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Validated stability-indicating derivative and derivative ratio methods for the determination of some drugs used to alleviate respiratory tract disorders and their degradation products

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Derivative and derivative ratio methods are presented for the determination of butamirate citrate, formoterol fumarate, montelukast sodium, and sodium cromoglycate. Using the second derivative ultraviolet (UV) spectrophotometry, butamirate citrate and formoterol fumarate were determined by measuring the peak amplitude at 260.4 and 261.8 nm, respectively, without any interference of their degradation products. Butamirate citrate degradation product, 2-phenyl butyric acid, was determined by the measurement of its second derivative amplitude at 246.7 nm where butamirate citrate displays zero crossing. Formoterol fumarate degradation product, desformyl derivative, could be evaluated through the use of the first derivative at peak amplitude of 264.8 nm where interference of formoterol fumarate is negligible. In the first mode, the zero-crossing technique was applied at 305 nm for the determination of montelukast sodium in the presence of its photodegradation product, cis-isomer. The derivative of ratio spectra of montelukast sodium and its cis- isomer were used to determine both isomers using the first derivative of the ratio spectra by measuring the amplitudes of the trough at 305 nm and the peak at 308 nm, respectively. The later technique was also used for the determination of a ternary mixture of sodium cromoglycate and its two degradation products using zero-crossing method. In the derivative ratio spectra of the ternary mixture, trough depths were measured at 271.6, 302.8 and 302.2 nm, using the second, the first, and the second mode to evaluate sodium cromoglycate, degradation product (1) and degradation product (2), respectively. All the methods were applied successfully to the pharmaceutical preparation and were validated according to ICH guidelines.

Keywords: respiratory tract drugs; degradation products; derivative spectrophotometry; derivative ratio spectrophotometry; stability indicating methods.

Introduction

Respiratory tract drugs include antitussive, anti-asthmatic and anti-allergic agents.[1] These drugs can alleviate illness of varied etiology, ranging from common cold to chronic obstructive pulmonary disease.[2] Butamirate citrate (BC) as a cough suppressant, formoterol fumarate (FF) as a β_2-adrenoceptor agonist, montelukast sodium (Mont. Na) as a selective leukotriene D_2 receptor antagonist, and sodium cromoglycate (NaCr) as a mast cell stabilizer, are various examples of such class used in this work.[3,4]

In the literature, derivative spectroscopy was used to determine BC,[5,6] as well as high performance liquid chromatogprahy (HPLC)[6] and capillary zone electrophoresis.[7] Neither of these methods considered the degradation product; 2-phenylbutyric acid (PBA). Various methods were used for the determination of FF including non-aqueous titration,[8] UV spectroscopy,[9] gas chromatography,[10] HPLC methods with UV detection,[11,12] tandem mass detection,[13] or electrochemical detection.[14] Capillary zone electrophoresis,[15] and quantitative nuclear magnetic resonance (NMR)[16] methods were also described. Only one stability-indicating method for the determination of FF and its related substance, the desformyl derivative (Des), using HPLC with UV detection, was described.[17] Several methods for evaluation of NaCr were described including spectroscopy,[18] thin layer chromatography (TLC),[19] HPLC using UV detection[20] and electrochemical methods[21] where other methods were used for drug determination in the presence of two or three impurities.[22–25] Mont. Na was determined via different techniques *viz.*, spectrophotometry,[26] fluorimetry,[27] HPLC with chiral stationary phase,[28] stability-indicating HPLC method using UV detection,[29] and HPLC using fluorescence detection.[30,31]

Obviously, all the reported methods did not include stability-indicating methods for the selected drugs, using derivative spectrophotometry or derivative of the ratio spectra methods, which are considered low cost methods and less complicated procedures.

The present work utilized, derivative and derivative of the ratio spectra spectrophotometry were used to determine the targeted drugs and/in the presence of their degradation products. The proposed methods were compared with spectroscopic[6] (for BC), official[8] (for FF and NaCr) and HPLC methods[29] (for Mont. Na).

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The proposed methods are designed to be suitable for the quality assessment of these compounds in pharmaceutical products.

Experimental

Apparatus

Schimadzu UV/visible recording spectrophotometer 1600 PC double beam with a fixed slit width 2 nm connected to a computer loaded with Shimadzu UV probe software and a HP LaserJet P2015 model printer was used for all the spectrophotometric measurements and treatment of data. Quartz cell 1 cm was used all over.

Second derivative curves of the zero spectra of BC and PBA solutions were recorded using scaling factor 100, over the range 255–267 nm, $\Delta\lambda = 4$ nm, and 242–251 nm, $\Delta\lambda = 2$ nm, respectively. The first and second derivative curves of the zero spectra of FF and Des solutions were recorded, using scaling factor 200 over the range 240–325 nm, $\Delta\lambda = 8$ nm, and 250.28–277.77 nm, $\Delta\lambda = 4$ nm, respectively.

The first derivative curves of the zero spectra of Mont. Na and its cis-isomer (Cis-mont) were recorded using scaling factor 25 over the range of 280–335 nm, $\Delta\lambda = 10$ nm, whereas a range 250–415 nm, $\Delta\lambda = 10$ nm, scaling factor 10 and 245–400 nm, $\Delta\lambda = 10$ nm, scaling factor 20 were selected to read the analytical signals for Mont. Na and Cis-mont, respectively, in the derivative of the ratio spectra.

Ratio spectra derivative spectrophotometry range were selected as 250–342 nm, $\Delta\lambda = 8$ nm, scaling factor 1.0; 274–316 nm, $\Delta\lambda = 8$ nm, scaling factor 100 and 258–335 nm, $\Delta\lambda = 8$ nm, scaling factor 50 for reading the analytical signals of NaCr, Deg1 and Deg2, respectively.

Material

BC and FF were kindly donated by Novartis Pharma Company (Cairo, Egypt). Purity were 99.84 ± 0.415 (n=6), and 99.76 ± 0.939 (n=6), respectively, according to the comparison methods. NaCr and Mont. Na were kindly donated by Amoun Pharmaceutical Company (Al-Obour City, Egypt). Purity were 99.79 ± 0.722 (n=6), and 99.85 ± 0.0602 (n=6), respectively, according to the comparison methods. The structures of the investigated compounds together with the degradation products cited in this work are shown in Figure 1. Sodium hydroxide, diethyl ether, and sodium sulfate anhydrous were obtained from El-Nasr Co., Cairo, Egypt. Hydrochloric acid was obtained from Reidel-de Haën Co., Hanover, Germany. Methanol was obtained from Allianco Bio Co., CA, USA.

Pharmaceutical preparations

Sinecod® syrup (Batch No. Y0012, containing 1.5 mg/ml of BC) and Foradil® capsules for inhalation (Batch No. 238/u0036, containing 12 mg of FF per capsule), of Novartis Pharma Company (Cairo, Egypt) under license from Novartis Pharma AG. (Basle, Switzerland) were purchased from the market.

Nasotal® drops (Batch No. 2205, containing 2g NaCr per 100 ml) and Clear air® tablets (Batch No. 4385, containing 10 mg of Mont. Na per tablet), of Amoun Pharmaceutical Company (Al-Obour City, Egypt) were purchased from the market.

Solutions

Stock solutions of 1.2 mg/ml BC, 0.9 mg/ml PBA, 0.24 mg/ml NaCr, 0.1 mg/ml Deg1 and 0.1 mg/ml Deg2 were prepared in 0.01M sodium hydroxide. Stock solution of 0.05 mg/ml FF in 0.01M hydrochloric acid was prepared. A solution of Des in 0.01M hydrochloric acid containing Des equivalent to 0.005 mg/ml FF was prepared. Stock solutions of 0.1 mg/ml (for derivative spectrophotometry), 0.08 mg/ml (for derivative of the ratio spectrophotometry) of Mont. Na and 0.08 mg/ml of Cis-mont were prepared in methanol. Des,[13] Deg1,[32] Deg2,[33] and Cis-mont[29,34] were prepared according to the reported methods.

Procedure

For derivative spectrophotometry

The value of the D_2 amplitudes were measured at 260.4 nm ($\Delta\lambda = 4$ nm) (zero crossing of PBA) and 246.7 nm ($\Delta\lambda = 2$ nm) (zero crossing of BC) for the determination of BC and PBA, respectively. The value of D_2 amplitudes were measured at 261.8 nm (zero reading value of Des) and that of D_1 at 264.8 nm (zero crossing of FF) for the determination of FF and Des, respectively. The value of D_1 amplitudes were measured at 305 nm (zero reading value of Cis-mont) for the determination of Mont. Na.

For the derivative of the ratio spectrophotometry

According to the theory of the ratio spectra derivative method,[35–36] the stored UV absorption spectra of standard solutions of the ternary mixture were divided wavelength by wavelength by a standard spectrum of Deg2 (20 μg/ml) for the determination of NaCr. The first derivative was then calculated with $\Delta\lambda = 8$ nm. The trough amplitudes at 271.6 nm were measured and found to be linear to the concentration of NaCr. For Deg1; the same was applied using NaCr (10 μg/ml) as a divisor. The second derivative was calculated with $\Delta\lambda = 8$ nm, the trough amplitudes were measured at 302.8 nm and found to be linear to the concentration of Deg1. For Deg2; the same was applied using NaCr (120 μg/ml) as a divisor. The first derivative was calculated with $\Delta\lambda = 8$ nm and the trough amplitudes were measured at 302.2 nm and found to be linear to the concentration of Deg2.

For the determination of Mont. Na, the stored UV absorption spectra of standard solutions of Mont. Na were divided wavelength by wavelength by a standard spectrum of Cis-mont (40 μg/ml). The first derivative was calculated with $\Delta\lambda = 10$ nm. The trough amplitudes were measured at 305 nm and found to be linear to the concentration of Mont Na. For Cis-mont, the same was applied using Mont. Na (20 μg/ml) as a divisor. The first derivative was calculated with $\Delta\lambda = 10$ nm, the peak amplitudes were measured at 308 nm and found to be linear to the concentration of Cis-mont.

Procedure for the pharmaceutical preparations

For syrup (BC)

A volume of syrup equivalent to 60 mg BC was introduced into a 250-ml separating funnel and diluted with water (10 ml), followed by 1M sodium hydroxide (15 ml). The solution was extracted with ether (5 × 15 ml). The ether extract was collected in a beaker through a funnel and Whatman No. 42 filter paper, filled with anhydrous sodium sulfate. The extract was dried on a water bath, and then the residue was dissolved in 0.01M hydrochloric acid (3 × 10 ml) and transferred quantitatively into a 50-ml volumetric flask. The volume was then completed with the same solvent. Aliquots of this solution equivalent to BC (1.68–4.08 mg) were introduced into a series of 10-ml volumetric flasks; the volumes were completed with 0.01M sodium hydroxide, and measured as mentioned earlier.

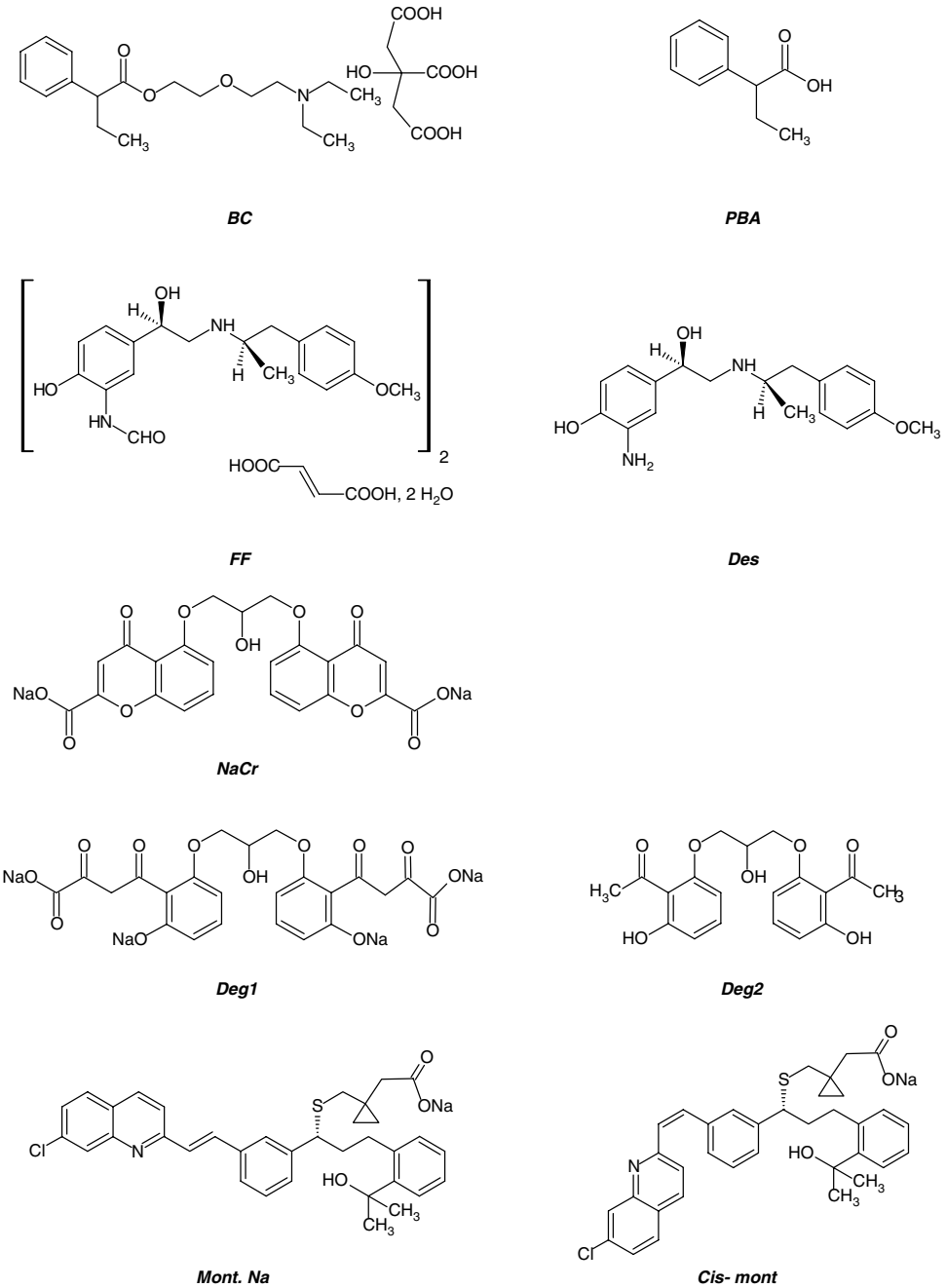

Figure 1. Chemical structure of: butamirate citrate (BC), 2-phenylbutyric acid (PBA), formoterol fumarate (FF), desformoterol fumarate (Des), sodium cromoglycate (NaCr), the open ring intermediate of NaCr (Deg1), the bisacetophenone derivative of NaCr (Deg2), montelukast sodium (Mont. Na) and the cis-isomer of Mont. Na (Cis-mont).

For capsules (FF)

The content of 30 capsules equivalent to 360 µg FF was introduced into a 50-ml volumetric flask, dissolved and diluted to volume with 0.01M hydrochloric acid. Aliquots of this solution equivalent to FF (20.16–57.60 µg) were introduced into a series of 10-ml volumetric flasks, the volumes were completed with 0.01M hydrochloric acid, and measured as mentioned earlier.

For nasal drops (NaCr)

A volume of nasal drops equivalent to 100 mg NaCr was introduced into a 250-ml volumetric flask and diluted to volume with 0.01M sodium hydroxide. Aliquots of this solution equivalent to NaCr (320–880 µg) were introduced into a series of 10-ml volumetric flasks, the volumes were completed with 0.01M sodium hydroxide, and measured as mentioned earlier.

For tablets (Mont. Na)

A quantity of the powdered tablets equivalent to Mont. Na (10 mg) was introduced into a beaker and extracted with methanol (20 ml) then filtered into a 50-ml volumetric flask. The residue was washed with methanol (4 × 5 ml). The combined filtrate and washings were completed to volume with methanol. An aliquot (20 ml)

of this solution (4 mg) was transferred into a 50-ml volumetric flask and the volume was completed with methanol to prepare a solution of Mont. Na (0.08 mg/ml). The experiment was carried out on aliquots equivalent to (96–320 μg) Mont. Na for D_1 method and on aliquots equivalent to (88–264 μg) Mont. Na. for DD_1 method, and measured as mentioned earlier for methods D_1 and DD_1.

Percent recovery study

To study the accuracy of the proposed methods and to check the interference of excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of each drug to a known concentration of the commercial pharmaceutical dosage form. The resulting mixtures were analyzed as described earlier.

Results and Discussion

Derivative spectrophotometry

*For **BC***

As reviewed in the literature, possible ester hydrolysis can be achieved by reflux in alkaline aqueous solution for 3 h.[37] BC (500 mg) was refluxed with 0.1M sodium hydroxide (50 ml) at 100 °C for 10 h till complete disappearance of BC as monitored by TLC using chloroform: methanol (9 : 1, *v/v*) as the developing system. Subsequently, the solution was acidified using 1M hydrochloric acid and extracted with chloroform (4x10 ml). The aqueous layer was discarded. The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness to yield white precipitate of PBA. The assignment of the degradation product as PBA was based on mass spectrum of PBA showing a peak at *m/z* 164 representing the molecular ion peak, IR spectrum lacking the characteristic ester C═O stretching band of BC at 1733.2 cm^{-1}, and ^{1}H-NMR spectrum lacking the characteristic signals of 2-(diethylamino)-ethoxyethyl chain protons.

Literature survey revealed that no method is reported for the determination of BC and its alkaline degradation product, PBA. Derivative spectroscopy opens up possibilities, not only for increasing selectivity,[38] but also for increasing sensitivity.[39] In this investigation, a study of BC stability in 0.01M sodium hydroxide revealed that BC was stable in 0.01M sodium hydroxide for at least 3 h. The second derivative spectra ($\Delta\lambda = 4$) of BC and PBA showed that BC in 0.01M sodium hydroxide exhibited a peak at 260.4 nm with a zero crossing point of PBA (Figure 2A). On the other hand, in the second derivative spectra ($\Delta\lambda = 2$), PBA exhibits a trough at 246.7 nm where BC displays zero reading (Figure 2B). BC could be easily determined by measuring the amplitude at 260.4 nm, while the amplitude at 246.7 nm could be used for the determination of PBA without any interference of BC.

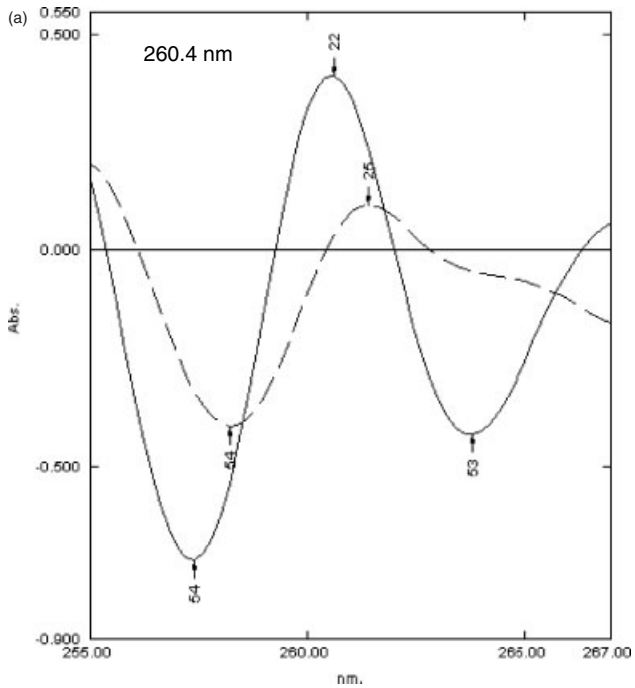

Figure 2a. Second derivative spectra ($\Delta\lambda = 4$) of BC (408 μg/ml) ——— and PBA (81 μg/ml) - - - - in 0.01 M sodium hydroxide.

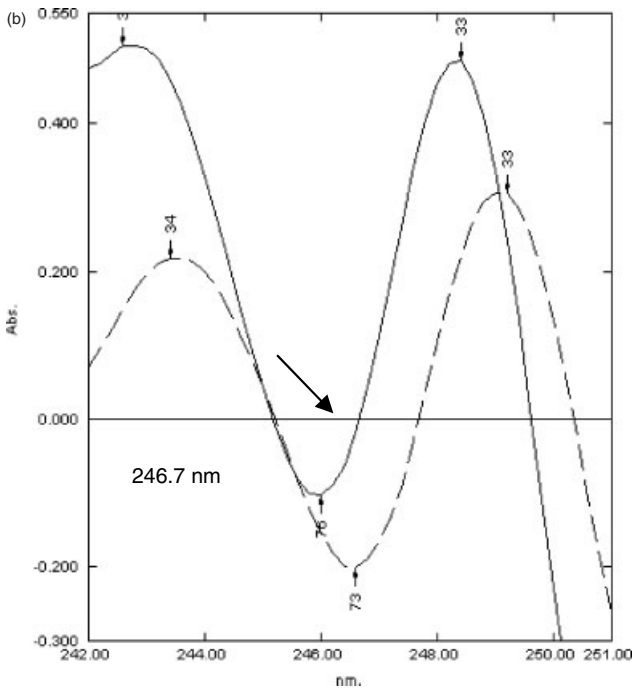

Figure 2b. Second derivative spectra ($\Delta\lambda = 2$) of BC (408 μg/ml) ——— and PBA (81 μg/ml) - - - - in 0.01 M sodium hydroxide.

For FF

Des is one of the related substances of FF listed in the European Pharmacopoeia (2004).[8] It was prepared by adopting the published procedure[13] and was confirmed by applying the reported stability indicating HPLC procedure.[17] In this investigation, a study of FF stability in different molarities of hydrochloric acid revealed that FF was stable in 0.01M hydrochloric acid for at least 4 h, while it was unstable in higher molarities of hydrochloric acid. The zero-order spectra of FF and Des in 0.01M hydrochloric acid are completely overlapped. In the second mode, FF in 0.01M hydrochloric acid exhibits a peak at 261.8 nm ($\Delta\lambda = 8$), where Des displays no reading (Figure 2C). First derivative spectra of FF and Des showed that Des exhibits a peak at 264.8 nm ($\Delta\lambda = 4$), where FF displays zero crossing (Figure 2D).

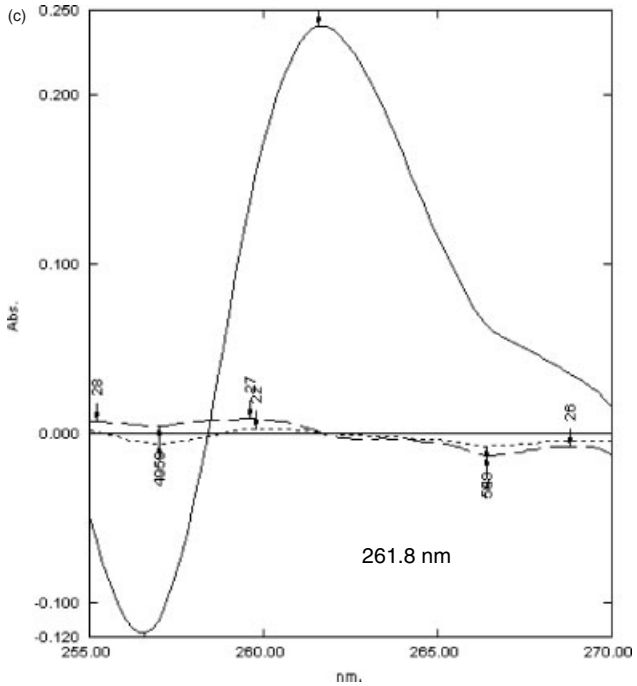

Figure 2c. Second derivative spectra of FF (5 μg/ml) ——— Des (2 μg/ml) - - - - and lactose (30 mg/ml) · · · · . in 0.01 M hydrochloric acid.

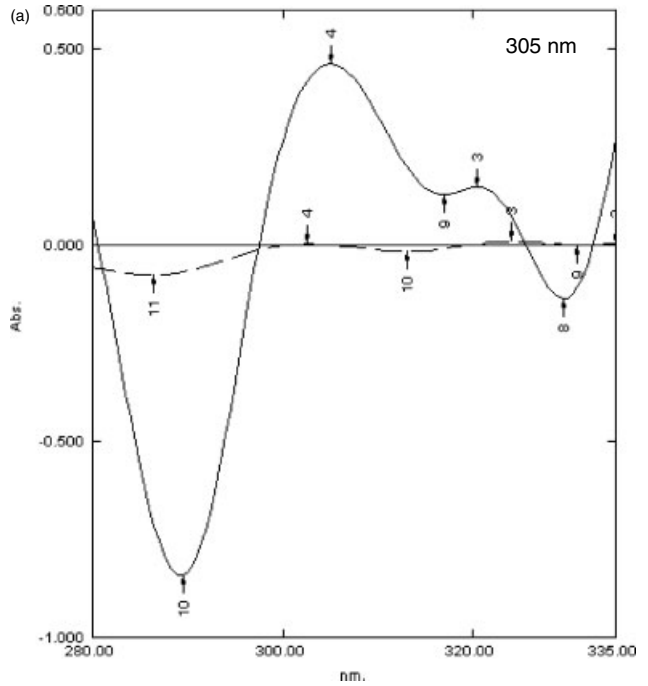

Figure 3a. First derivative spectra of Mont. Na (40 μg/ml) ——— and its cis-mont (8 μg/ml) - - - - in methanol.

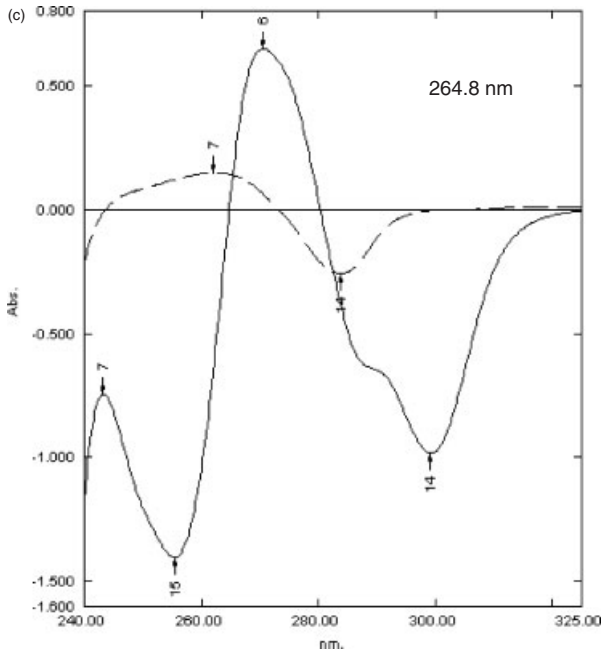

Figure 2d. First derivative spectra of FF (10 μg/ml) ——— and Des (2 μg/ml) - - - - in 0.01 M hydrochloric acid.

For Mont. Na

Mont. Na is a light-sensitive compound and this necessitates special handling precautions to protect it from light, in solution and in solid state.[29,34] Upon exposure to light, the (E) - ethenyl moiety of Mont. Na readily rotates to the (Z) geometric configuration leading to the formation of its cis-isomer.[34] Based on this knowledge, a solution of Mont. Na in methanol was exposed to light. Complete isomerization was confirmed after 30 days of applying the reported stability-indicating HPLC procedure.[34] There is no published research proposing the determination of Mont. Na in the presence of its inactive photo degradation product, Cis-mont, using the derivative spectrophotometric method. The zero-order spectra of Mont. Na and Cis-mont in methanol showed complete spectral interference preventing the determination of either of them. The first mode was used to determine Mont. Na in the presence of its photodegradation product by measuring the amplitude of the peak at 305 nm (A_{Mont}) ($\Delta\lambda = 10$), where Cis-mont displays zero reading (Figure 3A). Value of that amplitude was used for calculating the concentration of Mont. Na in the mixture.

Derivative of the ratio spectrophotometry

For NaCr

The most likely type of NaCr degradation was considered to be hydrolytic, analogous to the reaction of flavones in alkaline solution. Such degradation would involve the opening of the γ-pyrone ring of the chromone nucleus, followed by the loss of a two-carbon fragment as oxalate, and the formation of a bisacetophenone derivative. A thorough look in the literature revealed the presence of a general method for carrying out partial degradation of the chromone ring using alcoholic and not aqueous alkaline solution.[32] That method was used to prepare Deg1. IR spectra of NaCr and Deg1 were not able to differentiate between the two compounds because the OH stretching and CO stretching bands appear nearly at the same frequency (NaCr 3394, 1638 cm-1) (Deg1 3397, 1633 cm-1); thus mass spectrum of Deg1 was used to confirm the successful preparation of Deg1 showing a peak at *m/z* 592 corresponding to its molecular ion peak. Moreover a successful application of khellin hydrolysis[33] was used to prepare Deg2 based on the similarity of the chromone nucleus in both compounds (NaCr and khellin).

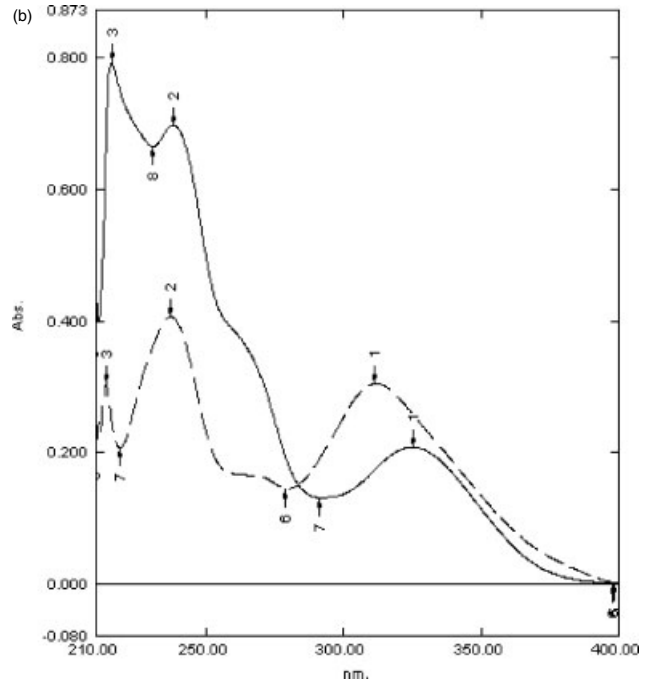

Figure 3b. Absorption spectra of NaCr in 0.1M sodium hydroxide at zero time ——— and after 3 h - - - - .

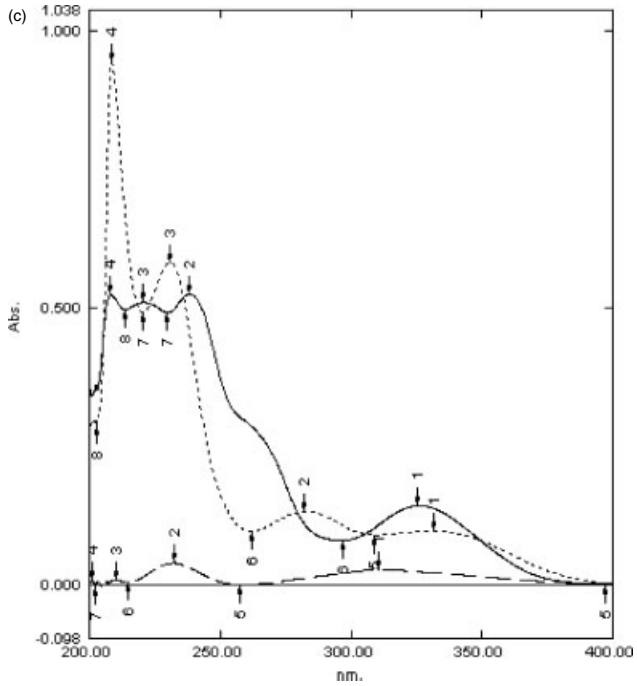

Figure 3c. Zero-order spectra of NaCr ———, Deg1 - - - - and Deg2 · · · · . in 0.01 M sodium hydroxide.

Deg2 is one of the related substances of NaCr listed in the European Pharmacopeia (2004),[8] thus it was deemed useful to determine NaCr in the presence of its two degradation products, Deg1 and Deg2, using the derivative ratio spectrophotometric method. In this investigation, a study of NaCr stability in sodium hydroxide revealed that NaCr is stable in 0.01M sodium hydroxide for at least 3 h, while dissolving NaCr in 0.1M sodium hydroxide leads to increasing the rate of hydrolysis to the open ring structure, Deg1, causing hyper- and hypsochromic shift (λ_{max} decreases from 326 to 310 nm gradually with time) (Figure 3B).

A complete overlap between the zero-order spectra of NaCr, Deg1 and Deg2 in 0.01M sodium hydroxide is shown in Figure 3C. Potentiality of the derivative ratio spectrophotometry as an analytical technique and its usefulness in providing an accurate, rapid, simple way of quantification[40] can be used to solve the case problem.

The main parameters that affect the shape of the derivative ratio spectra are the concentration of the standard solution used as a divisor and the wavelength intervals over which the derivative is obtained ($\Delta\lambda$). These parameters need to be optimized to give a well-resolved large peak with good selectivity and higher sensitivity in the determination.[41]

All the divisors were chosen for the reproducible results. The obtained ratio spectra were differentiated with respect to wavelength to afford the suitable derivative ratio spectra where that of the third component displays zero crossing point. The first derivative of the ratio spectra of NaCr are shown in Figure 3D; where Deg1 displays zero reading at the trough at 271.6 nm. The second derivative of the ratio spectra of Deg1 is shown in Figure 4A; where that of Deg2 displays zero crossing at the trough at 302.8 nm. The first derivative of the ratio spectra of Deg2 is shown in Figure 4B; where that of Deg1 displays zero crossing at the trough at 302.2 nm. The effect of wavelength intervals revealed that $\Delta\lambda = 8$ was the most suitable interval for measurement of the three mixture components, whereas using other intervals revealed either corrupted or irreproducible results.

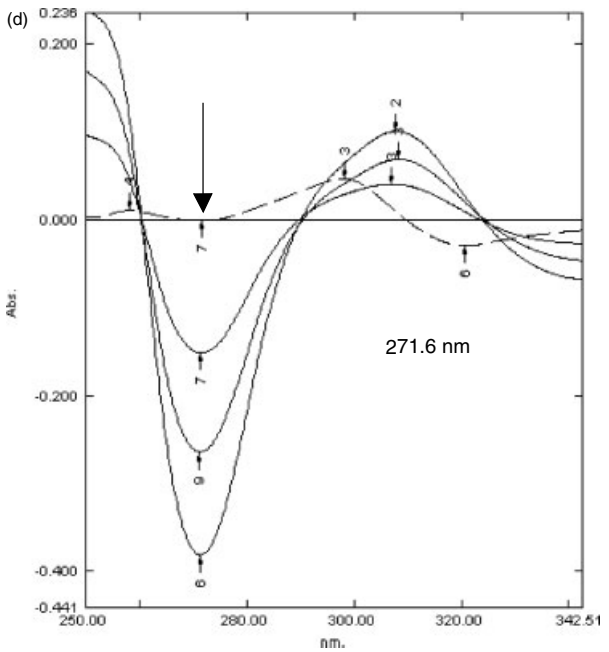

Figure 3d. First derivative ratio spectra (DR_{1C}) of NaCr (20, 40, 60 µg/ml) ——— and Deg1 (40 µg/ml) - - - - (divisor: 20 µg/ml Deg2).

For Mont. Na

The absorption spectra of Mont. Na and its photodegradation product, Cis-mont, in 0.1M hydrochloric acid: methanol (1 : 1, *v/v*), are greatly overlapped as shown in Figure 4C. First derivative method presented in this work could only determine Mont. Na in

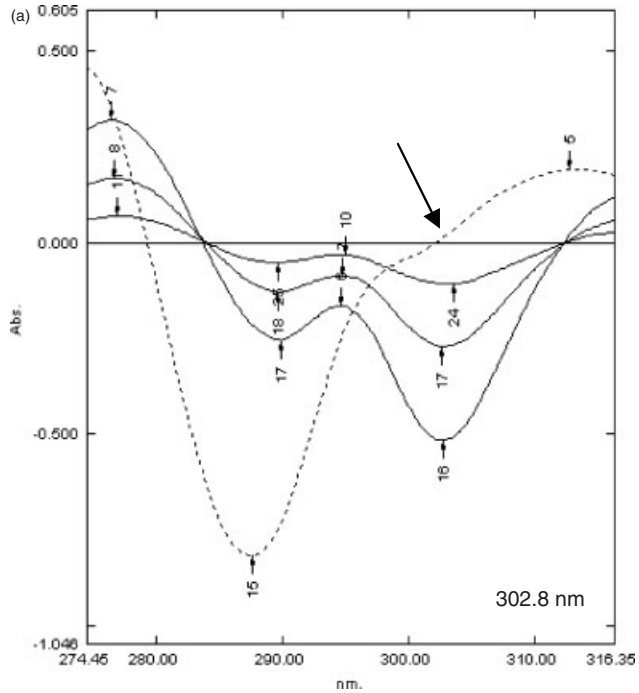

Figure 4a. Second derivative ratio spectra (DR_{2D1}) of Deg1 (10, 20, 40 μg/ml) ——— and Deg2 (10 μg/ml) - - - - (divisor: 10 μg/ml NaCr).

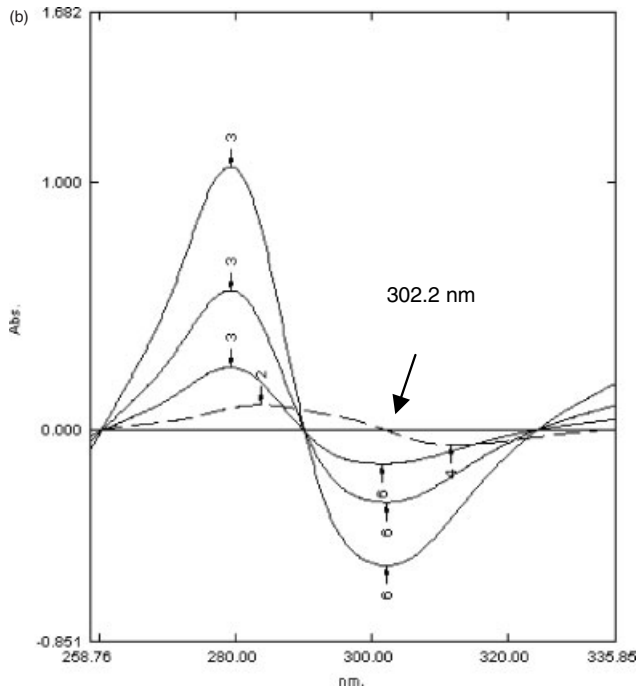

Figure 4b. First derivative ratio spectra (DR_{1D2}) of Deg2 (10, 20, 40 μg/ml) ——— and Deg1 (10 μg/ml) - - - - (divisor: 120 μg/ml NaCr).

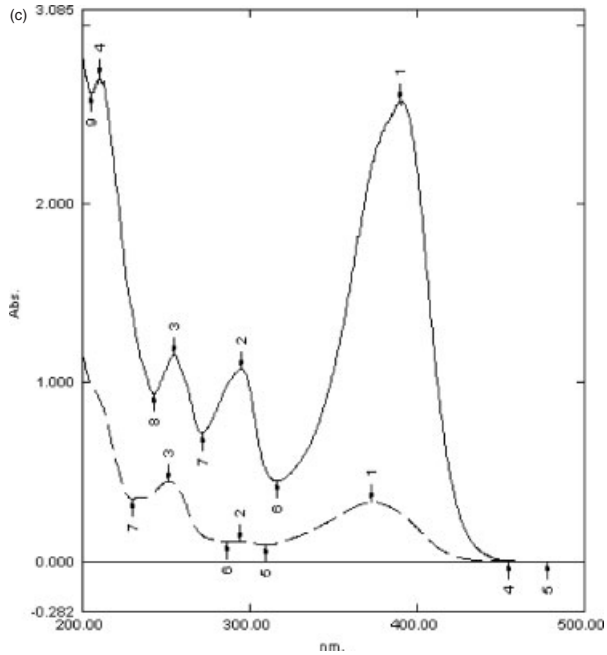

Figure 4c. Zero order spectra of Mont. Na (40 μg/ml) ——— and Cis-mont (10 μg/ml) - - - - in 0.1M hydrochloric acid: methanol (1 : 1, *v/v*).

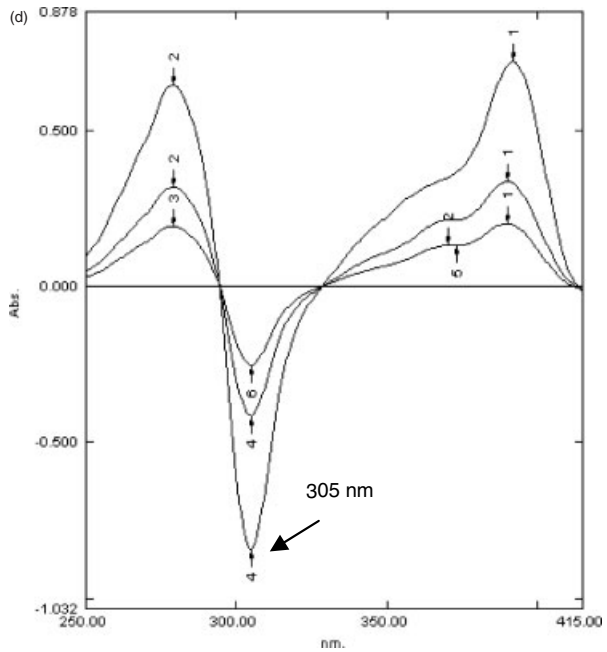

Figure 4d. First derivative ratio spectra (DR_{1M}) of Mont. Na (10, 20, 40 μg/ml) (Divisor: 40 μg/ml Cis-mont).

the presence of its photodegradation product. This promotes the author to think about a modification which allows the determination of both isomers. This could be done using the first derivative of the ratio spectra in 0.1M hydrochloric acid: methanol (1 : 1, *v/v*). The order of derivative used, the effect of wavelength intervals, and the concentration of the divisors were studied. The obtained ratio spectra in both cases were differentiated with respect to wavelength to afford the first derivative ratio spectra (DR_{1M}, DR_{1C}). Good measurements could be obtained at the trough 305 nm and peak 308 nm for Mont. Na and Cis-mont, respectively (Figures 4D and 5A). Effect of the wavelength intervals revealed that $\Delta\lambda = 10$ was the most suitable interval for measurement of both isomers. Increasing that interval led to a less sensitive peak.

For each of the proposed methods, regression equations and correlation coefficients are shown in Tables 1 and 2.

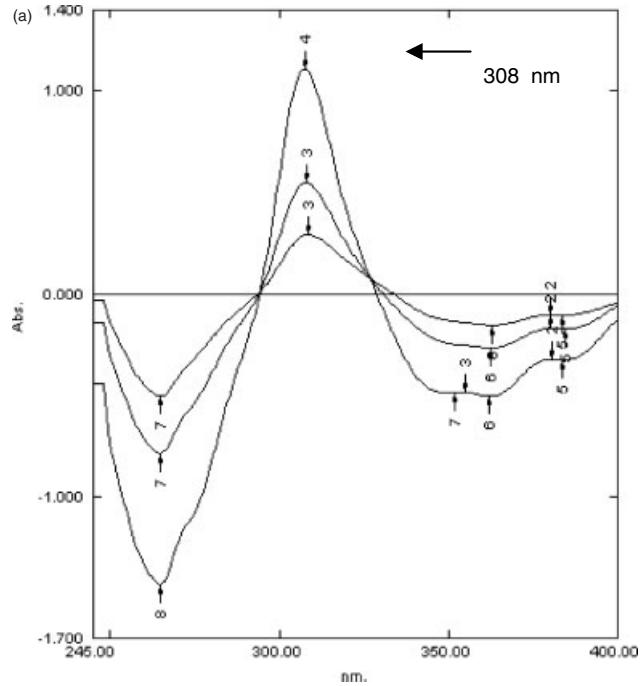

Figure 5a. First derivative ratio spectra (DR_{1C}) of Cis-mont (10, 20, 40 µg/ml) (Divisor: 20 µg/ml Mont. Na).

Method validation

The proposed methods were validated as per ICH guidelines.[42]

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The method's specificity was assessed by comparing the spectra obtained from the commercial formulations and the synthetic mixture from standard solutions. The absorption spectra were similar by the applied procedures after resolving all arising problems.

Linearity and range

A linear relationship should be evaluated across the range of the analytical procedure. In this work for the establishment of linearity, six concentrations were used, each was repeated three times. Data from the regression line itself are provided and used for mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept and slope of the regression line are provided. In addition, an analysis of the deviation of the actual data points from the regression line, the confidence limit of the slope and that of the intercept are also given. All these data are provided as helpful tools for evaluating linearity. The range for each drug was optimized. The correlation coefficient of the standard curve for each method was greater than 0.999 (Tables 1 and 2).

Accuracy

The accuracy was determined by standard addition methods. Different levels of standards were spiked to commercial pharmaceutical formulations in triplicate. The mean of the percentage recoveries of each drug was found acceptable (Table 3).

Precision

The repeatability of the methods was determined by assaying six standard solutions of each drug at the listed concentration (Table 4). The RSD% of each was accepted and was listed in the same table.

Table 1. Beer's Law data and statistical analysis for the calibration graphs using the derivative spectrophotometric proposed methods

Item	BC	PBA	FF	Des	Mont. Na
Derivative	^{2}D	^{2}D	^{1}D	^{2}D	^{1}D
Solvent used	0.01 NaOH	0.01 NaOH	0.01 HCl	0.01 NaOH	Methanol
λmax of measurements	260.4 nm	246.7 nm	261.8 nm	264.8 nm	305.0 nm
Concentration range	60–900 µg/mL	45–315 µg/mL	1.5–17.5 µg/mL	0.5–5.0 µg/mL	5–60 µg/mL
Regression equation	0.0009 C +0.0085	0.0022 C +0.005	0.043 C −0.006	0.067 C −0.002	0.0116 C −0.0015
Regression coefficient (r^2)	0.9998	0.9994	0.9998	0.9994	0.9999
^1S_b	5.30×10^{-6}	2.37×10^{-5}	3.05×10^{-4}	3.07×10^{-4}	5.97×10^{-5}
^2S_a	2.93×10^{-3}	4.76×10^{-3}	3.41×10^{-3}	9.56×10^{-4}	2.15×10^{-3}
^{3}***LOD***	9.767	6.491	0.238	0.043	0.556
^{4}***LOQ***	32.555	21.636	0.793	0.143	1.853
Confidence limit of the slope	$0.0009 \pm 1.30 \times 10^{-5}$	$0.0022 \pm 6.09 \times 10^{-5}$	$0.043 \pm 7.84 \times 10^{-4}$	$0.067 \pm 7.89 \times 10^{-4}$	$0.0116 \pm 1.53 \times 10^{-4}$
Confidence limit of the intercept	$0.0085 \pm 7.18 \times 10^{-3}$	0.005 ± 0.012	$-0.006 \pm 8.76 \times 10^{-3}$	$-0.002 \pm 2.46 \times 10^{-3}$	$-0.0015 \pm 5.52 \times 10^{-3}$
Standard error of the estimation	4.12×10^{-3}	5.64×10^{-3}	4.22×10^{-3}	1.22×10^{-3}	2.99×10^{-3}

^1S_b = Standard deviation of the slope.
^2S_a = Standard deviation of the intercept.
^{3}***LOD*** = Limit of detection.
^{4}***LOQ*** = Limit of quantification.

Table 2. Beer's Law data and statistical analysis for the calibration graphs using the ratio derivative spectrophotometric proposed methods

Item	Na Cr	Deg 1	Deg 2	Mont. Na	Cis-mont
Derivative ratio	^{1}DR	^{2}DR	^{1}DR	^{1}DR	
Solvent used		0.01 NaOH		Methanol : 0.1M HCl (1 : 1 v/v)	
λmax of measurements	271.6 nm	302.8 nm	302.2 nm	305 nm	308 nm
Concentration range	24–120 µg/ml	4–46 µg/ml	4–46 µg/ml	4–40 µg/ml	2–20 µg/ml
Regression equation	0.0062 C −0.0012	0.0115C −0.0177	0.0136 C −0.0012	0.021 C +0.0035	0.0279 C −0.0089
Regression coefficient (r^2)	0.9995	0.9997	0.9999	0.9999	0.9999
^1S_b	6.71×10^{-5}	8.38×10^{-5}	6.46×10^{-5}	7.92×10^{-5}	1.13×10^{-4}
^2S_a	5.31×10^{-3}	2.39×10^{-3}	1.84×10^{-3}	2.11×10^{-3}	1.38×10^{-3}
^3LOD	2.569	0.623	0.406	0.301	0.148
^4LOQ	8.564	2.078	1.353	1.005	0.495
Confidence limit of the slope	$0.0062 \pm 1.86 \times 10^{-4}$	$0.0115 \pm 2.05 \times 10^{-4}$	$0.0136 \pm 1.58 \times 10^{-4}$	$0.021 \pm 2.20 \times 10^{-4}$	$0.0279 \pm 3.14 \times 10^{-4}$
Confidence limit of the intercept	-0.0012 ± 0.0148	$-0.0177 \pm 5.85 \times 10^{-3}$	$-0.0012 \pm 4.51 \times 10^{-3}$	$0.0035 \pm 5.86 \times 10^{-3}$	$-0.0089 \pm 8.17 \times 10^{-3}$
Standard error of the estimation	5.39×10^{-3}	3.26×10^{-3}	2.51×10^{-3}	2.47×10^{-3}	1.77×10^{-3}

^1S_b = Standard deviation of the slope.
^2S_a = Standard deviation of the intercept.
^3LOD = Limit of detection.
^4LOQ = Limit of quantification.

Table 3. Results of the determination & the recovery analysis of commercial dosage forms using the proposed methods

Derivative methods	Claimed (µg/ml) BC 'Sinecod' Syp	added	Recovery % Syp	added	Claimed (µg/ml) FF 'Foradil' Caps.	added	Recovery % Caps	added	Claimed (µg/ml) Mont. Na 'Clair air' Tab	added	Recovery % Tab	added
1	168	96	97.55	98.38	2.016	5	98.06	100.92	9.6	8	99.23	100.21
2	216	144	95.42	99.54	2.016	2	98.06	98.80	16.0	10	98.87	100.00
3	264	396	96.17	99.89	3.456	7	98.23	100.67	20.8	32	100.09	100.48
4	312	300	96.33	98.89	3.456	3	98.23	100.00	24.0	24	98.96	99.49
5	360	204	97.07	99.13	5.760	4	97.71	100.00	28.8	20	100.42	100.00
6	408	456	95.45	99.66	5.760	9	97.71	100.25	32.0	16	99.81	98.59
Mean			96.33	99.25			98.00	100.11			99.56	99.79
S.D.			±0.856	±0.559			±0.265	±0.739			±0.639	±0.674
S.E.			±0.349	±0.228			±0.153	±0.302			±0.260	±0.275

Derivative Ratio methods	Na Cr 'Nasotal' Dps	added	Dps	added	Mont. Na 'Clair air' Tab	added	Tab	added
1	32	60.0	107.46	99.73	8.8	7.2	100.37	99.21
2	40	79.2	106.93	100.19	10.4	10.4	100.96	100.73
3	60	50.4	108.39	99.85	16.8	17.6	100.48	100.92
4	68	45.6	106.55	100.45	18.4	15.2	99.25	99.62
5	80	38.4	107.50	99.55	24.8	9.6	100.71	100.20
6	88	26.4	106.34	99.58	26.4	13.6	100.56	100.14
Mean			107.19	99.89			100.39	100.14
S.D.			±0.749	±0.358			±0.594	±0.647
S.E.			±0.306	±0.146			±0.242	±0.264

Table 4. Results of the determination and the recovery analysis of laboratory-made mixtures using the proposed methods

	Claimed (µg/ml)			Recovery %		Claimed (µg/ml)			Recovery %	Claimed (µg/ml)			Recovery %	
	BC					FF				Mont. Na				
D methods	BC	PBA	PBA%	BC	PBA	FF	Des	Des%	FF	Des	Mont. Na	Cis-mont	%	Mont. Na
1	240	63	20.79	98.38	101.01	3	0.90	23.08	100.00	99.44	16	4.0	20.00	98.87
2	480	144	23.08	100.58	99.43	5	1.75	25.92	100.00	98.91	24	2.4	9.09	98.96
3	600	117	16.32	100.09	99.07	8	4.00	33.33	101.16	99.62	36	9.6	21.05	99.97
4	720	108	13.04	100.54	100.59	12	2.40	16.67	100.77	98.25	42	12.0	22.22	99.03
5	804	81	9.15	99.71	99.32	14	2.10	13.04	100.16	100.90	48	7.2	13.04	99.05
6	900	45	4.76	99.57	98.99	15	0.75	4.76	99.38	99.47	60	3.2	5.06	100.65
Mean				99.81	99.73				100.24	99.43				99.42
S.D.				±0.814	±0.851				±0.631	±0.879				±0.724
S.E.				±0.332	±0.347				±0.258	±0.359				±0.295

	Na Cr							Mont. Na					
	Claimed (µg/ml)			Recovery %				Claimed (µg/ml)			Recovery %		
DR methods	Na Cr	Deg1	Deg2	Deg1%	Deg2%	Na Cr	Deg1	Deg2	Mont. Na	Cis-mont	%	Mont. Na	Cis-mont
1	40.8	12	10	22.73	19.68	101.28	100.51	100.88	16.0	8.0	33.33	100.74	99.86
2	50.4	9	15	15.15	22.93	100.23	100.19	100.59	21.6	5.6	20.59	100.86	99.78
3	69.6	14	11	16.75	13.65	99.92	101.6	99.06	24.0	9.6	28.57	99.7	100.39
4	91.2	23	18	20.14	16.48	101.02	99.32	100.98	32.0	6.4	16.67	99.48	100.19
5	110.4	11	6	9.06	5.15	100.83	100.94	100.73	36.0	4.0	10.00	99.14	100.27
6	120.0	6	12	4.76	9.09	99.49	99.57	99.38	37.6	2.4	6.00	99.23	99.92
Mean						100.46	100.26	100.27				99.68	100.07
S.D.						±0.694	±0.711	±0.830				±0.756	±0.248
S.E.						±0.283	±0.290	±0.339				±0.309	±0.101

Table 5. Results of tests of significance of the proposed methods

	BC		FF		Na Cr		Mont. Na		
Drug	*Reference method***	^{2}D method	*Reference method****	^{2}D method	*Reference method****	^{1}DR *method*	^{1}D *method*	*Reference method*****	^{1}DR *method*
Statistical Term									
Mean	99.84	99.81	99.76	100.24	99.79	100.46	99.42	99.85	99.86
SD±	0.415	0.814	0.939	0.631	0.722	0.694	0.724	0.602	0.756
SE±	0.169	0.332	0.383	0.258	0.295	0.283	0.295	0.246	0.309
%RSD	0.416	0.815	0.941	0.629	0.723	0.691	0.728	0.603	0.757
N	6	6	6	6	6	6	6	6	6
V	0.172	0.662	0.882	0.398	0.521	0.482	0.524	0.362	0.571
t (2.228)*	0.080		1.039		1.639		1.119		
								0.025	
F (5.050)*	3.849		2.216		1.081		1.447		
								1.577	

* Figures in parentheses are the theoretical t and F values at (p= 0.05).
** First derivative spetrophotometric method[6].
*** Non aqueous titration[8].
**** RP-HPLC method[29].

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of each method were calculated as 3.3 S/M and 10 S/M, respectively, where S is the standard deviation of the absorbance (n=3) and M is the slope of the calibration curve. The data were presented in Tables 1 and 2.

Statistical analysis

Statistical comparison between the results obtained by the proposed methods and those of the reference methods using Student *t*-test, F-ratio and one way ANOVA showed no significant difference as given in Table 5. It can be concluded that the

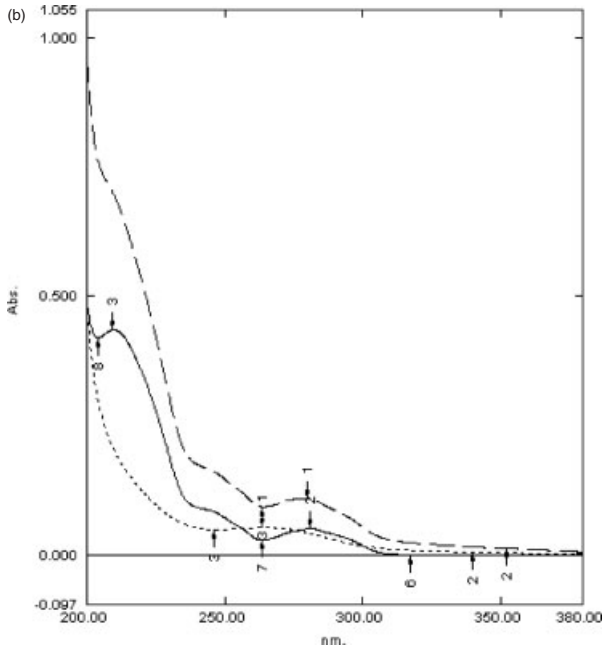

Figure 5b. Zero-order spectra of FF ———, Foradil extract - - - - and lactose · · · · . in 0.01 M hydrochloric acid.

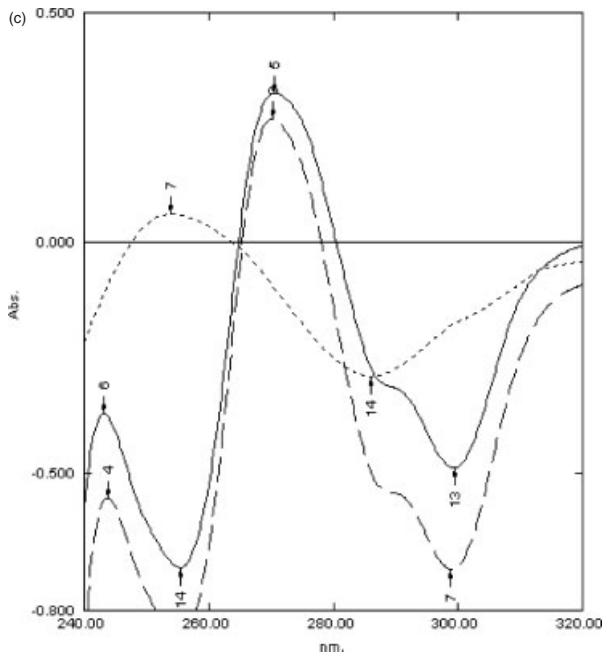

Figure 5c. First derivative spectra of FF ———, Foradil extract - - - - and lactose · · · · . in 0.01 M hydrochloric acid.

proposed analytical methods were sufficiently accurate and precise.

Application on the pharmaceutical preparation

For Syrup (BC)

On application of the proposed method to the analysis of BC in its pharmaceutical dosage form, Sinecod® syrup, very high recovery percentages were obtained. This was attributed to the presence

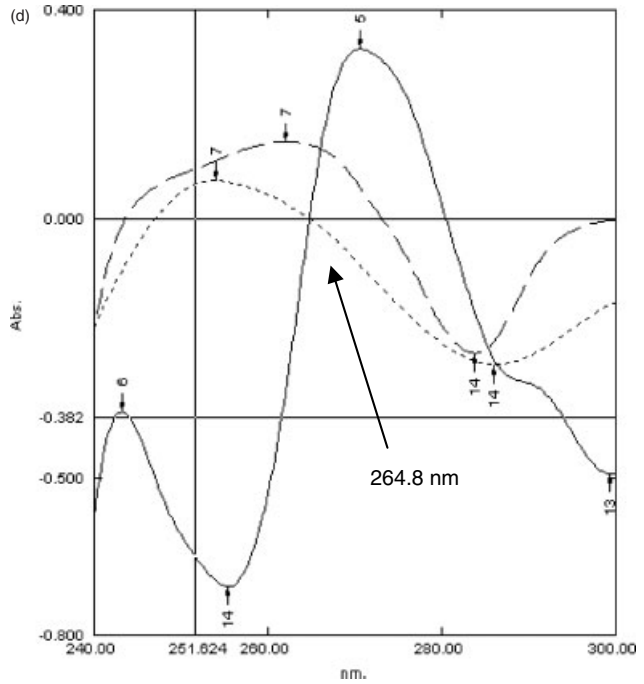

Figure 5d. First derivative spectra of FF (5 μg/ml) ———, Des (2 μg/ml) - - - - and lactose (30 mg/ml) · · · · . in 0.01 M hydrochloric acid.

of saccharin sodium and vanillin, with interfering UV absorbance. BC is a salt of weak base. Depending on this fact, a trial was done to extract the free base thus eliminating the interference and overcoming the problem. Ether and chloroform were suggested and tried as organic solvents for extraction. Ether gave better recoveries and thus was chosen to be used. A study of BC stability in 1M sodium hydroxide was also carried out. This study revealed that BC is stable in 1M sodium hydroxide for 1 h. The residue remained after evaporation was only soluble in 0.01M hydrochloric acid. Thus, stock solutions were prepared in 0.01M hydrochloric acid and aliquots taken were completed to volume with 0.01M sodium hydroxide. This procedure was checked by application on authentic BC, where a mean percentage recovery of 99.63 ± 0.632 was obtained. Moreover, when the same extraction procedure was applied on fortified Sinecod® syrup with known amounts of PBA, it was apparent from percentage recoveries obtained that PBA was not fully extracted. This finding was consistent with the fact of being PBA an organic acid with higher solubility in alkaline aqueous phase than organic phase. Besides, the validity of the suggested procedure was also assessed by applying the standard addition technique (Table 3).

For capsules (FF)

Applying the first mode for the determination of FF in Foradil® capsules resulted in an unexpected increase in percentage recoveries even after switching the solvent of extraction from 0.01M hydrochloric acid to methanol. A brief study of the spectra of each of the capsule constituents (according to the manufacturer information) revealed that the problem arises from the presence of lactose. Upon comparing the zero-order and the first derivative spectra of FF, Foradil® extract and lactose solution in 0.01M hydrochloric acid (Figures 5B and 5C), it can be deduced that the existent interference was mostly attributed to the presence of lactose in Foradil® extract. Using different extraction solvents did

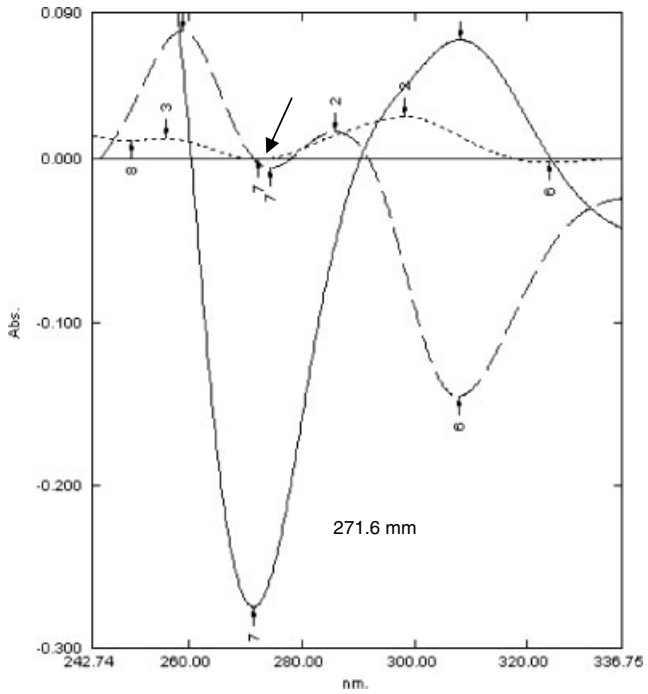

Figure 6. First derivative ratio spectra (DR1C) of NaCr (40 µg/ml) ———, excipients mixtures (20 µg/ml of each excipient) - - - - and Deg1 (40 µg/ml) · · · · . (Divisor: 20 µg/ml Deg2) in 0.01 M sodium hydroxide.

not overcome the existent problem. Upon experimental trial of the second mode, the second derivative spectra of FF, Des and lactose revealed a peak of FF at 261.8 nm; where Des and lactose exhibited zero reading (Figure 2C). For this reason, amplitude at 261.8 nm (A_{261}) could be used to calculate the concentration of FF. The method was successfully applied to Foradil® capsules. Assessment of the suggested method was done by applying the standard addition technique which revealed a mean percentage recovery of 100.11 ± 0.739 for the added authentic FF as shown in Table 3.

It is noteworthy to mention that Des can be estimated in Foradil® capsules on applying first derivative mode by measuring the amplitude at 264.8 nm, where lactose exhibits no reading (Figure 5D). Method was able to detect Des in the presence of FF in the range of 4.76 to 33.33% *w/w*. No degradation product was found in the pharmaceutical formulation. Another important hint is the trough of FF that appeared using the first mode at 299 nm (Figure 2D) could not be used for the determination of FF because of the aforementioned lactose interference (Figure 5C).

For nasal drops (NaCr)

The method was successfully applied on Nasotal® drops with a mean percentage recovery of 107.19 ± 0.749 and 99.89 ± 0.358 for the labelled and added authentic NaCr, respectively (Table 3). Excipients of Nasotal® drops were found to be methylparaben sodium (0.03 g% *w/v*) and propylparaben sodium (0.003 g% *w/v*). Slight increase in the percentage recoveries of NaCr in the drops was checked by recording the first derivative ratio spectrum of a mixture of the two excipients using the same instrumental parameters mentioned earlier for the determination of ***NaCr.*** The two excipients exhibit zero crossing at the wavelength of measurement of ***NaCr*** (271.6 nm) (Figure 6). Hence, the drug could be determined in the pharmaceutical formulation without any interference, while its two degradation products could not be determined in Nasotal® drops due to the interference of the excipients at 302.8 and 302.2 nm. Careful study of different parameters to find out another wavelength of measurement in order to avoid this interference failed due to complete overlap throughout.

For tablets (Mont. Na)

For derivative method. The method has been applied for the determination of Mont. Na in Clear air® tablets. Assessment of the suggested method by applying the standard addition technique, revealed a mean percentage recovery of 99.79 ± 0.674 for the added Mont. Na (Table 3).

For derivative of the ratio spectra method. The method was successfully applied on Clear air® tablets with percentage of 100.14 ± 0.647 for the added authentic Mont. Na (Table 3). No photodegradation product was found in the pharmaceutical formulation. The method can detect Cis-mont when present with Mont. Na in the range of 6 to 33.33% *w/w*.

Conclusion

The proposed derivative spectrophotometric and derivative of the ratio spectra spectrophotometric methods could be successfully applied for the determination of BC, FF, Mont. Na, and NaCr without any interference and with good accuracy and precision, either in laboratory-prepared mixture samples or in pharmaceutical dosage forms. The proposed methods demonstrate how analytical techniques can pass over any interference which arises during designing or applying analytical procedures. All the proposed procedures are rapid, precise, and work without solving equations. One of the advantages of the ratio spectra method produced over the zero-crossing derivative method for Mont. Na, is the possibility of determining Cis-Mont. Other advantages of the derivative ratio assays in comparison with the derivative spectrophotometric method are the easy measurements of the separate peaks, higher values of analytical signals and no need to work only at zero-crossing point. The proposed methods are suitable for quality control laboratories, where economy and time are essential.

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