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Application of an optical compensation method to the simultaneous determination of butamirate citrate and sodium benzoate by derivative spectrophotometry in the ultraviolet

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Abstract: Butamirate citrate and sodium benzoate are determined simultaneously by derivative ultraviolet spectrophotometry. As a consequence of the high absorbance of the sodium benzoate at the wavelengths where butamirate is determined, it is necessary to resort to an optical compensation method in order to maximize the signal to noise ratio. The method is validated for its application to pharmaceutical dosage forms. Good accuracy and precision are obtained. Some interactions found are discussed.

Keywords: Derivative spectrophotometry; butamirate citrate; sodium benzoate; optical compensation.

Introduction

Butamirate {α-Ethylbenzeneacetic acid 2-[2-(diethylamino)ethoxy]ethyl ester} is a modern cough-supressant drug.

$C_6H_5\ CH(CH_2CH_2)\ CH_2\ CH_2O\ CH_2\ CH_2N(CH_2\ CH_3)_2$

It is employed under the form of citrate (BC) in syrups, at a concentration of 2 g dm^{-3}.

Its absorptivity is extremely low (a = 0.42 L g^{-1} cm^{-1} at 257 nm), as could be expected from its chemical structure. The presence of an UV-absorbing matrix composed of preservatives, sweeteners, and flavoring and coloring agents, used in the preparation of liquid pharmaceutical forms preclude the direct spectrophotometric determination of this drug because of the extensive spectral overlap, as shown in Figure 1A, where the zero-order spectra of the two substances and of an artificial syrup in acid aqueous solution are presented. The first-, second-, and fourth-derivative spectra are shown in Figures 1B, 1C, and 1D respectively.

The same spectral overlap hinders the determination of sodium benzoate (SB) which is often used as a preservative in liquid pharmaceutical formulations.

The literature is scarce in methods for quantitative determination of BC. Some methods have been proposed for identification purposes, such as reverse phase high-performance liquid chromatography (HPLC) [1] and thin-layer chromatography (TLC) [2] but little else can be found in the literature.

Chromatographic methods, even though accurate and highly selective, may be rather slow and expensive, whereas ion-pair colorimetric determinations usually depend on liquid-liquid extractions in chloro-

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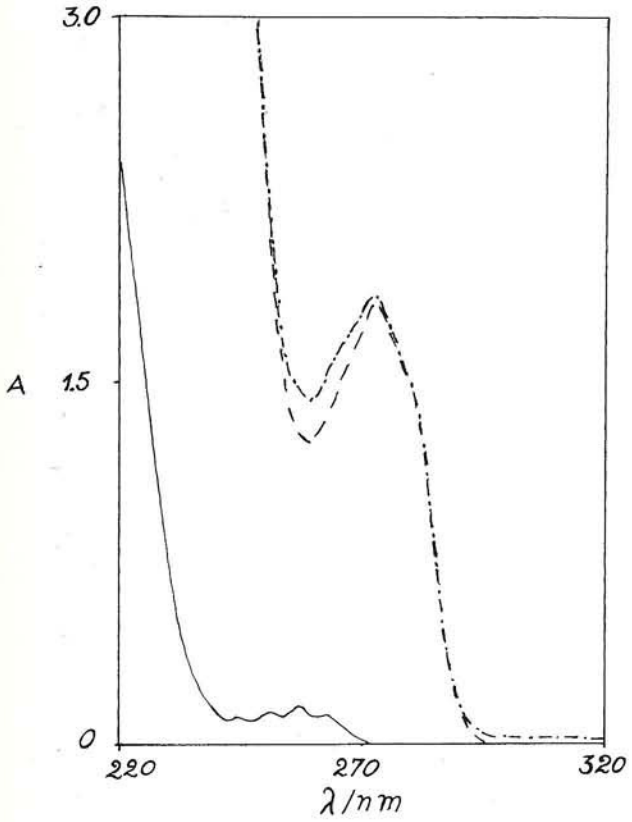


Figure 1A: Spectra of:
(_____) butamirate citrate (411 mg dm^{-3})
(----------) sodium benzoate (297 mg dm^{-3})
(-·-·-·-) mixture containing placebo (prepared as stated under "Reagents" and diluted (1+4)), butamirate citrate 411 mg dm^{-3}, sodium benzoate, 297 mg dm^{-3}.

Solvent was 0.2 M HCl.

form or other toxic solvents, so that a fast spectrophotometric method may be desirable for routine quality control.

One of the resources that can be used in such situation is derivative spectrophotometry [3-7]. The impressive development of digital electronics and the widespread implementation of this advances in commercial instruments have made this useful technique available to many analytical chemists. A rapid survey in the literature will show that it has been employed satisfactorily in various analytical areas to cope with additive spectral interferences in pharmaceutica [l8-15] and other areas.

Briefly, it consists of derivating the analytical signal (transmittance, absorbance, etc.) with respect to wavelength. Any interfering spectrum can be described with the desired approximation by a polynomial of degree N, hence its contribution to the total spectrum will be eliminated by derivating the spectrum N+1 times. If a given spectrum presents a fine structure, as an immediate consequence it will be enhanced in the process, while those spectra with little or no fine structure will tend to be cancelled. This characteristic is helpful when

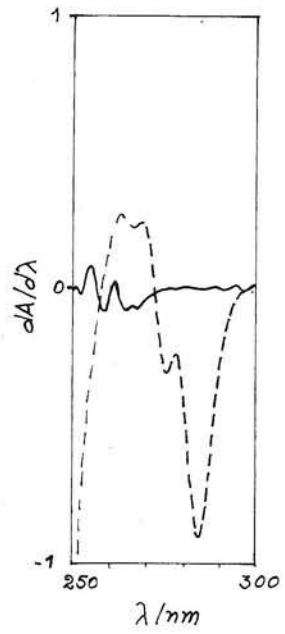


Figure 1B: First-derivative spectra of:
(_____) butamirate citrate (411 mg dm^{-3}),
(----------) sodium benzoate (297 mg dm^{-3}),
in 0.2 M HCl.

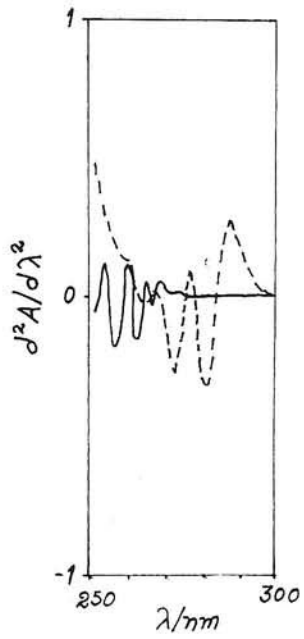


Figure 1C: Second-derivative spectra of:
(_____) butamirate citrate (411 mg dm^{-3}),
(----------) sodium benzoate (297 mg dm^{-3}),
in 0.2 M HCl.

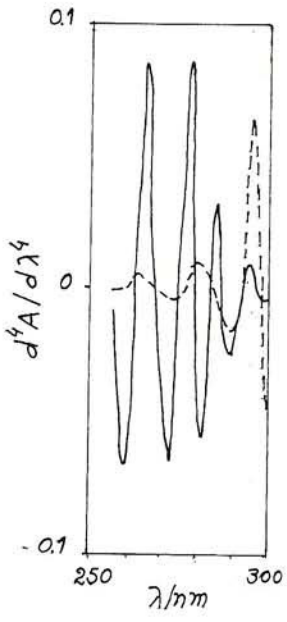


Figure 1D: Fourth-derivative spectra of:
(_____) butamirate citrate (411 mg dm^{-3}),
(----------) sodium benzoate (297 mg dm^{-3}),
in 0.2 M HCl.

dealing with spectral overlap in qualitative and quantitative analysis.

Derivative spectrophotometry has been used for over 20 years for the supression of spectral interferences in a number of analytical situations and is now firmly established as a highly useful alternative method, especially in the pharmaceutical industry.

In the present work, the determination of BC is performed by fourth-derivative ultraviolet spectrophotometry, while SB is determined by second-derivative spectrophotometry. As the spectral interference of SB on BC is very strong, and the sum of the two absorbances rather high, for the determination of the latter an optical compensation method described elsewhere [16-17] is employed, thus enhancing the signal to noise ratio of the measurements.

Experimental

Instruments

Shimadzu UV-240 spectrophotometer (Shimadzu Corp., Kyoto, Japan) fitted with Option Program Interface OPI-2 (Shimadzu), a constant-temperature cell holder (Shimadzu) connected to a constant-temperature water bath (Tecam) operated at 35 °C.

1-cm silica cells were used throughout.

Variable beam-attenuator (Beckman, Fullerton CA, USA, No. 104186).

Operating conditions

For determination of BC (fourth derivative):

Wavelength range:	240-280 nm.
Effective bandwidth:	1 nm.
Differentiation interval:	2 nm.
T/A range:	-0.1 ... +0.1.
Lambda scale:	10 nm cm^{-1}.
Scan speed:	slow.
Derivative mode:	mode 542 (fourth-derivative, memorized, $\Delta\lambda$ = 2 nm).

For determination of SB (first- and second-derivative):

Wavelength range:	250-300 nm.
Effective bandwidth:	1 nm.
Differentiation interval:	1 nm.
T/A range:	-1 ... +1.
Lambda scale:	10 nm cm^{-1}.
Scan speed:	slow.
Derivative mode:	modes 511 (first-derivative, memorized, $\Delta\lambda$ = 1 nm) and 521 (second-derivative, memorized, $\Delta\lambda$ = 1 nm).

Peak derivative values were obtained by means of the "Peak-pick" option program.

Reagents

Butamirate citrate and sodium benzoate of pharmaceutical grade were used as received. Other reagents were of Analytical -Reagent Grade.

Placebo excipient, containing sugar (500 g dm^{-3}), glucose (230 g dm^{-3}), glycerine (150 g dm^{-3}), ethyl alcohol (10 % v/v) and water as solvent.

Procedure

Standard solutions: prepare a solution of BC (0.4 g dm^{-3}) in 0.2 M hydrochloric acid, and a solution of SB (0.3 g dm^{-3}) in the same solvent.

Commercial syrup: dilute in 0.2 M hydrochloric acid so as to obtain concentrations of 0.4 g dm^{-3} of BC (with the commercial formulations studied, this corresponds to a concentration of 0.3 g dm^{-3} of SB).

Determination of BC: record and memorize the spectral baseline using air instead of the reference cell. Obtain the fourth-derivative spectrum of the standard solution according to the operating conditions stated above. Measure the amplitude of the peak at around 264 nm. Proceed concomitantly with problem solutions, installing previously the attenuator in the reference-beam path. The attenuator should be adjusted previously so that the absorbance at 264 nm when the sample cell is loaded with problem solution equals ca. 0.8.

Determination of SB: obtain the second-derivative spectrum according to the operating conditions stated above. Measure the amplitude between maximum and minimum at around 289 and 282 nm respectively. Proceed concomitantly with standard and problem solutions.

Results and discussion

1. Validation

Mutual interference

This was studied using an experimental design based on the central composite design [18]. Synthetic mixtures of BC, SB and placebo excipient were prepared with concentrations varying according to Table 1. Variation range for BC and SB was ±40% of the nominal analytical concentrations (stated under "Procedure"), whereas the placebo was maintained always at the nominal concentration and therefore is not mentioned.

The relative concentration of each analyte was determined according to the proposed procedures. In the case of SB, both first- and second-derivative were used and compared.

Simple linear regression ($y = a\,x + b$) was applied for each relative concentration found ("y") as a function of the corresponding "put" value ("x"). The com-

Table 1

BC put	SB put	Relative Concentrations found (%)		
(%)	(%)	BC	SB-1D	SB-2D
60	100	61.7	101.8	100.8
80	80	81.9	82.2	77.8
80	100	81.1	101.6	102.7
80	120	83.9	120.2	120.6
100	60	100.1	62.3	59.6
100	80	100.1	81.8	79.9
100	100	101.1	101.2	100.9
100	120	100.7	120.8	120.0
100	140	99.8	138.3	140.7
120	80	120.0	81.7	79.5
120	100	120.6	101.0	100.2
120	120	121.8	120.2	119.8
140	100	140.9	101.5	99.8

Relative concentrations found for butamirate citrate, fourth-derivative (BC), sodium benzoate, first-derivative (SB-1D) and sodium benzoate, second-derivative (SB-2D) for several synthetic mixtures of the two analytes. "Put" and "Found" values are relative to the nominal analytical concentrations ("100%"), which in this instance were: BC, 416 mg dm^{-3}; SB, 296 mg dm^{-3}. "Found" values are the average of two analytical replications. Placebo was added at the nominal analytical concentration and kept constant in all instances.

parison of the experimental slope with the theoretical value (= 1) was carried out by means of Student's "t" test. The "t" values found, as well as the regression parameters are shown on Table 2.

According to these results, it can be accepted that the slopes do not differ significantly from 1 in the case of BC and of SB, second derivative ($t_{calc} < t(0.025, f)$, f = degrees of freedom). In the case of SB, first derivative, the slopes differ significantly ($t_{calc} > t(0.025,f)$), hence first derivative is not deemed appropriate.

The intercepts are below 2 % of the nominal analytical concentrations of the method and thus can be considered to be non-significant.

Table 2

	BC	SB-1D	SB-2D
a	0.9784	0.9563	1.0177
b	3.2229	5.5264	-1.6197
tcalc	1.52	6.32	1.19

Regression parameters a and b (y = a x + b) for recovery experiments where found concentrations (y) are represented as a function of put concentrations (x). x and y are relative concentrations expressed respect to the nominal analytical concentrations ("100%"), which in this instance were - BC, 416 mg dm^{-3}; SB, 296 mg dm^{-3}. t_{calc} is the calculated Student's "t" for the slope "a", as compared with the theoretical value of 1 (t (0.025, 11) = 2.20).

Accuracy and precision

Analytical accuracy and precision were evaluated by determination of the two analytes in a synthetic syrup prepared as described before, containing known concentrations of the two substances.

In the case of BC, precision obtained with and without the use of optical compensation was compared.

Results found are listed in Table 3.

Table 3

Analyte/method	Mean recovery (%)	RSD (%)
BC-OC	100.8	1.6
BC	102.3	2.8
SB-1D	100.1	0.2
SB-2D	100.0	0.5

Comparison of recovery and precision (N = 5) in the determination of BC and SB in synthetic mixtures (N = 5).

2. Linearity

It was verified for BC (0-0.8 g dm^{-3}) and SB (0-0.6 g dm^{-3}) in the presence of each other. In each instance one of the substances was maintained at the nominal analytical concentration while the other was varied within the specified concentration range. In the case of SB, measurements were performed by means of first- and second-derivative spectrophotometry.
Results are shown on Table 4.

3. Discussion

When employing spectrophotometric methods for simultaneous determinations, the analyst should be aware not only of additive interferences (such as spec-

Table 4

	Butamirate citrate	Sodium Benzoate	
		First Derivative	Second Derivative
a	1.993×10^{-4}	3.034	2.147
b	4.048×10^{-4}	21.733	10.666
r	0.9999	0.9992	0.9994
Sa	1.3×10^{-6}	6.8×10^{-2}	3.5×10^{-2}
Sb	6.2×10^{-4}	20.3	13.0

Straight-line least-squares parameters (y = a x + b) and correlation coefficients for the calibration curves of butamirate citrate (fourth derivative) and sodium benzoate (first- and second- derivative). Sa and Sb are the standard deviations of a and b respectively (N = 5).

tral overlap) but also of interactions (chemical and other) between the analyte and the various substances present in the mixture.

In this work, a significant interaction was detected between BC and SB. This interaction was significant when the pH was neutral or basic, but became negligible when the pH was acid. Figures 2 and 3 show the effect of pH on the interactions.

As a consequence of these results, 0.2 M hydrochloric acid was chosen as the solvent.

For the determination of BC, derivation orders lower than four could not be used because of the severe spectral overlap. One disadvantage of using high derivation orders is the sensitivity loss, inherent to the differentiation process. One more consequence is the dramatic

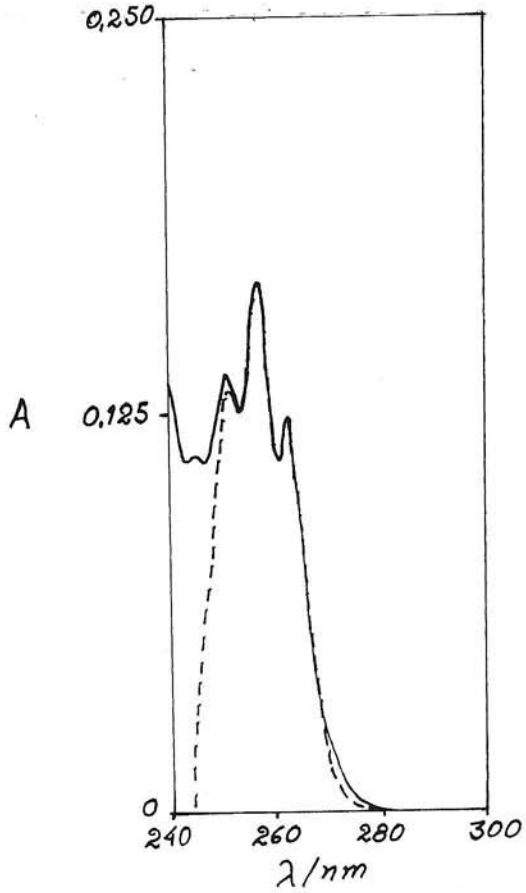
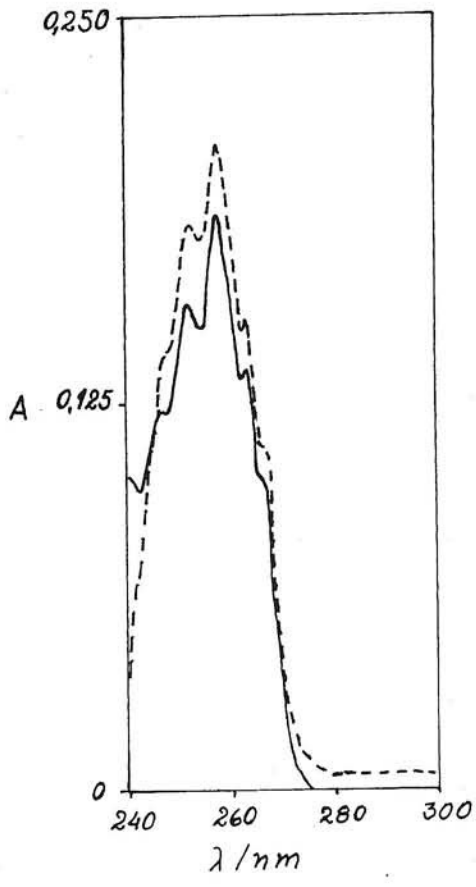


Figure 2.
(_____) True spectrum of butamirate citrate (411 mg dm^{-3}).
(----------) Spectrum obtained numerically by substracting the spectrum of sodium benzoate (297 mg dm^{-3}) from that (not shown) of the mixture of butamirate citrate (411 mg dm^{-3}) and sodium benzoate (297 mg dm^{-3}).
Solvent in all instances was 0.2 M HCl. Spectral substraction was carried out by using the spectral processing capabilities of the spectrophotometer.



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Solvent in all instances was 0.1 M KOH. Spectral substraction was carried out by using the spectral processing capabilities of the spectrophotometer.

deterioration of signal to noise (S/N) ratio, which is in turn a consequence of the differentiation of instrumental noise. For this reason, it is of the utmost importance that noise level be kept as low as possible during the data acquisition, so that the final S/N ratio may still be reasonably high.

This was partially obtained by selecting compromise values for spectral bandwidth, and differentiation interval, while scanning at low speeds. However, the high absorbance values found when scanning the spectra of BC-SB mixtures (values in excess of 2) and the concomitant increase of noise level led us to the use of the optical compensation method described before [16] in order to enhance the S/N ratio. This was carried out by using a variable beam-attenuator in the reference path of the spectrophotometer as described in the mentioned paper [16]. The increase in the S/N ratio, measured by the decrease in the relative standard deviation of the results (Table 2, see BC and BC-OC), shows the usefulness of this approach.

In all instances, the spectral baseline was memorized using air as the reference, instead of a cell filled with solvent. This was necessary because of the use, when measuring the sample, of a beam attenuator in the path of the reference beam, which precludes the simultaneous installation of a reference cell in the light path. The digital spectrophotometer used will memorize the spectral baseline, and later substract it automatically from the absorbance values measured. Thus, it is not necessary to use a cell with the solvent or blank in the path of the reference-beam, but a cell with solvent (or blank) only can be inserted in the path of the sample-beam during the process of baseline memorization, which is then made taking air as the reference.

The temperature in the cell was kept constant in order to minimize the effect of this variable [19,20]. Previous experiments demonstrated us that errors in excess of 10% could arise from temperature variations of a few degrees. This is especially serious in high-order derivatives such as the fourth derivative used in this work.

Conclusions

The proposed method seems to be easy to use, and accurate enough as to be used in routine quality control as a substitute for the more involved HPLC methods.

The different spectral structure of the substances and the slight wavelength shift presented by the spectra allows the simultaneous determination by careful selection of derivation order.

The use of optical compensation seems to be an efficient solution when higher derivation orders are employed in conditions of high absorbance, and low noise levels are required.

Reference

1. Daldrup, T.; Michalke, P; Boehme, W.; Chrom. Newsl. **1982** 10 1-7 through Chem. Abs. **1983** 98 84433c.
2. Musumarra, G.; Scarlata, G.; Romano, G.; J. Anal. Toxicol. **1987** 11 154-163 through Chem. Abs. **1987** 107 126383j.
3. Olson, E.C. and Alway, C.D.; Anal. Chem. **1960** 32 370.
4. O'Haver, T.C., and Green, G.L., Anal. Chem. **1976** 48 312.
5. Talsky, G., Mayring, L., and Kreuzer, H., Angew. Chem. Int. Ed. Engl. **1978** 17 785.
6. Cahill, J.E., Amer. Lab. **1979** 11 79.
7. Levillain, P., and Fompeydie, D., Analusis **1986** 14 1.
8. Jones, R., and Marnham, G., J. Pharm. Pharmacol. **1981** 33 458.
9. Hassan, S.M., and Davidson, A.G., J. Pharm. Pharmacol. **1984** 36 7.
10. Kitamura, K., and Majima, R., Anal. Chem. 55 54 **1983**.
11. Davidson, A.G. and Hassan, S.M., J. Pharm. Sci. **1984** 73 413.
12. Fasanmade, A.A., and Fell, A.F., Analyst **1985** 110 1117.
13. Dol, I., and Altesor, C., SAFYBI Buenos Aires **1988** 28, 2660.
14. Dol, I., Knochen, M., and Altesor, C., Boll. Chim. Farm. **1989** 128 18.
15. Knochen, M., Altesor, C., and Dol., I., Analyst **1989** 114 1303.
16. Dol, I., Knochen, M., and Altesor, C., Analyst **1991** 116 69.
17. Knochen, M., and Dol, I.; Analyst **1992** 117 1385.
18. Bayne, C.K., and Rubin, I.B. "Practical Experimental Designs and Optimization Methods for Chemists". VCH Publishers, Deerfield Beach, Florida, USA., **1986**, Chapter 6, pp 150 - 152.
19. Davidson, A.G., Analyst **1983** 108 728.
20. Davidson, A.G., J. Pharm. Biomed. Analysis. **1985** 3 329.