isomers that may be present as well as related compds., such as piperettine and piperylin, that also absorb at 340–345 nm.

**B. Apparatus and Reagents**

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

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

(c) *Ethylene dichloride.*—Reagent grade 1,2-dichloroethane (J.T. Baker, Inc., No. H0763). Make all dilns with this solv.

(d) *Piperine.*—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$_2$H$_4$Cl$_2$, 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 spectrophtr with C$_2$H$_4$Cl$_2$, and read A of each final diln at max. 342–345 nm using UV light source and C$_2$H$_4$Cl$_2$ in ref. cell. Divide each reading by mL used in final diln to convert A to concns of 1/10$^6$ (µg/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 calcns.

**D. Procedure**

(a) *Black and white pepper.*—Grind and screen sample to pass 30 mesh NIST sieve and blend uniformly. Accurately weigh (± 0.0001 g) 0.5 g sample and transfer to 125 mL erlenmeyer. Protect from light. Add ca 70 mL C$_2$H$_4$Cl$_2$. Reflux and stir 1 h., cool to room temp., and filter quant. thru paper into 100 mL vol. flask. Transfer rest of extd residue to filter, wash thoroly, 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 oleoresin.*—Accurately weigh (± 0.0001 g) 1.0 g well mixed sample and transfer to 100 mL vol. flask. Dil. to vol. with C$_2$H$_4$Cl$_2$. 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 (± 0.0001 g) 2.0 g sample and transfer to 100 mL vol. flask. Add ca 70 mL C$_2$H$_4$Cl$_2$, 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**

$$\% \text{ Piperine} = [(A_S \times F \times V)/(W_s \times 10^6)] \times 100$$

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)

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**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$_2$O, place on steam bath until mixt. 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 0.1N AgNO$_3$ (usually 30 mL is enough). Mix thoroly, 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 time, rotating flask after each addn to mix contents. Add ca 50 mL H$_2$O and filter into 200 mL vol. flask. Wash fiber free of AgNO$_3$ and dil. to vol. with H$_2$O. Mix thoroly and titr. 100 mL aliquot with 0.1N KSCN, using 2 mL satd Fe alum soln as indicator. Calc. chlorides as NaCl.

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

CAS-7647-14-5 (sodium chloride)

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**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$_2$O bath until mixt. 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.

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

Weigh 10 g sample into 200 mL vol. flask, dil. to vol. with H$_2$O, shake, filter thru dry paper, and det. acidity in 100 mL by titrn with 0.1N alkali, using phtln. Express result as HOAc. 1 mL 0.1N alkali = 0.0060 g HOAc.

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**Table of Contents Aoac Methods Volume 2 Pdf Curious**

1. Understanding the eBook Aoac Methods Volume 2 Pdf Curious
    - The Rise of Digital Reading Aoac Methods Volume 2 Pdf Curious
    - Advantages of eBooks Over Traditional Books
2. Identifying Aoac Methods Volume 2 Pdf Curious
    - Exploring Different Genres
    - Considering Fiction vs. Non-Fiction
    - Determining Your Reading Goals
3. Choosing the Right eBook Platform
    - Popular eBook Platforms
    - Features to Look for in an Aoac Methods Volume 2 Pdf Curious
    - User-Friendly Interface

4. Exploring eBook Recommendations from Aoac Methods Volume 2 Pdf Curious
    ○ Personalized Recommendations
    ○ Aoac Methods Volume 2 Pdf Curious User Reviews and Ratings
    ○ Aoac Methods Volume 2 Pdf Curious and Bestseller Lists
5. Accessing Aoac Methods Volume 2 Pdf Curious Free and Paid eBooks
    ○ Aoac Methods Volume 2 Pdf Curious Public Domain eBooks
    ○ Aoac Methods Volume 2 Pdf Curious eBook Subscription Services
    ○ Aoac Methods Volume 2 Pdf Curious Budget-Friendly Options
6. Navigating Aoac Methods Volume 2 Pdf Curious eBook Formats
    ○ ePub, PDF, MOBI, and More
    ○ Aoac Methods Volume 2 Pdf Curious Compatibility with Devices
    ○ Aoac Methods Volume 2 Pdf Curious Enhanced eBook Features
7. Enhancing Your Reading Experience
    ○ Adjustable Fonts and Text Sizes of Aoac Methods Volume 2 Pdf Curious
    ○ Highlighting and Note-Taking Aoac Methods Volume 2 Pdf Curious
    ○ Interactive Elements Aoac Methods Volume 2 Pdf Curious
8. Staying Engaged with Aoac Methods Volume 2 Pdf Curious
    ○ Joining Online Reading Communities
    ○ Participating in Virtual Book Clubs
    ○ Following Authors and Publishers Aoac Methods Volume 2 Pdf Curious
9. Balancing eBooks and Physical Books Aoac Methods Volume 2 Pdf Curious
    ○ Benefits of a Digital Library
    ○ Creating a Diverse Reading Collection Aoac Methods Volume 2 Pdf Curious
10. Overcoming Reading Challenges
    ○ Dealing with Digital Eye Strain
    ○ Minimizing Distractions
    ○ Managing Screen Time
11. Cultivating a Reading Routine Aoac Methods Volume 2 Pdf Curious
    ○ Setting Reading Goals Aoac Methods Volume 2 Pdf Curious
    ○ Carving Out Dedicated Reading Time

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