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GLC of Cycloserine

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Abstract □ A GLC assay is described for cycloserine (D-4-amino-3-isoxazolidinone). The sample is dissolved in pyridine-water (2:1), an aliquot is evaporated, and the residue is treated with *N,O*-bis(trimethylsilyl)acetamide and trimethylchlorosilane. After heating for a few minutes, chromatography is performed using a column containing methyl vinyl silicone, a relatively nonpolar liquid phase. Favorable quantitation is achieved by using hexamethylbenzene as the internal standard. The observed peak is the *N,N'*-bis(trimethylsilyl) derivative of cycloserine. The procedure eliminates interference from the cycloserine dimer, which does not chromatograph under these conditions.

Keyphrases □ Cycloserine—GLC analysis using trimethylsilyl derivatives □ GLC—analysis of cycloserine trimethylsilyl derivatives □ Trimethylsilyl derivatization—GLC analysis of cycloserine

Cycloserine (D-4-amino-3-isoxazolidinone, I) equilibrates with its dimer, (+)-*cis*-3,6-bis(aminoxymethyl)-2,5-piperazinedione (II) even in the solid state (1, 2). In solution, pH extremes cause degradation of the dimer to 3,6-dimethylene-2,5-piperazinedione and to β -aminooxalanine (3, 4). At or near neutral pH, dimerization predominates (4).

Quantitative determination of cycloserine in pharmaceutical preparations has been done by the nitroprusside method (5), titrimetrically and by UV spectrophotometry (6), or microbiologically against suitably susceptible microorganisms. The chemical treatment and solvents required for these assays cause formation of degradation products (1-4), which may interfere with

the assay or give inaccurate results. This report presents a more specific GLC method for the quantitative analysis of cycloserine.

EXPERIMENTAL

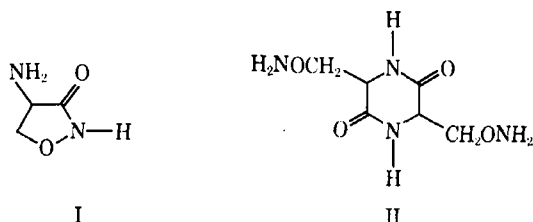
Equipment—A gas chromatograph¹, equipped with a flame-ionization detector, was used. The detector signal was fed to a computer² for peak integration and to a 1-mv. recorder³ with a chart speed of 15 in./hr. and a 1-sec. fullscale response. Samples were injected with a 10- μ l. syringe⁴. Helium was used as the carrier gas, and electrolytic hydrogen and oxygen were used in the detector. The stationary phase was 3.8% UC W98 on Diatoport S⁵ (80-100 mesh) packed in borosilicate glass columns 0.91-m. \times 0.64-cm. o.d. All chemicals used were reagent grade or the best quality available.

Operating Conditions—The column was operated isothermally at 115° with the detector block and injection port at 150°. The helium flow rate was 55 ml./min. with an inlet pressure of 40 p.s.i. The electrometer range was 100 with an attenuation of 32. Sample injections of between 1 and 5 μ l. were made.

Internal Standard—Hexamethylbenzene, 1 mg./ml., in chloroform was used.

Cycloserine Analysis—Solutions of cycloserine (I) reference standard or samples were prepared with pyridine-water (2:1) mixed prior to use and cooled to room temperature. Enough material was dissolved to provide a 1-mg./ml. solution. After mixing, excipient material was allowed to settle for 15 min. A 1-ml. aliquot of each was transferred by pipet to small screw-capped vials with Teflon septa. A stream of filtered dry air was used to evaporate the solvent. A 1-ml. portion of the internal standard solution was pipeted into each vial. The caps were screwed on firmly, and 100 μ l. of *N,O*-bis(trimethylsilyl)acetamide and 10 μ l. of trimethylchlorosilane⁶ were added with a tuberculin syringe. After heating the vials for 10 min. on a heating block at 80°, the samples were ready for chromatography.

UV Spectra—Solutions of cycloserine reference standard and of 3,6-bis(aminoxymethyl)-2,5-piperazinedione (II) were prepared



¹ Hewlett-Packard model 402.

² IBM 1800.

³ Honeywell Elektronik 16.

⁴ Hamilton No. 701.

⁵ Hewlett-Packard, Avondale, Pa.

⁶ Pierce Chemical Co.

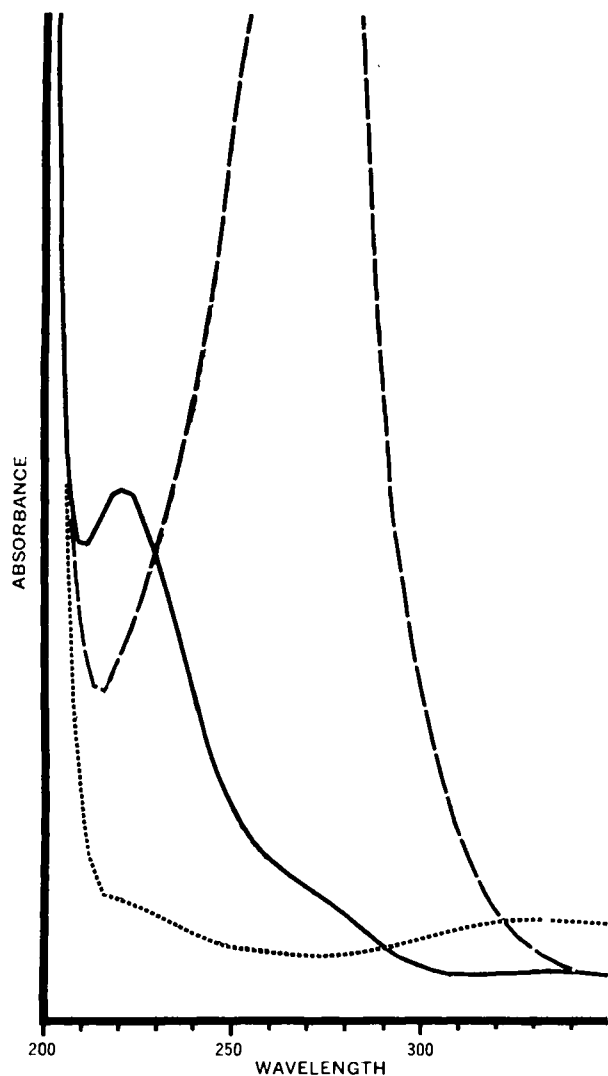


Figure 1—UV spectra of cycloserine (—) and of 3,6-bis(amino-methyl)-2,5-piperazinedione base treated (- - -) and untreated (· · ·).

according to the procedure described in the British Pharmacopoeia (6). Since this treatment converts II to 3,6-bismethylene-2,5-piperazinedione, solutions of II were also prepared in 0.1 *N* hydrochloric acid. UV spectra were obtained on a recording spectrophotometer⁷.

GLC—Mass Spectrometry—Mass spectra of the peak observed on GLC were obtained on a mass spectrometer⁸ coupled with a gas chromatograph⁹ by means of a separator¹⁰. The sample was ionized under the influence of 70 eV.

RESULTS AND DISCUSSION

The UV spectra of cycloserine monomer (I) and dimer (II), after treatment with sodium hydroxide as described in the British Pharmacopoeia (6), are presented in Fig. 1. Obviously, a mixture of the two would not yield valid data for the assay of cycloserine using the 219-nm. reading. Also shown is the spectrum of an equivalent concentration of II not treated with base. Although significantly less absorbance at 219 nm. is exhibited, readings taken on mixtures of monomer and dimer would not be completely free of error.

A typical gas chromatogram is presented in Fig. 2. The first peak is a by-product of silylation. The second peak is the trimethylsilyl derivative of cycloserine, which was identified as *N,N'*-bis-(trimethylsilyl)-cycloserine by GLC—mass spectrometry. The fragmen-

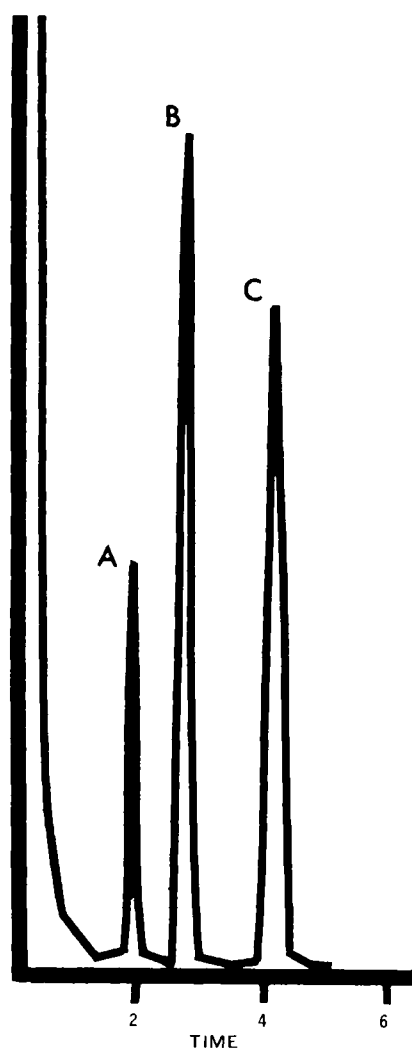


Figure 2—Typical gas chromatogram of trimethylsilyl-derivatized cycloserine. Key: A, derivatization by-product; B, *N,N'*-bis-(trimethylsilyl)-cycloserine; and C, hexamethylbenzene.

tation pattern was analogous to that described for various cycloserine derivatives (7). The third peak in the chromatogram is hexamethylbenzene, the internal standard. Relative to hexamethylbenzene, the retention time of cycloserine is 0.67.

Accuracy and precision were determined by analyses of five replicate samples. A residual standard deviation of $\pm 0.5\%$ and a relative error of $\pm 2.0\%$ were obtained.

Samples of II and of 3,6-bismethylene-2,5-piperazinedione were taken for GLC under conditions identical to those for I. An insoluble residue remained after treatment with silylating reagents, and no peaks were detectable on chromatography even after extended time periods and temperature elevation. These compounds interfere with UV analysis of I.

A sample of β -aminoxyalanine, treated in the same manner as I, gave a peak with a relative retention time of 4.0 compared to hexamethylbenzene. Thus, the known degradation products of I have been shown not to interfere with GLC analysis.

Aged and degraded samples of cycloserine capsules were evaluated by this method. The assay values showed a decrease *versus* the age of the sample and were in agreement with microbiological data. No interferences or extraneous peaks were observed on the chromatograms.

SUMMARY

A GLC procedure for the specific determination of cycloserine was developed. The method is precise to within $\pm 0.5\%$. The determination of cycloserine in a pharmaceutical preparation and in

⁷ Cary model 14.

⁸ Hitachi Perkin-Elmer RMN-6D.

⁹ Hewlett-Packard 402 F & M.

¹⁰ Biemann-Watson.

the presence of degradation products was performed. The method may also find application in kinetic studies of cycloserine degradation.

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Selective Determination of Belladonna Alkaloids by GLC

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Abstract □ Atropine/hyoscyamine and scopolamine, in dosage forms in which the scopolamine represented less than 9% of the total belladonna alkaloids, were isolated as free bases from phenylpropanolamine, chlorpheniramine, and interfering excipients by a series of pH- and solvent-controlled extractions and were quantitated by GLC. Homatropine was used as the internal standard. Precision and accuracy data are represented, along with assay results on three commercial capsule lots.

Keyphrases □ Belladonna alkaloid mixtures with phenylpropanolamine and chlorpheniramine, low scopolamine content—GLC analysis, atropine/hyoscyamine and scopolamine □ Atropine/hyoscyamine and low-content scopolamine—GLC analysis in mixtures with phenylpropanolamine and chlorpheniramine □ Scopolamine (low content) and atropine/hyoscyamine—GLC analysis in mixtures with phenylpropanolamine and chlorpheniramine □ GLC—analysis, atropine/hyoscyamine and scopolamine in mixtures with phenylpropanolamine and chlorpheniramine

Because of its specificity, sensitivity, and resolving properties, GLC has been increasingly used for belladonna alkaloid analysis (1-6). The USP collaborative study for the assay of atropine and scopolamine dosage forms by GC (7) indicated that these two substances, when found individually in tablet dosage forms, could be assayed in this manner with an acceptable degree of precision.

However, the case becomes less clear, even with the high resolving power of GC, when belladonna is found in the presence of a preponderant concentration of other amines and/or in dosage forms with high background contributions. The problem is further complicated when both atropine and scopolamine must be determined together with, as is many times the case, the scopolamine only a small fraction of the atropine.

This paper describes a GLC procedure in which hyoscyamine sulfate, atropine sulfate, and scopolamine hydrobromide in dosage forms are determined in the

presence of chlorpheniramine maleate and phenylpropanolamine hydrochloride, and also where the scopolamine content is less than 9% of the total belladonna present.

EXPERIMENTAL

Materials—Buffer—Dissolve 34.8 g. of dibasic potassium phosphate in 900 ml. of water, adjust to pH 9.0 with 1 N hydrochloric acid or sodium hydroxide, and dilute to 1 l. with water.

Methylene Chloride—Distill reagent grade methylene chloride and store in a tightly closed container.

Atropine Sulfate Standard Solutions—Prepare solutions of USP Atropine Sulfate Reference Standard (or NF Hyoscyamine Sulfate Reference Standard), dried at 105° for 4 hr., in 0.01 N sulfuric acid with concentrations of 0.70, 0.65, and 0.60 mg./ml.

Scopolamine Hydrobromide Standard Solutions—Prepare solutions of USP Scopolamine Hydrobromide Reference Standard, dried at 105° for 4 hr., in 0.01 N sulfuric acid with concentrations of 0.070, 0.065, and 0.060 mg./ml.

Internal Standard Solution—Prepare a solution of USP Homatropine Hydrobromide Reference Standard, dried at 105° for 4 hr., in 0.01 N sulfuric acid with a concentration of 0.50 mg./ml.

Procedure—For this study, the contents of six capsules, each containing 50 mg. of phenylpropanolamine hydrochloride, 4 mg. of chlorpheniramine maleate, and 0.25 mg. of combined belladonna alkaloids (0.0375 mg. atropine sulfate, 0.0219 mg. scopolamine hydrobromide, and 0.1906 mg. hyoscyamine sulfate) were triturated with 0.01 N sulfuric acid. The slurry was quantitatively transferred to a 50-ml. volumetric flask, 2.0 ml. of internal standard was added, and it was diluted to volume with 0.1 N sulfuric acid. The contents of the flask were transferred to a 50-ml. centrifuge bottle and centrifuged at 2000 r.p.m. for 15 min., and 25.0 ml. of clear supernatant solution was transferred to a 150-ml. separator.

The solution was extracted twice with 25-ml. portions of chloroform, and the chloroform extracts along with any emulsion were discarded. Vigorous shaking was avoided in this step to prevent excessive emulsion formation, so losses of belladonna alkaloids and internal standard were minimized. The pH of the solution was adjusted to 8.0 ± 0.1 by the addition of 25 ml. of pH 9.0 buffer and the dropwise addition of 1 N sodium hydroxide; then the solution was extracted with three 25-ml. portions of cyclohexane, and the cyclohexane extracts were discarded. The solution was further