

isomers that may be present as well as related compts., such as piperettine and piperlylin, that also absorb at 342–345 nm.

#### B. Apparatus and Reagents

(a) *UV spectrophotometer*.—Beckman A-25 (replacement instrument model DU-64), or equiv., with 1 cm sq. silica cuvetts.

(b) *Volumetric flasks*.—100 mL g.s., amber (No. 161-9057, Luxco Scientific). Amber flask is required to reduce photo-degradation of piperine in soln.

(c) *Erythronol dichloride*.—Reagent grade 1,2-dichloroethane (J.T. Baker, Inc., No. H0766). Make all dilns with this soln.

(d) *Piperone*.—Aldrich Chemical Co., No. P4,900-7.

#### C. Determination of Absorbance Factor

Weigh 0.1000 g piperine into 100 mL vol. flask, add ca 70 mL  $C_2H_2Cl_2$ , shake to dissolve, and dil. to vol. Pipet 10 mL into 100 mL vol. flask and dil. to vol. Into six 100 mL vol. flasks, pipet 1, 2, 3, 4, 5, and 6 mL aliquots, resp., and dil. each to vol. Zero spectrophot. with  $C_2H_2Cl_2$ , and read *A* of each final diln at max. 342–345 nm using UV light source and  $C_2H_2Cl_2$  in ref. cell. Divide each reading by mL used in final diln to convert *A* to concns of  $1/10^3$  ( $\mu\text{g}/\text{L}$ ). Det. av. of 6 values. Reciprocal of this av. is concn, mg/mL, of piperine required for *A* of 1.000, and is factor (*F*) used for calcs.

#### D. Procedure

(a) *Black and white pepper*.—Grind and screen sample to pass 30 mesh NIST sieve and blend uniformly. Accurately weigh ( $\pm 0.0001$  g) 0.5 g sample and transfer to 125 mL erlenmeyer. Protect from light. Add ca 70 mL  $C_2H_2Cl_2$ . Reflux and stir 1 h, cool to room temp., and filter quant. thru paper into 100 mL vol. flask. Transfer rest of exhd residue to filter, wash thoro., and dil. to vol. Pipet 2 mL of this soln into 100 mL vol. flask and dil. to vol. Record *A* at max. 342–345 nm.

(b) *Pepper essences*.—Accurately weigh ( $\pm 0.0001$  g) 1.0 g well mixed sample and transfer to 100 mL vol. flask. Dil. to vol. with  $C_2H_2Cl_2$ . Shake well until dissolved. Pipet 10 mL into 100 mL vol. flask and dil. to vol. Pipet 1 mL of this soln into 100 mL vol. flask and dil. to vol. Record *A* at max. 342–345 nm.

(c) *Soluble pepper seasonings*.—Accurately weigh ( $\pm 0.0001$  g) 2.0 g sample and transfer to 100 mL vol. flask. Add ca 70 mL  $C_2H_2Cl_2$  and let stand 20 min, swirling occasionally. Dil. to vol. and let settle. Pipet 1 mL into 100 mL vol. flask and dil. to vol. Record *A* at max. 342–345 nm.

#### E. Calculations

$$\% \text{ Piperine} = [(A_s \times F \times V)/(W_s \times 10^3)] \times 100$$

where  $A_s$  = absorbance of sample;  $F$  = factor derived from piperine std;  $V$  = diln vol., mL;  $W_s$  = sample wt., g.

Ref.: JAOAC 70, 112 (1987).

CAS-94-62-2 (piperine).

#### 920.170 Prepared Mustard Preparation of Sample Procedure

Transfer entire contents of container to dish large enough to permit thoro stirring and make whole mass homogeneous. Preserve in g-s bottle. Stir well each time before removing portion for analysis.

#### 920.171 Solids in Prepared Mustard Gravimetric Method Final Action

Weigh 5 g sample into flat-bottom Pt dish with approximately 5 g dry sand; distribute evenly over bottom of dish with little  $H_2O$ , place on steam bath until moist. appears dry, and heat in oven at 100° to min. wt.

#### 941.13 Chlorides (Total) in Prepared Mustard Titrimetric Method Final Action

Weigh 3–4 g sample from weighing bottle, place in 300 mL erlenmeyer, and add excess std 0.1*N*  $AgNO_3$  (usually 30 mL is enough). Mix thoro., add 15 mL  $HNO_3$ , and bring to boil on hot plate. To boiling mixt., add 15 mL 5%  $KMnO_4$  soln, 5 mL at a time, rotating flask after each addn to mix contents. Add ca 50 mL  $H_2O$  and filter into 200 mL vol. flask. Wash filter free of  $AgNO_3$  and dil. to vol. with  $H_2O$ . Mix thoro. and titr. 100 mL aliquot with 0.1*N*  $KSCN$ , using 2 mL std Fe alum soln as indicator. Calc. chlorides as  $NaCl$ .

Refs.: JAOAC 24, 703 (1941); 25, 97 (1942).

CAS-7647-14-5 (sodium chloride)

#### 920.172 Ether Extract of Prepared Mustard Gravimetric Method Final Action

(*Caution*: See safety notes on monitoring equipment, distillation, flammable solvents, and diethyl ether.)

Weigh 10 g sample into  $SiO_2$ , Al, or porcelain drying dish and mix with ca 30 g sand. Heat on  $H_2O$  bath until moist. appears dry; then finish drying in oven at 100°. Grind until all lumps are broken up, and det. ether ext by extg. 16 hr with anhyd. ether in Soxhlet extractor with Whatman single thickness or other close-texture thimble. Dry ext 30 min at 100°, cool, and weigh.

#### 920.173 Nitrogen (Total) in Prepared Mustard Improved Kjeldahl Method Final Action

Det. N as in 955.04C, using 5 g sample.

#### 920.174 Acidity of Prepared Mustard Titrimetric Method Final Action

Weigh 10 g sample into 200 mL vol. flask, dil. to vol. with  $H_2O$ , shake, filter thru dry paper, and det. acidity in 100 mL by titr. with 0.1*N* alkali, using phenols. Express result as HClAc. 1 mL 0.1*N* alkali = 0.0060 g HClAc.

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