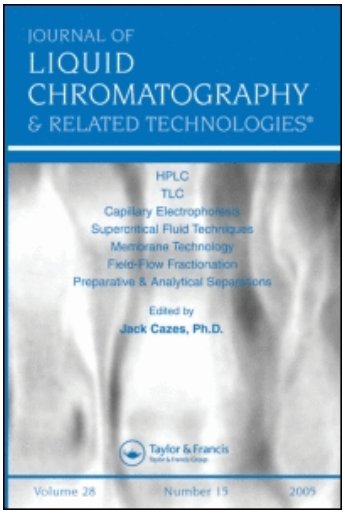

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Determination of Physostigmine and Its Degradation Products in Pharmaceutical Dosage Forms

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To cite this Article Stewart, James T. and Quinn, Karen D.(1989) 'High Performance Liquid Chromatographic Determination of Physostigmine and Its Degradation Products in Pharmaceutical Dosage Forms', Journal of Liquid Chromatography & Related Technologies, 12: 4, 673 — 683
To link to this Article: DOI: 10.1080/01483918908051766
URL: http://dx.doi.org/10.1080/01483918908051766

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(4), 673-683 (1989)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PHYSOSTIGMINE AND ITS DEGRADATION PRODUCTS IN PHARMACEUTICAL DOSAGE FORMS

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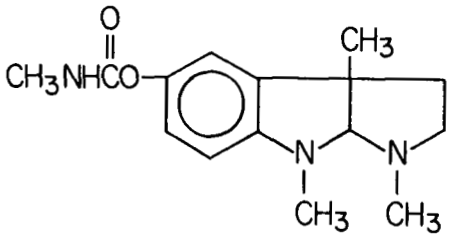
ABSTRACT

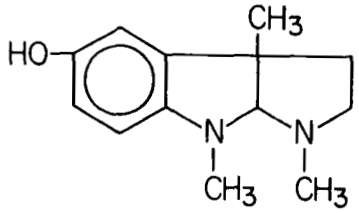
A high performance liquid chromatographic method for the simultaneous determination of physostigmine and its degradation products, eseroline and rubreserine, in pharmaceutical ointment and injection dosage forms is described. The compounds are separated isocratically on an octadecylsilane column using a 60:40 aqueous phosphate buffer pH 4.0-acetonitrile mobile phase containing 0.6% sodium dodecyl sulfate at a 1.0 ml/min flow rate. The column effluent was monitored by ultraviolet absorbance at 310 nm. Accuracy and precision of the method were in the 0.03 - 2.62 percent and 0.1 - 1.2 percent ranges, respectively, for the analytes studied. The method is linear for physostigmine, eseroline, and rubreserine in the 5-1000 ug/ml range.

INTRODUCTION

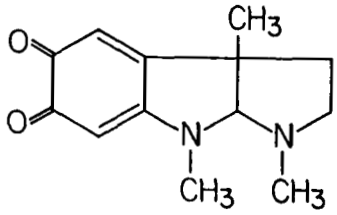
Physostigmine is an anticholinesterase drug used in the treatment of glaucoma and, more recently, for Alzheimer's disease



Physostigmine



Eseroline



Rubreserine

Figure 1. Chemical structures of physostigmine and its degradation products, eseroline, and rubreserine.

(1,2). It is hydrolyzed enzymatically or in the presence of alkali to eseroline, which can also be oxidized to rubreserine as shown in Figure 1 (3). Degradation of physostigmine to rubreserine is noted by the appearance of a red color to the sample. Reported methods for physostigmine in dosage forms include acidi-

metric (4), UV-VIS absorption (4,5), and chromatography (6,7). The acidimetric and UV-VIS spectrophotometric procedures are non-specific and are not stability-indicating. The chromatographic methods are HPLC based, but only assay for physostigmine and not eseroline and/or rubreserine in dosage forms.

This paper describes an HPLC reverse phase ion-pair method for the concurrent determination of physostigmine, eseroline, and rubreserine. The procedure is shown applicable to ointment and injection dosage forms where concentrations of physostigmine and the degradation products can be determined with excellent accuracy and precision.

EXPERIMENTAL

Materials

Physostigmine sulfate, physostigmine salicylate, and procaine hydrochloride (internal standard) were obtained from Sigma Chemical Co., St. Louis, MO 63178. Eseroline and rubreserine were synthesized by the procedures of Salway and Ellis (8,9) and Ellis (9), respectively. Sodium dodecyl sulfate was obtained from Bio-Rad Laboratories (Richmond, CA 94804). Physostigmine sulfate ophthalmic ointment, 0.25 percent USP (Lot. No. 1887), and physostigmine salicylate injection, 1 mg/ml (Lot No. 85A056, O'Neal, Jones and Feldman, Inc., St. Louis, MO 63043) were purchased from a local hospital pharmacy. Acetonitrile (HPLC grade, J. T. Baker Chemical Co., Philipsburg, NJ 07055) was used in preparation of the mobile phase. All other chemicals were commercially available

and were utilized as received. All water was double-distilled in house.

Solutions

a. HPLC mobile phase

A solution was prepared by mixing 600 ml of 0.05 M sodium dihydrogen phosphate pH 4 (adjusted with concn phosphoric acid) containing 0.6 percent sodium dodecyl sulfate with 400 ml of acetonitrile. The solution was filtered through a nylon membrane (0.45 µm MicronSep Magna Filter, Westborough, MA 01581) and sonicated for 20 min prior to use.

b. Stock solutions of drugs and degradation products

Stock solutions (10mg/ml) of physostigmine sulfate, physostigmine salicylate, eseroline, and rubreserine were prepared as solutions in 0.1 M aqueous sodium dihydrogen phosphate pH 4.0. Serial dilutions of these stock solutions were used in the preparation of working standards for calibration curves. The solutions were stored at ambient temperature protected from light.

c. Internal standard solution

A 100 µg/ml solution of procaine hydrochloride was prepared in 0.1 M aqueous sodium dihydrogen phosphate pH 4.0.

Preparation of Dosage Form Samples

a. Physostigmine sulfate ophthalmic ointment

An accurately weighed sample of approximately 400 mg of 0.25 percent physostigmine sulfate ophthalmic ointment was dissolved in 20 ml of n-hexane and transferred to a 60 ml

separatory funnel where it was extracted four times with 20 ml aliquots of acetonitrile. The acetonitrile extracts were then collected in a 100 ml volumetric flask containing 10 ml of a 100 µg/ml solution of procaine hydrochloride in pH 4 phosphate buffer. Acetonitrile was then added to volume.

b. Physostigmine Salicylate Injection

One milliliter of the 1 mg/ml injection was accurately pipetted into a 10 ml volumetric flask containing 1 ml of a 100 µg/ml solution of procaine hydrochloride in pH 4 phosphate buffer. Acetonitrile was then added to volume.

Instrumentation

The liquid chromatograph consisted of a Beckman Model 110B pump (Fullerton, CA), a Rheodyne Model 9125 injector (Cotati, CA), a Kratos Model 757 variable wavelength UV-VIS detector (Ramsey, NJ) and a Hewlett-Packard Model 3390A integrator (Avondale, PA).

Operating parameters - flow rate 1.0 ml/min., detection wavelength 310 nm, 20 µl loop, ambient temperature.

HPLC Column

A Brownlee (Santa Clara, CA) octadecylsilane cartridge column 5 µm, 10 cm x 4.6 mm i.d. was used.

RESULTS AND DISCUSSION

A reverse phase HPLC method based on ion-pair chromatography was developed for the analysis of physostigmine and its degradation products, eseroline and rubreserine, and applied to pharma-

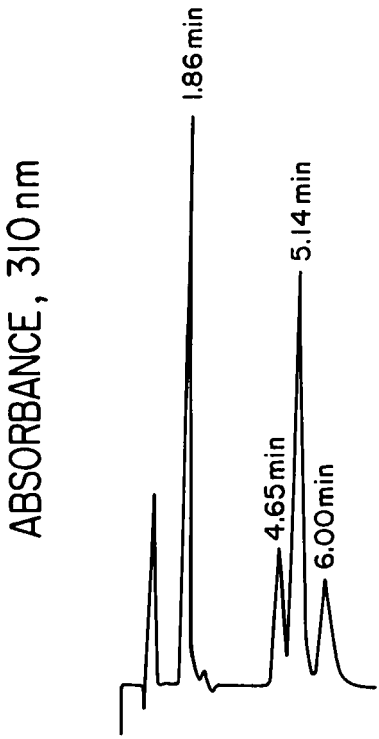


Figure 2. Typical chromatograms of rubreserine (1.86 min), eseroline (4.65 min) procaine HCl (5.14 min), and physostigmine (6.0 min) on a 5 μm ODS column using 60:40 aqueous phosphate buffer pH 4.0-acetonitrile containing 0.6% sodium dodecyl sulfate at a 1.0 ml/min flow rate with UV detection at 310 nm.

ceutical dosage forms. The separation of the three compounds and procaine hydrochloride (internal standard) was achieved on a 5 μm octadecylsilane column using a 60:40 aqueous phosphate buffer pH 4.0 - acetonitrile mobile phase containing 0.6 percent sodium dodecyl sulfate at a flow rate of 1 ml/min. A typical chromatogram of the separation using UV detection at 310 nm is shown in Figure 2. The elution order was rubreserine, eseroline, procaine

hydrochloride, and physostigmine with retention times of 1.86, 4.65, 5.14, and 6.0 min., respectively. Capacity factors (k') for the four compounds in this chromatographic system were 1.0, 3.7, 4.2, and 5.1, respectively. The following resolution values were calculated for each pair of compounds: rubreserine - eseroline, 8.2; eseroline-procaine, 1.3; procaine-physostigmine, 1.5. The linearity of the procedure was determined using standard mixtures containing 5 - 1000 µg/ml of physostigmine sulfate or salicylate, eseroline, and rubreserine. Linear regression analyses of analyte-/internal standard peak areas versus analyte concentration gave correlation coefficients of 0.9999 (n = 5), 0.9993 (n = 5), 0.9997 (n = 6), and 0.9999 (n = 6) for physostigmine sulfate, physostigmine salicylate, eseroline, and rubreserine, respectively. Accuracy and precision of the method were calculated based on

Table 1

Accuracy and Precision Data for Spiked Samples of Physostigmine Sulfate, Physostigmine Salicylate, Eseroline and Rubreserine

	Accuracy, %[a]		Precision, % RSD[b]	
Compound	75 µg/ml	750 µg/ml	75 µg/ml	750 µg/ml
Physostigmine Sulfate	1.82	0.96	0.6	0.3
Physostigmine Salicylate	1.52	0.03	1.0	0.3
Eseroline	2.62	0.41	1.0	0.1
Rubreserine	1.33	0.23	1.2	0.4

[a]Based on n = 5

[b]Based on n = 5

assay of spiked samples of each analyte at 75 and 750 µg/ml levels (see Table 1). The data indicate that the method provides excellent accuracy and precision for all of the analytes and should be suitable as a stability-indicating assay for physostigmine in dosage forms.

Commercially available physostigmine sulfate ointment and physostigmine salicylate injection dosage forms were analyzed using the HPLC assay developed herein. The data shown in Table 2 indicate that the method gives acceptable recovery of drug from both dosage form matrices. Solutions of each dosage form in acetonitrile were then degraded by the addition of 10 µl of 10

Table 2

Assay of Physostigmine Sulfate and Physostigmine Salicylate in Commercial Dosage Forms

Dosage Form	Labeled Amt.	Amt. Found in non-degraded sample[a]	Amt. Found in degraded sample[b]
Physostigmine Sulfate Ointment	0.25%	0.245 ± 0.005%	0.120%
Physostigmine Salicylate Injection	1.0 mg/ml	0.948 ± 0.005 mg/ml	0.879 mg/ml

[a]Based on triplicate determinations.

[b]Based on a single determination of sample degraded with base for 10 min. at ambient temperature.

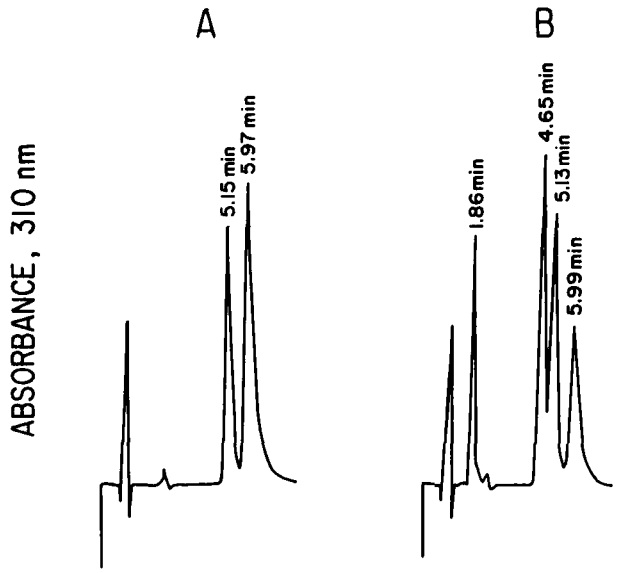


Figure 3. Chromatogram of physostigmine sulfate ophthalmic ointment dosage form (A) before degradation (B) after degradation with 10% sodium hydroxide for 10 min. at ambient temperature.

percent aqueous sodium hydroxide solution for 10 min at ambient temperature. Chromatograms of the physostigmine sulfate ophthalmic ointment sample before and after degradation with base are shown in Figure 3. The physostigmine salicylate injection sample also gave comparable chromatograms before and after degradation with base. Table 2 lists the amounts of physostigmine sulfate or salicylate found in the base-degraded sample using our HPLC method.

In summary, a stability-indicating HPLC assay for physostigmine and its degradation products using ion-pairing techniques has been developed and shown to be applicable to commercially

available ointment and injection dosage forms. The method gives excellent accuracy and precision data for all the compounds studied and could be used to estimate quantities of eseroline and rubreserine degradation products in a physostigmine dosage form if desired.

ACKNOWLEDGEMENTS

K. D. Quinn expresses her appreciation to the United States Pharmacopeial Convention, Inc., for a USP Fellowship during 1986-87.

REFERENCES

1. Remington's Pharmaceutical Sciences, Hoover, J. E., Ed., 14th ed., Mack Printing Co., Easton, PA. 10842, p. 905.

2. Davis, K. L., Mohs, R. C., and Tinklenker, J. Enhancement of Memory by Physostigmine, New Eng. J. Med. 301, 946c, 1979.

3. DeWildt, D. J., Porsius, A. J., and van Rooy, H. H., Quantitative Determination of the Cholinesterase Inhibitor Physostigmine in Brain Tissue Samples Using Reversed-Phase High-Performance Liquid Chromatography, J. Chromatogr., 225, 381, 1981.

4. The United States Pharmacopeia, Twentieth Revision, United States Pharmacopeial Convention, Inc., Rockville, Md., 1980, Mack Publishing Co., Easton, PA. 10842, Physostigmine Salicylate Injection, p. 624, Physostigmine Sulfate Ophthalmic Solution and Physostigmine Sulfate Ophthalmic Ointment, p. 625.

5. Fletcher, G. and Davies, D. J. G., The Effect of pH and Sodium Metabisulphite on the Stability of Physostigmine Sulphate Solutions to Heat and Ionizing Radiation, J. Pharm. Pharmacol., 20, 1085, 1968.

6. Tymes, N. W., High Pressure Liquid Chromatographic Determination of Physostigmine Salicylate and Physostigmine Sulfate in Liquids and Ointments: A Collaborative Study, J. Ass. Off. Anal. Chem., 68, 132, 1982.

7. The United States Pharmacopeia, Twenty-first Revision, Second Supplement, United States Pharmacopeial Convention, Inc., Rockville, Md., 1985, Mack Publishing Co., Easton, PA. 18042, Physostigmine Salicylate Injection, p. 1878 and Physostigmine Sulfate Ophthalmic Ointment, p. 1879.

8. Salway, A. H., Researches on the Constitution of Physostigmine, J. Chem. Soc., 101, 978, 1912.

9. Ellis, S., Studies on Physostigmine and Related Substances IV. Chemical Studies on Physostigmine Breakdown Products and Related Epinephrine Derivatives, J. Pharm. Expt. Ther., 79, 364, 1943.