

Quantitative Applications of Fluorescence and Ultraviolet Scanning Densitometry for Compositional Analysis of Petroleum Products in Thin-Layer Chromatography

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Abstract

Direct application of the fluorescence response enhancement (derived from the interaction of the berberine cation with saturated hydrocarbons) to petrochemical analysis is achieved. Hydrocarbon types are successfully determined in middle (gasoil) and heavy (lubricants, vis-breaking fuel, heavy oil) petroleum distillates with adequate precision and sensitivity. The novelty of this method resides in the fact that a single fluorescence scanning is only needed for the determination of saturates and aromatics in the same chromatogram. In spite of their chemical inertness, saturated hydrocarbons can be quantitatively determined using thin-layer chromatographic densitometry. The fluorescent response depends on the alkane structure. A reliable analysis depends on the adequate choice of variables that influence fluorescence response, precision, and sensitivity: sample load, application volume, beam size, and impregnation conditions (berberine concentration and impregnation time). Sensitivity of the analysis can be tailored to a certain extent through control of the last parameter. The quantitative results agree with those provided using other well-established techniques in the petrochemical industry.

Introduction

Fluorescence enhancement of a broad variety of solutes has been used for a long time in thin-layer chromatography (TLC) (1). If a TLC silicagel plate is impregnated with a solution contain-

ing berberine and an alkane is applied on the thin layer, an enhancement of the fluorescence signal in the visible region occurs when the system is irradiated with monochromatic ultraviolet (UV) light (2,3). This phenomenon was reported in a qualitative manner (visual inspection) in 1947 and 1980, when the modern technology of densitometers was not available. In a previous work (4), the present authors proposed a theoretical explanation for it, laying the groundwork for designing new interaction systems of analytical interest. A direct application can be the analysis of compounds in the field of petroleum derivatives.

Analysis of compound classes, also called hydrocarbon group-type analysis, is important in petrochemistry and carbochemistry for the choice of process conditions, process monitoring, compliance with environmental regulations, and evaluation of fuel quality, catalyst performance, and feed processability (5). This analysis involves the separation and quantitation of the corresponding hydrocarbon types in the sample; therefore, chromatographic techniques are frequently used. The boiling point range and polarity of the sample to be analyzed (6) are used as selection criteria for a particular chromatographic technique and method.

Among the available chromatographic techniques, TLC (or planar chromatography) has the advantages of being comparatively simple, rapid, robust, and inexpensive. Nevertheless, it has not been widely used for petroleum analysis, mainly because it is not considered quantitative or sensitive enough, and the detection of saturated hydrocarbons (classically inert molecules that do not exhibit UV or fluorescence spectra in the usual wavelength ranges) is not immediate using scanning densitometry.

However, TLC presents several advantages: high sample throughput, simultaneous processing of standards and sam-

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ples, analysis of the whole sample without previous fractionation or deasphalting, and the possibility of physically storing the developed plates.

Conventional TLC silicagel and alumina plates (with developments using solvents of increasing polarity) have been mostly used for either qualitative and semiquantitative hydrocarbon-type analysis or the preparative isolation of hydrocarbon families in heavy distillates in order to carry out either further analysis or the gravimetric determination of collected fractions (7–12). In general, nonquantitative analyses have involved the visual detection of colorless substances using inspection under UV illumination or the use of chromogenic or fluorogenic spray reagents (13). At present, only few works deal with quantitative hydrocarbon-type analysis of fossil fuels using UV and fluorescence scanners (14,15).

In our opinion, TLC has been underfrutilized in the case of petroleum. The recent progress (16–18) in layer manufacturing (including small-sized high-performance TLC plates), sample application devices, development instrumentation, and automated UV and fluorescence scanning densitometers should allow an improvement to be achieved with regard to the separation and quantitation of petroleum-derived samples.

In 1991, Marsh and Hiekane (14) carried out an analysis of bitumen using fluorescence (for saturates) and UV densitometry (for the other peaks). However, neither the nature nor the influence of the different analytical parameters were studied, and the possible application to other kinds of samples was not considered.

A more complete study of the application of TLC–UV–fluorescence scanning densitometry to the compositional analysis of petroleum products, including middle distillates as gasoil, is presented in this work. Quantitative, sensitive, and precise methods based exclusively on fluorescence enhancement for the compositional analysis of saturates and aromatics are presented, along with alternative methods for aromatics using UV detection. The variables that influence sample response, precision, and sensitivity of analysis have been studied.

Experimental

Samples

Pure *n*-alkanes (Fluka, Basel, Switzerland; Ultrascientific, North Kingstown, RI) were used. The gasoil, synthetic lubricant, and vis-breaking (a thermal treatment for fuels that decreases viscosity without additives) fuel were provided by CEPESA (Madrid, Spain). The heavy oil was a deasphalted oil from a 450°+ vacuum distillate Brent residue (19).

In the case of gasoil, the content in aromatics was provided by CEPESA using a high-performance liquid chromatography–refractive index (HPLC–RI)-based IP 391 standard (20).

The synthetic lubricant was prepared from the main fractions (saturated and aromatic) of the original lubricant by mixing these fractions in known proportions in order to validate the results. Different lubricants obtained by mixing dif-

ferent proportions were analyzed. Saturates and aromatic fractions were obtained from CEPESA from the raw lubricant by the strict application of a liquid chromatography-based ASTM D2007 standard method (21).

In the case of the vis-breaking fuel, compound class composition was also provided by CEPESA using TLC–flame ionization detection (FID). Detailed descriptions of the technology, method, and quantitative conditions can be found elsewhere (19).

Analytical TLC separation

Silicagel plates (aluminium sheets, 20 × 20 cm, 5- to 25- μ m particle size, 60- \AA pore size, 0.2-mm layer; Panreac, Barcelona, Spain) and silicagel-impregnated plates with berberine sulfate (Across Chimica, Geel, Belgium) were used. A solution of berberine sulphate in methanol (6 mg/100 mL) was used for impregnation for 20 s. Plates were subsequently dried horizontally overnight at 40°C.

Samples were spotted at least in duplicate onto TLC plates using an SES 3202/IS-02 automatic spotter (Germany). Applied volumes of 0.4 μ L were typical. Sample loads usually ranged from 1 to 20 μ g.

After application, samples were eluted using a sequence of eluants. The normal elution scheme for the lubricant and gasoil was *n*-hexane (9 min) and dichloromethane (DCM) (4.5 min). A normal elution scheme for the vis-breaking fuel and the heavy oil was *n*-hexane (30 min), toluene (8 min), and DCM (3 min). These eluants were obtained from Scharlau (Barcelona, Spain). In the cases where short development times were employed, both sides of the plates were often used. Plates were dried at room temperature after hexane elution. Plates were always dried at room temperature when toluene was not used as eluent. When using toluene, plates were dried at 100°C for 3 min.

Fluorescence and UV scanning densitometry

After development, a CS9301 TLC scanner (Shimadzu, Japan) was used in either the fluorescence or UV mode to provide detection of the peaks.

In the case of berberine, fluorescence scanning densitometry was used, usually under the following conditions: λ_{exc} , 365 nm; beam size, 1 × 1 mm; filter 3 (visible zone); linear scanning.

In the cases of both nonimpregnated silicagel and coated (impregnated) plates, UV scanning densitometry was used. The wavelength was 250 nm. Linear or zigzag scanning and different beam sizes were used in order to obtain adequate sensitivity.

Peak positions and distances of spotting between the different lanes were determined using either UV bidimensional scanning (CS9301 software) or conventional scanning (perpendicular to the direction of development, at the *y*-coordinate corresponding to undeveloped peaks).

Peak area data were taken from the scanner, and all data were collected, displayed, and stored using Shimadzu CS9310 PC software. The Kubelka–Munk equation (22) was applied through the software to each data point in order to linearize the area–mass response. An SX = 3 factor, recommended by the manufacturer for 0.2-mm-thick silicagel plates, was used.

Preparative isolation of calibrating fractions and calibration procedure

Quantitative determination was carried out in each case by external calibration using fractions from the corresponding fuel itself.

Preparative TLC for the isolation of saturate and aromatic fractions from the studied products (except the lubricant) was carried out using glass silicagel TLC plates (20 × 20 cm, 2-mm layer, 5- to 25- μm particle size; 60- \AA pore size). After sample application, *n*-hexane was allowed to develop to the end of the plate. The separation edge between saturates and aromatics in a preparative separation was monitored by either fluorescence scanning using a berberine-impregnated plate or UV scanning. After choosing the cutting point, the preparative isolation was carried out in a nonimpregnated silicagel plate. After development, the zones corresponding to saturates and aromatics were scratched and subsequently extracted from the silicagel in a Soxtec apparatus (Tecator, Sweden) under mild conditions (dichloromethane solvent) and dried under N_2 until constant weight. Purity of the fractions was monitored by fluorescence and UV scanning densitometry (vide infra).

The saturate and aromatic fractions from the lubricant were provided by CEPESA. They were obtained by the application of the ASTM D2007 standard method.

Results and Discussion

Quantitative determination of saturates using TLC–fluorescence scanning densitometry

Berberine sulfate is a fluorescent, aromatic, heterocyclic alkaloid. If a TLC silicagel plate is impregnated with a solution containing the berberine cation and an alkane is applied to the thin layer, an enhancement of the fluorescence signal in the visible region occurs when the system is irradiated with monochromatic UV light (e.g., $\lambda = 365 \text{ nm}$) (2,3,14). Figure 1 shows chromatograms corresponding to the saturates and aromatics determined in different petroleum products. Saturates are detected using fluorescence after elution with *n*-hexane. Aromatics are detected by UV after elution with toluene.

It was demonstrated in a previous work using computational calculations that an ion–molecule interaction between alkanes and berberine cation is responsible for this phenomenon (4). The role of the alkane is to provide an apolar environment for the excited berberine cation that hinders alternative relaxation mechanisms and favors fluorescence emission. The phenomenon is also observed for compounds that have a partially saturated structure, as long as they adopt extended conformations along the main berberine axis. Therefore, alkyl-substituted aromatics should also be detectable, provided the length of the alkylic chain is long enough. This suggests the

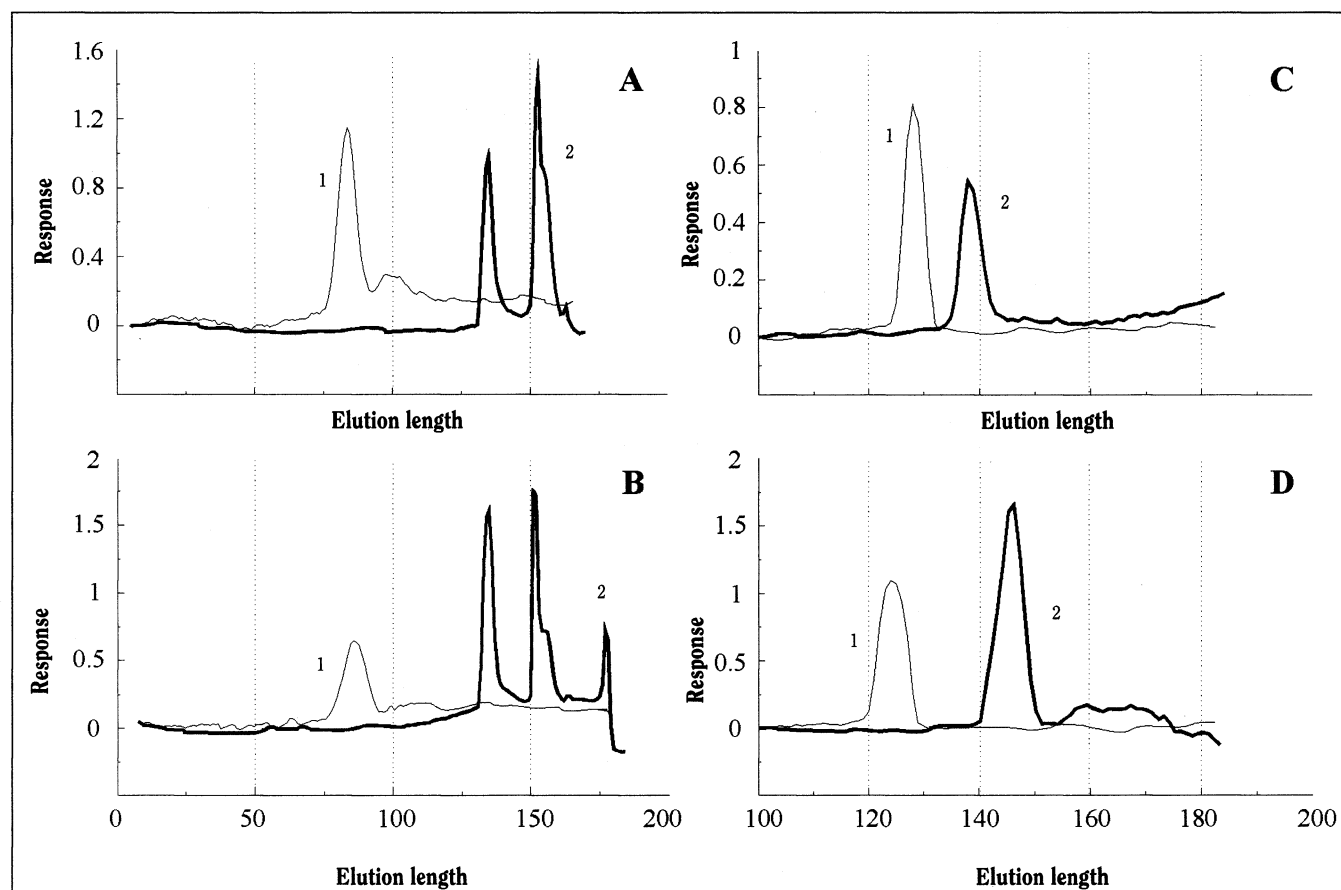


Figure 1. TLC chromatograms corresponding to saturate fractions determined using fluorescence scanning densitometry on berberine-coated silicagel plates (1) and aromatic fractions determined using UV scanning densitometry on the same plates (2): heavy oil (A), vis-breaking fuel (B), lubricant (C), gasoil (D).

possibility of performing a complete analysis of petrochemicals using TLC-fluorescence scanning densitometry, as will be explained later.

Variables that influence fluorescence, chromatographic response, precision, and sensitivity of determinations are sample load, length of paraffinic chain, application volume, development length, beam size, and impregnation conditions.

For a given wavelength and concentration of berberine in the plate, the fluorescent response is proportional to the mass of the alkane applied and also depends on the alkane structure.

The addition of each new $-CH_2-$ group results in an increase in the fluorescent response. The curves of response corresponding to the fractions of the saturates from the analyzed petrochemicals show coherence with regard to the results obtained from the *n*-alkanes (Figure 2). The lubricant-saturate fraction that consists, on average, of long alkanes ($> C_{24}$) provided higher response than C_{24} . In turn, the response of the gasoil-saturate fraction is near the C_{18} response. Therefore, the map of the fluorescence responses of *n*-alkanes may be used to obtain some clues about their nature, an interesting possibility for unknown environmental samples in order to identify types of pollutants.

The scanning beam size and the impregnation conditions have a clear influence on the sensitivity of the fluorescent detection and also affect precision. The larger the beam size, the lower the sensitivity. For the volumes (0.4 μ L), sample loads, and chromatographic conditions typically used, 1×1 mm was the minimum beam size that allowed the totality of the peak to be scanned. As expected, it provided the highest sensitivity, as seen in Figure 3. Smaller beam sizes did not cover the peaks and therefore were not included in the study. Development length also has a clear influence on peak width. Therefore, elution and detection conditions must be chosen with some care in order to achieve a good compromise between peak separation and sensitivity for a given sample.

Because the fluorescence response of berberine was used to detect saturated hydrocarbons, the berberine concentration on silicagel resulting from the impregnation process will directly influence the response. The sensitivity of the analysis will be determined by berberine concentration in the impregnating solution and impregnation time. Three concentrations of berberine (2, 4, and 6 mg/100 mL methanol) and two impregnating times (20 and 50 s) were studied in duplicate for a gasoil saturate fraction (Figure 4). Plates impregnated for 50 s gave worse repeatabilities and baselines than those obtained using 20-s impregnated plates, probably because of a deterioration of the silicagel-aluminium sheets. In the case of the 20-s impregnation time, the saturation of fluorescence signal occurred at sample loads higher than 100 μ g for concentrations of 2 and 4 mg berberine/100 mL methanol and at 40 μ g for 6 mg berberine/100 mL methanol.

The simultaneous selection of impregnating conditions, application volume (in this case, 0.4 μ L), development time (e.g., 9 min), and beam size (allowing the totality of

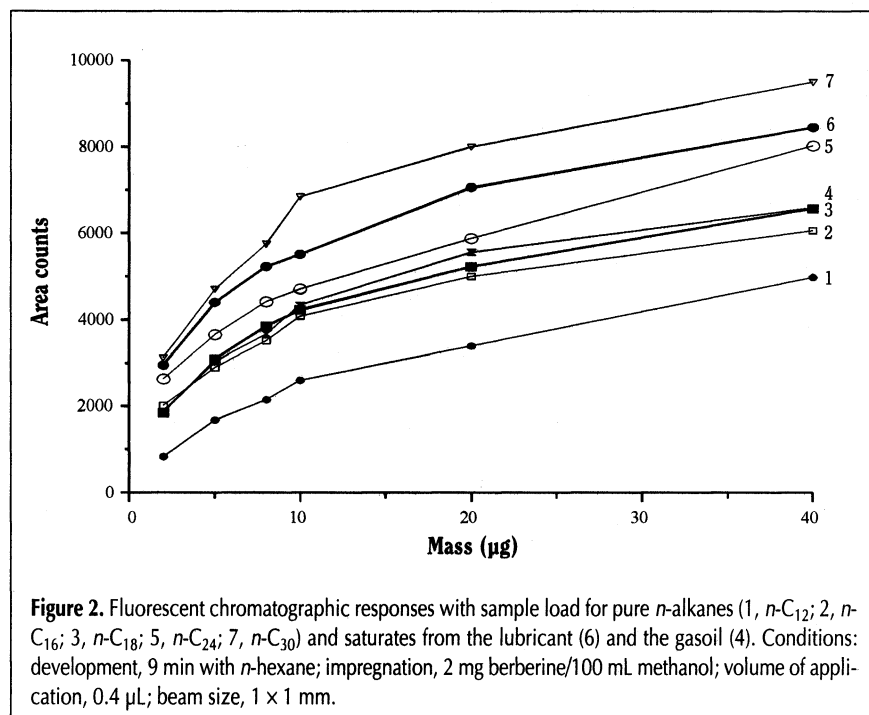


Figure 2. Fluorescent chromatographic responses with sample load for pure *n*-alkanes (1, *n*- C_{12} ; 2, *n*- C_{16} ; 3, *n*- C_{18} ; 5, *n*- C_{24} ; 7, *n*- C_{30}) and saturates from the lubricant (6) and the gasoil (4). Conditions: development, 9 min with *n*-hexane; impregnation, 2 mg berberine/100 mL methanol; volume of application, 0.4 μ L; beam size, 1×1 mm.

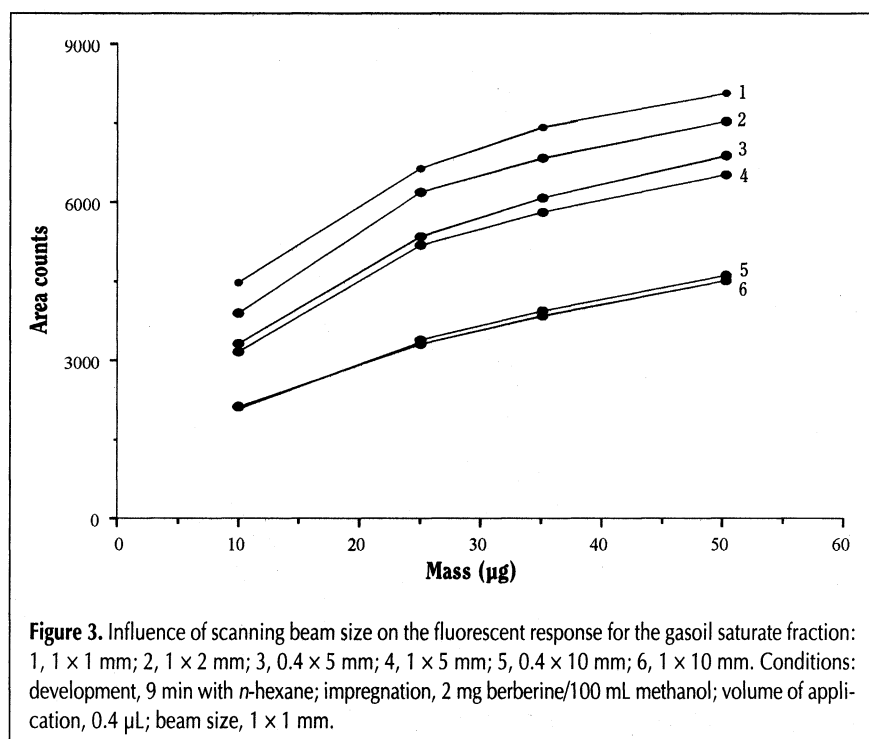


Figure 3. Influence of scanning beam size on the fluorescent response for the gasoil saturate fraction: 1, 1×1 mm; 2, 1×2 mm; 3, 0.4×5 mm; 4, 1×5 mm; 5, 0.4×10 mm; 6, 1×10 mm. Conditions: development, 9 min with *n*-hexane; impregnation, 2 mg berberine/100 mL methanol; volume of application, 0.4 μ L; beam size, 1×1 mm.

sample to be scanned) provides adequate area–mass linear regressions and precisions. (Tables I and II).

The direct determination of saturates is possible with this method on other samples as well. Table I shows results concerning the quantitative determination of saturates for a lubricant, a heavy oil, and a vis-breaking fuel. In each case, the application of different sample loads of the corresponding preparative fraction of saturates was used to perform the calibration curve.

Quantitative results agree quite well with those using TLC–FID for the studied products. Under the impregnation conditions used (6 mg/100 mL methanol), sensitivity is adequate, and the linear mass range for these products is between 3 and 16 µg.

Quantitative compound class analysis of middle and heavy petroleum distillates using TLC–fluorescence scanning densitometry

Typical analyses of petrochemicals include the determination of saturates, aromatics, and polar materials. Figure 1 shows the chromatograms of several analyzed products. In this case, samples were scanned twice: saturates were obtained using fluorescence scanning under the conditions already described, and the other peaks were obtained using UV scanning at 250 nm. In essence, this procedure is a direct application of the previous work of Marsh and Hiekane (14) to different samples.

Nevertheless, TLC–fluorescence scanning densitometry on berberine-impregnated plates can also be used to perform a quantitative determination of both saturates and aromatics in these samples, because the aromatic fraction usually includes alkyl aromatic compounds, removing the need to scan the plates twice. The particular characteristics of the alkylic chains in the alkyl aromatics will greatly influence the detection limit for these compounds. In this case, polars are determined by difference. The aromatic fraction could also show an intrinsic fluorescence response, especially in the case of samples that include condensed aromatics. These samples can be analyzed as well, although the origin of the fluorescence signal would be different.

Figure 5 shows the complete chromatograms corresponding to the separation of the lubricant and gasoil studied with this method. The linearity intervals of the fluorescence response for the aromatic fraction of these samples were similar to those found for saturates (Table III). In all the cases, per-

cent relative standard deviation (for 5 measurements) was lower than 5.

Under the impregnation conditions employed, fluorescence responses were linear with adequate precision at least in the range of 1–20 µg for saturates and 1–13 µg for aromatics.

Table III also shows that the saturate and aromatic contents from the fluorescence chromatograms agree quite well with

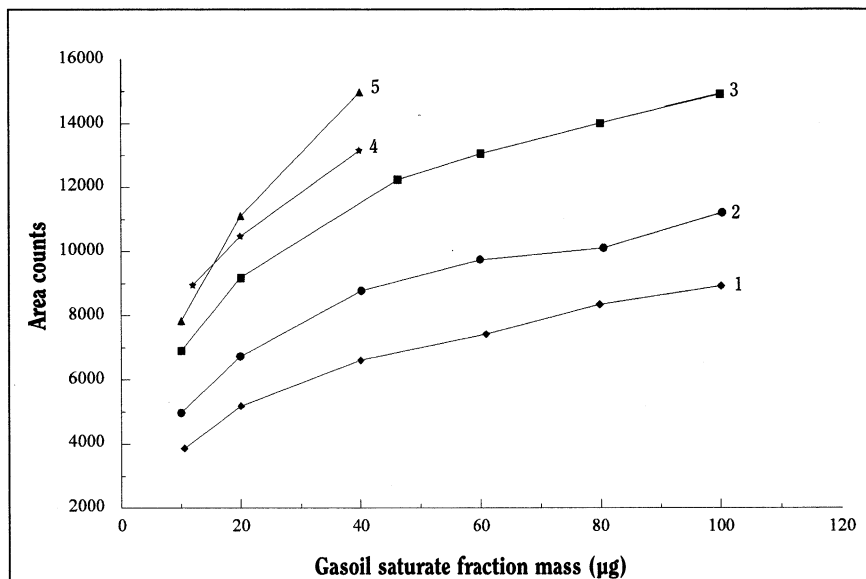


Figure 4. Influence of impregnating conditions on the fluorescent response for the gasoil saturate fraction: 1, 2 mg berberine/100 mL methanol (time, 20 s); 2, 2 mg berberine/100 mL methanol (time, 50 s); 3, 4 mg berberine/100 mL methanol (time, 20 s); 4, 6 mg berberine/100 mL methanol (time, 20 s); 5, 4 mg berberine/100 mL methanol (time, 50 s).

Table I. Percentage of Saturates Fraction (Weight %) of Different Petroleum Products Determined Using TLC–Scanning Fluorescence Densitometry with Berberine-Coated Silicagel Plates* and Other Techniques

Sample	TLC–fluorescence	Other techniques
Heavy oil	35.2	36.1 (TLC–FID)
Vis-breaking fuel	12.3	12.1 (TLC–FID)
Lubricant (spiked sample [†])	56.2	56.8 (Spiked)

* Impregnation, 6 mg/100 mL methanol.

[†] Samples spiked from their previously isolated saturate and aromatic fraction according to ASTM D2007.

Table II. Precision and Linear Regression Data for TLC–Fluorescence Analysis of Different Petroleum Products Using Berberine-Coated Silicagel Plates* and Other Techniques

Sample	% RSD	Linear interval	Equation [†]		
			<i>m</i>	<i>b</i>	<i>r</i>
Heavy oil	6.85	3–16	567.5	4574	0.999
Vis-breaking fuel	1.85	4–20	979.7	4876	0.996
Lubricant (spiked sample [‡])	2.21	4–16	306.8	2464	0.993

* Impregnation, 6 mg/100 mL methanol.

[†] Area = *m* • mass + *b*. Regression coefficient = *r*.

[‡] Sample spiked from their previously isolated saturate and aromatic fraction according to ASTM D2007.

those found using other reference techniques. In the particular case of gasoil, reference values are obtained using HPLC-RI, according to the IP391 standard (20). This standard allows the calculation of the saturate content by difference, whereas the present method, using detection based on the fluorescence response, provides a direct determination of this value. It should be noted that quantitative analysis of compound classes of gasoils using TLC have not previously been reported in the literature, as far as the authors of this work know.

According to the previously proposed explanation, the observed higher response of aromatics from the lubricant, when compared with those from gasoil, will show the contribution of the presence of longer alkylic chains. The particular characteristics of the alkylic chain (in the case of alkyl aromatics) will also greatly influence the detection limit for these compounds.

Alternative determination of aromatics in middle and heavy distillates using TLC-UV scanning densitometry

The complete analysis of the sample using only fluorescence scanning has clear, practical advantages, but it obviously depends on the nature and abundance of the aromatic fraction. It cannot be assumed that the aromatic fraction will always be detectable, although impregnation conditions allow the magnitude of response to be tailored to a certain degree, and the aromatic fraction might also have an intrinsic fluorescence response. In any case, the alternative determination of aromatics using UV detection remains available because it is clearly suitable for this kind of detection, and there is no need to perform a second analysis using a nonimpregnated plate because the aromatic fraction can also be quantitated in the berberine-impregnated plates. Figure 6 and Table IV illustrate this possibility.

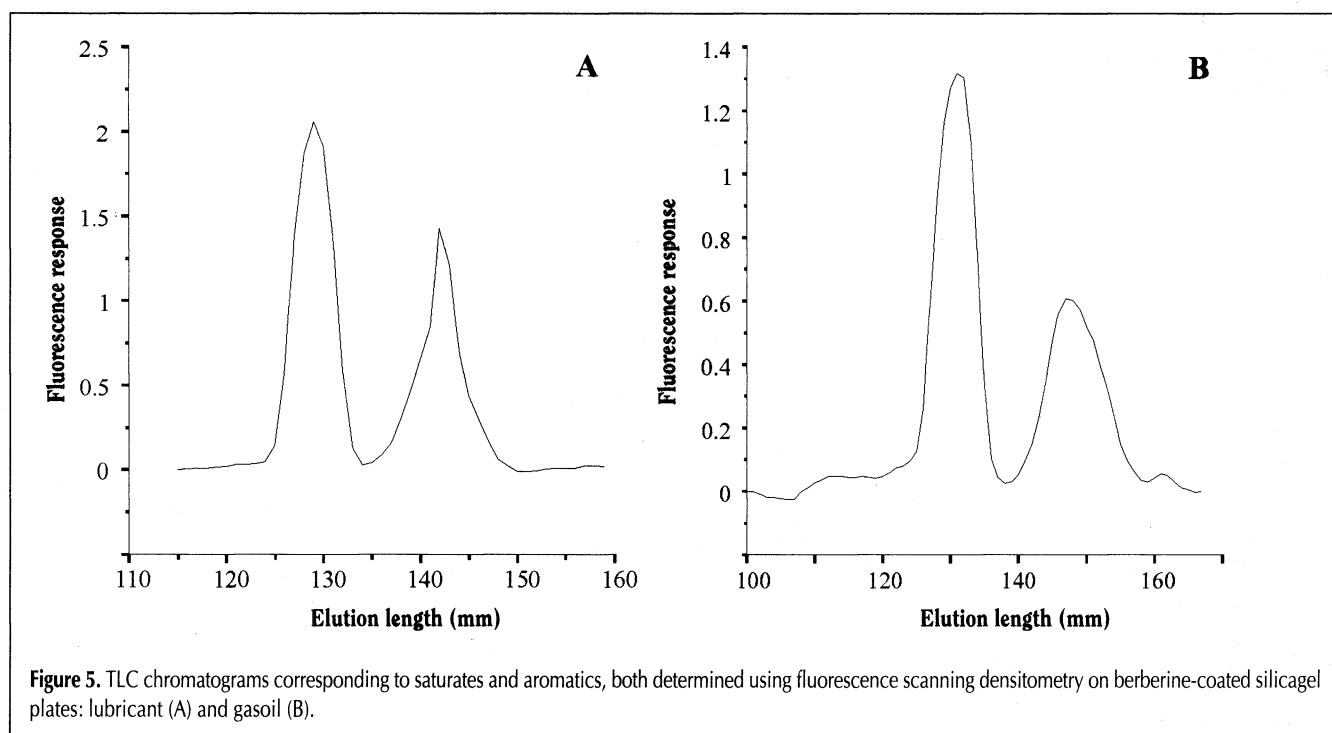


Figure 5. TLC chromatograms corresponding to saturates and aromatics, both determined using fluorescence scanning densitometry on berberine-coated silicagel plates: lubricant (A) and gasoil (B).

Table III. Quantitative (Weight %) Determination of Compound Classes for the Studied Lubricant and Gasoil Using TLC-Scanning Fluorescence Densitometry on Berberine-Coated Silicagel Plates*

Sample compound classes	Reference values (weight %)	TLC-fluorescence (weight %)	Linear interval (μg)	Equation [†]		
				<i>m</i>	<i>b</i>	<i>r</i>
<i>Lubricant</i>						
saturates	56.8	56.2	4-16	306.8	2464	0.993
aromatics	43.2	43.8	3-16	432.5	4754	0.992
<i>Gasoil</i>						
saturates	70.8 [‡]	69.8	5-20	357.6	4818	0.990
aromatics	29.2 [‡]	30.2	1-13	242.7	212	0.98

* Conditions: development, 9 min with *n*-hexane and 4.5 min with dichloromethane-methanol (95:5, v/v); impregnation, 6 mg berberine/100 mL methanol; volume of application, 0.4 μL ; beam size, 1 \times 1 mm.

[†] Area = *m* • mass + *b*. Regression coefficient = *r*.

[‡] Determined using HPLC-RI according to standard IP391; aromatics are directly determined by this technique, but saturates are obtained by difference.

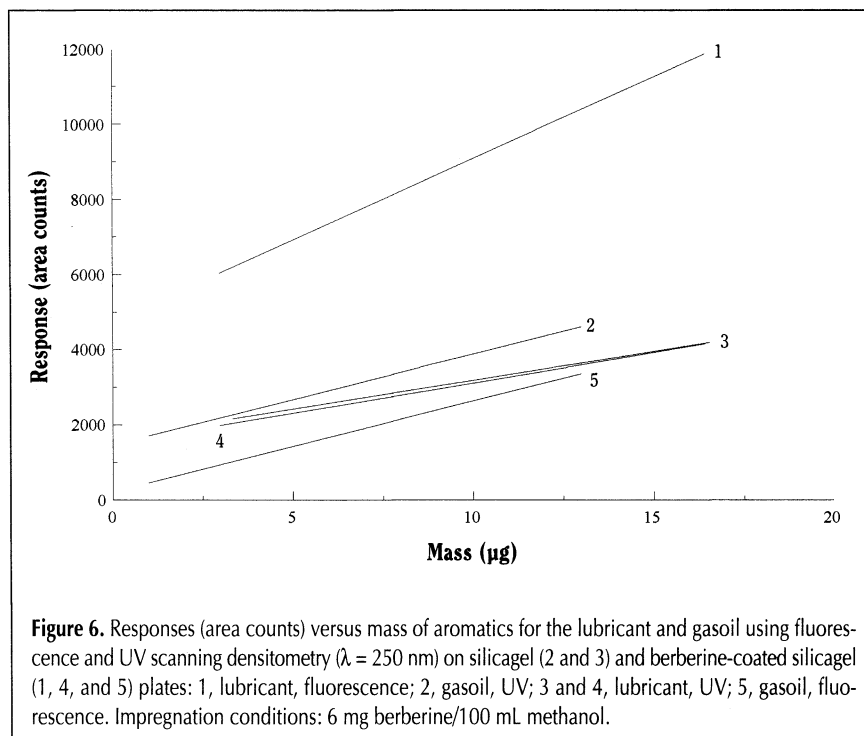


Figure 6. Responses (area counts) versus mass of aromatics for the lubricant and gasoil using fluorescence and UV scanning densitometry ($\lambda = 250$ nm) on silicagel (2 and 3) and berberine-coated silicagel (1, 4, and 5) plates: 1, lubricant, fluorescence; 2, gasoil, UV; 3 and 4, lubricant, UV; 5, gasoil, fluorescence. Impregnation conditions: 6 mg berberine/100 mL methanol.

Table IV. Comparison Between Aromatics Determination (Weight %) Using Either Fluorescence or UV Scanning Densitometry

Sample	TLC-fluorescence*	TLC-UV†
Lubricant	43.8	42.1
Gasoil	30.2	27.0

* On berberine plates (6 mg/100 mL methanol).
† On berberine plates (6 mg/100 mL methanol); UV zigzag scanning (beam size, 0.4 × 0.4 mm)

Conclusion

A weak interaction between the berberine cation and saturated hydrocarbons allows them to be detected and quantitated using TLC-fluorescence scanning densitometry, as was demonstrated in a previous work. The proposed theoretical explanation for this phenomenon predicts fluorescent response for other analytes (e.g., alkyl-aromatics) with partially saturated structure, adopting extended conformations along the main axis of the berberine molecule. Therefore, direct application of this phenomenon to petrochemical analysis has been achieved in this work. Hydrocarbon types have been successfully determined in middle (gasoil) and heavy (lubricants, vis-breaking fuel, heavy oil) petroleum distillates with adequate precision and sensitivity. The novelty of this method resides on the fact that only a single fluorescence scanning is needed for the determination of saturates and aromatics in the same chromatogram.

Quantitative results agree with those provided using other well-established techniques: TLC-FID in the case of heavy distillates and HPLC-RI, according to IP391 standard, in the case of gasoil. In this last case, it should be highlighted that our flu-

orescence method does allow the determination of saturates to be directly performed without the technical problems associated with the use of an RI detector.

Although the fluorescent response depends on the chain length and amount of the corresponding hydrocarbon, the magnitude of the response can be modulated, if necessary, using an adequate scanning beam size or through the modification of the conditions of berberine impregnation.

As explained previously, in the case of aromatics determination, at least a part of the fluorescent response comes from the alkylic chain. Even in the case in which fluorescence sensitivity is not adequate for determining short-chain or low-abundance alkyl-aromatics in some products, an alternative UV scanning reading allows aromatics to be detected on the same berberine-coated silicagel plate.

Acknowledgments

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