Journal of Chromatography, 261 (1983) 111–116
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,686

ASSAY OF MIXTURES OF PHENYLPROPANOLAMINE HYDROCHLORIDE AND CAFFEINE IN APPETITE SUPPRESSANT FORMULATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

HENRY S. I. TAN* and GERALDINE C. SALVADOR
College of Pharmacy, University of Cincinnati-Medical Center, Cincinnati, OH 45267 (U.S.A.)
(Received January 10th, 1983)

SUMMARY

A simple assay method for the quality control of appetite suppressant products containing phenylpropanolamine hydrochloride and caffeine has been developed. A methanolic extract of the product was evaporated to dryness and the residue was treated with aqueous solutions of sodium metaperiodate and disodium phosphate. Following addition of methyl *p*-hydroxybenzoate as internal standard and subsequent filtration, an aliquot of the filtrate was subjected to high-performance liquid chromatography on a 10-μm Partisil ODS-2 column with acetonitrile–water (30:70) as mobile phase. The drug: internal standard peak height ratio at 254 nm was linear over the ranges 0.3–2.0 μg of phenylpropanolamine hydrochloride and 1.1–16.0 μg of caffeine injected. All peaks were well-resolved. The heights equivalent to a theoretical plate (± S.D.) for phenylpropanolamine (as benzaldehyde) and caffeine were (n = 10) 0.42 ± 0.08 and 1.31 ± 0.23 mm, respectively. Overall per cent recoveries (± S.D.) from simulated formulations (n = 6) were 100.7 ± 1.6% phenylpropanolamine hydrochloride and 99.1 ± 1.4% caffeine. The method was applied to marketed products and is also applicable for single dose analysis.

INTRODUCTION

Over the past few years an increasing number of over-the-counter (OTC) appetite suppressant (anorectic) products containing 1-phenyl-2-aminopropan-1-ol (phenylpropanolamine) hydrochloride and caffeine have been marketed. This has necessitated the development of a simple and reliable assay method to aid in the quality control of these OTC products, particularly since the combination of phenylpropanolamine hydrochloride and caffeine has been the subject of review by the U.S. Food and Drug Administration (FDA)[1]. The FDA review stipulated among other things that the total daily dose of phenylpropanolamine hydrochloride may

* Presented at the *130th Annual Meeting of the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, New Orleans, LA, U.S.A., April 1983.*

0021-9673/83/$03.00

not exceed 75 mg. At present, marketed products contain up to 35.5 mg of this drug in immediate-release preparations and 75 mg in time-release products. A close monitoring of the drug content in these products is, therefore, necessary. In addition, the FDA recently decided that triple products containing phenylpropanolamine hydrochloride, caffeine and ephedrine are not included in the above review and are thus considered as new drugs and as such are required to be subject to approval of a new drug application (NDA) prior to marketing[2].

Chromatographic assay methods for the determination of phenylpropanolamine hydrochloride or caffeine have been reported[3–13]. Most of these methods, however, dealt with one of the above compounds in cold and cough preparations in combination with antihistamines and analgesics. The gas–liquid chromatographic (GLC) methods involved, in general, an extraction and a derivatization step and often required relatively long analysis times[3–6,12]. Several of the available high-performance liquid chromatographic (HPLC) procedures involved ion-pair, ion-exchange or regular reversed-phase modes[7–11]. The GLC and HPLC assays of caffeine are generally straightforward[12,13].

The phenylpropanolamine hydrochloride content in appetite suppressant products can be as low as 25% of that of caffeine. This presents problems in HPLC assays with UV detection because the molar absorptivity of phenylpropanolamine is very low whereas that of caffeine is high. Attenuation changes are generally necessary during the HPLC runs. This problem can be circumvented by oxidizing phenylpropanolamine to the highly UV-absorbing benzaldehyde by means of metaperiodate[14–17].

Assay methods for the determination of mixtures of phenylpropanolamine hydrochloride and caffeine have been reported[12,18]. Nonzioli[18] developed a spectrophotometric method in which the phenylpropanolamine was first oxidized with metaperiodic acid. A GLC method was developed by De Fabrizio[12] for mixtures of these two drugs with other compounds, utilizing the acetylderivative of phenylpropanolamine.

This report presents a reversed-phase HPLC method for the assay of phenylpropanolamine hydrochloride and caffeine after treatment with sodium metaperiodate in alkaline medium; methyl *p*-hydroxybenzoate is employed as internal standard.

EXPERIMENTAL

Apparatus

The following were used: an Altex Model 330 liquid chromatograph with a Model 210 sampling injection valve (20-μl loop), Model 110A pump and a Model 153 fixed-wavelength UV detector (254 nm) (Beckman, Fullerton, CA, U.S.A.); and a Kipp & Zonen BD 40 strip-chart recorder (Kipp & Zonen, Delft, The Netherlands).

Reagents and materials

The following reagents and materials were used: phenylpropanolamine hydrochloride (Sigma, St. Louis, MO, U.S.A.); caffeine and methyl *p*-hydroxybenzoate (Fisher Scientific, Fair Lawn, NJ, U.S.A.); a 1.0% aqueous sodium metaperiodate solution; and a 1.5% aqueous disodium phosphate solution. All other chemicals used were analytical grade.

HPLC conditions

A 25 cm × 4.6 mm I.D. 10-μm Partisil PXS ODS-2 column (Whatman, Clifton, NJ, U.S.A.) was used at ambient temperature. A Beckman 4 cm × 4.6 mm I.D. guard column packed with 25–37 μm Whatman Co:Pell ODS preceded the analysis column. An isocratic mobile phase system of acetonitrile–water (30:70 v/v) was delivered at the rate of 1.7 ml/min. This mobile phase was filtered through a 0.45-μm membrane filter (Nylon 66; Rainin Instruments Inc., Woburn, MA, U.S.A.) and degassed before use. The detector was attenuated to 0.16 a.u.f.s.

Internal standard solution

A solution of 30.0 mg of methyl *p*-hydroxybenzoate in 25 ml of methanol.

Standard solution

About 50 mg of phenylpropanolamine hydrochloride and 150 mg of caffeine were weighed accurately, transferred into a 50-ml volumetric flask and dissolved in 30 ml of methanol. The volume was made up with chloroform, and 1.0 ml of the solution was subjected to the same assay procedure as described for the 1.0 ml filtrate of the sample solution.

Sample solution

Tablets. Twenty tablets were accurately weighed and finely powdered. An amount of the powder equivalent to 25–75 mg of phenylpropanolamine hydrochloride was weighed accurately, transferred into a 50-ml volumetric flask and dissolved in 30 ml of methanol. The volume was made up with chloroform, and the solution was filtered through dry filter-paper, discarding the first 5 ml of filtrate. Exactly, 1.0 ml of the filtrate was evaporated to dryness in a 25-ml beaker on a steam-bath. Five millilitres of 1.0% sodium metaperiodate and 4.0 ml of 1.5% disodium phosphate solutions were added to the residue, the mixture was briefly (≈ 15 sec) sonicated and then allowed to stand for 30 min. Following addition of 1.0 ml of internal standard solution, the mixture was quantitatively transferred into a 25-ml volumetric flask. The beaker was rinsed with three 5-ml portions of methanol and the rinsings were combined in the volumetric flask. The volume was made up with methanol and filtered through a 0.45-μm membrane filter, discarding the first 5 ml of filtrate.

Capsules. The contents of twenty capsules were weighed accurately and mixed to a homogeneous powder in a mortar. An amount of the powder equivalent to 25–75 mg of phenylpropanolamine hydrochloride was weighed accurately and subjected to the same procedure as described for the tablet powder.

Chromatographic procedure

By means of the sampling valve, 20 μl of the prepared sample or standard solution were chromatographed under the operating conditions described above. Quantitation was based on relating the compound:internal standard peak height ratio of the sample to that of the standard.

RESULTS AND DISCUSSION

Preliminary studies with the Partisil ODS-2 column and a number of mobile phases such as acetonitrile–water and buffer solutions with organic modifiers showed

TABLE I

RECOVERY DATA FROM SIMULATED FORMULATIONS

Sample	*Amount (mg/dose)*		*Amount found* (mg/dose)*		*Recovery (%)*	
	Phenylpropanolamine–HCl	*Caffeine*	*Phenylpropanolamine–HCl*	*Caffeine*	*Phenylpropanolamine–HCl*	*Caffeine*
Tablet 1	27.5	101.5	27.6	100.6	100.4	99.1
Tablet 2	24.2	99.5	25.0	97.1	103.3	97.6
Tablet 3	29.6	70.6	29.5	71.8	99.7	101.7
Capsule 1	75.8	100.0	74.7	99.0	98.5	99.0
Capsule 2	74.1	200.0	74.4	197.0	100.4	98.5
Capsule 3	72.7	199.8	73.9	196.6	101.6	98.4
Overall recovery (%)					100.7	99.1
Coefficient of variation (%)					1.6	1.4

* Average from duplicate runs.

TABLE II

RECOVERY DATA FROM COMMERCIAL APPETITE SUPPRESSANT PRODUCTS

Sample	*Label claim (mg/dose)*		*Amount found* (mg/dose)*		*Label claim (%)*	
	Phenylpropanolamine–HCl	*Caffeine*	*Phenylpropanolamine–HCl*	*Caffeine*	*Phenylpropanolamine–HCl*	*Caffeine*
Tablet 1	25.0	66.0	25.4	55.2	101.5	84.2
Tablet 2	25.0	66.0	26.4	55.8	105.7	85.6
Capsule 1	50.0	200.0	51.4	199.4	102.7	99.7
Capsule 2	50.0	200.0	51.2	200.8	102.3	100.4
Capsule 3	75.0	200.0	77.3	201.6	103.1	100.8
Capsule 4	75.0	200.0	78.3	198.4	104.4	99.2

* Average from duplicate runs.

that phenylpropanolamine hydrochloride eluted near the solvent front. Also, a substantial amount of the drug was required to give a fair sized peak. The latter observation is not surprising since the ephedrines have low molar absorptivities. The molar absorptivity of phenylpropanolamine is similar to that of norpseudoephedrine and is approximately 150 and 190 liter/mole · cm in methanol at 252 and 258 nm, respectively[19]. The molar absorptivity of caffeine in methanol is about 57 times higher[20]. This very low molar absorptivity coupled with the low content with respect to caffeine in anorectic products necessitated an attenuation change during the chromatographic run. This situation is undesirable since it would require continuous operator attendance.

Oxidation of phenylpropanolamine by means of metaperiodate in an alkaline medium resulted in the formation of benzaldehyde which has a molar absorptivity of about 1.44×10^4 liter/mole · cm at 241 nm in hydrocarbon solvents[16]. The increase in sensitivity upon conversion to benzaldehyde allowed the chromatography to be run at one attenuation. In addition, benzaldehyde is much more strongly retained on the column. Under the proposed experimental conditions, caffeine, methyl *p*-hydroxybenzoate and benzaldehyde were eluted as fairly symmetrical peaks and were well-resolved from one another (Fig. 1). Their retention times were ≈ 3.8, ≈ 6.5 and ≈ 10.2 min, respectively. The average height equivalent to a theoretical plate (HETP) of the column, ± S.D., was 0.42 ± 0.08 and 1.31 ± 0.23 mm for phenylpropanolamine (as benzaldehyde) and caffeine, respectively (n = 10). Additional studies

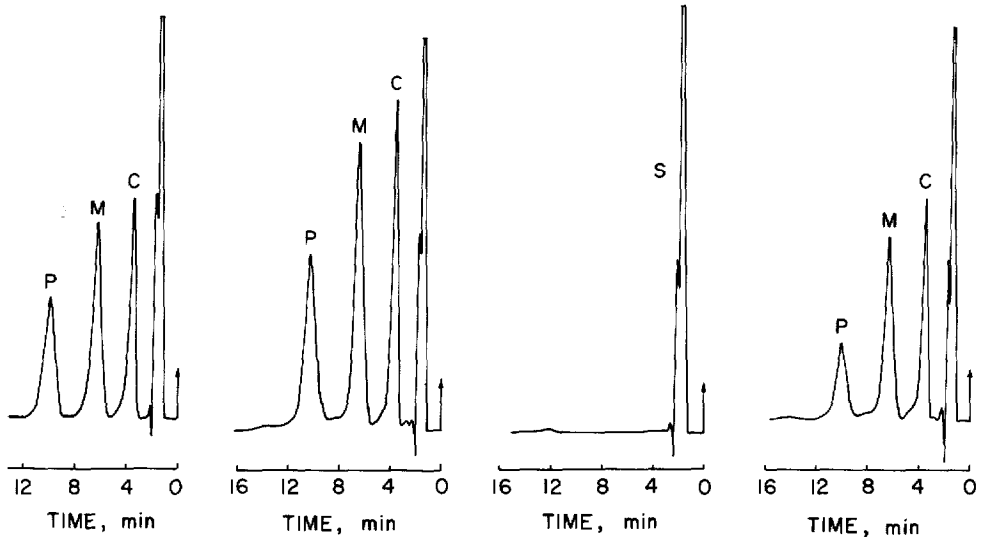

Fig. 1. Liquid chromatogram of a standard solution run under conditions described in the text. Peaks: C = caffeine; M = methyl *p*-hydroxybenzoate and P = phenylpropanolamine (as benzaldehyde).

Fig. 2. Liquid chromatogram of the extract from a simulated tablet formulation run under conditions described in the text. Peaks as in Fig. 1.

Fig. 3. Liquid chromatogram of an extract from a tablet placebo run under conditions described in the text. Peak: S = Metaperiodate.

Fig. 4. Liquid chromatogram of an extract from a commercial product (Capsule 2) run under conditions described in the text. Peaks as in Fig. 1.

showed that under the conditions described caffeine and methyl *p*-hydroxybenzoate were stable toward metaperiodate. The methyl *p*-hydroxybenzoate was nevertheless added after the oxidation reaction was complete, but prior to the transfer of the mixture from the reaction vessel into the volumetric flask to account for losses, if any, during the transfer.

The relationship between compound:internal standard peak height ratio and amount of compound injected was linear over the concentration range of 0.3–2.0 μg of phenylpropanolamine hydrochloride and 1.1–16.0 μg of caffeine injected. Typical regression equations $H = 0.147\ C + 0.002$ for phenylpropanolamine hydrochloride and $H = 0.336\ C + 0.555$ for caffeine, where H = compound:internal standard peak height ratio and C = μg of compound injected. The correlation coefficients, r, were 0.9999 for phenylpropanolamine hydrochloride and 0.9997 for caffeine. A linear relationship existed also between the compound:internal standard peak area ratio and amount of compound injected, but the correlation coefficients were only 0.9950 and 0.9715 for phenylpropanolamine hydrochloride and caffeine, respectively.

Recovery studies were performed on simulated formulations of the two drugs. Tablet and capsule excipients used consisted of mannitol, starch, sucrose, carboxymethylcellulose, magnesium stearate and/or talcum. Fig. 2 shows a typical chromatogram from a simulated formulation. For comparison, the liquid chromatogram of a tablet placebo run under identical conditions is shown in Fig. 3. The overall per cent recovery (± S.D.) (n = 6) was 100.7 ± 1.6% for phenylpropanolamine hydrochloride and 99.1 ± 1.4% for caffeine (Table I).

The method was applied to the assay of these two drugs in some commercial appetite suppressant products. Table II shows the recovery data. A typical liquid chromatogram of a commercial product is shown in Fig. 4.

The proposed assay procedure was designed to allow individual dosage unit assay for the usual dosages in tablet and capsule forms of phenylpropanolamine hydrochloride and caffeine. The method is simple and reliable. The chromatographic run can be completed within 12 min.

REFERENCES

1 Food and Drug Administration, *Fed. Reg.*, 47 (1982) 8466.
2 Food and Drug Administration, *Fed. Reg.*, 47 (1982) 35344.
3 B. R. Rader and E. S. Aranda, *J. Pharm. Sci.*, 57 (1968) 847.
4 C. Hishta and R. G. Lauback, *J. Pharm. Sci.*, 58 (1969) 745.
5 R. E. Madsen and D. F. Magin, *J. Pharm. Sci.*, 65 (1976) 924.
6 D. V. Canfield, P. Lorimer and R. L. Epstein, *J. Forensic Sci.*, 22 (1977) 429; *C.A.*, 88 (1978) 69909n.
7 I. L. Honigberg, J. T. Stewart and A. P. Smith, *J. Pharm. Sci.*, 63 (1974) 766.
8 T. L. Spieck, *J. Pharm. Sci.*, 63 (1974) 591.
9 V. Das Gupta and A. G. Ghanekar, *J. Pharm. Sci.*, 66 (1977) 895.
10 G. B. Cox, C. R. Loscombe and K. Sugden, *Anal. Chim. Acta*, 92 (1977) 345.
11 G. W. Halstead, *J. Pharm. Sci.*, 71 (1982) 1108.
12 F. DeFabrizio, *J. Pharm. Sci.*, 69 (1980) 854.
13 V. Das Gupta, *J. Pharm. Sci.*, 69 (1980) 110.
14 N. H. Brown and G. A. Portmann, *J. Pharm. Sci.*, 60 (1971) 1229.
15 K. C. Heimlich, D. R. MacDonnell, T. L. Flanagan and P. D. O'Brien, *J. Pharm. Sci.*, 50 (1961) 232.
16 L. Chafetz, *J. Pharm. Sci.*, 60 (1971) 291.
17 C. C. Clark, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 100.
18 A. C. Nonzioli, *SAFYBI*, 15 (1975) 865; *C.A.*, 83 (1975) 209460h.
19 J. Holubek (Editor), *Spectral Data and Physical Constants of Alkaloids*, Vol. VII, Academia Publishing House of the Czechloslovak Academy of Sciences, Prague, 1972, No. 873.
20 J. Holubek and O. Strouf (Editors), *Spectral Data and Physical Constants of Alkaloids*, Vol. I, Academia Publishing House of the Czechoslovak Academy of Sciences, Prague, 1965, No. 60.