

ETHYL LAUROYL ARGINATE

New specifications prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw was established at the 69th JECFA (2008).

SYNONYMS Lauric arginate ethyl ester, lauramide arginine ethyl ester, ethyl-N^α-lauroyl-L-arginate-HCl, LAE, INS No. 243

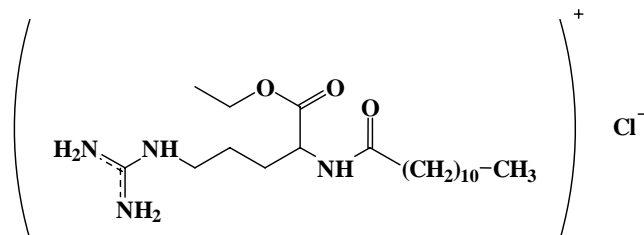
DEFINITION Ethyl lauroyl arginate is synthesised by esterifying arginine with ethanol, followed by reacting the ester with lauroyl chloride. The resultant ethyl lauroyl arginate is recovered as hydrochloride salt and is a white, solid product which is filtered off and dried.

Chemical name Ethyl-N^α-dodecanoyl-L-arginate-HCl

C.A.S. number 60372-77-2

Chemical formula C₂₀H₄₁N₄O₃Cl

Structural formula



Formula weight 421.02

Assay Not less than 85% and not more than 95%

DESCRIPTION White powder

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

pH (Vol.4) 3.0-5.0 (1% solution)

Solubility (Vol. 4) Freely soluble in water, ethanol, propylene glycol and glycerol

Chromatography The retention time for the major peak in a HPLC chromatogram of the sample is approx. 4.3 min using the conditions described in the Method of Assay.

PURITY

<u>Total ash</u> (Vol. 4)	Not more than 2% (700°)
<u>Water</u> (Vol. 4)	Not more than 5% (Karl Fischer Titrimetric Method, "General Methods, Inorganic Components")
<u>N^α-Lauroyl-L-arginine</u>	Not more than 3% See description under TESTS
<u>Lauric acid</u>	Not more than 5% See description under TESTS
<u>Ethyl laurate</u>	Not more than 3% See description under TESTS
<u>L-Arginine·HCl</u>	Not more than 1% See description under TESTS
<u>Ethyl arginate·2HCl</u>	Not more than 1% See description under TESTS
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

N^α-Lauroyl-L-arginine Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the conditions described in the Method of Assay. NOTE: The retention time of N^α-lauroyl-L-arginine is approx. 2.2 min.

Calculate the percentage of N^α-lauroyl-L-arginine in the test sample as follows:

$$\% \text{ N}^{\alpha}\text{-Lauroyl-L-arginine} = \frac{C (\mu\text{g/ml}) \times 50 (\text{ml})}{W (\text{mg}) \times 1000} \times 100$$

where:

C= N^α-lauroyl-L-arginine concentration detected (μg/ml)

W= weight of sample (mg)

Lauric acid and ethyl laurate

Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the following conditions.

Chromatography

Liquid chromatograph equipped with a spectrophotometric detector.
Column: Symmetry C18, 150 x 3.9 mm, 5μm (Waters) or equivalent

Column temperature: room temperature
Mobile phase: acetonitrile/water (85:15) containing 0.1% trifluoroacetic acid
Flow rate: 1 ml/min
Wavelength: 212 nm
Injection volume: 10 µl

Standard solution

Weigh accurately about 125 mg of lauric acid standard and 75 mg ethyl laurate standard into a 50-ml volumetric flask. Dissolve and dilute with the mobile phase to obtain a solution of about 2500 µg/ml of lauric acid and 1500 µg/ml of ethyl laurate. Take 5, 10 and 15 ml of the solution and dilute to 50 ml with mobile phase for the standard curves.

Sample solution

Weigh accurately about 500 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure

Inject the standard and sample solutions into the chromatograph and measure their concentration (C µg/ml) from their peak area and their standard curves.
NOTE: The retention time of lauric acid is approx. 3.65 min and that of ethyl laurate is approx. 11.2 min.

Calculate their percentage in the test sample as follows:

$$\% \text{ Lauric acid or ethyl laurate} = \frac{C (\mu\text{g/ml}) \times 50 (\text{ml})}{W (\text{mg}) \times 1000} \times 100$$

where:

C= lauric acid or ethyl laurate concentration detected (µg/ml)
W= weight of sample (mg)

L-Arginine·HCl and ethyl arginate·2HCl

Determine by HPLC in Volume 4 (under “Analytical Techniques, Chromatography”) using the following conditions:
NOTE: Use deionized water

Chromatography

Liquid chromatograph equipped with a post-column derivatization and a spectrophotometric detector.

Column and packing: µ Bondapak C18, 300 x 3.9 mm, 10µm (Waters) or equivalent

Mobile phase: A-B-C-D (1:1:1:1.5)

A: 15 mmole/l sodium heptanesulphonate, B: 27 mmole/l phosphoric acid solution, C: 3 mmole/l sodium di-hydrogen phosphate solution, D: methanol

Flow rate: 0.8 ml/min

Flow rate of reagent solution: 0.8 ml/min

Column temperature: 65°

Wavelength: 340 nm

Injection volume: 10 µl

Standard solution

L-Arginine·HCl: Weigh accurately about 40 mg of L-arginine·HCl standard into

a 100-ml volumetric flask. Dissolve and dilute to 100 ml with water to obtain a solution of about 400 µg/ml of L-arginine-HCl.

Ethyl arginate-2HCl: Weigh accurately about 40 mg of ethyl arginate-2HCl standard into a 100-ml volumetric flask. Dissolve and dilute to 100 ml with water to obtain a solution of about 400 µg/ml of ethyl arginate-2HCl.

Take 2, 4, 6 and 8 ml of each solution and dilute to 25 ml with mobile phase separately for the standard curves.

Sample solution

Weigh accurately about 200 mg of test sample into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water.

Derivatizing solution

Mix 1 liter of 0.2M borate buffer solution (pH 9.4) with 0.8 g of o-phthalaldehyde dissolved in 5 ml of methanol and 2 ml of 2-mercaptoethanol. The solution is stable 48 h at room temperature and without additional preventive measure but It is advisable to keep the solution under nitrogen and to prepare it freshly every 24-48 h.

Procedure

Inject the standard and sample solutions into the chromatograph and measure the area of the peak.

NOTE: The retention time of L-arginine-HCl is approx. 5.03 min and ethyl arginate-2HCl is approx. 6.70 min.

Calculate the percentage of L-arginine-HCl and ethyl arginate-2HCl in the test sample as follows:

$$\% \text{ L-Arginine-HCl or ethyl arginate-2HCl} = \frac{C (\mu\text{g/ml}) \times 50 (\text{ml})}{W (\text{mg}) \times 1000} \times 100$$

where:

C= L-arginine-HCl and ethyl arginate-2HCl concentration detected (µg/ml)

W= weight of sample (mg)

METHOD OF ASSAY Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the following conditions:

NOTE: Use deionized water

Standards

Ethyl-N^α-lauroyl-L-arginate-HCl standard

N^α-lauroyl-L-arginine standard

(available from Laboratorios Miret, S.A, Géminis 4, Políg. Ind. Can Parellada, 08228 Terrassa, Spain)

Chromatography

Liquid chromatograph equipped with a spectrophotometric detector.

Column and packing: Symmetry C18, 150 x 3.9 mm, 5µm (Waters) or equivalent

Column temperature: room temperature

Mobile phase: acetonitrile/water (50:50) containing 0.1% trifluoroacetic acid

Flow rate: 1 ml/min
Wavelength: 215 nm
Injection volume: 10 µl

Standard solution

Weigh accurately about 25 mg of N^α-lauroyl-L-arginine standard into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with mobile phase (solution A). Weigh accurately about 150 mg of ethyl-N^α-lauroyl-L-arginate·HCl standard into a 50-ml volumetric flask and dissolve with some milliliters of the mobile phase. Then, add 5 ml of solution A and dilute to 50 ml with mobile phase to obtain a solution of about 3000 µg/ml of ethyl-N^α-lauroyl-L-arginate·HCl and 100 µg/ml of N^α-lauroyl-L-arginine (solution B). Take 2, 4, 6, 8 and 10 ml of solution B and dilute to 25 ml with mobile phase for the standard curves.

Sample solution

Weigh accurately about 50 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure

Inject the standard and sample solutions into the chromatograph and measure the area of the peak.

Note: The retention time of ethyl-N^α-lauroyl-L-arginate·HCl is approx. 4.3 min.

Calculate the percentage of ethyl-N^α-lauroyl-L-arginate·HCl in the test sample as follows:

$$\% \text{ Ethyl-N}^{\alpha}\text{-lauroyl-L-arginate}\cdot\text{HCl} = \frac{C (\mu\text{g/ml}) \times 50 (\text{ml})}{W (\text{mg}) \times 1000} \times 100$$

where:

C= ethyl-N^α-lauroyl-L-arginate·HCl concentration detected (µg/ml)

W= weight of sample (mg)