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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 811–818

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Simultaneous spectrophotometric determination of phenilpropanolamine HCL, caffeine and diazepam in tablets

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Received 11 March 2002; received in revised form 25 March 2002; accepted 30 March 2002

Abstract

A rapid, reliable and specific UV spectrophotometric method was developed to determine Phenilpropanolamine Hydrochloride (**I**), Caffeine (**II**) and Diazepam (**III**) formulated in tablets. This method was validated and compared with a liquid chromatography (LC) procedure used for the simultaneous quantitative analysis of the drugs. The established linearity ranges by both methods for compounds **I**, **II** and **III** were 0.36–0.88, 0.012–0.028 and 0.036–0.084 mg/ml, respectively. The correlation coefficients by HPLC were $r^2_{\mathbf{I}} = 0.997$, $r^2_{\mathbf{II}} = 0.999$, $r^2_{\mathbf{III}} = 0.999$ and by the UV spectrophotometric method were $r^2_{\mathbf{I}} = 0.998$, $r^2_{\mathbf{II}} = 0.996$, $r^2_{\mathbf{III}} = 0.999$. LC and UV methods showed excellent precision and accuracy. As regards precision, LC showed CV values range of 0.2–0.9 and UV 0.15–0.72. On the other hand, accuracy was obtained with CV values range of 0.1–1.8 and 0.32–1.11 for LC and UV, respectively. The recoveries of **I**, **II** and **III** were $> 98.04\%$ for both methods over the linear range. The UV and HPLC methods have been successfully used to determine the **I**, **II** and **III** content in tablets of different origin.

Keywords: Analysis; HPLC; Spectrophometric; Phenilpropanolamine hydrochloride; Caffeine; Diazepam

1. Introduction

A commercial product as appetite suppressants contain phenilpropanolamine hydrochloride (**I**), caffeine (**II**) and diazepam (**III**). Compound **I** is used as an anorectic agent in the management of obesity, **II** [3,7-dihydro-1,3,7-trimethyl-1 H-purine-2,6-dione] is used as a central nervous stimulant and **III** [7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one] is used as a tranquillizer.

This pharmaceutical preparation is widely used in the Province of Córdoba (Argentina) and no spectrophotometric method has been described in literature for the determination of **I**, **II** and **III** in tablets. One of the basic requirements for the effective quality control is adequate and validated test procedure. Since spectrophometric procedures [1] are less time consuming, less expensive and require less operational training than the HPLC [2,3] we consider it important to develop a UV spectrophometric method and its comparison with

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00130-9

an RP-HPLC method recently described by our research group [4]. This new analytical procedure was able to accurately quantify the three active principles with a previous extraction procedure since the direct measurement of its first and second derivative did not resolve the combination of active principles.

Both methods were compared statistically and were validated in compliance with the analytical performance parameters [5–8].

2. Experimental

2.1. Instrumentation

The absorbance of the standard spectra and the first and second derivatives of absorbance of **I**, **II** and **III** were recorded over the wavelength range 200–350 nm in order to identify absorption maximums and the overlapping that might occur among the drugs. For this purpose, a 1 cm quartz cells and a Shimadzu UV–VIS spectrophotometer model UV 260 were used to obtain the spectral curves.

The HPLC analysis was performed on a Konik 500 G liquid chromatograph with an UV spectrophotometric detector of variable wavelength UVIS-204 that it was set at 254 nm.

Water was prepared daily by reversed osmosis and deionization using a Milli Rho Milli-Q System and it was used in mobile phase and sample preparations.

All analytical weighings were performed with a Voyager balance (OHAUS) and an Electrobalance model G.

2.2. Materials

The compounds **I**, **II** and **III** were purified following the previous procedure [7,9–11]. All the other chemicals, reagents, and solvents used were of analytical grade.

Acetonitrile of HPLC-grade was supplied by J.T. Baker (Phillipsburg, USA).

The tablets were commercially obtained, from Tratobes RS, manufactured in Argentina by Disprovent SA (batch 795) containing 50 mg of **I** and 50 mg of **II** per tablet. The reference tablets were prepared in our laboratory and the common tablet excipients were obtained from the local market. Tablets prepared in Pharmacy Office were A Tablets and B Tablets. These together with Reference Tablets containing 100 mg of **I**, 50 mg of **II** and 5 mg of **III** per tablet, were analyzed to determine their precise components.

All the studied tablets contained lactose, magnesium stearate and carboximethylcellulose sodium as excipients.

2.3. Test solutions, standard solutions and calibration graph

2.3.1. Sample preparation

A total of 10 tablets containing **I**, **II** and **III** as the active ingredient were weighed and finely powdered. A portion of the powder equivalent to 6.0 mg **I**, 3.0 mg **II** and 0.3 mg **III** were accurately weighed, transferred to a flask and suspended in 5 ml of petroleum ether; then, the flask was placed in ultrasonic water bath. The insoluble product which corresponded to a combination of **I** and **II** was filtered, and in the organic phase the active principle **III** was separated (test solution of III). The solid mixture of **I** and **II** was suspended in 25 ml of chloroform; the insoluble material corresponding to **I**, was dissolved in 10 ml of water (test solution of **I**) and the chloroformic layer permitted the separation of the drug **II** (test solution of **II**). Different volumes of the test solutions obtained from the development of the extraction were accurately transferred to volumetric flasks and the volume was completed with the same extraction solvent. These solutions were later analyzed by UV spectrophotometry at 256 nm for **I**, 275 nm for **II** and 315 nm for **III**.

For HPLC analysis, the procedure was the same as recently described by our research group [4].

2.3.2. Spectrophotometric procedure

A stock solution was prepared for each compound **I**, **II** and **III** dissolving them in water, chloroform and petroleum ether, respectively, to obtain concentrations of 0.40, 0.30 and 0.49 mg/ml, respectively.

The standard solutions for UV spectra, were prepared by dilution of the stock solution in the extraction solvent to reach concentration ranges of 0.36–0.84, 0.012–0.028 and 0.036–0.084 mg/ml for compounds **I**, **II** and **III**, respectively. The curves of the working standard solutions were scanned in the range of 200–350 nm against water, chloroform and petroleum ether as a blank by **I**, **II** and **III**, respectively.

The pure compounds **I**, **II** and **III** demonstrated to be stable in the extraction solvents during the spectrophotometric analysis.

2.4. Methods

2.4.1. UV method for content uniformity

Individual tablets were pulverized and the compounds **I**, **II** and **III** were extracted of each pharmaceutical preparation according to the extraction method previously described (Section 2.3.1). An aliquot of each solution was filtered and transferred to 5 ml volumetric flasks using the same extraction solvent to adjust the final volume and then were analyzed for quantitation by the spectrophothometric procedure.

Ten replicate commercial tablets were analyzed for statistical evaluation of the assay.

3. Results and discussion

3.1. Ultraviolet spectrophotometry

Standard solutions of **I**, **II**, **III** in distilled water were run individually on the UV spectrophotometer and gave the absorption (zero-order) UV spectras shown in Fig. 1 (curve *a* for compound **I**, curve *b* for compound **II** and curve *c* for compound **III**). As it can be seen, there is a significant interference in the conventional spectrophotometric determination, as indicated by the overlapping that appears in the spectral bands of **I**, **II** and **III**. Due to the overlapping spectra of these compounds, normal UV spectroscopy cannot be used for simultaneous quantitation if three compounds are present.

However, we think that the application of the derivative spectrophotometric technique will allow the simultaneous determination of three active principles.

Figs. 2 and 3 show the first and second order spectra of **I**, **II** and **III** standard solutions. The first-derivative UV spectra of these three compounds are shown in Fig. 2. Sharp bands of large amplitudes (compound **I** curve *a* and compound **II** curve *b*) are produced, however, because of the overlap of the spectral bands of the two compounds with spectra of compound **III**, first-derivative UV spectrophotometry cannot be used for their determination in mixtures. As it can be seen (Fig. 3), there was a considerable overlapping between the spectra of compound **I** (curve *a*) and the spectra of compounds **II** (curve *b*) and **III** (curve *c*). The relation of concentrations used for the simultaneous determination of components in the mixture is critical since this relation is the same that would be found in formulations.

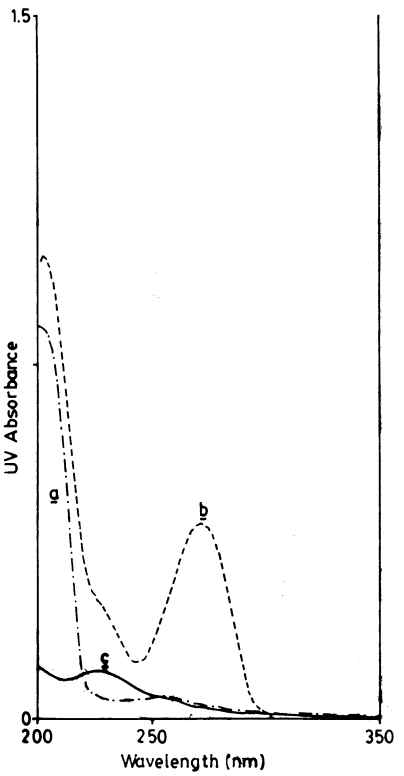

Fig. 1. Absorption spectra of (a) phenilpropanolamine (0.02 mg/ml); (b) caffeine (0.01 mg/ml) and (c) diazepam (0.001 mg/ml) in distilled water.

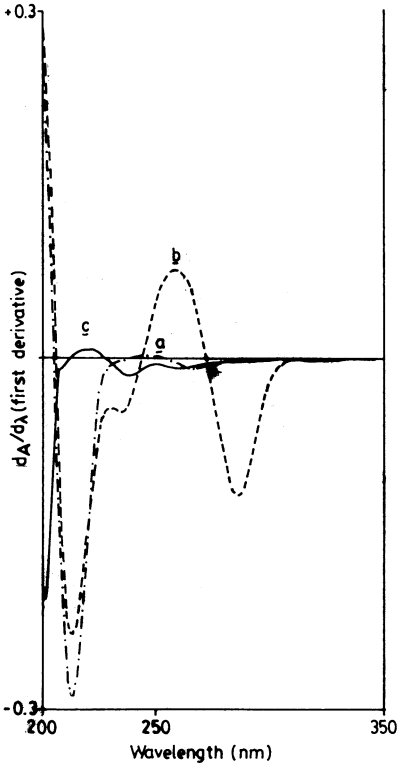

Fig. 2. First derivative UV scan of Compound **I** curve *a* (…), Compound **II** curve *b* (_ _) and Compound **III** curve *c* (—).

The derivative transformation of spectral data has been proved not to be a valuable procedure for the simultaneous quantitation of **I**, **II** and **III**. Unfortunately, neither the first nor the second derivative spectra were suitable to accurately quantify the three active principles from dosage forms of this kind. For this reason, it was necessary the separation of the three compounds being the simples assay method the direct measurement of the UV absorption at the maximum of each derivative.

3.2. Method validation

For the spectrophotometric procedure, standard curves were determined for each of the components separately. The linearity of response of the standard solutions was assessed using six solutions by varying the analyte concentration over range of 60–140% of the nominal working concentration. In all cases, plots of absorbance versus concentration, at 256 nm for **I**, 275 nm for **II** and 315 nm for **III** were linear in the concentration ranges examined, showing adherence to Beer's Law. Linear regression analysis of the responses (y) on the theoretical concentration (x) gave the following equations: $y = 0.931x - 0.014$, $y = 53.7x - 0.574$ and $y = 5.58x - 0.034$ for **I**, **II** and **III**, respectively. The determination coefficients, $r^2_{\mathbf{I}} = 0.998$, $r^2_{\mathbf{II}} = 0.996$, $r^2_{\mathbf{III}} = 0.999$ confirmed the linearity of methods over the concentration range analyzed.

The sensitivity of the method was calculated for each derivative as the slope of the calibration line. The results obtained, expressed as mg/ml, were 0.931, 53.7 and 5.58 for **I**, **II** and **III**, respectively.

The limit of detection (LOD) and the limit of quantification (LOQ) of **I**, **II** and **III** were calculated in accordance with the International Conference on Harmonization (ICH) Guideline [8].

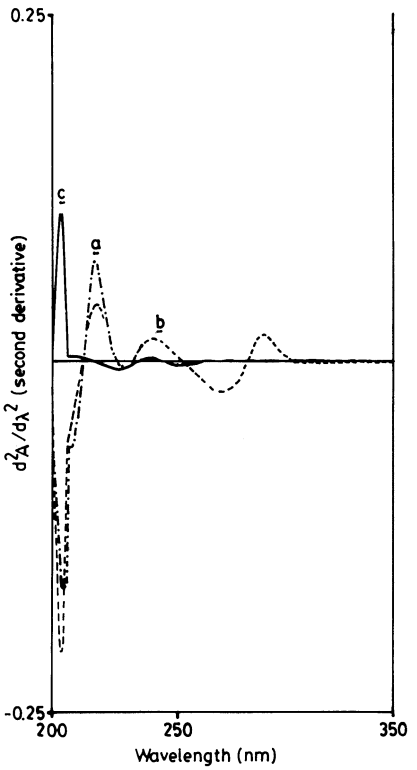

Fig. 3. Second derivative of absorbance of Compound **I** curve *a* (…), Compound **II** curve *b* (_ _) and Compound **III** curve *c* (—).

Table 1
Accuracy of spectrophotometric method determined by the recovery of **I**, **II** and **III**

Theoretical Concentration μg/ml $\times 10^{-1}$			Recovery (%)[a] ± CV		
Compounds					
I	**II**	**III**	**I**	**II**	**III**
36.00	1.48	3.60	98.04 ± 0.66	98.3 ± 2.2	99.1 ± 1.1
44.00	1.68	4.40	99.42 ± 0.67	98.2 ± 2.2	98.08 ± 0.49
68.00	2.24	6.72	98.62 ± 0.32	102.50 ± 0.79	101.07 ± 0.51
76.00	2.52	7.56	98.36 ± 0.39	102.60 ± 0.62	99.68 ± 0.80
Average			98.61	110.4	99.5
R.S.D. (%)			0.51	2.2	1.1

[a] Mean for five determinations.

The LOD and LOQ for the compound **I** was found to be 0.049 and 0.16 mg/ml, respectively. The values of LOD and LOQ for the compound **II** were 1.86×10^{-4} and 8.4×10^{-4} mg/ml, respectively. The results obtained for the compound **III** were 3.08×10^{-3} and 10×10^{-3} mg/ml for the LOD and LOQ, respectively.

The accuracy of the method was determined by investigating the recovery of active principles at four levels ranging from 60 to 140% of the amount of **I**, **II** and **III** in the dosage form. The results are shown in Table 1; which indicate excellent recoveries ranging from 98.04 to 102.60%. The average recovery for the four levels were 98.61% (R.S.D. = 0.51%), 100.4% (R.S.D. = 2.2%) and 99.5% (R.S.D. = 1.1%) for compounds **I**, **II** and **III**, respectively.

The measurement precision was determined by calculating the R.S.D. (CV) for five solutions at two levels of concentration of each active principle.

The results obtained, expressed as μg/ml ± CV, for compound **I** were 44.26 ± 0.70 and 74.69 ± 0.39 for theoretical concentrations of 44.00 and 76.00, respectively. The precision obtained for the compound **II** was 1.62 ± 0.31 and 2.59 ± 0.15 and the theoretical concentrations were 1.68 and 2.52, respectively. The precision of the method for the compound **III** to the following theoretical concentrations, 3.60 and 6.72 were 3.54 ± 0.72 and 6.83 ± 0.35, respectively.

The system suitability test was assessed within short intervals of time at two concentrations and the results, which were in all cases below 0.95%, were expressed as R.S.D. (CV). The assayed values are presented in Table 2.

3.3. Ultraviolet spectrophotometry for content uniformity

The optimized UV method was used to carry out the content uniformity test, which is considered as the most important quality control for individual doses in solid dosage forms.

The results of content uniformity test for three dosage forms of different origin were determined by UV at maximum absorption wavelength of each compound and are given in Table 3. The UV means values of ten separate tablets were 134 ± 18% for **I** and 119.5 ± 4.2% for **II** in Tratobes RS

Table 2
System suitability results

Compound	Theoretical concentration[a] (μg/ml $\times 10^{-1}$)	Calculated concentration[a] ± CV (μg/ml $\times 10^{-1}$)
I	44.00	44.14 ± 0.99
	76.00	74.96 ± 0.61
II	1.68	1.61 ± 0.94
	2.52	2.60 ± 0.59
III	3.60	3.98 ± 0.39
	6.72	6.77 ± 0.26

[a] Mean for eight determinations.

Table 3
The results of content uniformity by spectrophotometric method

Sample	Percent label claim [a] ± CV							
	Tablet number							
	Tratobes RS[b]		A Tablets			B Tablets		
	I	**II**	**I**	**II**	**III**	**I**	**II**	**III**
1	107.4 ± 6.0	115.6 ± 4.6	103.9 ± 1.3	105.0 ± 8.7	106.8 ± 7.6	94.9 ± 7.7	113.6 ± 3.0	104.0 ± 0.5
2	114.6 ± 6.4	112.8 ± 6.4	105.2 ± 1.5	104.2 ± 6.2	100.0 ± 8.1	90.8 ± 9.1	116.0 ± 0.5	116.0 ± 4.2
3	118.2 ± 4.8	115.4 ± 5.5	105.6 ± 2.3	99.2 ± 1.6	101.4 ± 4.4	100.4 ± 3.1	122.8 ± 2.0	125.8 ± 2.5
4	148.4 ± 1.6	118.4 ± 5.4	104.8 ± 7.2	104.8 ± 5.9	102.4 ± 5.3	104.9 ± 8.2	150.8 ± 2.4	107.2 ± 5.1
5	159.2 ± 6.1	130.6 ± 3.5	109.1 ± 4.1	106.2 ± 8.5	99.6 ± 4.1	102.2 ± 8.1	158.6 ± 1.1	143.2 ± 4.0
6	109.8 ± 5.4	123.4 ± 4.8	103.9 ± 4.1	109.4 ± 7.7	132.6 ± 2.6	96.3 ± 6.3	173.8 ± 5.2	161.0 ± 0.2
7	150.8 ± 7.4	117.6 ± 6.6	113.7 ± 4.7	120.0 ± 8.8	118.8 ± 9.9	106.7 ± 5.0	174.4 ± 5.8	92.4 ± 0.7
8	182.0 ± 4.5	118.4 ± 5.9	116.1 ± 7.3	114.6 ± 6.5	132.2 ± 5.5	91.7 ± 4.4	124.0 ± 4.5	116.6 ± 6.7
9	109.8 ± 9.2	118.4 ± 5.5	122.6 ± 5.4	110.2 ± 1.9	130.8 ± 3.5	91.7 ± 1.1	146.4 ± 8.7	120.0 ± 2.0
10	143.4 ± 9.1	124.4 ± 5.4	119.3 ± 4.1	103.6 ± 2.2	151.8 ± 5.2	108.5 ± 7.4	139.6 ± 4.2	116.6 ± 3.2
Average	134	119.5	110.4	106.9	117	98.8	142	120
CV	18	4.2	6.0	4.1	15	6.4	15	15

[a] Mean for three determinations.
[b] Tablets do not contain compound **III**.

tablets that do not contain the compound **III**. The means values obtained for the active principles **I**, **II** and **III** in A Tablets were 110.4 ± 6.0, 106.9 ± 4.1 and 117 ± 15%, respectively. In the same order, the determined means and R.S.D. in B Tablets were 98.8 ± 6.4, 142 ± 15 and 120 ± 15. A significative fluctuation was observed in the doses of **I**, **II** and **III** in A tablets, B tablets and Tratobes RS tablets. As it can be seen these results exceed the permissible limits given by the pharmacopoeias consulted [7]. This variability may be due to the processes involved in the manufacturing or the small amount of drug available at the time of preparing the tablets, which made exact dosage difficult to achieve.

3.4. Comparison of results by HPLC and UV methods

The amount of each component in ten individual tablets was determined by using the spectrophotometer method and by comparing the results obtained using an HPLC method developed and validated in our laboratory [4]. The assayed formulation tablets contained excipients which did not interfere with the UV and HPLC analysis. Therefore, it was not necessary to run a placebo formulation along with the standard. Table 4 compares the average recovery from the label claim amount by the HPLC and the proposed UV method. The percentage recovery was 117.8 and 113.6 for the UV and the HPLC methods by compound **I**, respectively, and their coefficients of variation were 4.5 and 4.1, respectively, in tablets of Tratobes RS labeled to contain 50 mg of this compound. The results obtained for Compound **II** in A tablets were 149.1 and 143.1 for the UV and the HPLC methods, respectively, and their coefficients of variation were 0.8 and 1.1, respectively. These results are not satisfactory with the label claim and indicate that there was no significant difference between the methods compared.

The reference tablets containing **I**, **II**, **III** and excipients were prepared and subjected to the described UV and HPLC assays to determine the content of active principles. The results obtained for reference tablets and B tablets were satisfactory for the three drugs, indicating a good agreement with the label claims.

Table 4
Recovery of **I**, **II** and **III** from commercial tables and reference tablets by UV and HPLC methods

Dosage form	Active ingredients mean found ± CV[a]					
	UV			HPLC		
	I	**II**	**III**	**I**	**II**	**III**
Tratobes RS[b] (Disprovent SA)	117.8 ± 4.5	98.8 ± 1.2	–	113.6 ± 4.1	107.6 ± 1.4	–
A Tablets	99.2 ± 2.6	149.1 ± 0.8	92.8 ± 0.3	96.3 ± 1.1	143.1 ± 1.1	94.6 ± 2.9
B Tablets	107.3 ± 2.0	101.2 ± 1.1	107.4 ± 1.6	105.2 ± 1.8	106.4 ± 1.7	100.0 ± 2.4
Reference tablets	103.9 ± 2.7	104.3 ± 2.8	100.8 ± 0.4	98.6 ± 4.5	108.6 ± 0.5	107.8 ± 1.6

[a] Mean and CV for five determinations, percentage recovery from the label claim amount.
[b] Tablets do not contain compound **III**.

The analytical results confirm that UV and HPLC methods offer accuracy and precision when applied to tablets.

From these data, it can be deduced that the developed spectrophotometer procedure is suitable for the routine analysis of **I**, **II** and **III** in multicomponents tablets. In stability studies, in which these active ingredients may exit with other decomposition or related substances, the preferred method would be the HPLC.

4. Conclusions

Comparative study of methods for the analysis of multicomponent tablets formulation containing phenilpropanolamine hydrochloride (**I**), caffeine (**II**) and diazepam (**III**) is presented.

These commercially available tablets were analyzed using a spectrophotometric method and a HPLC method that was chosen as the analytical reference method. The intent of this study was to provide basic experimental results for two simple test procedures for the assay of **I**, **II** and **III** in solid dosage forms.

The described spectrophometric method was found to be linear, reproducible, accurate and capable of quantifying **I**, **II** and **III** in tablets, but required an extraction procedure. This spectrophotometric method has many advantages over multicomponent analysis by HPLC. It is fast, inexpensive and easy to perform, without sacrificing accuracy.

Finally no significant differences were found among the results obtained by UV and HPLC for the same batch consequently we consider that UV method can be used as an excellent alternative to HPLC for the routine analysis.

Acknowledgements

This work was partly supported by Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT).

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