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# DETERMINATION OF DEMECARIUM BROMIDE AND RELATED COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

LAWRENCE J. COHN*, VINCENT J. GREELY and DONALD L. TIBBETTS
*Quality Control Technical Services, Merck Sharp and Dohme, West Point, PA 19486 (U.S.A.)*
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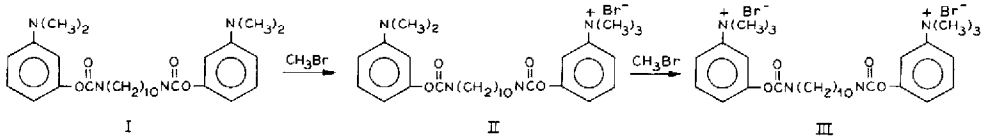
## SUMMARY

A high-performance liquid chromatographic method for the determination of demecarium bromide and related process intermediate and companion products is described. The compounds of interest are separated by isocratic reversed-phase chromatography on a μBondapak CN column using UV detection. The reproducibility of the method and stability of demecarium bromide is demonstrated. Applications presented for the method include quantitation of demecarium bromide in aqueous solutions and control of the raw material.

## INTRODUCTION

Demecarium bromide is a bis-carbamic acid ester cholinesterase inhibitor used in the treatment of glaucoma. Aqueous solutions are administered by topical application to the eye.

The synthesis of demecarium bromide was patented in 1957[1]. N,N,N′,N″-Tetramethyldecamethylenediamine is reacted with phosgene to produce decamethylene-bis(N-methyl carbamic chloride). The carbamic chloride is reacted with *m*-dimethylaminophenol to produce N,N′-bis[3-dimethylaminophenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine (I). The diamine (I) can be reacted with one or two moles of methyl bromide to produce the corresponding quaternary compounds. The bis-quaternary compound, N,N′-bis[3-trimethylammoniumphenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine dibromide (III) is demecarium bromide.




I II III

The compendial method of analysis[2] of demecarium bromide involves the base-catalyzed hydrolysis of the carbamate to produce *m*-trimethylammoniophenol bromide. At high pH, this phenol is converted into the corresponding phenoxide, which is measured at 292 nm. Analysis of demecarium bromide by gravimetric[3] and

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colorimetric[4,5] methods has also been reported in the literature.

A reversed-phase liquid chromatographic procedure has been developed for the determination of the active ingredient demecarium bromide and the preservative benzalkonium chloride in an ophthalmic solution. This procedure separates demecarium bromide from its degradates, process impurities, and from the preservative benzalkonium chloride.

## EXPERIMENTAL

### *Reagents and chemicals*

Selected lots of demecarium bromide and benzalkonium chloride raw materials were used in the standard solutions. N,N′-bis[3-dimethylaminophenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine (I) was obtained from Österreichische Stickstoffwerke, Austria.

Dimethylaminophenol was purchased from Eastman Organic Chemicals (Rochester, NY, U.S.A.) and was recrystallized twice from ligroine. Trimethylammoniophenol iodide was synthesized by reacting dimethylaminophenol with methyl iodide and was purified by recrystallization from ethanol.

High-performance liquid chromatographic (HPLC) grade acetonitrile and reagent-grade hydrous sodium acetate (Fisher Scientific, Fair Lawn, NJ, U.S.A.) were used in the mobile phase.

### *Mobile phase*

Sodium acetate buffer (0.1 *M*) was prepared by adding 13.6 g of sodium acetate to 1.0 l of distilled water. Glacial acetic acid was added with stirring until the pH was 4.5.

The mobile phase was prepared by mixing equal amounts of sodium acetate buffer and HPLC-grade acetonitrile. This solution was degassed by vacuum filtration through a 5.0-$\mu$m PTFE filter (LSWP 04700, Millipore, Bedford, MA, U.S.A.).

### *Chromatography*

The chromatographic system consisted of a Waters 710B WISP autosampler connected to an Altex 110A solvent-metering pump. An LDC Spectromonitor III Variable Wavelength Detector operated at 254 nm was used. Separation was carried out on a $\mu$Bondapak CN column (30 × 0.39 cm I.D., 10-$\mu$m particle size) (Waters Assoc., Milford, MA, U.S.A.) at room temperature, with the flow-rate set at 1.5 ml/min.

### *Standard and sample preparation*

The sample and standard solutions were prepared in HPLC-grade water at a demecarium bromide concentration of 1.25 mg/ml and a benzalkonium chloride concentration of 0.2 mg/ml.

## RESULTS AND DISCUSSION

Demecarium bromide ophthalmic solutions were assayed by this HPLC method. A typical chromatogram is shown in Fig. 1. The peak at 3.5 min (III) is due

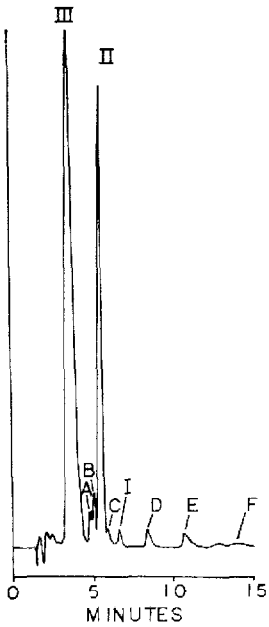


Fig. 1. Separation of demecarium bromide from benzalkonium chloride. Peaks: III = demecarium bromide; II = N-[3-dimethylaminophenoxylcarbonyl]-N′-[3-trimethylammoniumphenoxyl]-N,N′-dimethyldecamethylenediamine cation; I = N,N′-bis[3-dimethylaminophenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine; A, B and C = unidentified companions of demecarium bromide which are present in the raw material; D = benzalkonium chloride $C_{12}$ homologue; E = benzalkonium chloride $C_{14}$ homologue; F = benzalkonium chloride $C_{16}$ homologue.

to the demecarium cation. Peaks at 5.6 (II) and 6.9 (I) min were present in all samples. The area of these peaks does not correlate with age of the sample; however, it does correlate with the lot of demecarium bromide used to prepare the topical solution. These peaks are present in the chromatograms of the demecarium bromide raw material and they appear to be attributable to incomplete quaternization of the diamine (I) during synthesis of demecarium bromide. The peak at 6.9 min has been identified as N,N′-bis[3-dimethylaminophenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine (I) by retention coincidence. In samples which contain the free amine and the bis-quaternary compound, the mono-quaternary compound should also be present. The peak at 5.6 min has the expected retention time and relative area and has been attributed to the N-[3-dimethylaminophenoxylcarbonyl]-N′-[3-trimethylammoniumphenoxylcarbonyl]-N,N′-dimethyldecamethylenediamine cation (II).

These assignments were confirmed by reacting an impure sample of demecarium bromide with methyl iodide. As expected, compounds I and II were converted into the demecarium cations; the peaks at 5.6 (II) and 6.9 (I) min decreased in area and there was a corresponding increase in the size of the peak at 3.5 (III) min. The molar absorptivity at 254 nm of I and III was determined from the UV spectra of the compounds. The molar absorptivity of II was calculated using the values obtained for I and III and assuming that the chromophore 3-dimethylaminophenoxylcarbonyl and the chromophore 3-trimethylammoniumphenoxylcarbonyl are electronically in-

TABLE I

DEMECARIUM BROMIDE OPHTHALMIC SOLUTION

| *Lot No.** | *Age (months)* | *Compendial assay demecarium bromide (%)* | *HPLC assay demecarium bromide (%)* | *Companions*** | |
|---|---|---|---|---|---|
| | | | | *I (%)* | *II (%)* |
| 1 | 19 | 107 | 104 | 0.01 | 0.4 |
| 2 | 40 | 106 | 107 | 0.02 | 1.2 |
| 3 | 45 | 107 | 105 | – | 0.2 |
| 4 | 54 | 104 | 108 | 0.04 | 2.0 |
| 5 | 12 | 101 | 103 | 0.04 | 1.8 |
| 6 | 18 | 107 | 103 | 0.01 | 1.6 |
| 7 | 20 | 106 | 102 | 0.01 | 0.5 |
| 8 | 41 | 107 | 105 | 0.03 | 1.5 |
| 9 | 47 | 106 | 104 | – | 0.1 |
| 10 | 56 | 106 | 107 | 0.04 | 1.9 |

* Lots 1–4 were formulated at a demecarium bromide concentration of 1.25 mg/ml; lots 5–10 were 2.5 mg/ml. Samples were stored at 22–25°C.

** Expressed as demecarium bromide equivalent.

dependent. The molar absorbances of I, II, and III are 32,900, 16,900, and 918, respectively. The molar absorptivity ratio of I to III was 36:1; the ratio of II to III was 18:1; these ratios were used to estimate companion levels in routine samples.

Two hydrolysis products could be expected in degraded samples of demecarium bromide. Trimethylammoniophenol bromide would be produced by hydrolysis of (III) and dimethylaminophenol would be produced by hydrolysis of (I). Trimethylammoniophenol bromide was produced using the compendial procedure[2]; trimethylammoniophenol iodide was synthesized from dimethylaminophenol and methyl iodide. These compounds were chromatographed and the retention time of the trimethylammoniophenol cation was 2.2 min; dimethylaminophenol gave a retention time of 2.7 min. Neither of these compounds were found in aged samples of demecarium bromide ophthalmic solutions.

Demecarium bromide solutions are extremely stable when stored at room temperature and no detectable degradation was found (Table I).

TABLE II

REPRODUCIBILITY OF HPLC METHOD USING STANDARDS CONTAINING ALL EXCIPIENTS

| *% Added* | *% Found demecarium bromide* |
|---|---|
| 80 | 79.4, 81.8, 79.9 |
| 100* | 99.9, 101.3, 99.8 |
| 120 | 119.0, 118.7, 117.7 |
| $\bar{x}$** | 99.8 |
| R.S.D.** | 1.26 |

* The 100% standard had a concentration of 1.25 mg/ml.

** Calculated on results expressed as a percentage of target.

TABLE III

LINEARITY OF THE HPLC METHOD FOR DEMECARIUM BROMIDE

Slope = 0.9994; intercept = 0.0003; correlation coefficient = 0.9999.

| *Actual concentration of demecarium bromide (mg/ml)* | *Observed HPLC results* | *Recovery (%)* |
|---|---|---|
| 0.0 | 0.0 | – |
| 0.75 | 0.750 | 100 |
| 1.00 | 1.005 | 100 |
| 1.25 | 1.254 | 100 |
| 1.50 | 1.481 | 99 |
| 1.88 | 1.888 | 100 |

The reproducibility, accuracy, and linearity of the method was determined by assaying a series of known solutions (Tables II and III). There are no observable interferences, and the accuracy and precision of the method are satisfactory.

This HPLC method is specific and stability-indicating, and separates demecarium bromide from its process intermediates, companions, and degradates. The method is accurate and precise and can be used to control the raw material as well as monitor the stability of demecarium bromide in aqueous solutions.

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