Photometric Microtitration of Catechols (Epinephrine)

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Catechols in a buffered aqueous solution may be quantitatively titrated with ferrous ethylenediamine sulfate standard solution. The photometric end point is determined at 530 mμ, the wavelength of maximum absorption for the purple complex formed between ferrous ion and catechols. Advantages over the U.S.P. assay for epinephrine are (*a*) sensitivity is increased and (*b*) an epinephrine reference standard is not needed.

IN THE ASSAY of dosage forms it is customary to seek a representative average sample by combining 20 or more dosage units. Occasionally, however, it is necessary to assay a single dosage unit or part of such a unit. As part of a project for the assay of single dosage units, a paper concerning anesthetics derived from aminobenzoic acid in cartridges for dental anesthesia was published in 1959 (1). The present paper proposes a method for assay of the catecholamine vasoconstrictor frequently present in such preparations.

The proposed method is based on the U.S.P. colorimetric epinephrine assay (2) which is adapted from a paper by Doty (3). In this method, epinephrine in a solution suitably buffered about pH 8.5 forms with ferrous ion a color having maximum absorbance at 530 mμ. Doty reports that the color is given by all catechols tested but not by phenols or by dihydroxy phenols in which the phenolic groups are not *ortho* to each other. The reaction is stoichiometric, 0.5 mole of ferrous ion being required per mole of catechol.

The U.S.P. requires about 200 mcg. of epinephrine in the sample. The color developed is compared with color developed in a similar manner with U.S.P. epinephrine bitartrate reference standard. By using a suitable standard, acceptable accuracy may be obtained with a sample as small as 50 mcg. When epinephrine is used as a vasoconstrictor in preparations for dental anesthesia, the recommended concentrations are 1:50,000 (20 mcg./ml.) or 1:100,000 (10 mcg./ml.). As high as 1:20,000 (50 mcg./ml.) are found in commercial preparations. Only at the highest concentration is the colorimetric method, as specified in the U.S.P., satisfactory for a 1-ml. sample.

METHOD

Reagents.—Epinephrine bitartrate reference solution, dissolve 18.2 mg. of U.S.P. epinephrine bitartrate reference standard plus 200 mg. $NaHSO_3$ in enough water to make 50.0 ml. Epinephrine working standard, dilute 5.00 ml. of epinephrine bitartrate reference solution with enough water to make 50.0 ml. (1:50,000). Buffer stock solution, dissolve 42 Gm. of $NaHCO_3$ and 50 Gm. of $KHCO_3$ in about 180 ml. of water, dissolve 37.5 Gm. of aminoacetic acid and 17 ml. of strong ammonia solution, U.S.P. (28% NH_3) in about 180 ml. of water, mix the solutions, and dilute to 500 ml. Buffer solution, pH 9.0, dilute 10 ml. of the buffer stock solution to 100 ml. Standard ferrous salt solution, dissolve 47.8 mg. of ferrous ethylenediamine sulfate $FeC_2H_4(NH_3)_2(SO_4)_2 \cdot 4H_2O$, standard of reference grade (G. Frederick Smith Chemical Co., 1867 McKinley Ave., Columbus, Ohio), 5 ml. 0.1 *N* hydrochloric acid, and 0.5 Gm. $NaHSO_3$ in water to make 100.0 ml. Each ml. contains 1.25 μm. of ferrous ion equivalent to 2.50 μm. of catechol.

Equipment.—Gilmont micropipet-buret, 0.1-ml. size (Manostat Corp., 20 N. Moore St., New York 3, N. Y.). Beckman B spectrophotometer with 1/4 in. wooden blocks in cell compartments to raise cells in light path and reduce required volume.

Procedure.—Pipet into the reference cell 1.00 ml. water, 1.0 ml. of buffer solution, and 0.5 ml. of diluent, and mix. Pipet into the sample cell 1.00 ml. of sample, 1.0 ml. of buffer solution, and 0.5 ml. of diluent, and mix. With the wavelength setting at 530 mμ and a sensitivity of 1, the slit width required for balancing the instrument with the reference solution in the light path is about 0.18 mm. Record the absorbance of the sample solution. Add at least three aliquots of standard ferrous salt solutions so that the total added is less than 50% of the volume calculated to be required at the end point, with no aliquot less than 0.005 ml., recording the absorbance of the sample after each addition. Add enough standard ferrous salt solution so that the total added is 1.5 times the amount calculated to be required by the sample, record the absorbance of the sample, then add at least two additional 0.01-ml. aliquots, recording the absorbance after each addition. Plot total volumes added *vs.* absorbance. The first set of points will define a line of positive slope; the second set, a horizontal line. The intersection of the two lines defines the end point (4). Calculate the amount of catechol present in the sample. A complete discussion of photometric titration, including typical curves, is given by Goddu and Hume (4).

Assays of commercial lots were run in the same manner using isopropyl alcohol as the diluent to keep the anesthetic bases in solution.

Results of assays are given in Tables I and II.

DISCUSSION

The maximum observed absorbance for 1:100,000 solutions was about 0.040 with 0.005-ml. increments of titrant producing increments in absorbance of about 0.007. The maximum observed absorbance for 1:50,000 solutions was about 0.080 with 0.01-ml. increments of titrant producing increments of absorbance of about 0.015. The maximum observed absorbance for 1:20,000 solution was about 0.190 with 0.02-ml. increments of titrant producing increments in absorbance of about 0.040. Despite the low photometric accuracy in each individual observation that might be expected at these low ab-

Received June 14, 1961, from the Department of Chemistry, School of Pharmacy, Temple University, Philadelphia 40, Pa.

Accepted for publication October 5, 1961.

Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.

TABLE I.—ASSAY OF ALIQUOTS OF REFERENCE SOLUTION

Series A. Water diluent	Epinephrine, mcg. Theory	Found	Recovery, %	
1	20.0	19.3	97	
2		19.4	97	
3		19.3	97	
4		19.0	95	
				Av. 19.2 mcg.
				S.D. 0.2 mcg.
Series B. Isopropyl alcohol diluent				
5		19.1	95	
6		20.4	102	
7		20.3	101	
				Av. 19.8 mcg.
				S.D. 0.8 mcg.
Series C. Acetone diluent				
8		19.7	99	
9		20.6	103	
				Av. 20.1 mcg.
				S.D. 0.7 mcg.

Average of 9 determinations 19.7 mcg., S.D. 0.5 mcg.

TABLE II.—ASSAY OF COMMERCIAL LOTS

Lot	Anesthetic Present	Claimed, mcg.	Found, mcg.	
A	2% Procaine · HCl (epinephrine 1:50,000)	20.0	22.2	
			22.0	
			22.4	
				Av. 22.2 mcg.
B	2% Lidocaine · HCl (epinephrine 1:100,000)	10.0	12.5	
			12.7	
			12.3	
			12.6	
				Av. 12.5 mcg.
				S.D. 0.2 mcg.
C	2% Lidocaine · HCl (epinephrine 1:100,000)	10.0	11.8	
			11.8	
			11.5	
				Av. 11.7 mcg.
				S.D. 0.2 mcg.
D[a]	2% Mepivacaine · HCl (epinephrine 1:20,000)	50.0	49.0	
			46.9	
			47.6	
				Av. 47.8 mcg.
				S.D. 1.3 mcg.

[a] 1-Nordefrin, an isomer of epinephrine, present as vasoconstrictor.

sorbance readings, the relative standard deviation of the results did not exceed 5% in any determination and was usually about 2%. It may be concluded, therefore, that the reproducibility of the assay using 10 mcg. of epinephrine in the sample is of about the same order as the U.S.P. photometric method using 200 mcg. of epinephrine in the sample.

The actual titration requires about 10 minutes. If preparation of solutions and calculations of results are included, three samples may be completed in an hour.

The method allows the smaller laboratory to assay all catechols without the necessity of maintaining a reference standard for each. The single ferrous salt standard is all that is required. The primary standard grade of ferrous ethylenediamine sulfate is stable and has an infinite shelf life. Both in stability and cost it is to be preferred to a catecholamine reference compound.

The proposed method also has served as a check on the U.S.P. epinephrine bitartrate reference standard. By comparison with the standard ferrous salt, the reference standard is not less than 99.5% pure.

REFERENCES

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