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International Journal of Pharmaceutics 189 (1999) 67–74

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Simultaneous determination of chlordiazepoxide and clidinium bromide in pharmaceutical formulations by derivative spectrophotometry

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Received 4 January 1999; received in revised form 9 July 1999; accepted 9 July 1999

Abstract

A direct and simple first derivative spectrophotometric method has been developed for the simultaneous determination of clidinium bromide and chlordiazepoxide in pharmaceutical formulations. Acetonitrile was used as solvent for extracting the drugs from the formulations and subsequently the samples were evaluated directly by derivative spectrophotometry. Simultaneous determination of the drugs can be carried out using the zero-crossing method for clidinium bromide at 220.8 nm and the graphical method for chlordiazepoxide at 283.6 nm. The calibration graphs were linear in the ranges from 0.983 to 21.62 mg/l of clidinium bromide and from 0.740 to 12.0 mg/l of chlordiazepoxide. The ingredients commonly found in commercial pharmaceutical formulations do not interfere. The proposed method was applied to the determination of these drugs in tablets.

Keywords: Chlordiazepoxide; Clidinium bromide; Simultaneous determination; First derivative spectrophotometry; Pharmaceutical formulations

1. Introduction

Chlordiazepoxide (7-chloro-*N*-methyl-5-phenyl-3H-1,4-benzodiazepin-2-amina-4-oxide) is a sedative-hypnotic drug widely employed as a tranquilizer and antidepressant (Gasparic and Zimak, 1983) and clidinium bromide (3-[(hydroxydiphenylacetyl)-oxy]-1-methyl-1-azoniabicyclo-[2.2.2] octane bromide) is effective for anxiety-related conditions including spastic colon (Rudy and Senkowski, 1973).

Tensoliv and Libraxim are pharmaceutical formulations which contain both drugs. Some chromatographic techniques, including liquid chromatography (Yuen and Lehr, 1991) and a reversed-phase high performance liquid chromatography (Jalal et al., 1987) have been reported for their simultaneous determination. The pro-

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00238-0

posed method by US Pharmacopoeia 23 (Anon., 1995) is a liquid-chromatographic method, involving an UV detector and a 4-mm × 25-cm column that contains packing L1. This simultaneous determination by HPLC requires careful preparation of the column and it is expensive. On the other hand the derivative spectrophotometry is more simple, inexpensive and has been used in simultaneous determination of organic compounds (Toral et al., 1996a,b; Wrobel-Zasada et al., 1996) and inorganic compounds (Toral et al., 1993, 1997; El-Sayed and Khalil, 1996).

In this work a simple, accurate, precise, rapid and inexpensive method by derivative spectrophotometry is proposed. Simultaneous determination of both compounds was carried out using acetonitrile as solvent for extracting the drugs from the formulations and subsequently the samples were evaluated directly by first order derivative spectrophotometry.

A study of the spectral behavior of these compounds in different solvents, the optimization of the spectral variables, the determination of pKa values and the application of the proposed method to the determination of these drugs in tablets are included in this work.

2. Materials and methods

2.1. Instruments

A Shimadzu UV-160 spectrophotometer with 10-mm cells was used for measurements of the absorbance and derivative absorption spectra. For all solutions, the first derivative spectra were recorded, over a range of 400–200 nm against solvent, at a scan speed of 480 nm/min with a $\Delta\lambda = 3.2$ nm. The spectra derivatives were obtained digitally by software incorporated in the Shimadzu UV-160 spectrophotometer.

2.2. Reagents

All reagents were of analytical reagent grade. Clidinium bromide (I) and chlordiazepoxide (II) were kindly provided by Laboratorio Chile, Santiago, Chile.

Stock solutions of clidinium bromide and chlordiazepoxide were prepared, by dissolving 21.62 ± 0.01 and 14.98 ± 0.01 mg of each compound in acetonitrile in order to give 1×10^{-3} M solution, corresponding to 432.4- and 299.8-mg/l solutions of each specie. Other ranges of concentrations were prepared by appropriate dilution using the same solvent. The tablets containing clidinium bromide and chlordiazepoxide were also dissolved with the same solvent and assayed.

2.3. Calibration procedure for determination of I and II in mixtures

Aliquots of the stock solution of I and II were simultaneously diluted in acetonitrile over the concentration range 0.9–22.0 mg/l. The calibration procedure was carried out for each compound in the presence of 8.6 mg/l clidinium bromide solution and 6.0 mg/l chlordiazepoxide solution, respectively. In all cases the corresponding absolute values of the first derivative spectra at 220.8 and 283.6 nm for clidinium bromide and chlordiazepoxide, respectively, were obtained and were plotted against the corresponding concentrations.

2.4. Procedure for determination of I and II in tablets

A total of 20 tablets of each formulation were weighed and powdered. A quantity of powder equivalent to 10–35 mg of tablet containing I and II was accurately weighed and dissolved in acetonitrile, transferred into separate 100 ml calibrated flasks and diluted to mark. The contents of the flasks were shaken for 20 min and then the first derivative spectra were obtained.

3. Results and discussion

The structures of chlordiazepoxide and clidinium bromide are shown in Fig. 1. As can be see the structures of these drugs are quite different. Because of this, the spectral behavior of both compounds can be expected to be quite different. The spectral behavior of chlordiazepoxide in dif-

ferent solvents such as methanol, ethanol, dimethylsulfoxide, dimethylformamide, acetonitrile and formamide, is similar. In all cases the molar absorptivities are near to 4000 l(mol × cm)$^{-1}$. In contrast, clidinium bromide shows a different spectral behavior because the molar absorptivities are considerably lower, with values between 100 and 200 l(mol × cm)$^{-1}$. Only when acetonitrile is used as solvent does the molar absorptivity of this compound increase to 4000 l(mol × cm)$^{-1}$.

According to results obtained from this spectral study, acetonitrile was selected, because only in this solvent is the ratio of the molar absorptivities for both compounds near to one. Under this condition, the simultaneous determination is more effective and the sensitivity for the determination of clidinium bromide is improved. Further, in this solvent both drugs are stable for at least 24 h.

3.1. pKa Determination

The determination of pKa of chlordiazepoxide in Britton Robinson Buffer ranging between pH 0.0 and 11.0 was carried out by the first derivative technique, plotting the derivative unit at $\lambda = 260.6$ and $\lambda = 289.4$ nm versus pH. These derivative units correspond to the absorption of the acid and the basic forms, respectively (Fig. 2). As can be see in Fig. 2A,B, a pKa of 4.9 was obtained: this value is consistent with the pKa = 4.8 reported in the literature. In the case of clidinium bromide, the pKa determination was also carried out in Britton Robinson Buffer, ranging between pH 0.0 and 13.0. The first derivative technique was used, plotting the derivative unit at $\lambda = 213.0$ nm (acid form) and $\lambda = 227.6$ nm (basic form) versus pH. Fig. 2C,D shows that the pKa of this compound is near 11. This value is also in accordance with the low characteristic acidity of clidinium bromide. For the pKa determination of both compounds, it was necessary to use the derivative spectrophotometry technique because, in both cases, the bands of the acid forms and the basic forms were very overlapped.

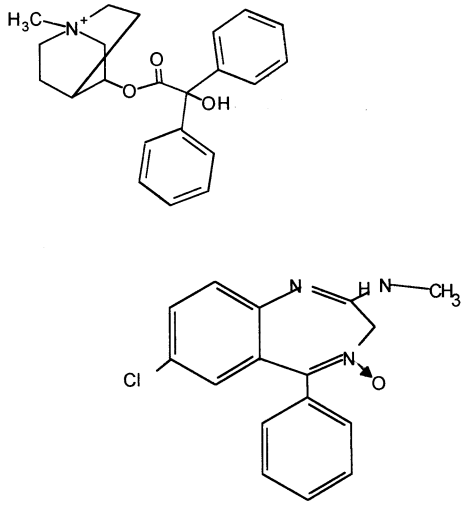

Fig. 1. The structure of chlordiazepoxide and clidinium bromide. (a) Chlordiazepoxide (7-chloro-*N*-methyl-5-phenyl-3H-1,4-benzodiazepin–2-amina-4-oxide); (b) clidinium bromide (3-[(hydroxydiphenylacetyl)-oxy]-1-methyl-1-azoniabicyclo-[2.2.2] octane bromide).

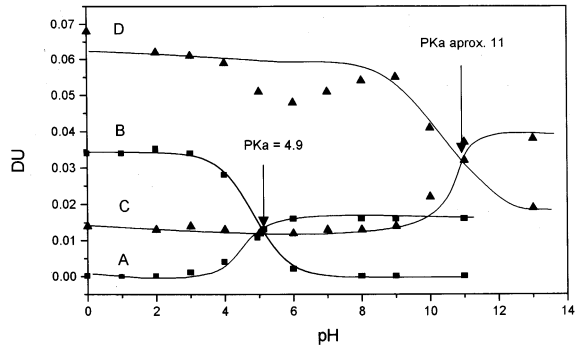

Fig. 2. Determination of pKa of chlordiazepoxide and clidinium bromide in Britton Robinson Buffer. Chlordiazepoxide: (A) derivative unit at 260.6 nm versus pH; (B) derivative unit at 289.4 nm versus pH. Clidinium bromide: (C) derivative unit at 213.0 nm versus pH; (D) derivative unit at 227.6 nm versus pH.

3.2. Spectral features

The zero-order spectra of both analytes (Fig. 3), using acetonitrile as solvent, show that only chlordiazepoxide could be determined directly between 240 and 350 nm. However, it is not possible to determine clidinium bromide directly in mixtures, because its spectrum is totally overlapped by that of chlordiazepoxide (Fig. 3). In order to carry out simultaneous determinations of multi-

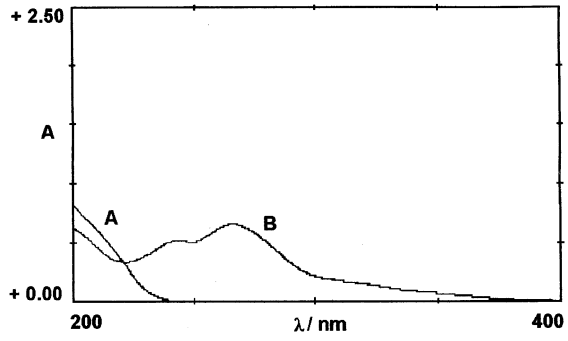

Fig. 3. Spectra of chlordiazepoxide and clidinium bromide dissolved in acetonitrile measured against acetonitrile. (A) Clidinium bromide, 8.6 mg/l; (B) chlordiazepoxide, 6.0 mg/l.

components when the spectra overlap, there are some chemiometric approaches which have been successfully applied to spectrophotometric signals. Derivative spectrophotometry and multiwavelength evaluation methods are well-know examples of these types of approaches. The latter case has been proposed in the resolution of mixtures of PAHs (Rossi and Pardue, 1985), which present some problems when separated by chromatography.

In this work, we adopted derivative spectrophotometry for resolution of analyte bands, because this approach is simple and it does not require many data mathematical processes.

3.3. Selection of spectral variables

3.3.1. Derivative order

To choose the optimum derivative order, the first, second, third and fourth derivative spectra of the solution containing separately the respective analytes were recorded (Fig. 4a–d).

As can be seen in Fig. 4a–d, the second, third and fourth derivatives are more resolved, but these also have more noise than the first deriva-

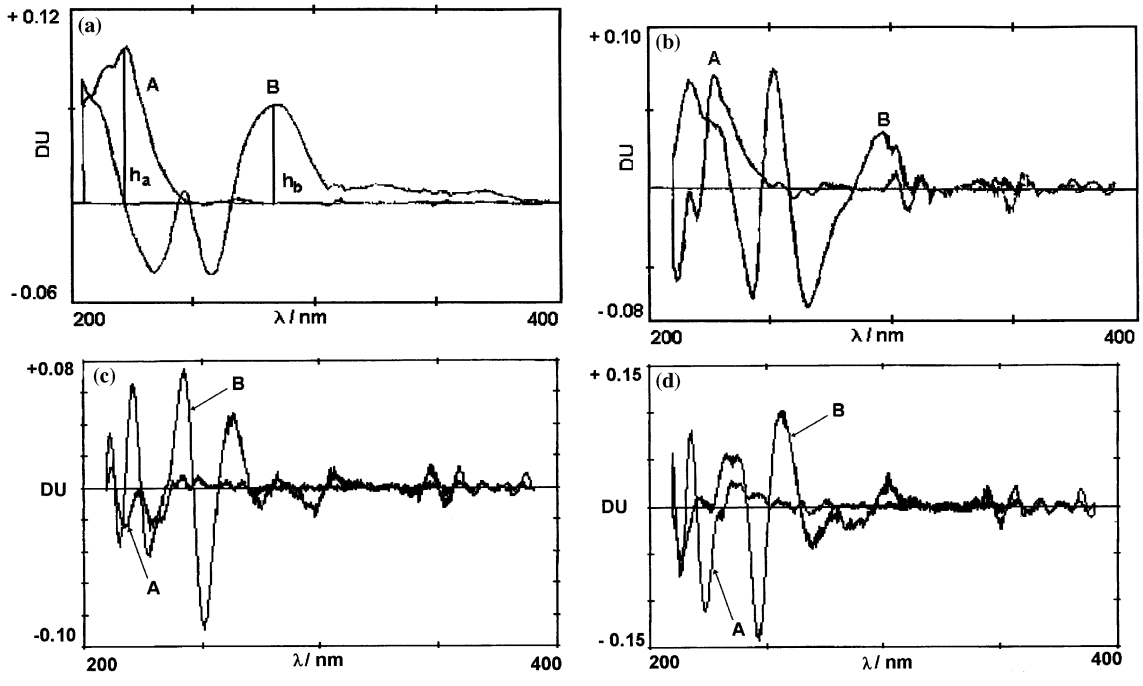

Fig. 4. (a) First derivative spectra of chlordiazepoxide and clidinium bromide dissolved in acetonitrile measured against acetonitrile. (A) Clidinium bromide, 8.6 mg/l; (B) chlordiazepoxide, 6.0 mg/l. (b) Second derivative spectra of chlordiazepoxide and clidinium bromide dissolved in acetonitrile measured against acetonitrile. (A) Clidinium bromide, 8.6 mg/l; (B) chlordiazepoxide, 6.0 mg/l. (c) Third derivative spectra of chlordiazepoxide and clidinium bromide dissolved in acetonitrile measured against acetonitrile. (A) Clidinium bromide, 8.6 mg/l; (B) chlordiazepoxide, 6.0 mg/l. (d) Fourth derivative spectra of chlordiazepoxide and clidinium bromide dissolved in acetonitrile measured against acetonitrile. (A) Clidinium bromide, 8.6 mg/l; (B) chlordiazepoxide, 6.0 mg/l.

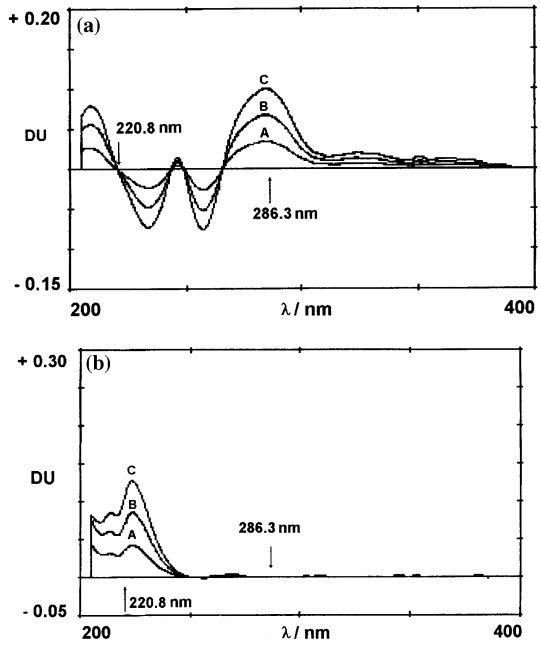

Fig. 5. (a) Effect of chlordiazepoxide over the first derivative spectra: (A) 1.0×10^{-5} mg/l; (B) 2.0×10^{-5} mg/l; (C) 3.0×10^{-5} mg/l. (b) Effect of the clidinium bromide concentration over the first derivative spectra: (A) 1.0×10^{-5} mg/l; (B) 2.0×10^{-5} mg/l; (C) 3.0×10^{-5}.

tives. In this context, the first derivative was selected in the simultaneous determination of these compounds, because the ratio signal/noise is higher.

3.3.2. Selection of the analytical wavelengths

The selection of the analytical wavelengths was carried out taking into account the first derivative spectra of both analytes separately (Fig. 4a). Fig. 5a shows that the graphical method (O'Haver, 1982) at 283.6 nm can be used for determination of chlordiazepoxide and Fig. 5b shows that the zero crossing method (O'Haver, 1982) can be used for the determination of clidinium bromide at 220.8 nm. The selection of the analytical wavelengths was confirmed by recording a series of the first derivative spectra of acetonitrile solution containing chlordiazepoxide and clidinium bromide separately, between a concentration range of 1.0×10^{-5} and 3.0×10^{-5} M of each analyte. Fig. 4a shows that at 286.3 nm, the distance, h_B is only proportional to the concentration of chlordiazepoxide. In contrast the clidinium bromide does not absorb in this wavelength (Fig. 5b). Similarly, the measurement of the derivative spectrum at an abscissa value of 220.8 nm (h_A), corresponding to the zero-crossing point of the derivative spectrum of chlordiazepoxide, can be satisfactory to determine clidinium bromide (Fig. 4a) without interference of chlordiazepoxide.

3.3.3. Selection of $\Delta\lambda$ value

In order to select the $\Delta\lambda$ value for differentiation, a series of first derivative spectra of a mixture of 6.0 mg/l chlordiazepoxide with increasing concentration of clidinium bromide ranging from 2.16 to 15.13 mg/l, were evaluated under the selected conditions, at different $\Delta\lambda$ values. In all cases good linearly was obtained (Table 1).

The sensitivity increase was proportional to the increase in $\Delta\lambda$, between 1.6 and 6.4 nm. However in the selection of $\Delta\lambda$ it is also necessary to study the effect of the presence the clidinium bromide on the chlordiazepoxide signal (Fig. 6a). Simi-

Table 1
Effect of $\Delta\lambda$ over the sensitivities

Analyte	Sensitivity	Intercept	R	$\Delta\lambda$/nm
Chlordiazepoxide	0.005	0.0007	0.999	1.6
	0.010	0.0014	0.999	3.2
	0.015	0.0012	0.999	4.8
	0.020	0.0017	0.999	6.4
Clidinium bromide	0.004	0.002	0.997	1.6
	0.008	0.003	0.997	3.2
	0.012	0.004	0.996	4.8
	0.016	0.003	0.997	6.4

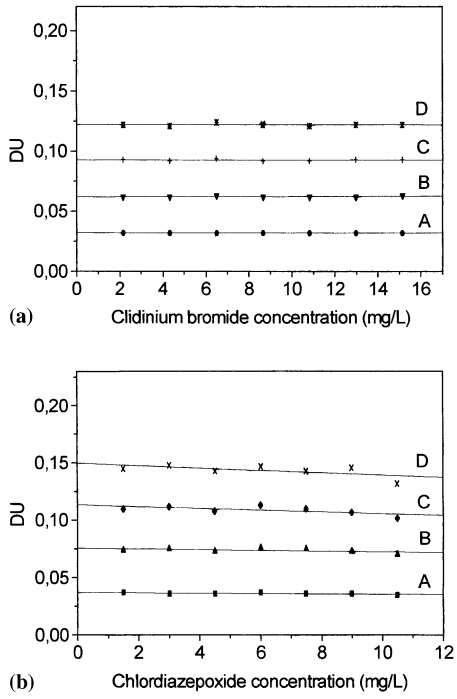

Fig. 6. (a) Effect of concentrations of clidinium bromide over the signals to analytical wavelength of 6.0 mg/l chlordiazepoxide at different $\Delta\lambda$ values. (A) $\Delta\lambda = 1.6$ nm; (B) $\Delta\lambda = 3.2$ nm; (C) $\Delta\lambda = 4.8$ nm; (D) $\Delta\lambda = 6.4$ nm. (b) Effect of concentrations of chlordiazepoxide over the signals to analytical wavelength of 8.6 mg/l clidinium bromide at different $\Delta\lambda$ values. (A) $\Delta\lambda = 1.6$ nm; (B) $\Delta\lambda = 3.2$ nm; (C) $\Delta\lambda = 4.8$ nm; (D) $\Delta\lambda = 6.4$ nm.

larly, a series of first derivative spectra of 8.65 mg/l clidinium bromide with increasing concentrations of chlordiazepoxide ranging from 1.5 to 10.49 mg/l were evaluated under the selected conditions, at different $\Delta\lambda$ values.

The sensitivities at different $\Delta\lambda$ are show in Table 1 and the effect of chlordiazepoxide concentration over signals of clidinium bromide at different $\Delta\lambda$ values are show in Fig. 6b. For clidinium bromide, the sensitivity increase is also proportional to the increase in $\Delta\lambda$, in same range mentioned before.

According to Fig. 6a,b when $\Delta\lambda$ is equal to 1.6 and 3.2 nm, in both cases the signals of the analytes that remain constant are not affected when the concentration of the variable analyte increases. When $\Delta\lambda$ value equal to 3.2 nm was selected, the sensitivity increased but the accuracy and the precision were not affected, because under these conditions there was no interference between the analytes.

3.4. Analytical features

Under the working conditions stated, a linear relation was observed between corresponding derivative units and the respective clidinium bromide and chlordiazepoxide concentrations. The calibration graphs ($n = 8$) were obtained by plotting the first-derivative value h_A for clidinium bromide at 220.8 nm and h_B for chlordiazepoxide at 283.6 nm using a $\Delta\lambda = 3.2$ nm value for differentiation, versus the analyte concentrations. The equations of the regression line obtained were:

clidinium bromide: $h_A = 0.008 \times C(\text{mg/l}) + 0.003 \; r = 0.997$ (1)

chlordiazepoxide: $h_B = 0.010 \times C(\text{mg/l}) + 0.0014 \; r = 0.999$ (2)

where h is in derivative units and C corresponds to analyte concentration in mg/l.

The determination ranges were found to be 0.983–21.62 mg/l for clidinium bromide and 0.740–12.0 mg/l for chlordiazepoxide. The detection limits (calculated by using the 3σ recommendation) were found to be 0.295 mg/l for clidinium bromide and 0.222 mg/l for chlordiazepoxide.

The repeatability of the determination expressed as the relative standard deviation of 11 replicates of solutions containing 8.6 mg/l clidinium bromide and 6.0 mg/l chlordiazepoxide, and the relative standard deviations were 2.4 and 1.3%, respectively.

In order to establish the ratios at which one substance can be accurately measured one in presence of the other, a study of the recoveries of compound was carried out in standard mixtures of clidinium bromide and chlordiazepoxide at different ratios. The results (Table 2) show that the content of each compound can be reliably determined in mixtures at different molar ratios, using this first derivative spectrophotometric method.

According to the results obtained, the simultaneous determination of both compounds in

Table 2
Determination of clidinium bromide and chlordiazepoxide in different standard mixtures

Ratio I:II	Stated concentration per mg/l		Found concentration per mg/l[a]	
	I	II	I	II
3:1	6.00	2.00	6.10 ± 0.2	2.06 ± 0.3
1:2	2.00	4.00	2.00 ± 0.2	3.97 ± 0.2
1:2	4.00	8.00	3.96 ± 0.3	7.95 ± 0.4
1:4	2.00	8.00	1.97 ± 0.4	7.93 ± 0.3
1:5	2.00	10.00	1.95 ± 0.3	9.86 ± 0.4

[a] Mean of ten determinations.

Table 3
Simultaneous determination of chlordiazepoxide and clidinium bromide by first derivative spectrophotometry

Dosage form	Amount found per mg	
	Chlordiazepoxide	Clidinium bromide
Tensoliv (Laboratorio Chile)	4.90 ± 0.09	2.46 ± 0.05
Libraxim (Roche)	4.93 ± 0.10	2.42 ± 0.04

tablets was possible, taking into account that the usual ratio of clidinium bromide and chlordiazepoxide in commercial formulations is 1:2.

3.5. Application

The accuracy of the method was tested for both drugs by analysis of synthetic formulations. Samples of 5.0 mg of chlordiazepoxide and 2.5 mg of clidinium bromide were accurately weighed and mixed with 96.1 mg of excipients. The excipients in the synthetic mixtures approximately contained magnesium stearate + gelatine 3–5% and lactose-starch 95%. The recoveries were found to be $97.8 \pm 0.5\%$ and $101.0 \pm 0.5\%$ for chlordiazepoxide and clidinium bromide, indicating that the common excipients do not interfere. The method was applied to different tablet samples (Tensoliv, Libraxin, Lerogin) in which the nominal contents of all the dosage forms are 5 mg chlordiazepoxide and 2.5 mg of clidinium bromide. The amounts found for these different samples are shown in Table 3.

4. Conclusion

The structures of chlordiazepoxide and clidinium bromide are quite different. Because of this, the spectral behavior of both compounds can be expected to be quite different. In this work, after a study of the spectral behavior of chlordiazepoxide and clidinium bromide it was possible to select a solvent in which the ratio of the molar absorptivities for both compounds was near to one. In these conditions the simultaneous determination is more effective and it is possible to obtain accurate results.

In addition, an accurate, precise, direct, and inexpensive method by first derivative spectrophotometry has been developed for the simultaneous determination of clidinium bromide and chlordiazepoxide in pharmaceutical formulations. The ingredients commonly found in commercial pharmaceutical formulations do not interfere. The proposed method is a good alternative for the simultaneous determination of both drugs in pharmaceutical formulations.

Acknowledgements

The authors are grateful to the National Fund for Development of Sciences and Technology (FONDECYT), project 1961024, for financial support and Laboratory Chile for supply of the drugs.

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