SEPARATION, DETERMINATION AND COMPOSITION PROFILE OF LIPIDS IN BIODIESEL USING HYPHENATION OF GRADIENT-HPTLC WITH FLUORESCENCE DETECTION BY INTENSITY CHANGES AND MASS SPECTROMETRY

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Introduction

Biodiesel is a lipid-based renewable fuel mostly composed of methyl esters of fatty acids. It is used as a total or partial substitute for diesel fuel, either in its pure form (B100), or blended with diesel in different proportions (BX, with X being the volume percent of B100 in the mixture) without requiring any essential modification in the ignition engines.

A fatty acid methyl ester content lower than 98 wt% indicates inappropriate reaction conditions for biodiesel production and, therefore, the presence of impurities in the final product which include mono- (MG), diglycerides (DG) and fatty acids (FA)

HPTLC has scarcely been used for petrochemical analysis. However, it is a suitable technique for these types of samples, especially when compound classes rather than individual species are to be determined. Likewise, as all compounds in a sample are stored in the plate after chromatographic development, a quantitative analysis may be possible. This is an advantage over column-based techniques in which polar and/or high molecular weight compounds in fuels may be irreversibly adsorbed on the stationary phase.

HPTLC experienced a great instrumental development in recent years, and is now a fully automated and computer-controlled analytical technique that makes it possible to design original hyphenated instrumental methods well suited to a particular analytical issue [1]. As an example, fine-tuned separations can be achieved using AMD, an automated technique that combines incremental multiple development and solvent gradient elution [2]. Likewise, separations can be on-line coupled to MS [3].

We report here an on-line, hyphenated procedure that provides separation and quantitative determination of lipids in BX, as well as their composition profile in the sample. All this information is obtained from a single plate.

Experimental

Plates. HPTLC plates, 20 x 10 cm (Merck) were pre-conditioned by developing 90 mm with tetrahydrofuran (THF).

Standard and sample application. Ten bands of 4 mm-length of the corresponding lipid standard were applied using CAMAG ATS-4, with volumes between 0.1-2.5 µL (concentration: 1 mg mL-1), track distance 2 mm, distances from lateral edge and lower plate edge 10 mm. One track was left empty, as a blank run. Three bands of 12 mm-length of a BX were also applied with track distance 16 mm. Sample load (BX, diesel fuel) was between 2500 and 5000 (volume: 25 µL; concentration:100 mg mL-1). An additional track was left empty, as a blank run.

Chromatography. It was performed with an AMD2 system. A 3-step gradient was developed to separate MG from BX (Gradient 1, Table 1). A 4-step gradient was for optimizing lipid separation in BX (Gradient 2, Table 2).

Table 1. 3-Step gradient for separation of monoglycerides

	t-butyl methyl ether (vol %)	DCM (vol %)	*n*-heptane (vol %)	Migration distance m.d. (mm)
Step 1	100	0	0	40
Step 2	0	80	20	60
Step 3	0	60	40	90

Table 2. 4-Step gradient for separation of MG, DG, and FA

	t-butyl methyl ether (vol %)	DCM (vol %)	*n*-heptane (vol %)	Migration distance m.d. (mm)
Step 1	100	0	0	25
Step 2	0	90	10	70
Step 3	0	80	20	80
Step 4	0	60	40	90

Densitometric detection. The plate was post-impregnated by dipping it into a solution of primuline in MeOH (0.02 w/v %) using a Camag Immersion Device III. A TLC Scanner 3 in fluorescence mode was used for peak detection by excitation at 365 nm. Emission was collected at longer wavelengths than 400 nm

Coupling with MS. Peaks from the primuline-impregnated plate were transferred to an ion-trap Bruker Esquire 3000 Plus system operating in positive (for MG and DG) or negative (for FA) ESI mode, using a CAMAG elution-based TLC-MS interface (oval elution head 4 x 2 mm). Methanol was used at a flow of 0.2 mL min-1. Blank portions of silica gel and primuline-impregnated plates were extracted as control.

Results and Discussion

Sequential steps of hyphenation include: AMD gradient separation of lipid classes, followed by a post-chromatographic impregnation of the plate with primuline, and fluorescence densitometric detection (and quantification). Finally, the peaks are directly transferred to an ESI-MS spectrometer using an elution-based interface.

AMD chromatograms show three zones:

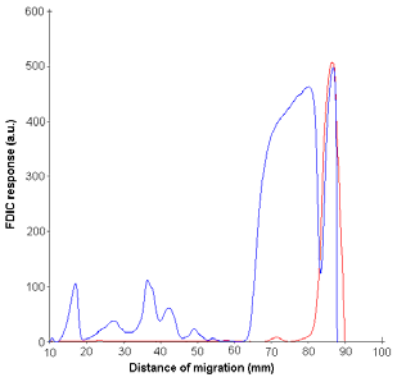

Figure 1. AMD chromatogram (gradient 1) of B100 (blue) and pure diesel (red)

- A single peak migrating near the end of the plate (80-90 mm) This peak corresponds to the diesel component in the BX blend, as shown in red in Figure 1. Diesel is not an interference for lipid impurities determination in BX under the described conditions.
- An intense zone (60-90 mm for gradient 1, Figure 1; 80-90 mm for gradient 2, not shown) which corresponds to fatty acid methyl esters.

- A zone which corresponds to other lipid material in BX (10-60 mm for gradient 1, Figure 1, and 10-80 mm for gradient 2).

Gradient 1 was developed to separate MG as a single peak at 16 mm. AMD allows a fine tuning of separation. Gradient 1 was modified to gradient 2 for separating other BX-related lipid families. Desired but unresolved zones of the chromatograms can be directly related to gradient composition in a given step. Thus it becomes possible to make changes in solvent composition and/or number of steps to obtain extended targeted zones in the chromatogram. The addition of one step (DCM 90 vol %-heptane 10 vol %; gradient 2) and modification of migration distances allowed the separation of MG (15.4 mm), FA (24.2 mm) and DG (41.4 mm) to be carried out. In all cases, peaks were identified by ESI-MS, as shown below.

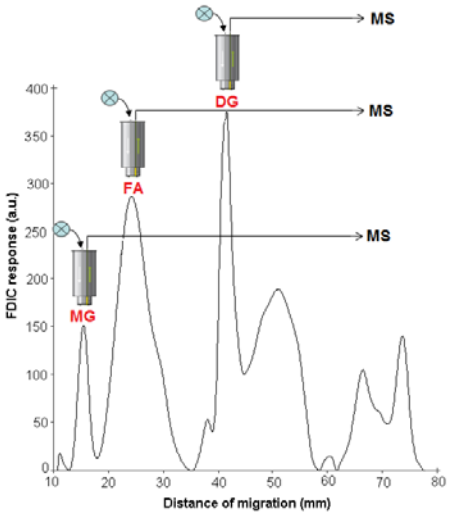

Figure 2. Detail of lipid-part of AMD chromatogram (gradient 2). MG, FA and DG peaks are to be transferred to MS.

Densitometric detection was performed using primuline-impregnated plates, from the increases in fluorescence emission experienced by primuline in the presence of lipids and saturated hydrocarbons. All components of BX provide increases in fluorescence with regard to the baseline provided by primuline.This detection procedure is applicable, in general, to molecules bearing long-hydrocarbon chains, and was referred to as FDIC (Fluorescence Detection by Intensity Changes) [4].

The use of primuline is not a derivatization because it only involves non-covalent interactions, and is compatible with the use of ESI-MS. Therefore the peaks at 15.4, 24.2, and 41.4 mm in B100 sample (Figure 2) were directly transferred from the primuline impregnated-plate to ESI-MS using the elution-based interface.

The nature of the peak at 15.4 mm was evidenced from the corresponding ESI spectrum and its comparison with that of the corresponding MG standard (Figure 3). In the case presented in the figure, $[M+Na]^+$=379.3 ion, 1-oleoyl glycerol is the most abundant one. Likewise, ESI spectrum of peak displays other ions corresponding to other MG in the sample. Therefore, the fatty-acid profiles of the corresponding MG in BX can be obtained.

Peak at 24.2 mm corresponded to FA, with a preponderant fragment in ESI spectrum of at $[M-H]^-$= 281.0 (oleic acid). Other present FA in B100 can be identified in the Figure 3. FA were detected using negative ESI mode as non-sodiated ions.

Peak at 41.4 mm can be attributed to DG according to ESI spectrum (Figure 3). Ion at 647.6 corresponded to $[M+Na]^+$ of glyceryl distearate. ESI fragments derived from this peak are coherent with the presence of DG coming from mixtures of saturated and unsaturated C18 and C20 fatty acid-chains. Quantitative determination can be done from the primuline-FDIC chromatograms. As an example, a calibration curve per plate was obtained for MG by plotting the fluorescent response (in Area counts) vs standard load. Thus, its percentage in biodiesel was calculated from its corresponding calibration curve. In the examples of figures, 1-oleoyl glycerol standard was used as external standard. However, after inspection of ESI-MS profiling, other standard or mixture of standards may be used. Results indicate that FDIC is suitable for determining monoglycerides in BX (X≥5) at low concentrations, and sensitive enough to determine them according to current standards.

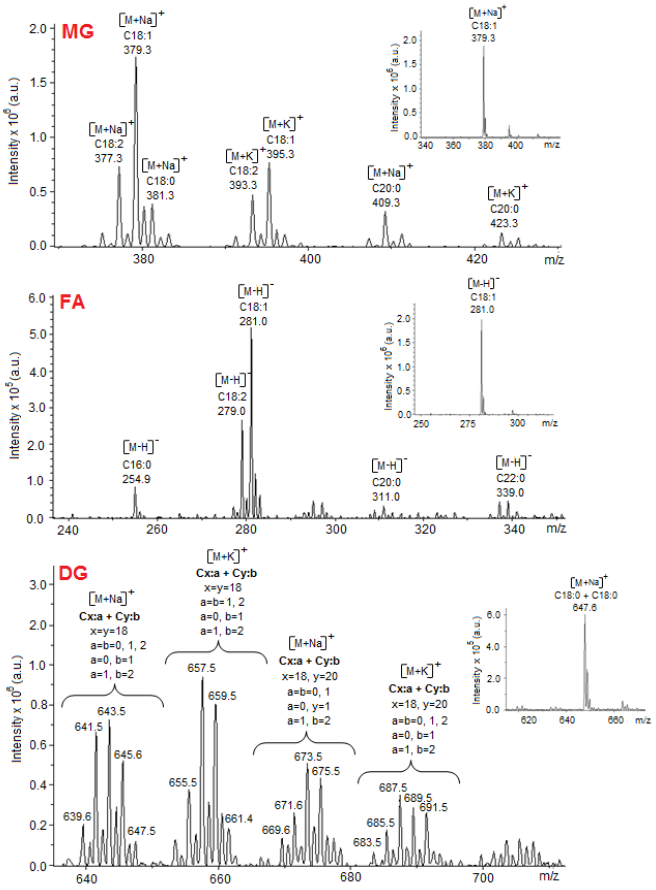

Figure 3. ESI-MS of MG, DG and FA peaks (Standards, top right)

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