Microanalysis of Piperazine

HERMAN F. BECKMAN and LUTRELL FELDMAN

State Chemist Laboratory, Texas Agricultural Experiment Station, College Station, Tex.

A method of analysis for piperazine (diethylenediamine) which requires a minimum of time and sample handling is described. The material is easily extracted from animal feeds, and when the extract is suitably treated—a portion made alkaline and then reacted with 1,4-benzoquinone—a red-orange color is produced. The color reaction is specific for piperazine in feeds, as there are no related materials added to feeds that will interfere.

MEDICINALLY, piperazine has been used as an anthelmintic in humans and animals. Many uses have been developed and suggested for piperazine derivatives (*4*, *8*). Leng (*9*) describes an analytical method for determining piperazine in feeds, which, however, requires rather large samples and is too long for routine use. Cavett (*2*) and Perlmutter (*10*) investigated a method of analysis which uses steam distillation to isolate the piperazine and an acid medium for color development between the drug and *p*-quinone.

Several semimicromethods for the analysis of piperazine have been published, but all lack the sensitivity required for parts per million analysis. The use of chloroanil (*3*) (tetrachloro-1,4-benzoquinone) and *p*-quinone (*6*) has been reported for the identification of the presence of certain amines.

Reactions between carbonyl compounds and certain amines are discussed by Fuson (*7*). The necessary ingredients for such reactions appear to be an amine, an alkali, and a carbonyl compound. Some of the products are colored. In the case of *p*-quinone and piperazine, the quinoidation is enhanced by the alkaline medium and a colored complex is formed. The reaction of a quinone with an amine is also analogous to that described by Feinstein (*5*) for pyrethrins and allethrin.

Experimental

The color reaction of *p*-quinone with piperazine was found to be quantitative if certain precautions and techniques were followed.

Piperazine and its salts are highly water-soluble; hence this approach was tried first. However, a dilute acid would remove piperazine from feeds much better than water alone. Settling or centrifugation failed to give a truly clear solution for further use. Filtering the aqueous extract through a mat of Super-Cel gave a clear solution. A suitable aliquot could then be taken for color development. The color reagent is prepared largely as described by Foucry (*6*). The *p*-benzoquinone is purified by steam distillation under a hood. Then, 0.10 gram of the compound is dissolved in 2.5 ml. of glacial acetic acid in a 100-ml. volumetric flask and diluted to the mark with 95% ethyl alcohol. This solution should be freshly prepared each time.

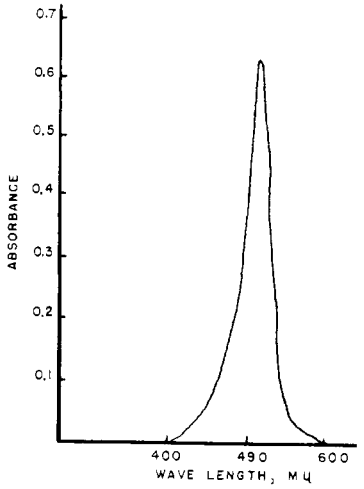

Figure 1. Absorption curve for piperazine

The concentration of potassium hydroxide solution chosen for use in the procedure provides a sufficient excess of alkali to catalyze the reaction between the piperazine and the quinone and maintain an alkaline pH.

Method

Standard Curves. Prepare a standard solution of piperazine by weighing 200 mg. of pure piperazine, or the equivalent of its salt, into a 100-ml. volumetric flask. Dilute to volume with distilled water and mix well. Transfer 0-, 5-, 10-, 15-, and 20-ml. aliquots to 100-ml. volumetric flasks; to each add 0.3 ml. of 12*N* sulfuric acid. Dilute each to volume with distilled water and mix well. Transfer a 20-ml. aliquot from each to a 200-ml. volumetric flask and dilute to volume with distilled water and mix well. Take a 5.0-ml. aliquot from each of these and place it in a test tube. Add 5.0 ml. of the quinone reagent to each tube and mix. Add 1.0 ml. of 1*N* potassium hydroxide in 50% ethyl alcohol to each tube and again mix. Heat the tubes in an 80° C. water bath for 10 minutes. Remove the tubes from the water bath and place them in a larger beaker of cold water. As soon as the tubes have cooled to room temperature, determine the absorbance of each sample. Use the zero concentration tube for a blank. The spectrophotometer is set at 490 mμ for maximum absorbance.

To prepare a standard curve in the

Table I. Recovery of Piperazine Added to Blank Feed

Piperazine, γ			
Added		Recovered	Recovery, %
50		54	108
		56	112
		48	96
		49	98
		52	104
	Av.	51.8	103.6
100		97	97
		97	97
		99	99
		99	99
	Av.	98	98.0
150		154	103
		154	103
		146	97
		147	98
	Av.	150.2	100.1
200		203	102
		200	100
		196	98
		193	96
	Av.	198	99.0
250		250	100
		250	100
		243	97
		253	101
	Av.	249	99.6

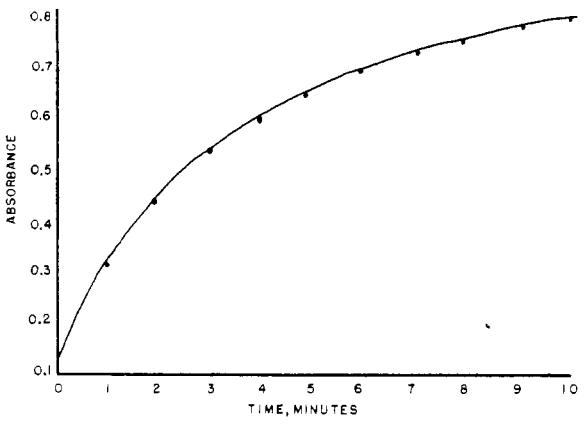

Figure 2. Effect of time on color concentration, 80° C.

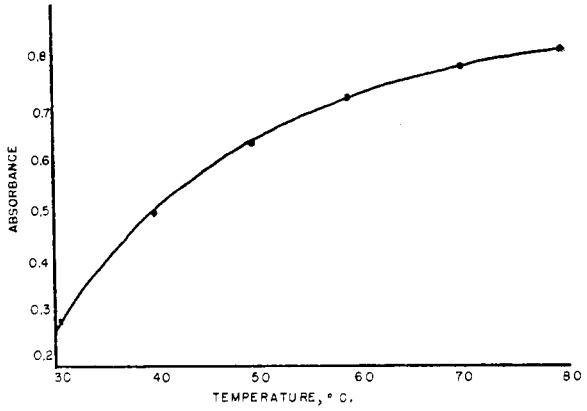

Figure 3. Effect of temperature on color concentration, 10 minutes

presence of feeds, weigh 10.0-gram samples of a blank feed into six 250-ml. Erlenmeyer flasks. A suitable blank feed should be prepared as has been previously described (*1*). Add 0-, 5-, 10-, 15-, and 20-ml. aliquots of the standard piperazine solution to the flasks. Add 0.3 ml. of 12*N* sulfuric acid to each and add sufficient distilled water to each flask to total 100 ml. of liquid. Shake the flasks intermittently for 30 minutes.

Allow the heavy particles to settle. During the settling period (10 to 15 minutes), fit a Büchner funnel into each of six 250-ml. suction flasks. Add a filter paper (S & S No. 597) to each of the funnels and cover the paper with a $^1/_4$-inch layer of Hyflo Super-Cel. Spread the Super-Cel evenly and apply suction. Pour a few milliliters of the extract from each flask over the Super-Cel mats taking care to wet them all over. Release the suction and discard the filtrate. Reconnect the flasks and suction, and pour the balance of the extract onto the mat. The filtrate must be clear; refilter if it is not. Take a 20.0-ml. aliquot from each filter flask and transfer to 200-ml. volumetric flasks, diluting to the mark with distilled water. After mixing, transfer a 5.0-ml. aliquot to a test tube and follow directions under Standard Curves beginning with ". . . add 5.0 ml. of the quinone reagent . . ."

Analysis of Samples. Weigh feed samples containing piperazine in such a way that the final solution in the determination will contain approximately 100 γ of piperazine. For feeds containing 0.05% piperazine, weigh 10.0-gram samples. To ensure reliability of results, run a reagent blank with each set of samples and use it as the blank for setting the spectrophotometer. The analysis of samples follows the preparation of a standard curve in the presence of feed, omitting the addition of the standard piperazine solution.

Results and Discussion

To test the method for applicability, several effects were studied. Feeds with varying amounts of protein and fat, as well as specific ingredients such as corn, soybean meal, cottonseed meal, meat scraps, fishmeal, alfalfa, feathers, and milk by-products, were analyzed against the laboratory prepared blank. No interference was found from any of the above listed ingredients. Recoveries of piperazine added to the above feeding ingredients ranged between 98.0 and 101.0%. The presence of medicaments such as nicarbazine, arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid, sulfaquinoxaline, and furazolidone in feeds does not interfere with the analysis. The data recorded in Table I show that piperazine added in solution in graded amounts to a blank feed may be recovered nearly quantitatively. Figure 1 shows the shape of the absorption curve using a solution containing 100 γ of piperazine in the final volume of 11.0 ml. Figures 2 and 3 show the effects of time and temperature on color development of solutions containing 150 γ of piperazine in the final aliquot and indicate that a maximum is approached at 10 minutes at 80° C. This time and temperature must be carefully controlled in the analysis of samples.

The procedure described requires a minimum of time for analysis and a minimum of sample handling. One point in the procedure requires careful attention: The temperature must be carefully controlled and the time of heating must be exact. Experiments have shown that the color increases with both heat (up to the boiling point of the alcohol) and time. The choice of the 10-minute heating period and 80° C. temperature is explained by examination of Figures 2 and 3, which show that a plateau is reached at this specific time and temperature. Heating beyond this time does not materially alter the color concentration, and a higher temperature would cause a loss of alcohol from the solution.

Experimental results were obtained using a Beckman DK-2 ratio-recording spectrophotometer. Other instruments whose wave length may be set at 490 mμ should be suitable for use.

The conditions described in the procedures and methods were maintained throughout the experimental phases of the work to develop the curves and to obtain the data presented. The alkali reagent, the experimental feed, and the quinone reagent used were equivalent for all the work reported here.

An $E^{1\%}_{1cm.}$ value of 590 has been obtained using the procedure described and Beer's law is followed over the concentration range used.

Literature Cited

(1) Beckman, H. F., J. Agr. Food Chem. **6,** 130 (1958).
(2) Cavett, J. W., Heotis, J. P., *J. Assoc. Offic. Agr. Chemists* **41,** 323 (1958).
(3) Crippa, A., *Ist. botan, univ. lab. crittogram., Pavia, Atti.* **10,** 173 (1953).
(4) Dow Chemical Co., Midland, Mich., ADC Inform. Bull. **106,** August 1956.
(5) Feinstein, L., *Science* **115,** 245 (1952).
(6) Foucry, M., *J. pharm. chim.* **20,** 116 (1934).
(7) Fuson, R. C., "Advances in Organic Chemistry," pp. 370 ff., Wiley, New York, 1950.
(8) Jefferson Chemical Co., Inc., Houston, Tex., Piperazine Tech. Bull., 1958.
(9) Leng, M. L., *J. Assoc. Offic. Agr. Chemists* **40,** 1059 (1957).
(10) Perlmutter, S. H., *Ibid.*, **41,** 506 (1958).

Received for review August 18, 1958. Accepted January 7, 1960. Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959. Approved as technical article No. 2909 by the Texas Agricultural Experiment Station as part of Project 1039.