Mass Spectrometry in the Analysis of Non-ionic Detergents

III. Analysis of polyethylene glycol esters of fatty acids

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High performance liquid chromatography coupled with mass spectrometry using external standardization was applied for the quantitative analysis of polyethylene glycol esters of fatty acids.

Keywords: nonionic emulsifier, mass spectrometry, mass fragmentations

It was demonstrated that the widespread use of ethoxylated nonylphenol and its uncontrolled escape into the environment have a harmful effects on the vertebrates [1]. Therefore, it appeared the interest to replace it with other non-toxic emulsifiers and thus the polyethylene glycol esters of fatty acids with polyethoxylated alcohols were produced and used. In our previous papers [2, 3], we have already demonstrated that the molecular mass distribution of polyethyleneglycol PEG 400 in the analyzed polyethoxylated compounds spreads between 3 and 14 units with the maximum concentration of 8 units. One of the problems raised by the employment of the emulsifiers consists in their analysis, which can be done either before the use or in the medium in which they are present. Taking into account the complexity of the mixtures obtained at the emulsifier production, the characteristics of their UV absorbance, as well as, the lack in specificity of UV-VIS detector, the liquid chromatographic analysis can not be used successfully for this aim. As a continuation, herein we propose a method for qualitative and quantitative analysis of the emulsifiers based on polyethoxylated vegetable oils by using liquid chromatography coupled with mass spectrometer as detector. The sample size is not a critical part of our protocol analyses. For qualitative analysis we performed only screening to inspect the composition. We could not expect the same response factors for each component, despite of great structural similarities of the oily mixtures. Based on calibration with reference substances obtained by syntheses, we made quantitative analyses of samples of ethoxylated fatty acids.

Experimental part

Materilas and methods

We have analyzed two samples of polyethoxylated vegetable oils, sample **1** and sample **2**, obtained from Rhodia and were defined as mainly polyethoxylated fatty acid esters. Commercial methanol, acetonitrile, chloroform polyethylene- glycole (PEG 400, from Fluka), lauric, palmitic, oleic, stearic, as well as, thionyl chloride and pyridine were used. Ultrapure water (18.2 MW) was obtained by an Elix purification system from Millipore. Acids chlorides were synthesized by the known procedure starting from fatty acids, which were treated with thionyl chloride and, then purified by distillation under the deep vacuum. To obtain the fatty acids mono esters of PEG 400, we have adapted the Einhorn method, as follows: acid chloride (1 g) was added to a solution of dry pyridine (1 mL) and PEG 400 (10 g) in dry acetonitrile (25 mL). The mixture was stirred for 30 minutes at 50 °C under argon, then 50 mL water was added and the product was extracted with chloroform (30 mL) in a separatory funnel. The organic extract was washed four times with water (4x50 mL), dried with anhydrous sodium sulfate and concentrated in vacuum. It was obtained about 1 g of mono ester. To obtain the fatty acids diesters with PEG 400, acid chloride (2 g) was added to a solution of dry pyridine (0.05 mL) in dry acetonitrile (50 mL) containing the equivalent amount of PEG 400 (for example, 2.64 g when stearoyl chloride was reacted). The same work-up as above was used. The diesters were obtained in high purity, free from fatty acid or PEG 400.

The liquid chromatography was accomplished using a pump Prostar 240, a Prostar 410 automatic injector, and a mass spectrometer triple quadrupole 1200 L/MS/MS fitted with an electrospray ionization interface (ESI) or an atmospheric pressure chemical ionization interface (APCI), all from Varian. Fragmentation was performed by collision with argon at 1.5 mTorr in the second quadrupole. For chromatographic separation was used a Hypersil Gold column from Thermo with dimensions (length x i.d.): 50 x 4.6 mm, 3 μm particles. Mobile phase consisted in 20 % water and methanol and had a flow rate of 0.6 mL/min for screening. After the column, a splitter divided the mobile phase, allowing to only 10% from the column eluent to reach the interface.

Results and discussions

The first step for the evaluation of the qualitative composition of emulsifier samples **1** and **2**, consisted in a liquid chromatography with an isocratic mobile phase. From the reported data [4] it can be estimated that polyethylene glycol will be the first eluted compound, followed by the mono esters and, finally, by the diesters; unsaturated esters eluted before saturated esters with the same number of ethoxy groups.

The chromatogram obtained for a commercially product declared as polyethoxylated vegetable oil (sample **1**) was represented in figure 1. This chromatogram contains four major peaks and several minor peaks (table 1). The major peaks (in terms of area) correspond to palmitic, linoleic, oleic and stearic acids esters. From this screening we did not noticed diesters.

For the analyzed emulsifiers, the major characteristic ion observed for all chromatographic peaks was the “vinyl ester” of the corresponding acid with the structure as suggested in scheme 1 for oleic ester. It is generated by

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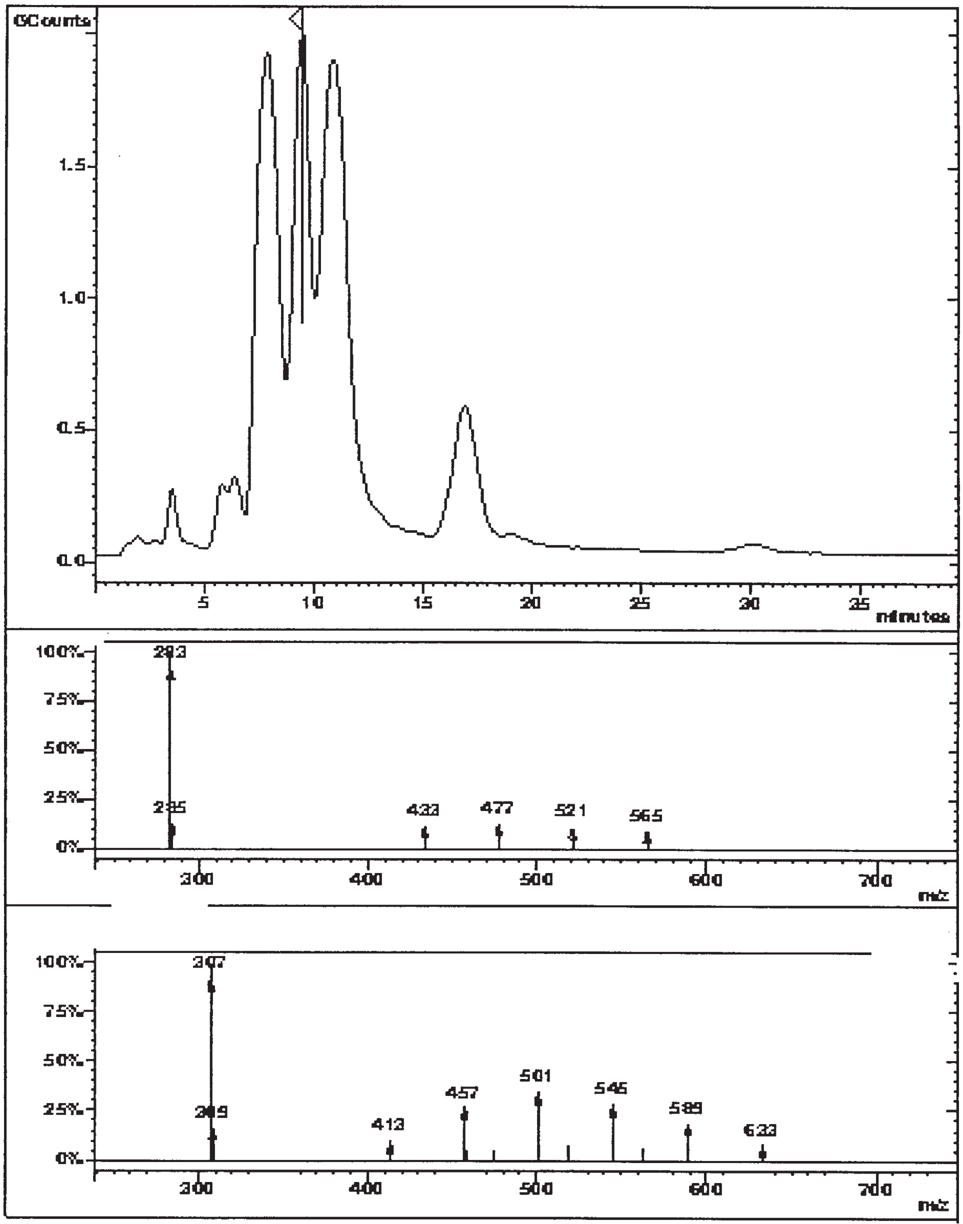


Fig. 1. Total ion chromatogram of sample **1** and spectra of ethoxylated palmitic acid with characteristic ion m/z=283 and ethoxylated linoleic acid with characteristic ion m/z= 307

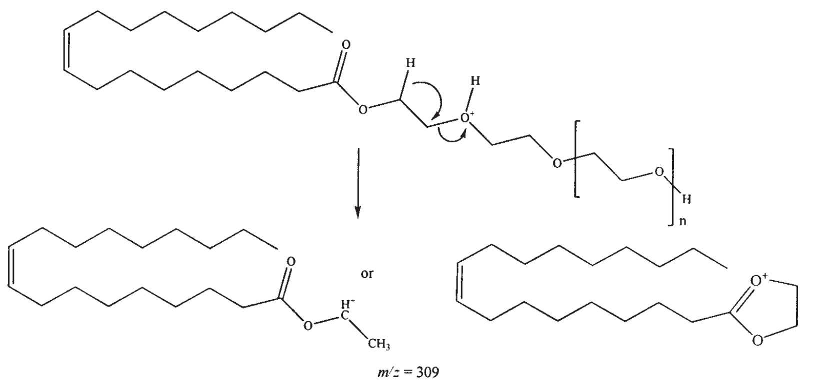
Table 1
DETECTED POLYETHOXYLATED FATTY ACIDS (MONO ESTERS) IN THE COMMERCIAL POLYETHOXYLATED VEGETABLE OIL (SAMPLE 1)

Polyethoxylated fatty acid	Retention time (min)	Characteristic fragment (*m/z*)	Protonated molecular ions serie (*m/z*)
lauric	3.53 (minor peak)	227	377, 421, 465, 509, 553
linolenic	5.87 (minor peak)	305	411, 455, 499, 543, 587
palmitoleic	6.37 (minor peak)	281	387, 431, 475, 519, 563
linoleic	7.86 (major peak)	307	413, 457, 501, 545, 589, 633
palmitic	9.44 (major peak)	283	389, 433, 477, 521, 565, 609
oleic	11.66 (major peak)	309	415, 459, 503, 547, 591, 635
stearic	16.99 (major peak)	311	417, 461, 505, 549, 593
arachidic	29.98 (minor peak)	339	445, 489, 533, 577, 621

the splitting of the esters *in-source* and is accompanied by the smaller peaks having the mass of the acid esters with polyethylene glycols containing 3-7 units CH_2CH_2O.

For the esters with palmitic, palmitoleic, linoleic, linolenic, stearic and arachidic acid, characteristic ions with m/z = 283, 281, 307, 305, 311 and 339 respectively, were found.

For the mass spectrometer calibration, we have synthesized the mono- and di- esters of PEG 400 with lauric, palmitic, stearic and oleic acids, in order to use these esters as reference samples. A program of selected ions monitoring, corresponding to each product and component, was used. For instance, for polyethylene glycol mono



Scheme 1

oleate, we have selected ions starting from *m/z* = 415 (n = 3) to *m/z* = 855 (n = 14) with an interval of 44 atomic mass units, corresponding to the -[CH_2-CH_2-O]- sequence. Therefore, in the mass spectrometer program we used a number of 112 ions. The instrument calibrations curves were built by injecting solutions with known concentrations for each reference material and the results are summarized in table 2. From the obtained data, a good linearity can be observed, however the response factors showed a relative large dispersion that is specific for the detection by mass spectrometry. For the saturated fatty acids series, we can notice a decrease of calibration curve slope as following: lauric > palmitic ≈ stearic >> oleic. From the resulted data, we can conclude that the response factor of the unsaturated acids esters was five times lower than that of the saturated esters. Thus, despite of similar peak areas corresponding to palmitic and oleic acid from figure 1, the real composition we expect to be dominated by the oleic acid monoesters.

The same chromatographic conditions and mass spectrometer program were used for the analysis of the commercial samples, **1** and **2** and the obtained compositions were summarized in table 3. These compositions referred only to calibration component and ignored other main components for which we have not available reference samples.

From the table 3 results that the polyethylene glycol mono oleate represents the main component of the mixture while diesters are almost absent. Unfortunately, because

Table 2
CALIBRATION CURVES
y = a*x+b WHERE
E x = CONCENTRATION µg/mL,
y = RESPONSE EXPRESSED
BY PEAK AREA

Reference	a	b	Slope intercept % to the c4 level	Relative standard deviation %	Correlation coefficient r^2
PEG monolaurate	158.9	-2033.9	8.25	18.48	0.9975
PEG monopalmitate	141.7	262.8	0.93	11.57	0.9989
PEG monooleate	21.8	-542.6	8.75	27.1	0.9995
PEG monostearate	135.3	168.2	0.64	8.70	0.9982
PEG dilaurate	27.5	-233.8	4.36	21.12	0.9971
PEG dipalmitate	37.1	-234.1	4.36	21.20	0.9971

Table 3
TWO COMMERCIALLY
AVAILABLE PRODUCTS
UNCORRECTED COMPOSITION
BASED ON AVAILABLE
REFERENCE MATERIALS*

Component	Sample **1**		Sample **2**	
	Content (%)	Relative standard deviation (%)	Content (%)	Relative standard deviation (%)
PEG mono laurate	1.1	6.3	0.8	15.4
PEG mono palmitate	9.5	1.5	0.4	22.0
PEG mono oleate	54.0	4.3	45.9	6.3
PEG mono stearate	4.8	1.1	0.6	9.0
PEG di laurate	0.0	-	0.0	-
PEG di palmitate	0.4	25.3	0.0	-

* For the esters of unsaturated acids we have considered the same response factor with oleic esters and for arachidic acid we have considered the same response factor with stearic acid esters, due to lack of reference sample.

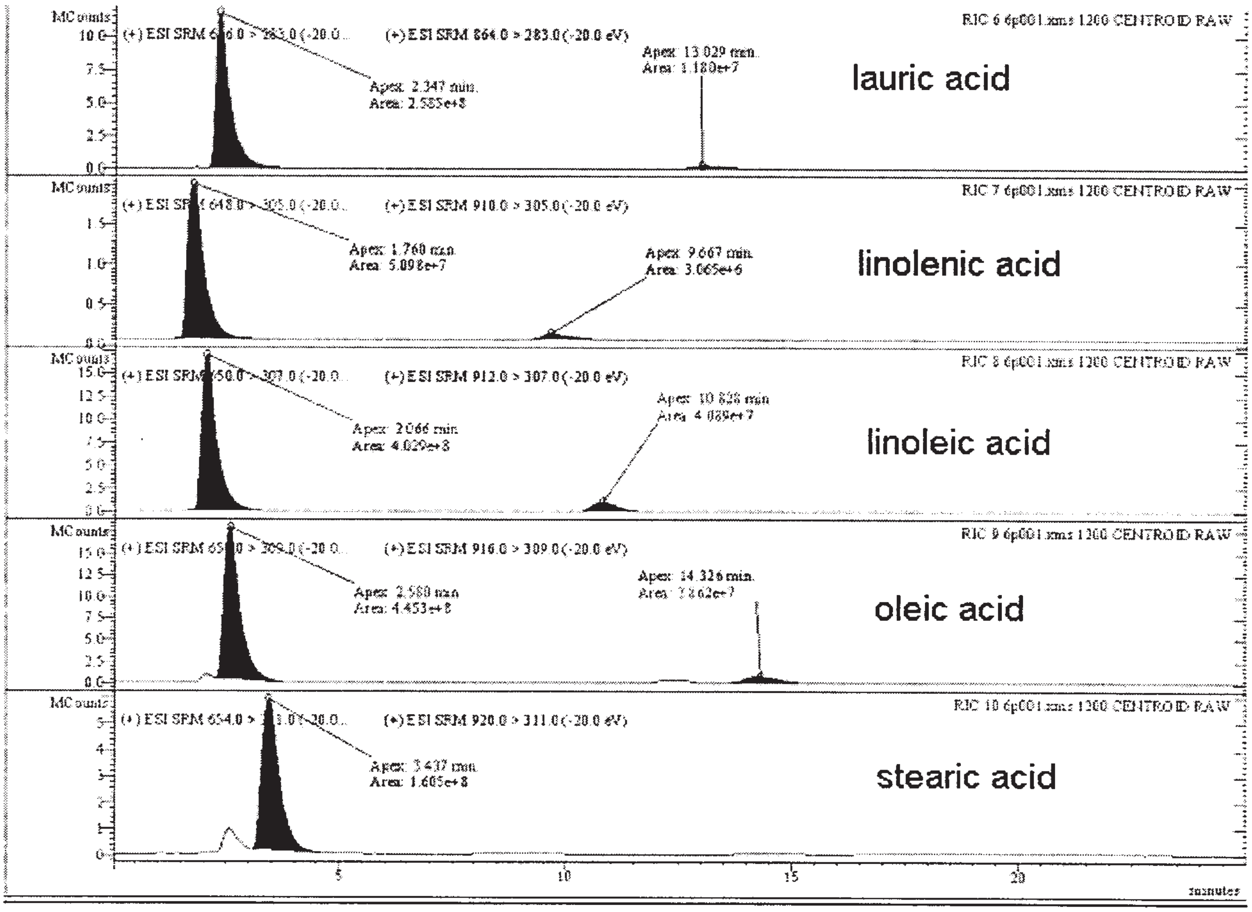


Fig. 2. Time-windows simplified detection of ethoxylated fatty acids monoesters (first 6 min) and diesters (rest time until 30 min)

for the mass spectrometer calibration we have not the reference samples for all components implicated in the samples composition, several simplifications were taken into account. Thus, as it is described above (scheme 1), each kind of ester can be characterized by specific fragments. The strength for the ester bound was considered independent of the acid as the first simplification. Therefore, the abundance of these characteristic fragments corresponds to the amount of each ester into the sample mixture. Thus, instead of the program which implies the monitoring of 112 selected ions, another program was used for the monitoring of multiple reactions using as parent ions the protonated molecular ion $[M+H]^+$ and the corresponding characteristic fragments as daughters. However, this means a number of 112 reactions for each ester, which represents too much for the scanning power of the mass spectrometer. Therefore, we have performed a second simplification taking into account only the component with a degree of ethoxylation of n=8 that is the most abundant in polyethylene glycol 400 and, therefore, was the most important in the molecular mass distribution of each ethoxylated acid. This new program supposes only 9 reactions, one for each component. To increase in the source ions yield, we have performed the chromatography with water and ammonia, while for favouring the protonated molecular ions, an elevated drying gas temperature was used. For the next experiments the ionization source was the electrospray.

With the same reference solutions used in our previous experiment, we have again calibrated and analyzed the products and the results were corrected by a factor for each component. This corrections factor represents the ratio between the real composition based on the first set of calibration and the new composition from the normalized response factors. For instance, for oleic acid its mono ester with polyethylene glycol, the real composition is higher than that obtained by normalization of response factors.

However, as results from table 3, the found composition ignores the presence of some components that we really expected as the products of natural vegetable fatty acids esterification with PEG 400. Therefore, we used again a program based on multiple reactions starting, this time, from protonated molecular ion of each ester of fatty acids present in vegetable oil. With a time windows program we have expected the elution of mono esters in the first 6 minutes and the rest time till to 30 min for diesters. The program in the first 6 min consisted in the multiple reactions monitoring of mono esters as following (first number is m/z for parent ion, second number is m/z for daughter ion): for lauric acid 570>227, for palmitic acid 626>283, for palmitoleic acid 624>281, for oleic acid 652>309, for linoleic acid 650>307, for linolenic acid 648>305, for stearic acid 654>311 and for arachidic acid 682>339. The program in the rest time till to 30 min consisted in the multiple reactions monitoring of diesters, as following (first number is m/z for parent ion, second number is m/z for daughter ion): for lauric acid 752>227, for palmitic acid 864>283, for palmitoleic acid 860>281, for oleic acid 916>309, for linoleic acid 912>307, for linolenic acid 908>305, for stearic acid 920>311 and for arachidic acid 976>339. Under these conditions we have performed the chromatography and the mass spectrometry detection as represented in figure 2.

Major amounts were obtained only for the following carboxylic acids monoesters: lauric, linolenic, linoleic, oleic and stearic and the final results are reported in the table 4. We have found that the diesters almost were missing and

Table 4
FINAL CORRECTED COMPOSITION OF THE ANALYZED EMULSIFIERS*

PEG monoester	Final corrected composition (%)	
	Sample **1**	Sample **2**
lauric	0.1	0.1
palmitic	10.7	1.0
palmitoleic	0.6	0.9
stearic	5.4	9.3
oleic	41.6	55.2
linoleic	36.1	30.7
linolenic	4.5	2.0
arachidic	0.5	0.4

* For the esters of palmitoleic, linoleic, linolenic and arachidic acids

they can be ignored at all. The found esters composition for the samples **1** and **2** corresponds to that of the ratio between the fatty acids in the vegetable oil with a high content of unsaturated acids. Therefore, it is to attempt that the composition of raw vegetable oil to be reflected by the composition of ethoxylated derivatives. For all vegetable oils, the content in palmitic acid is higher than in stearic acid. However, a clear difference in the amount of palmitic acid monoesters for the samples **1** and **2** can be observed in table 4 suggesting either a different source of raw used vegetable oil or a possible processing of the raw vegetable oil before etoxylation.

After Griffin, surfactants could be characterized by HLB (hydrophilic-lipophilic balance) defined as follows:

$$HLB = 20 * Mh / M$$

where Mh is the molecular mass of the hydrophilic portion of the molecule, and M is the molecular mass of the whole molecule. While a value from 7 to 11 indicates water in oil emulsifier, a value from 12 to 16 indicates oil in water emulsion. We can calculate the HLB value from molecular mass distribution and data from table 4. The analyzed samples have HLB values from 7 to 14 with a value of 12 to the maximum ethoxylation degree, n=8. These two samples provide a wide range of HLB values and are, therefore, used successfully in the formulations of pesticides type EC (emulsifiable concentrate). In this way pesticides can be dosed in very large limits by using different amounts of water. At the same time, there is an explanation for the absence of diesters in the emulsifier compositions, the HLB values for diesters are lower than 7. Therefore, these products have the antifoam action.

Conclusions

In the present paper the composition of detergents belonging to the classes of polyetoxylated esters of vegetable fatty acids was analyzed. We have already proved that the analysis by liquid chromatography, coupled with mass spectrometry, can be considered for the elucidation of the composition of nonionic detergents. As a result of such analysis, we have concluded that there is a concordance between the composition of liquid nonionic polyethoxylated vegetable oils and the corresponding original vegetable fatty acid oil used for the detergent preparation. This is true, however, only for the mono esters of polyethylene glycol. Several simplifications were undertaken for a quick and reliable analysis of the emulsifiers. The character of the obtained information about the composition is mainly qualitative because of the lack of reference samples for all the compounds contained in the analyzed detergent. However, the qualitative information is sufficient for surfactants profiling. Structural analyses coupled with quantitative data allowed us to predict the HLB values. Our researches will be continued with the analysis of other classes of nonionic detergents.

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Manuscript received: 20.01.2012