EN 15779 Determination of Polyunsaturated (≥ 4 double bonds) fatty acid methyl esters (PUFA)

- **Compliant to EN 15799:2009**
- **Included QC materials**
- **Dedicated Software for Biodiesel specific reporting**

Keywords:
FAME, PUFA, EN 15779, All-in-one Biodiesel, Polyunsaturated Fatty Acids

Cas nr.	Chemical Name	Abbreviation
2566-89-4	Methyl eicosatetraenoate (Methyl Arachidonoate)	C20:4 (n-6)
2734-47-6	Methyl eicosapentaenoate	C20:5 (n-3)
108698-02-8	Methyl docosapentaenoate	C22:5 (n-3)
18061-46-3	Methyl docosahexaenoate	C22:6 (n-3)

Table 1: Four predominant PUFA methyl esters

INTRODUCTION

Biodiesel is the "green" equivalent for petroleum diesel or petrodiesel. Biodiesel is a renewable fuel derived from algae, vegetable oils, animal fats or cooking oils. Recent developments trend towards Algae-based fuels that do not impact our food supply chain.

Quality requirements of biodiesel for use as pure biofuel or blending stock for diesel fuel are defined in ASTM D6751 and EN 14214 specification. The standards ensure that among others the following important factors in the fuel production process are satisfied: complete reaction, removal of glycerin, absence of Polyunsaturated fatty acids, removal of alcohol and absence of free fatty acids.

Polyunsaturated fatty acid methyl esters, which are much more prominent in marine based biodiesels compared to land based sources, are considered critical as they exhibit lower oxidation stability and are more susceptible to polymerization reactions, with the potential hazard of engine fouling and fuel line or filter blocking.

Method EN 15779 is now mandated for the determination of the PolyUnsaturated (≥ 4 double bonds) Fatty Acid (PUFA) methyl esters content of FAME.

SOLUTION

Based on its Biodiesel All in One Solution, PAC has developed an application which fully complies with EN 15779:2009. After addition of internal standard solution (C23:0 in Heptane), the sample is introduced into the Split/Splitless inlet, where it is mixed with clean carrier gas. One part of the blend will be directed to the split vent, while the other part enters the analytical column. The capillary column separates the individual components in a temperature programmed oven after which they are detected by the FID. Calculation of the percentage of PUFA's is achieved with internal standard calibration. The method as described in EN 15779:2009 covers the quantification of the predominant four polyunsaturated fatty acid methyl esters listed in *Table 1).*

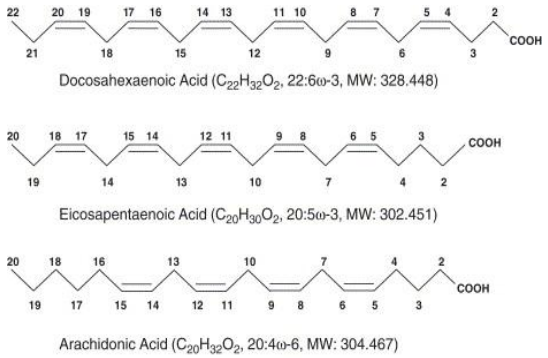

Figure 1: Structural formula's of common PUFA's

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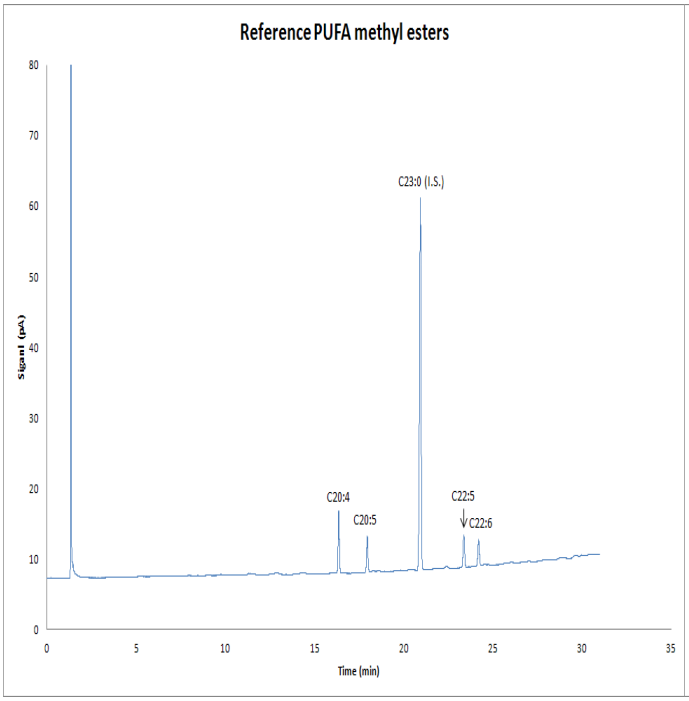

Figure 2: Chromatogram of Reference sample, containing the four predominant PUFA methyl esters and Internal standard (C23:0)

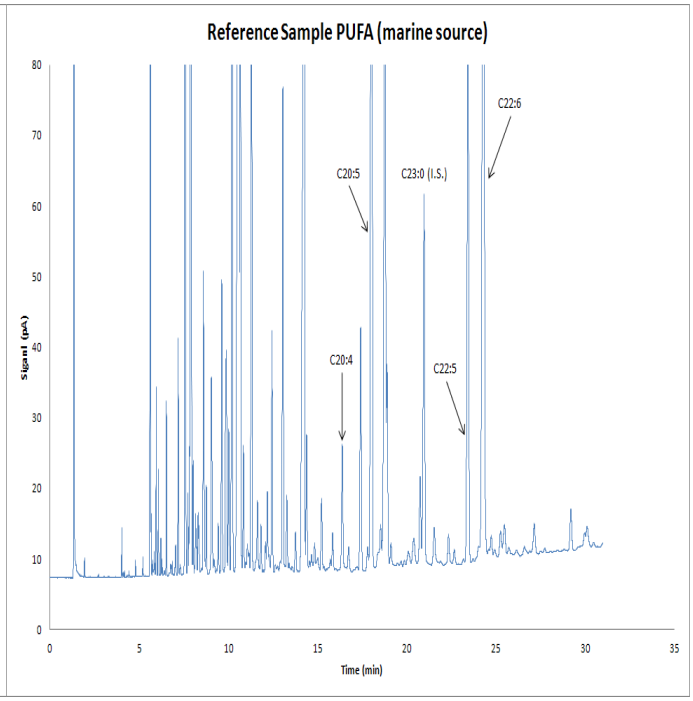

Figure 3: Reference sample PUFA (marine source). Sample contains approximately 16.0 % total PUFA methyl esters.

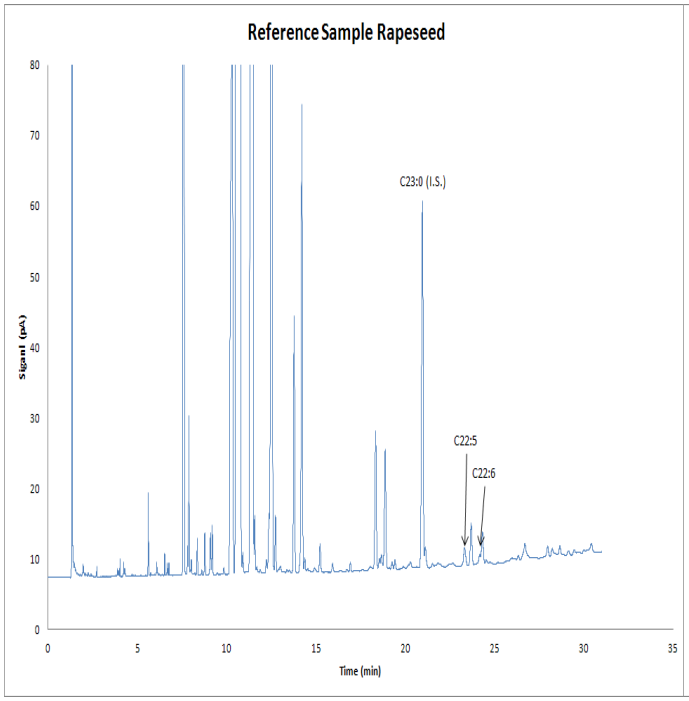

Figure 4: Reference biodiesel sample (rapeseed source). Sample contains < 0.1 % PUFA methyl ester.

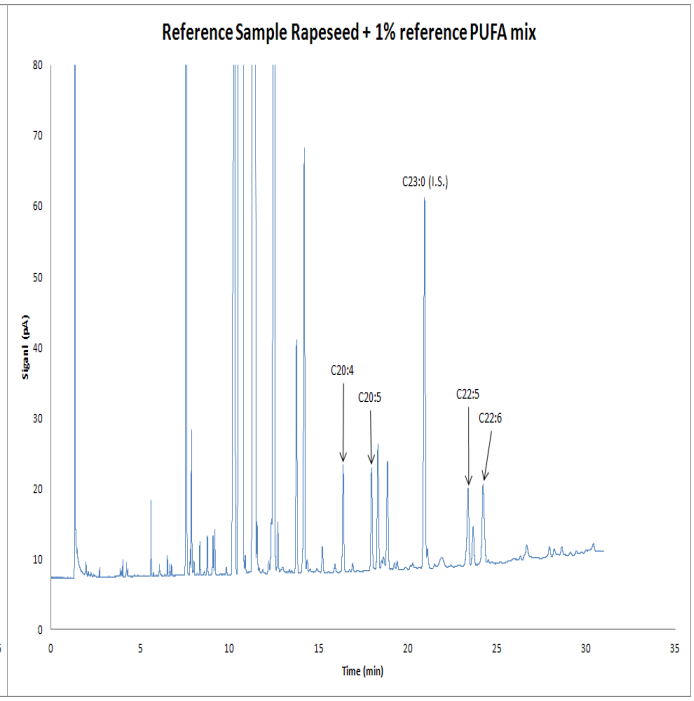

Figure 5: Reference biodiesel sample (rapeseed source) + 1 % added Reference PUFA mixture (0,25 % each PUFA methyl ester).

Parameter	Setting
Inlet	S/SL
Injection Volume	1 µl
Split Ratio	1:50
Injection Temp	220 °C
Oven initial Temp.	150 °C
Oven initial Time	1 min
Ramp 1	15 °C / min
Final Temperature 1	200 °C
Ramp 2	2 °C / min
Final Temperature 2	250 °C
Final Time	2 min
Flow mode	Constant flow
Flow	2 ml/min
Column dimensions	30 m x 0.25 mm x 0.25µm
Column type	Polyethylene Glycol (Wax)
Detector	FID
Detector Temp	275 °C
H_2 flow	35 ml/min
Air flow	350 ml/min
Make-up flow (N_2)	20 ml/min

Table 2: Chromatographic conditions

#	C20:4 (n-6)	C20:5 (n-3)	C22:5 (n-3)	C22:6 (n-3)	Total PUFA
1	0.247%	0.249%	0.270%	0.324%	1.090%
2	0.247%	0.249%	0.270%	0.322%	1.088%
3	0.248%	0.249%	0.271%	0.324%	1.093%
4	0.248%	0.249%	0.270%	0.325%	1.093%
5	0.248%	0.249%	0.270%	0.325%	1.091%
6	0.248%	0.249%	0.272%	0.324%	1.092%
7	0.248%	0.249%	0.271%	0.323%	1.091%
8	0.248%	0.249%	0.271%	0.323%	1.091%
Average	**0.247%**	**0.249%**	**0.270%**	**0.323%**	**1.091%**
stdev	**0.00057%**	**0.00023%**	**0.00081%**	**0.00093%**	**0.00167%**
RSD	**0.23%**	**0.09%**	**0.30%**	**0.29%**	**0.15%**

Table 3: Typical system repeatability data of biodiesel sample (rapeseed source), spiked with ≈ 1 % PUFA's (≈ 0,25 % each).

EXPERIMENTAL

European Standard EN 14214 specifies the maximum PUFA methyl esters content of FAME as 1,00 %. EN 15779 specifies a method for the determination of PUFA methyl esters as a whole between 0,6 % (m/m) and 1,5 % (m/m). Commercially available samples, like PUFA from marine source (figure 3), contain very high amounts of PUFA (≈ 16 %) and therefore fall outside the scope of the method.
To obtain a suitable reference sample inside the proper range, a common sample (rapeseed source, figure 4) is spiked with ≈ 1 % PUFA's (≈ 0,25 % of each of the four predominant PUFA methyl esters, chromtaogram in figure 5).

A 0,1 mg / ml reference solution of the four PUFA methyl esters was prepared in n-heptane containing 1.0 mg/mL on the internal standard (C23:0).

This reference standard is used to deter-mine the retention times for each PUFA methyl ester (figure 2).

The spiked reference sample is prepared by weighing 100 mg of the sample in a 2 ml auto sampler vial and adding 1.0 mL of the C23:0 internal standard solution followed by mixing. The sample has been run 8 times on the analyzer using the chromatographic conditions as mentioned in table 2. Quantification of the PUFA methyl esters was done using the theoretical response factors for each PUFA methyl ester published in the EN15779 method. The response factors were corrected using the detector response of the C23:0 Internal Standard. Results are shown in table 3.

System repeatability (r) of total PUFA content is 0.005 % which exceeds repeatability specifications in the method (0.07 %).

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CONCLUSION

The performance of the AC Biodiesel All in One is demonstrated for EN 15779:2009. All requirements as stated in method are met.

The AC Biodiesel All in One is also an excellent choice to analyze FAME feedstock according to EN 14103, EN 14105, EN 14110, prEN 16300 and ASTM 6584. Its innovative dual programmable oven design ensures optimal availability and flexibility towards various methods as no column changes are required when switching applications and the system is 'always ready', regardless the method that's needed for the sample .

The included reference materials & chemicals and the dedicated reporting makes the AC Biodiesel All in One very user friendly and easy to use for fastest adoption in any biodiesel lab.

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