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# Optimization of parameters for the supercritical fluid extraction in the determination of N-nitrosamines in rubbers

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**Abstract**

The study of the possibilities of supercritical fluid extraction (SFE) with N-nitrosamines in rubbers has been carried out. Home-made materials fortified with several N-nitrosamines were prepared in order to optimize the SFE parameters. A Plackett–Burman design was employed to evaluate the influence of those parameters to be controlled in SFE, such as pressure, temperature, static and dynamic time, restrictor temperature and volume of modifier while $CO_2$ was used as the extraction fluid. An extra central composite design for the main factors (according to the previously obtained results) was also developed in order to refine the best supercritical conditions for the extraction of N-nitrosamines from rubbers. Gas chromatography with a nitrogen and phosphorus sensitive detector was used to achieve sensitivity and limits of detection for the concentrations expected in plastic materials. The proposed analytical method has shown to be useful in the determination of N-nitrosamines even for complex matrices. 

*Keywords:* Chemometrics; Rubbers; Supercritical fluid extraction; Nitrosamines

## 1. Introduction

Magee and Barnes [1] first indicated the carcinogenicity of N-nitrosamines in 1956. These compounds are potentially mutagenic and carcinogenic for animals and humans even at low concentrations [2]. N-Nitrosamines are formed from different precursors and under a wide variety of conditions. They can be formed by the reaction of secondary amines with nitrosating agents, such as nitrite or nitrate [3]. Volatile N-nitrosamines are found in drinking water [4], drug formulations [5,6], foods [3,7–10], tobacco [11–14] and other materials. In polymeric materials, such as rubbers [15–19], these compounds are present when the accelerators and stabilizers used in the vulcanization process are alkylamines derivatives [18].

Determination of volatile N-nitrosamines has been usually carried out by gas chromatography (GC) using thermal energy analysis (TEA) [3–6,8–19], although nitrogen–phosphorus detection (NPD or FTD) has also been used for the determination of N-nitrosamines with good results [7,20]. Supercritical fluid extraction (SFE) was shown to be useful in the selective removal of analytes in different types of samples. Moreover, SFE minimizes sample handling, provides fairly clean extracts, expedites sample preparation and reduces the use of environmentally toxic solvents [21,22]. Examples of SFE applications include, semivolatile compounds [23], polychlorinated biphenyls and polycyclic aromatic hydrocarbons from environmental samples [24], phthalates in

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poly(vinyl chloride) [25] and aromatic amines in finger-paints [26]. The use of SFE in removing N-nitrosamines from different samples includes food [8–10], tobacco [12–14], etc.

The use of a formal approach is necessary to study new systems where several factors interact and more information is obtained with few runs by varying several factors at once [27,28]. For this reason, factorial designs are interesting in SFE applications and have been used for different samples [26,29–34]. In this case, a Plackett–Burman design was used to study the influence of several parameters on SFE in terms of recovery. An extra central composite design was also developed to define the response surface as a function of the significant parameters obtained from the previous one and to choose the final optimal conditions for SFE of N-nitrosamines.

The main goal of this approach in the study of SFE of N-nitrosamines was to elucidate the mechanisms involved in the extraction process in supercritical conditions, as well as the establishment of a fast and useful method to determine these analytes in rubber products.

## 2. Experimental

### 2.1. Materials and chemicals

Four N-nitrosamines were chosen among those normally found in rubber products, two of them aliphatic [N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) and two alicyclic (N-nitrosopyrrolidine (NPYR) and N-nitrosopiperidine (NPIP)]. All N-nitrosamines were obtained from Sigma (St. Louis, MO, USA). Stock solutions (3000 μg/g) of each one were prepared in methylene chloride (analytical grade) purchased from Normapur (Prolabo, Barcelona, Spain). Home-made probes were prepared using polyisoprene (Revultex® Lan 960) as polymer matrix and a solution of cyclohexylamine in ethanol as coagulant, both provided by Nuky Baby (Alicante, Spain). The probes were made by pouring alternating thin layers of liquid polymer and coagulant solution into a 6-cm diameter aluminium vessel. After the coagulant solution layer, the adequate quantity of the stock solutions of each N-nitrosamine was added. This procedure was repeated twice, finishing with a liquid polymer and coagulant solution layer. Finally, the probes were cured at 60 °C for 7 h, resulting in a final concentration of 100 μg/g for each N-nitrosamine. Then, the probes were cut in small pieces of 2×2 mm to be used later.

### 2.2. Supercritical fluid extraction

SFE was performed (off-line mode) using an ISCO model SFX-220 extraction system (ISCO, Lincoln, NE, USA) consisting of an SFX-220 extractor, a SFX-200 controller and a 100DX-syringe pump. Supercritical-grade $CO_2$ was obtained from Abelló Linde (Valencia, Spain). Then, 0.20 g of each probe were introduced in a stainless steel cartridge (internal volume, 2.5 ml). When necessary, the modifier (methanol) was added directly to the cartridge before extraction, with a small amount of quartz wool, which helps to minimize the dead volume of the

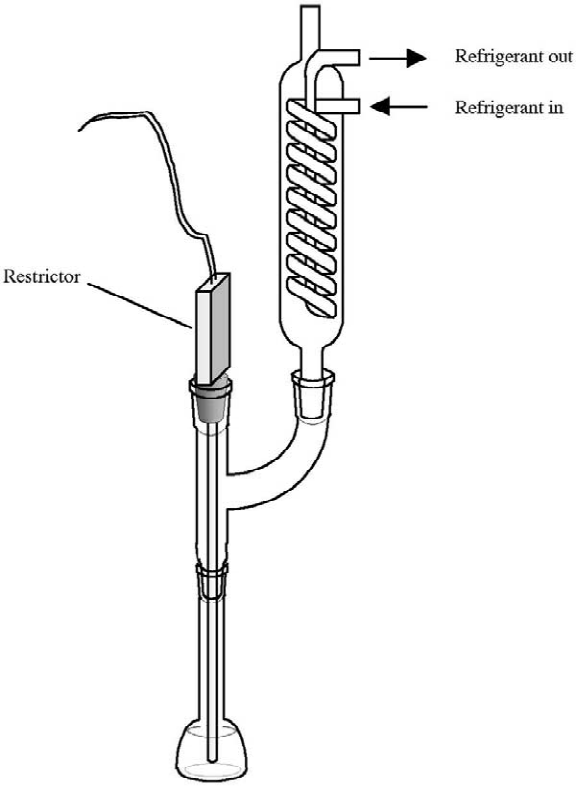


Fig. 1. Liquid trap for collection in off-line SFE.

Table 1
Codification of factors

| Levels | *P* (MPa) | *T* (°C) | *S* (min) | *D* (min) | *R* (°C) | *M* (μl) |
|---|---|---|---|---|---|---|
| −2 | 13.8 | 50 | 2.0 | 2.0 | 150 | 0 |
| −1.68179 | 17.1 | 56 | 3.0 | 3.0 | 156 | 8 |
| −1 | 24.1 | 68 | 5.2 | 5.2 | 169 | 25 |
| 0 | 34.5 | 85 | 8.5 | 8.5 | 188 | 50 |
| 1 | 44.8 | 102 | 11.8 | 11.8 | 206 | 75 |
| 1.68179 | 51.9 | 114 | 14.0 | 14.0 | 219 | 92 |
| 2 | 55.2 | 120 | 15.0 | 15.0 | 225 | 100 |

cartridge. The collection system was a capillary restrictor coaxially heated. In order to trap the extracted analytes, the outlet of the restrictor was introduced in a liquid trap consisting of a piece of glass with two inlets, one to introduce the restrictor and the other one to be coupled to a Dimroth glass condenser, and one outlet to be coupled to a 10-ml volumetric flask with 8 ml of methylene chloride (Fig. 1). All extractions were carried out in the static/dynamic mode, with the use of the selected static and dynamic extraction times while iced-water from a reservoir was circulated along the Dimroth glass condenser in order to avoid the loss of analytes. Finally, the extract was diluted to 10 ml with methylene chloride.

### 2.3. Gas chromatography

Analysis of extracts was carried out by using a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) equipped with a Shimadzu AOC-20i auto-injector, a TR-WAX.DB capillary column (30 m× 0.25 mm I.D. and 0.2 μm film thickness) (Teknokroma, Barcelona, Spain), a split–splitless injector and a NPD system. Helium was used as the carrier gas, with a linear velocity of 65 cm/s and a head pressure of 165 kPa. The injector temperature was 225 °C and the detector temperature was 300 °C with a 50-pA current. Column temperature was programmed in three steps from 50 °C (hold 5 min) to 95 °C (hold 0 min) at 5 °C/min, to 120 °C (hold 0 min) at 20 °C/min, to 210 °C (hold 0 min) at 5 °C/min. A 2-μl sample was injected in the splitless mode (1.5 min splitless time). Quantification of each N-nitrosamine was performed by comparing their chromatographic peak areas for sample extracts with those of standards in the same concentration range.

Table 2
List of experiments in the Plackett–Burman design

| Experiment | *P* | *T* | *S* | *D* | *R* | *M* |
|---|---|---|---|---|---|---|
| P–B-1 | 1 | −1 | 1 | −1 | −1 | −1 |
| P–B-2 | 1 | 1 | −1 | 1 | −1 | −1 |
| P–B-3 | −1 | 1 | 1 | −1 | 1 | −1 |
| P–B-4 | 1 | −1 | 1 | 1 | −1 | 1 |
| P–B-5 | 1 | 1 | −1 | 1 | 1 | −1 |
| P–B-6 | 1 | 1 | 1 | −1 | 1 | 1 |
| P–B-7 | −1 | 1 | 1 | 1 | −1 | 1 |
| P–B-8 | −1 | −1 | 1 | 1 | 1 | −1 |
| P–B-9 | −1 | −1 | −1 | 1 | 1 | 1 |
| P–B-10 | 1 | −1 | −1 | −1 | 1 | 1 |
| P–B-11 | −1 | 1 | −1 | −1 | −1 | 1 |
| P–B-12 | −1 | −1 | −1 | −1 | −1 | −1 |
| P–B-Central | 0 | 0 | 0 | 0 | 0 | 0 |

### 2.4. Design of experiments

A fractional factorial Plackett–Burman design for the fortified probes was carried out to distinguish the significant parameters affecting the supercritical process. The results of this design were used to plan a subsequent higher order design (central composite design), which was performed with the same procedure. All statistical calculations were developed with Statgraphics Plus 4.0 for Windows by Statistical Graphics Corp.

## 3. Results and discussion

The initial parameters to be included in the study of SFE for N-nitrosamines in rubber products were $CO_2$ pressure (*P*), extraction temperature (*T*), static and dynamic extraction time (*S* and *D*), restrictor temperature (*R*) and volume of modifier (*M*) (metha-

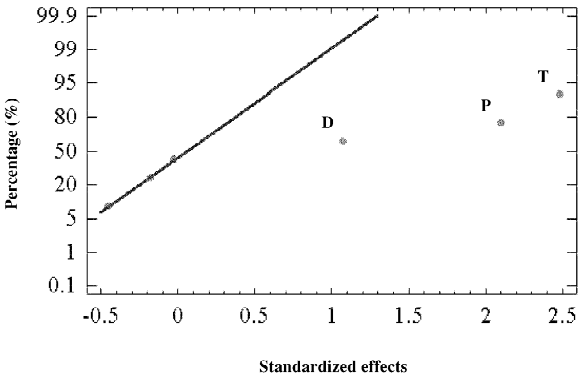


Fig. 2. Normal probabilistic plot of cumulative probability density function vs. calculated effects for $Y = (\%\ W)^{0.15}$.

nol). The low and high values for each parameter were selected according to the experimental limitations and codified to be −2 and +2 from the centre of the design (0 for each parameter). With the use of this transformation, every parameter is independent of the measure units. Table 1 lists the values of each factor and their corresponding codified value. The effect of the different variables affecting the SFE of N-nitrosamines was studied by a Plackett–Burman design with two levels (−1 and +1) for the six factors previously indicated. This design requires 12 experiments, performed randomized. It is assumed that the main factors are those significant for the process [27,28]. An extra experiment was included to have an estimation of the response in the centre of the design. The evaluated responses were mass loss (% $W$), which includes all those coextracted components, mainly cyclohexylamine, and recovery (% $R$) for each N-nitrosamine. The conditions of SFE in this design are presented in Table 2.

In order to stabilize the variance of results, an appropriate power transformation ($Y = y^{\lambda}$) of either response ($y = \%\ W$ or $y = \%\ R$) was carried out before the analysis of results. A suitable transformation of the response is recommended when large differences between the values of response are found [28]. The best transformation is achieved when the sum of squares of residuals is the lowest as a function of the exponent $\lambda$. The optimum transformation for mass loss [$Y = (\%\ W)^{\lambda}$] was obtained for $\lambda = 0.15$. The significant factors were identified

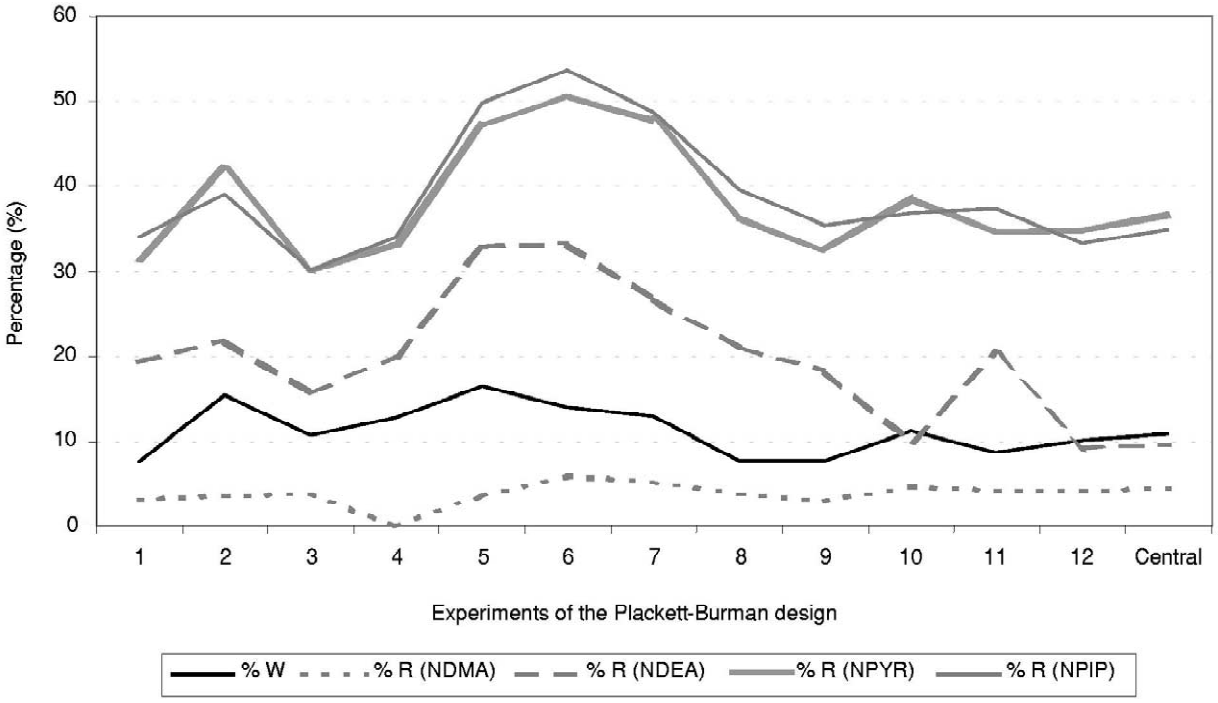


Fig. 3. Results of the Plackett–Burman design.

Table 3
List of experiments in the central composite design

| Experiment | *P* | *T* | *D* |
|---|---|---|---|
| CC-1 | −1 | −1 | −1 |
| CC-2 | 1 | −1 | −1 |
| CC-3 | −1 | 1 | −1 |
| CC-4 | 1 | 1 | −1 |
| CC-5 | −1 | −1 | 1 |
| CC-6 | 1 | −1 | 1 |
| CC-7 | −1 | 1 | 1 |
| CC-8 | 1 | 1 | 1 |
| CC-9 | −1.68179 | 0 | 0 |
| CC-10 | 1.68179 | 0 | 0 |
| CC-11 | 0 | −1.68179 | 0 |
| CC-12 | 0 | 1.68179 | 0 |
| CC-13 | 0 | 0 | −1.68179 |
| CC-14 | 0 | 0 | 1.68179 |
| CC-Central | 0 | 0 | 0 |

by using normal probabilistic plots, where all negligible factors are expected to be located along a straight line. By contrast, points that fall well off the line would suggest the existence of a significant influence [28]. Fig. 2 shows the normal probabilistic plot for $Y = (\%\ W)^{0.15}$. The figure shows three significant factors, *P*, *T* and *D*, with positive effects, that means % *W* can be improved by an increase of these factors, although this consequence should be taken with caution because interactions between factors are not considered in a Plackett–Burman design.

A similar analysis was carried out for the recovery of each N-nitrosamine resulting in no significant factors for any of them. Nevertheless, the similar relation between % *W* and % *R* for all N-nitrosamines (see Fig. 3) led to choose *P*, *T* and *D* as those factors to be included in the next higher order design. It can be observed that, although the maximum recovery is lower than 55% (NPIP), the higher the N-nitrosamine molecular mass the higher the recovery, especially in the case of alicyclic compounds. This can be explained according to a decrease in the polarity of these analytes with increasing molecular mass. Although a polarity modifier was added, supercritical $CO_2$ is basically nonpolar showing a higher affinity for low-polarity compounds [21,22].

Therefore, a central composite design was carried

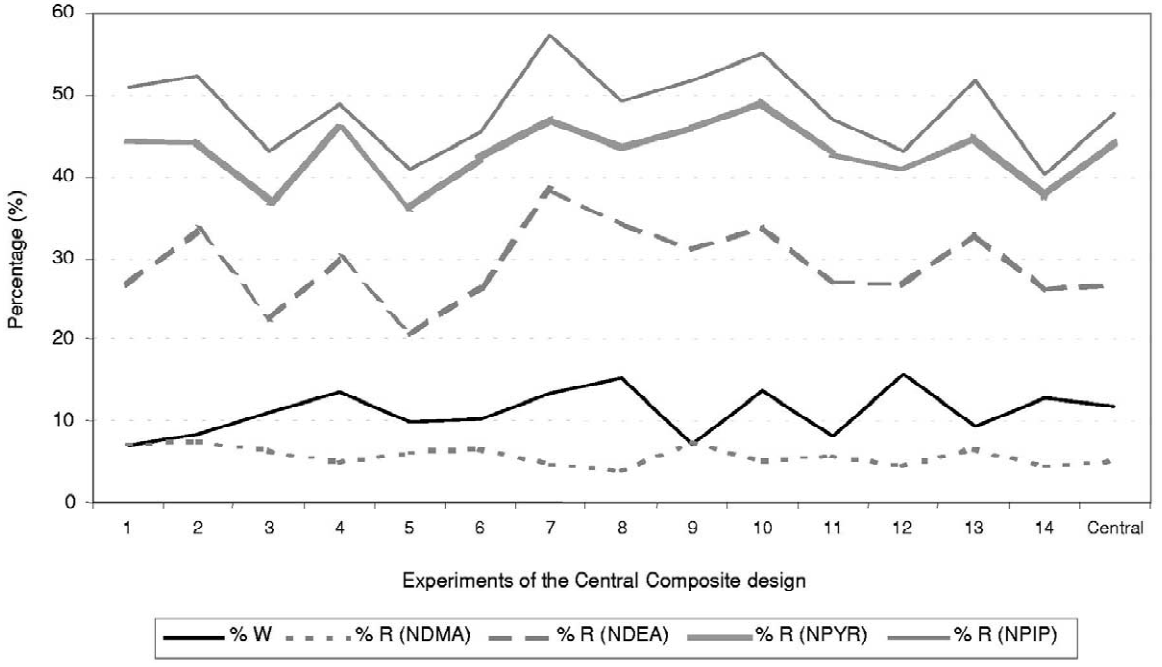


Fig. 4. Results of the central composite design.

out for those three factors in order to refine the optimum supercritical fluid conditions for the extraction of N-nitrosamines. This design was constructed by the addition of a full factorial design $2^3$ and a star design, with a central experiment and others located at an adequate distance from the centre of the design. This distance was selected to be equal to $-1.68179$ and $+1.68179$ to assure the rotatability condition of the central composite design. Rotatability makes the uncertainty of the design only dependent on the distance to the centre of the working range [28]. The required experiments are presented in Table 3, where previous factors considered as not significant (*S*, *R* and *M*), are maintained at their central value (0). The obtained results are presented in Fig. 4. Attending to the mathematical model assumed for this design, the predicted recoveries for each N-nitrosamine were represented as a function of the corresponding decodified values of *P* and *T* for several values of *D*, resulting in response surfaces for each N-nitrosamine. Fig. 5 shows an example of these response surfaces for NDEA. From that figure, it can be seen that the behaviour changes with the value of *D*, showing two possibilities to get the maximum recovery. When *D* is low, the best result is found for a high *P* and a low *T* (option A). However, when *D* is high, a good recovery is obtained for a low *P* and a high *T* (option B). This is a consequence of the interactions between these three factors, one positive (*T*–*D*) and two negatives (*P*–*T* and *P*–*D*), as it can be concluded from the statistical analysis of the results. That means that an increase of *T* and/or *D* produces a synergistic effect on the other factor, resulting in a higher improvement of the recoveries, while the modifications of *P* have an opposite effect on the other two factors. The existence of two options to obtain the best recoveries can be explained attending to the mechanisms that influence the SFE process. Therefore, option A could be related to a process where solubility of analytes in supercritical $CO_2$ is the main step in SFE, because the polarity of $CO_2$ is higher when *P* increases and *T* decreases. Moreover, as *D* is longer the added modifier is evacuated of the extraction cell contributing to the decrease of polarity. By contrast, in option B, the main step in the supercritical process should be the diffusion of the analyte through the matrix, which is higher at high

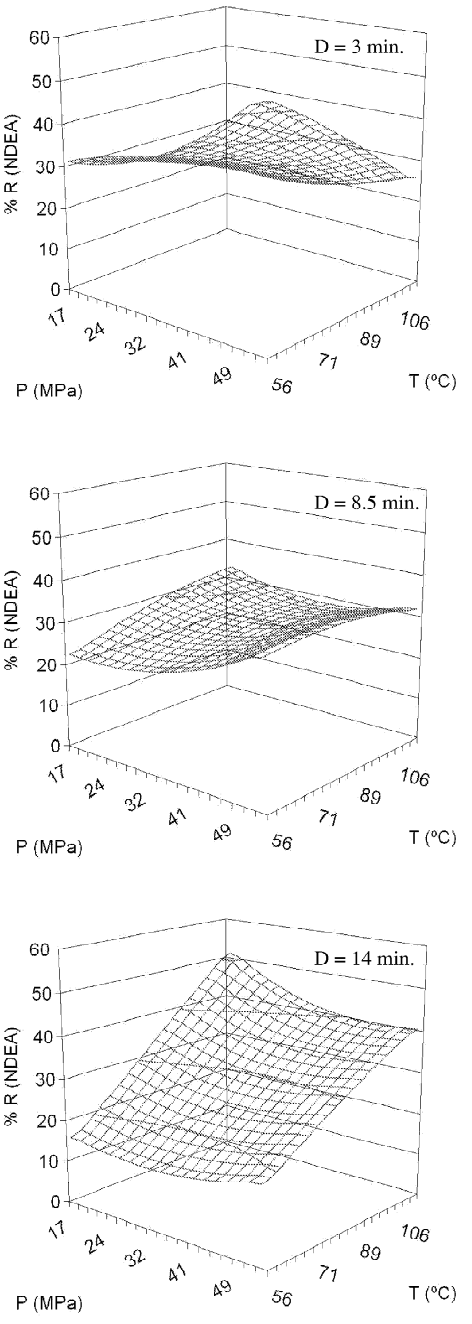


Fig. 5. Example of response surfaces for NDEA from the central composite design. Recovery of NDEA vs. pressure and temperature for dynamic extraction time equal to 3, 8.5 and 14 min.

temperatures and low pressures. In this case, longer dynamic extraction times are convenient in order to remove the analytes from the matrix–$CO_2$ interface and increasing the concentration gradient of analyte along the matrix and, in consequence, increasing the diffusion rate [21,22,24]. Therefore, recoveries lower than 58% could be explained as it is not possible to obtain a good solubility and a good diffusion at the same time. Moreover, an extraction in two stages, one with maximum solubility and the other one with maximum diffusion, was rejected because the use of SFE modifies considerably the features of the sample, making impossible the application of the previous conclusions to the next SFE. A choice between option A and B is therefore necessary in order to continue the optimization. Option A was selected because of the quantity of coextracted analytes is considerably lower (mainly cyclohexylamine), making the SFE more selective and permitting a better integration of the NDMA peak, as it can be seen from Fig. 6.

As 50 °C and 3 min were low values, only $P$ was increased up to 55.2 MPa. The results showed that no significant improvement on recoveries was found, being the best recoveries obtained 8, 38, 49 and 58% for NDMA, NDEA, NPYR and NPIP, respectively, with relative standard deviations between 2 and 9%. Thus, the optimum conditions for the SFE of N-nitrosamines from rubber products were established to be $P=55.2$ MPa, $T=50$ °C, $S=8.5$ min, $D=3$ min, $R=185$ °C, and $M=50$ μl of methanol.

## 4. Conclusions

SFE has demonstrated that is a good extraction technique for N-nitrosamines in rubber products that permits fast analysis with a reduction in solvent waste, time and manipulation. Although recoveries are not good, specially for the smaller N-nitrosamines, SFE could be considered a useful tool to determine these analytes, considering that it provides quite clean extracts in one step, due to its selectivity. Moreover, the high toxicity of N-nitrosamines makes their presence in a sample unacceptable at any level. For this reason, SFE and GC–NPD combination can permit a first quick method in order to decide if a rubber sample should be rejected if presence of N-nitrosamines is detected.

On the other hand, factorial designs can be

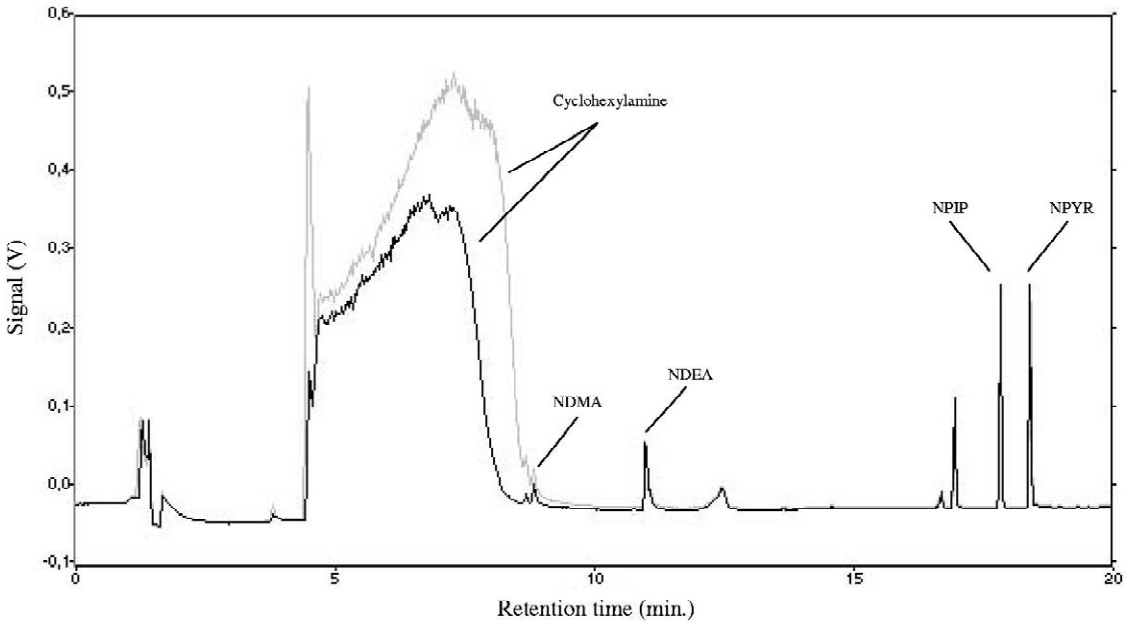


Fig. 6. Comparison between the chromatograms obtained with the option A (black) and option B (grey) conditions.

considered as an effective approach to study the influence of the parameters affecting SFE, and they permit the acquisition of more robust results with a reduced number of experiments when compared to the classical one-to-one parameter approach. So, a better understanding of the mechanisms taking place in the supercritical extraction process is possible, providing a larger base to improve the analytical results.

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