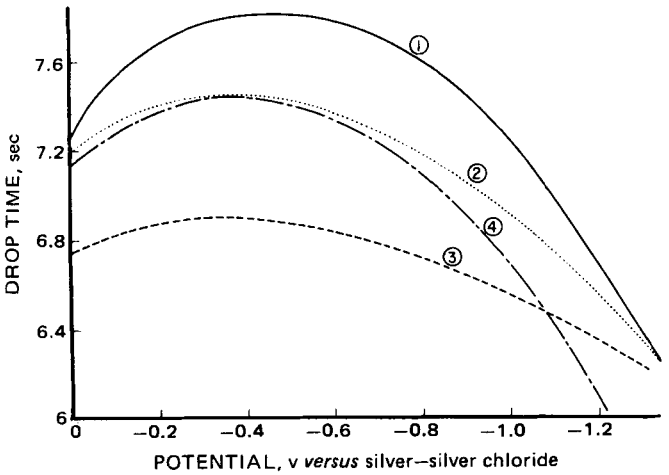

Figure 3—*Electrocapillary maximum curves of 0.1* N *HCl (curve 1), 0.8 mM chlordiazepoxide in 0.1* N *HCl (curve 2), 0.8 mM amitriptyline in 0.1* N *HCl (curve 3), and 0.8 mM clidinium bromide in 0.1* N *HCl (curve 4).*

Other polarographic systems, *i.e.*, amitriptyline with diazepam and clidinium bromide with chlordiazepoxide, underwent similar $E_{1/2}$ shifts and reductions in limiting current. The action of surface-active excipients on the polarography of chlordiazepoxide was studied by Oelschlaeger *et al.* (5) who showed that they all produced, to various degrees, results similar to the action of amitriptyline. It is highly unlikely that all of these dissimilar chemicals would form complexes with chlordiazepoxide.

Solid electrode evidence also points to adsorption as the underlying cause. The $E_{1/2}$ of the single chlordiazepoxide wave observed on carbon electrodes, where no adsorption is expected, was not significantly affected by the presence of amitriptyline in ratios as high as 2:1 of amitriptyline to chlordiazepoxide. On the dropping mercury electrode, the $E_{1/2}$ shift with amitriptyline concentration was most pronounced with ratios below 2:1. Thus, a solution interaction between amitriptyline and chlordiazepoxide appears improbable. If such an interaction exists, it does not significantly contribute to the observed polarographic phenomena.

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High-Pressure Liquid Chromatographic Separation and Determination of Anomeric Forms of Streptozocin in a Powder Formulation

PHILIP J. OLES

Received September 15, 1977, from *The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication January 20, 1978.

Abstract □ A high-pressure liquid chromatographic assay for streptozocin in a sterile powder formulation (1.0 g/vial) is described. The method effectively separates the α- and β-anomeric forms of streptozocin. Quantitative results are presented for the drug based on the use of an internal standard and peak height measurements.

Keyphrases □ Streptozocin—high-pressure liquid chromatographic analysis and separation of anomers in a commercial dosage form □ High-pressure liquid chromatography—analysis and separation of anomers of streptozocin in a commercial dosage form □ Antineoplastic agents—streptozocin, high-pressure liquid chromatographic analysis and separation of anomers in a commercial dosage form

Streptozocin is used for the treatment of malignant insulinoma. An assay was sought for this drug that would satisfy quality control requirements with respect to accuracy, precision, and specificity. The described high-pressure liquid chromatographic (HPLC) technique separates the two anomeric forms of streptozocin, 2-deoxy-2-(3-methyl-3-nitrosoureido)-α(and β)-D-glucopyranose. The mutarotation of streptozocin was studied by using HPLC and optical rotation. With these data, the optimum experimental conditions could be chosen for routine assay of both bulk drug and formulated product.

EXPERIMENTAL

Apparatus—A commercial liquid chromatograph[1] was used at an ambient temperature with UV detection at 254 nm. The column was stainless steel (type 316), 4 × 300 mm, prepacked with 10-μm microparticulate C_{18} bonded to silica gel[2]. Chromatographic recordings were made with a standard 1-mv, commercially available recorder[3].

Reagents and Solutions—The mobile phase was 0.1 *M* acetic acid in water–methanol (97:3). The pH was adjusted to 4.0 with 50% NaOH. The internal standard was a 2-mg/ml solution of potassium acid phthalate. A 1-mg/ml reference standard solution of streptozocin was prepared in 0.1 *M* acetate buffer (pH 4.0). Exactly 5.0 ml of this reference standard solution and 5.0 ml of the internal standard solution were mixed

[1] Model ALC202, Waters Associates, Milford, Mass.
[2] μBondapak C_{18}, Waters Associates, Milford, Mass.
[3] Model HP7123A, Hewlett-Packard, Palo Alto, Calif.

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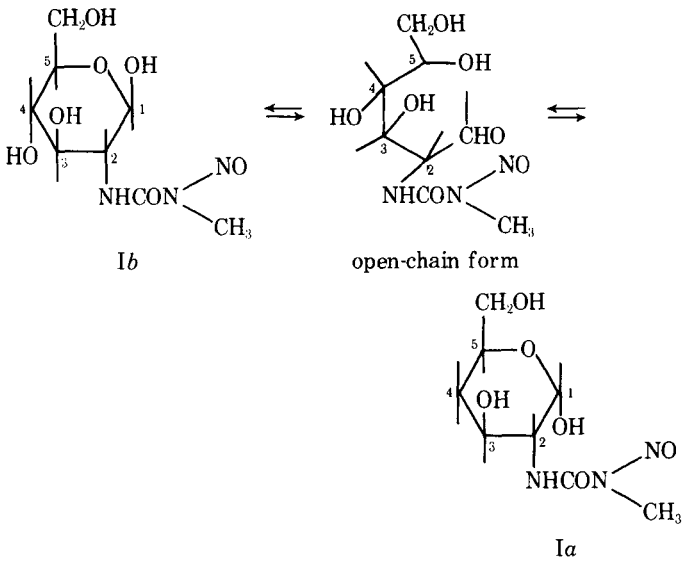

Scheme I

in a suitable container, and the resultant solution served as the standard preparation; 60–90 min elapsed before injection.

Sample Preparation—*Bulk Drug*—A 1-mg/ml solution of streptozocin bulk drug was prepared in 0.1 *M* acetate buffer (pH 4.0). Exactly 5.0 ml of this sample preparation and 5.0 ml of the internal standard solution were mixed in a suitable container, and the resultant solution served as the sample preparation; 60–90 min elapsed before injection.

Sterile Powder—Distilled water, 10–15 ml, was added to the vial to dissolve the contents. This solution was transferred quantitatively to a 1-liter volumetric flask and diluted to volume with water. Exactly 5.0 ml of this sample preparation and 5.0 ml of the internal standard solution were mixed in a suitable container, and the resultant solution served as the sample preparation.

HPLC—Approximately 4 μl of a prepared sample was injected onto the column. The chromatograph was operated at 0.5 ml/min (about 1500 psig) with the detector set at 0.16 absorbance unit full scale. The retention times of the β-anomer, α-anomer, and internal standard were 5, 7, and 21 min, respectively.

Calculations—The amount of streptozocin was calculated by the internal standard–reference standard method using peak height ratios. The peak heights of both anomeric forms of streptozocin were added together in the calculation.

RESULTS AND DISCUSSION

Streptozocin is produced primarily in the α- (Ia) or β- (Ib) form. A typical chromatogram of a freshly prepared solution of streptozocin is shown in Fig. 1A. Subsequent chromatograms of the same solution at various time intervals are shown in Figs. 1B–1D. When the second eluting peak was trapped and reinjected after about 5 min, the chromatogram shown in Fig. 2A resulted. A subsequent injection of the trapped fraction after about 60 min resulted in the chromatogram shown in Fig. 2B.

The phenomenon responsible for this chromatographic behavior is mutarotation of the glucopyranose ring (Scheme I). To assign correctly the proper anomeric form to each peak, the mutarotation reaction of two lots of bulk drug was followed using a polarimeter[4]. Each lot, when freshly prepared as a solution, was composed of principally one form or the other as shown by the chromatograms. The mutarotation reaction followed the time pathway depicted in Fig. 3. Lot A had the higher positive specific rotation, which is generally considered to be attributed to the α-anomer conformation (1). Lot A, when chromatographed, was observed to be primarily composed of the second eluting fraction. Therefore, the order of elution is considered to be the β-anomer first followed by the α-anomer. The mutarotation reaction arrived at an equilibrium state after approximately 60–90 min. The composition of the mixture at equilibrium,

Table I—Recovery of Streptozocin from Spiked Excipients

Added, g/Vial	Found, g/Vial	Recovery, %
0.799	0.802	100.4
0.899	0.879	97.8
0.998	0.985	98.7
1.098	1.073	97.7
1.198	1.180	98.5
	Average	98.6
	RSD	1.1

Table II—Assay Results for Streptozocin in a Sterile Powder

Vial	Found, g/Vial	Percent of Theory
1	0.961	96.1
2	0.977	97.7
3	0.956	95.6
4	0.958	95.8
5	0.955	95.5
	Average	96.1
	RSD	0.9

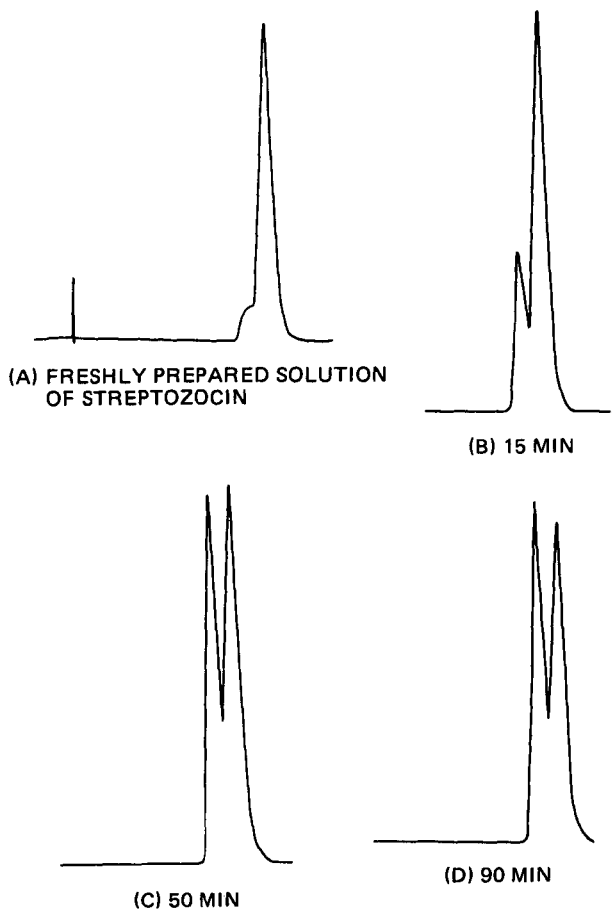

Figure 1—*Mutarotation of streptozocin in aqueous solution as observed by HPLC.*

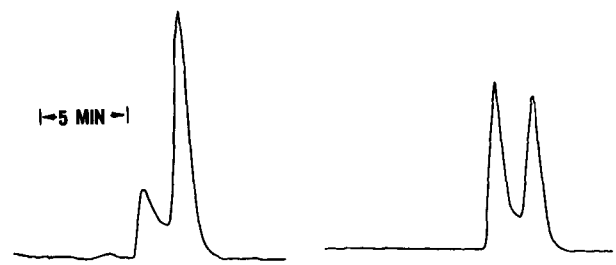

Figure 2—*Chromatograms of second HPLC fraction of streptozocin.*

[4] Model 141, Perkin-Elmer, Norwalk, Conn.

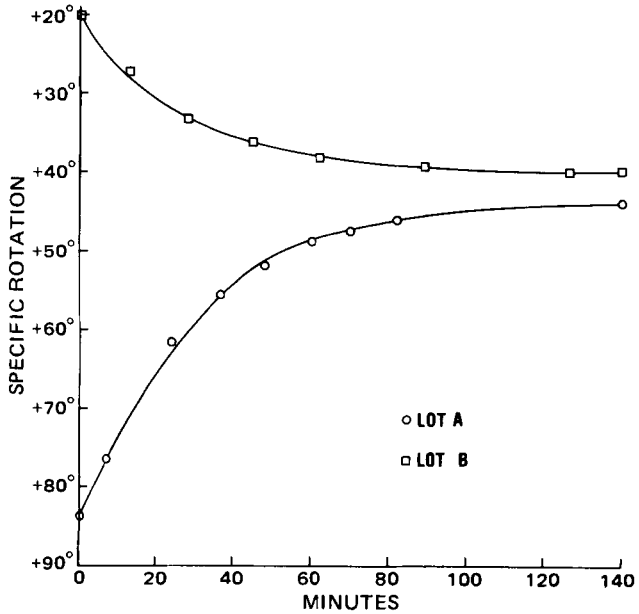

Figure 3—*Change in specific rotation of two anomeric forms of streptozocin with time.*

determined by HPLC using peak area integration, was approximately 1:1. There was a slight pH dependence upon the rate of attainment of equilibrium (Fig. 4).

When streptozocin is formulated as a sterile powder, the drug is in solution for 4–6 hr before freeze drying. Therefore, irrespective of the form of the drug used in manufacture, the product contains the equilibrium mixture of the α- and β-anomers.

The different anomeric forms of streptozocin show different response factors when peak height measurements are used for quantitative analysis. Therefore, when standard preparations or bulk drug sample preparations are chromatographed, 60–90 min should elapse before injection.

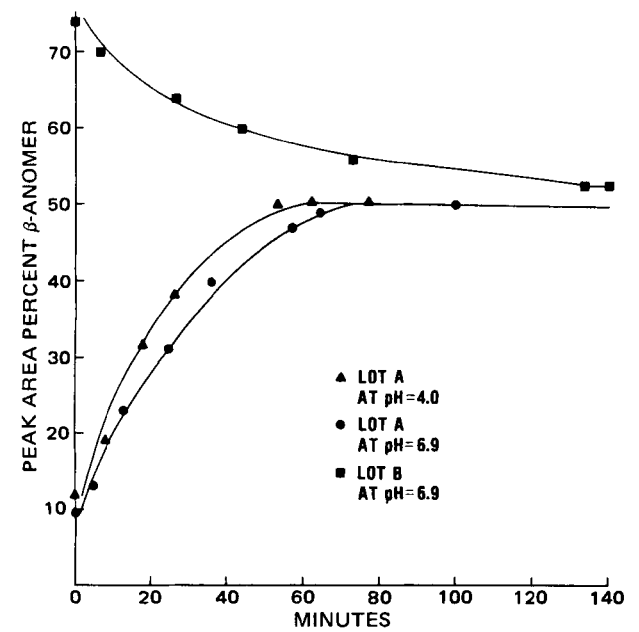

Figure 4—*Change in solution composition of the two forms of streptozocin with time.*

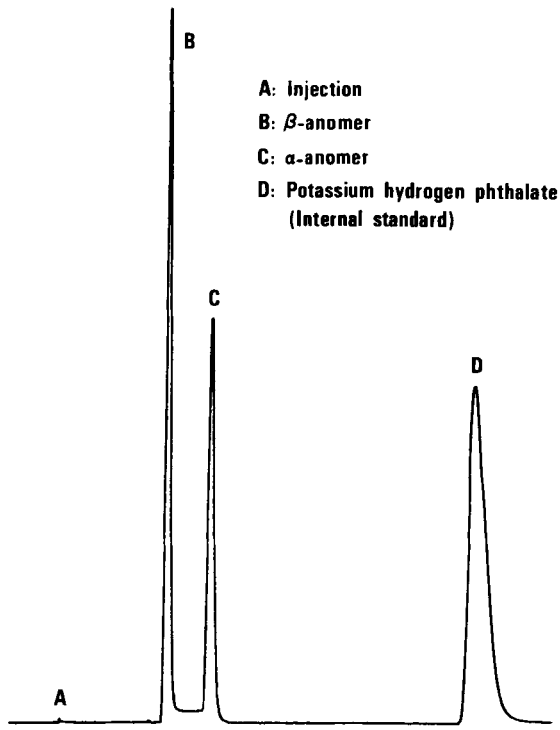

Figure 5—*Chromatogram of streptozocin and internal standard.*

This procedure will ensure that all measurements are taken with the equilibrium mixture of the α- and β-anomers.

A series of placebos consisting of sodium citrate–citric acid was spiked with different amounts of streptozocin to demonstrate the recovery and precision of the chromatographic procedure (Table I). A typical chromatogram is shown in Fig. 5.

These results illustrate the effectiveness of the HPLC procedure for the quality control of the drug, both in bulk and formulation. Streptozocin is not stable for an appreciable time in unbuffered aqueous solution. In buffered solutions, it is stable at room temperature for several days but decomposes rapidly above 70°. Literature reports concerning the degradation of *N*-nitrosamines (2) and *N*-nitrosamides (3) suggest that the majority of degradation products arising from streptozocin have lost the *N*-nitroso group, *i.e.*, the UV chromophore. Similar results also were attained in this laboratory for the degradation of *N*-nitrosoureas.

This effect was verified experimentally by heating a solution of streptozocin at 80° for 30 min and then analyzing an aliquot of the solution for the drug. While the peak heights of the streptozocin anomers decreased significantly (~16%), extraneous peaks due to degradation products were not present in the chromatogram. Furthermore, UV results of the solution for streptozocin were in agreement with the HPLC results for the same solution. Although these data support the UV assay as a specific stability-indicating assay, the HPLC assay was adopted since it provides additional information with respect to the α- and β-anomer composition of bulk drug and product.

Assay results for streptozocin in a sterile-powder, 1 g/vial, are given in Table II.

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