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Identification and semi-quantitative determination of anti-oxidants in lubricants employing thin-layer chromatography-spray mass spectrometry☆

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ABSTRACT

A quick and simple method for identification and semi-quantitative determination of nine antioxidants commonly used in lubricants is presented. A dual step thin-layer chromatography (TLC) separation, removes in a first step the oil matrix whereas in a second step the antioxidants are separated. Cutting the spots out of the TLC-plate in the form of triangles allows direct-spray mass spectrometric (MS) measurements, providing MS and MS^n spectra (if an appropriate MS instrument is employed) of the antioxidants, allowing their identification but also giving information about potential oxidation or degradation of these additives. Calibration curves within the concentration range relevant for the analysis of real oil samples (0.2–1.2 g L^{-1}) were constructed with R^2 values above 0.98 (when using an appropriate internal standard). This allowed the semi-quantitative determination of the selected antioxidants in real oils samples. Comparison with results from HPLC-UV measurement showed acceptable agreement for all analytes.

1. Introduction

A novel group of mass spectrometric (MS) techniques commonly summarized under the name "ambient mass spectrometry" has gained increasing interest over the last years [1,2]. One main advantage all these MS-methods have in common is that they are simple, quick and in most cases allow a straightforward analysis of the sample without any sample pretreatment. The most widely known ambient MS techniques are direct analysis in real time (DART) introduced by Cody [3] and desorption electrospray ionization (DESI) presented by the group of Cooks [4]. Only a few years later a new sub-group named "direct ionization" or "direct analysis" MS was introduced whereby a spray was generated for example from a piece of paper [5] (soaked with the analytes) or directly from biological tissue such as leaves [6].

Although largely replaced by HPLC in routine analysis, thin-layer chromatography (TLC) still is widely used as it allows the chromatographic separation of mixtures with very simple instrumentation [7]. Additionally, when analyzing "dirty" samples without any previous clean-up, the fact that TLC plates are cheap and designed for "single use only" can be seen as a distinct advantage of this technique. Detection in TLC is usually performed with photometric techniques but in recent years some approaches allowing obtaining mass spectra from TLC spots have been presented. These comprise the analysis of TLC plates by electrospray assisted laser desorption ionization MS [8], MALDI [9,10], DART [11], DESI [12] or by a dedicated instrumentation for automatic elution of the analyte from the TLC spot with subsequent MS analysis [13]. Employing a "direct ionization" approach, spraying from narrow TLC stripes with a sharpened tip [14] and just recently from spots cut from developed TLC plates was introduced [15] allowing the coupling of TLC with MS detection.

Lubricants are important technical products and a wide range of different types of lubricants, each specially designed for its field of application, are commercially available to meet the requirements by all sorts of industries. Besides the so called basestock (i.e. the actual oil component), lubricants contain a wide range of additives determining their final properties. Thereby, several classes of additives influencing different parameters can be distinguished: viscosity control additives, antiwear and extreme-pressure additives, corrosion inhibitors, dispersants, detergents and antioxidants [16]. Depending on the field of application, the total amount of additives is typically between 5% (w/w) and 30% (w/w) [17]. Analysis of such additives in oil samples has been done by gas-chromatography (GC) [18–21], HPLC [19,22] and MS methods [23–26].

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In this work we present a simple and straightforward approach for the qualitative and semi-quantitative analysis of antioxidants in lubricant samples based on TLC-spray MS. Oil sample can be directly (if necessary after dilution with hexane) spotted onto the TLC-plate, whereby a first step development removes the oil-phase and in a second step the additives are separated. Finally direct spray MS is possible for any spot of interest after cutting from the TLC plate.

2. Materials and methods

2.1. Chemicals and materials

Tetrakismethylene-(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate)methane (Irganox L101), 2,2′-thiobis(4-methyl-6-*tert*-butylphenol) (Irganox 1081), dilauryl thiodipropionate (Irganox PS 800), alpha-tocopherol (Irganox E201) and 2,2′-thiodiethylen-bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)proprionate (Irganox L115) were provided by Ciba (Basel, Switzerland); 2,5-di-tert-butyl-4-hydroxy-octadecyl-hydrocinnamate (Irganox L107), hexane-1,6-bis[3-(3,5-di-*tert*-butyl-4-hydroxy-phenyl)propionate] (Irganox L109), 2,5-di-*tert*-butyl-4-hydroxy-(C_{10}-C_{14})-isoalkyl-thioacetate (Irganox L118) and 2,5-di-*tert*-butyl-4-hydroxy-(C_7-C_9)-hydrocinnamate (Irganox L135) were obtained from BASF (Ludwigshafen, Germany), tris[(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl) methyl]-1,3,5-triazinane-2,4,6-trione (Cyanox 1790) from Cytec Industries Inc (Woodland Park, NJ, USA) and 4,4′-bis(α,α-dimethylbenzyl)-diphenylamine (Naugalube AMS) from Chemtura (Philadelphia, PA, USA).

Acetone, toluene, acetonitrile and *n*-hexane, all analytical grade, were supplied by VWR (Vienna, Austria).

Several mineral oil based model oils were provided by Fuchs Europe Schmierstoffe GmbH (Mannheim, Germany).

For TLC separations, silica gel 60 F_{254} coated aluminum TLC plates (200 × 200 mm; Merck, Darmstadt, Germany) were developed in a standard TLC chamber (250 × 250 × 100 mm; Desaga, Heidelberg, Germany). Pure *n*-hexane and an *n*-hexane/acetone mixture (91% (v/v) n-hexane) were used as mobile phase. To apply the oil samples on TLC plate a 25 μL syringe (Hamilton, Reno, NV, USA) was employed.

2.2. Instrumentation and Methods

2.2.1. Thin-layer chromatography

For visualization of the colorless spots a multi-purpose equipment for UV 254/366 nm and daylight illumination (Desaga) was applied using a wavelength of 254 nm.

2.2.2. Mass spectrometry

For MS measurements a G2440A MSD Ion-Trap from Agilent (Waldbronn, Germany) was employed in positive ion mode with a capillary voltage of 3 kV. Based on the optimization results (see Section 3.2) the drying gas flow rate was set to 3 L min^{-1} and the drying gas temperature to 150 °C for all further measurements.

2.3. Thin-layer chromatography

5 μL of sample were applied to a TLC Plate 5 cm from the bottom edge using a 25 μL syringe. The plate was then developed in a standard TLC chamber using 50 mL of *n*-hexane. When the solvent front reached a height of 9 cm the plate was removed from the chamber. Subsequently, the lower part of the plate is cut off at a height of 6 cm from the bottom edge. Afterwards, the polarity of the mobile phase was increased by adding 5 ml of acetone. The lower part of the TLC plate was then rotated by 180° and inserted into the TLC chamber for another chromatographic separation. As soon as the solvent front reached a height of 5.8 cm, the plate was removed and investigated under UV-light at a wavelength of 254 nm.

2.3.1. Mass spectrometric analysis of TLC spots

From the TLC plate the desired spot was cut out in a way that its center is located 3 mm below a 60° tip. For this reason, the shape of an equilateral triangle was used for the experiments, with a side length of 10 mm. The analyte-containing triangle was then placed directly in front of the MS inlet with a distance of 2 mm between the TLC material and the orifice of the spray shield. Thereby it was fixed with a grounded clamp which can be adjusted with three setscrews that enable exact, fast and reliable positioning in all spatial directions. After mounting the triangle, the capillary voltage was switched on. Dispensing 20 μL of solvent resulted in the formation of an electrospray and the observation of respective mass spectra.

For semi-quantitative measurements an internal standard was added. This was achieved by spotting 10 μl of a Irganox PS 800 solution in acetone (c = 200 mg L^{-1}) onto the TLC triangle. For subsequent quantitative evaluation, peak areas of extracted ion traces were integrated.

2.4. Analysis of antioxidants in lubricants by HPLC-UV after SPE pre-treatment

For conditioning, the SPE cartridge (in lab prepared glass cartridges (13 mm i.d.), packed with 0.8 g silica gel, for column chromatography, with a particle size of 63–200 μm supplied by Merck) was flushed with 5 ml of an 1:1 *n*-hexane/acetone mixture (v/v) and another 5 ml *n*-hexane. Around 600 mg of oil were diluted with 200 μl toluene and vortexed for 2 min. 100 μl of this mixture were applied on a conditioned SPE cartridge, followed by removal of the oil matrix with 5 ml of *n*-hexane using a flow rate of approximately 0.5 ml min^{-1}. Subsequently the analytes were eluted employing 2 ml of the same 1:1 mixture of *n*-hexane and acetone previously used for conditioning at a flow rate of 0.5 ml min^{-1}. The obtained eluate was diluted with acetonitrile 1:10 (90% (v/v) acetonitrile, 10% (v/v) eluate).

HPLC-UV analyses were performed on an Agilent 1260 Infinity LC System equipped with vacuum degasser, quaternary pump, autosampler and photodiode array detector. For the separation, a Kinetex C_{18} column (50 × 3 mm; particle size: 2.6 μm; Phenomenex, Aschaffenburg, Germany) with a binary acetonitrile/water gradient at a flow rate of 1.2 ml min^{-1} was used. The specific parameters of the employed method were based on previous work by Hintersteiner et al. [27].

3. Results and discussion

3.1. Sample preparation and TLC separation

Antioxidants typically employed in oils and included in the current study are displayed in Fig. 1. In order to analyze them in lubricants, they must be isolated from the oil-matrix first. In this study, the spiked oil samples were diluted and applied to a TLC plate, followed by a dual separation approach (see Fig. 2): in a first step a chromatographic isolation of the antioxidants from the matrix was accomplished. In a second step, the composition of the mobile phase was changed and the additives themselves were separated. More specifically, 100 μL of oil were diluted with 600 μL toluene and vortexed for two minutes at 2000 rpm. This dilution step was conducted in order to decrease the rather high concentration of the stabilizers in the oil-samples and also to decrease the viscosity, which in turn allows an easy application of the oil onto the TLC plate. 5 μL of this mixture were applied 5 cm from the