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Colorimetric Determination of Cephradine, a Cephalosporin Antibiotic

JOEL KIRSCHBAUM

Abstract □ The quantitative colorimetric determination of microgram quantities of cephradine is detailed. An aqueous solution of this compound is reacted with sodium hydroxide, partially neutralized, and subsequently reacted with 5,5′-dithiobis(2-nitrobenzoic acid); the absorbance of the resulting yellow solution is then measured. Other cephalosporins tested give a similar color. Studies with various cephalosporins showed that the development of maximum color intensity was attributable to the presence of the R—$CHNH_2$—CO—cephalosporin nucleus, in which R is a mono-, di-, or trienyl cyclohexyl ring. Molar absorptivities of penicillin solutions tested by the same procedure were found to be 5–15% of those of the cephalosporins.

Keyphrases □ Cephradine—colorimetric analysis □ Cephalosporins and penicillins—colorimetric determination □ Penicillins and cephalosporins—colorimetric determination □ 5,5′-Dithiobis(2-nitrobenzoic acid)—reagent for colorimetric determination of cephalosporins and penicillins □ Colorimetry—analysis, cephalosporins and penicillins

This report describes a colorimetric method for the quantitative determination of cephradine (1), a cephalosporin antibiotic, utilizing treatment with base followed by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (I), a compound known to react with sulfhydryl groups. The procedure appears generally applicable to cephalosporins and penicillins.

EXPERIMENTAL

Reagents—The following were used: sodium hydroxide–sodium carbonate solution (dilute 20 ml of 0.5 *M* sodium carbonate and 8 ml of 10 *M* sodium hydroxide to 100 ml); 2 *N* sulfuric acid; 0.5 *M* sodium carbonate buffer, pH 9.2 ± 0.05; and 5,5′-dithiobis(2-nitrobenzoic acid)[1] (I) color reagent, 15.5 mg/50 ml of 95% ethanol (prepared fresh daily).

Procedure—Pipet 5 ml of an aqueous solution containing 30–600 μg of cephradine into a test tube. Then pipet 5 ml of sodium hydroxide–sodium carbonate solution. Cover the tube loosely and place it in a boiling water bath for 75 min. Rapidly cool the test tube to room temperature by immersing it in an ice bath, and then add 2 *N* sulfuric acid to adjust the contents to pH 9.2 ± 0.1 (pH meter). Transfer the solution to a 25-ml volumetric flask. Into the same flask, pipet first 10 ml of 0.5 *M* sodium carbonate buffer and then 3 ml of I color reagent. Dilute to volume with water and mix. Determine spectrophotometrically[2] the absorbance of the 412-nm peak between 5 and 20 min after addition of the color reagent, using a reagent blank for comparison.

RESULTS AND DISCUSSION

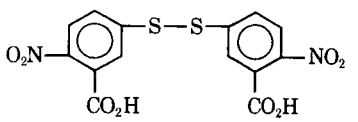
Beer's law is followed by solutions containing 1–30 μg of cephradine/ml (25–750 μg/25-ml flask). The sensitivity of this assay of 0.2 μg/ml indicates its utility, although the error of quantitation is inversely proportional to cephradine concentration. Base treatment with acetone, as described by Marrelli (2) for the estimation of cephalexin, another cephalosporin antibiotic, has a sensitivity of 0.1–2 mg/ml and does not follow Beer's law. Under the experimental conditions employed here, I is as sensitive to sulfide and sulfite (3) and to such mercaptans as cysteine and mercaptoethanol as it is to base-degraded cephradine. The thioether methionine does not interfere with the assay.

Related reagents that react with base-degraded and partially neutralized cephradine are 2,2′-dithiodipyridine and 4,4′-dithiodipyridine, but their maximal absorbance is in the same UV region of the spectrum as are the absorbance peaks of possible interfering substances, and the color development cannot be followed visually.

The results of the investigation of several variables of this procedure are discussed here.

Effect of Heating Time—Samples heated for 60–90 min and then processed as described had absorbances that did not differ from each other by more than 1%. Heating the sample for 120 min or longer resulted in a reduction of absorbance.

Effect of pH—Base-degraded solutions adjusted to pH < 9.2

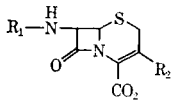


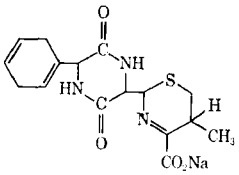
I

[1] Aldrich Chemical Co.

[2] A Beckman DU spectrophotometer was used.

Table I—Molar Absorptivities of Various Cephalosporins, Penicillins, and Related Compounds Carried through the Experimental Procedure

Compound	R_1	R_2	$\epsilon \times 10^{-4}$
			
Cephalosporins:			
I, cephradine	$\Delta^{1,4}$-C_6H_7—$CHNH_2$—CO—	—CH_3	1.30
II	Δ^{1}-C_6H_9—$CHNH_2$—CO—	—CH_3	1.31
III, cephalexin	C_6H_5—$CHNH_2$—CO—	—CH_3	1.29
IV, cephaloglycin	C_6H_5—$CHNH_2$—CO—	—CH_2OCOCH_3	1.28
V	C_6H_5—CH_2—CO—	—CH_3	0.53
VI, cephalothin	2(—C_4H_3S)—CH_2—CO—	—CH_2OCOCH_3	0.45
VII	C_6H_5—OCH_2—CO—	—CH_2OCOCH_3	0.39
VIII	C_6H_5—OCH_2—CO—	—CH_3	0.44
IX, 7-aminodesacetoxycephalosporanic acid	H—	—CH_3	0.11
X, 7-aminocephalosporanic acid	H—	—CH_2OCOCH_3	0.29
Penicillins:			
Methicillin			0.15
Oxacillin			0.22
Epicillin			0.08
Ampicillin			0.09
Penicillin G			0.08
Sodium cloxacillin			0.08
Phenoxymethyl penicillin			0.06
Related compounds:			
6-Aminopenicilloic acid			0.17
2-(1,4-Cyclohexadienyl)glycine			0.0
Phenylglycine			0.0
Penicillamine			0.12
XI			1.30



were less intense in color than those at pH 9.2. Above pH 9.2, the dilute carbonate interacted with the reagent to give an increased intensity of the reagent blank. When the pH remained 9.2, however, a fourfold increase in the carbonate concentration had no effect on color intensity, within the limits of 1% experimental error.

Effect of Concentration of Color Reagent—When the concentration was kept at 800 μg cephradine/25 ml, the absorbance increased as the volume of I color reagent was increased to 3 ml. Additional volumes of color reagent caused no increase in absorbance.

Effect of Time on Color Intensity—The color intensity reached a maximum within 5 min after the addition of the color reagent, remained constant for approximately 20 min, and then decreased slowly. In the single set of experiments performed, the faded colors of several samples were proportional to the diminished intensity of the standard. Waiting 3 hr after cooling the solutions, before proceeding with the remainder of the assay, also decreased the absorbance of both sample and standard, to about 60% of the original intensity.

Effect of Nitrogen—Bubbling nitrogen through the cephradine solution prior to and during degradation of the antibiotic with base had no effect on the intensity of the final color, within the limits of a 1% experimental error.

Effect of Distilled Water—The use of distilled water instead of deionized[3] distilled water resulted in a 3% reduction in absorbances.

Molar Absorptivities of Related Compounds—Table I lists the values for some cephalosporins, penicillins, and related compounds assayed as described under *Experimental*. The variables in the procedure were only investigated for cephradine.

The development of maximum color intensity appears to depend on the presence of the α-amino group in the cephalosporin side chain. This is also true for the procedure of Marrelli (2). However, subjecting cephradine to the procedure of Marrelli (base treatment in the presence of acetone) results in a peak absorbance at 500 nm, which is 40% that of cephalexin. The absorbance maximum of cephalexin is 520 nm.

The isolated base degradation product of cephradine, XI (4), also gives maximum color development. If it is assumed that the reaction with I involves a sulfhydryl group generated by the rupture of a C—S bond, the cephradine rearrangement compound, XI, most likely is an intermediate in this reaction.

This procedure is capable of producing reproducible results from day to day using bulk solids. The relative standard deviation of six samples analyzed on 2 days was 0.81%.

Two-milliliter samples of normal human serum and urine had absorbances of 0.60 and 0.75, respectively, when assayed by this procedure. Although the dilution of serum and urine samples containing cephradine would give correspondingly lower absorbances of the blanks, the extent of such dilutions would depend on the cephradine contents of the samples. The effect of such variables as diet, disease, and time of sample collection has not been determined. For these reasons, a separation of cephradine from a body fluid is recommended prior to analysis.

[3] A Barnstead-type HN cartridge demineralizer, mixed bed type, was used.

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PHARMACEUTICAL TECHNOLOGY

Comparison of Dissolution Profiles of Tablets and Capsules from the USP, Levy, and Magnetic Basket Methods

T. E. NEEDHAM, Jr.[x], and L. A. LUZZI

Abstract □ Single-batch lots of pentobarbituric acid tablets and sodium butabarbital capsules were dissolved in the USP, Levy beaker, and three different size magnetic basket dissolution apparatuses. Each method was compared using an analysis of variance and other statistical tests to ascertain if significantly different dissolution profiles were produced. Variation in terms of standard deviation of drug released by the different methods was also compared. The five different dissolution methods produced significantly different dissolution profiles at various selected times for both the tablets and capsules studied. However, differences in variations produced by the different dissolution methods upon repeated dissolution of either tablets or capsules seemed to be of the same order.

Keyphrases □ Dissolution profiles (pentobarbituric acid tablets and sodium butabarbital capsules)—comparison of USP, Levy beaker, and three different size magnetic basket apparatuses, statistical analysis □ Tablets, dissolution (pentobarbituric acid)—comparison of USP, Levy beaker, and magnetic basket apparatuses, statistical analysis □ Capsules, dissolution (sodium butabarbital)—comparison of USP, Levy beaker, and magnetic basket apparatuses, statistical analysis

The adoption by the compendia of an official dissolution apparatus (1) has produced much controversy for and against that method. Many new methods and adaptations of older methods have been reported (2). Usually each method has been presented with *in vitro* data to substantiate its effectiveness in following tablet and/or capsule dissolution as well as its ability to differentiate between common manufacturing variables. Many suggestions have been made to "improve" or replace the official method or to establish more than one official method. Some *in vitro* comparisons of the different dissolution methods also have been reported (3). However, little has been done to determine if the methods themselves actually produce significantly different dissolution profiles or if the variations seen in the dissolution apparatuses are of such magnitude as to produce overlapping curves that are essentially similar.

Table I—*F* Values for Pentobarbituric Acid Tablets at Selected Times

Minutes	*F* Value: Repeated Tablets	*F* Value: Different Dissolution Methods	$F(4,16)_{0.99}$
8	0.462	15.29	4.77
12	1.38	36.25	4.77
18	0.987	21.86	4.77
30	0.391	7.13	4.77

This study compared several different dissolution methods to determine if they produced significantly different dissolution profiles for a drug from the same dosage form. Variation in terms of standard deviation of drug released as produced by the different dissolution methods was compared, as well as the significant changes in drug availability caused by changing the impeller speed for both capsule and tablet dosage forms.

EXPERIMENTAL

Materials—Each tablet was formulated to contain 25 mg pentobarbituric acid[1], 236 mg fast-flow lactose[2], 43.5 mg microcrystalline cellulose[3], 35 mg starch[4], and 10.5 mg stearic acid[5]. Tablets were compressed using a 16-station rotary tablet press[6] equipped with an induced die feeder. Standard concave punches,

[1] Abbott Laboratories.
[2] Foremost Dairy, San Francisco, Calif.
[3] FMS Corp., Newark, Del.
[4] Ruger Chemical Co., Irving, N.J.
[5] Fisher Chemicals, Fairlawn, N.J.
[6] Model 216-RP, Cherry-Burrell.