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Simultaneous determination of chlorzoxazone and acetaminophen in combined dosage forms by an absorbance ratio technique and difference spectrophotometry

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Abstract: Two spectrophotometric methods have been developed for the simultaneous determination of chlorzoxazone and acetaminophen in their combined dosage forms. No preliminary separation step is required in either method. The first, an absorbance ratio technique using the isoabsorptive point as one of the wavelengths, together with "Q curve" analysis gives accurate and reproducible results for both drugs. The second, a difference spectrophotometric method is based on measurement of absorbance of an alkaline solution relative to that of an acidic solution of identical concentration of the sample at two different wavelengths. The method is suitable for routine analysis of such combinations of the drugs.

Keywords: *Difference spectrophotometry; absorbance ratio technique; "Q curve" analysis; chlorzoxazone–acetaminophen determination; muscle relaxants.*

Introduction

Chlorzoxazone (5-chloro-2-benzoxazolinone) and acetaminophen (paracetamol; 4-hydroxyacetanilide) in combined dosage formulations (capsules and tablets) are official in the USP [1] and are widely used as muscle relaxants. The assay method and dissolution-rate procedure described in the USP involve HPLC determination of each component. Honigberg *et al.* [2] and Stewart *et al.* [3] have also reported HPLC methods for assay of this combination.

In the present investigation, the absorbance ratio method of Pernarowski *et al.* [4] and the difference spectrophotometric method [6] have been applied for simultaneous determination of both the components without prior separation.

Experimental

Apparatus and reagents

A Hitachi 150-20 recording spectrophotometer with 1-cm matched silica cells was used. Reference acetaminophen and chlorzoxazone stock solutions: 25 mg/100 ml of

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acetaminophen IP and chlorzoxazone USP in methanol, separately. Glass distilled water (pH ~7). All reagents were of analytical grade.

Procedure

Absorbance ratio method

Standard preparation. A 2-ml aliquot of each of the stock solutions was diluted to 50 ml with 0.02 M hydrochloric acid and the absorbance was measured at 270 and 280 nm against methanol (4%, v/v) in 0.02 M hydrochloric acid as the solvent blank.

Sample preparation. An accurately weighed quantity of the powdered tablets, equivalent to 25 mg of chlorzoxazone, was transferred to a 100-ml volumetric flask and dissolved as completely as possible by shaking with about 70 ml of methanol. The solution was filtered through a sintered-glass funnel under suction; the residue was washed with small portions of methanol and the combined filtrates were diluted to volume. A 2-ml aliquot was diluted to 50 ml with 0.02 M hydrochloric acid and the absorbance was measured as above against the solvent blank. The respective concentrations of both components were calculated by solving the following two analogous equations:

$$C_{\mathrm{I}} = \frac{Q_{\mathrm{o}} - Q_{\mathrm{II}}}{Q_{\mathrm{I}} - Q_{\mathrm{II}}} \cdot \frac{A}{a_i}; \tag{1}$$

$$C_{\mathrm{II}} \frac{Q_{\mathrm{o}} - Q_{\mathrm{I}}}{Q_{\mathrm{II}} - Q_{\mathrm{I}}} \cdot \frac{A}{a_i}. \tag{2}$$

Where C_{I} and C_{II} are the concentrations in g l^{-1} of chlorzoxazone and acetaminophen, respectively; Q_{o} is the ratio of the absorbance values of the sample solution at the two wavelengths (280 and 270 nm); Q_{I} and Q_{II} are the absorbance ratio values of the chlorzoxazone and acetaminophen standard solutions, respectively at the two wavelengths; A and a_i are the absorbance value of the sample solution and the absorptivity value of the components, respectively, both at the isoabsorptive wavelength (270 nm).

Difference spectrophotometric method

Standard preparation. Aliquots (2 ml) of each of the stock solutions were separately diluted to 50 ml with 0.02 M sodium hydroxide and 0.02 M hydrochloric acid, and the absorbance difference (ΔA) of the alkaline solution was measured at 246.5 and 267.5 nm, using the acidic solution of the corresponding drug as blank. Solvent blank corrections were carried out.

Sample preparation. The sample solution was prepared in methanol as described under the absorbance ratio method. The preparation of the acidic and alkaline solutions and measurement of the absorbance difference (ΔA) of the solutions was carried out as described under standard preparations with necessary blank corrections.

The chlorzoxazone content was calculated by direct correlation of the absorbance difference (ΔA) of the sample solution to that of the standard solution at 246.5 nm. The acetaminophen content was calculated according to the following formula:

$$\text{Acetaminophen content} = [\Delta A_{267.5} - (\Delta A_{246.5} \cdot \Delta A_{\mathrm{c};267.5}/\Delta A_{\mathrm{c}246.5})] \cdot C_{\mathrm{p}}/\Delta A_{\mathrm{p};267.5}, \tag{3}$$

where $\Delta A_{267.5}$ and $\Delta A_{246.5}$ are the absorbance differences of the sample solution at the respective wavelength; $\Delta A_{c;267.5}/\Delta A_{c;246.5}$ is the ratio of the absorbance difference of the reference chlorzoxazone solution at the respective wavelength; $\Delta A_{p;267.5}$ and C_p are the absorbance difference and concentration of the reference acetaminophen solution, respectively.

Results and Discussion

For analysis of the binary mixture, the absorbance ratio method of Pernarowski *et al.* [4] involving the use of the isoabsorptive point, was applied depending on the nature of the curve. Chlorzoxazone and acetaminophen exhibit an isoabsorptive point at 270 nm and maxima at 280 and 244 nm, respectively, in 0.02 M hydrochloric acid (Fig. 1). It was observed that the combined spectrum of the drugs (Fig. 1) shows a shoulder at 244 nm and minimum and maximum absorbance at 270 and 280 nm, respectively. The simultaneous equation technique [5] using 244 and 280 nm as the measuring wavelength was eliminated because it was found to introduce a greater degree of error. Slight interference due to irrelevant absorption of excipients was observed at 244 nm, whereas no such interference was observed at 270 and 280 nm. Thus the isoabsorptive point at 270 nm and the absorption maximum of chlorzoxazone (280 nm) were chosen for analysis of the mixture. Both the components obey Beer's law in the concentration range 0.006–0.02 mg ml^{-1} at the above wavelengths.

The "Q curve" [4] for this mixture was constructed from the data accumulated on mixtures containing known amounts of chlorzoxazone and acetaminophen. Using the method of least squares [7], the following regression equation was derived representing Q_o values corresponding to the fraction of chlorzoxazone (F_1) in the mixture:

$$Q_o = 1.04\ F_1 + 0.652, \tag{4}$$

for which the correlation coefficient is 0.9994. The fraction of chlorzoxazone can also be calculated by application of the equation.

For difference spectrophotometry [6], both the chlorzoxazone and acetaminophen display a bathochromic shift together with a hyperchromic effect from acidic to alkaline

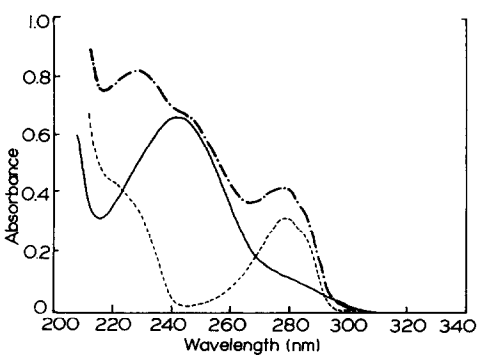

Figure 1
UV absorption spectra (0.01 mg ml^{-1}) of chlorzoxazone (-----), acetaminophen (———) and a binary mixture (- · - · -, 0.01 mg ml^{-1} of each) in 0.02 M hydrochloric acid containing 4%, v/v, methanol.

media, whereas no spectral change is observed from acidic to aqueous (neutral) media. This shift is attributed to the measurement of the ionic form of the drug (in alkaline media) against its molecular form (in acidic media). The difference absorption spectra (Fig. 2) of the alkaline solution (0.02 M sodium hydroxide) of acetaminophen relative to its identical solution in 0.02 M hydrochloric acid shows a maximum absorbance difference (ΔA) at 267.5 nm and an isoabsorptive point of zero absorbance at 246.5 nm; in contrast, chlorzoxazone under identical conditions exhibits almost maximum absorbance at 246.5 nm and negative absorbance at 267.5 nm. Thus at 267.5 nm, total absorbance of the mixture of the two components is the vector sum of the positive absorbance due to acetaminophen and the negative absorbance due to chlorzoxazone. Thus chlorzoxazone is calculated by direct correlation of the absorbance difference of the test solution at 246.5 nm to that of a standard solution; acetaminophen is calculated by measuring the absorbance difference at 267.5 nm, and application of equation (3). Both the drugs exhibit a linear relationship between ΔA versus C, within a concentration range of 0.004–0.02 mg ml^{-1} at the respective wavelength. The choice of pH of the solvents is based on the pK_a values of the compounds (8.0 for chlorzoxazone and 9.5 for acetaminophen) to provide a maximum difference absorbance. Thus 0.02 M hydrochloric acid (pH ~2) and 0.02 M sodium hydroxide (pH ~12) were selected to satisfy the experimental conditions.

To confirm the validity and applicability of the proposed methods, three synthetic mixtures at different drug ratios and three commercial dosage forms were analysed by both the proposed methods. The validity of the derived equations were assessed by calculating the recovery of the synthetic mixtures. The methods were also subjected to recovery studies by adding known amount of the drugs to the preanalysed samples. The analytical results (Tables 1 and 2) show that the standard deviations are low, and that the mean per cent recoveries varied from 98.95 to 99.49, indicating that the proposed methods are precise, accurate and reproducible. Common excipients and adjuvants do not interfere. To confirm further any interference from the formulation matrix in the difference spectrophotometric method, graphs of log ΔA versus λ were plotted for the sample as well as for authentic solutions. The graphs were completely superimposable

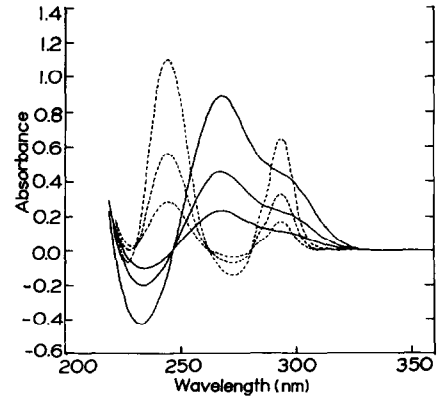

Figure 2
Difference absorption (ΔA) spectra (0.02, 0.01 and 0.005 mg ml^{-1}) of chlorzoxazone (-----) and acetaminophen (———); alkaline (0.02 M sodium hydroxide) versus acidic (0.02 M hydrochloric acid) solution as blank.

Table 1
Determination of chlorzoxazone and acetaminophen by the absorbance ratio method

Formulation	Chlorzoxazone Claimed (mg/tablet)	Found* (% claim)	Added (mg)	Recovery (%)	Acetaminophen Claimed (mg/tablet)	Found* (% claim)	Added (mg)	Recovery (%)
Tablets								
1	250	100.72 ± 0.27	20	97.75	300	101.85 ± 0.20	20	99.25
2	250	101.66 ± 0.31	25	100.56	300	98.96 ± 0.29	25	98.48
3	250	100.35 ± 0.33	30	98.53	300	102.06 ± 0.37	30	99.66
Mean ± SD				98.95 ± 1.45				99.13 ± 0.6
Synthetic mixtures								
1	25	98.68 ± 0.29	—	—	25	99.52 ± 0.41	—	—
2	30	98.87 ± 0.35	—	—	20	98.85 ± 0.31	—	—
3	20	99.70 ± 0.28	—	—	30	98.93 ± 0.34	—	—

*Mean of five determinations ± relative standard deviation (%).

Table 2
Determination of chlorzoxazone and acetaminophen by the difference spectrophotometric method

Formulation	Chlorzoxazone Claimed (mg/tablet)	Found* (% claim)	Added (mg)	Recovery (%)	Acetaminophen Claimed (mg/tablet)	Found* (% claim)	Added (mg)	Recovery (%)
Tablets								
1	250	100.18 ± 0.23	20	98.15	300	101.24 ± 0.30	20	98.40
2	250	101.68 ± 0.25	25	100.20	300	98.97 ± 0.35	25	99.28
3	250	100.76 ± 0.33	30	100.13	300	102.48 ± 0.29	30	100.10
Mean ± SD				99.49 ± 1.16				99.26 ± 0.85
Synthetic mixtures								
1	25	98.96 ± 0.30	—	—	25	99.52 ± 0.29	—	—
2	30	99.70 ± 0.21	—	—	20	98.40 ± 0.36	—	—
3	20	97.99 ± 0.21	—	—	30	99.73 ± 0.31	—	—

*Mean of five determinations ± relative standard deviation (%).

indicating that the method nullifies any non-specific irrelevant absorption due to the formulation matrix, which may affect the accuracy of the result.

Applying the *t*-test and *F*-test for comparison of the two methods, the calculated *t*-values and *F*-values (acetaminophen, $t = 1.94$, $F = 2.53$; chlorzoxazone, $t = 1.75$, $F = 1.42$) do not exceed the theoretical *t*-value (2.31 at $\alpha = 0.05$) and *F*-value (6.39 at $\alpha = 0.05$), indicating that the difference between the results and the reproducibility of the two methods are insignificant. Thus both the methods are free from matrix interference and are simple to use for routine and control analysis.

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