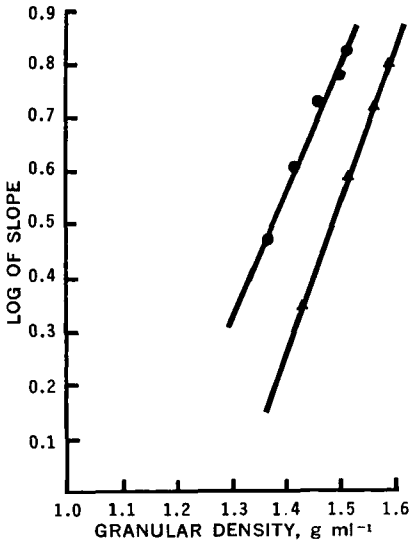

Figure 6—*Plot of logarithm of slope of first falling rate period* versus *granular density of sulfathiazole and lactose granulation. Key:* ▲, *sulfathiazole granulations prepared using acacia mucilage as binder; and* ●, *lactose granulations prepared using acacia mucilage as binder.*

mic-type relationship existed. This phenomenon could be easily explained based upon mechanism of moisture movement within the granules during this phase of drying.

Linear relationships were also observed between the slopes of the falling rate period and the statistical diameters of granulations prepared using the same binding agent (Figs. 3–5). A reduction in the size of the granules led to an increase in the slope because for smaller granules the first falling rate period starts at a lower moisture content so moisture is depleted at a faster rate and, furthermore, the moisture movement to the surface of the granules occurs faster.

A logarithmic linear relationship was observed between the slopes of the first falling rate period and the granular density for both lactose and sulfathiazole granules (Fig. 6). As the granular density decreased, the moisture movement within the granules became more restricted and the rate of moisture depletion from the surface increased. The granular density affects the first critical points as well as the slope of the first falling rate period and, therefore, the drying rate curve of the granulations. Having established the effect of physical parameters upon the drying rate during the constant rate period, falling rate periods, and critical points, one could draw a drying rate curve for a given formulation and determine the drying time from the initial to a desired final moisture content.

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* Present address: Department of Pharmacy, University of Manchester, Manchester, England M13 9PL.

[x] To whom inquiries should be directed. Present address: Pharmaceutical Products Division, Pharmaceutical Research and Development, Abbott Laboratories, North Chicago, IL 60064

Automated Method for Determining Calcium Disodium Edetate in Iodinated Contrast Media Parenterals

HOWARD W. ZIEGLER [x], WILMA L. ANDERSON, and JOHN T. SHADE

Abstract □ An automated method, based on the chelating reaction of calcium disodium edetate with zirconium and the subsequent determination of excess zirconium reacted with xylenol orange, was developed. The procedure is applicable to parenterals consisting of iodinated contrast media. Familiar modules of an automated analyzer were used, but the method can be performed manually if the sample load does not warrant automation. The pH should be controlled between 0.3 and 0.5. No interferences were encountered. Twenty samples per hour can be run on prepared sample solutions. The precision of a single determination, at the 95% confidence level, was ±0.008 mg/ml with a limit of detection near 0.40 mg/ml.

Keyphrases □ Calcium disodium edetate—automated analysis in iodinated contrast media parenterals □ Sequestering agents—automated analysis of calcium disodium edetate in iodinated contrast media parenterals □ Automated analysis—calcium disodium edetate in iodinated contrast media parenterals

Calcium disodium edetate USP (I) is commonly added to iodinated contrast media parenterals (*e.g.*, sodium and meglumine iothalamates USP) as a sequestering–stabilizing agent. It is also used as an antioxidant in some foods and beverages such as salad dressings, margarine, barbecue sauce, beer, and wine. As a sequestering agent, it is added to complex traces of metals, thus preventing oxidation (catalyzed by trace metals) or possible discoloration.

Several colorimetric methods have been reported

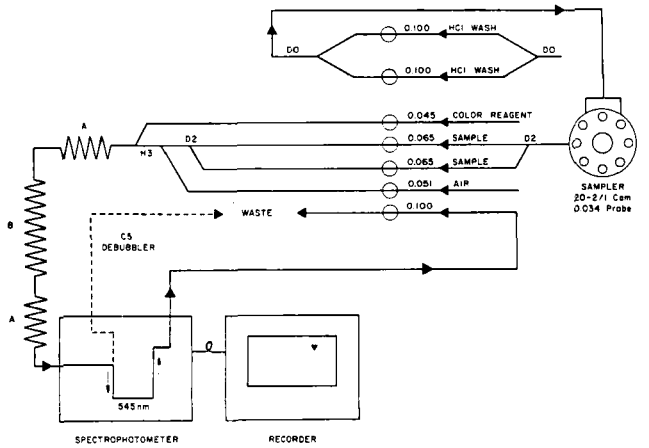

Figure 1—*Flow diagram of automated system for determining calcium disodium edetate. All tubing is standard. Key: A, 14-turn mixing coil, 2 mm i.d.; and B, 28-turn mixing coil, 2 mm i.d.*

for the determination of ethylenediaminetetraacetic acid (II). A method was reported that measures excess nickel with dimethylglyoxime after reaction with II (1). A procedure also was described using iron; the excess was reacted with thiocyanate (2). The use of a copper complex was reported (3), as was a procedure using chromium (4). The FDA "Food Additives Analytical Manual" (5) lists a method based on the chelating reaction of I on zirconium and subsequent determination of excess zirconium with xylenol orange. In the present study, this zirconium–xylenol orange (III) method, originally applied to the analysis of foods, was evaluated, adapted, and automated to analyze iodinated contrast media parenterals.

EXPERIMENTAL

Apparatus—A standard automated analyzer system[1] consisting of the following modules was used (Fig. 1): *(a)* a liquid sampler II[1] with 0.034 probe and 20-2/1 cam, *(b)* a proportioning pump II[1], *(c)* a spectrophotometer[2] modified with a 1-cm flowthrough cell, and *(d)* a strip-chart recorder[3].

Reagents and Solutions—*Zirconium Stock Solution*—Prepare by dissolving 1.77 g of zirconium oxychloride octahydrate in 100 ml of hydrochloric acid and dilute to 1000 ml with water in a volumetric flask.

Zirconium–Xylenol Orange (III) Color Reagent—Dissolve 50 mg of xylenol orange tetrasodium salt in 75 ml of water in a 500-ml volumetric flask. Add 5.0 ml of zirconium stock solution and 225 ml of 1 *N* HCl and dilute to volume with water. Age 24 hr and filter through a medium-porosity paper prior to use.

Hydrochloric Acid Wash Solution—Dilute 70 ml of concentrated hydrochloric acid to 2000 ml with water.

Calcium Disodium Edetate Stock Solution A—Prepare by dissolving 2.00 g of anhydrous (dried at 105° for 4 hr) calcium disodium edetate USP in water, adjust the pH to 7.0–7.5 with diluted sodium hydroxide or hydrochloric acid solution, and dilute to volume in a 100-ml volumetric flask with water.

Standard Solution B—Prepare by diluting 25.0 ml of Stock Solution A to 1000 ml with water

Diluted Standard Solution C (0.025 mg/ml of I)—Prepare by diluting 25.0 ml of Standard Solution B to 500 ml with water.

Transfer aliquots (usually five) of diluted Standard Solution C, ranging from 0.050 to 0.350 mg/100 ml, to 100-ml volumetric flasks; add 36 ml of 1 *N* HCl to each flask and dilute to 100 ml with water. Carry the standard solutions through the entire procedure with prepared sample solutions.

Procedure—Transfer a 2.0-ml aliquot of parenteral sample solution to a 100-ml volumetric flask. Add 20 ml of water and 40 ml of 1 *N* HCl, dilute to 100 ml with water, and mix. Let stand for at least 15 min with occasional swirling and allow the precipitate to settle. Filter a portion of the clear supernate through a dry, medium-porosity paper, discarding the first 10 ml. Fill an 8.5-ml turntable sample cup with filtrate and place in the sampler turntable.

Position the series of standard solutions in 8.5-ml sample cups ahead of the sample solutions and place one between every sixth sample. Insert the color reagent tube into the hydrochloric acid wash reservoir. Establish a baseline at zero absorbance by pumping hydrochloric acid wash through the system. Remove the color reagent tube and place it in the color reagent reservoir. Establish a steady "reagent blank" baseline, which will occur at about 0.3 absorbance unit.

Table I—Reproducibility of Automated Method for Calcium Disodium Edetate in Iodinated Contrast Media

Product	Day[a]	Calcium Disodium Edetate, mg/ml Lot[b] A	Lot B	Lot C	Lot D
C-Y	1	0.035	0.035	0.042	0.034
	2	0.045	0.045	0.045	0.043
	3	0.040	0.042	0.040	0.042
	4	0.038	0.038	0.043	0.036
	5	0.036	0.037	0.037	0.037
		Pooled *SD* of replications within lots = 0.00377			
A	1	0.098	0.095	0.095	0.107
	2	0.103	0.104	0.102	0.084
	3	0.106	0.099	0.101	0.082
	4	0.097	0.096	0.096	0.078
	5	0.099	0.097	0.099	0.080
		Pooled *SD* of replications within lots = 0.00348			
C	1	0.100	0.075	0.095	0.076
	2	0.110	0.086	0.109	0.087
	3	0.106	0.079	0.101	0.082
	4	0.102	0.076	0.097	0.078
	5	0.104	0.077	0.099	0.080
		Pooled *SD* of replications within lots = 0.00450			
C-4	1	0.115	0.085	0.082	0.093
	2	0.113	0.094	0.095	0.097
	3	0.111	0.090	0.090	0.092
	4	0.114	0.088	0.084	0.093
	5	0.107	0.088	0.087	0.090
		Pooled *SD* of replications within lots = 0.00366			

[a] A fresh sample was carried through the entire procedure each day. [b] Four different production lots of each product were arbitrarily lettered.

Table II—Recovery of Calcium Disodium Edetate from Iodinated Contrast Media[a]

Sample[b], ml	Detected, mg/ml	Added, mg/ml	Found, mg/ml	Recovery, %
1	0.102	0.050	0.152	100
0.5	0.060	0.050	0.105	88
1	0.111	0.050	0.156	90
1	0.108	0.050	0.158	100
0.5	0.038	0.050	0.089	102
1	0.066	0.050	0.114	96
			Average	96

[a] Determined by the manual procedure. [b] One-half- and 1-ml samples were taken to keep the total calcium disodium edetate content at approximately the level in the contrast media being analyzed.

[1] Technicon Autoanalyzer Model I, Technicon Corp., Ardsley, N.Y.
[2] Beckman model DB.
[3] Technicon Corp., Ardsley, N.Y.

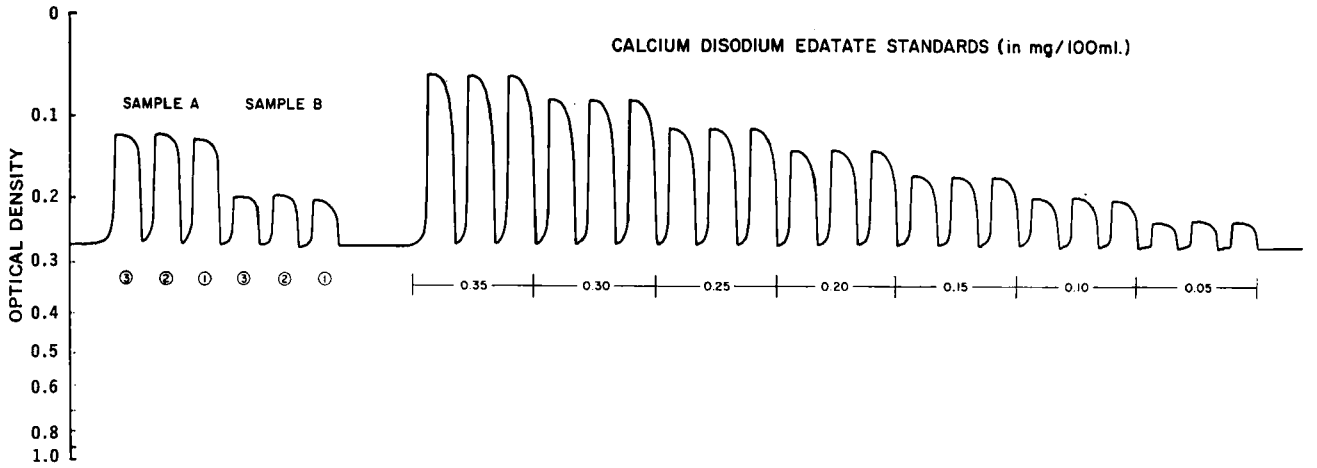

Figure 2—*Typical curves for the automated determination of calcium disodium edetate showing reproducibility of standards and actual readings of two different commercial parenterals on triplicate samples, individually prepared.*

Run the standards and sample solutions through the automated apparatus. Subtract the absorbances of the standards readings from the absorbance of the reagent blank baseline from the recorded peaks. Prepare a standard calibration curve by plotting the calculated absorbances *versus* the mg/100 ml of I taken for each standard. Calculate the milligrams per milliliter of I contained in a sample by dividing the milligrams read from a standard calibration curve by 2.

RESULTS AND DISCUSSION

Figure 2 shows a typical recording of a repeated series of standards. Typical curves for two commercial contrast media containing different levels of I are also shown in Fig. 2; triplicate samples, individually prepared, were used. Results of day-to-day reproducibility analyses are shown in Table I. A statistical evaluation of the results, from five replicates run on different days using four commercial products, indicates that the reproducibility at the 95% confidence level for a single determination is 0.008 mg/ml I (at the 0.1-mg/ml level).

The method is useful on formulations consisting of differing matrixes (*e.g.*, prepared with meglumine, sodium hydroxide, or saline). Several parameters were evaluated to ascertain if there were any interferences with the test. The experiments revealed the following:

1. The pH has an effect on optimum color development and should be controlled between 0.3 and 0.5. Alternatively, pH can be between 0.2 and 1.0 provided the pH of the sample and that of the standard solutions are identical. There is little or no color developed at pH <0.2. Investigations above pH 1.0 were not made.

2. The I level cannot be greater than 0.4 mg/100 ml, where full bleaching is achieved. This amount is equivalent to about 0.2 mg/ml in a typical iodinated contrast media parenteral solution or about double the level customarily added.

3. The wavelengths of the absorbance maximum shift slightly with concentration. The optimum wavelength was 545 nm, where a linear plot for all concentrations was obtained. The curve does not generally pass through the origin.

4. A phosphate salt is often added to parenterals as a buffer. No interference was found with as much as 0.2 mg/100 ml of sodium phosphate added to standard solutions.

5. Meglumine has no noticeable effect or interference on color development or other parameters of the test.

6. Fluoride ion interferes by bleaching and color complexation. If fluoride is suspected to be present, it can be masked by reaction with magnesium chloride (10 ml of a 25% solution) at pH 9 and then acidification with phosphoric acid (5 ml) (5) prior to color reaction.

7. Recovery studies using the manual procedure show an overall recovery of 96% (Table II).

The FDA procedure (5) calls for the preparation of separate xylenol orange and zirconium solutions to be used separately just prior to color measurement in standard and sample solutions. In this investigation, it was found that the reagents can be mixed and used as a single reagent. The mixed reagent should age about 24 hr and then be filtered. The solution is stable for several weeks. The mixed reagent is used essentially as a reagent blank to establish a working baseline in the automated procedure. This method is convenient since the reaction in the test is a bleaching of the highly colored solution. When using the technique, a positive slope can be obtained.

The method is fast, simple, and reproducible. Twenty prepared samples per hour can be run. The procedure can be performed manually as well. The method has been applied to other types of samples (*e.g.*, saline and plasma) with adaptations to remove or mask interferences. Meglumine iothalamate USP, sodium iothalamate USP, and combination formulas containing meglumine and sodium iothalamates and sodium acetrizoate have been run.

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[x] To whom inquiries should be directed.