SIMULTANEOUS DETERMINATION OF PROCAINE AND BENZOIC ACID BY DERIVATIVE SPECTROMETRY

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abstract: A derivative spectrometric method has been developed for the determination of benzoic acid in presence of procaine. The method is precise and accurate and ensures the assay of both compounds benzoic acid and procaine in finished drugs.

Introduction

Procaine, (2-diethylaminoethyl-4-aminobenzoate hydrochloride), belongs to local anesthetics and is the active substance of Romanian drugs Aslavital and Gerovital H3 used in treatment of several disease related to aging, asthma and arthritis [1]. Finished products with procaine contain up to 6% benzoic acid as major excipients. In the present study a rapid, precise and accurate derivative spectrometric method for direct and simultaneous determination of both compounds procaine and benzoic acid has been developed. Such a method is required in quality control of finished drugs as the official monographs are based on a classical volumetric method for benzoic acid [2] and on a potentiometric titration for procaine [2]. These official methods can not be used for direct determination of both compounds. The research of the literature reveals that electrochemical [3÷5], ultraviolet-visible spectrometry [6], fluorimetry [7], high-performance liquid chromatography [8,9] and gas chromatography [10] methods were developed for the determination of procaine both in pharmaceuticals and biological fluids. Only one method has been reported for the determination of procaine in presence of benzoic acid [11].

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Analele Universității din Bucureşti – Chimie, Anul XIV (serie nouă), vol. I-II, pg. 401-406

Experimental

Reagents

Procaine hydrochloride and benzoic acid were commercially available and were of analytical grade (obtained from Aldrich). The water used was double distilled. Stock solutions of procaine (2 $mg.mL^{-1}$) and benzoic acid (0.12 $mg.mL^{-1}$) were prepared by dissolving the appropriate quantity of each of them in double distilled water. Working solutions were prepared by dilution of stock solutions in water.

Apparatus

All measurements were performed on a Jasco V 530 spectrometer coupled with a PC computer, running the Jasco software. The measurements have been made in quartz cells of 1 cm path length and conditions for recording the spectra were: wavelength range, 200-400 nm; scan speed, 100 nm/min; slit width, 2 nm; wavelength interval 1nm; smooth, 5. The ORIGIN program (Micro Cal Inc, version 6.0) was employed for the linear regression analysis.

Procedure

The UV spectra of the working solutions are recorded in the range 200-400 nm. Second derivative spectra of the zero order spectra were obtained by means of Jasco software. The zero-crossing method has been used to select the optimum wavelength for quantitative simultaneous determination of both compounds.

Linearity and range

For the calibration graph successive dilutions of working solutions were performed using 25 mL volumetric flasks. The zero order spectrum of each solution was recorded against a corresponding as blank. The second derivative spectrum was obtained using the Jasco software.

Accuracy and Precision

Six spectra of different concentration of each compound were recorded on the same day and the values of RSD were calculated to determine the intra-day precision. The same procedure was also performed on different days and the inter-days precision was determined. In mixtures of both compounds the recovery of each of them was calculated.

Detection and quantification limits

According to ICH [12] rules the LOD and LOQ have been determined by experiment.

Ruggedness

The ruggedness was established through the spectrometric studies by different analysts on the same apparatus.

Results and discussion

The zero order spectra recorded for procaine (0.01 mg.mL^{-1}), benzoic acid (0.005 mg.mL^{-1}) and a 2 : 1 mixture of both compounds are shown in figure 1. It is not possible to determine the benzoic acid in presence of procaine and not even the procaine in presence of benzoic acid as both spectra are very similar between 200 nm and 250 nm and overlapped in the range 250 – 350 nm. The problem was solved using the second derivative spectrometry.

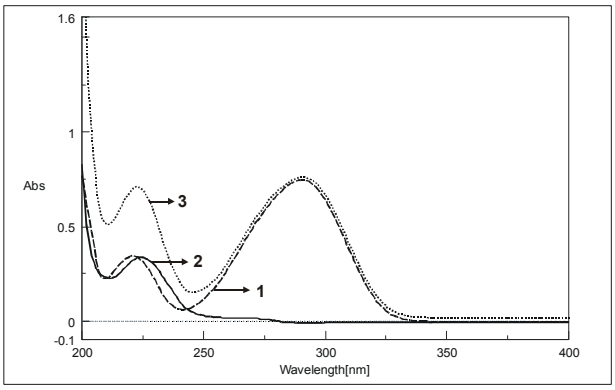

Fig. 1: *Zero order spectra for 1 – procaine, 2 - benzoic acid and 3 - mixture of both compounds*

The second derivative spectra of procaine and benzoic acid are presented in figure 2. As it shown the 231 nm was selected as the optimum working wavelength for determination of benzoic acid in presence of procaine while 238 nm for determination of procaine in presence of benzoic acid.

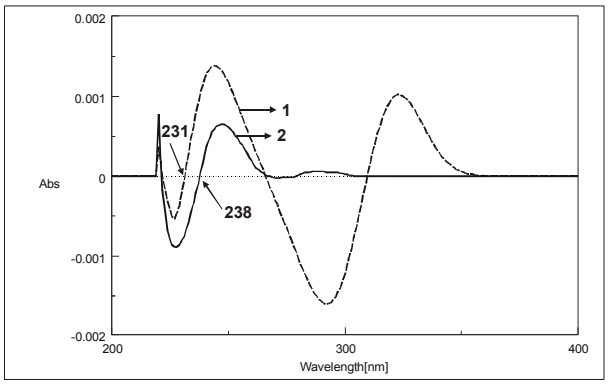

Fig. 2: *Second derivative spectra for: 1 – procaine and 2 - benzoic acid*

Validation of the method

Linearity and range

The spectra recoded during the linearity studies are presented in figure 3 a and b. The results of linearity and range for procaine and benzoic acid respectively are presented in Table 1.

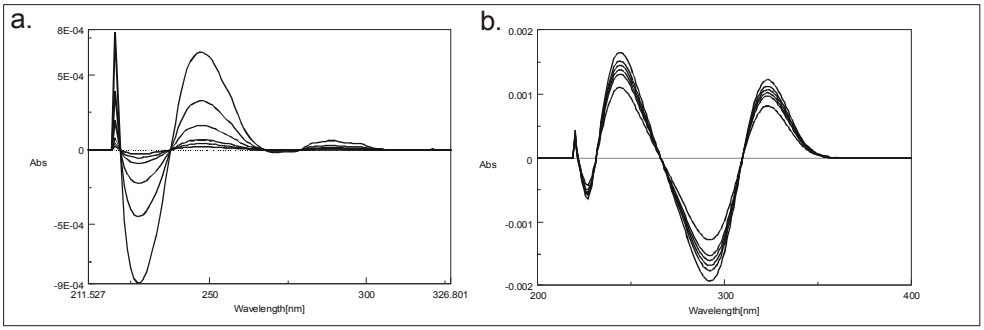

Fig. 3: *Spectra recoded during the linearity studies for: a – benzoic acid b – procaine*

As it is presented in Table 1 good linearity has been obtained for both compounds. The Student's t distribution was calculated and the values obtained were 2.46 and 2.18 for procaine and benzoic acid respectively. These values do not exceed the tabulated data of 2.77 for a probability of 95% means the method is free from the procedural errors.

Table 1 Results of linearity, LOD and LOQ studies

Compound	Range	I = a + bC			LOD	LOQ
	($\mu g.mL^{-1}$)	a	b	R	($\mu g.mL^{-1}$)	($\mu g.mL^{-1}$)
Procaine	8.00 – 13.00	1.65E-6	0.009	0.9992	0.03	0.10
Benzoic acid	0.50 – 0.78	4.32E-7	–138.8	0.9990	0.08	0.27

Note: I – second derivative intensity, C – concentration ($g.mL^{-1}$) R – correlation coefficient.

LOD and LOQ

Detection limits and the quantification limits, determined as 3 : 1 and 10 : 1 signal to noise ratio respectively, are presented in Table 1 for both compounds, also.

Selectivity of the method

The selectivity of the method has been checked in presence of potasium metabisulfite and disodium phosphate. Both compounds are used in formulation of finished product. Neither the former nor the latter interfere with procaine and benzoic acid. Thus the method is selective by regard to inorganic excipients found in finished formulation.

Accuracy and Precision

The accuracy has been established by six replicate determination made on each of five solid mixture containing procaine (100%) and benzoic acid (6%). Percentage recovery was calculated and the values obtained are in the range 99.2 – 100.8% for procaine and 98.7 – 101,6 % for benzoic acid. The RSD values for intra-day precision and for the inter-day precision are both bellow 2% for each compound. These results confirm that the method is precise.

Stability of the solutions

The stock solutions of the each compound were stored in light at room temperature for 4 days. The solutions were anlysed each day. The results obtained by the proposed method were compared with those obtained using methods described in pharmacopoeia for the assay[2]. The solutions are stable during this time.

Ruggedness

Ruggedness was performed to confirm that the assay of each compound was satisfactory under conditions external to the method. Good results were obtained during this study confirming that the method is accurate and precise for both compounds under tested conditions.

Applications

The method has been tested on synthetic samples containing procaine and benzoic acid. The results obtained are presented in Table 2. As it is shown the results obtained for benzoic acid when the volumetric titration with sodium hydroxide is performed are not in good agreement with those obtained with the proposed method. The alkaline conditions determine the hydrolysis of procaine with formation of *para*-aminobenzoic acid that interferes in benzoic acid titration.

Table 2 Determination of procaine and benzoic acid in synthetic samples

No.	Quantity					
	actual (mg)	Procaine determined (%)		actual (mg)	Benzoic acid determined (%)	
		proposed method	reference method		proposed method	reference method
1	100.0	100.23 ± 0.55	101.87 ± 1.89	6	99.17 ± 0.56	103.26 ± 1.89
2	100.0	100.44 ± 1.16	102.06 ±1.29	6	99.57 ± 0.87	105.46 ± 1.42
3	100.0	99.86 ± 0.97	101.64 ± 0.88	6	100.42 ± 1.26	101.23 ± 1.58
4	100.0	99.32 ± 0.21	98.36 ± 1.74	6	98.85 ± 1.04	102.85 ± 1.36
5	100.0	100.67 ± 1.05	99.62 ± 1.53	6	98.99 ± 0.22	104.54 ± 1.53
6	100.0	99.47 ± 0.68	101.58 ±1.88	6	101.05 ± 0.85	102.09 ± 1.95

Note: each value represents an average of three determinations ± standard deviation.

The method has been tested on real samples of Gerovital H3. Sugar coated tablets of Gerovital H3 (supplier Sicomed, Romania) have been analyzed only by proposed method. One tablet of Gerovital H3 contains 100 mg procaine, benzoic acid 6 mg, 5 mg potasium metabisulfite and 0.5 mg disodium phosphate. The results obtained are in good agreement with the nominal value of each compound.

Conclusion

A simple and rapid derivative spectrometric method has been developed for simultaneous determination of procaine and benzoic acid in formulated drugs. The method was validated and it is accurate and precise. The major advantage of this method is related to the determination of benzoic acid in presence of procaine with very good results as there are no official methods for this purpose.

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