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Monitoring carboxylic acid formation in engine oils by liquid chromatography with fluorescence detection

Simon W. Lewis[a], Paul J. Worsfold *,[a], Euan H. McKerrell[b]

[a] *Department of Environmental Sciences, University of Plymouth, Plymouth PL4 8AA, UK*

[b] *Shell Research Ltd., Thornton Research Centre, P.O. Box 1, Chester CH1 3SH, UK*

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Abstract

Straight chain aliphatic carboxylic acids (C_6–C_{22}) were selectively derivatized in oxidised engine oils with the fluorescent label 9-anthracenemethanol after dialysis of the oils to remove polymeric additives, organometallic oxidation products and solid debris. The ester derivatives were separated by reversed-phase liquid chromatography and quantified using fluorescence detection (λ_{ex} = 251 nm; λ_{em} = 412 nm). Calibrations over the range 1–4 mg ml^{-1} were linear ($0.9987 \leq r^2 \leq 1.0000$). The limit of detection ($S/N = 3$) for octadecanoic acid was 85 μg ml^{-1} in *n*-heptane (4.3 ng on-column). Results showed that carboxylic acids are formed during the oxidation of oils in car engines and that their concentrations are directly related to the degree of oil degradation.

1. Introduction

Oxidation of an engine oil occurs when the hydrocarbon constituents in the oil react with dissolved oxygen under the extreme conditions of heating and agitation within the engine. This process is promoted by the affects of contact with the metal construction of the engine and the presence of contaminants in the oil [1,2]. The performance of the oil is degraded by the formation of suspended solids and sludges which block filters, oil lines and lubrication grooves.

Formation of these sludges is a complex process which is not fully understood [3]. It is known that oxidation involves a radical chain mechanism with primary products that include alcohols, aldehydes, ketones, carboxylic acids and water. Condensation polymerisation and further oxidation leads to an increase in the average molecular size of the products and the formation of sludges [4]. It is therefore desirable to monitor the intermediates and products of oxidation *e.g.* aldehydes, ketones and carboxylic acids, to aid the elucidation of the oxidation pathways.

Techniques such as thin-layer chromatography (TLC) and Fourier transform infrared spectroscopy (FT-IR) have been used to quantify total oxidised species and to identify functional groups respectively. This information has been correlated with results from traditional methods such as titrimetry [5,6] and viscosity determinations.

The present emphasis in used oil analysis, however, is the identification and quantification of individual species at the mg ml^{-1} level. Carboxylic acids are major primary products of

* Corresponding author.

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oil oxidation in engines and their presence can be used to monitor the degree of degradation. Gas chromatography (GC) has been used to determine carboxylic acids in a variety of matrices including crude oils [7], petroleum [8] and edible oils [9]. Analysis of used engine oils by GC however is aggravated by the presence of additives in the oil which lead to complex chromatograms. In addition, due to the nature of the sample matrix, extensive cleanup is required. Liquid chromatography (LC) with pre-column derivatization is one possible approach to achieve the required selectivity for the determination of carboxylic acids.

A wide range of labelling reagents for ultraviolet (UV) [10,11], fluorescence [12–21] and chemiluminescence (CL) detection [22–26] have been reported for the determination of carboxylic acids. Most of these derivatization reactions have been developed for clinical applications and are not suitable for the analysis of used oils because they are incompatible with the matrix.

One possible candidate as a fluorescence label for non-aqueous media is 9-anthracenemethanol [27], which has been used in conjunction with CL detection for the analysis of carboxylic acids in used engine oils [28]. The derivatives were separated by isocratic reversed-phase LC and detected using a post-column peroxyoxalate CL reaction. This method separated acid derivatives (C_8–C_{16}) in 45 min but this is a longer runtime than would be desirable for routine analysis. The separation of these acids could be achieved in a shorter time using gradient elution but the peroxyoxalate CL reaction is sensitive to changes in mobile phase composition [28,29].

This communication describes a procedure for the determination of individual carboxylic acids (C_6–C_{22}) in oxidised engine oils by pre-column derivatization with 9-anthracenemethanol followed by gradient elution LC with fluorescence detection. Polymeric additives, organometallic oxidation products and solid debris were removed from samples by dialysis prior to derivatization. The method was applied to the analysis of a series of oxidised oils sampled from a test engine after different periods of operation.

2. Experimental

2.1. Reagents

High-quality deionized water from a Milli-Q system (Millipore) and analytical-grade reagents were used unless otherwise stated. Acetonitrile (ACN), dichloromethane (DCM), *n*-heptane, petroleum spirit (b.p. 60–80°C) and tetrahydrofuran (THF) were of HPLC grade (Rathburn).

Solutions of 9-anthracenemethanol (Fluka) and 4-pyrrolidinopyridine (Aldrich) were prepared daily in DCM. Dicyclohexylcarbodiimide (DCC; Fluka) was used as the solid. Solutions of hexanoic (C_6), heptanoic (C_7), octanoic (C_8), nonanoic (C_9), decanoic (C_{10}), dodecanoic (C_{12}), hexadecanoic (C_{16}), octadecanoic (C_{18}), eicosanoic (C_{20}) and docosanoic (C_{22}) acids (all Aldrich) were prepared in *n*-heptane. All carboxylic acids were reagent grade.

2.2. Sample pretreatment

The oil samples were initially fractionated using a continuous dialysis system as shown in Fig. 1. Solutions of oil in light petroleum (b.p. 60–80°C) were contained within a semipermeable membrane around which warm petroleum spirit (b.p. 60–80°C) was continually circulated. The membrane was a dry, hypo-allergenic incontinence sheath rubber membrane (London Rubber Co.; product No. Q100-251, C10103900).

The dialysis proceeded for 24 h to allow low-molecular-mass material to diffuse through the membrane (the nominal molecular mass cutoff was 1000). Solvent was removed from the dialysate by rotary evaporation.

2.3. Precolumn derivatisation

Carboxylic acid standard solutions and oil dialysate samples, both unspiked and spiked with mixtures of acids (250 μl) were separately added to a mixture of dicyclohexylcarbodiimide (0.25 g), 9-anthracenemethanol in DCM (1 ml, $2.1 \cdot 10^{-2}$ *M*), 4-pyrrolidinopyridine in DCM (1 ml,

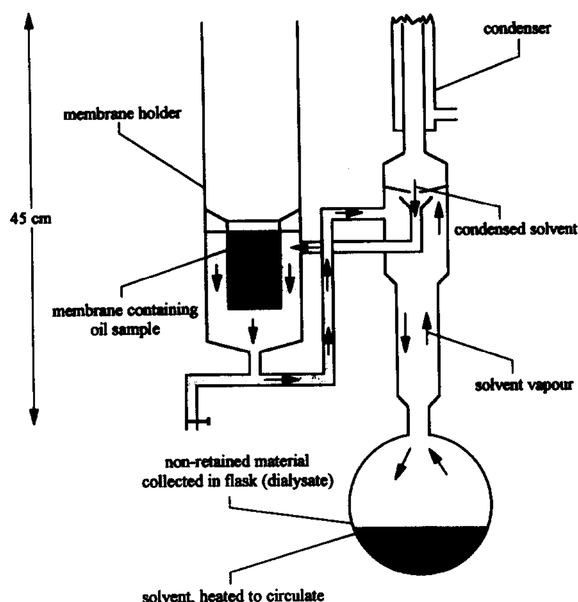

Fig. 1. Schematic diagram of continuous dialysis system. The dialysis solvent was petroleum spirit (b.p. 60–80°C) and the membrane was a dry, hypo-allergenic incontinence sheath rubber membrane (London Rubber Co.; product No. Q100-251, C10103900).

$1.0 \cdot 10^{-2}$ *M*) and *n*-heptane (15 ml) which was heated under reflux for 15 min. The reaction mixture was allowed to cool for 5 min before the solvent was removed by rotary evaporation. The residue was dissolved in ACN–THF (40:60, v/v) (25 ml).

2.4. Liquid chromatography

LC was carried out using a liquid chromatograph (Hewlett-Packard HP1090M) with a dual grating fluorescence detector (Hewlett-Packard HP1046A). To measure the excitation and emission spectra of the label and the derivatives, species were isolated in the fluorescence detector flow cell by a manual switching valve (Rheodyne). The optimum wavelengths for excitation and emission were 251 and 412 nm, respectively.

The instrument, containing an integral ternary solvent pump with helium sparging, an automated injector and a column oven (40°C) was controlled by a Hewlett-Packard Chemstation. The Chemstation was also used to control the detector and acquire and manipulate the data generated.

Separation of the carboxylic acid derivatives was achieved with two separate columns (stainless steel, 150 × 4.6 mm I.D.; Hichrom) packed with (1) Spherisorb S5 C_8 and (2) Hichrom RPB, a reversed-phase base-deactivated material. The solvent gradient was as follows; 0.0 min, water–ACN–THF (40:50:10, v/v/v); 5.0 min, water–ACN–THF (40:50:10); 15.0 min, ACN–THF (60:40); 20.0 min, ACN–THF (60:40); 21.0 min water–ACN–THF (40:50:10); 25.0 min water–ACN–THF (40:50:10). The injection volume was 5 μl and the flow-rate was 1.0 ml min^{-1}.

3. Results and discussion

All concentrations quoted relate to the original sample.

3.1. Dialysis

A lubricating oil comprises of a base oil plus additives and dialysis is used for the bulk fractionation of such into a dialysis residue (high-molecular-mass species, usually $M_r > 1000$) and a dialysate (base oil plus low-molecular-mass species).

The procedure is commonly used as a de-oiling process, but in this case it is used to remove higher-molecular-mass compounds and debris that could interfere with the pre-column derivatisation of the carboxylic acids.

3.2. Liquid chromatography

Carboxylic acids lack a chromophore or a fluorophore and so require derivatization prior to LC to achieve enhanced selectivity and sensitivity. Fluorescence labelling reagents based on 7-methoxycoumarin, especially 4-bromomethyl-7-methoxycoumarin (BrMMC) [12], aryldiazoalkanes [15] and halogenobenzofurazans [19] have

all been used for the analysis of carboxylic acids. Coumarin derivatives [24,25], 3-aminoperylene [22] and N-(bromoacetyl)-N′-[5-(dimethylamino)-naphthalene-1-sulphonyl]piperazine [26] have also been used as selective labels for carboxylic acids in conjunction with peroxyoxalate CL detection. These procedures are unsuitable for the analysis of carboxylic acids in used oils as the derivatization reactions and the separations either used polar solvents that are incompatible with the used oil matrix, or, as in the case of 3-aminoperylene, used a solvent (benzene) that is undesirable for health reasons.

9-Anthracenemethanol can be directly coupled with carboxylic acids in non-polar media using DCC as a coupling agent. Separation of the 9-anthracenemethanol esters on a Spherisorb S5 ODS2 column using a water–ACN–THF mobile phase has been achieved, under both isocratic [28] and gradient conditions [30], with CL detection. However, the isocratic method was unable to provide sufficient resolution for oxidised oil samples and the gradient method was not used for quantification due to the effect of varying mobile phase composition on the response factors.

The same label has been used with fluorescence detection for the determination of carboxylic acids [27]. It has been shown that the sensitivities of fluorescence and CL detection for these derivatives are of the same order of magnitude [31] although these figures are clearly instrument dependent. However, the attraction of using this label with fluorescence detection (as opposed to CL detection) is its greater tolerance to changes in mobile phase composition. To determine the optimum excitation and emission wavelengths for the ester derivatives, a mixture of three acids [hexanoic (C_6), decanoic (C_{10}) and octadecanoic (C_{18}) acids] were derivatised and separated using the gradient profile given above. As the excess label peak and each acid derivative was eluted in turn, it was isolated in the fluorimeter flow cell and the emission and excitation wavelengths scanned. On the basis of these profiles the excitation wavelength was set at 251 nm and the emission wavelength was set at 412 nm for all subsequent work.

Separation of 9-anthracenemethanol derivatives has been carried out on columns packed with Spherisorb S5 ODS2 [28,30]. Spherisorb S5 C_8 columns are less retentive of these derivatives due to the shorter C_8 alkyl chains of the stationary phase [32]. Several different water–ACN–THF gradients were investigated and the conditions which gave the shortest runtime with baseline resolution of the acid derivatives (C_6–C_{22} straight-chain acids) are presented in Table 1. Preliminary results showed that the Hichrom RPB column could also be used for this application and gave lower capacity factors (*e.g.* 9.7 *vs.* 13.0 for octadecanoic acid) and narrower baseline peak widths (*e.g.* 0.09 *vs.* 0.15 min for octadecanoic acid) than the S5 C_8 column.

3.3. Oil analysis

Two oil formulations, oil 1 and oil 2, differing only in the presence of an anti-oxidant additive in oil 2, were tested in a car engine. The oil in the sump of the engine was sampled after 0, 16, 40 and 64 h continuous running of the engine. At 64 h, oil 1 had thickened and degraded to beyond the point of use while oil 2 still held its lubricating properties.

When analysed by TLC–flame ionisation detection, the concentration of polar oxidation products in the dialysates of both oils was shown to increase with time from a negligible amount at 24 h to a high proportion (30%, m/m) at 64 h. FT-IR spectra of the 64-h dialysates indicated the presence of acid carbonyl groups.

Calibration data

The fresh (0 h) oil dialysate of oil 1 was spiked at varying concentration levels (0–4 mg ml^{-1}) with a mixture of straight-chain carboxylic acids (C_6–C_{22}) in *n*-heptane prior to derivatization. Separation of the derivatives was achieved on the Spherisorb S5 C_8 column within 20 min, with negligible interference from the oil dialysate matrix (except on the hexanoic acid derivative). Three components with retention times between 10.5 and 12 min interfere with the hexanoic acid derivative. These peaks are not present in the reagent blank (derivatization mixture without a

Table 1
Retention data for carboxylic acid-9-anthracenemethanol derivatives in *n*-heptane and fresh oil 1 dialysate matrices, identification of carboxylic acids in 64-h used oil dialysates of oil 1 and oil 2

Acid derivative	Carbon number	Capacity factor (k')[a]			
		Solvent matrix	Fresh oil dialysate matrix	Oil 1, 64-h dialysate	Oil 2, 64-h dialysate
Hexanoic acid	6	8.0	8.0	Not found	Not found
Heptanoic acid	7	8.9	8.9	Not found	Not found
Octanoic acid	8	9.6	9.6	9.6	Not found
Nonanoic acid	9	10.2	10.2	Not found	Not found
Decanoic acid	10	10.7	10.7	10.7	10.7
Dodecanoic acid	12	11.5	11.5	11.5	11.5
Hexadecanoic acid	16	12.6	12.6	12.6	12.6
Octadecanoic acid	18	13.0	13.0	13.0	13.0
Eicosanoic acid	20	13.3	13.3	13.3	13.3
Docosanoic acid	22	13.6	13.6	13.6	13.6

[a] $t_0 = 1.24$ min.

spike of oil dialysate or acid standard) and so are probably due to acidic additives in the oil, *e.g.* salicylic acid-based detergents. The reagent blank is compared with the fresh oil dialysates in Fig. 2 whilst the fresh oil 1 dialysate and the fresh oil 1 dialysate spiked with the complete mixture of acids at the 2 mg ml^{-1} level are compared in Fig. 3. Retention data for the acid derivatives in the fresh oil dialysate and in pure solvent are presented in Table 1. As can be seen by comparison of the capacity factors (k') the oil dialysate matrix does not affect the retention times of the derivatives.

Calibration graphs for all of the acids over the range of interest (1–4 mg ml^{-1}) were linear (Table 2), with correlation coefficients (r^2) over the range 0.9987–1.0000. The limit of detection ($S/N = 3$) for octadecanoic acid (determined using standards covering the range 0–0.15 mg ml^{-1}) was 85 μg ml^{-1} in *n*-heptane (4.3 ng on-column) which is more than adequate for this application. Recoveries for the derivatisation

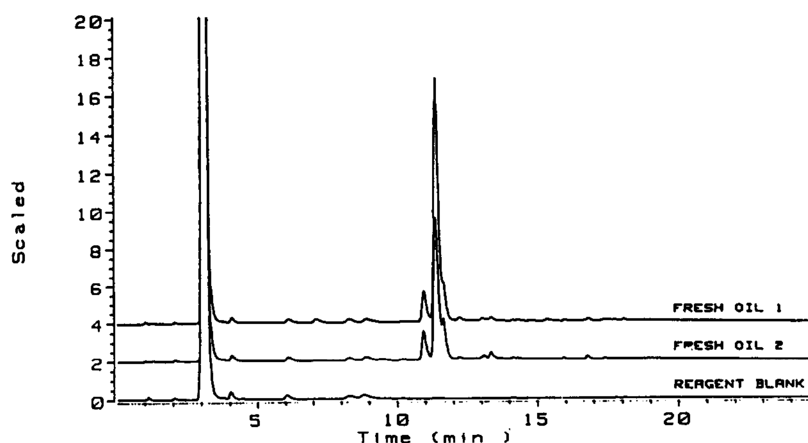

Fig. 2. Chromatograms of a reagent blank (derivatization mixture without spike of oil dialysate or acid standard) compared with chromatograms of fresh oil dialysates of oil 1 and oil 2.

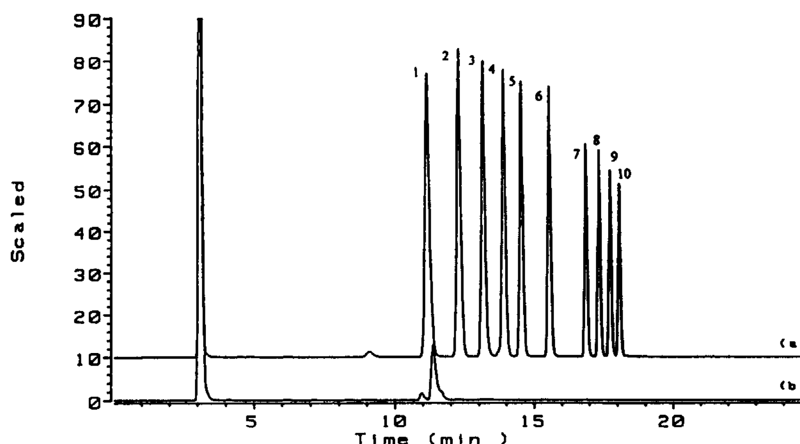

Fig. 3. Comparison of chromatograms of (b) fresh oil dialysate of oil 1 and (a) of the same dialysate spiked with a mixture of carboxylic acids at the level of 2 mg ml^{-1}. Peak assignment; 9-anthracenemethanol, (1) hexanoate (C_6), (2) heptanoate (C_7), (3) octanoate (C_8), (4) nonanoate (C_9), (5) decanoate (C_{10}), (6) dodecanoate (C_{12}), (7) hexadecanoate (C_{16}), (8) octadecanoate (C_{18}), (9) eicosanoate (C_{20}) and (10) docosanoate (C_{22}).

reaction were in the range 100–103% (Table 2) for the fresh oil dialysate (oil 1) spiked with the mixture of acids (C_6–C_{22}) at the 3 mg ml^{-1} level.

To determine whether the derivatives have a similar detector response, the calibration data were recalculated with the concentrations of the acids converted from mg ml^{-1} to *M*. The slopes of the lines of best fit for ten acids covering the range C_6–C_{22} are very similar (in the range 35 495–37 367, mean 36 640), with a relative standard deviation of 1.8%. It can be inferred from this that the detector response of the 9-anthracenemethanol esters of straight-chain aliphatic carboxylic acids is independent of chain length and the solvent gradient.

Comparison of engine test oils

The two series of dialysates for oil 1 and oil 2 over the time course of the engine test were derivatised and analysed and gave very similar results; the chromatograms for the entire series

Table 2
Linear fit and recovery data for carboxylic acids in fresh oil 1 dialysate matrix

Acid	Slope [concentration (mg ml^{-1})]	Intercept	Concentration found in fresh oil dialysate spiked with mixture of acids (mg ml^{-1})	Concentration of acid standard spike (mg ml^{-1})	Recovery (%)
Hexanoic acid	318.5	−185.5	3.0	2.9	103
Heptanoic acid	277.6	−168.5	3.2	3.2	100
Octanoic acid	257.6	−148.0	3.1	3.0	103
Nonanoic acid	232.9	−141.0	3.2	3.1	103
Decanoic acid	216.6	−125.7	3.0	3.0	100
Dodecanoic acid	185.2	−108.1	3.2	3.1	103
Hexadecanoic acid	143.2	−81.9	3.0	2.9	103
Octadecanoic acid	128.5	−76.2	3.1	3.1	100
Eicosanoic acid	116.6	−67.8	3.0	3.0	100
Docosanoic acid	105.7	−62.2	3.0	3.0	100

for oil 1 and the two 64-h samples are presented in Fig. 4. The peaks tentatively identified as additives disappear from the oil dialysates between 0 and 24 h of the test, as would be expected, as they are used up in the course of carrying out their functions. No measurable carboxylic acids appear until 64 h, at which time an homologous series of acids starting at C_8 and going beyond C_{22} can be clearly seen superimposed on a "hump" of unresolved components. The carboxylic acids identified by capacity factors (and confirmed by spiking) in the two 64-h samples are shown in Table 1.

The difference between the two 64-h samples was calculated by summing the areas of the peaks with retention times between 15.07 and 18.31 min and dividing by the mass of oil dialysate taken. This time window was selected because it contained the bulk of the carboxylic acids and was free of interference from the label and oil additives. It gives an indication of total carboxylic acid content and allows direct comparison of different oils. The area/mg is greater in oil 1 (2.8 area units mg^{-1}) than oil 2 (2.3 area units mg^{-1}), indicating that the hydrocarbon fraction of oil 1 has been oxidised to a greater extent than that of oil 2. This is in agreement with the physical characteristics of the two oils. The most important physical characteristic is thickening (change in viscosity) and the cut-off point is a 270% increase in viscosity compared to that of the fresh oil at the start of the engine test.

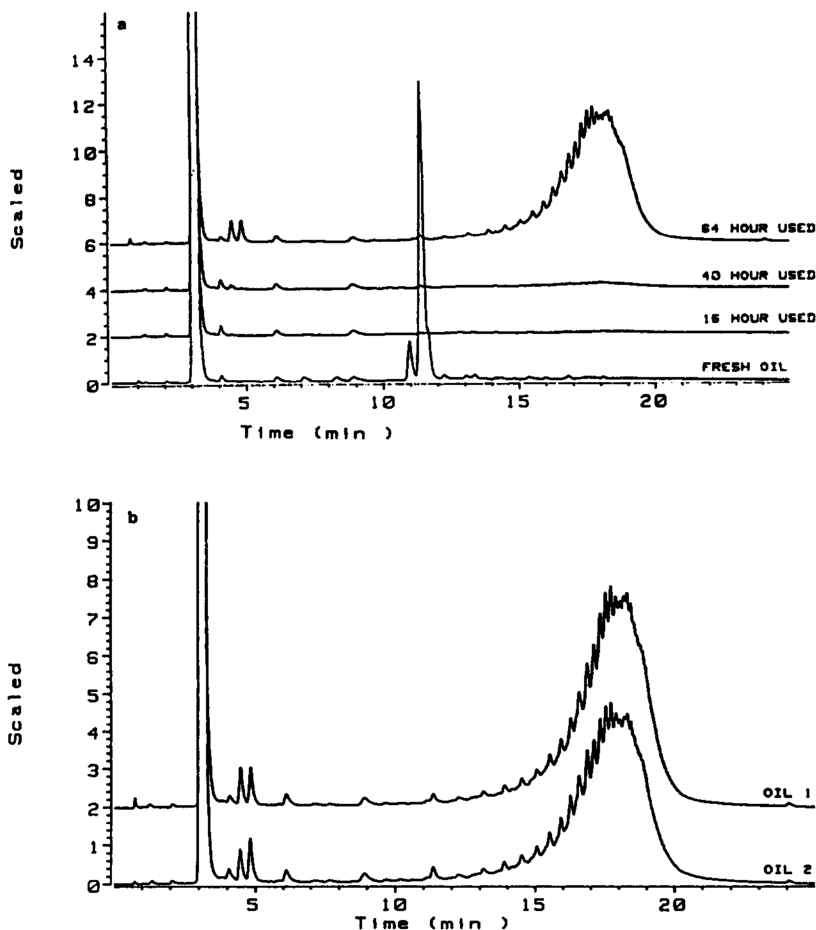

Fig 4. Chromatograms of derivatised engine oil dialysates (a) oil 1 engine test (b) 64-h used engine oils.

In the case of oil 1 there was a 950% increase in viscosity after 64 h as compared with a 215% increase for oil 2.

4. Conclusions

This procedure allows for the rapid and simple determination of aliphatic carboxylic acids (C_6–C_{22}) in used oil matrices by selective derivatization with 9-anthracenemethanol and gradient reversed-phase LC with fluorescence detection. Calibrations over the range 1–4 mg ml^{-1} were linear ($0.9987 \leq r^2 \leq 1.0000$). The limit of detection ($S/N = 3$) for octadecanoic acid was 85 μg ml^{-1} in *n*-heptane (4.3 ng on-column). It was shown by analysis of two series of oils from engine tests that carboxylic acids are formed during the oxidation of engine oils and that their concentrations are directly related to the degree of degradation. Work in this laboratory is now aimed at developing procedures for the determination of species intermediate in the oxidation mechanism, *e.g.* aldehydes.

5. Acknowledgement

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