Titel der Diplomarbeit

Developement of a fast analytical methodology based on HS-PTV-GC-MS to determine antioxidants in motor oils

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Contents

Abbreviations

API American Petrol Institute

DL detection limit

EI electron impact (ionization)

FAB fast atom bombardment

FGC fast gas chromatography

FI/FD flame ionization/flame detector

FID flame ionization detector

GC gas chromatography

HPLC high performance liquid chromatography

HS head space

ICP/MS inductive coupled plasma/mass spectrometry

MALDI matrix assisted laser desorption ionization

MS mass spectrometry

NPD nitrogen phosphor detector

P&T Purge and Trap

PAHs polycyclic aromatic hydrocarbons

PC personal computer

PI photo ionization

PTV programmable temperature vaporizer

QL quantitation limit

RI refractive index (detector)

RSD relative standard deviation

S/N signal/noise ratio

SEC size exclusion chromatography

SEM secondary electron multiplier

SIMS secondary ion mass spectrometry

TOF time of flight

Chapter 1

Introduction - Techniques [1]

1.1 Headspace generation (HS)

The generation of a headspace (HS) is a technique to separate the volatile compounds of a sample from its matrix. The gas phase in equilibrium with the sample is named headspace.
This possibility of separation can be coupled with various analytical techniques, but has found most use in the coupling with gas chromatography, because it is an appropriate separation technique for the analyses of gas samples [1].
In HS-GC the gas phase is in equilibrium with the sample (liquid or solid) and is analyzed with gas chromatography. The headspace generation can either be static or dynamic.

In static HS-GC the sample is put into a vial leaving a certain volume (usually at least half of the vials volume) empty. The vial is sealed hermetical and then heated in an oven. There the volatile compounds are separated from the matrix and after some time depending on the sample an equilibrium between the gas phase and the matrix phase is established. When this point is reached a portion of the headspace is injected into the gas chromatograph.
Based on this procedure, which is also named HS in one step, there have been explored variations of this technique. One of them to mention is the addition of an absorption trap which separates the volatile analytes from the rest of the compounds in the gas phase.
In 1989 Pawliszyn and his research group introduced solid phase micro extraction (SPME) [2, 3]. A fiber of fused-silica, whose surface was covered with an immobilised stationary phase, was introduced into the vial containing the sample. The fiber can submerge into the liquid or stay above the sample, in the headspace. After a certain time the volatile compounds were absorbed in the fiber and were then introduced into the vaporization chamber of the injector of the gas chromatograph. The analytes were transferred to the

[1] adapted from (with permission from the author): "Determinación de Trihalometanos en aguas mediante el acoplamiento de un generador de espacio de cabeza con un inyector de temperatura programada", Sara Herrero Martín, 2007, Universidad de Salamanca

column by thermical desorption. This technique is named HS-SPME and was first used in 1993 [4] and has resolved various analytical problems since.

The continuous or dynamic headspace generation was proposed for the first time in 1962 by Swinnerton and coworkers [5, 6]; Bellar and Lichtenberg [7] developed a technique which they named purge and trap (P&T). With this mode an equilibrium is not established between the two phases of the vial, but the separation is achieved by continuingly sucking in the gas phase until the volatile analytes are separated from the sample. An inert gas flow over the solid or liquid sample, or bubbling the inert gas through the liquid sample, is used. The purged volatiles are diluted in the extracting gas and need to be focused in a trap before they are introduced into the column. In order to focus the volatiles a cold trap can be used, although in general a tube packed with absorbing material is employed. From there the volatile analytes are transferred to the chromatographic column by thermical desorption.

The headspace generation permits one to analyze the volatile compounds of a sample without interference of the non volatile compounds of the sample matrix. This is a simple extraction, which minimizes the step of sample treatment, thereby reducing considerably the errors associated with this step, the time and the price per sample analyses.

The static headspace generation is the fastest and most simple alternative, which can be easily automatized. The coupling of the static mode presents sometimes the disadvantage of a broad initial band when large sample volumes are used to increase sensibility. Although, there have been described some modifications which reduce this effect by techniques of cryogenic focusing [1], thus permitting sensibilities equal to purge and trap and HS-SPME.
The sensibility of HS-GC depends, apart from the detector, on the capacity of the chromatographic column, which is dependent on the initial band and the consecutive disminuation of the chromatographic resolution [8].
Independent from the HS-technique selected, the headspace consists of a more or less diluted gas sample. Thus the problem resulting is: How to introduce into the chromatographic column the largest sample volume possible, in order to reach the desired sensibility, and furthermore, how to speed up this process to reduce the broadness of the initial band of the chromatogram.
When a headspace generator is coupled with a gas chromatograph with a conventional injector the usual injection modes are:

- split injection: injects quickly a portion of the generated volatiles into the chromatographic column; with this injection mode the necessary sensibility for trace analyses is generally not achieved.

- splitless injection: practically injects all of the generated volatiles, permitting higher sensibilities, but the injection speed is slower, therefore showing a lower

chromatographic resolution in the initial zone of the chromatogram.

In the book recently published by the investigation group of Bruno Kolb, some techniques are presented which have been developed in the last years, in order to solve the stated problem [1].
These techniques try to enrich the analytes by cryogenic focusing, thus achieving preconcentration and posterior fast injection into the chromatographic column.
The first devices produced were metal or glas traps in U-form, which were held in a liquid nitrogen or argon bath. Once the analytes were condensed in the tube, the bath was removed manually and the trap was heated (electrically in general) to evaporate the condensed analytes.
Later, advances to eliminate the manual removement of the cryogenic liquid were assayed. In place of a bath around the U-formed trap a teflon tube surrounding the trap was used, and after the condensation through the liquid nitrogen hot water heated the trap. Further, traps cooled with a flow of gas and the posterior evaporation of the analytes by a hot gas stream arose. With the goal to evade the flow of hot gas, the idea to introduce the device into the interior of the gas chromatograph came to mind, so that once the cool gas flow stopped the trap would rapidly reach the temperature of the GC oven.
This strategy was carried out initially with a flow of nitrogen gas (previously cooled outside the chromatograph) circulating in the teflon tube surrounding the first part of the column. Also liquid nitrogen and liquid CO_2 were used. The sample condenses in the head of the column and later by steady increasing the temperature of the oven the solvent eluates rapidly, while the analytes with higher evaporation points stay in a small band.
This system has certain limitations. Firstly the (venting) flow is limited by the flow of the column. Secondly the entrapment in the head of the column at low temperatures not only traps the analytes but also traps impurities or undesired compounds, which requires once in a while to cut the column to eliminate these interfering compounds. Lastly the preconcentration at temperatures below zero degrees centigrade may lead to flow blocking in capillary columns by frozen water [9, 10, 11].
An alternative is the use of pre-columns, which are deactivated chromatographic columns, connected to the initial part of the separating column. These devices are more easily replaced, although they require an appropriate connection to the chromatographic column.
Besides the blocking of the gas flow due to frozen water, presence of water in the GC may cause distortions in the peak, especially at the peaks, which eluate together with water. To solve this problem various techniques to eliminate water vapour before injection into the column were developed. In dynamic HS, semipermeable membranes and reflux condensators are used. When static HS is used, less water vapour is produced and can be absorbed by an inert salt for example.

The principal problem of these cryogenic focusing devices is their manual fabrication and the high qualification necessary to operate them. This concludes the necessity of

automatized and easy-to-use devices which can solve the coupling of HS-GC, in order to reach appropriately low levels of sensibility for trace-analytics, without compromising the initial zone of the chromatogram.

A possible alternative is the use of commercial devices named programmable temperature vaporizers (PTV). This way was assayed in the present work.

1.2 Programmable temperature vaporizer (PTV)

The concept of injecting the sample at programmed temperature was described in 1964 by K. Abel [12]. In 1979, Vogt and coworkers presented for the first time a PTV system [13, 14].
At the beginning of the 1980s the research groups of Poy [15] and Schomburg [16] explored the possibilities of cold injection and developed devices which were universally applicable.
From that moment on various authors have investigated methods to use injection with PTV. The number of publications about this type of injection in GC shows the interest and its potential, which can be seen in the bibliographic revision of Engewald and coworkers in 1999 [17].

Initially it has been applied to injectors of liquids due to the advantages, which are involved respectively to the conventional GC injections.
Traditionally the injection techniques of liquid samples in GC can be divided into techniques with prior evaporation and techniques with (direct) injection into the column.
In the first one the injector is a liner or vaporization chamber which is held at high temperatures (up to 350°C), to facilitate the fast evaporation of the sample. These injectors allow two types of injection: split and splitless.
The technique of (direct) injection into the column is usually used for samples which decompose at temperatures above their boiling point [18]. The solution is injected directly into the column without passing a hot injector. Thereby thermic degradation as well as discrimination of the analytes (possible in the vaporization chamber) is evaded.
Unfortunately, in the case of samples with considerably quantity of non volatile analytes or samples containing much water, a diminished efficiency and stability of the chromatographic separation was observed [17, 19, 20]. Furthermore, the retention of impurities in the chromatographic column shortens its life.
This technique has been used for the injection of large liquid sample volumes ($> 2\mu$L) in GC in combination with pre-columns. In this device the separation of solvent and analytes, at an appropriate temperature, is achieved, followed by transference of the analytes to the chromatographic column.
Besides permitting injection of large sample volumes, the pre-column also retains parts of the impurities of the sample. Nevertheless, some keep being injected into the column. Therefore this technique is not considered the appropriate one for the analyses of sam-

ples with high concentrations of non volatile compounds [19, 21].

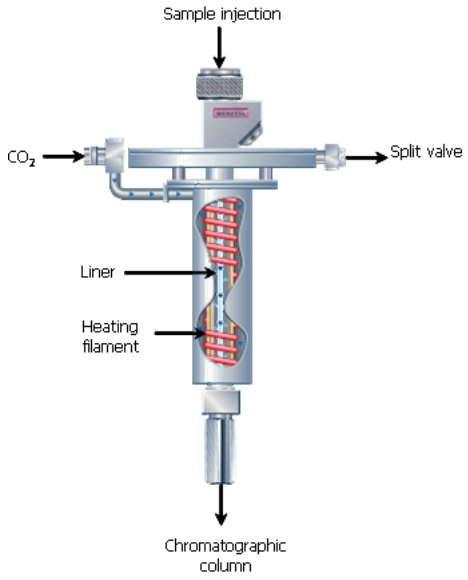

Figure 1.1: Programmable temperature vaporizer (PTV); *from ref [21]*

The PTV consists of the same elements of a traditional injector, but is equipped with an efficient cooling and heating system. This permits the programmed control of the temperature of the injector during the injection process, thus permitting a great variety of the injection mode, including the classic split and splittless injection:

- hot split injection
- cold split injection
- hot splitless injection
- cold splitless injection
- solvent vent injection

Using the appropriate liner, injection of liquid samples is also possible. The results are comparable with conventional injection techniques, but with the advantage that the PTV permits one to work with samples of high concentrations of non volatile compounds, due to the controlled temperature [17, 10].
One of the advantages of a PTV over conventional injection modes is the cold injection. The injector is at low temperatures when the sample is injected and, once the injection is complete, the liner is heated continuously, thus controlling the vaporization of the compounds with different boiling points. So, with this technique the discrimination due

to the different boiling points of the analytes [15, 16, 21, 22], which can occur in the conventional vaporizing chambers, is eliminated. On the other hand the thermic degradation occurs in less proportion, because the analytes do not undergo a thermical shock and heating is only necessary up to that temperature so that they are transferred to the column [21, 22].

The prime advantage of a PTV injector is the large volume injection (LVI) [13, 14]. With this device, injection of sample volumes higher than 100μL into the chromatographic system is possible. This is a major advantage, because a significant increase of the sensibility of the analytical method is achieved compared to conventional injectors, where sample volumes range between 1 and 2μL [19, 10].
The elimination of the solvent (purging) at low temperatures, before transferring the sample to the column, permits the introduction of large sample volumes. This injection mode is known as solvent vent (SV).
In general the PTV is programmed in a way that the liner is kept at a temperature a little lower than the boiling point of the solvent when the sample is injected and the split valve is open. Thereby the solvent is eliminated through the split valve while the analytes (with a higher boiling point) remain condensed in the liner. Once the solvent is eliminated, the valve is closed (splitless mode) and the analytes are transferred to the column by fast heating of the liner [17].

To maximize the retention effect and to minimize the loss of the analytes in the liner during the purging of the solvent different packing materials were explored. The group of Herraiz has described the use of Volasphere A-2, Tenax and glass wool [23]. Mol and coworkers studied alternatives for glass wool, like polytetrafluorethylene (PTFE), polyimide and Tenax-TA® in the analyses of a solution of 27 compounds, with a wide range of polarities and thermical stabilities [24]. PTFE and Tenax-TA® showed good retention capacities.

The injection process can be repeated many times with a process named multiple injection. In this case the solvent is purged many times consecutively and in the end the injections in the PTV are heated until all analytes are in the chromatographic column. Another way of multiple injection is the injection at controlled speed. In this case the repeated injection of small portions of sample is replaced by a continuous process. Thus the injection and the evaporation of the solvent at the same time creates an equilibrium between the liquid phase being injected and the vapor phase being purged. This technique permits injection volumes above 1000μL [21].

The advantages of a PTV in combination with the commercial accesability of these types of injectors have made it very attractive for the trace analyses. The principal application of PTV injectors was the injection of large liquid sample volumes in the solvent vent injection mode.

This method has been employed recently in the determination of cannabinoides in biological fluids [25], phenols [26], polybromated compounds [27], polycyclic aromatic hydrocarbons (PAHs) [28, 29], pentaclorobenzylhydroxylamines [30] and pesticides [10, 31], among others.
Also other applications of a PTV were developed, like the posibility to use it as sample preparation step, combining microextracion techniques or derivatization processes. Thereby this step was simplified and an online-coupling of GC with sample preparation steps was achieved. Furthermore the direct injection of aqueous samples was possible due to the use of hidorfobic adsorbents in the liner and occasionally it was used as a thermoreactor [17].

Kolb [1] describes the different possibilities of cryogenic trapping. In the present work cryogenic focusing was applied.

1.3 PTV as injection system of headspace in GC

Unfortunately in the consulted bibliography hardly any works related to HS-PTV-GC were found. The devices used for cryogenic focusing on sample introduction into the GC column were mainly homemade and not very automatizable (see section 2.2).
Some technical notes from suppliers of these devices were found [32]. Furthermore, J. Efer published an article in 1993, where static HS-PTV-GC was used to determine indirectly Ethephon (2-chlorethylphoshonic acid). The HS used had a manual injection system, where the volatiles were extracted in a syringe and then injected into the liner of the PTV [33].

Regarding dynamic HS, also only a few applications of P&T-PTV have been found in the consulted bibliography. R. Eiden and coworkers published in 1998 a study of automatized determination of stannous organic compounds in water by P&T-PTV-GC-MS [34].

In the present work the possibilities of coupling static HS-PTV-FGC-MS to determine antioxidants in lubricant oils were studied.

1.3.1 Hot and cold injection

The programmable control of the temperature in the PTV permits cold and hot injection, each possible in split and splitless mode. The sequence of steps involved in sample analysis by HS-PTV-GC after the HS generation is shown in Figure 1.2. The typical temperature profiles for the syringe, the liner in the PTV and of the chromatographic column are shown. Figure 1.2a is the profile for hot, and Figure 1.2b the profile for cold injection.
The first step is identical in both injection modes, because the syringe holding the HS is kept at a higher temperature than where the HS generation was conducted, in order

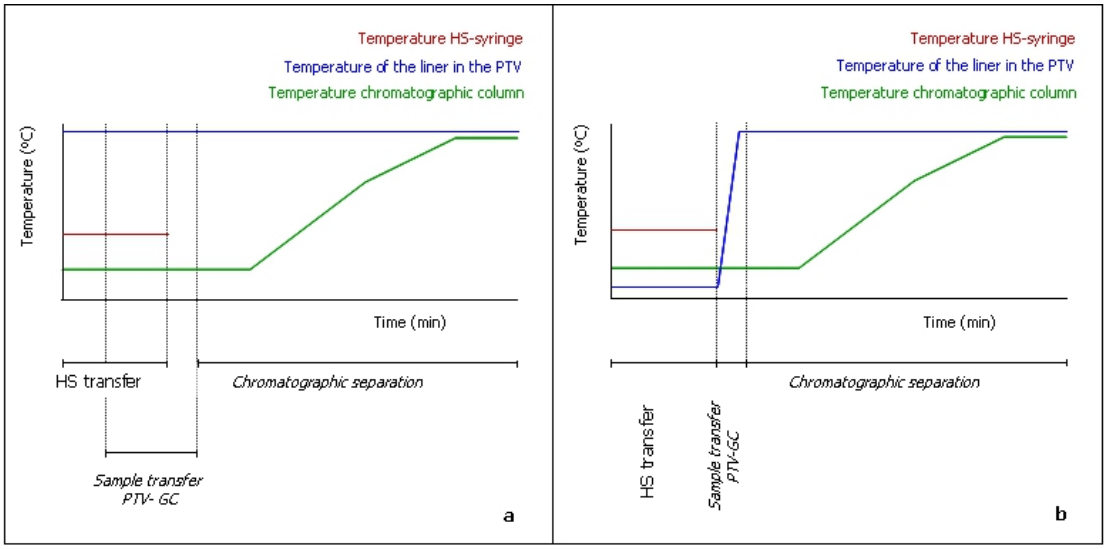

Figure 1.2: Temperature sequence of hot (a) and cold (b) injection

to prevent condensation of the compounds whilst transferred. Although the transfer of the analytes to the chromatographic column differs in the presented injection modes.

In hot injection (Figure 1.2a) the injector is just transferring the injected sample. This is because it is held at a constant high temperature (over 200°C) throughout the analysis. Therefore the analytes are not retained in the liner and are directly transferred to the chromatographic column.
The times of transference from the HS generation to the PTV and from the PTV to the column overlap. Thus the transference is progressive and slow.

In cold injection (Figure 1.2b) the injector is held at a low temperature throughout the transference of the sample from the HS generator to the PTV, so the compounds condense in the liner. Once all the HS is injected into the PTV, the liner is flash heated, thus transferring the compounds from the PTV to the GC.
With this technique a preconcentration in the liner can be achieved and furthermore the injection is faster than the hot injection mode.

1.3.2 PTV-injection modes

Apart from programming the temperature of the injector, a PTV device also permits different injection modes. The main difference is the time that the split valve is open in the injection process and the gas flow around it. These variables determine how much sample volume is finally injected into the chromatographic column.

Split injection

In the split injection mode the split valve is kept open throughout the injection time, which permits venting of analytes and thereby sample division. So only a smaller portion than the injected volume reaches the chromatographic column. The ratio of the division of the sample is controlled by the gas flow into the column and the flow at the split valve [35]. The split ratio is usually between 1:50 and 1:6000 and the sample volume injected is smaller than 1μL.
However, this technique has the inconvenience that the injected volume is very small, thus not permitting trace analysis. But it is very useful when the amount of analytes in a sample is more than 0.1%.
Due to the gas flow the volatiles circle with great velocities in the liner, and therefore it is a rapid injection technique which gives small initial bands.

- **Hot split injection** The split valve is open throughout the whole analysis and the liner of the PTV is at a high temperatures. So the analytes, once injected, pass the liner and the divided sample reaches the column (Figure 1.3a).

- **Cold split injection** The split valve is open throughout the whole analysis and the liner of the PTV is at low temperatures. So the difference to the previous injection mode is that the analytes are preconcentrated at low temperatures before they reach the column by flash heating of the liner (Figure 1.3b).

Splitless injection

In the splitless injection mode the split valve is closed during the injection to the chromatographic column. So the analytes of the sample reach the column without division. Then the split valve is opened to wash the liner and prepare it for the next injection.

- **Hot splitless injection** The sample transference from the PTV to the chromatographic column is very slow (Figure 1.4a), because the sample is transferred with the same gas flow which is applied to the column (about 1 mL/min). Therefore the initial band of the chromatogram is broadened.

- **Cold splitless injection** With this injection mode the principal problem of hot split injection can be excluded. The analytes are concentrated in the liner and the transference to the column is fast. Thus the initial band of the chromatogram is narrowed. Consecutively the form of the peaks is improved, without having to change the chromatographic program (Figure 1.4b).

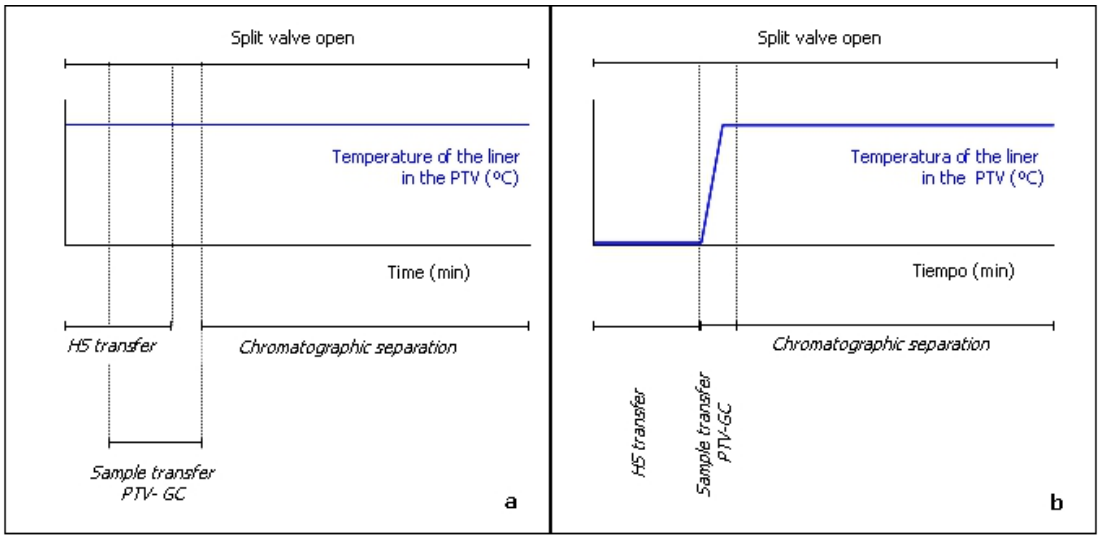

Figure 1.3: Hot (a) and cold (b) split injection

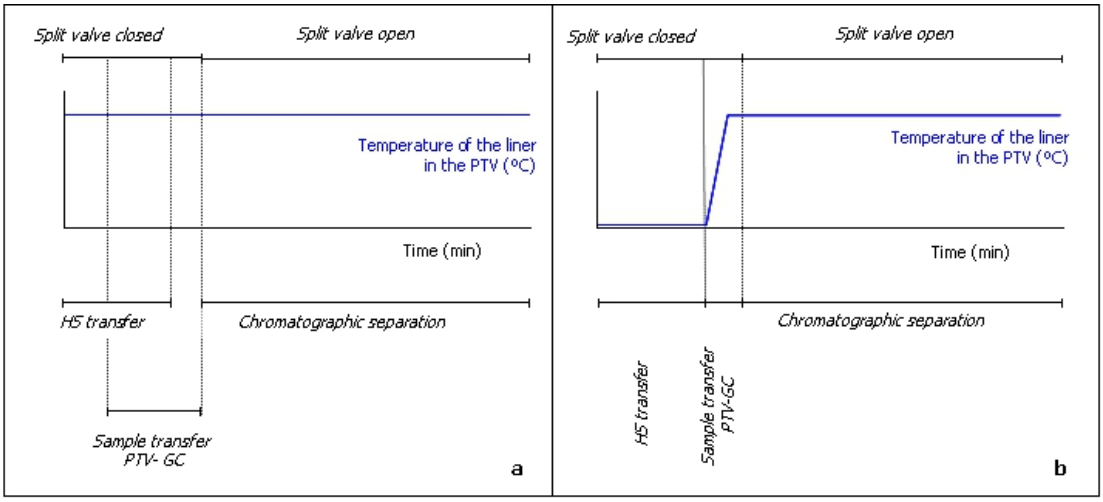

Figure 1.4: Hot (a) and cold (b) splitless injection

Solvent vent injection

This technique combines the cold injection with the controlled vaporization. The liner is kept at a low temperature during the transference process from HS to PTV and the split valve is open. So the solvent of the analytes is purged through the valve, while the analytes are retained in the liner. At the end of the purge time, when the transfer from HS to PTV is complete, the split valve is closed and the liner is flash heated, like in the splitless mode. When the analytes are considered to be fully transferred to the column the split valve is opened again and the liner is purged (at high temperature) to prepare the liner for the next injection.
Usually the purpose of the purging the liner is to eliminate the solvent at a low temperature, because the boiling point of the analytes is higher. The greater the difference of the boiling point, the better the outcome of this technique. Furthermore, this technique can be applied to analytes which have lower boiling points than the solvent. Then the separation is achieved by using liners packed with adsorbent material, which retains the compounds of interest [36].
Thus, with this injection mode the problems of the conventional injection modes of HS-GC can be overcome: a cold injection is possible and better sensibility can be achieved, due to the elimination of the solvent before the analytes are injected to the column.

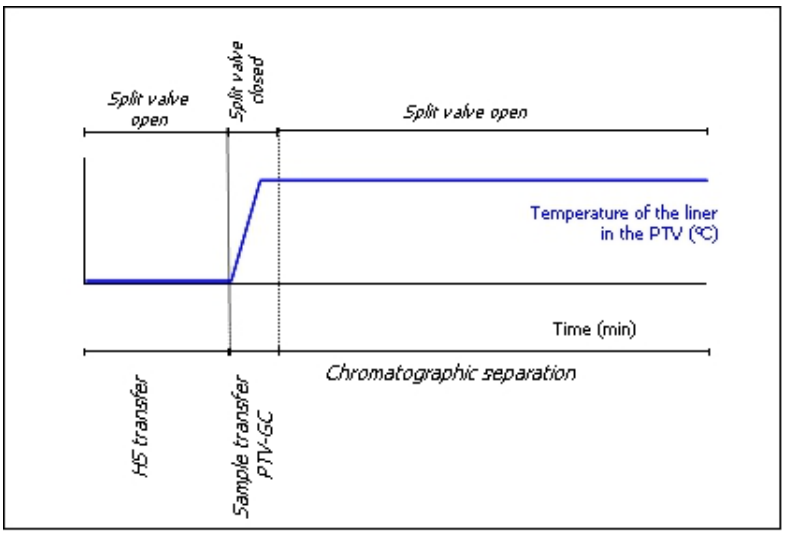

Figure 1.5: Solvent vent injection

1.4 Fast gas chromatography (FGC)

Since gas-liquid chromatography was first described by James and Martin in 1952 [37], gas chromatography has undergone a great development, both in the technical aspect and in the numerous applications which were developed.
The first major step was the introduction of capillary columns in 1958 [38]. These improved considerably the chromatographic resolution, increased the analyses speed and showed better sensibility compared to the packed columns, although the capacity of the column was smaller. So, capillary gas chromatography is the most efficient analyses method for volatiles and semivolatiles [39].
The major concern for gas chromatography ever since was to achieve the desired resolution of the compounds in the quickest time possible. In conventional capillary gas chromatography the inner diameter of the column is between 0.2 and 0.32 mm and the time necessary for the analyses of one sample is found to be in the range from 10 to 60 minutes (depending on the sample, the number of analytes to be separated and the experimental conditions).
Due to the aim to shorten analyses time, fast gas chromatography (FGC) evolved. The first studies about capillary columns with inner diameters around 0.1 mm to achieve fast separation date back to 1962 [40], but it was not until the 1990s when they found their way into the laboratories of analyses as commonly applicable. The reduction of analyses time implicated an increase of the sample's capacity, savings of time and money and thereby increased productivity of the laboratories.
On-line process controlling and quality control of food can be found within the range of the applications of FGC, so that results as fast as possible are required.
Another advantage is the possibility to repeat various times the analyses (replica) of a sample in the same time that one sample would be analyzed in conventional chromatography. This concludes a greater amount of analytical data and thereby implies greater precision of the results [41, 42].

The analyses time for fast GC depends fundamentally on the complexity of the samples. Very complex samples need more time to be separated (minutes) than simple mixtures (milli-seconds/ms). Thus, various classifications of FGC have been made, depending on analyses time or on the peak width at half height [39, 41, 42]:

- **fast gas chromatography** - separation of the compounds between 1 and 10 minutes, peak width at half height 1 to 3 seconds
- **very fast gas chromatography** - separation of the compounds in the interval of 0.1 and 1 min, peak width at half height 30 to 200 ms
- **ultra fast gas chromatography** - separation in times less than 0.1 min, peak width at half height 5 to 30 ms

However, one of the major limitations for the use of thin capillary columns was the lack of compatible instrumentation for trace analyses. These types of columns need very

small injection volumes, due to their small inner diameter, to evade distortion of the initial peaks in the chromatogram. This would have a negative effect on the sensibility of the analytical method [31, 43, 44, 45].
A PTV injector permits the introduction of large volumes of sample in fast GC, because the solvent can be eliminated at low temperatures. The combination of GC with a PTV permits rapid separation with results that exceed the results of conventional gas chromatography concerning the resolution and sensibility. This is because of the better peak definition and better signal to noise ratios (S/N) in FGC [44, 45].
This configuration has been employed in many different types of analyses, solving the problems of classic GC and the traditional injectors in GC [31, 44, 45, 46].

1.5 Mass Spectrometer (MS)

A mass spectrometer (MS) is an analytical device which achieves the separation of the analytes by their charge. Naturally, only a few compounds are charged, so in order to use the separation capabilities of an MS all the atoms and molecules have to be charged/ionized.
To ionize molecules a number of ionization techniques are known, such as matrix assisted laser desorption ionization (MALDI), electron impact ionization (EI), field ionization/desorption (FI/FD), fast atom bombardment (FAB), photo ionization (PI), ionization by inductive coupled plasma (ICP/MS) and secondary ion mass spectrometry (SIMS) [47].
The ionization technique used in the MS device (HP 4440) is electron impact ionization. Here, a wire of Tungsten (W) is heated and thereby emits an electron. On impact with the molecules, an electron with energy of about 70 eV is struck out, resulting in a radical-cation.
One disadvantage of this ionization technique is the possibility of molecule fragmentation, which occurs in so called “hard ionization techniques”. Many characteristic bond breaks are known, so relative distribution can often be predicted.
Now charged, the molecule is accelerated by the gradient of voltage and enters the separator. The separator (a mass analyzer) used was a quadrupole, but others such as sector field mass analyzers or time of flight (TOF) analyzers are often used.
In quadrupole analyzers the voltage gradient is usually between 5 and 15 Volts and the charged radical-cations oscillate in the inner space of four bar-electrodes. Depending on the alternating current applied to the bars only one m/z ratio has a trajectory which is not intercepted by the bars.
At the end of the detector unit a secondary electron multiplier (SEM) transforms the ions into an electrical signal which is registered by a personal computer (PC). The ions, which pass through the quadrupole, impact on the cathode where an electron is emitted. Due to the (high) voltage between anode and cathode the emitted electrons impact on the nearest dynode where not only one, but more electrons are emitted. This is repeated many times. Therefore these secondary electrons multiply until they reach the anode,

where the potential difference between the electrodes is then converted into a signal. Thus, this detector permits just the registration of one ion at a time, and therefore has to work very fast to permit real time detection.

The output of an MS unit is a mass spectrum, where on the X-axis (abscissa) the mass per charge (m/z) ratio is found and the Y-axis (ordinate) is a relative scale, shaped to the most abundant ion. An example is given in figure A.8.
Not only fragmentation but also the fact that there exist isotopes of most of the chemical elements, produce the mass spectrum. So according to the relative occurrence of each isotope, isotope-peaks only differ in one m/z unit.
If the resolution of the mass spectrometer is high enough, a molecule can be identified by its mass spectrum. To do this, the mass spectrum is compared with a reference in a library. By an algorithm, the program used for analysis calculates a percentage of correlation, indicating which compound is most likely to be detected here. In general, correlation coefficients higher than 90 % are acceptable and the compound can be considered to be identified.

A mass spectrometer can work in two different modes. More modern devices, such as the one used, can operate in these two modes simultaneously. But a signal loss, when operated in these modes simultaneously has to be taken into account.

- **SCAN** In this mode a mass range in which the ions are expected is defined and this mass range is measured over the whole analysis time (is scanned).

- **SIM** In the **s**ingle **i**on **m**onitoring mode only a few ions in a certain time window are measured. This permits better sensitivity and if in combination with GC, it focuses on the compounds of interest, while the others are not measured.

If in SCAN mode, the number of measurement repetitions per second may be adjusted to its needs. Narrowing the m/z range permits (intuitively) the augmentation of the number of measurements (cycles per second). But if the device should cover a wide range of m/z naturally fewer measurements can be carried out in the same time, resulting in less precision.
In SIM mode another parameter occurs, which has to be adjusted for best results: the dwell time. It is the time the quadrupole voltage "dwells" on each ion specified. So the more time a m/z is measured the more dependable is the corresponding result, but there is also a disadvantage. Since one m/z ratio is measured fewer times, less points define a peak. On the other hand, if the dwell time is shorter the noise will increase, because of the uncertainties of the ion measured arising. Thus a compromise between this two extremes has to be found.

Chapter 2

Objectives

2.1 Impact

2.1.1 Lubricant oils

In motor engines the piston of the cylinder moves when a fuel air mix is combusted. But not only is it moved in one direction but also in the other one, when the fuel air mix needs to be compressed. Due to this, the piston is always in movement when the motor is operating. Naturally friction occurs when the surface of the piston and the cylinder move against each other, which produces heat in addition to the heat of the combustion. If no counter measurements are taken the temperature can even reach the point of fusion of the metal.
Logically this has to be prevented, so lubricant oils are used, which form a thin film between the piston and the cylinder wall impeding friction. But further tasks are subscribed to the lubricant oils [48].

As motor oils are heavy petroleum products they are commonly used to reduce the friction between surfaces, to prevent the corrosion, to remove heat and contaminants and to clean the motor. Conventional motor oils are mainly comprised of base oil and up to 10 % additives [49]. The major component (>90 %) is the base oil and it is generally produced by the refining of crude oil. Gas chromatography with flame ionization (GC-FID) or mass spectrometry detector (GC-MS) [50], comprehensive two dimensional gas chromatography (GCxGC-FID) [50], high performance liquid chromatography-differential refractometer detector (HPLC-RI) [51] and HPLC-GCxGC-FID [49] have been used for characterization and determination of hydrocarbons present in motor (base) oils. Non-petroleum base fluids are employed when special properties are necessary, when petroleum base oils are in short supply or when substitution of natural products is practical or desirable [52].

The requirements a lubricant oil has to meet are various, but the most important is to guarantee the operability of the motor, to impede the friction. Once an oil is selected

to be used in a motor, it has to be kept in mind that the operating temperature is not always the same. This will have an effect on the viscosity which is controlled by the temperature (decreases at higher temperatures). Therefore it is desirable to use an oil which does not change the viscosity at different temperatures.
The oils obtained by the refining of petrol oil have different viscosity properties. Thus, the mixing of different oils permits one to define the working temperature range of the lubricant oil.
Before the 1970s motor oils composing only of one oil were used, but since then a mixture of oils with different properties has been used.
An option to classify lubricant oils is proposed by the American Petrol Institute (API). Their method is to classify the oil by the temperature range in which a certain viscosity can be maintained. An example is given:
Oil: *Repsol Super Elite 20W50, SJ/SHCF*

- "20" indicates the viscosity when cold (60.000 $mPa * s$ at T= -20°C)
- "W" indicates that it can be used in winter
- "50" indicates the viscosity when hot (16,3 $mm^2 * s$ at T= 100°C) ⇒ cinematic viscosity
- "S" indicates the use in gasoline motors, "H" or "F" indicates the quality (higher is better)
- "C" indicates the use in diesel combustion engines, "F" indicates the quality (higher is better)

But not only different oils are mixed to achieve better viscosity properties. Additives are also used to improve oil performance and life.

2.1.2 Additives in lubricant oils

Degradation reactions take place in the combustion chamber, since there is oxygen/air present and the temperatures are highly elevated. In order to prevent the oxidation of the hydrocarbon chains composing the oil, additives preventing this are the logical step to take here [48].

Base oil requires additives (dispersants, detergents, oxidation inhibitors and anti-wear agents) to satisfy the lubricating needs of an engine and to increase the useful lifetime of the oil. One of the most important aspects of lubricating oils is that the oxidation stability be maximized. Generally, engine oil compounds have a relatively high thermal/ oxidative stability in order to avoid the degradation of hydrocarbons which are exposed to oxygen and heat. Antioxidants [53] are the key additives that protect the lubricant from this degradation, allowing the fluid to meet the demanding requirements for use in engines and industrial applications. Some of the main classes of oil-soluble organic

antioxidants are sulfur and phosphorous compounds, aromatic amines and hindered phenolic compounds. A synergistic effect has been found when different types of inhibitors have been used together [54]. Thus, the useful lifetime of the additives is extended beyond the expected performance of each additive if they were used separately.
Oil composition changes during engine operation. The oxidation of oils in engines is one of the aging processes that results in the formation of polar oxidation products including alcohols, aldehydes, ketones and carboxylic acids [55, 56]. These compounds undergo further reactions to form sludges which degrade the performance of the oil. The depletion of antioxidants can be seen as a form of "titration" where oxidation products are formed when additives are depleted. In that sense, the conditional maintenance becomes a task of determining the "end point" in this titration [57]. Other common contaminants found in the oil are fuel, soot, water and ethylene glycol (a major component in antifreeze). Polycyclic aromatic hydrocarbons (PAHs) are known to be present in used lubricating oils. The presence of all these compounds can cause detrimental changes in engine oil and they accelerate the depletion process of additives. The occurrence of unburned fuel, for example, reduces oil viscosity and its flash point. Several methods [58, 59, 60, 61] have been developed to determine these contaminants in lubricants.

So, the "real-life" application of this study is to be able to decide when a lubricant oil has to be changed; when it is necessary to know when the properties can no longer be maintained. This already implies the objective of the present work.

2.2 Previous studies

A number of different studies has been carried out on that matter, all of them applying different analysis techniques to determine the antioxidants in lubricants [48].

Determination of antioxidants in lubricant has been performed with infrared spectroscopy (IR) [62], size exclusion chromatography-RI (SEC-RI) [63], HPLC-MS [64], GC-MS [64] and GC-nitrogen-phosphorous detector (GC-NPD) [64]. When gas chromatography is used, the capillary columns are susceptible to degradation by the types of contaminants present in used oils. A possible alternative to direct injection of the oil is to use headspace analysis (HS) which does not involve any additional sample treatment. Accordingly, a method based on HS-GC-MS [57] has been used to identify the principal gaseous components of engine oil and to establish the concept of oil condition monitoring via the vapor phase.

Consecutively this technique was yet to be explored for analysis of antioxidants in motor oils.

2.3 Objective of the present work

In this work a new method is explored to determine eight antioxidants (2-tert-butylphenol, 3-tert-butylphenol, 4-tert-butylphenol, di-tert-butylphenol, 3-tert-butyl-4-hydroxyanisol (BHA), 2,6-di-tert-butyl-4-methyl-phenol (BHT), 1-naphthol and diphenylamine(DPA)) in new and used lubricant oils. The aim was to create a sensitive method that furthermore would give results in short time and that would be easily automatized.
To make the sample preparation as easy as possible a headspace auto-sampler was employed, also fulfilling the automatisation demand. This implies that the species of interest would be present in the headspace, which is the case in this work.
The use of a programmable temperature vaporizer has the advantage that different injection modes into the gas chromatograph are possible and manual cryogenic focusing techniques, which are difficult to manipulate, are evaded.
With the aim of reducing analysis time the possibilities of fast gas chromatography were studied. The column of small intern diameter provides this possibility, permitting high gas flows and steep temperature ramps.
The use of a MS detector coupled to a gas chromatograph permits the analysis of very small concentrations, even more when operated in SIM mode. Thus the sensitivity can be considered very good [65, 66, 48].
So the present work is a novel method for the determination of antioxidants in lubricant oils by HS-PTV-GC-MS. As far as the author knows, this technique has never been used before to determine antioxidants in lubricant oils.

The research was carried out over eight months at the Universidad de Salamanca, Spain, as part of an Erasmus-scholarship from September 2009 until June 2010.

Chapter 3

Experimental Section

3.1 Reagents and Samples

The eight antioxidants (2-tert-butylphenol, 3-tert-butylphenol, 4-tert-butylphenol, 2,6-di-tert-butylphenol, 3-tert-butyl-4-hydroxyanisol (BHA), 2,6-di-tert-butyl-4-methyl-phenol (BHT), 1-naphthol and diphenylamine(DPA)), methanol and propylacetate, used as solvents, were supplied by Sigma-Aldrich (Steinheim, Germany) with purities of at least 99%.
The antioxidants studied were selected because some were present in real oil samples and some were mentioned in the bibliography [52, 53, 57] to be possible antioxidants in lubricant oils.
In order to prepare the stock solutions seven of the antioxidants were weighed and dissolved in methanol or propylacetate. 2-tert-butylphenol, which is a liquid at room temperature, was simply diluted in the solvents. Parting from these concentrated stock solutions, the working solutions and calibration solutions were prepared. The classification of the chemicals used is given in Table 3.1.
Sample preparation and manipulation was carried out in an extractor hood and latex gloves were used for protection.

The oil samples were bought at gas stations (in Spain). The used oil samples were extracted from cars at gas stations (in Spain).
For the calibration of the new oils, an oil bought at a gas station (new oil 1) was used because none of the studied antioxidants were found.

3.2 Instrumental Setup

The instrumental setup used was a headspace auto-sampler coupled to a programmable temperature vaporizer injector of the gas chromatograph and a quadrupole mass spectrometer detector (HS-PTV-GC-MS), as shown in figure 3.1. While the PTV, the GC and the MS unit were controlled by software over a personal computer (PC), the HS

Table 3.1: Formula, retention times, boiling points, hazard classification and m/z ratios selected for the eight antioxidants studied and the solvents used.

	Compounds	Formula	t_R (min)	Boiling point (° C)	R-phrases	m/z	
						Quantitation ion	Qualifier ions
(1)	2-tert-butyl-phenol	$C_{10}H_{14}O$	3.441	224	R21/22-R23-R34	135	107, 150
(2)	3-tert-butyl-phenol	$C_{10}H_{14}O$	3.530	*	R34	135	107, 150
(3)	4-tert-butyl-phenol	$C_{10}H_{14}O$	3.530	236-238	R37-R41-R51/R53	135	107, 150
(4)	2,6-di-tert-butyl-phenol	$C_{14}H_{22}O$	4.108	253	R22-R52/53	191	57, 206
(5)	3-tert-butyl-4-hydroxyanisole (BHA)	$C_{11}H_{16}O_2$	4.178	264-270	-	165	137, 180
(6)	2,6-di-tert-butyl-4-methylphenol (BHT)	$C_{15}H_{24}O$	4.334	265	R22-R36-R37-R38	205	57, 220
(7)	1-naphthol	$C_{10}H_8O$	4.477	278-280	R21/22-R37/R38-R41	144	115, 116
(8)	di-phenylamine (DPA)	$C_{12}H_{11}N$	5.025	302	R23/24/25-R33-R50/53	169	167, 168
	methanol	CH_4O		64.7	R11-R23/24/25-R39/23/24/25		
	propylacetate	$C_5H_{10}O_2$		102	R11-R36-R66-R67		

* not found

sampler had to be programmed independently.

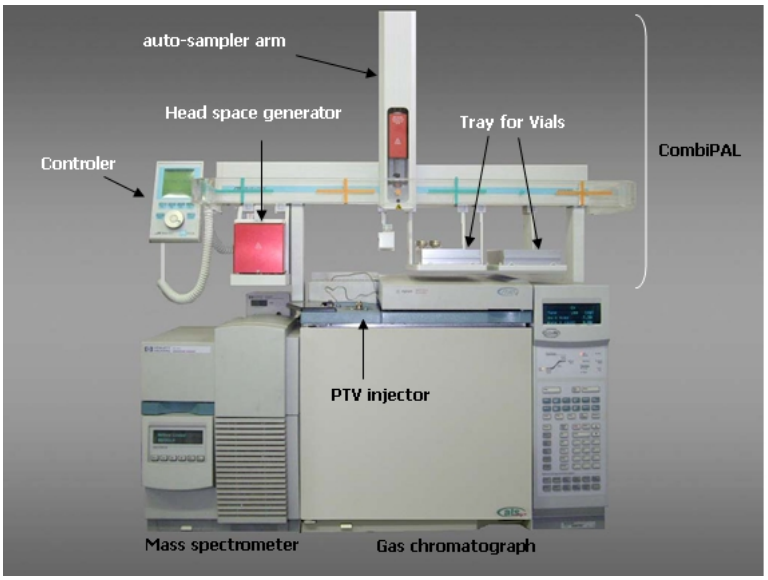

Figure 3.1: Instrumental setup HS-PTV-GC-MS: headspace auto-sampler (HS), programmable temperature vaporizer (PTV), gas chromatograph (GC) and quadrupole mass spectrometer (MS);
photo taken by author

3.2.1 Headspace sampler

The headspace generator used was a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). This sampler permits analysis of 32 consecutive samples (in the headspace configuration) and has an oven with positions for six vials.
The syringe (2.5ml HS-syringe) can be heated from 30 to 150°C and the arm, where it is implemented, also transports the vials from the tray to the oven and back, after analysis. Furthermore the syringe can be purged with inert gas. **The oven** can be set to temperatures between 30 and 200°C and the vials in the oven can be agitated during the headspace generation (250-750 rpm).
When a sample is analysed, it is first put into the oven to generate the headspace for a certain time. After the equilibrium of the gas phase and liquid phase (oil) is reached the syringe pierces the septum of the vial and takes an aliquote of the HS. That is then directly injected into the gas chromatograph, in this case the PTV.

3.2.2 Programmable temperature vaporizer

The PTV inlet used was a CIS-4 from Gerstel (GERSTEL, MD, USA) and it was equipped with a liner of 71 mm x 2.0 mm. Among the different liners that can be assayed, are a liner packed with a material called Tenax-TA® (which is a porous polymere of 2,6-di-phenyloxide), a liner filled with glass wool and an empty baffled liner.
The Tenax-liner is of hydrophobic character, therefore it is recommended to be used to trap volatile and semivolatile organic compounds. The liner filled with the deactivated glass wool is more polar, and therefore less retardant for organics. By using an empty baffled liner no species is preferred as practically no interaction occurs, and the entrapment is merely thermal.
A scheme of the used device was already shown in figure 1.1. The cooling was accomplished with liquid CO_2.

3.2.3 Gas chromatograph

The column used in the Agilent GC 6890 device was a DB-VRX (20 m x 0.18 mm x 1 μm). It has a temperature range of -20°C to 260°C, and a bonded and cross-linked stationary phase of low polarity [67]; the exact composition of the stationary phase is considered proprietary information [68]. In addition to the small intern diameter, the column length and the possibility of applying high gas flows, these characteristics all together permit a fast chromatographic separation (for the studied compounds).

3.2.4 Mass spectrometer

The mass spectrometer used (HP 4440) has a quadrupole mass analyzer and an electron impact ionization source. The ionization source was operated at 70 eV at a temperature of 230°C, while the quadrupole was kept at 150°C.
The MS detector can be operated in SCAN and in SIM mode.

A scheme of the instrumental setup is given in figure 3.2.

3.3 Method Setup

3.3.1 Headspace generation

2.0 g of oil plus solvent or a solution of the antioxidants in the solvent (methanol or propylacetate) are placed in a 10 mL glass vial (Agilent technologies, DE, Germany), which is sealed with a teflon/silicone septum. The process of headspace generation consists in heating the samples, along with agitation for mixing, at a determined temperature (in this case 95°C). After 15 minutes (in this case) it can be considered that an equilibrium between the liquid and the gas phase is reached, and the gas phase contains a fraction of the volatile compounds.
Then an aliquot (2.4 mL) of the gas phase is taken with a headspace syringe (2.5 mL).

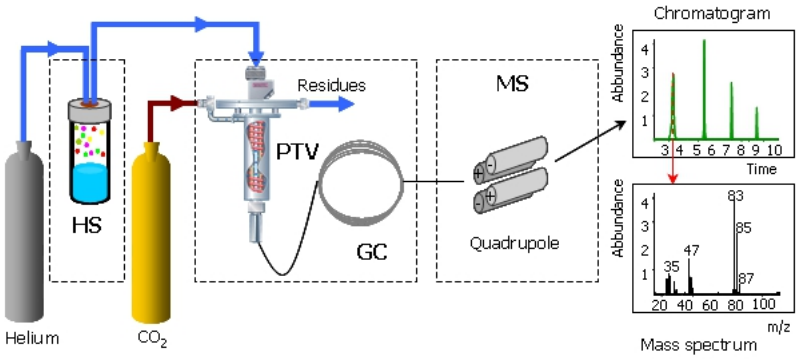

Figure 3.2: Scheme of the HS-PTV-GC-MS instrumental setup; *adapted from ref [69]*

This is then injected into the PTV, where the introduction into the chromatographic column takes place.
The optimized conditions are summarized in table 3.2.

3.3.2 Programmable temperature vaporizer

Here, different liners as well as different injection techniques were studied. The results of these studies will be discussed in chapter 4.
The optimized method was to use a liner filled with Tenax-TA® and use the solvent vent injection mode. The venting temperature was kept at 60°C for 0.6 min, where the solvent was purged, followed by flash heating to 325°C of the vaporization chamber as the split valve was closed. After one minute of injection the split valve was opened again and the liner was kept at 325°C for another 7.90 minutes, thus eliminating the remaining compounds in the liner.
The optimized conditions are summarized in table 3.2.

3.3.3 Gas chromatograph

The chromatographic program used in the beginning of the study differed very much from the final experimental conditions, for the parameters were changed subsequently. The initial column temperature, the temperature ramp(s) and the runtimes in each step were optimized to give the best results in the least time. This optimization process shall be discussed in chapter 4.
The final conditions were: an initial temperature of 110°C held for one minute, followed by the steepest ramp allowed (65°C/min) until 175°C, where the ramp changed to 45°C/min, which was the maximum ramp that could be achieved with the chromatographic oven. When the final temperature of 240°C is reached it is held for 2 min. The

resulting chromatographic runtime was 5.44 min.
The optimized conditions are summarized in table 3.2.

3.3.4 Mass spectrometer

Once the separation was optimized, a solvent delay of 2.5 min was set in order to prevent detector saturation from the solvent and the resulting data acquisition time was 2.94 min. In order to identify the ions, SCAN acquisition mode was used, covering a mass range from 25 to 300 amu at a repetition rate of 5.91 cycles per second. This mass range covers all the ions of the studied antioxidants. Their spectra were compared to the spectra of the NIST'05 mass spectra database (NIST/EPA/NIH Mass Spectral Library, version 2.0) and if the match factor was above 90% the compound can be considered to be identified.
In the SIM mode (used for quantification) the repetition rate depends on the ions selected in the time windows and the dwell times selected:
The three most abundant ions of each antioxidant were selected and the detector was set to work in SIM mode. Five time windows were selected. The first window from 2.5 to 3.7 min covered the zone of the three isomers studied (o-, m-, p-tert-butylphenol) . From 3.7 to 4.25 min 2,6-di-tert-butylphenol and BHA were measured. Group three from 4.25 to 4.42 min covered BHT. From 4.42 to 4.80 min the ions for 1-naphthol were measured. In the final group the ions for DPA were acquired.
The dwell time used for all the ions was 10 ms in order to give good peak definition along with a low noise level, at a repetition rate of 19.33 cycles/s. The optimized conditions are summarized in table 3.2.

This setup was then used to establish the calibration slopes and to determine the detection and quantification limit of the method.

Table 3.2: Optimized experimental conditions

Headspace sampler		
Oven	95°C	
Syringe	100°C	
Headspace generation	15.00 min	
Agitation	750 rpm	
Syringe flushing (He)	2.00 min	
Interval between samples	12.00 min	
Sample volume	2.4 mL	
Programmable temperature vaporizer		
Injection mode: solvent vent	Purge flow	150 mL/min
	Purge time	0.60 min
	Purge temperature	60°C
	Injection time	1.00 min
	Rate	12°C/s to 325°C (5.44 min)
Gas chromatograph		
Carrier gas	Helium	(1.4 mL/min)
Oven	Initial temperature	110°C (1 min)
	Ramp 1	65°C/min to 175°C
	Ramp 2	45°C/min to 240°C (2 min)
		(total run time 5.44 min)
Mass spectrometer		
Data acquisition mode: SCAN	m/z 25-300	
	cycles per second: 5.95	
Data acquisition mode: SIM	Dwell time	10 ms
	cycles per second: 19.33	
	Group 1	m/z (107, 135, 150)
		2.50 - 3.70 min
	Group 2	m/z (57, 137, 165, 180, 191, 206)
		3.70 - 4.25 min
	Group 3	m/z (57, 205, 220)
		4.25 - 4.42 min
	Group 4	m/z (115, 116, 144)
		4.42 - 4.80 min
	Group 5	m/z (167, 168, 169)
		4.80 - 5.44 min

Chapter 4

Results and Discussions

4.1 Optimization of the HS-PTV-GC-MS conditions

4.1.1 Sample preparation

There were two variables to be fixed concerning sample preparation: the amount of oil and the volume of the solvent added, which would be necessary for calibration.
So oil samples between 0.2 g and 2.0 g were analyzed along with different amounts of solvent added. These studies were carried out in solvent vent and hot split injection mode.
While oil samples where no solvent was added showed the same signals (figure 4.1a) independent of the amount of oil, a considerable decrease in signal was observed when to 2.0 g of oil propyl acetate was added (figure 4.1b). This effect was even greater for smaller sample amounts (0.2 g oil, see figure 4.1d). The combination that did not show this effect was 2.0 g of oil and 20 μL of solvent and was therefore chosen to be used from there on (figure 4.1c).

4.1.2 Headspace generation

The first step in the method optimization was to overcome the analyte contamination that occurred. When an oil sample was measured using a glass wool liner in the PTV and afterwards a sample of methanol (20 μL) was measured, the antioxidants studied were present in this methanol chromatogram (see figure 4.2). So the possible parameters were changed subsequently.
Neither the reduction of sample volume nor the rise in the syringe flushing time could resolve this problem, so another liner was assayed. But even an empty baffled liner could not overcome this contamination effect. Also the sample volume of the headspace that is taken was reduced, but this did not show the desired effect of a clean methanol chromatogram after an oil measurement.
Thus, it was concluded that the contamination could neither be a result of the liner, nor the injection mode, nor the sample volume but had to be correlated with the syringe. It

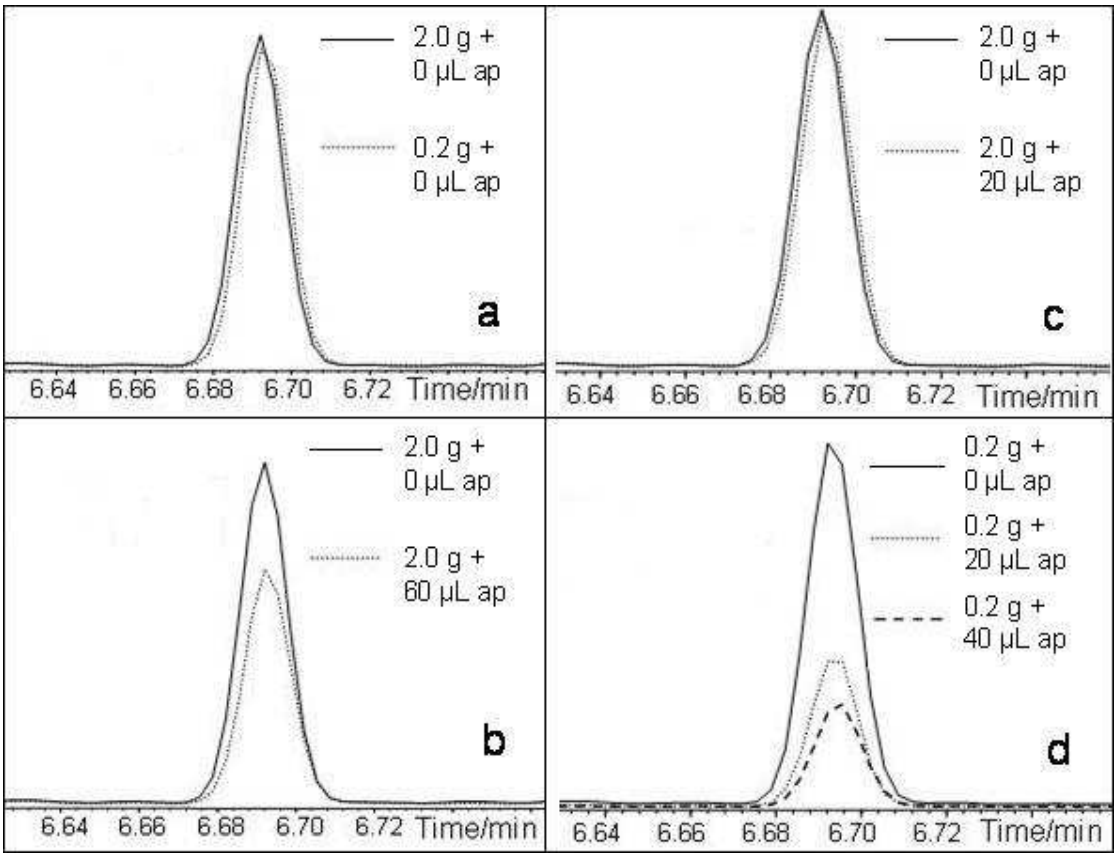

Figure 4.1: Comparative study of the amount of oil and solvent. The oil used for this study contained 2,6-di-tert-butylphenol, so the peaks corresponding to this antioxidant are shown here. All chromatograms shown were recorded in scan mode and the following chromatographic program was applied: 50°C (1 min), 35°C/min, 240°C (3 min)
In figure **a** 2.0 g and 0.2 g of oil without adding solvent are shown. The signals are of the same quality.
Figure **b** shows an amount of 2.0 g of oil with 0 μL and 60 μL propyl acetate (ap), respectively. A considerable signal loss can be seen.
The signal for 2.0 g and 0 μL and 20 μL of propyl acetate (ap) is shown in figure **c**. No signal loss occurs here by adding solvent.
In figure **d** the signals for 0.2 g oil are shown. A drastic loss of response occurs here when propyl acetate (ap) is added.

then occurred that even though the syringe was flushed for 8.00 minutes with He gas, the syringe was not cleaned properly. So another approach was taken to wash the syringe. After the injection of an oil sample, the solvent in liquid form (methanol) was taken out of a vial, which was not subjected to headspace generation, and directly injected into the waste of the headspace autosampler. As the syringe was held 5°C above the headspace generation oven (100°C) in order to avoid condensation, and the boiling point of methanol being at 65°C, this washing could only be executed very poorly due to the evaporation of the solvent. So another solvent was assayed, which had a higher boiling point: propyl acetate (102°C).
Now the contamination could be eliminated successfully, when the sample volume at the washing step was set to 2.5 mL, whereas the sample volume at the sample injection was set to 2.4 mL to guaranty the complete washing of the syringe.
The He flush times were set to 2 minutes after sample injection and 1 minute after the washing step.

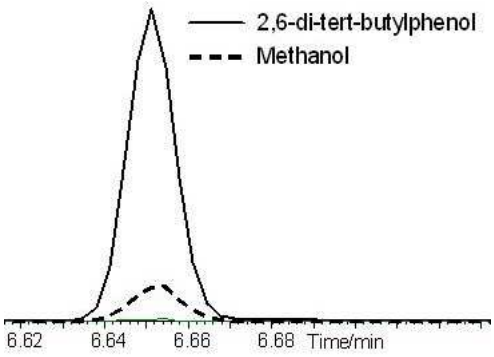

Figure 4.2: The dashed line is the peak of the methanol chromatogram, after the injection of an oil sample, that contained 2,6-di-tert-butylphenol (continuous line).

4.1.3 Programmable temperature vaporizer

A comparative study of the signals obtained for the compounds studied in different injection modes was performed. Solvent vent and hot split were assayed. Also the signals of different liners were compared. In the comparison of the liner filled with Tenax-TA®, which is designed to trap organic compounds, and the liner filled with deactivated glass wool (figure 4.3), it is obvious that the Tenax-TA® liner is best suited for this method. The only exception is 1-naphthol, which gives similar high signals when a glass wool liner is assayed. But apart from that, all the other studied antioxidants could best be analyzed in solvent vent injection mode and a liner filled with Tenax-TA® (figure 4.3). The first setup was a liner temperature at 70°C for 0.60 min. Then the flash heating of the liner with a rate of 12°C/s until it reached 325°C. The injection time onto the chromatographic column, or in other words the time the split valve was closed, lasted

from 0.50 to 1.50 min after the injection of the headspace sampler. A purge flow to split vent of 150 mL/min at 5.0 psi was applied.

The following PTV parameters for solvent vent injection were optimized: the venting temperature and the injection time.
Venting temperatures between 40°C and 100°C were tested. The aim was to find the best temperature that would eliminate as much solvent as possible while the compounds of interest would be retained in the liner and thus give high signals. So to purge the solvent a higher temperature would be desirable and for the antioxidants to be retained at a lower temperature. Thus the compromise was found at 60°C of purging temperature, where the signal of propyl acetate was diminished compared to that of 40 or 50°C, while the antioxidants gave still signals of the same peak height and area. At higher temperatures, especially at temperatures above 70°C, the signals of the compounds studied also started to diminish. So a venting temperature of 60°C was chosen.
When injection time was studied intervals from 0.60 to 1.50 min were tested. Less time to inject from the PTV into the column would imply less signal as the time is too short for the desorption of the analytes. Whereas longer injection time would increase the amount of analytes in the column.
The signals obtained showed clearly that for an injection time of 0.60 min not all the compounds were transferred to the column and for 1.50 min no better results than for an injection time of 1.00 min were obtained. Thus the injection time was set to 1.00 min.

4.1.4 Gas chromatograph

All the previous studies were carried out with a slower chromatographic program: 50°C for 1.0 minute, followed by a ramp of 35°C/min until the temperature reached 240°C which was held for 3.0 minutes. The resulting chromatographic runtime was 9.43 minutes. So the parameters that could be optimized in that part of the system were on the one hand the temperatures and temperature ramps and, on the other hand, the times that the temperatures were applied.
Firstly the initial temperature was changed. The temperature range from 50 to 150°C was studied and the signal output was compared. These temperatures were applied for 1.0 minute. As figure 4.4 shows the analysis time was shortened, which would be a desirable effect, but also the peaks broadened at temperatures above 110°C. (The reduced signal at 90°C is designated to irreproducibility of the equipment.) Thus, the initial temperature was set to be 110°C for further experiments, shortening considerably analysis time without a quality loss.

Then the temperature ramp was changed to the highest that the oven could achieve (65°C/min until 175°C, then 45°C/min) and the analytes still remained baseline separated in the extracted ion chromatogram. So the final step was to reduce the time of the final temperature to be held. The initial 240°C for 3.00 min were shortened to 2.00 min, which still allowed all the analytes to elute. Figure 4.5 shows the total ion

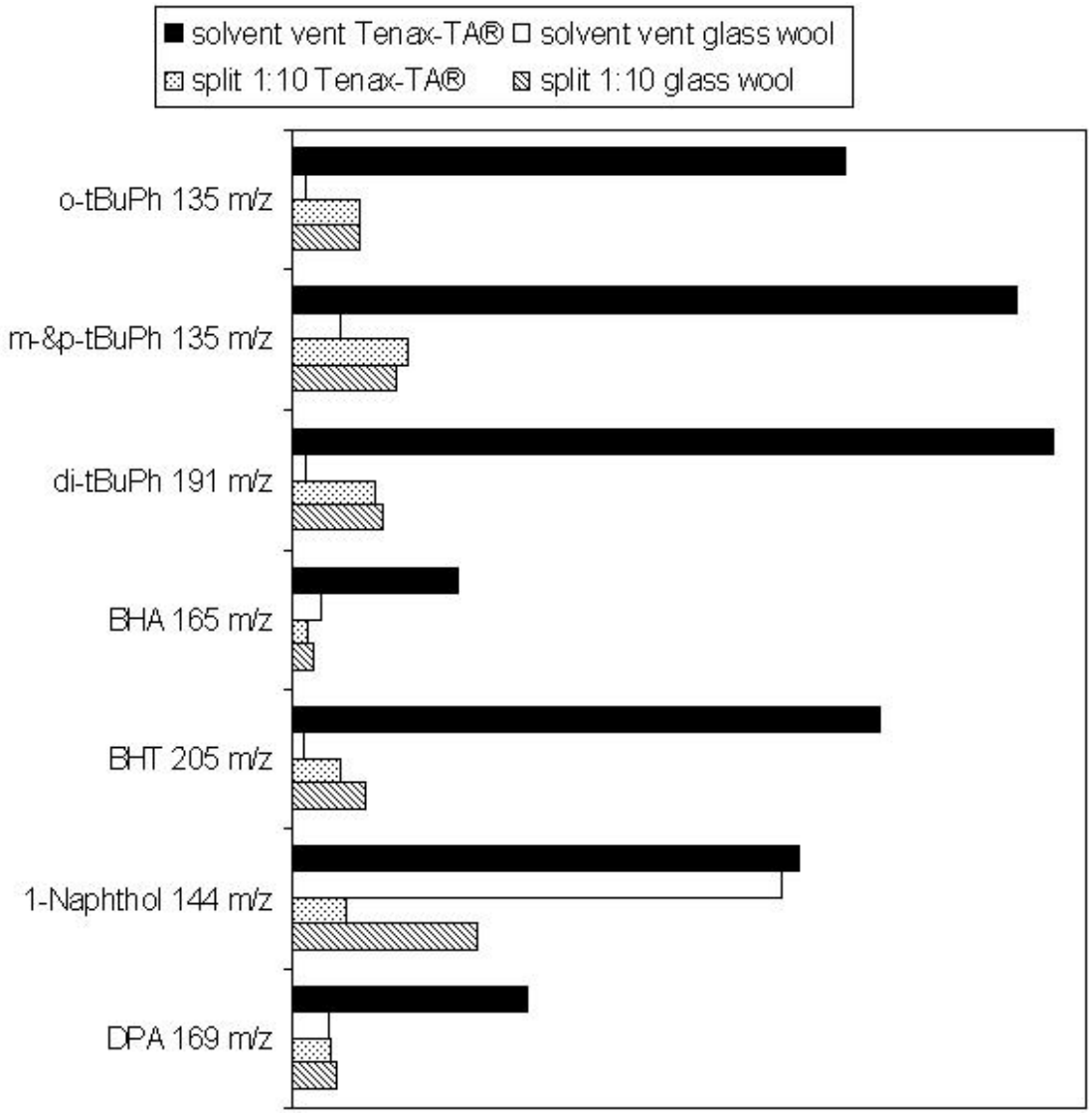

Figure 4.3: Comparative study of the signal obtained for the antioxidants studied (peak areas for the characteristic m/z ratios) when two different liners were employed (one filled with glass wool, the other with Tenax-TA®) in solvent vent injection mode and hot-split (1:10).

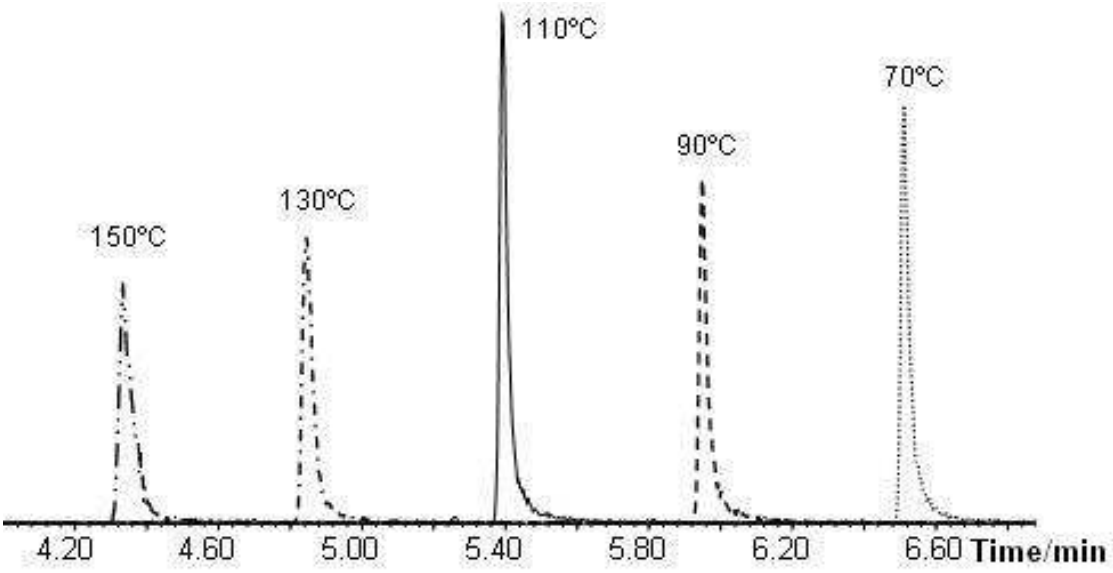

Figure 4.4: Comparative study of the initial chromatographic temperatures in the extracted ion chromatogram of 1-naphthol (144 m/z).

chromatogram of an oil sample spiked with an analyte solution, measured with the final chromatographic conditions.

In all the previously described setups the separation of 3- and 4-tert-butylphenol could not be achieved. To check the possibility of their separation an even longer chromatographic program was applied: initial oven temperature 35°C (3 min), ramp 12°C/min to 240°C (3 min). The resulting chromatographic runtime was 23.08 min. However, separation of these two isomers could not be achieved. So in the further work they were treated as one compound and their concentration being the sum of their individual concentration.

4.1.5 Mass spectrometer

In order to increase the S/N ratio and thereby achieve better detection and quantitation limits, the data acquisition mode of single ion monitoring (SIM) was explored.
Because the elution times of the compounds of interest were already known from the measurements in the SCAN mode only the dwell time and the time windows had to be fixed. Initially SIM windows were assayed as follows:

The dwell time used for this experiment was set to 10 ms, for being a good value in previous studies with this equipment. The same measurements were made with dwell times of 1 ms and 50 ms. In figure 4.6 an amplified section of the chromatogram (the

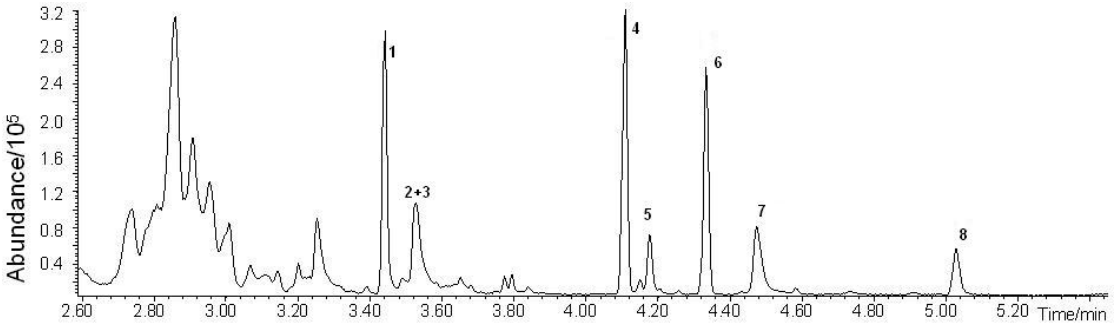

Figure 4.5: Total ion chromatogram of an oil sample (oil 1) spiked with an analytes solution (50 ppm 1-naphthol, 5 ppm all the other compounds) recorded in SCAN mode. The numbers indicate the peaks corresponding to the analytes in order of their retention time; see table 3.1.

Table 4.1: Initially assayed SIM time windows. Most abundant ions are emphasized.

Compound	m/z	time (min)
o-, m- & p-tert-butylphenol	107, **135**, 150	3.37 - 3.70
2,6-di-tert-butylphenol	57, **191**, 206	3.70 - 4.15
BHA	137, **165**, 180	4.15 - 4.25
BHT	57, **205**, 220	4.25 - 4.42
1-naphthol	115, 116, **144**	4.42 - 4.80
DPA	167, 168, **169**	4.80 - 5.44

peak for BHT) is shown. It can be clearly seen that at 1 ms of dwell time the peak is much better defined but also the peak height is lower and the noise is by far greater than in the other chromatograms. Meanwhile at 50 ms of dwell time the peak is defined by only a few points, thus not giving an accurate signal, but also resulting in less noise.

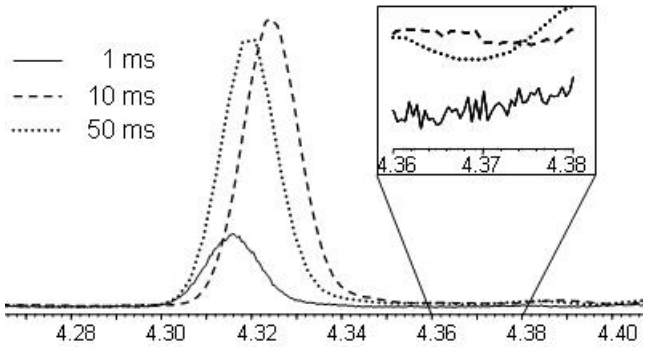

Figure 4.6: Total ion chromatogram for BHT (m/z: 57, 205, 220) of a oil sample (oil 1) spiked with an analytes solution recorded in SIM mode. In the amplified window the noise amplitude varying with dwell time can be observed.

The compromise of 10 ms dwell time satisfies both needs of good peak definition and a good S/N ratio.
As the peaks of 2,6-di-tert-butylphenol and BHA were very close, a time window including both compounds was set.

4.2 Calibration and analytical characteristics of the optimized method

The oil used as basis was new oil 1, because it did not contain any of the antioxidants of interest, though it might contain other antioxidants, to which the proposed method is not applicable. Over this oil the solutions of the analytes in propyl acetate were added.

4.2.1 Calibration

The concentration range studied for the selected antioxidants ranged between 28 ppb and 88 ppm, though the ranges were not the same for all. This is because the method is not equally sensitive to all compounds. 1-naphthol, for example, is not very volatile and therefore only a small fraction is found in the headspace. In table 4.2 the concentration ranges for each anlyte are listed. Due to the lack of possibility to separate the isomers 3- & 4-tert-butylphenol as previously discussed in section 4.1.4, these were calibrated and

quantified as their sum.

Table 4.2: Calibration range, number of levels measured and number of levels used for linear regression

Compound	Concentration (mg/L)	levels measured
2-tert-butylphenol	0.029 - 1.00	7
3- & 4-tert-butylphenol	0.014 - 3.90	7
2,6-di-tert-butylphenol	0.10 - 20.0	7
BHA	0.50 - 8.11	7
BHT	0.067 - 10.0	7
1-naphthol	8.88 - 88.7	4
DPA	3.00 - 40.6	7

The analytical signals used for the calibration were the areas of the extracted ion chromatogram peaks. The ions selected were the most abundant ones for each analyte (see table 4.1). All concentration levels were analyzed in triplicate.

Before these concentration ranges were selected, the chromatograms obtained in the method optimization step (spiked oil samples of new oil 1, which did not contain any of the studied antioxidants) were compared with chromatograms of oil samples to be predicted. So, in order to be able to predict the oil samples, thereby the upper limits for the contained compounds were fixed. As it is desirable in most cases of analytical methods, the lowest concentrations possible were tried to be achieved. Further it was attempted to cover at least one order of magnitude for all analytes.
In figure 4.7 two examples of the linear calibration curves used for prediction are presented for 2,6-di-tert-butylphenol and BHT. The regression parameters and their correlation coefficients are given in table 4.3. The form of the linear equation used is:

$$y = b \cdot x + a$$

In order to validate the constructed linear models the data was subjected to the test for lack of fit (or F-test) [70].

Lack of fit testing

Linear calibration functions are used for almost all analytical procedures. But often this model is only applicable in a certain concentration range. And though there is no test for linearity as such, statistical tests can be applied to data obtained in a calibration experiment to test for significant lack of fit of the linear regression model. For the correlation coefficient can be misleading, the “F-test”, as it is often called, can be used to

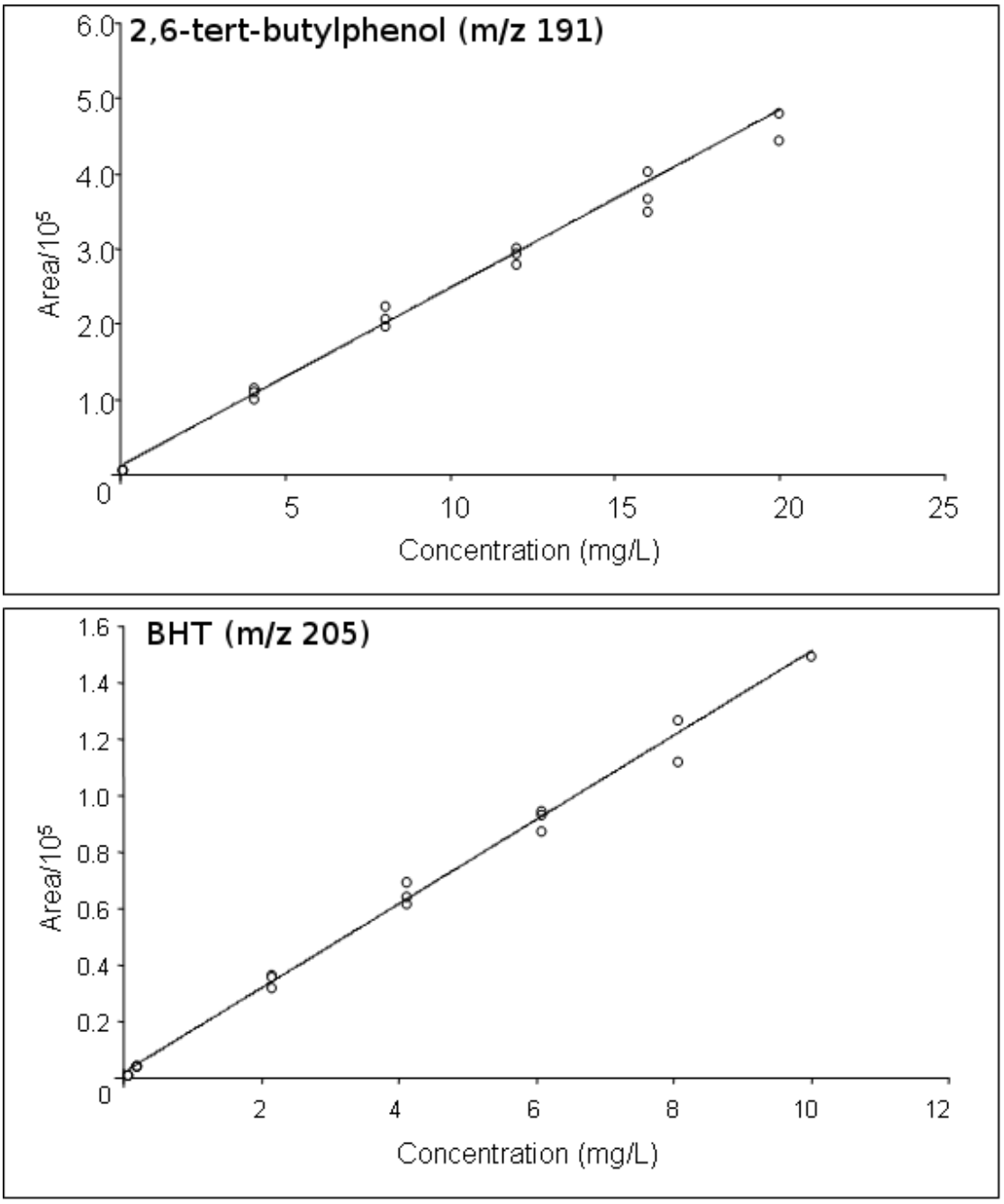

Figure 4.7: Calibration curves for 2,6-di-tert-butylphenol and BHT for the concentration range 0.10-20.0 ppm and 0.067-10.0 ppm, respectively.

Table 4.3: Parameters of the linear regression of all antioxidants and the correlation coefficients of their corresponding concentration range (see table 4.2).

Compound	Slope (b)	Confidence interval (b)	Intercept (a)	Confidence interval (a)	Correlation coefficient (R^2)
2-tert-butylphenol	(92	$\pm$ 4) $\cdot 10^3$	(17	$\pm$ 26)$\cdot 10^2$	0.9919
3-&4-tert-butylphenol	(57	$\pm$ 2) $\cdot 10^3$	(43	$\pm$ 45)$\cdot 10^2$	0.9946
2,6-di-tert-butylphenol	(23	$\pm$ 1) $\cdot 10^3$	(12	$\pm$ 14)$\cdot 10^3$	0.9900
BHA	(44	$\pm$ 2) $\cdot 10^2$	(-2	$\pm$ 11)$\cdot 10^2$	0.9941
BHT	(148	$\pm$ 5) $\cdot 10^2$	(13	$\pm$ 27)$\cdot 10^2$	0.9947
1-naphthol	118	$\pm$ 9	(-3	$\pm$ 5)$\cdot 10^2$	0.9916
DPA	(30	$\pm$ 2)$\cdot 10^2$	(25	$\pm$ 44)$\cdot 10^2$	0.9914

test in a given confidence interval.

Measuring a concentration c will give a response R. It is assumed that the measurements made are independent and random from an infinite population of possible results. The responses will vary around the actual response in a frequency distribution, which is often described by the standard deviation σ.
Two cases of distribution of the data can be distinguished: the homoscedastic (one σ for all levels) and the heteroscedastic (different σ for the levels) case.
The calibration function (for n concentration levels and p repeated measurements of each level) was estimated by linear regression from the data obtained and the heteroscedastic case was assumed in the present work.

The variables in the linear regression ($R = a + b \cdot c$), a and b, are estimated by minimizing

$$\sum_{ij} w_i r_{ij}^2$$

w_i being σ_i^{-2}, where σ_i is the standard deviation of the responses at the concentration c_i, and r_{ij} being the residuals $(R_{ij} - \overline{R}_i)$.
As for the heteroscedastic case the regression is fitted best by weighted least squares, where rough estimates of the weights will suffice. Then the weighted residual sum of squares can be calculated by

$$SS_{(w)r} = \sum_{ij} w_i r_{(w)ij}^2$$

which has $np - 2$ degrees of freedom. The sum of squares due to pure error is given by

$$SS_{(w)\epsilon} = \sum_{ij} w_i (R_{ij} - \overline{R}_i)^2$$

with $n(p-1)$ degrees of freedom ($\overline{R}_i = \sum_{ij} R_{ij}/p$). The sum of squares associated to the lack of fit has $n-2$ degrees of freedom and is

$$SS_{(w)lof} = SS_{(w)r} - SS_{(w)\epsilon}$$

To test now for lack of fit, the mean squares are calculated and the variance ratio is formed:

$$MS_{(w)\epsilon} = \frac{SS_{(w)\epsilon}}{n(p-1)}$$

$$MS_{(w)lof} = \frac{SS_{(w)lof}}{n-2}$$

$$F = \frac{MS_{(w)lof}}{MS_{(w)\epsilon}}$$

The critical value for $F_{n-2,n(p-1)}$ is given in a table (see Appendix, table A.1). If the value for F is significantly greater than the tabulated value, the goodness of fit can be rejected, within a given confidence interval.

The data in the present work, subjected to the above described test, did not show any significant lack of fit within the confidence interval of 95%.

4.2.2 Detection and quantification limits

The detection limit (DL) as well as the quantification limit (QL) were calculated by the following equations:

$$DL = \frac{3.3 \cdot \sigma}{b}$$

$$QL = \frac{10 \cdot \sigma}{b}$$

Here σ is the standard deviation of a signal at a S/N ratio of approximately three (3), obtained by n repeated measurements of a concentration given in table 4.4 for the studied compounds, b is the slope of the calibration function and 3.3 is the Student's t-factor for 99% probability and $n-1$ degrees of freedom (in this case 9). The value 10 in the QL results from the QL being the threefold of the DL.
The analytical signal used was the area of the peaks in the extracted ion chromatogram for each analyte (recorded in SIM mode).
Detection limits as low as 0.6 ppb for 2-tert-butylphenol, but also much higher values like for 1-naphthol (1282 ppb = 1.282 ppm) or DPA (93.4 ppb) were obtained, as these compounds are less volatile. This results can already be estimated from the values of their calibration slopes being less steep than 2-tert-butylphenol, e.g.
So this method permits the determination of most of the compounds in the low ppb range.

Table 4.4: Detection limit (DL), quantification limit (QL) and relative standard deviation (RSD) of the method

Compound	2-tert-butylphenol (m/z 135)	3-&4-tert-butylphenol (m/z 135)	2,6-di-tert-butylphenol (m/z 191)	BHA (m/z 165)	BHT (m/z 205)	1-naphthol (m/z 144)	DPA (m/z 169)
Concentration (ppm)	0.0096	0.0138	0.0511	0.5010	0.0678	8.8876	3.0094
Area	601	3513	994	460	604	1524	1429
	557	3348	962	469	593	1646	1533
	580	3386	999	490	675	1583	1711
	604	3314	967	469	613	1526	1661
	583	3168	981	452	604	1594	1606
	563	3268	954	458	608	1519	1599
	586	3269	930	449	615	1555	1627
	572	3303	959	470	599	1545	1634
	564	3019	887	453	584	1533	1569
	586	3022	969	438	585	1486	1488
Standard deviation (σ)	15.8	154.8	32.5	14.4	25.8	45.8	83.8
Slope (b)	91881	57472	22733	4404	14818	117.9	2961
DL (ppb)	**0.6**	**8.9**	**4.7**	**10.8**	**5.8**	**1281.9**	**93.4**
QL (ppb)	**2**	**27**	**14**	**33**	**17**	**3884**	**283**
RSD (%)	**2.7**	**4.7**	**3.4**	**3.1**	**4.2**	**3.0**	**5.3**

4.2.3 Reproducibility

In order to determine the reproducibility of the analytical method the Relative Standard Deviation (RSD) in % was calculated. It expresses the relative deviation of the signals obtained (x_i) to the mean of these values ($\overline{x}$). To calculate it, the following equation was used:

$$RSD = \frac{\sqrt{\frac{\sum_{i=1}^{n}(x_i-\overline{x})^2}{n-1}}}{\overline{x}} \cdot 100$$

The reproducibility was evaluated at the concentrations corresponding to a S/N ratio of approximately 3. The results are given in table 4.4.
In all cases values of the RSD minor than 5.3% were achieved and therefore the reproduciblity can be considered highly satisfactory.

4.3 Quantification of antioxidants in lubricant oil samples

All oil samples (new: 1-8 and used: 10-12) are intended to be used in automobile combustion engines, except for new oil 9. This oil sample is intended to be used in tractor engines.

4.3.1 Prediction of oil samples

In order to test if the previously described calibrations were applicable to predict the concentrations of lubricant oil samples, the oils were tested for matrix effect. This test was carried out by spiking oil 1 (20W50), oil 2 (5W40), oil 3 (10W40), oil 9 (5W40, tractor engine oil), used oil 10 (15W40) and used oil 11 (15W40) with a solution that contained the antioxidants at an intermediate concentration level. The resulting peak areas were compared: the peak area of spiked oil 1, which did not contain any of the studied antioxidants and was therefore used as "base" oil, was added to the area the unspiked oil (to be tested for matrix effect) gave. If no matrix effect occurred, this sum had to be similar to the spiked oil of interest. In figure 4.8 this principle is shown for new oil 2 and used oil 10.
By this procedure, it was determined that the new oil samples 2 and 3 had no matrix effect, while new oil sample 9 did show matrix effect. For the used oil samples (10 and 11) very strong matrix effect was observed.
From these results the conclusion was drawn that new oil samples intended for use in automobile engines could be predicted with the linear calibration models. But the determination of the studied compounds by prediction via the linear calibrations was not possible for the oils that showed matrix effect, so standard addition method was applied to predict their antioxidant concentration.

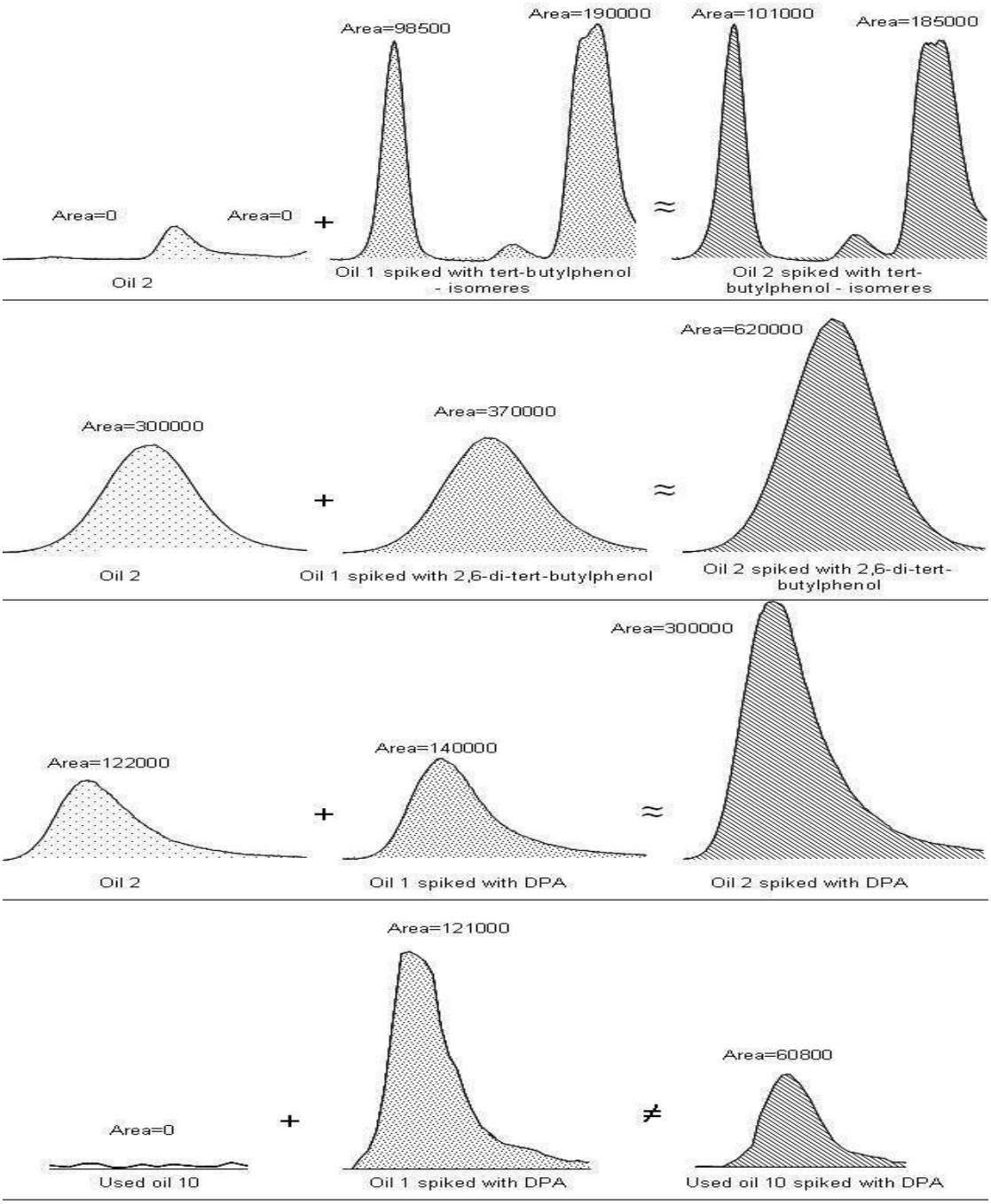

Figure 4.8: Matrix effect. The peak areas of the unspiked oil sample 2 and the spiked oil 1 are summed and coincide with the peak area of the spiked oil sample 2. The concentrations were 0.416, 1.76, 8.02, 4.33, 4.10, 16.1, 21.7 mg/L for 2-tert-butylphenol, 3- and 4-tert-butylphenol, di-tert-butylphenol, BHA, BHT, 1-naphthol, and DPA respectively. For the used oil 10 the areas do not coincide and therefore matrix effect has to be assumed.

New oil samples

For the new oil samples, where no matrix effect was found (see section 4.3.1), the linear calibration models were applied (see section 4.2.1). In table 4.5 the results of the prediction are shown. Also the viscosity range for each oil is given.

Used oil samples

As previously discussed, matrix effect was found when the used oils were tested for it. So the standard addition method was applied to determine their antioxidants concentration. In the intervals given in table 4.5 four uniformly distributed concentration levels were measured. They were analyzed in triplicate.
In all cases, calibrations devoid of lack of fit (see section 4.2.1) were obtained, with R^2 coefficients equal or greater than 0.99 . The resulting calibration slopes were different in the three used oil samples, which means that it is not possible to predict antioxidants in these types of oils using the same strategy described for new ones.
Used oil 10 corresponds to new oil 8 and it was removed from the car at 10000 km. As one can see in table 4.5 and figure 4.9, the total depletion of BHT occurred in this oil. This was the only determined antioxidant in new oil 8.

Table 4.5: Predicted concentration (mg/L) and confidence intervals (95% probability) for the eight new oils and the three used oil samples. Oil 9 corresponds to a tractor engine

Sample	Compounds			
	3- and 4-tert-butylphenol	2,6-di-tert-butyl-phenol	BHT	DPA
Oil 2 (5W40)	-	8.0 ± 0.3	< QL	22 ± 2
Oil 3 (10W40)	-	< QL	< QL	18 ± 1
Oil 4 (15W40)	-	8.1 ± 0.4	1.80 ± 0.07	< QL
Oil 5 (10W40)	-	8.4 ± 0.4	0.48 ± 0.07	6.9 ± 0.7
Oil 6 (5W40)	< QL	< QL	1.9 ± 0.2	-
Oil 7 (15W40)	-	4.1 ± 0.2	0.23 ± 0.04	5.4 ± 0.6
Oil 8 (15W40)	< QL	< QL	0.6 ± 0.06	-
Oil 9* (5W40)	-	119 ± 8 (0-100)**	18 ± 3 (0-12)**	45 ± 5 (0-54)**
used oil 10* (15W40)	-	-	-	-
used oil 11* (15W40)	-	-	39 ± 2 (0-78)**	17 ± 2 (0-195)**
used oil 12* (15W40)	-	-	47 ± 4 (0-78)**	7 ± 1 (0-195)**

* standard addition method was used for quantification

** standard addition concentration range in brackets

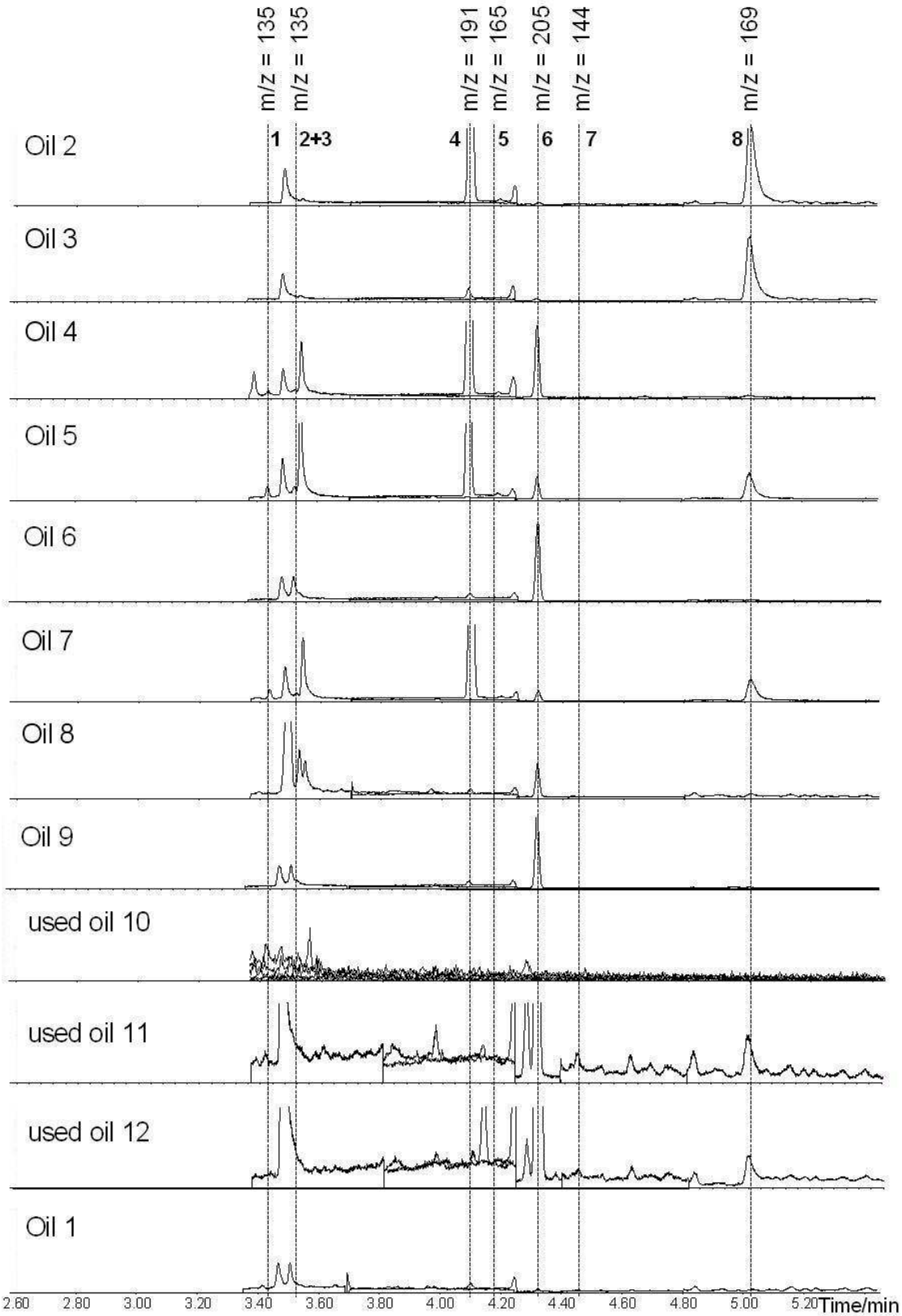

Figure 4.9: Extracted ion chromatograms of the new oil samples (oil 2-9), used oil samples (used oil 10-12), and the oil used for the calibration in SIM mode. Dashed lines indicate the retention times of 2-tert-butylphenol (1), 3- and 4-tert-butylphenol (2+3), di-tert-butylphenol (4), BHA(5), BHT (6), 1-naphthol (7) and DPA (8). This figure is conclusive with the results given in table 4.5.

4.3.2 Accuracy of the prediction

To check the possibilities of the methodology for those compounds that could not be quantified, the samples were spiked with them. The recovery rates were calculated by comparison with the real concentration. The results of this study are shown in table 4.6. The results obtained clearly show that the proposed methodology is applicable to the determination and quantification of the studied antioxidants in new and used lubricant oils.

Table 4.6: Accuracy of the method. Samples were spiked with those antioxidants which were not detected or were below quantification limits.

Compounds	New oils			Used oils			
	Added (mg/L)	Recovery Rate (%)		Standard addition (mg/L)	Added (mg/L)	Recovery Rate (%)	
		Oil 2 (5W40)	Oil 3 (10W40)			Oil 10	Oil 11
2-tert-butylphenol	0.416	109	104	4.40 - 17.6	4.40	107	90
3- and 4-tert-butylphenol	1.76	89	103	7.23 - 29.0	7.23	99	89
2,6-di-tert-butylphenol	-	-	96	4.43 - 17.7	4.43	105	90
3-tert-butyl-4-hydroxyanisole (BHA)	4.33	102	99	87.0 - 351	87.0	93	96
2,6-di-tert-butyl-4-methylphenol (BHT)	4.10	80	90	19.5 - 78.0	19.5	106	-
1-naphthol	16.1	86	103	212 - 584	212	92	94
di-phenylamine (DPA)	-	-	-	48.0 - 195	48.0	93	-

Chapter 5

Conclusions

In the present work antioxidants in lubricant oils were successfully determined by the proposed method, consisting in the coupling of a HS generator to a PTV injection system followed by chromatographic separation and detection with a mass spectrometer.
The HS sampler permits the introduction of the sample without previous sample treatment, the errors possibly committed in this step thereby being eliminated. Also the analysis time for a sample is not prolonged by preparation steps.
Usage of a PTV with a liner filled with Tenax-TA® allows cryogenic focusing of the analytes and venting of the solvent in the solvent vent injection mode. The sensitivity of the method is increased compared to other injection techniques, because the analytes are retained in the liner.
The chromatographic column used permits fast separation, for the peak widths at half height range for the antioxidants between 0.78 (2-tert-butylphenol) and 1.68 (1-naphthol) seconds. The total chromatographic runtime is 5.44 minutes.
Finally, the use of an MS detector unit permits the identification of the compounds of interest by comparison with a mass spectra data base when operated in SCAN mode, but also permits the SIM mode. When operated in this mode the sensitivity can be improved by a factor of 10.

The quantification of antioxidants in new oils was carried out using an external standard, from calibration in one of the oils which does not contain any of the studied compounds. This means that both time and costs can be reduced since it is not necessary to generate individual calibration models for each type of new oil. Summarizing the analytical characteristics of the proposed method, it can be stated that the HS-PTV-fastGC-MS method revealed good precision and accuracy with detection limits for most of the compounds at μg/L level. So this is an adequate method for the determination of antioxidants in new lubricant oils, for most of the studied compounds in the low ppb range.
However, standard additions method will be required for quantification of analytes in the used oils due to the matrix effect, where the calibration slopes in new oil samples were not applicable. Also a calibration in a used oil would not have overcome this difficulty,

since the slopes obtained by standard addition method in the used oil samples did not coincide. This indicates that matrix effect is different in used oil samples, depending on many factors (age of the motor, gas used, etc.).

It should be stressed that all the studied antioxidants have high boiling points which means that headspace analysis could not be, a priori, a suitable sampling technique for the analysis. However, the use of solvent vent (Tenax-TA®liner) in the PTV and SIM detection mode in the MS provided satisfactory results beside the benefits of using HS. Concluding all, it is an easy to use method, for the instrumentation as well as the sample preparation is simple and the detection and quantification limits achieved were satisfactory low to quantitate antioxidants in real lubricant oil samples. Furthermore the repeatability study carried out showed good reproducibility and accuracy for the proposed method.

Concerning the objectives of the present work, all requirements were met.

Appendix A

Supplementary Information

A.1 Risk phrases

Risk phrases:

R11 Highly flammable

R22 Harmful if swallowed

R23 Toxic by inhalation

R33 Danger of cumulative effects

R34 Causes burns

R36 Irritating to eyes

R37 Irritating to respiratory system

R38 Irritating to skin

R41 Risk of serious damage to eyes

R66 Repeated exposure may cause skin dryness or cracking

R67 Vapours may cause drowsiness and dizziness

Combinations:

R21/22 Harmful in contact with skin and if swallowed

R23/24/25 Toxic by inhalation, in contact with skin and if swallowed

R37/38 Irritating to respiratory system and skin

R39/23/24/25 Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed

R50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

A.2 F-test values

$\nu_2 \backslash \nu_1$	1	2	3	4	5	6	7	8	9	10
1	161.448	199.500	215.707	224.583	230.162	233.986	236.768	238.882	240.543	241.882
2	18.513	19.000	19.164	19.247	19.296	19.330	19.353	19.371	19.385	19.396
3	10.128	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494
17	4.451	3.592	3.197	2.965	2.810	2.699	2.614	2.548	2.494	2.450
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348

Table A.1: Upper critical values of the F distribution for ν_1 numerator degrees of freedom and ν_2 denominator degrees of freedom,
5% significance level: $F_{0.5(\nu_1,\nu_2)}$

A.3 MS-Spectra

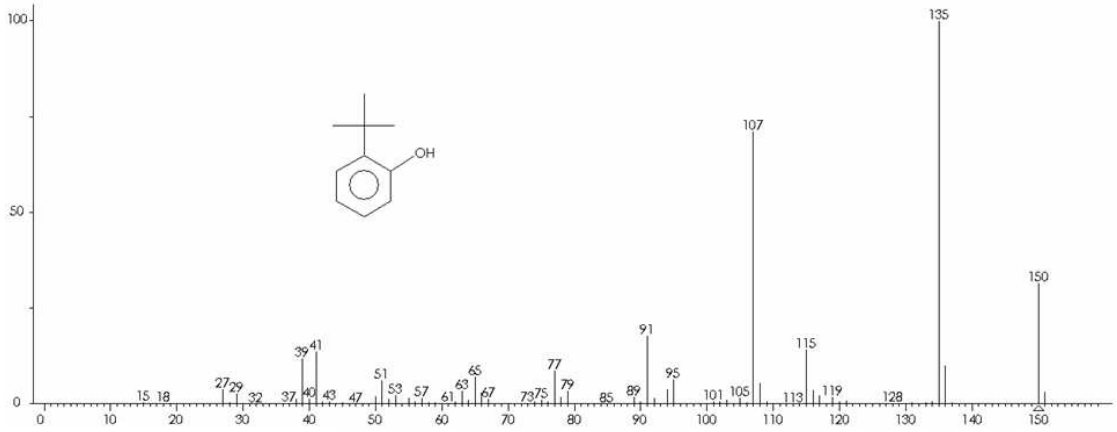

Figure A.1: Mass spectrum of 2-tert-butylphenol

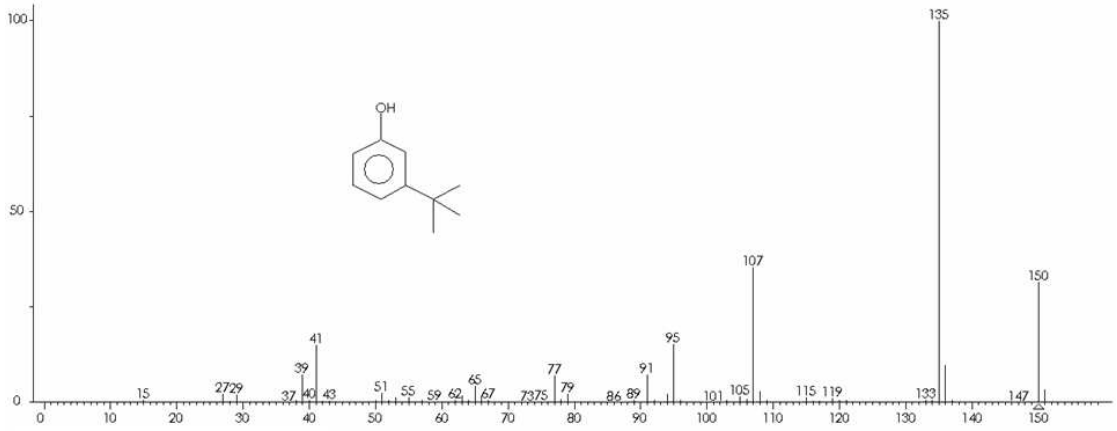

Figure A.2: Mass spectrum of 3-tert-butylphenol

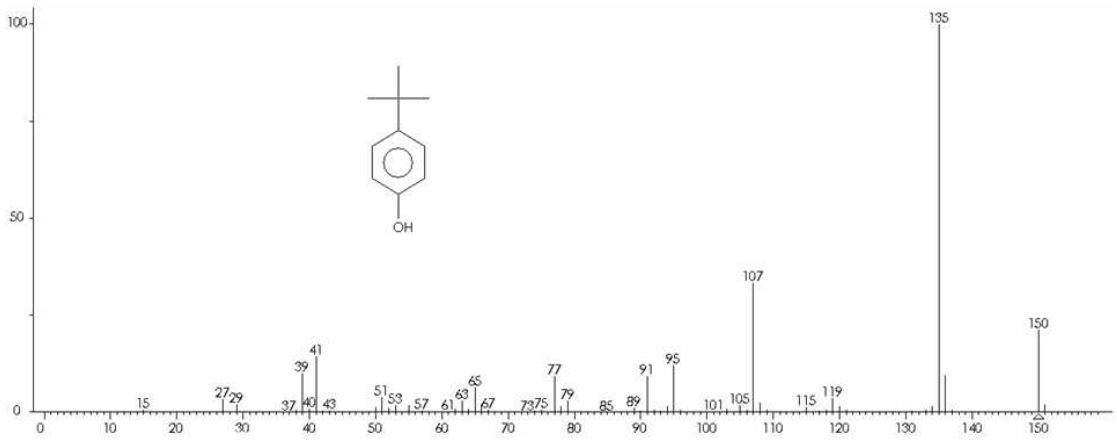

Figure A.3: Mass spectrum of 4-tert-butylphenol

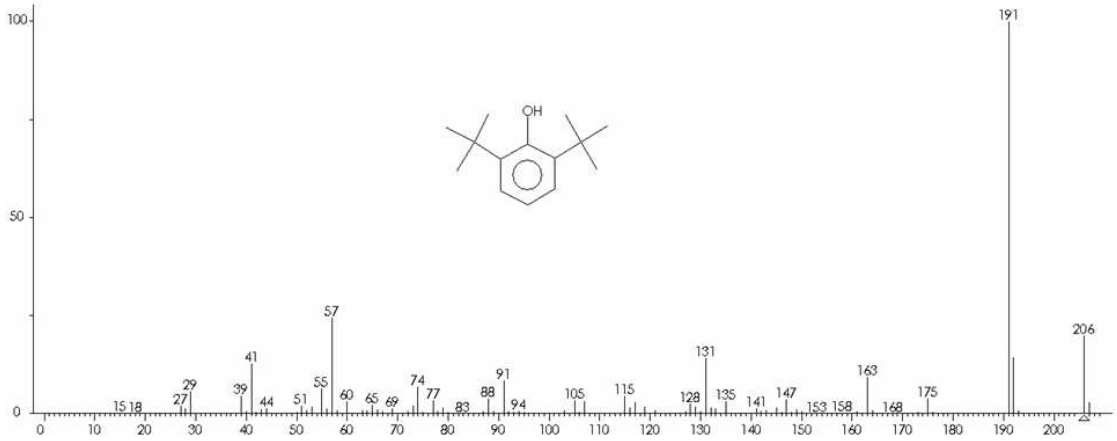

Figure A.4: Mass spectrum of 2,6-di-tert-butylphenol

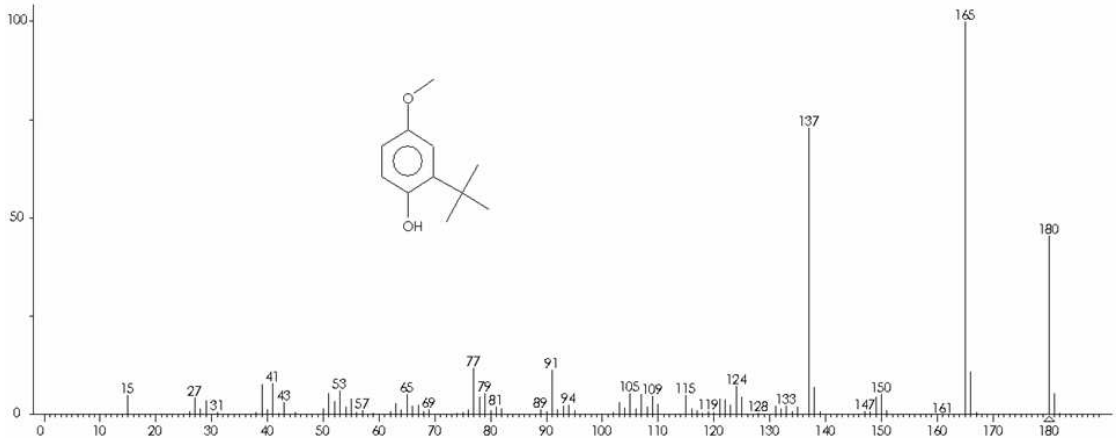

Figure A.5: Mass spectrum of 3-tert-butyl-4-hydroxyanisol (BHA)

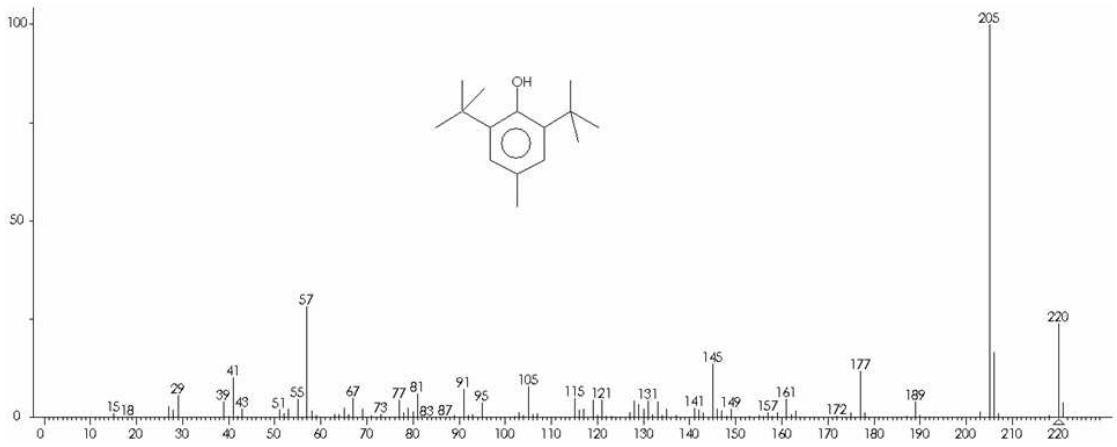

Figure A.6: Mass spectrum of 2,6-di-tert-butyl-4-methyl-phenol (BHT)

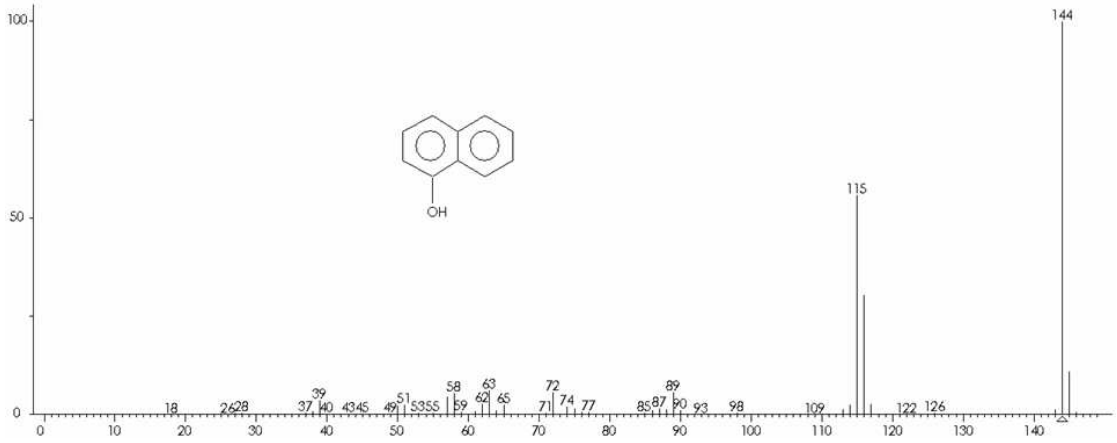

Figure A.7: Mass spectrum of 1-naphthol

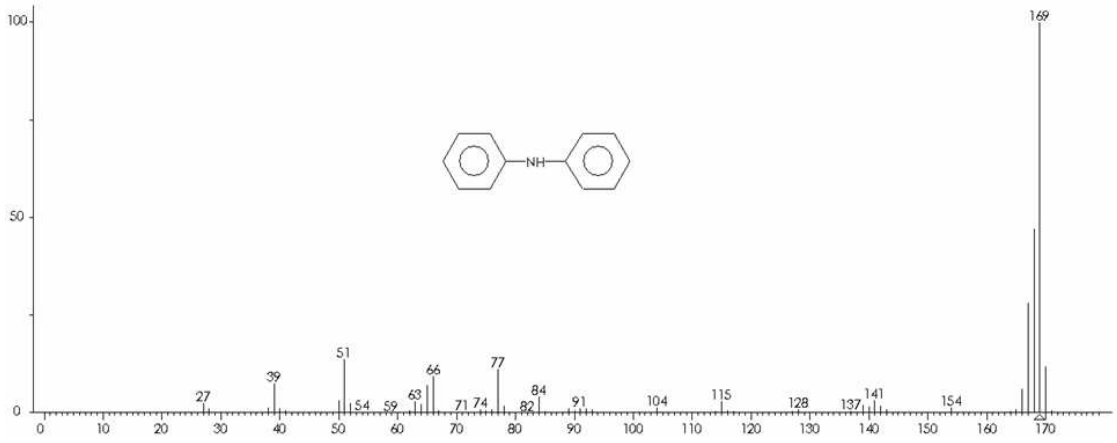

Figure A.8: Mass spectrum of di-phenylamine

Appendix B

Abstract

A sensitive method is presented to determine antioxidants (2-, 3- and 4- tert-butylphenol, 2,6-di-tert-butylphenol, 3-tert-butyl-4-hydroxyanisol, 2,6-di-tert-butyl-4-methyl-phenol, 1-naphthol and di-phenylamine) in new and used lubricant oil samples. Research was carried out on a GC device equipped with a headspace-sampler (HS), a programmed temperature vaporizer (PTV) and a MS detector unit.
The proposed method does not require sample treatment prior to analyses, hence eliminating possible errors occurring in this step. Sample preparation is reduced to placing the oil sample (2.0 g) in the vial and adding propylacetate (20 μL) as solvent. Solvent vent injection mode permits a preconcentration of the compounds of interest in the liner filled with Tenax-TA®, while venting other species present in the headspace. Thereby both the life of the liner and the capillary column is prolonged and unnecessary contamination of the detector unit is avoided.
Calibration was performed by adding different concentrations of analytes to a new oil which did not contain any of the studied compounds. Limits of detection as low as 0.57 μg/L (2-tert-butylphenol) with a precision lower or equal to 5.3% were achieved. Prediction of the antioxidants in new oil samples of different viscosities (5W40, 10W40, 15W40) was accomplished with the previous calibration and the results were highly satisfactory. To determine antioxidants in used engine oils standard addition method was used due to the matrix effect.

Appendix C

Zusammenfassung

Die gegenständige Arbeit ist eine sensitive Methode zur Bestimmung von Antioxidantien (2-, 3- and 4- tert-Butylphenol, 2,6-di-tert-Butylphenol, 3-tert-butyl-4-Hydroxyanisol, 2,6-di-tert-butyl-4-methyl-Phenol, 1-Naphthol und di-Phenylamin) in neuen und gebrauchten Motorölen. Die Messungen wurden an einem Gaschromatographen durchgeführt der mit einem headspace sampler (HS), einem Temperatur-programmierbaren Verdampfer (PTV) und einer MS-Detektionseinheit ausgestattet ist.
Die vorgeschlagene Methode benötigt keinen Proben Vorbereitungsschritt, wodurch mögliche Fehler in diesem Schritt eliminiert werden. Es müssen lediglich die Ölprobe (2.0 g) und Propylacetat als Lösungsmittel (20 μL) in das Glasfläschchen gegeben werden. Solvent vent Injektion erlaubt die Konzentrierung der Analyte im Liner, der mit Tenax-TA®gefüllt ist, während andere Komponenten des Headspace ventiliert werden. Dadurch wird das Leben von Liner und Kappillarsäule verlängert und unnötige Verschmutzung der Detektionseinheit wird vermieden.
Die Kalibration wurde in einem neuen Öl durchgeführt, das keine der genannten Analyte enthält, indem unterschiedliche Konzentrationen derselben zugegeben wurden. Detektionsgrenzen bis hin zu 0.57 μg/L (2-tert-Butylphenol) mit einer Präzision unter oder gleich 5.3% wurden erreicht. Die Konzentrationen in neuen Ölproben unterschiedlicher Viskosität (5W40, 10W40, 15W40) wurden mit Hilfe der vorherigen Kalibration bestimmt und die Ergebisse waren sehr zufriedenstellend. Die Antioxidantien in gebrauchten Ölen wurden aufgrund von Matrixeffekt mittels Standard-Addition bestimmt.

List of Figures

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ORIGINAL PAPER

Determination of antioxidants in new and used lubricant oils by headspace-programmed temperature vaporization–gas chromatography–mass spectrometry

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Abstract A sensitive method is presented to determine antioxidants (2-, 3-, and 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 3-*tert*-butyl-4-hydroxyanisol, 2,6-di-*tert*-butyl-4-methylphenol, 1-naphthol, and diphenylamine) in new and used lubricant oil samples. Research was carried out on a GC device equipped with a headspace sampler, a programmed temperature vaporizer, and an MS detector unit. The proposed method does not require sample treatment prior to analyses, hence eliminating possible errors occurring in this step. Sample preparation is reduced when placing the oil sample (2.0 g) in the vial and adding propyl acetate (20 μL). Solvent vent injection mode permits a pre-concentration of the compounds of interest in the liner filled with Tenax-TA®, while venting other species present in the headspace. Thereby, both the life of the liner and the capillary column is prolonged, and unnecessary contamination of the detector unit is avoided. Calibration was performed by adding different concentrations of analytes to a new oil which did not contain any of the studied compounds. Limits of detection as low as 0.57 μg/L (2-*tert*-butylphenol) with a precision lower or equal to 5.3% were achieved. Prediction of the antioxidants in new oil samples of different viscosities (5W40, 10W40, and 15W40) was accomplished with the previous calibration, and the results were highly satisfactory. To determine antioxidants in used engine oils, standard addition method was used due to the matrix effect.

Keywords Antioxidants · HS–PTV–GC–MS · Lubricant oil

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Introduction

Motor oils are heavy petroleum products commonly used to reduce the friction between surfaces, to prevent the corrosion, to remove heat and contaminants, and to clean the motor. Conventional motor oils are mainly comprised of base oil and up to 10% additives [1]. The major component (>90%) is the base oil, and it is generally produced by the refining of crude oil. Gas chromatography with flame ionization (GC–FID) or mass spectrometry detector (GC–MS) [2], comprehensive two-dimensional gas chromatography (GC×GC–FID) [2], high-performance liquid chromatography–differential refractometer detector (HPLC–RI) [3], and HPLC–GC×GC–FID [1] have been used for characterization and determination of hydrocarbons present in motor (base) oils. Non-petroleum base fluids are employed when special properties are necessary, when petroleum base oils are in short supply, or when substitution by natural products is practicable or desirable [4].

Base oil requires additives (dispersants, detergents, oxidation inhibitors, and antiwear agents) to satisfy the lubricating needs of an engine and to increase the useful lifetime of the oil. One of the most important aspects of lubricating oils is that the oxidation stability be maximized. Generally, engine oil compounds have a relatively high

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thermal/oxidative stability in order to avoid the degradation of hydrocarbons which are exposed to oxygen and heat. Antioxidants [5] are the key additives that protect the lubricant from this degradation. Some of the main classes of oil-soluble organic antioxidants are sulfur and phosphorous compounds, aromatic amines, and hindered phenolic compounds. A synergistic effect has been found when different types of inhibitors have been used together [6]. Thus, the useful lifetime of the additives is extended beyond the expected performance of each additive if they were used separately.

Oil composition changes during engine operation. The oxidation of oils in engines is one of the aging processes that results in the formation of polar oxidation products including alcohols, aldehydes, ketones, and carboxylic acids [7, 8]. These compounds undergo further reactions to form sludges which degrade the performance of the oil. The depletion of antioxidants can be seen as a form of "titration" where oxidation products are formed when additives are depleted. In that sense, the conditional maintenance becomes a task of determining the "end point" in this titration [9]. Other common contaminants found in the oil are fuel, soot, water, and ethylene glycol (a major component in antifreeze). Polycyclic aromatic hydrocarbons are known to be present in used lubricating oils. The presence of all these compounds can cause detrimental changes in engine oil, and they accelerate the depletion process of additives. Several methods [10–13] have been developed to determine these contaminants in lubricants.

Determination of antioxidants in lubricant has been performed with infrared spectroscopy [14], size-exclusion chromatography–RI [15], HPLC–MS [16], GC–MS [16], and GC–nitrogen–phosphorous detector (GC–NPD) [16]. When gas chromatography is used, the capillary columns are susceptible to degradation by the types of contaminants present in used oils. A possible alternative to direct injection of the oil is to use headspace analysis (HS) which does not involve any additional sample treatment. Accordingly, a method based on HS–GC–MS [9] has been used to identify the principal gaseous components of engine oil and to establish the concept of oil condition monitoring via the vapor phase.

Here, we propose a new method for the determination of antioxidants in new and used engine oil samples. This sensitive method employs a headspace autosampler in combination with a gas chromatograph equipped with a programmable temperature vaporizer and an MS detector in the selected ion monitoring acquisition mode (HS–PTV–GC–MS (SIM)). The use of a programmed temperature vaporizer is an attractive option that allows different modes of injection into the gas chromatograph, including solvent vent. This setup is a pre-concentration technique in the injection port that offers benefits in terms of simplicity and automation possibilities, together with very good sensitivity [17]. As far as we know, to date, the HS–PTV combination has not been proposed in motor oil analysis.

Experimental section

Chemicals

The eight antioxidants studied (Table 1) and propyl acetate were supplied by Sigma-Aldrich (Steinheim, Germany) with purities of at least 99%.

Standard solutions and samples

A set of stock solutions of the eight antioxidants were prepared in propyl acetate, with concentrations ranging from 2.97 to 104.1 g/L. Working solutions and calibration standards were prepared by dilution of the stock solutions with propyl acetate.

To perform the calibration measurements, 2.0 g of a lubricant oil which does not contain any of the compounds of interest (oil 1, 20 W50) plus 20 μL of antioxidant solution were placed in a 10-mL glass vial (Agilent Technologies, DE, Germany) sealed with Teflon/silicone septa (Agilent Technologies, DE, Germany). Each sample was analyzed in triplicate (three vials; one injection per vial).

Eight new motor oil samples (oils 2–9) of varying viscosity (5W40: oils 2, 6, and 9; 10W40: oils 3 and 5; 15W40: oils 4, 7, and 8) and three used motor oil samples (collected from passenger cars after 10,000 km) were analyzed (oils 10–12). Seven of the new oils (oils 2–8) correspond to automobile engines, and the other one is a tractor engine oil (oil 9). To perform the measurements of the new oils, 2.0 g of the motor oil plus 20 μL of propyl acetate were placed in the vial.

In case of the used lubricant oil samples, standard addition method was used, and to carry out the measurement, 2.0 g of the oil plus 20 μL of antioxidant solution were placed in the vial.

All the prediction samples were analyzed in triplicate.

None of normalization procedures for the chromatograms was used because the injection volume variations were negligible.

HS–PTV–fast GC–MS

All the experimental conditions are summarized in Table 2, including headspace autosampler, programmed temperature vaporizer, gas chromatograph, and mass spectrometer.

Headspace sampling

HS sampling was executed with a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). This sampler per-

Table 1 Target compounds, formula, retention times, boiling points, and *m*/*z* ratios selected (SIM mode) for the eight compounds studied

Compounds	Formula	t_R (min)	Boiling point (°C)	*m*/*z*	
				Quantitation ion	Qualifier ion
(1) 2-*tert*-butylphenol	$C_{10}H_{14}O$	3.441	224	135	107,150
(2) 3-*tert*-butylphenol	$C_{10}H_{14}O$	3.530	–[a]	135	107,150
(3) 4-*tert*-butylphenol	$C_{10}H_{14}O$	3.530	236–238	135	107,150
(4) 2,6-di-*tert*-butylphenol	$C_{14}H_{22}O$	4.108	253	191	57,206
(5) 3-*tert*-butyl-4-hydroxyanisole (BHA)	$C_{11}H_{16}O_2$	4.178	264–270	165	137,180
(6) 2,6-di-*tert*-butyl-methylphenol (BHT)	$C_{15}H_{24}O$	4.334	265	205	57,220
(7) 1-naphthol	$C_{10}H_8O$	4.477	278–280	144	115,116
(8) Diphenylamine (DPA)	$C_{12}H_{11}N$	5.025	302	169	167,168

[a] Not found

mits analyses of 32 consecutive samples and has an oven with positions for six vials. Oven temperature was kept at 95 °C, and the equilibration time was 15 min. The injected volume from HS to PTV was 2.4 mL (syringe at 100 °C).

Programmed temperature vaporizer

All experiments were carried out with a PTV inlet (CIS-4: Gerstel, Baltimore, MD, USA). Two different liners (71 mm×

Table 2 Optimized experimental conditions

Headspace sampler			
Oven	95 °C	Syringe washing	
Syringe	100 °C	Syringe temperature	100 °C
Headspace generation	15.00 min	Sample volume	2.5 mL
Agitation	750 rpm	Solvent	Propyl acetate
Syringe flushing (He)	2.00 min	Syringe flushing (He)	1.00 min
Interval between vials	12.00 min		
Sample volume	2.4 mL		
Programmable temperature vaporizer			
Injection mode: solvent vent	Purge flow	150 mL/min	
	Purge time	0.60 min	
	Purge temperature	60 °C	
	Injection time	1.00 min	
	Rate	12 °C/s to 325 °C (5.44 min)	
Gas chromatograph			
Carrier gas	Helium	(1.4 mL/min)	
	Initial temperature	110 °C (1.00 min)	
Oven	Ramp 1	65 °C/min to 175 °C	
	Ramp 2	45 °C/min to 240 °C (2.00 min)	
		(Total run time 5.44 min)	
Mass spectrometer			
Data acquisition mode: SCAN	*m*/*z* 25–300. 5.95 cycles per second		
	Dwell time	10 ms	
Data acquisition mode: SIM	Group 1	*m*/*z* (107,135,150) 2.50–3.70 min	
	Group 2	*m*/*z* (57,137,165,180,191,206) 3.70–4.25 min	
	Group 3	*m*/*z* (57,205,220) 4.25–4.42 min	
	Group 4	*m*/*z* (115,116,144) 4.42–4.80 min	
	Group 5	*m*/*z* (167,168,169) 4.80–5.44 min	

2 mm) packed with glass wool or with a chemical sorbent (Tenax-TA®) designed to trap organics were assayed. Hot-split injection and solvent-vent injection were compared.

When hot-split injection was used, the injector temperature was at 325 °C throughout the analyses time (split ratio 1:10, split flow 7.0 mL/min). In the solvent-vent injection mode, the injector venting temperature was 60 °C, with a flow of 150 mL/min at a pressure of 5.0 psi (34,474 Pa). After 0.60 min, the split valve was closed and flash-heated at a rate of 12 °C/s to 325 °C, thus transferring the analytes to the column. At 1.60 min, the split valve was opened again (split flow 150 mL/min), and the liner temperature was held at 325 °C until the end of the analyses. In the optimized method, solvent-vent injection and a liner packed with Tenax-TA® was used. Cooling was accomplished with liquid CO_2.

Gas chromatography

To perform the gas chromatographic measurements, an Agilent 6890 GC device equipped with a low-polarity DB-VRX capillary column (20 m×0.18 mm×1 μm) from Agilent J&W was used. The initial oven temperature was 110 °C for 1.00 min, followed by a ramp of 65 °C/min to 175 °C. After that, the rate changed to 45 °C/min, until 240 °C, and this temperature was held for 2.00 min. The total chromatographic run time was 5.44 min. The carrier gas was helium N50 (99.995% pure; Air Liquide), and the flow rate was 1.4 mL/min.

Mass spectrometry

The detector was a quadrupole mass spectrometer (HP 5973 N) equipped with inert ion source. It was operated in electron-impact mode using an ionization voltage of 70 eV. The ion source temperature was 230 °C, and the quadrupole was set at 150 °C. The analyses were performed in the scan and SIM mode.

The scan detection mode was only used for identification of the compound in the samples covering a *m*/*z* range of 25–300 amu. The different compounds were identified by comparison of the experimental spectra with those of the NIST'05 database (NIST/EPA/NIH Mass Spectral Library, version 2.0).

The SIM detection mode was used for quantification of the compounds, and the *m*/*z* ratios used were the most abundant ones of the compounds studied (Table 1). Five SIM windows were used, and the ions were acquired with a dwell time of 10 ms. (Table 2)

Data analyses

Data collection was performed with Enhanced ChemStation software [18] from Agilent Technologies.

Results and discussions

Preliminary study of HS–PTV–GC–MS data

Headspace sampling

A contamination effect between samples was observed when different oils were measured. The signal when an empty vial (only air) was measured after an oil sample contained *m*/*z* characteristic of the antioxidants from previous oil. This effect produces an important irreproducibility between replicates.

HS syringe and liner in the PTV are possible instrumental zones where the sample could remain between injections. Typical experimental conditions to clean both the HS syringe (helium flush flow 0.5 bar, 2 min) and the liner (final temperature 325 °C for 5.44 min) were not enough to eliminate contamination. To check contamination in the vaporizer chamber, three different liners were assayed. Two of them contained a packing material (glass wool and Tenax-TA®), and the last one was an empty liner. In all cases, contamination effects persisted even when empty and new liners were used. In order to ensure that the contamination effect was due to lack of cleaning in the HS syringe, a new step was added in the analysis. The syringe was cleaned with propyl acetate between samples (see conditions in Table 2). After that, memory effect was removed.

The quantity of oil sample in the vial and the amount of propyl acetate added were also studied. Propyl acetate addition in the vial is necessary to build the calibrations for the studied antioxidants. Regarding the first variable, amounts between 0.20 and 2.0 g of oil sample (oil 7) were assayed without adding propyl acetate. The analytical signal for the studied compounds was similar in all cases, so the preferable amount of oil could be 0.20 g in order to minimize sample quantity for analysis. However, when this study was repeated with different amounts of propyl acetate (20–60 μL) in vials containing 0.20 g of oil, the analytical signal diminished in comparison to the previous study. The only combination for both variables that did not show signal decrease was 2.0 g of oil and 20 μL of propyl acetate. For further analyses, these sample parameters were used.

PTV parameters

Initially, a comparative study of the signal obtained for the compounds studied was performed using two liners packed with different materials (glass wool and Tenax-TA®). Both were found to exhibit different analyte absorption capacity. Moreover, with each type of liner, two injection modes were assayed: hot split and solvent vent.

Figure 1 shows the results obtained when a laboratory-prepared sample containing all the antioxidants in propyl acetate (50 mg/L for 1-naphthol and 5 mg/L for all the other compounds) was measured. The highest signals were obtained when solvent-vent injection mode was used with a liner filled with Tenax-TA®. This is because of the interactions of the analytes with the filling of the liner which traps the studied compounds while propyl acetate and other compounds are vented. When a glass-wool-packed liner was used (solvent vent), the analytes are less strongly retained in the liner. Hot-split injection provides signals of similar intensities for both liners because their absorption capacities disappear at high temperatures.

Different venting temperatures (40–100 °C) were assayed, and 60 °C was selected as a final temperature because it allows the removal of a propyl acetate fraction while the compounds of interest are retained in the liner during the venting step. This temperature allows the removal of the lightest compounds in the oil that elute in the initial part of the chromatogram of the sample. In this way, the life of the liner is prolonged, preventing its rapid saturation, and cleaner injections devoid of interferences are obtained. The analytical signal of some antioxidants (2-, 3-, and 4-*tert*-butylphenol) decreased when the venting temperature was higher than 60 °C.

Three different injection times (from PTV to GC) were studied (0.6, 1.0, and 1.5 min). The injection time chosen was 1.0 min. When this time was shorter, the desorption of the analytes retained in the liner was incomplete. An increase in this variable does not elicit a significant increase in the studied analytes.

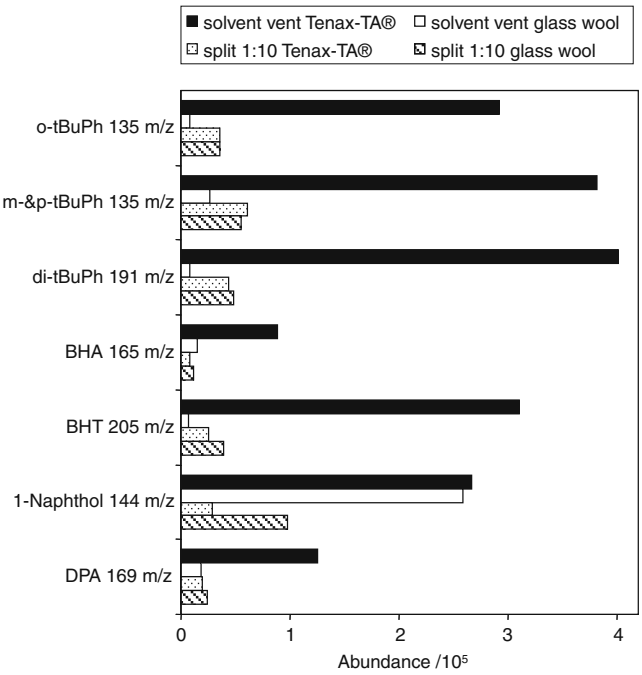

Fig. 1 Comparative study of the signal obtained for the antioxidants studied (peak areas for the characteristic *m*/*z* ratios) when two different liners were employed (glass wool and Tenax-TA®) in solvent-vent and hot-split (1:10) injection modes

GC parameters

In order to perform the separation of the studied antioxidants by fast gas chromatography, the maximum temperature ramps permitted by the oven of the chromatograph and the capillary column were chosen (see "Experimental"). Under these conditions, the only variable to be optimized was the initial column temperature. Values ranging between 70 and 150 °C were studied. The results showed that as the initial temperature was increased a slight peak broadening along with diminishing peak height occurred (for 130 and 150 °C, respectively). Also, the time necessary to recover the initial chromatographic conditions increased considerably as the initial temperature of the column decreased, thus prolonging analyses time. The time invested to cool down the capillary column from 240 to 150, 110, and 70 °C were 0.9, 1.8, and 3.0 min, respectively. According to the results obtained, the initial temperature was set to 110 °C, allowing adequate separation of the analytes without excessively prolonging the analysis time.

Figure 2 shows the chromatogram obtained on analyzing the oil sample 1, which does not contain any of the studied antioxidants, spiked with the studied antioxidants. Retention times, boiling points, and the characteristic and most abundant *m*/*z* ratios of the analytes studied are given in Table 1. All peaks indicated in Fig. 2 had widths at half height ($W_{1/2}$) of less than 2 s (ranging from 0.78 s for 2-*tert*-butylphenol to 1.68 s for 1-naphthol). In fast GC, the usual value [19] for peak widths at half height is 0.2–3 s and the typical runtimes range from 1 to 10 min. Thus, this case is a fast GC application for the studied compounds.

As it can be seen, the separation of 3-*tert*-butylphenol and 4-*tert*-butylphenol could not be achieved with the chromatographic program used. In order to check if separation was possible, the following chromatographic program was used: initial oven temperature 35 °C (3 min), ramp of 12 °C/min to 240 °C (3 min). The resulting chromatographic runtime was 23.08 min. However, separation of these two isomers could not be achieved.

In view of the above results, the chromatographic program involving an initial temperature of 110 °C and the maximum temperature ramps allowed was used.

MS parameters

Different dwell times (1–50 ms) in the SIM mode were assayed. A dwell time of 50 ms showed a bad peak definition due to the few points that defined it. However,

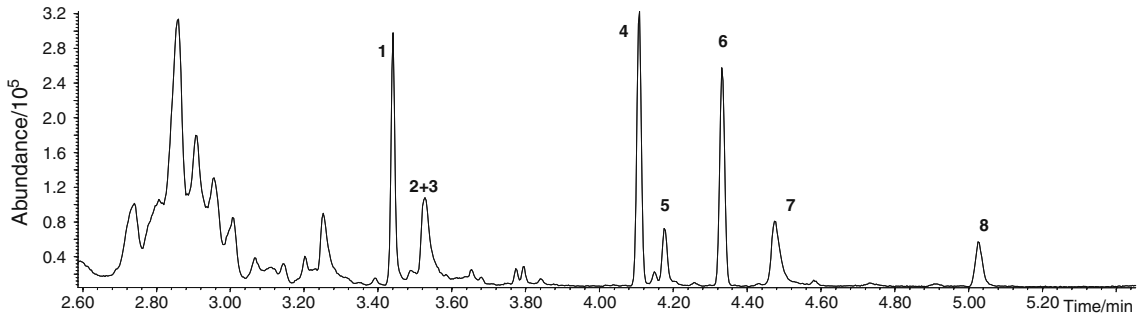

Fig. 2 Total ion chromatogram (scan mode) of spiked oil 1 at an intermediate calibration level. The peaks of the analytes are labeled with numbers according to Table 1

1 ms of dwell time provided better peak definition but the noise increased. A compromise for both parameters was found when a dwell time of 10 ms was used.

HS–PTV–GC–MS method evaluation

Oil 1 represented the anomaly of none of the common antioxidants present in the sample. However, there is the possibility [9] that other additives may be present and were not detected by the instrumental configuration used.

The linearity of the method was evaluated from triplicate injections of samples of oil 1 containing the antioxidant solution across the concentration range studied (six uniformly distributed concentration levels, Table 3). The variables used in the calibrations were the chromatographic peak area of 2-, 3-, and 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 3-tert-butyl-4-hydroxyanisol, 2,6-di-tert-butyl-4-methylphenol, 1-naphthol, and diphenylamine in the extracted ion chromatogram for *m*/*z* 135, 191, 165, 205, 144, and 169, respectively. All calibrations showed good linear behavior, with values of the coefficient of determination (R^2) above 0.99 (Table 3). The intercept included zero in all cases, and the models generated did not show a lack of fit.

For the set of compounds, repeatability (n=10) was evaluated at a level corresponding to an S/N ratio of approximately 3. Repeatability was satisfactory, with an RSD equal or less than 5.3%.

The detection limits (DLs) were calculated using the following equation:

$$\mathrm{DL} = \frac{3.3\sigma}{S}$$

where σ is the standard deviation of peak response for ten replicates (n=10) corresponding to an S/N ratio of approximately 3, S is the slope of the calibration curve, and 3.3 is Student's t factor for n−1 degrees of freedom (99% probability). DLs as low as 0.57 μg/L for 2-*tert*-butylphenol and 4.7 μg/L for 2,6-di-*tert*-butylphenol were obtained. The DLs of the other analytes were similar, except for 1-naphthol (1.28 mg/L) which has a high boiling point.

The quantitation limits (QLs) were calculated using the following equation:

$$\mathrm{QL} = \frac{10\sigma}{S}$$

The results for precision, detection, and quantitation limits are summarized in Table 3.

Determination of antioxidants in lubricant oils

Matrix effect

In order to check the possible existence of a matrix effect in the analysis of the oil samples, oil 1 (20W50), oil 2

Table 3 Figures of merit for the HS–PTV–GC–MS method

Compounds	Linearity		Precision RSD (%)	Detection limit (μg/L)	Quantitation limit (μg/L)
	Concentration (mg/L)	R^2			
2-*Tert*-butylphenol	0.0290–1.00	0.9919	2.7	0.57	1.7
3- and 4-*tert*-butylphenol	0.0138–3.90	0.9946	4.7	9.0	27
2,6-Di-*tert*-butylphenol	0.102–20.0	0.9900	3.4	4.7	14
3-*Tert*-butyl-4-hydroxyanisole (BHA)	0.503–8.11	0.9941	3.1	11	33
2,6-Di-*tert*-butyl-methylphenol (BHT)	0.0678–10.0	0.9947	4.2	5.8	18
1-Naphthol	8.87–88.7	0.9916	3.0	1,282	3,884
Diphenylamine (DPA)	3.01–40.6	0.9914	5.3	93	283

(5W40), oil 3 (10W40), and two used oil samples (oils 10–11) were spiked with a solution of the compounds studied. Further, these oils were measured without spiking. If no matrix effect occurs, the sum of the signal corresponding to the unspiked oil and the spiked oil 1 (which did not contain any of the analytes studied) should provide the same area response as the corresponding spiked oil.

As shown in Fig. 3, for the unused oil samples, no matrix effect was observed, and it allows the prediction of the analytes using the calibration in oil 1. The sum of the two individual areas (oil 2 and spiked oil 1) did not present any trend, and positive and negative values with respect to the spiked oil 2 were obtained. In all cases, the differences were lower than 15%. However, the used oil samples showed a matrix effect, and standard additions method is required as quantification technique. These results were confirmed by experiments with different concentrations.

New oils

The detection of the studied antioxidant was performed in eight new oils (oil 2–9) with scan data acquisition mode. 3- and 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 2,6-di-*tert*-

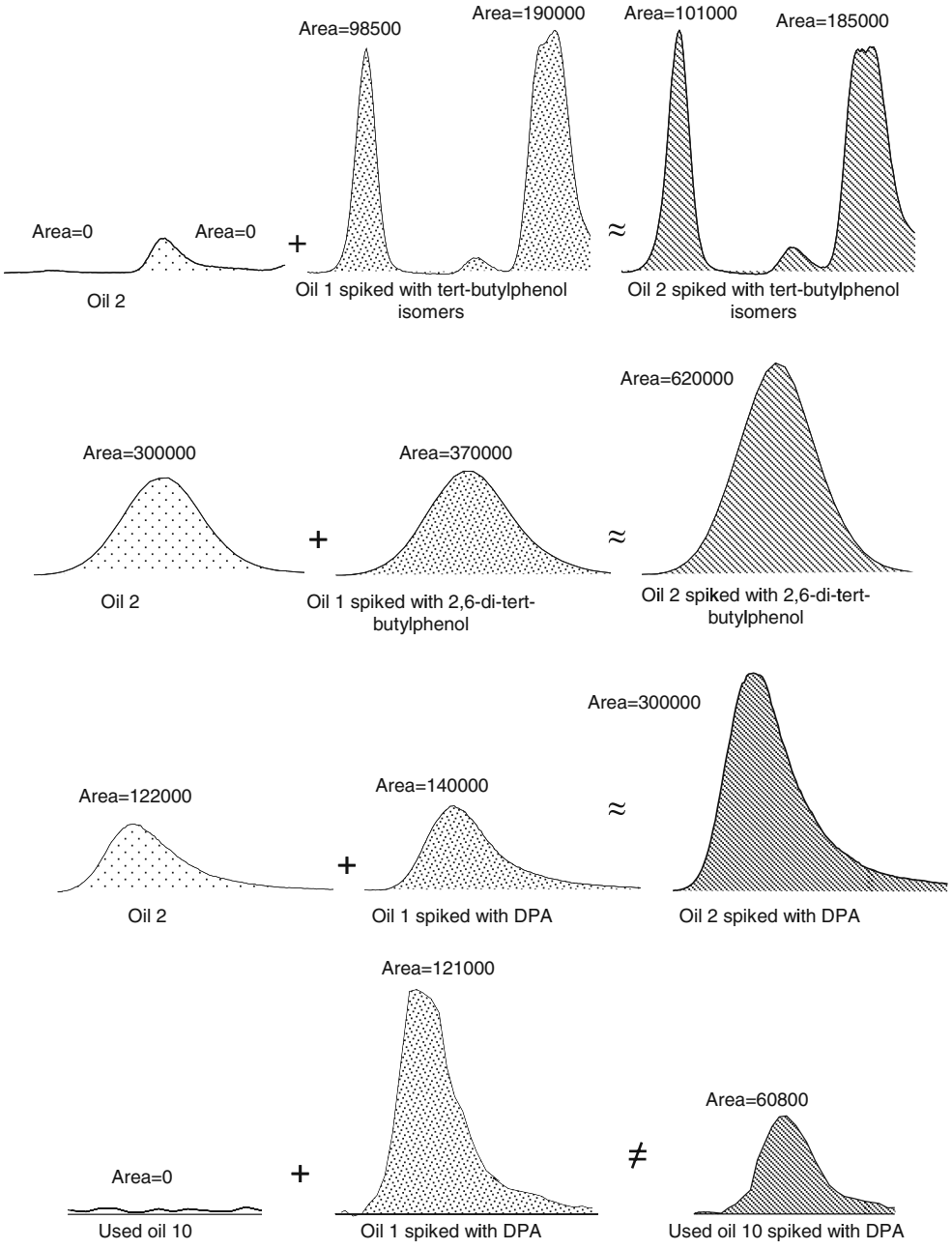

Fig. 3 Matrix effect. The peak areas of the unspiked oil sample 2 and the spiked oil 1 are summed and coincide with the peak area of the spiked oil sample 2. The concentrations were 0.416, 1.76, 8.02, 4.33, 4.10, 16.1, and 21.7 mg/L for 2-, 3-, and 4-*tert*-butylphenol, di-*tert*-butylphenol, BHA, BHT, 1-naphthol, and DPA, respectively. For the used oil 10, the areas do not coincide, and therefore matrix effect has to be assumed

butyl-4-methylphenol, and diphenylamine were found in the samples (see Fig. 4). Prediction was carried out using an external standard with the calibration for oil 1 previously shown. SIM data acquisition mode was used. The concentrations obtained and their confidence intervals (95% probability and 16 degrees of freedom) are shown in Table 4. To check the possibilities of the methodology for those compounds that were not detected or were below quantification limits, the samples were spiked with them. Table 5 shows the recovery rates for two oil samples (oils 2 and 3). They ranged from 80% to 109%, which can be considered acceptable. Similar results were obtained for all samples. These results confirm the applicability of the proposed methodology for the quantification of all the studied antioxidant in new oil samples.

This methodology was also applied in oil 9 which corresponds to a sample from a tractor engine, and matrix effect was found. Table 4 shows the results obtained when standard additions method was employed for quantification. The concentrations found were always higher than those in automobile engines.

Used oil samples

By the time the antioxidants are consumed, the engine oil reaches the end of its useful lifetime. In the used oils, only two

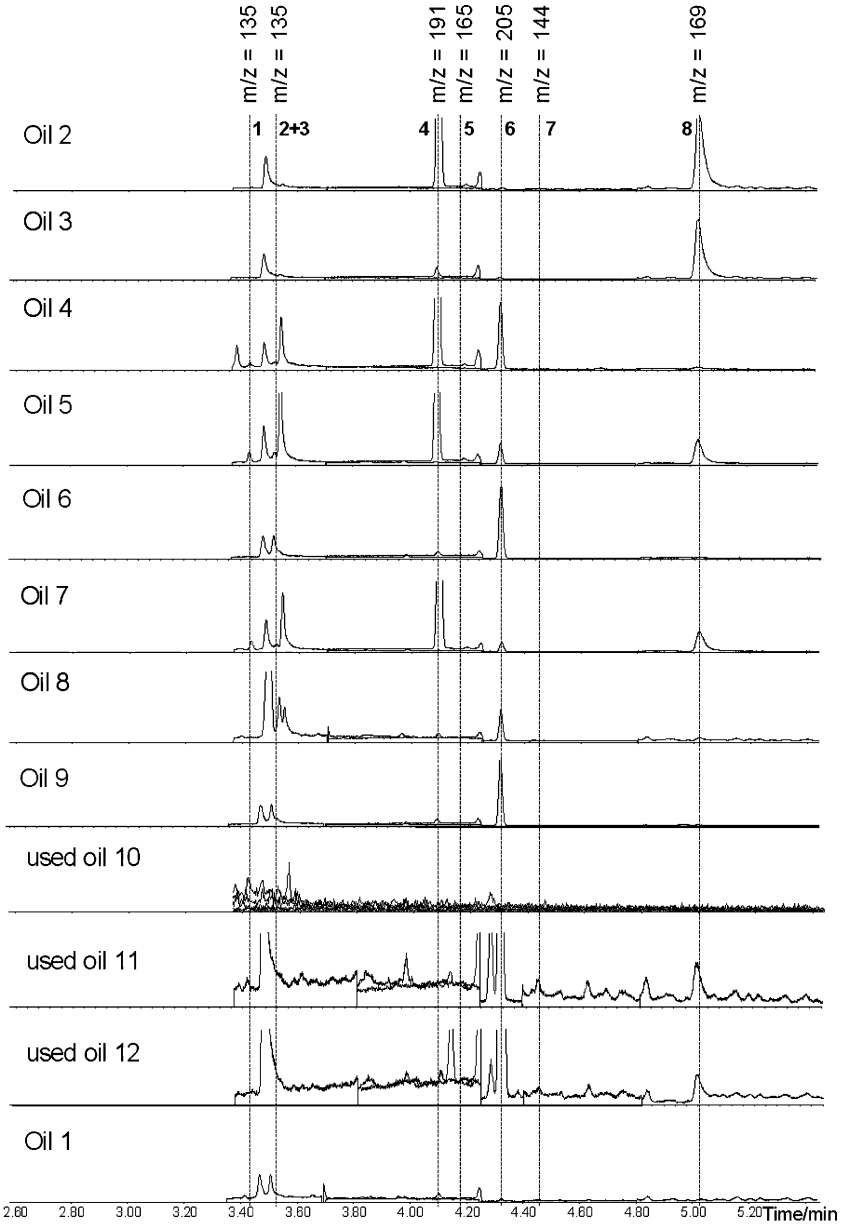

Fig. 4 Extracted ion chromatograms of the new oil samples (oils 2–9), used oil samples (used oils 10–12), and the oil used for the calibration in SIM mode. *Dashed lines* indicate the retention times of 2-*tert*-butylphenol (1), 3- and 4-*tert*-butylphenol (2+3), di-*tert*-butylphenol (4), BHA (5), BHT (6), 1-naphthol (7), and DPA (8). This figure is conclusive with the results given in Table 4

Table 4 Concentration (mg/L) and confidence intervals (95% probability) for the eight new oils and the three used oil samples

Sample	Compounds			
	3- and 4-*tert*-butylphenol	2,6-di-*tert*-butylphenol	BHT	DPA
Oil 2	–	8.0±0.3	< QL	22±2
Oil 3	–	< QL	< QL	18±1
Oil 4	–	8.1±0.4	1.80±0.07	< QL
Oil 5	–	8.4±0.4	0.48±0.07	6.9±0.7
Oil 6	< QL	< QL	1.9±0.2	–
Oil 7	–	4.1±0.2	0.23±0.04	5.4±0.6
Oil 8	< QL	< QL	0.60±0.06	–
Oil 9[a]	–	119±8 (0–100)[b]	18±3 (0–12.0)[b]	45±5 (0–54.0)[b]
used oil 10[a]	–	–	–	–
used oil 11[a]	–	–	39±2 (0–78.0)[b]	17±2 (0–195)[b]
used oil 12[a]	–	–	47±4 (0–78.0)[b]	7±1 (0–195)[b]

Oil 9 corresponds to a tractor engine

[a] Standard addition method was used for quantification

[b] Standard addition concentration range in brackets

antioxidant were detected: 2,6-di-*tert*-butyl-4-methylphenol and diphenylamine (Fig. 4). The concentration range for the set of standard additions (five concentration levels uniformly distributed for each compound) is shown in Table 4. In all cases, calibrations devoid of lack of fit were obtained, with R^2 coefficients equal or greater than 0.99. The concentrations obtained and their confidence interval (95% probability and 13 degrees of freedom) are shown in Table 4. In addition, these samples were also spiked with those antioxidants which were not detected or were below quantification limits as previously described. The recovery rates for the used oils range from 89% to 107% and were similar in all used oils, which is acceptable (Table 5).

The calibration slopes obtained with the standard addition method are different in the three used oils, which means that it is not possible to predict antioxidants in this type of oils using the same strategy described for new ones.

Used oil 10 corresponds to new oil 8, and it was removed from the car at 10,000 km. As one can see in Table 4 and in Fig. 4, the total depletion of BHT occurred in this oil. This was the only determined antioxidant in new oil 8.

Conclusions

The proposed method has been successfully applied in new and used lubricant oil samples.

The use of headspace sampling has the advantage that no prior treatment of the sample is required, thus minimizing the creation of analytical artifacts and the errors associated with this step of the analytical process.

Solvent-vent injection mode removes most of the solvent and lightest volatile compounds in the sample. The use of

Table 5 Accuracy of the method

Compounds	New oils			Used oils			
	Added (mg/L)	Recovery rate (%)		Standard addition (mg/L)	Added (mg/L)	Recovery rate (%)	
		Oil 2 (5 W40)	Oil 3 (10 W40)			Used oil 10	Used oil 11
2-tert-butylphenol	0.416	109	104	4.40–17.6	4.40	107	90
3- and 4-tert-butylphenol	1.76	89	103	7.23–29.0	7.23	99	89
2,6-di-tert-butylphenol	–	–	96	4.43–17.7	4.43	105	90
3-tert-butyl-4-hydroxyanisole (BHA)	4.33	102	99	87.0–351	87.0	93	96
2,6-di-tert-butyl-methylphenol (BHT)	4.10	80	90	19.5–78.0	19.5	106	–
1-naphthol	16.1	86	103	212–584	212	92	94
diphenylamine (DPA)	–	–	–	48.0–195	48.0	93	–

Samples were spiked with those antioxidants which were not detected or were below quantification limits

Tenax-TA® as packing material in the liner allows the analyte of interest to be retained during the venting process. This enrichment technique has the advantage of simplicity over other pre-concentration alternatives such as purge and trap.

The quantification of antioxidants in new oils was carried out using an external standard, from calibration in one of the oils which does not contain any of the studied compounds. This means that both time and costs can be reduced since it is not necessary to generate individual calibration models for each type of new oil. However, standard additions method will be required for quantification of analytes in the used oils due to the matrix effect.

The HS–PTV–fastGC–MS method revealed good precision and accuracy with detection limits for most of the compounds at the microgram per liter level.

It should be stressed that all the studied antioxidants have high boiling points which means that headspace analysis could not be, a priori, a suitable sampling technique for the analysis. However, the use of solvent vent (Tenax-TA® liner) in the PTV and SIM detection mode in the MS provided satisfactory results beside the benefits of using HS.

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