

LAMPIRAN

Lampiran 1 Metode FSSAI (2016)

Manual of Methods of Analysis of Foods (Food Additives)

- Qualitative Methods: Ferric Chloride Test

Acidify the food product with hydrochloric acid (1+3) and extract with diethyl ether. Evaporate the solvent on a hot water bath removing last traces of solvent under a current of air. Dissolve the residue in few mL of hot water and add few drops of 0.5% ferric chloride solution. Salmon colour precipitate of ferric benzoate indicates the presence of benzoic acid.

Lampiran 2 Metode AOAC 960.38 (2000)

AOAC 17th (2000) official method 960. 38 Benzoic acid in nonsolid food and beverages- spectrophotometric method.

- Principle:

Benzoic acid is extracted from prepared sample using diethyl ether and the absorbance of the ether layer is measured at 272 nm, 267.5 nm and 276.5 nm in the UV region. From the corrected absorbance and the calibration graph obtained using standard benzoic' acid solution, the amount of benzoic acid is determined.

- Reagents:

1. Diethyl ether distilled
2. Hydrochloric acid (1+3)
3. Saturated sodium chloride solution
4. Ammonium hydroxide (0.1%)
5. Standard benzoic acid

- Procedure:

Preparation of standard curve:

Prepare solution of benzoic acid in ether containing 50 mg/l. Determine absorbance of this solution in tightly stoppered cell in Beckman DU or recording spectrophotometer between 265 and 280 nm at 1 nm

intervals. Plot absorbance against wavelength and record wavelength of minimum at approximately 267.5 nm as point B. Other minimum at approximately 276.5 nm as point D and highest maximum at approximately 272 nm as point C.

Prepare solution of benzoic acid in ether containing 20, 40, 60, 80, 100 and 120 mg/L. Determine absorbance of these solutions in a spectrophotometer at points B, C and D. For each concentration average absorbance at Band D subtract from absorbance at C. Plot difference against concentration to get the standard curve.

- Preparation of sample:

Mix sample thoroughly. Transfer 10 gm or 10 mL to separator and dilute to 200 mL with saturated sodium chloride solution. Make solution definitely acidic to litmus with hydrochloric acid and mix well.

- Determination:

Extract prepared solutions with 70, 50, 40, and 30 mL portions of diethyl ether, shaking well to ensure complete extraction (break emulsions by standing, stirring or centrifuging). Drain and discard aqueous phase. Wash combined ether extracts with 40 and 30 mL portions hydrochloric acid (1+1000) and discard hydrochloric acid washings (if extraction requires no purification, proceed to next para). Extract ether solution with 50, 40, 30, and 20 mL portions of 0.1% ammonium hydroxide and discard ether. Neutralize combined ammonium hydroxide extracts with hydrochloric acid and add 1 mL excess. Extract the acidified solution with 70, 50, 40 and 30 mL ether.

Dilute combined ether extracts to 200 mL with ether and determine absorbance in stoppered cell in spectrophotometer at wavelengths B, C and D, diluting with ether if necessary to obtain optimum concentration of 20-120 mg/L. Average the absorbance's at B and D, subtract this value from absorbance at C. Determine the concentration of benzoic acid from standard curve correcting for dilutions.

Benzoic acid x 1.18 = Sodium benzoate.

Lampiran 3 Prosedur yang berubah

1) Prosedur Sampel 4 dan Sampel 5

Ditimbang sampel sebanyak 1 gram dan dimasukkan dalam gelas beaker 100 mL, ditambahkan dengan 20 mL NaCl jenuh dan dilarutkan. Ditambahkan HCl 1M hingga larutan bersifat asam (uji dengan kertas lakmus). Dimasukkan dalam corong pisah dan dilakukan ekstraksi dengan dietil eter sebanyak 7,5 mL. Terbentuk 2 lapisan yaitu lapisan eter dan lapisan air. Ekstrak eter dimasukkan dalam corong pemisah dan dicuci dengan 5 mL HCl 0,1 %. Lapisan bawah dibuang dan lapisan atas dicuci kembali dengan 4 mL HCl 0,1% dan seterusnya dilakukan pencucian kembali dengan 3 mL HCl 0,1%. Ekstrak eter dimasukkan dalam labu ukur 50 mL dan ditanda bataskan dengan etanol 70% lalu dihomogenkan. Larutan diuapkan diatas penangas air dengan suhu 80°C dalam lemari asam, residu yang diperoleh dilarutkan dengan etanol p.a. lalu dimasukkan labu ukur 50 mL dan ditanda bataskan dengan etanol p.a. Dipipet larutan sampel sebanyak 5 mL, dimasukkan dalam labu ukur 10 mL dan ditanda bataskan dengan etanol p.a. Selanjutnya larutan diukur absorbansinya dengan spektrofotometer UV-Vis pada Panjang gelombang maksimum

2) Prosedur Sampel 6

Ditimbang sampel sebanyak 1 gram dan dimasukkan dalam gelas beaker 100 mL, ditambahkan dengan 20 mL NaCl jenuh dan dilarutkan. Ditambahkan HCl 1M hingga larutan bersifat asam (uji dengan kertas lakmus). Dimasukkan dalam corong pisah dan dilakukan ekstraksi dengan dietil eter sebanyak 7,5 mL. Terbentuk 2 lapisan yaitu lapisan eter dan lapisan air. Ekstrak eter dimasukkan dalam corong pemisah dan dicuci dengan 5 mL HCl 0,1 %. Lapisan bawah dibuang dan lapisan atas dicuci kembali dengan 4 mL HCl 0,1% dan seterusnya dilakukan pencucian kembali dengan 3 mL HCl 0,1%. Ekstrak eter dimasukkan dalam labu ukur 50 mL dan ditanda bataskan dengan etanol 70% lalu dihomogenkan. Larutan diuapkan diatas penangas air dengan suhu 80°C dalam lemari asam, residu yang diperoleh dilarutkan dengan etanol p.a. lalu dimasukkan labu ukur 50 mL dan ditanda bataskan dengan etanol p.a. Dipipet larutan sampel sebanyak 2,5 mL, dimasukkan dalam labu ukur 10 mL dan

ditanda bataskan dengan etanol p.a. Selanjutnya larutan diukur absorbansinya dengan spektrofotometer UV-Vis pada Panjang gelombang maksimum

Lampiran 4 Perhitungan

- **Perhitungan konsentrasi asam benzoat dalam sampel**

1) Sampel 1

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,743

$$y = 0,0908x + 0,0077$$

$$0,743 = 0,0908x + 0,0077$$

$$x = \frac{0,743 - 0,0077}{0,0908}$$

$$x = 8,09802 \text{ ppm}$$

2) Sampel 2

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,206

$$y = 0,0908x + 0,0077$$

$$0,206 = 0,0908x + 0,0077$$

$$x = \frac{0,206 - 0,0077}{0,0908}$$

$$x = 2,18392 \text{ ppm}$$

3) Sampel 3

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,718

$$y = 0,0908x + 0,0077$$

$$0,718 = 0,0908x + 0,0077$$

$$x = \frac{0,718 - 0,0077}{0,0908}$$

$$x = 7,82269 \text{ ppm}$$

4) Sampel 4

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,588

$$y = 0,0908x + 0,0077$$

$$0,588 = 0,0908x + 0,0077$$

$$x = \frac{0,588 - 0,0077}{0,0908}$$

$$x = 6,39097 \text{ ppm}$$

5) Sampel 5

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,716

$$y = 0,0908x + 0,0077$$

$$0,716 = 0,0908x + 0,0077$$

$$x = \frac{0,716 - 0,0077}{0,0908}$$

$$x = 7,80066 \text{ ppm}$$

6) Sampel 6

Diketahui:

persamaan garis linear : $y = 0,0908x + 0,0077$

absorbansi : 0,783

$$y = 0,0908x + 0,0077$$

$$0,783 = 0,0908x + 0,0077$$

$$x = \frac{0,783 - 0,0077}{0,0908}$$

$$x = 8,53855 \text{ ppm}$$

• **Perhitungan kadar asam benzoat dalam sampel**

1) Sampel 1

Diketahui: C : 8,09802 mg/L

V : 0,05 L

Fp 1

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{8,09802 \text{ mg/L} \times 0,05 \text{ L} \times 1}{0,001 \text{ kg}}$$

$$kadar = 404,901 \text{ mg/kg}$$

2) Sampel 2

Diketahui: C : 2,18392 mg/L

V : 0,05 L

Fp 1

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{2,18392 \text{ mg/L} \times 0,05 \text{ L} \times 1}{0,001 \text{ kg}}$$

$$kadar = 109,196 \text{ mg/kg}$$

3) Sampel 3

Diketahui: C : 7,82269 mg/L

V : 0,05 L

Fp 1

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{7,82269 \text{ mg/L} \times 0,05 \text{ L} \times 1}{0,001 \text{ kg}}$$

$$kadar = 391,1345 \text{ mg/kg}$$

4) Sampel 4

Diketahui: C : 6,39097 mg/L

V : 0,05 L

Fp : 10/5 = 2

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{6,39097 \text{ mg/L} \times 0,05\text{L} \times 2}{0,001 \text{ kg}}$$

$$kadar = 639,097 \text{ mg/kg}$$

5) Sampel 5

Diketahui: C : 7,80066 mg/L

V : 0,05 L

Fp : 10/5 = 2

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{7,80066 \text{ mg/L} \times 0,05\text{L} \times 2}{0,001 \text{ kg}}$$

$$kadar = 780,066 \text{ mg/kg}$$

6) Sampel 6

Diketahui: C : 8,53855 mg/L

V : 0,05 L

Fp : 10/2,5 = 4

W : 0,001 kg

$$kadar = \frac{C \times V \times Fp}{W}$$

$$kadar = \frac{8,53855 \text{ mg/L} \times 0,05\text{L} \times 4}{0,001 \text{ kg}}$$

$$kadar = 1707,71 \text{ mg/kg}$$

- **Kadar natrium benzoat pada sampel**

1) Sampel 1

Diketahui: kadar asam benzoat pada sampel : 404,901 mg/kg

BM natrium benzoat : 144,11

BM asam benzoat : 122,12

$$\text{kadar Na. benzoat} = \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}}$$

$$\text{kadar Na. benzoat} = 404,901 \times \frac{144,11}{122,12}$$

$$\text{kadar Na. benzoat} = 477,81103 \text{ mg/kg}$$

2) Sampel 2

Diketahui: kadar asam benzoat pada sampel : 109,196 mg/kg

BM natrium benzoat : 144,11

BM asam benzoat : 122,12

$$\text{kadar Na. benzoat} = \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}}$$

$$\text{kadar Na. benzoat} = 109,196 \times \frac{144,11}{122,12}$$

$$\text{kadar Na. benzoat} = 128,85879 \text{ mg/kg}$$

3) Sampel 3

Diketahui: kadar asam benzoat pada sampel : 391,1345 mg/kg

BM natrium benzoat : 144,11

BM asam benzoat : 122,12

$$\text{kadar Na. benzoat} = \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}}$$

$$\text{kadar Na. benzoat} = 391,1345 \times \frac{144,11}{122,12}$$

$$\text{kadar Na. benzoat} = 461,56561 \text{ mg/kg}$$

4) Sampel 4

Diketahui: kadar asam benzoat pada sampel : 639,097 mg/kg

BM natrium benzoat : 144,11

$$\begin{aligned}
 & \text{BM asam benzoat} && : 122,12 \\
 \text{kadar Na. benzoat} &= \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}} \\
 \text{kadar Na. benzoat} &= 639,097 \times \frac{144,11}{122,12} \\
 \text{kadar Na. benzoat} &= 754,17842 \text{ mg/kg}
 \end{aligned}$$

5) Sampel 5






$$\begin{aligned}
 \text{Diketahui: kadar asam benzoat pada sampel} & : 780,066 \text{ mg/kg} \\
 & \text{BM natrium benzoat} && : 144,11 \\
 & \text{BM asam benzoat} && : 122,12 \\
 \text{kadar Na. benzoat} &= \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}} \\
 \text{kadar Na. benzoat} &= 780,066 \times \frac{144,11}{122,12} \\
 \text{kadar Na. benzoat} &= 920,53154 \text{ mg/kg}
 \end{aligned}$$






6) Sampel 6

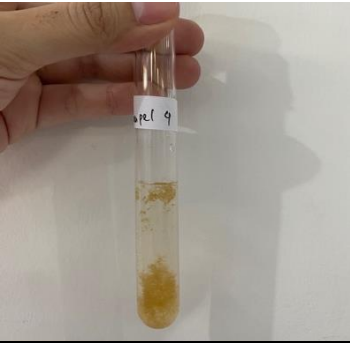


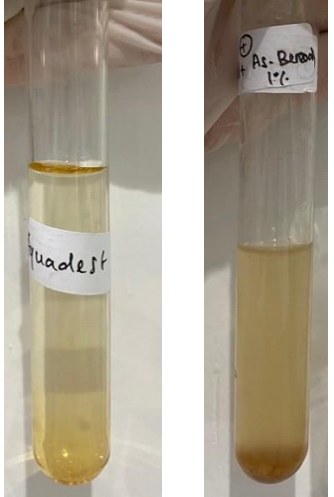
$$\begin{aligned}
 \text{Diketahui: kadar asam benzoat pada sampel} & : 1707,71 \text{ mg/kg} \\
 & \text{BM natrium benzoat} && : 144,11 \\
 & \text{BM asam benzoat} && : 122,12 \\
 \text{kadar Na. benzoat} &= \text{kadar As. benzoat} \times \frac{\text{BM Na. benzoat}}{\text{BM As. benzoat}} \\
 \text{kadar Na. benzoat} &= 1707,71 \times \frac{144,11}{122,12} \\
 \text{kadar Na. benzoat} &= 2015,21526 \text{ mg/kg}
 \end{aligned}$$



Lampiran 5 Dokumentasi Penelitian

	Sampel 1
	Sampel 2
	Sampel 3
	Sampel 4

	<p>Sampel 5</p>
	<p>Sampel 6</p>
	<p>Penimbangan sampel untuk preparasi uji kualitatif</p>
	<p>Sampel setelah penambahan NaOH 10%</p>
	<p>Penyaringan larutan sampel</p>

	<p>Ekstraksi larutan sampel</p>
	<p>Penguapan larutan sampel di atas penangas air</p>
	<p>Hasil uji kualitatif sampel 1</p>
	<p>Hasil uji kualitatif sampel 2</p>
	<p>Hasil uji kualitatif sampel 3</p>

	<p>Hasil uji kualitatif sampel 4</p>
	<p>Hasil uji kualitatif sampel 5</p>
	<p>Hasil uji kualitatif sampel 6</p>
	<p>Pembandingan kontrol negatif (aquadest) dan kontrol positif (sampel+asam benzoat)</p>

	<p>Larutan baku induk</p>
	<p>Larutan sampel</p>