ANALYSIS OF ETHYL-N$^{\alpha}$-LAUROYL-L-ARGINATE HCl IN LIQUID MATRICES

DOCUMENT No.
ID-11-2494
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REVISION:0

1. BASIS

This method describes the necessary steps to determine the residual content of ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl in liquid matrices. The type of the food matrix from which ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl is going to be extracted determines which method is used.

Once ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl is extracted, it is analysed by Reserved Phase High Performance Liquid Chromatography (HPLC-RP) and quantified using an external standard curve. This method of analysis describes two conditions of chromatographic analysis, one at isocratic conditions and the other one using gradient conditions. The use of one of them depends on the type of food matrix that is going to be analysed. Thus, it is convenient to have control samples (without ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl content) in order to check the absence of chromatographic interferences in the analytical conditions studied. The examples studied have demonstrated that gradient conditions are more robust than isocratic conditions and for this reason gradient conditions are recommended.

Liquid food matrices from which this method was successfully applied are:

- Pineapple juice
- Coffee liquor
- Liquefied egg product
- Orgeat (a cold drink made of tiger nuts)
- Juice concentrates
- Soft drinks: carbonated flavoured drinks, non carbonated flavoured drinks, sport drinks.

LAS, which comes from the hydrolysis of ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl, is also detected in this method of analysis. Although quantification of LAS is possible, it is not the goal of this analysis.

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15/06/2003	06/02/2006	06/02/2006	07/02/2006

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2. INTERFERENCES

In the assayed studies, no interferences have been found in the analysis except the matrix itself.
However, if the level of sulphites are high, chromatographic interferences could be observed (the HPLC-RP column could be damaged). In these cases, it is advisable to add to the sample a little amount of hydrogen peroxide*.

3 REAGENTS AND MATERIALS

- Analytical balance, precision ≥ ± 0.1 mg
- High Performance Liquid Chromatography
- Column: Symmetry® C_{18} 5µm 150 mm x 3.9 mm
- Volumetrics flasks and measuring pipettes, A class
- Nylon filters, 0.45 µm
- Magnetic stirring rods
- Sparkle vials 20 mL
- Acetonitrile (ACN) HPLC grade
- Water MiliQ® Ultrapure grade
- Trifluroacetic acid (TFA) syntesis grade
- LAE a.i. (ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl) standard (Q-98.250, purity ≥95%)
- LAS standard (N$^{\alpha}$-lauroyl-L-arginine) (Q-98.251, purity ≥98%)
- Sonicator

4. CHROMATOGRAPHIC CONDITIONS

The most suitable detector for this type of analysis is the photodiode detector at the range of wavelength between 190 – 300 nm.

* In these analysis, a sample with 500 ppm of LAE a.i. and 5000 ppm of metabisulphite has analysed. The analyte has been extracted with ACN + 0.1% TFA and 84 µL of hydrogen peroxide (purity 30%) has been added to 2 mL of the extract. The reaction is fast and virulent.

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4.1. Isocratic chromatographic conditions:

Column: Symmetry® C_{18} 5 µm 150 x 3.9 mm (Waters)
Solvent: ACN/H_2O (50/50 v/v) + 0.1% TFA[1]
Flow rate: 1 mL/min
Wavelength: 215 nm (range 190 – 300 nm)
Injection volume: 10 µL
Retention time (R_T) for ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl 4.3 minutes (± 0.2)[2]
Retention time (R_T) LAS: 2.3 minutes (± 0.2)[2]
Total time: 10 minutes

4.2. Gradient chromatographic conditions:

Column: Symmetry® C_{18} 5 µm 150 x 3.9 mm (Waters)
Solvent A: H_2O + 0.045% TFA[3]
Solvent B: ACN + 0.036% TFA[4]
Wavelength: 215 nm
Injection volume: 20 µL
Retention time (R_T) ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl: 19 minutes (± 0.3)[5]
Retention time (R_T) LAS: 16.2 minutes(± 0.3)[5]
Total time: 40 minutes (32 minutes of gradient + 8 minutes of equilibration)

Time (minutes)	Flow (mL/min)	% A	% B
0	1.0	70	30
5	1.0	70	30
25	1.0	30	70
27	1.3	0	100
30	1.3	0	100
32	1.0	70	30

[1] 500 mL of H_2O + 500 mL of ACN + 1mL TFA.
[2] The retention times (R_T) for ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl and LAS were obtained at LAMIRSA laboratory under the reported chromatographic conditions. The indicated interval is due to R_T variations observed when the concentration of analyte was altered.
[3] 1L H_2O + 450 µL TFA.
[4] 1L ACN + 360 µL TFA.
[5] The retention times (R_T) for ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl and LAS were obtained at LAMIRSA laboratory under the reported chromatographic conditions. The indicated interval is due to R_T variations observed when the concentration of analyte was altered.

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5. PROCESS

5.1. Preparation of Standard Solutions

Standard ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl and LAS solutions are prepared by adding powdered substrate to ACN/H_2O (50/50 v/v + 0.1% TFA). The range of substrate concentrations used to create a standard curve must bracket the substrate concentration expected to be present in the analysed food sample. At least, four different substrate concentrations ("standards") must be prepared and analysed to create the standard curve. The volume of standard solution injected is 10 μL.

5.2. Sample Preparation[6]

Due to unpredictable chromatographic interferences from new food matrices treated with ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl, the availability of different methods of sample preparation is required. It is difficult to determine *a priori* which sample preparation method will completely extract residual ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl from a new food matrix to be analysed. In order to make this determination, a known concentration of ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl is added to a sample of the food matrix of interest.

The suggested mass and volume values presented in this analytical method are appropriate for extracted ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl concentrations in the range of 10 to 1,000 mg/L.

6. SAMPLE ANALYSIS

6.1. Analysis from a liquid food matrix

The assayed conditions in the different matrices are described in the following table. The indicated quantities are calculated in order that the analyte is in a calibrate curve between 10-1000 mg LAE a.i./L.

[6] The values of mass and volume reported in this document are only indicatives and suitable for the analysis of a food matrix with an approximate concentration of ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl equal to 77 mg/kg. Mass, volumes and conditions used for analysis may need to be adjusted for each new case.

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Matrix	LAE a.i. Content (%)	Solvent	Sample weight	Stirring time (min)	Final Volume (mL)	Cromatographic conditions
Pineapple juice	0.1	50/50 v/v*	10-20 g	--	25-50	Isoc. / Grad
Coffee liquor	0.1	50/50 v/v*	10-20 g	--	25-50	Isoc. / Grad
Liquid egg	0.1	ACN + 0.1% TFA	5 g	10	25	Isocratic
Orgeat	0.04	50/50 v/v*	6-12 g	20	50	Isocratic
Mouthwash	0.1	ACN + 0.1% TFA	1 mL	--	2	Gradient
Fruit concentrates	-	50/50 v/v*	5 mL	5	25	Gradient
Carbonated flavoured drinks	-	ACN + 1% TFA	-	10	-	Gradient
Non carbonated flavoured drinks		ACN + 0.1% TFA				
Sports drinks						

*(ACN/H_2O 50/50 v/v) + 0.1% TFA

After carbonated drinks are mixed with the solvent, it is important to put them in the sonicator to remove the carbon dioxide for 15 minutes.

The mixture of the solvent and the sample has to be filtered through 0.45 μm nylon filter. The filtered sample is then analysed using the appropriate chromatographic method.

7. QUANTIFICATION

A standard curve is created with at least 4 standard concentrations bracketing the expected ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl concentration in the food matrix and plotting standard concentration *versus* peak area. Chromatographic conditions are optimised by means of the instructions from the chromatographic software. The standard curve must have a correlation coefficient (r^2) > 0.998 and the slope must not be different from a minimum of two standard curves prepared under the

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same conditions with a percent relative standard deviation (%RSD) < 2%. If these two conditions were not fulfilled, then the standard curve must be discarded and a new standard curve with freshly prepared standards must be created.

The concentration of residual ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl in the analysed food is calculated as shown below.

$$C_{LAE} = \frac{C_{LAE\ obt}\ x\ V}{W}$$

C_{LAE}: Concentration of residual ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl found in the analysed food (mg/kg)
$C_{LAE\ obt}$: Concentration of ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl in the analysed phase (mg/L)
V: Total volume of solvent used for extration (L)
W: Mass of the treated sample (kg)

Note: Any additional dilutions employed must be included in the final quantification calculation.

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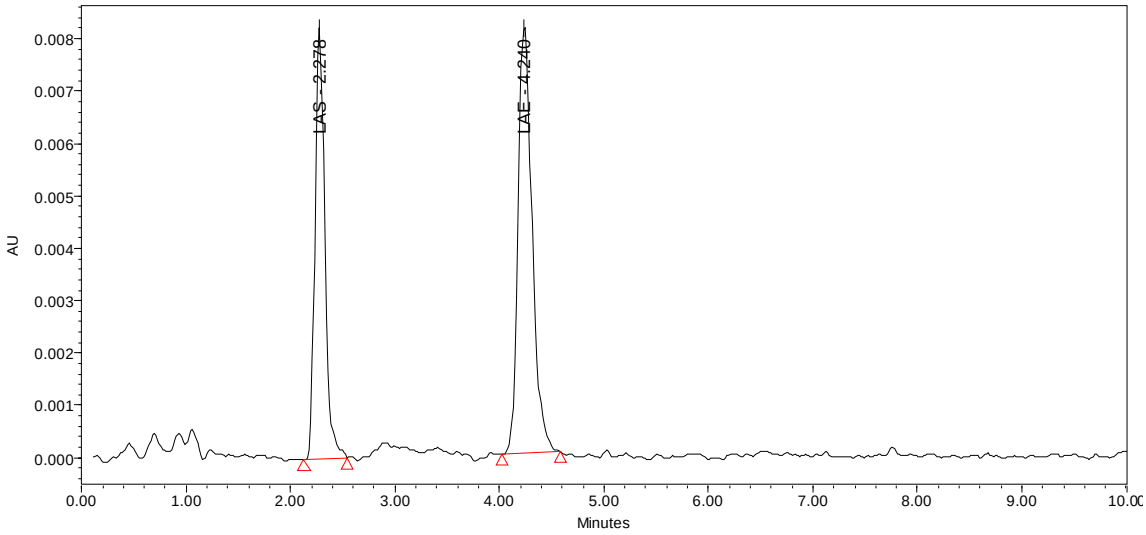
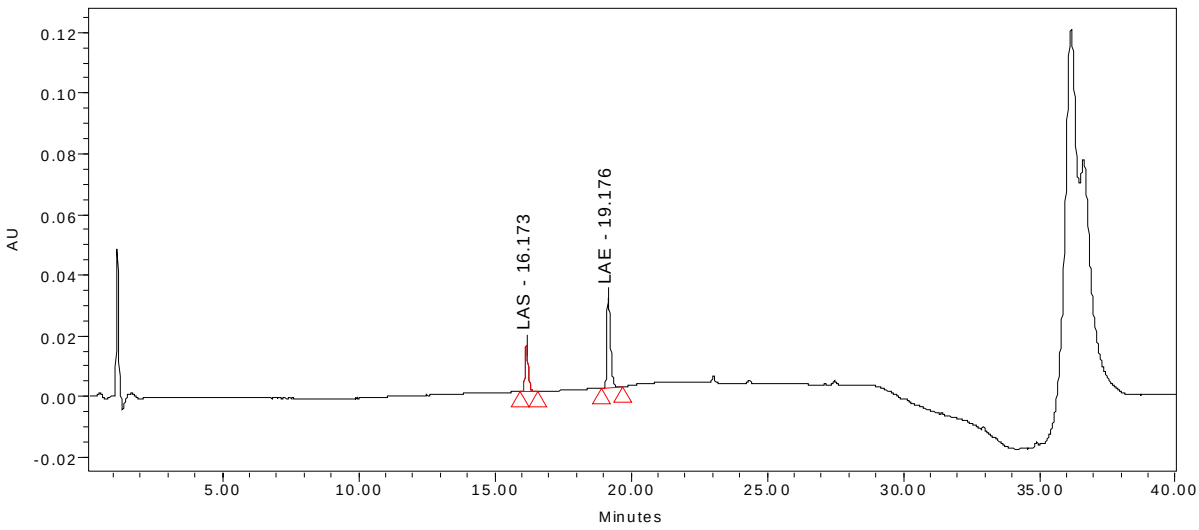
8. CHROMATOGRAPHIC PROFILES

Isocratic conditions



Gradient conditions



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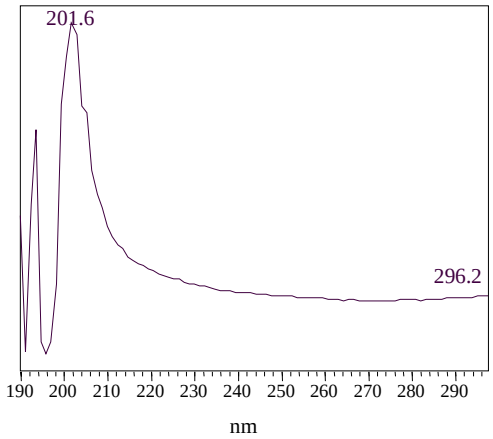
Ultraviolet profiles of LAS and ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl

LAS



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