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Simultaneous determination of compounds in Septalen® pellets by derivative spectrophotometry*

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In this paper, a second-derivative spectrophotometric method of assaying Septalen® pellets (Krka, Novo Mesto, Slovenia), which contain lidocaine 1 mg, and cetrimonium bromide 2 mg, is described. Lidocaine, 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-acetamide, is a local anesthetic with pronounced antiarhythmic and anticonvulsant properties. Cetrimonium bromide, *N,N,N*-trimethyl-l-hexadecanaminium bromide, is a topical antiseptic and cleansing agent. Lidocaine was determined at 250 nm using the "zero crossing" technique because the signals of centrimonium bromide and the colour ingredient are zero at this wavelength. Cetrimonium bromide was determined by correction of the peak amplitude at 215 nm according to lidocaine. In choosing the optimal magnitudes for the simultaneous determination of both drugs, the following criteria were considered: (1) the linearity of the calibration graphs as given by the correlation coefficients, (2) the intercept, (3) the sensitivity as given by the regression coefficient, (4) the degree of interference in the derivative measurement by the presence of the other compound, as given by the relative percent error and by the relative recovery, and (5) the reproducibility, as given by the coefficient of variation, calculated by recording the second-derivative spectra.

Keywords: second-derivative spectrophotometry, zero-crossing method, method with correction, lidocaine, cetrimonium bromide, Septalen® pellets.

INTRODUCTION

Septalen® pellets are a combination of the local anesthetic and topical antiseptic agents. The aim of these investigations was to develop a method for the simultaneous determination of the pharmaceutically active ingredients in Septalen® pellets.

Lidocaine is official in the USP 23[1] and British Pharmacopoeia,[2] but centrimonium bromide only in the British Pharmacopoeia. Titration in non-aqueous media with perchloric acid using the potentiometric determination of the end point is prescribed for lidocaine in British Pharmacopoeia, but in USP 23 it is the liquid chromatography. For cetrimonium bromide it is an iodometric titration.

* Dedicated to Professor Slobodan Ribnikar on the occasion of his 70th birthday

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Lidocaine has been determined in simple pharmaceutical dosage forms or in combinations with some other drugs. In the presence of cocaine[3–5] or some other pharmaceutical substances,[6,7] lidocaine was determined spectrophotometrically or by using the atomic absorption spectrophotometric method.[8,9] HPLC techniques have been used for the determination of lidocaine in human blood [10,11] or in some pharmaceuticals.[12] Of the electrochemical methods, ion-selective electrodes[13–15] are the most important. Cetrimonium bromide was analysed using fluorimetic[16] and spectrophotometric[17] method or by using liquid-membrane electrodes[18] and capillary electrophoresis.[19]

EXPERIMENTAL

Apparatus

A Beckman DU Series 600 spectrophotometer, with 1 cm quartz cells and the experimental conditions: $\Delta\lambda = 2$ nm and scan speed 2400 nm/min, was used. The second-derivative spectra ${}^2D = d^2A/d\lambda^2$ were recorded in the range from 200 nm to 300 nm against 0.1 mol/L hydrochloride acid.

Materials and reagents

All materials and reagents used were of analytical grade. Hydrochloric acid (Zorka Pharma d.d., Šabac) was used as the solvent for the spectrophotometric assay. Septalen® pellets (lidocaine 1 mg, and cetrimonium bromide 2 mg) is the official formulation by Krka, Novo Mesto, Slovenia. The USP standard substances of lidocaine and cetrimonium bromide, as well as the standards of colour ingredients and Septalen® pellets were obtained from Krka, Novo Mesto.

Procedure

Stock solutions were prepared by dissolving the respective standard substance in 0.1 mol/L hydrochloric acid to obtain a concentration of 0.25 mg/mL for lidocaine and 0.5 mg/mL for cetrimonium bromide. For the calibration curve, a series of eight solutions was prepared in the concentration range from 5.0 to 30.0 μg/mL for lidocaine and from 10.0 to 60.0 μg/mL for cetrimonium bromide.

To prove the validity and applicability of the proposed derivative spectrophotmetric method, a laboratory mixture of standard substances of lidocaine and cetrimonium bromide was made in the ratio which corresponded to that in the Septalen® pellets. A stock solution of the laboratory mixture was made by dissolving 10.0 mg lidocaine and 20.0 mg cetrimonium bromide with 0.1 mol/L hydrochloric acid in a 100 mL volumetric flask. The concentration of this solution was 100 μg/mL for lidocaine and 200 μg/mL for cetrimonium bromide. The declared colours were added to the laboratory mixture in the corresponding ratios. From this laboratory mixture solution, three series of ten solutions were made the concentrations of which were: 7.5 μg/mL, 10.0 μg/mL and 12.5 μg/mL of lidocaine and 15.0 μg/mL, 20.0 μg/mL and 25.0 μg/mL for cetrimonium bromide.Then, the second-derivative spectra were measured. In the same way, preliminary decolouration of the three mentioned series of dilutions of the laboratory mixture were made using activated carbon. The stock solution of the laboratory mixture was shaked with activated carbon for five minutes and then filtered.

To analyse the Septalen® pellets, ten pellets were accurately weighed and finally powdered. The powdered pellets were transferred into a 100 mL volumetric flask, 80 mL of 0.1 mol/L hydrochloric acid was added and the powder dissolved in an ultrasonic bath for 30 min. The volumetric flask was filled to the mark with 0.1 mol/L hydrochloric acid and the resulting solution filtered. The concentration of this solution was 100 μg/mL of lidocaine and 200 μg/mL of cetrimonium bromide. The diluted solutions was prepared by the same procedure as was used for the laboratory mixture.

The second-derivative spectra of the laboratory mixture and of the Septalen® pellets were recorded in the range from 200 nm to 300 nm against the 0.1 mol/L hydrochloric acid.

RESULTS AND DISCUSSION

The classical spectrophotometric methods are not suitable for analysing a multicomponent mixture because the absorption spectra of the components overlap. This is often further complicated by interference from the formulation matrix. Also, the energy changes in the molecules are very inhomogeneous, causing wide and complex absorption bands, which are not characteristic enough for the identification or quantification of the substances in a multicomponent mixture. Derivative spectroscopy is a simple technique for magnifying the fine structure of spectral curves. It consists of calculating the first, second, or higher order derivative of a spectrum with respect to wavelength or frequency and plotting this derivative rather that the spectrum itself. Higher order derivative spectrophotometry enables the problems of overlapping bands in the spectra to be solved. The resolution increases with the derivative order. It is most important to choose the optimal derivative order to resolve the absorption spectra. For a quantitative analysis it is necessary to measure the peak amplitude of the derivative spectra in the concentration range of the Lambert-Beer linearity.

The zero order, first, second, third and fourth-derivative spectra for all the investigated components of Septalen® pellets were recorded in the wavelength range from 200 nm to 300 nm. For the simultaneous determination the second-derivative order ^2D was chosen (Fig. 1).

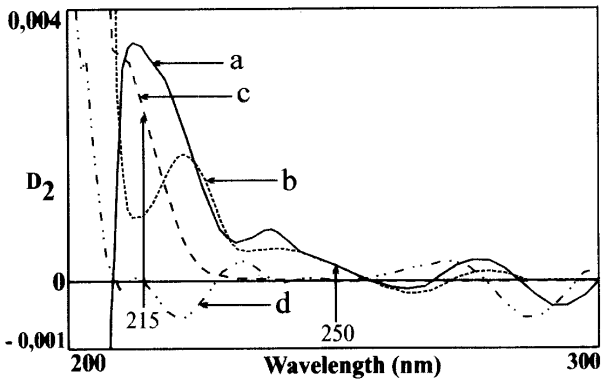

Fig. 1. Second-derivative spectra of a laboratory mixture which corresponds to Septalen® pellets a), lidocaine b), cetrimonium bromide c), and colour ingredients d), in 0.1 mol/L hydrochloric acid.

Lidocaine was determined at 250 nm, while cetrimonium bromide was determined at 215 nm. The signal at 250 nm corresponded to lidocaine, while the absorption of cetrimonium bromide and the colour ingredients at this wavelength are zero. For this reason the "zero crossing" technique for the determination of lidocaine in combination with cetrimonium bromide at 250 nm was chosen. However, the determination of cetrimonium bromide necessited a correction of the peak amplitude at 215 nm. It demanded the calculation of the participation factor (*correction factor*) for lidocaine at the wavelength for cetrimonium bromide determination. The factor shows the participation of one component in the mixture with

the other when the bands overlap. It could be calculated from the calibration spectra of lidocaine, because the ratios of the characteristic signals at the chosen wavelength (in our case: for lidocaine at λ = 215 nm) and the signals at the wavelength where cetrimonium bromide "was zero" (λ = 250 nm) were of constant value:

$$f = \frac{D_{250}^{L}}{D_{215}^{L}}$$

where $D_{250}{}^{L}$ is the peak amplitude for lidocaine in the second-derivative spectra at 250 nm and $D_{215}{}^{L}$ is the peak amplitude for lidocaine in the second-derivative spectra at 215 nm.

This means that the participation of lidocaine in the mixture with cetrimonium bromide at 215 nm is given by:

$$D_{215}^{L} = \frac{D_{250}^{L}}{f}$$

The total peak amlitude ($D_{215}{}^{S}$) of the mixture of lidocaine and cetrimonium bromide at 215 nm is:

$$D_{215}^{S} = D_{215}^{L} + D_{215}^{C}$$

where $D^{C}{}_{215}$ is the peak amplitude for cetrimonium bromide at 215 nm. The signals of the colour ingredients were zero at 215 nm.

Under the described experimental conditions, the calibration curves, obtained by plotting ^{2}D values *versus* concentration at the mentioned characteristic wavelengths, show linear relationships in the following concentration ranges: 5.0 to 30.0 μg/mL for lidocaine and 10.0 to 60.0 μg/mL for cetrimonium bromide. The calibration curves were in agreement with the Beer's law. The regression equations for the investigated substances were calculated including the correlation coefficient (r)(Table I).

TABLE I. Calibration curve for Septalen® pellets

Parameters	Lidocaine (λ=250 nm)	Cetrimonium bromide (λ=215 nm)
Concentration range (μg/mL)	5.0–30.0	10.0 – 60.0
$y = ax + b$	$y = 0.00002x + 0.0000039$	$y = 0.000094x + 0.00006$
Correlation coefficient	$r = 0.9991$	$r = 0.9994$
Limit of detection	LD = 0.075 μg/mL	LD = 0.083 μg/mL

n = 10

For the quantitive analysis of Septalen® pellets, three series of ten solutions were prepared. Table II presents the results of the determination of lidocaine and cetrimonium bromide in the Septalen® pellets under the described experimental conditions. The validation parameters (linearity, selectivity, accuracy, precision) were also determined. The derivative spectrophotometric method is selective be-

cause the excipient did not interfere during the determination of the components of the Septalen® pellets. The small values of the coefficient t_α show good accuracy and the coefficient of variation confirms the precision of the method.

TABLE II. Second-derivative spectrophotometric determination of Septalen® pellets

		Taken/(μg/mL)	Found/(μg/mL)	Recovery/%	CV/%
	Lidocaine	7.5	7.40±2*	99.2	2.8
		10.0	10.2±0.2	102.0	2.4
A		12.5	12.5±0.3	100.0	2.0
	Cetrimonium	15.0	15.0±0.3	100.0	2.2
	bromid	20.0	20.2±0.3	100.9	1.5
		25.0	25.0±0.5	100.2	2.2
	Lidocaine	7.5	7.4±0.2	99.2	2.8
		10.0	10.0±0.2	100.0	2.4
B		12.5	12.4±0.2	99.7	2.3
	Cetrimonium	15.0	14.9±0.3	99.8	1.4
	bromid	20.0	19.8±0.5	99.2	2.6
		25.0	24.8±0.3	99.2	1.5

* Sd (n = 10); A – Septalen® pellets without decolouration; B – Septalen® pellets after decolouration with activated carbon

CONCLUSION

The second-derivative spectra of the investigated substances is suitable for the simultneous determination of the composition of Septalen® pellets. The obtained results are accurate and precise which is confirmed by the statistical parameters (standard deviation, coefficient of variation and recovery values). There was no interference of the excipient in the pellets. The second-derivative spectrophotometric method can be proposed for the simple, rapid, simultaneous, selective, accurate and precise determination of the components of Septalen® pellets or a corresponding multicomponent mixture. The obtained results confirms that the aim of the investigations (to develop a method for the simultaneous determination of the pharmaceutically active ingredients in Septalen® pellets with a good accuracy and precision) was reached.

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И З В О Д

ОДРЕЂИВАЊЕ СЕПТАЛЕН® ПАСТИЛА ПРИМЕНОМ ДЕРИВАТИВНЕ СПЕКТРОФОТОМЕТРИЈЕ

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У овом раду описана је деривативна спектрофотометрија извода другог реда за одређивање Septalen® пастила (Крка, Ново Место, Словенија), које садрже лидокаин 1 mg, и цетримонијум-бромид 2 mg. Лидокаин, 2-(диетиламино)-*N*-(2,6-диметил-фенил)-ацетамид, је локални анестетик са изразитим антиаритмичним и антиконвулзивним особинама. Цетримонијум-бромид, N,N,N-триметил-1-хексадеканаминијум-бромид је површински антисептик и агенс за чишћење. Лидокаин је одређиван на таласној дужини од 250 nm коришћењем методе "нултог пресека", јер су сигнали за цетримонијум-бромид и боју били нула на тој таласној дужини. Цетримонијум-бромид је одређиван методом корекције на 215 nm у односу на лидокаин. Приказани су резултати одређивања активних компоненти Septalen® пастила у присуству боје или са претходним обезбојавањем. Под изабраним експерименталним условима одређени су и следећи критеријуми за оптимизацију предложене деривативне спектрофотометријске методе: (1) линеарност калибрационе криве дата је корелационим коефицијентом, (2) пресек са ординатом, (3) осетљивост је дата регресионим коефицијентом, (4) утицај присуства осталих компоненти на деривативна мерења дат је релативном грешком и "recovery" вредношћу и (5) репродуктивност је дата коефицијентом варијације.

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