ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 493–499

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Analysis of multicomponent formulations containing phenylpropanolamine hydrochloride, caffeine and diazepam by using LC

Carola Ferreyra, Cristina Ortiz *

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina

Received 8 July 2000; received in revised form 18 October 2000; accepted 27 October 2000

Abstract

A reverse phase high performance liquid chromatography assay was carried out for the simultaneous determination of three active principles present in tablets of different origin and wide commercial use in the Province of Córdoba (Argentina). Prescriptions, commercially available as appetite suppressants, very often include the active principles Phenylpropanolamine Hydrochloride (**I**), Caffeine (**II**) and Diazepam (**III**). Simultaneous determination of these three drugs: anorexic, central nervous stimulant and tranquilizer, respectively, in pharmaceutical dosage forms has not been reported. In this study these active principles are quantified. The only sample preparation necessary for the analysis was their dilution with acetonitrile. The resulting solution was filtered and analyzed on a column packed with Supelcosil LC-18 (5 μm) with acetonitrile:water (30:70 v/v) as initial mobile phase (0.4 ml min^{-1}) and the detection was performed at 254 nm. Then a linear gradient up to 100% acetonitrile in 18 min (3.0 ml min^{-1}) was applied. The procedure was simple and suitable for quality control. The calibration function was established in the ranges of 0.072–0.168 mg ml^{-1} for **I**, 0.036–0.084 mg ml^{-1} for **II** and 0.06–0.196 mg ml^{-1} for **III**. The detection limits of these compounds were 12.8, 4.1 and 11.0 μg ml^{-1}, respectively with linear response. No chromatographic interference from the tablet excipients was found. The method described in this paper was validated following the analytical performance parameters required by the USP XXIV, and was successfully applied to the commercial tablets.

Keywords: High performance liquid chromatography; Multicomponent formulations; Validation; Analysis quantitative

1. Introduction

From observation in the local market, it has been found that in recent years multicomponent dosage forms have been widely adopted and most of them are composed of numerous chemical constituents. The analysis of these pharmaceutical preparations is of special interest due to their intensive use in the Province of Córdoba (Argentina), to their pharmacological action and the high power of their active ingredients.

* Corresponding author. Tel.: +54-351-4334163; fax: +54-351-4334127.
E-mail address: crisar@dqo.fcq.unc.edu.ar (C. Ortiz).

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00599-9

Because of these reasons it was found necessary to develop a rapid and simple reverse phase high performance liquid chromatographic (HPLC) method for simultaneous determination of Phenylpropanolamine Hydrochloride (**I**), Caffeine (**II**) and Diazepam (**III**).

The pharmaceutical preparation of these active principles has not been described in the literature and a proper quality control that ensures the quality, purity and efficacy of drugs prescribed to the pacient is missing. These two facts in turn imply a potential risk for the health of the population.

The main objective of this research was then to develop and test the applicability of an analytical technique to the study of tablets prepared in the Pharmaceutical Industry, the Pharmacy Office and in our Laboratory [1–3].

For the validation process the following parameters were characterized: selectivity; linearity and range; limit of detection and quantitation; repeatability; percent recoveries; precision and reproducibility [4].

The results demonstrated that there are marked quantitative differences between the commercial products studied.

2. Experimental

2.1. Equipment

The chromatography was carried out using a Konik 500 G instrument equipped with an UV spectrophotometric detector of variable wavelength UVIS-204 operating at 254 nm during all the analyses with a sensitivity of 0.02 absorbance units full scale, and an injector Rheodyne model 7125.

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

A pH meter (Orion Model SA 520) fitted with combination electrodes was used for all pH measurements. The pH meter was standardized with the combination of standard buffer solutions at room temperature.

Melting points were determined on a Büchi 510 melting point apparatus and were uncorrected.

The IR spectra were recorded from potassium bromide discs with a FT-IR Spectrophotometer Bruker Model IFS 28.

2.2. Materials

Standards of **I**, **II** and **III** were prepared and their purity was checked and found to be 99.53, 99.76 and 99.81%, respectively [4–6].

HPLC grade acetonitrile was purchased from J.T. Baker (Phillipsburg, USA). Water was prepared daily by reverse osmosis and de-ionization using a Milli Rho Milli-Q System and Schleicher & Schuell filters were used in mobile phase and sample preparations.

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

2.3. Chromatographic system

A good deal of preliminary assays including changes in mobile phase composition and flow-rate were performed to determine the optimum analytical conditions [7].

All three compounds (**I**, **II** and **III**) were separated in less than 18 min with a Supelcosil LC-18, 5 μm (250 × 4.6 mm) analytical column operated at 0.4 and 3.0 ml min^{-1} flow which produced a maximum column back pressure of 230 atm. Chromatographic characteristics of **I**, **II** and **III** are shown in Table 1. Separations were performed at ambient temperature and chart speed 0.25 cm min^{-1}. The injection volume was 20 μl in all cases and a re-equilibration period of 3 min was used between each run.

The hold-up time (t_o) was determined by injecting acetonitrile and measuring the elution time of the disturbance peak. Quantitation was based on the peak area measurement.

2.4. Mobile phase

Initial experiments were carried out using mobile phases of water and acetonitrile in different proportions. The optimal condition selected was a linear gradient with acetonitrile (A) and water (W) (0 min 30:70–0.4 ml min^{-1}, 14 min 100:0–3.0 ml min^{-1}, 16 min 30:70–0.4 ml min^{-1}). The separation of **I** and **II** was performed with a flow rate of 0.4 ml min^{-1} and a binary gradient consisting of acetonitrile: water (30:70 v/v). The separation of compound **III** was performed with a flow rate of 3.0 ml min^{-1} and acetonitrile as mobile phase. The gradient elution program started with 30% A and 70% W which was held for 12 min and then changed linearly to 100% A between 12 and 14 min. The mobile phase was finally changed linearly to 30% A and 70% W which was held for 2 min. Mobile phases were prepared freshly each day and degassed before use.

2.5. Standard preparation

For HPLC analysis, each standard sample of **I**, **II** and **III** was accurately weighed using an Electrobalance model G with a sensitivity of 2×10^{-4} mg (Cahn Instrument, CA). Working standard solutions were prepared considering the solubility characteristics of each component [8]. The weighed sample was dissolved in acetonitrile and then injected separately onto the analytical column in order to determine the retention time for each compound. These solutions were used for preparation of standard curves samples and the validation of the assay. Samples were diluted to final concentrations in the ranges of 0.072–0.168 mg ml^{-1} for **I**, 0.036–0.084 mg ml^{-1} for **II** and 0.060–0.196 mg ml^{-1} for **III**.

2.6. Sample preparation

Ten tablets containing **I**, **II** and **III** as active ingredients were weighed and crushed to a fine powder. An accurately weighed portion of this powder equivalent to 2 mg was taken in a 5 ml volumetric flask using acetonitrile and then the mixture was sonicated for 2 min. The flask was completed to volume with acetonitrile. The resultant solution was analyzed by HPLC as previously described (Section 2.3) and the chromatographic peaks were identified using authentic standards of **I**, **II** and **III**.

None of the components in the sample, except **I**, **II** and **III**, absorbed at the wavelength recorded in the chromatogram (254 nm).

3. Results and discussion

3.1. Characteristics of the chromatographic peaks

An HPLC chromatogram demonstrating the separation of a mixture of **I**, **II** and **III** is shown in Fig. 1. It can be seen that the order of the retention time is Phenylpropanolamine Hydrochoride (**I**), Caffeine (**II**) and Diazepam (**III**) with a relative retention time of 4.15, 7.25 and 15.68 min, respectively. Sharp and symmetrical peaks

Table 1
Chromatographic characteristics of **I**, **II** and **III**[a]

Compound	RT (min) ± SD	CV (%)	Resolution	Wb (min)
I	4.44 ± 0.03	0.79		0.82
			2.14	
II	7.25 ± 0.07	0.91		1.81
			4.87	
III	15.68 ± 0.01	0.06		1.65

[a] RT, retention time in min; Wb, baseline width.

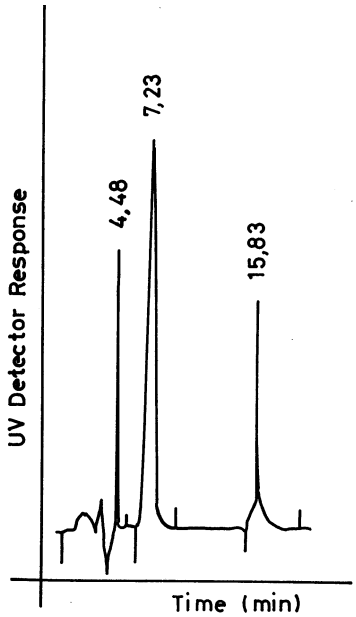

Fig. 1. HPLC chromatogram showing the separation of compounds **I**, **II** and **III**.

are obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area. Compounds **I** and **II** eluted in short time, particularly **I**, whose retention time was close to the void volume of the chromatographic system. Both peaks are well resolved and no interference was detected. Compound **III** eluted at the end of the chromatographic run. The HPLC method satisfactorily resolved the resulting mixture of **I**, **II** and **III**.

3.2. Method validation

An analytical procedure was developed and validated to successfully separate Phenylpropanolamine Hydrochloride (**I**), Caffeine (**II**) and Diazepam (**III**) in compliance with the analytical performance parameters required by the USP XXIV for LC method validation [4]. The following parameters were evaluated: selectivity; repeatability; linearity and range; limit of detection and quantitation; precision and percent recoveries [9–12].

3.2.1. Selectivity

The selectivity of the method for components **I**, **II** and **III** is adequate, since the peaks come out very separate (Fig. 1). For the excipients no interference was detected with these substances.

3.2.2. System suitability

To ascertain the effectiveness of the system suitability test, eight replicate injections of freshly prepared standard stock solutions of **I**, **II** and **III**, at two concentrations were injected into the chromatograph and the relative standard deviation was calculated [(CVs) are below 2.0%-USP XXIV limit is not more than 2%] [4]. The results are tabulated in Table 2.

3.2.3. Linearity of the method

Aliquots of the standard stock solution of **I**, **II** and **III** were taken in different standard volumetric flasks and diluted with acetonitrile to obtain the final concentrations; 20 μl of each solution was injected into the chromatograph. Peak areas were recorded for all the chromatograms.

Detector linearity was determined by linear regression analyses (model $y = ax + b$) of seven-level calibration curves for each analyte (measurement of each level in triplicate). A wide concentration range (60–140% of nominal concentration) was evaluated to establish a method dynamic range. The chromatographic analysis was linear in the working range for standard solutions containing up to 0.168 mg ml^{-1} of **I**, 0.084 mg ml^{-1} of **II** and 0.196 mg ml^{-1} of **III**.

Table 2
System suitability results

Compound	Theoretical concentration[a] mg ml^{-1} 10^2	Calculated concentration[a] ± CV mg ml^{-1} 10^2
I	7.2	7.4 ± 1.3
	8.6	9.2 ± 1.8
II	3.6	3.6 ± 0.6
	7.5	7.5 ± 0.1
III	6.0	5.6 ± 0.8
	9.8	9.8 ± 0.3

[a] Mean for eight determinations.

Table 3
Statistical analyses from calibration data at 254 nm in acetonitrile for compounds **I**, **II** and **III**

Compound	Slope (SD) 10^{-5}	Intercept 10^{-5}	r [a]	Sensitivity mg^{-1} ml	LOD μg ml^{-1}	LOQ μg ml^{-1}
I	5.66 (0.19)	−0.0042	0.997	5.7×10^5	12.8	42.8
II	879 (19)	0.55	0.999	8.8×10^7	4.14	13.8
III	515 (14)	−25	0.999	5.2×10^7	11.0	37.4

[a] Correlation coefficient.

After achieving linear regression equations, standard deviation (SD) and (CVs) were determined (Table 3).

The sensitivity of the method was calculated for each derivative as the slope of the calibration line (Table 3).

3.2.4. Limit of detection and quantitation

The method limit of detection (LOD) was calculated from the calibration curve, area versus concentration, according to $LOD = 3.3\ \delta/S$, with δ being the standard deviation of intercepts of regression line and S being the slope of the calibration curve.

The limits of quantification, defined here as $LOQ = 10\ \delta/S$, were determined on the basis of standard deviation of the response and the slope. The LOD and LOQ were calculated by means of seven determinations (Table 3).

3.2.5. Precision

The precision of the chromatographic system was determined by preparing five solutions of **I**, **II** and **III** at two different concentrations. The determinations were made by three consecutive injections.

The precision of the method is reported as percent relative standard deviation and, less than 2.4% for all analyses (Table 4).

3.2.6. Accuracy

The accuracy of the method was determined by recovery experiments. The percent recoveries assayed for **I**, **II** and **III** quantification were evaluated by comparison of the expected amounts and the amounts calculated expressed as percentage mean recovery ± relative standard deviations. Determinations were performed in quintuplicate at four different concentrations. Table 5 shows the results obtained for the three compounds, with relative standard deviations in the range of 0.14–3.30%, these results indicate the effectiveness of the analytical method.

3.3. Application to commercial formulations of tablets

The method was applied for the determination of **I**, **II** and **III** in tablets prepared in the Pharmaceutical Industry (Tratobes R.S., Disprovent S.A, Lot 795), Pharmacy Office (A Tablets and B Tablets), and in our Laboratory (Reference Tablets).

Table 6 lists the results obtained from 10 Reference Tablets and each pharmaceutical preparation commercially available, which were weighed and finally powdered. Each analytical sample was injected by quintuplicate into the HPLC system and the concentration was calculated by interpolating the area obtained in the working curve.

The dosage form of **I**, **II** and **III** is not described in the pharmacopeia so the results were compared with the Reference Tablets.

Table 4
Precision results

Compound	Theoretical concentration[a] mg ml^{-1} 10^2	Calculated concentration[a] ± CV mg ml^{-1} 10^2
I	7.2	7.1 ± 0.9
	8.6	8.9 ± 0.2
II	3.6	3.5 ± 0.9
	7.5	7.6 ± 0.3
III	6.0	5.2 ± 0.7
	9.8	10.0 ± 0.7

[a] Mean for five determinations.

Table 5
Recovery of I, II and III by HPLC method

Amount added (mg ml^{-1})			Recovery%[a] ± CV		
Compound **I**	Compound **II**	Compound **III**	Compound **I**	Compound **II**	Compound **III**
7.2	3.6	9.8	101.2 ± 1.6	100.4 ± 0.3	102.2 ± 1.8
8.6	5.1	14.7	104.4 ± 1.5	102.3 ± 1.5	99.0 ± 0.2
13.5	6.7	17.2	98.0 ± 0.8	103.0 ± 0.9	100.7 ± 0.2
15.0	7.5	19.6	101.3 ± 0.4	100.7 ± 0.1	99.8 ± 0.2

[a] Mean for five determinations.

As the release requirement for the dosage form is ± 10% of nominal label claim, the results with **I** and **III** in Tratobes RS and in B Tablets, respectively, were significantly different from the stated content of the formulation. This may be due to the type of excipients used or the processes involved in the manufacturing of these tablets. Further, it can also be observed that the assay results for each of the active substances in A Tablets and Reference Tablets were satisfactory. Therefore, the results obtained indicate that the commercial formulation (A Tablets) for the three drugs is in good agreement with the label claims.

4. Conclusions

A simple HPLC method has been developed for simultaneous quantification of the active principles present in the commercial formulations from the three different manufacturers without previous preparation of the sample. The method described in this paper meets the established pharmacopoeia requirements to be used as a routine method for the quality control of pharmaceuticals. The HPLC procedure was validated with respect to simple ternary mixtures of Phenylpropanolamine Hydrochloride (**I**), Caffeine (**II**) and Diazepam (**III**). The validation proved to be in compliance with the USP XXIV analytical performance parameters.

Run times were 25 min with spectrophometric detection. The limit of quantification was 42.8 μg ml^{-1} for **I**, 13.8 μg ml^{-1} for **II** and 37.4 μg ml^{-1} for **III**. These results indicate that the proposed HPLC method could be useful for routine analytical and quality control assays of dosage forms of **I**, **II** and **III**.

In conclusion, the analytical procedure is accurate, precise, and efficient and offers the advantage that the sample preparation is very simple. Also, the equipment is commercially available.

Acknowledgements

This work was supported in part by Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT). The authors are grateful to Professor Dra. María M. de Bertorello for her advice and assistance during this study.

Table 6
Determination of **I**, **II** and **III** in commercial tablets and reference tablets by HPLC method

Dosage Form	Active ingredients mean found ± CV[a]		
	I	**II**	**III**
Tratobes RS[b] (Disprovent SA)	112.56 ± 0.82	107.4 ± 2.5	–
A tablets	96.10 ± 0.12	91.0 ± 1.8	103.20 ± 0.55
B tablets	97.31 ± 0.41	98.8 ± 1.7	114.0 ± 1.4
Reference tablets	93.9 ± 2.6	94.75 ± 1.04	92.00 ± 0.62

[a] Mean and CV for five determinations, percentage recovery from the label claim amount.

[b] Tablets did not contain compound **III**.

References

[1] C. Barbas, A. García, L. Saavedra, M. Castro, J. Chromatogr. A 870 (2000) 97–103.
[2] N. Okamura, H. Moki, T. Hada, S. Yamashita, Y. Masaoka, Y. Nakamoto, M. Tsuguma, M. Yoshitomi, A. Yagi, J. Pharm. Biomed. Anal. 20 (1999) 363–372.
[3] J. Dolejsova, P. Solich, C.K. Polydorou, M.A. Koupparis, C.E. Efstathiou, J. Pharm. Biomed. Anal. 20 (1999) 357–362.
[4] United States Pharmacopoeia, XXIV, Rockville (2000) 2149–1923.
[5] K. Florey, Analytical Profiles of Drug Substances, 1991, pp. 80–99.
[6] The International Pharmacopoeia, Third Edition, World Health Organization (1979) 85, 86, 98, 99.
[7] G. Lunn, N. Schmuff, HPLC Methods for Pharmaceutical Analysis, John Wiley & Sons, Inc., 1997.
[8] The Merck Index, Twelfth edition, Merck & CO., Inc. Rahway, NJ, USA (1996) 268, 508, 1258.
[9] International Conference on Harmonisation, Guideline on the Validation of Analytical Procedure. Food and Drug Administration (1997).
[10] M. Swartz, I. Krull, Analytical Method Development and Validation, Marcel Dekker, Inc., 1997.
[11] C.M. Riley, T.W. Rosanske, Development and Validation of Analytical Methods, 1996.
[12] J.M. Green, Analytical Chemistry 68 (1996) 305A–309A.