added amount* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | CIN | BMV | | | | |
| CIN | BMV | CIN | BMV | $^0D_{327.0}$ | $ISO_{248.1}$ | DW $\Delta A$ (264.5 and 271.5 nm) | $RS_{240.0}$ | $^1DD_{253.0–271.0}$ | RD $\Delta P_{240.0-259.0}$ |
| 30.00 | 6.00 | 15.00 | 3.00 | 99.67 | 101.53 | 99.33 | 100.67 | 101.33 | 99.67 |
| | | 30.00 | 6.00 | 100.30 | 99.61 | 100.33 | 99.50 | 99.92 | 100.67 |
| | | 60.00 | 12.00 | 100.23 | 99.55 | 100.50 | 99.83 | 100.75 | 101.08 |
| Mean ± SD | | | | 100.07±0.35 | 100.23±1.13 | 100.05±0.63 | 100.00±0.60 | 100.67±0.71 | 100.47±0.73 |

* Each gram was labeled to contain 5.0 mg CIN and 1.0 mg BMV, Batch No. 0014. Average of three determinations.

TABLE 6. Determination of Cinchocaine Hydrochloride and Betamethasone Valerate in Pharmaceutical Dosage Form by the Proposed Multivariate Methods and the Standard Addition Technique

| Product | Drug | Standard Addition | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PLS | | | | PCR | | | |
| | | Found (%) ± SD of the claimed amount* | Claimed (μg/mL) | Added (μg/mL) | %Recovery from the added amount* | Found (%) ± SD of the claimed amount* | Claimed (μg/mL) | Added (μg/mL) | %Recovery from the added amount* |
| Supraproct-S® Ointment Each gram was labeled to contain 5.0 mg CIN and 1.0 mg BMV, Batch No. 0014. | CIN | 101.10±0.22 | 10.00 | 5.00 | 100.60 | 101.23±0.21 | 10.00 | 5.00 | 98.80 |
| | | | | 10.00 | 99.60 | | | 10.00 | 99.70 |
| | | | | 20.00 | 99.50 | | | 20.00 | 98.50 |
| | | | Mean ±SD | | 99.90±0.54 | | Mean ±SD | | 99.00±0.62 |
| | BMV | 98.80±0.53 | 5.00 | 3.00 | 100.67 | 100.27±1.45 | 5.00 | 3.00 | 101.00 |
| | | | | 6.00 | 99.67 | | | 6.00 | 100.33 |
| | | | | 12.00 | 100.25 | | | 12.00 | 100.25 |
| | | | Mean ±SD | | 100.20±0.50 | | Mean ±SD | | 100.53±0.41 |

* Average of three determinations.

For multivariate calibrations, linearity correlations are obtained upon plotting the value of the predicted concentration versus the original ones for both drugs. The concentration ranges and regression parameters are shown in Table 7. ICH guidelines [10] are followed to calculate LOD and LOQ and ensure the validity of the proposed methods.

Statistical comparison is performed between the results obtained by applying the proposed conventional and multivariate calibrations for the determination of CIN and BMV in bulk powder with the official methods [1] for both drugs. No significant difference is observed for either accuracy or precision upon using the student's *t* and *F*-values, as shown in Table 8.

TABLE 7. Regression of the Proposed Methods for the Determination of Cinchocaine Hydrochloride and Betamethasone Valerate by the Univariate Spectrophotometric Methods and the Determination of Both Drugs in the Presence of their Degradation Products by Multivariate Calibrations in the Validation Set.

| Method parameter | CIN | | | BMV | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $^0D_{327.0}$ | PLS | PCR | $ISO_{248.1}$ | DW $\Delta A$ (264.5 and 271.5 nm) | $RS_{240.0}$ | $^1DD_{253.0–271.0}$ | RD $\Delta P_{259.0–240.0}$ | PLS | PCR |
| Range (μg/mL) | 10–120 | 10–30 | | 5–50 | 5–90 | 5–50 | 5–60 | 5–50 | 5–45 | |
| Regression equations parameters | | | | | | | | | | |
| Intercept | | | | | | | | | | |
| SE of intercept | 0.0082 | –0.0598 | –0.0724 | –0.0074 | 0.0005 | 0.0085 | 0.0118 | 0.0099 | 0.1452 | 0.1401 |
| Slope | 0.0024 | 0.1005 | 0.0989 | 0.0024 | 0.00046 | 0.0028 | 0.0084 | 0.0038 | 0.2522 | 0.2520 |
| SE of slope | 0.0107 | 1.0036 | 1.0043 | 0.0274 | 0.0048 | 0.0318 | 0.0665 | 0.0402 | 0.9994 | 0.9995 |
| Correlation | $3.37\times10^{-5}$ | 0.0052 | 0.0051 | $7.91\times10^{-5}$ | $8.80\times10^{-6}$ | $9.12\times10^{-5}$ | 0.00023 | 0.00013 | 0.0080 | 0.0079 |
| Coefficient (r) | 0.9999 | 0.9999 | 0.9999 | 0.9998 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9997 | 0.9997 |
| Accuracy (Mean±SD) | 100.35±0.76 | – | – | 99.58±0.45 | 100.40±1.38 | 99.81±0.35 | 99.62±0.68 | 99.29±0.12 | – | – |
| Specificity[a] | 99.95±0.49 | – | – | 100.49±0.97 | 100.09±1.04 | 99.44±0.81 | 100.01±0.77 | 99.90±0.81 | – | – |
| Precision (± %RSD)[b] | 0.45 | – | – | 0.24 | 0.39 | 0.23 | 0.34 | 0.31 | – | – |
| (± %RSD)[c] | 0.62 | – | – | 0.67 | 1.43 | 1.01 | 0.98 | 0.76 | – | – |

[a] Recovery of CIN and BMV in laboratory-prepared mixtures.
[b] Intra-day precision [average of 3 different concentrations of 3 replicate each ($n$=9) within the same day].
[c] Inter-day precision [average of 3 different concentrations of 3 replicate each ($n$=9) repeated on 3 successive days of CIN and BMV (10.00, 20.00 and 40.00 μg/mL).

TABLE 8. Statistical Comparison of the Results Obtained by the Proposed Methods and Those Obtained by the Official Ones for the Analysis of Cinchocaine Hydrochloride and Betamethasone Valerate in their Pure Form.

| Value | CIN | | | BMV | | | | | | | Official Method [1] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^0D_{327.0}$ | PLS | PCR | $ISO_{248.1}$ | DW $\Delta A$ (264.5 and 271.5 nm) | $RS_{240.0}$ | $^1DD_{253.0–271.0}$ | RD $\Delta P_{259.0–240.0}$ | PLS | PCR | CIN[a] | BMV[b] |
| Mean | 100.10 | 99.95 | 99.89 | 99.87 | 100.64 | 99.94 | 100.15 | 99.90 | 100.51 | 100.50 | 100.05 | 100.03 |
| SD | 1.11 | 0.71 | 0.72 | 0.67 | 0.80 | 0.70 | 0.91 | 1.06 | 1.22 | 1.22 | 1.32 | 1.17 |
| %RSD | 1.11 | 0.71 | 0.72 | 0.67 | 0.79 | 0.70 | 0.91 | 1.06 | 1.21 | 1.21 | 1.32 | 1.17 |
| $n$ | 7 | 10 | 10 | 6 | 7 | 6 | 7 | 6 | 10 | 10 | 5 | 5 |
| Variance | 1.23 | 0.50 | 0.52 | 0.45 | 0.64 | 0.49 | 0.83 | 1.12 | 1.49 | 1.49 | 1.74 | 1.37 |
| Student's $t$ test | 0.691 (2.228) | 0.159 (2.160) | 0.253 (2.160) | 0.271 (2.262) | 1.010 (2.228) | 0.151 (2.262) | 0.177 (2.228) | 0.122 (2.262) | 0.738 (2.160) | 0.723 (2.160) | – | – |
| $F$ value | 1.41 (4.53) | 3.48 (3.63) | 3.34 (3.63) | 3.04 (5.19) | 2.14 (4.53) | 2.80 (5.19) | 1.65 (4.53) | 1.22 (5.19) | 1.09 (6.00) | 1.09 (6.00) | – | – |

[a] For cinchocaine hydrochloride: the titrimetric method against 0.1 M NaOH as a titrant using a mixture of 0.01 M HCl and alcohol as a solvent with potentiometric detection of the end point.
[b] For betamethasone valerate: spectrophotometric determination at 240 nm against 96% ethanol as a blank.
– The values in the parenthesis are the corresponding theoretical values of $t$ and $F$ at $P = 0.05$.

**Conclusion.** The univariate spectrophotometric methods based on the mathematical manipulation of spectral data have the advantage of being rapid and simple for the resolution of a CIN and BMV binary mixture and in their dosage form without any interference from the excipients. As for the development of stability-indicating methods, a much more complex spectral interference constitutes a problem. It is the multivariate calibration methods that solve this problem. From the previous discussion, it can be concluded that the proposed procedures are simple, time-saving, and do not require sophisticated techniques or equipment. The developed methods are applicable in laboratories lacking liquid chromatographic instruments.

**REFERENCES**

1. J. Morch, *Dansk. Farm*., **2** (1953), through *Analytical Profile of Drug Substances*, **12** (1983).
2. M. Y. Salem, S. M. Amer, H. A. Merey, B. A. El-Zeany, *Egypt. J. Pharm. Sci*., **49**, 1–24 (2008).
3. A. El-Gindy, M. A. Korany, M. F. Bedair. *J. Pharm. Biomed. Anal*., **17**, 1357–1370 (1998).
4. M. Li, M. Lin, A. Rustum, *J. Pharm. Biomed. Anal*., **48**, 1451–1456 (2008).
5. E. W. Smith, J. M. Haigh, I. Kanfer, *Int. J. Pharm*., **27**, 185–192 (1985).
6. H. Bundgaard, J. Hansen, *Int. J. Pharm*., **7**, 197–203 (1981).
7. Y. W. Yip, P. A. Li Wan, *J. Pharm. Pharmacol*., **31**, 400–402 (1979).
8. A. S. Fayed, M. R. Rezk, H. M. Marzouk, S. S. Abbas, *J. Chromatogr. Sci*., **55**, 518–527 (2017)
9. *International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology Q2* (R1), (2005).
10. *The British Pharmacopoeia*, Her Majesty's Stationary office, London (2012).
11. R. G. Brereton, *Analyst*, **122**, 1521–1529 (1997).
12. S. V. Erram, H. P. Tipnis, *Indian Drugs*, **31**, 65–68 (1994).
13. H. M. Lotfy, M. A. Hegazy, M. R. Rezk, Y. R. Omran, *Spectrochim. Acta: Mol. Biomol. Spectrosc*., **148**, 328–337 (2015).
14. M. G. El-Bardicy, H. M. Lotfy, M. A. El-Sayed, M. F. El-Tarras. *J. AOAC Int*., **91**, 299–310 (2008).
15. F. Salinas, J. J. Nevado, A. E. Mansilla, *Talanta*, **37**, 347–351 (1990).
16. H. M. Lotfy, M. A. Hagazy, *Spectrochim. Acta: Mol. Biomol. Spectrosc*., **96**, 259–270 (2012)