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Simultaneous determination of benzocaine and cetylpiridinium chloride in tablets by first-derivative spectrophotometric method

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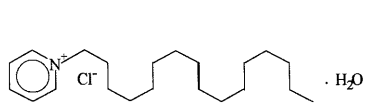
Abstract

A new spectrophotometric method for the simultaneous determination of benzocaine and cetylpiridinium chloride in pharmaceutical tablets, which does not require any preliminary separation or treatment of the samples, is described. The quantitative determination of both drugs was carried out using the first derivative values measured at 231.40 and 310.00 nm for benzocaine and at 220.70 nm for cetylpiridinium chloride using the zero-crossing method. The calibration graphs were linear in the ranges from 10 to 25 mg/l of benzocaine and from 4 to 20 mg/l of cetylpiridinium chloride. The developed method was successfully applied for the assay of pharmaceutical tablets and proved to be simple, sensitive and selective. Thermogravimetric techniques, Karl Fischer and loss on drying were also used for a stoichiometric evaluation of the substances studied.

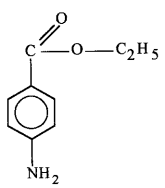
Keywords: Benzocaine; Cetylpiridinium chloride; Derivative spectrophotometry; Thermogravimetry; Pharmaceutical tablets.

1. Introduction

Pharyngitis, tonsillitis, colds and mouth and throat infections are illnesses regularly treated using antiseptic and analgesic medicines [1]. Cetylpiridinium chloride (**I**), an antiseptic quaternary ammonium, and benzocaine (**II**), used as a local anesthetic, are found associated in tablets [1,2].



I $C_{21}H_{38}ClN.H_2O$



II $C_9H_{11}NO_2$

Several methods have been used for qualitative and quantitative determination of cetylpiridinium chloride and benzocaine. They include thermogravimetry [3], volumetry [4–6] and spectrophotometry [3–6]. On the contrary of derivative spectrophotometric methods [8–17], these methods do not permit simultaneous analysis of both compounds without previous procedures of separation and extraction.

Thus, this paper describes the first derivative spectrophotometric method for the simultaneous analysis of benzocaine and cetylpiridinium chloride in tablets, without previous procedures of separation. Other techniques such as thermogravimetry, Karl Fischer and loss on drying [7] were also used for qualitative and quantitative evaluation of the raw materials used.

2. Experimental

2.1. Apparatus

The zero-order spectra and the respective derivatives were obtained on a spectrophotometer Shimadzu model

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UV-1601 PC double beam, with a slit width of 2 nm. The first derivative of the spectrum curves of the references and samples were recorded in a 1 cm quartz cells over the range of 200–350 nm ($\Delta\lambda = 1.0$ nm). The scan speed was of 8.3 nm/s (middle).

The TG–DTG curves were obtained using a Mettler TA 4000 thermoanalyzer system, with a heating rate of 20°C/min ranging from 25 to 900°C, a synthetic air in flow rate of 20 ml/min and an alumina crucible. The sample mass ranged from 6.0 to 9.0 mg.

The loss on drying was determined using an OHAUS model MB 200 dryer with infrared heating, temperature registration and a balance with a programming device. The sample mass was in the range of 10.00 ± 0.5 g.

The Karl Fischer equipment used to determine the water content in the samples was a Mettler model DL 18 Titrator employing a Merck reagent that corresponds to the following relationship: 1 ml = 5 mg H_2O.

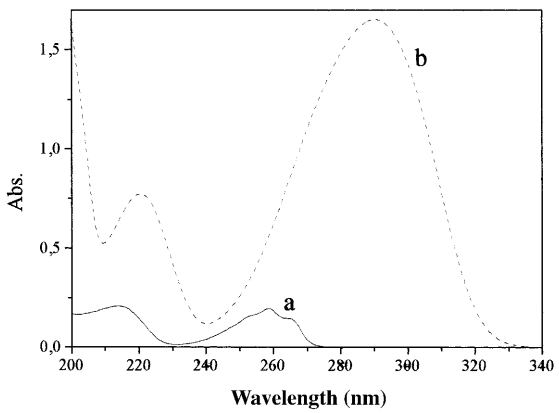

Fig. 1. (a) Zero-order absorption spectra of cetylpiridinium chloride (16 mg/l); and (b) benzocaine (15 mg/l), obtained in 1:1 water–alcohol (v/v) solution.

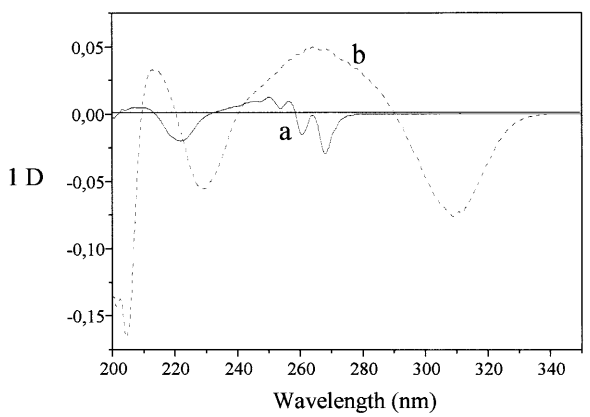

Fig. 2. First derivative absorption spectra of: (a) cetylpiridinium chloride; and (b) benzocaine obtained in 1:1 water–alcohol (v/v) solution.

2.2. Chemicals and pharmaceutical preparation

The cetylpiridinium chloride and the benzocaine pharmaceutical raw materials were acquired from the distributors Henrifarma and Synth, respectively (Brazil).

The used tablets were a well-known commercial product, Cetil Drops (Luper). The contents of the tablets were:

- Cetylpiridinium chloride 2.5 mg
- Benzocaine 2.0 mg

The alcohol used was absolute ethanol, an analytical reagent Merck (Germany) with a purity of 99.8%.

2.2.1. Standard solutions

2.2.1.1. Stock solutions. Benzocaine (10 mg) and cetylpiridinium chloride were separately dissolved in 1:1 water–alcohol (v/v). These solutions were used for the preparation of calibration curves and for spectra after appropriate dilution with the same solvent.

2.2.1.2. Working standard solutions. Using standard stock solutions, serial solutions were prepared containing 10–25 mg/l of benzocaine and 4–20 mg/l of cetylpiridinium chloride for derivative spectrophotometry measurements.

2.2.1.3. Mixed standard working solution. Using standard solutions, we prepared solutions of benzocaine at 12 mg/l while varying the concentrations of cetylpiridinium chloride (4, 8, 12, 15 and 20 mg/l). We also made solutions of cetylpiridinium chloride at 15 mg/l while varying the concentrations of benzocaine (10, 15, 20, 25 and 30 mg/l).

2.3. Procedure

2.3.1. Zero-order UV spectra

The zero-order spectra (Fig. 1) of the compounds were separately obtained in a 1:1 water–alcohol (v/v) solution containing cetylpiridinium chloride (16 mg/l) and benzocaine (15 mg/l).

2.3.2. First-derivative UV spectra

The first derivative spectra (Fig. 2) of the compounds was obtained separately starting from the zero order spectra of cetylpiridinium chloride (16 mg/l) and benzocaine (15 mg/l). Using the zero-crossing method, three points were observed for the cetylpiridinium chloride analyzes (209.60, 220.70 and 240.50 nm) and four points for the benzocaine analyzes (214.00, 231.40, 258.80 and 310.00 nm). These results suggest the possible absorption wavelengths that we could use to selectively dose these materials.

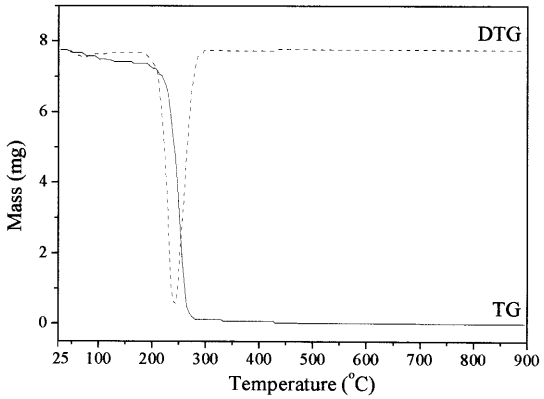

Fig. 3. The TG–DTG plot of cetylpiridinium chloride.

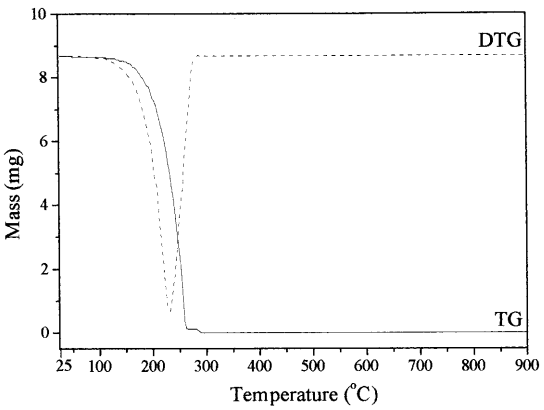

Fig. 4. The TG–DTG plot of the benzocaine.

2.3.3. Calibration graphs

The benzocaine calibration curves were made by plotting the derivative amplitude, ^1D, at 214.00, 231.40, 258.80 and 310.00 nm against the concentration (10, 15, 20 and 25 mg/l). The calibration curves of cetylpiridinium chloride were made by plotting the derivative amplitude, ^1D, at 209.60, 220.70 and 240.50 nm against the concentration (4, 8, 12, 16 and 20 mg/l).

2.3.4. Analysis of tablets

Tablets were accurately weighed and cut into small pieces in a mortar. A mass equivalent to one tablet was dissolved in 50 ml of the 1:1 water–alcohol (v/v) solution. This solution was filtered and 15 ml was diluted again in 50 ml 1:1 water–alcohol (v/v) to obtain the cetylpiridinium chloride (15 mg/l) and benzocaine (12 mg/l) solutions.

2.3.5. Tablets simulation

Tablet simulations were prepared in 1:1 water–alcohol (v/v) solutions with cetylpiridinium chloride and benzocaine, in agreement with the item 2.2.1.2. to obtain a cetylpiridinium chloride (15 mg/l) and benzocaine (12 mg/l) solution.

3. Results and discussion

3.1. Thermogravimetric (TG–DTG) experiments

The TG–DTG curves of the cetylpiridinium chloride (Fig. 3) show mass loss in two stages between 25 and 275°C. The first step is between 25 and 100°C, due to the loss of 5.1% as a result of the evaporation of 1.02 molecules of water. The second step is between 157 and 275°C, due to the loss of 93.57% for the burning of organic matter. The TG–DTG curves of the benzocaine (Fig. 4) shows mass loss in one step, between 86.5 and 277.5°C presenting loss mass of 99.36% of organic matter.

3.2. Karl Fischer

On the basis of the molecular structures of the products **I** and **II**, only cetylpiridinium chloride presents a molecule of water corresponding to 5% of its composition. However 6.5 and 0.15% of water in cetylpiridinium chloride and benzocaine, respectively, were found by the Karl Fischer technique.

3.3. Loss on drying

When cetylpiridinium chloride was submitted to a temperature of 110°C/30 min, a loss of 3.7% by evaporation of 0.73 molecules of water was observed. When submitted to a temperature of 140°C/30 min, a loss of 4.3% by evaporation of 0.85 molecules of water was observed. And when submitted to a temperature of 150°C/30 min, a loss of 4.7% by evaporation of 0.93 molecules of water was observed.

The results obtained by drying, Karl Fisher and thermogravimetric analysis were compared (Table 1) and showed small variations in the value of water depending on the technique used.

3.4. Derivative spectrophotometry

Direct UV absorption measurements (zero order spectra) were found to be inapplicable to the analyzes of benzocaine and cetylpiridinium chloride in binary mixture because of spectra interferences (Fig. 1). However the first-derivative spectra permitted the simultaneous determination of both compounds (Fig. 2).

Linear relationships between derivative amplitude and drug concentration were obtained over the concentration range 10–25 mg/l for benzocaine and 4–20 mg/l for cetylpiridinium chloride. Linear regressions were obtained at 209.60, 220.70 and 240.50 nm for cetylpiridinium chloride and at 214.00, 231.40, 258.80 and 310.00 nm for benzocaine. The best results for the tablets and your respective 'simulation' (Table 2) were obtained with linear regressions at 220.70 nm for

Table 1
Comparison of the amount of water found in cetylpiridinium chloride when analyzed by thermogravimetric analysis, loss on drying and Karl Fischer

Loss on drying			Thermogravimetric analysis			Karl Fischer	
Temperature (°C) (30/min)	%	H_2O molecules	Temperature (°C)	%	H_2O molecules	%	H_2O molecules
110	3.7	0.73					
140	4.3	0.85	25–100	5.1	1.02	6.5	1.29
150	4.7	0.93					

cetylpiridinium chloride (Fig. 5) and at 310.00 nm (Fig. 7) for benzocaine (see also Fig. 6).

The possible interference of excipients and associated drugs in commercial pharmaceutical formulations was investigated. Thus, by the comparison of the results obtained between the simulation and the tablets, it has been found that the excipients do not interfere with the determination of benzocaine and cetylpiridinium chloride by the presently proposed method.

The mean percentage RSD and percentage recovery could be considered to be very satisfactory, at least for the level of the examined concentrations of benzocaine and cetylpiridinium chloride.

The study of the concentration influence of one component on the other was performed by varying the concentrations of one and maintaining the other fixed (Tables 3 and 4).

The percentage RSD and percentage recovery values (Table 3) showed that at 231.4 and 310 nm, the proposed method has adequate sensibility and accuracy for benzocaine evaluation whereas, at 220.7 nm, the proposed method has adequate sensibility and accuracy for cetylpiridinium chloride evaluation.

The study of the disintegration–dissolution profile of the tablets containing cetylpiridinium chloride and benzocaine is in progress using this first derivative spectrophotometric method. The results of that research will be published shortly.

4. Conclusions

The proposed methodology based on first order derivative UV spectrophotometry, permitted identification and quantification of the combined benzocaine and cetylpiridinium chloride, in tablet preparations. Quantitative determination may be done at 231.5 and 310.00 nm for benzocaine and at 220.70 nm for cetylpiridinium chloride.

The method employs low cost reagents and requires a short time for analyzes. Reproducibility, accuracy and sensitivity of this method are satisfactory. The method may be considered suitable for routine analysis of large number of samples.

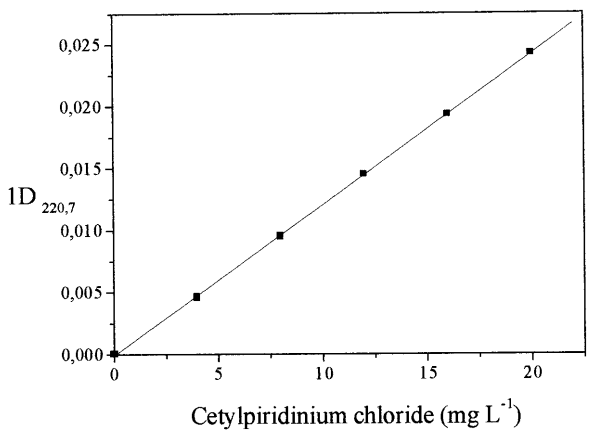

Fig. 5. Calibration graph of cetylpiridinium chloride obtained at 220.7 nm. The found linear regression was: $A = -1.33 \times 10^{-4} + 1.22 \times 10^{-3}$. c (mg/l), with $r = 0.9999$.

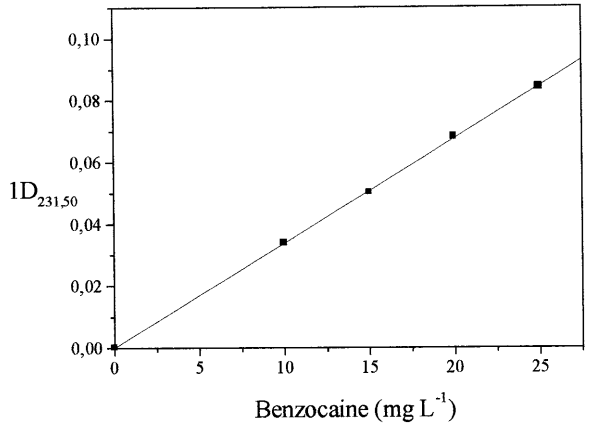

Fig. 6. Calibration graph of benzocaine obtained at 231.5 nm. The found linear regression was: $A = 3.51 \times 10^{-5} + 3.37 \times 10^{-3}$. c (mg/l), with $r = 0.9999$.

Table 2
Values found for benzocaine and cetylpiridinium chloride in binary mixture (simulation) and tablets

Drug	Simulation [a]				Tablets [a]			
	Theoretical value (mg)	Total amount found (mg)	Recovered (%)	RSD (%)	Theoretical value (mg)	Total Amount found (mg)	Recovered (%)	RSD (%)
Benzocaine $^1D_{231.40\ nm}$	2.00	1.95	97.50	0.44	2.00	2.20	110.00	0.24
Benzocaine $^1D_{310.00\ nm}$	2.00	1.93	96.50	0.28	2.00	2.00	100.01	0.52
Cetylpiridinium chloride $^1D_{220.70\ nm}$	2.50	2.56	102.40	1.15	2.50	2.42	96.80	1.31

[a] The values are the averages of four determinations.

Table 3
Values found for benzocaine and for cetylpiridinium chloride when the concentration of cetylpiridinium chloride was varied in the presence of benzocaine (12 mg/l)

Pharmaceutical raw material (mg/l)	Benzocaine (nm)								Cetylpiridinium chloride (nm)					
Theoretical value (mg/l)	Total amount found (mg/l)				Recovered (%)				Total amount found (mg/l)			Recovered(%)		
	214.0 [a]	231.4 [b]	258.8 [c]	310.0 [d]	214.0	231.4	258.8	310.0	209.6	220.7	240.5	209.6	220.7	240.5
Benzocaine/12 Cetylpiridinium chloride/4	11.61	12.24	11.84	12.13	96.75	102.00	98.67	101.08	<4	4.95	4.68		123.75	117.00
Benzocaine/12 Cetylpiridinium chloride/8	11.56	12.13	11.40	12.05	96.33	101.08	96.00	100.42	>12	9.45	9.55		118.12	119.38
Benzocaine/12 Cetylpiridinium chloride/12	11.84	12.42	11.63	12.31	98.67	103.50	96.92	102.58	12.44	13.06	11.98	103.67	108.83	99.83
Benzocaine/12 Cetylpiridinium chloride/15	11.65	12.40	11.70	12.13	97.08	103.33	97.50	101.08	<11	15.44	13.87	-	102.93	92.47
Benzocaine/12 Cetylpiridinium chloride/20	11.65	12.66	12.14	12.31	97.08	105.50	101.16	102.58	<10	19.70	17.93	-	98.50	89.65

[a] RSD = 0.91%.
[b] RSD = 1.63%.
[c] RSD = 2.33%.
[d] RSD = 0.97%.

Table 4
Values found for cetylpiridinium chloride and for benzocaine when the concentration of benzocaine was varied in the presence of cetylpiridinium chloride (15 mg/l)

Pharmaceutical raw material (mg/l)	Benzocaine (nm)								Cetylpiridinium chloride/nm					
Theoretical value (mg/l)	Total amount found (mg/l)				Recovered (%)				Total amount found (mg/l)			Recovered (%)		
	214.0	231.4	258.8	310.0	214.0	231.4	258.8	310.0	209.6	220.7 [a]	240.5 [b]	209.6	220.7	240.5
Cetylpiridinium chloride/15 benzocaine/10	9.66	10.28	9.64	10.22	96.60	102.80	96.40	102.20	<4	16.11	14.40		107.40	96.00
Cetylpiridinium chloride/15 benzocaine/15	14.23	15.39	14.44	15.66	94.87	102.60	96.27	104.40	>20	16.90	16.57		112.67	110.47
Cetylpiridinium chloride/15 benzocaine/20	19.57	20.46	19.58	20.44	97.85	102.30	97.90	102.20	>20	16.80	17.38		112.00	115.87
Cetylpiridinium chloride/15 benzocaine/25	24.50	25.45	24.75	25.78	98.00	101.80	99.00	103.12	>20	16.40	16.57		109.33	110.47
Cetylpiridinium chloride/15 benzocaine/30	29.20	31.00	30.05	30.92	97.33	103.33	100.17	103.07	>20	16.70	16.03		111.33	106.87

[a] RSD = 1.95%.
[b] RSD = 6.86%.

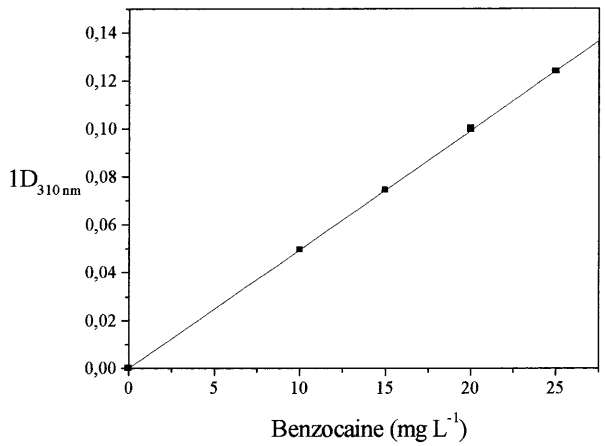

Fig. 7. Calibration graph of benzocaine obtained at 310.0 nm. The found linear regression was: $A = 2.16 \times 10^{-5} + 4.96 \times 10^{-3}$. c (mg/l), with $r = 0.9999$.

Acknowledgements

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