TABLE II.—ASSAY OF LIPASE-CONTAINING DOSAGE FORMS

Dosage Form	U. Lipase per Dosage Form	U. Lipase[a] Titrimetric Assay (1)	U.[a] Turbidimetric
Lipase 3000 powder	3400 u./Gm.	3400	3400
Lipase 3000 powder	3000 u./Gm.	3000	3000
Enteric coated tablet A	120 u./tablet	110	108
Enteric coated tablet B	130 u./tablet	110	120
Capsules A	100 u./capsule	105	105
Capsules B	200 u./capsule	190	200
Capsules C	250 u./capsule	230	240
Powder paper A	100 u./paper	100	105
Powder paper B	200 u./paper	195	205
Powder paper C	250 u./paper	240	240

[a] Mean values ten assays.

known is inversely related to the half-time of the standards: ($T^1/_2$ unknown/$T^1/_2$ standard) = (concn. of standard/concn. of unknown). The following data are used to illustrate the assay.

A sample labeled to contain 200 u. of lipase was diluted to 200 ml. A 1-ml. sample of the above suspension produced a half-time of 20 minutes. The half-time for 1 u./ml. of standard enzyme is 24 minutes. Then (20/24) = (1/X) and X = 1.2 u./ml. of lipase.

RESULTS AND DISCUSSION

Several commercial dosage forms were evaluated as well as several lipase concentrates employed in the manufacture of commercial dosage forms. Capsules and powder papers were also prepared from the concentrates in this laboratory and checked by this assay procedure (see Table II).

The rate of disappearance of the substrate turbidity is proportional to the release of fatty acids when compared to the method of Lazo-Wasem. Good recoveries of enzyme have been found with errors generally under 5%, but never exceeding 10%.

The substrate remains stable for a period of 2–3 weeks at 25°. It is advised that each new substrate batch be standardized with a standard lipase preparation, although duplications between batches have been good.

In assaying tablets of lipase the complete tablet is crushed and triturated to a fine powder. The material is then washed into a volumetric flask where it is brought to volume. The preparation is then homogenized in a blender.

One should be cautioned about the assay of lipase in a digestant tablet containing pepsin. The pepsin coat must be washed off prior to assay since the pepsin enzyme will inactivate lipase.

Caution in washing glassware is essential since it has been observed that certain surfactants will affect the rate of enzyme activity.

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Sensitivity of Color Tests for Nitrites, Nitrates, and Glyceryl Trinitrate I. Solutions in Distilled Water

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Threshold concentrations detected (limens) for nitrites, nitrates, and glyceryl trinitrate have been determined for solutions in distilled water with 16 reagents. Generally the most sensitive tests detected 10 to 100 parts per million of these products; except chromotropic acid detected 2 parts per million of nitrite.

NITROGLYCERIN (glyceryl trinitrate) investigations require delicate methods for the detection and determination of nitrites, nitrates, and glyceryl trinitrate in tissue fluids, viscera, and pharmaceutical products. Some hundred reagents have been suggested in the literature (1–22) but our investigations showed that only a few considered specificity and threshold limits for determination. Results with these reagents indicated that sixteen showed promise, although close attention to details are required for successful use with them. Based on findings in testing solutions in distilled water reported in Table I, additional tests are under way for determinations in various pharmaceuticals, as well as tissue fluids and viscera.

Preparation of Test Solutions.—A concentrated solution was prepared by dissolving: (*a*) 1.50 Gm. of reagent grade sodium nitrite; (*b*) 1.37 Gm. of reagent grade sodium nitrate; and (*c*) 10 Gm. of an analyzed mixture of 1 part glyceryl trinitrate and 9 parts of beta lactose, in distilled water and making up each volume to 100 ml. Just before use each stock solution was diluted from 1% (10,000 parts per million) to 5,000, 2,000, 1,000, 100, and 10 parts per million for test. The sodium nitrite solution was further diluted to 2 p.p.m. for the test with chromotropic acid. If a negative test was obtained within 5 minutes using a concentration of 10,000 p.p.m., additional tests were not undertaken. Such negative results are indicated by (10,000) in Table I. Otherwise, the figures represent the limen (threshold concentration detected) for each product with the chosen reagent. The colors recorded are the final colors observed at the end of 5 to 10 minutes.

Preparation of Reagent Solutions.—Reagent chemicals were obtained from Eastman Kodak Co., Rochester, N. Y. Solutions were prepared at the

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TABLE I.—LIMITS OF SENSITIVITY OF COLOR REACTIONS, P.P.M.

Reagent, No.	Nitrate	Nitrite	Glyceryl Trinitrate
1. Antazoline	5,000 red-yellow	100 yellow	5,000 red-yellow
2. Benzidine	(10,000)[b]	10 yellow-brown	(10,000)
3. Chromotropic acid[a]	100 yellow[a]	2 yellow[a]	1,000
4. Diphenylamine	1,000 blue-purple	1,000 violet-blue-green	5,000 blue
5. Guaiacol	(10,000)	10,000 red-orange	(10,000)
6. Indole	10,000 pink	5,000 orange	5,000 pink
7. Michler ketone	10,000 yellow	5,000 yellow	10,000 yellow
8. Chicago acid	10,000 brown-orange	(10,000)	(10,000)
9. Amino G acid[a]	(10,000)	10 violet-blue[a]	5,000 violet-blue
10. Neutral red	(10,000)	5,000 red-orange	(10,000)
11. Phenol	(10,000)	1,000 yellow-brown	(10,000)
12. *m*-Phenylaminediamine	(10,000)	100 orange-brown	(10,000)
13. Resorcinol	(10,000)	100 brown	(10,000)
14. Thioglycolic acid	(10,000)	5,000 orange	(10,000)
15. Thiourea	(10,000)	10,000 red-brown	(10,000)
16. 2,4-Xylenol	1,000 yellow	(10,000)	100 yellow[a]

[a] Reagent most characteristic in distinguishing among nitrites, nitrates, and glyceryl trinitrate; also most sensitive. [b] (10,000) indicates negative reaction.

time of use, thus avoiding possible changes with aging. In general one drop of the test solution was added to one drop of the reagent solution on a spot plate and changes observed over a period up to 10 minutes. The reagent solutions were prepared as follows:

1. Antazoline; 2 - (N - Benzoylanilinemethyl)-imidazoline HCl.—Mix one drop of 1% aqueous solution with one drop of concentrated H_2SO_4.

2. Benzidine.—Prepare 1% solution in glacial acetic acid.

3. Chromotropic Acid: 4,5-Dihydroxy-2,7-naphthalene-disulfonic Acid.—Dissolve 0.5 Gm. in 100 ml. of concentrated H_2SO_4.

4. Diphenylamine.—Dissolve 1 Gm. in 100 ml. of concentrated H_2SO_4.

5. Guaiacol.—Dissolve 1 Gm. in 100 ml. of dilute H_2SO_4.

6. Indole.—Dissolve 15 mg. in 100 ml. of water. Just before use, add 1 ml. of this solution to 0.75 ml. of concentrated H_2SO_4.

7. Michlers Ketone; Tetramethyldiaminobenzophenone.—Dissolve 1 mg. in three drops of concentrated H_2SO_4, add about 1 mg. of test product; after standing 5 minutes add 1 ml. of distilled water.

8. Chicago Acid; S-Amino-1-naphthol-5,7-disulfonic Acid.—Dissolve 1 Gm. in 100 ml. of water.

9. Amino G Acid; 2-Naphthylamine-6,8-disulfonic Acid.—Dissolve 200 mg. in 100 ml. of water; mix one drop of reagent solution with one drop of test solution, then add one drop of 1% α-naphthylamine in dilute hydrochloric acid.

10. Neutral Red: Toluylene Red CI 825; Aminodimethylaminotoluaminozine HCl.—Dissolve 100 mg. in 100 ml. of alcohol.

11. Phenol.—Dissolve 10 Gm. in 100 ml. of water.

12. m-Phenylaminediamine.—Dissolve 100 mg. in 100 ml. of 10% acetic acid.

13. Resorcinol.—Dissolve 1 Gm. in 100 ml. of water, and add three drops of 10% aqueous solution of $FeCl_3$.

14. Thioglycolic Acid.—Dissolve 4 Gm. in 100 ml. of water.

15. Thiourea.—Dissolve 1 Gm. in 100 ml. of water. Mix one drop of test solution with one drop of reagent solution, then add one drop of 10% aqueous solution of $FeCl_3$.

16. 2,4-Xylenol.—Dissolve 100 mg. in alcohol; mix 0.1 ml. of this solution with 10 ml. of concentrated H_2SO_4.

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