Journal of Chromatography, 237 (1982) 169–173
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,481

Note

Simultaneous determination of benztropine mesylate and benzophenone by high-performance liquid chromatography

STEPHEN BIFFAR* and DONALD TIBBETTS
Merck, Sharp & Dohme, West Point, PA 19486 (U.S.A.)
(Received October 13th, 1981)

Benztropine mesylate is an anticholinergic drug used in the therapy of parkinsonism. It may be administered orally or by intramuscular injection. Ion-exchange[1] or colorimetric[2] methods have been reported in the literature. Spectrophotometric[3] analyses are currently used for the analysis of benztropine mesylate tablets and injectables.

The compendial method[3] for benztropine mesylate injection specifies an extraction into ether and a back-extraction into dilute hydrochloric acid. The aqueous solution is read at 258 nm.

The compendial method[3] for tablets involves a dichromate oxidation solution of benztropine mesylate in acid solution. The resulting benzophenone is read at 247 nm.

This paper describes a high-performance liquid chromatographic (HPLC) procedure for the simultaneous determination of benztropine mesylate and benzophenone.

The mobile phase contains octylamine phosphate at a pH equal to 3.0. The *n*-octylamine then acts as a competing base by adsorbing to the unreacted silanol groups[4] or as an ion-repelling agent[5] by adsorbing to the octylsilane groups bonded to the base silica. A reduction in the octylamine concentration greatly increases the retention time of the benztropine mesylate.

The method is rapid, accurate, and precise. A comparison of the HPLC method with the current official method[3] indicates that the methods are equivalent for tablets and injectable solutions.

EXPERIMENTAL

Reagents and chemicals

USP reference standard benztropine mesylate and reagent-grade benzophenone (MCB, Plainfield, NJ, U.S.A.) were used in the standard solutions. HPLC acetonitrile, *n*-octylamine (Aldrich, Milwaukee, WI, U.S.A.) and reagent-grade phosphoric acid (85%) (Fisher Scientific, Fair Lawn, NJ, U.S.A.) were used in the mobile phase. Isopropanol and methanol (Fisher Scientific) were reagent-grade.

Octylamine phosphate buffer was prepared by adding 0.84 ml of *n*-octylamine to 1.0 l of deionized-distilled water. While stirring, phosphoric acid was added until the pH was equal to 3.0.

0021-9673/82/0000–0000/$02.75

Aqueous phosphoric acid–isopropanol solution was prepared by mixing 600 ml of deionized-distilled water, 400 ml of isopropanol, and 1.0 ml of phosphoric acid.

Mobile phase

The mobile phase was prepared by mixing 650 ml of HPLC acetonitrile with 350 ml of octylamine phosphate buffer. This solution was degassed by vacuum filtration through a 0.45-μm membrane filter (HVLPO4700; Millipore, Bedford, MA, U.S.A.).

Assay for tablets

Standard preparation. The standard solution contains both benztropine mesylate and benzophenone.

A solution of benzophenone was prepared by dissolving 25 mg of benzophenone in 1.0 l of methanol.

The standard solution was prepared by dissolving 50 mg of benztropine mesylate USP reference standard in 100 ml of deionized-distilled water in a 200-ml volumetric flask. A 5-ml volume of the benzophenone solution was added to the 200-ml volumetric flask, and the solution was diluted to volume with deionized-distilled water.

Assay preparation. Weigh and finely powder not less than 20 tablets (Merck, Sharp & Dohme, West Point, PA, U.S.A.). Transfer an accurately weighed portion of the powder equivalent to about 10 mg of benztropine mesylate to a 50-ml stoppered centrifuge tube. Pipet 40.0 ml of aqueous phosphoric acid–isopropanol solution, into the centrifuge tube. Shake by mechanical means for not less than 60 min. Centrifuge for 5 min. Filter each sample through a 0.45-μm membrane filter (HVLP01300, Millipore).

Assay for injectables

Standard preparation. Dissolve 100 mg of benztropine mesylate reference standard in 50 ml of deionized-distilled water in a 100-ml volumetric flask. Dilute to volume with deionized-distilled water.

Assay preparation. 1.0 mg/ml benztropine mesylate injection samples (Merck, Sharp & Dohme) were assayed with no sample preparation.

Solutions for linearity and precision. Standard solutions were prepared by adding 20 mg, 25 mg, 35 mg, 50 mg, 100 mg, and 150 mg of benztropine mesylate USP reference standard into separate 100-ml volumetric flasks. Each flask was diluted to volume with deionized-distilled water. These solutions were used to test the linearity and precision of the HPLC method.

Conditions for chromatographic quantification. The Varian Model 5060 liquid chromatograph was equipped with a loop-injector, a variable-wavelength detector (Spectromonitor III, Model 1204, LDC Instrument Co), and an octylsilane column (Ultrasphere Octyl (5 μm), 25 cm × 4.6 mm column; Altrex Co., Berkely, CA, U.S.A.). The mobile phase was pumped at a flow rate of 1.3 ml/min, at approximately 25°C. An injection volume of 25 μl was used for both the tablets and injectables. The peaks were detected at 239 nm.

RESULTS AND DISCUSSION

Benztropine mesylate tablets and injectables were assayed by this method. The benztropine mesylate is separated from its degradation product, benzophenone. When injected into the chromatograph, benzophenone is eluted first followed by benztropine mesylate at retention times of 5.20 and 7.60 min, respectively (Fig. 1).

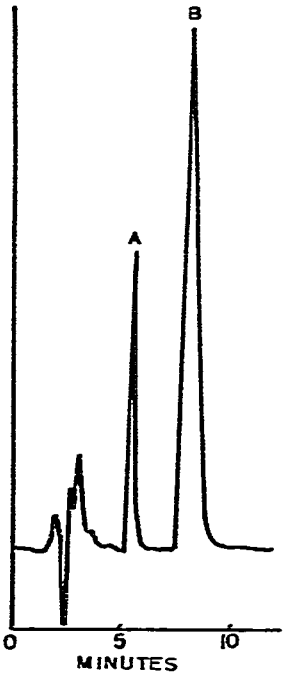

Fig. 1. The separation of benzophenone from benztropine mesylate. Peaks: A = benzophenone; B = benztropine mesylate.

TABLE I

LINEARITY AND PRECISION OF THE HPLC METHOD

Actual weighed concentration benztropine mesylate (mg/ml)	*Observed HPLC results (mg/ml)**	*Recovery (%)*	*Slope*	*Intercept*	*r***
0.202	0.203 ± 0.001	100.4	0.996	0.003	1.00
0.253	0.253 ± 0.001	100.1			
0.353	0.353 ± 0.001	99.9			
0.500	0.508 ± 0.001	101.7			
0.998	0.996 ± 0.002	99.9			
1.498	1.495 ± 0.002	99.8			

* Results based on six replicate injections. The value given is the mean ± S.D.

** Correlation coefficient between the actual and observed concentrations as determined by linear regression analysis.

Standard solutions of benztropine mesylate were chromatographed using the reversed-phase C_8 column. The concentration of each solution was calculated using a Spectra-Physics SP4100 programmable integrator.

A linear regression analysis of the data for the six concentration levels of benztropine mesylate is shown in Table I. This data shows that the benztropine mesylate response is linear from 0.0 to 1.50 mg/ml.

Three lots each of 0.5 mg, 1.0 mg, and 2.0 mg tablets were assayed by this method and the official procedure (USP XX). The results are shown in Table II. The

TABLE II

BENZTROPINE MESYLATE TABLETS COMPARISON OF THE HPLC AND OFFICIAL METHODS

Tablet strength (mg)	*Benztropine mesylate observed results*				*Benzophenone HPLC result*	
	*HPLC**		*USP*			
	mg/tablet	*% claim*	*mg/tablet*	*% claim*	*mg/tablet*	*% Benztropine mesylate equivalent*
0.50	0.486 ± 0.001	97.1	0.490	98.0	0.0007	0.31
	0.491 ± 0.003	98.1	0.491	98.2	0.0005	0.22
	0.499 ± 0.005	99.7	0.509	101.8	0.0001	0.04
1.0	1.007 ± 0.010	100.7	1.02	102.0	0.0012	0.27
	0.970 ± 0.007	97.0	0.985	98.5	0.0037	0.82
	1.003 ± 0.003	100.3	0.988	98.8	0.0020	0.44
2.0	2.036 ± 0.010	101.8	1.95	97.5	0.0039	0.43
	1.974 ± 0.004	98.7	1.92	96.0	0.0078	0.86
	2.040 ± 0.008	102.0	2.03	101.5	0.0017	0.19

* Result is based on two replicate injections. The value given is the mean ± S.D.

TABLE III

BENZTROPINE MESYLATE INJECTION COMPARISON OF THE HPLC AND OFFICIAL METHODS

Sample No.	*Benztropine mesylate observed results*				*Benzophenone HPLC result*	
	*HPLC**		*USP*			
	mg/ml	*% claim*	*mg/ml*	*% claim*	*mg/ml*	*% Benztropine mesylate equivalence*
1	1.009 ± 0.002	100.9	0.998	99.8	$<2.0 \cdot 10^{-5}$	≤0.01
2	1.014 ± 0.001	101.4	1.01	101.0	$<2.0 \cdot 10^{-5}$	≤0.01
3	1.005 ± 0.003	100.5	0.993	99.3	$<2.0 \cdot 10^{-5}$	≤0.01

* Result is based on four replicate injections. The value given is the mean ± S.D.

assay methods are comparable and the HPLC method indicates less than 1.0% degradation of the benztropine mesylate in any lot.

Three lots of 1.0 mg/ml injection were assayed by the HPLC method and the official procedure (USP XX) as shown in Table III. The injection samples all have less than $2.0 \cdot 10^{-5}$ mg/ml of benzophenone, indicating excellent stability in aqueous solution.

This HPLC method is rapid, quantitative, and can simultaneously assay benztropine mesylate and low levels of benzophenone in tablets and injectables.

REFERENCES

1 M. C. Stricklin and D. J. Smith, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 893.
2 M. C. Stricklin, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 681.
3 *The United States Pharmacopeia*, Mack Publishing Co., Easton, PA, 20th Rev., 1980, p. 78.
4 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
5 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.