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Experimental design approach for the optimisation of pressurised fluid extraction of additives from polyethylene films

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Abstract

A pressurised fluid extraction (PFE) and normal-phase-high performance liquid chromatography (NP-HPLC) method is proposed for the determination of additives in polyethylene films. The study of PFE variables was performed using a Plackett–Burman (PB) experimental design for screening and a central composite design (CCD) for optimising the main variables obtained from the Pareto charts. The studied variables were: temperature, time, cyclohexane (CHx) and tetrahydrofuran (THF) as modifiers, flush volume and extraction cycles, and an isopropanol:CHx (92.5:7.5) mixture twice at 105 °C for 15 min were the final conditions selected. The additives in the PFE extracts were separated by NP-HPLC using a silica column and a gradient *n*-hexane:dichloromethane:acetonitrile mobile phase. Additive solubility is higher in normal-phase solvents; thus, their separation can be carried out at room temperature. Finally, the method was applied to determine additives in several polyethylene films.

Keywords: Pressurised fluid extraction; Polyethylene; Additives; Experimental design; Normal-phase-HPLC

1. Introduction

Additives such as light stabilisers, antioxidants, UV-protectors and others [1] are required in polyethylene films in order to improve and preserve polymer properties. Additive content in polymers must be known for quality and regulatory reasons. Traditionally, their extraction from polymers has been carried out by Soxhlet extraction or by boiling under reflux, and more recently by microwave assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurised fluid extraction (PFE) [2,3]. Also, the use of two on-line high performance liquid chromatography (HPLC) columns connected in series, one for size-exclusion chromatography which separates additives from the polymer matrix and a second normal-phase (silica) column which separates additives between them, are proposed for determining additives after polymer dissolution [4]. The use of reverse-phase (RP) HPLC for analysing extracts is more common [5–9] than normal-phase (NP) HPLC [4]. When RP-HPLC is used, the extracts must be evaporated and re-dissolved in an appropriate solvent. Moreover, the slight solubility of additives in reverse phase mobile phase implies temperature control of the column while it is not necessary for normal-phases.

PFE is an extraction procedure that uses organic solvents at high pressures and therefore temperatures above boiling point can be used, resulting in increased efficiency and reduction of extraction times. Analyte diffusion and desorption occur at a faster rate because of the higher temperature used. PFE also allows analytes in pores to be more rapidly extracted than at room temperature and atmospheric pressure. Elevated pressure forces solvent into pores and increases solvent contact with the analytes, prompting them to be extracted more quickly. There are many parameters to optimise in PFE extractions: particle size, extraction solvent, pressure, swelling solvent, temperature, extraction time, flush volume and static cycles [9–11]. The selection of the solvent is the basic step in PFE extraction. Pressurised fluid extraction of additives from polyethylene has been carried out using different solvents, namely isopropanol [8], acetone [9], ethyl acetate or tetrahydrofuran (THF) [12] and mixtures of isopropanol and cyclohexane (CHx) [8,9,13]. Solvents used in Soxhlet extraction tend to dissolve the polymer at the high

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temperatures used in PFE, but at low concentrations, the polymer swells and extraction efficiency increases [8]. Therefore, CHx and THF are usually added to the solvent as modifier. Temperature is another important parameter to optimise because it increases extraction efficiency but too high a temperature can lead to polymer melting. Pressure is not a significant parameter in the extraction of non-volatile compounds, it is only required to maintain the extraction solvent in liquid state at a temperature above the atmospheric boiling point [9]. Samples are usually extracted several times in order to ensure complete extraction of analytes. Consequently, time and extraction cycles are parameters to be optimised.

Two approaches can be used to select the best conditions for PFE extraction: an univariate study where the variables are studied one by one; or an experimental design approach where all the variables are studied at the same time. This allows a reduction in the number of experiments with a complete exploration of the experimental domain to be studied. In the first stage of PFE optimisation, the relative influence of the factors can be established using a Plackett–Burman (PB) experimental design that indicates with minimum experimental effort the most significant variables in complex systems. Once the significant factors have been identified, the curvature of the response surface and the accurate position of the optimum can be evaluated by means of central composite design (CCD).

The variables affecting the PFE of additives in polymers were studied through univariate studies [8–10,13], but the application of a univariate study requires a higher number of experiments compared with experimental design studies for exploring the same experimental domain. The experimental design approach has previously been applied to the optimisation of variables in additive extraction by SFE [14], liquid–liquid extraction [15] and MAE [16]. It has also been used to optimise PFE variables in the extraction of many different analytes, such as pesticides in soils [17], PAH's in soils [18], polyhalogenated dibenzo-*p*-dioxins and benzo-*p*-furans in mineral and environmental matrixes [19], cocaine and benzoylecgonine in coca leaves [20], etc. In the field of polymer additives extraction by PFE, the experimental design approach has only been applied to the optimisation of variables in the extraction of Irganox 1076 in polyethylene granules before and after γ-irradiation [12]. After a screening study, ethyl acetate was chosen as solvent. An experimental design was applied to optimise the temperature and percentage of hexane in the ethyl acetate and 15 min of static extraction gave the highest yield.

The aim of this study was to develop a method to determine 11 additives in polyethylene films. We compared NP-HPLC and RP-HPLC for the separation of the additives. Sample treatment before PFE extraction was studied by means of a Newman–Keuls (NK) test in order to determine the best method for reducing sample size. Some PFE parameters, such as solvent, temperature, time, flush volume and extraction cycles were studied by experimental design, using a Plackett–Burman for screening and a central composite design for determining the optimum values for the significant variables. Finally, the proposed method was applied to the determination of additives in several polyethylene films.

2. Experimental

2.1. Materials and reagents

BHA (3-*tert*-butyl-4-hydroxyanisole), Irganox MD 1024 (2′,3-bis[[3-[3,5-di-*tert*-butyl-4-hydroxyphenyl] propionyl]]propionohydrazide), BMP (2,6-di-*tert*-butyl-4-methyl phenol), Irgafos 126 (bis(2,4-di-*tert*-butylphenyl)pentaerythriol diphosphite), HP 136 (reaction product between 5,7-di-*tert*-butyl-furan-2-one and o-xylene), Irganox 3114 (1,3,5-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione), Tinuvin 328 (2-(2H-benzotriazole-2-yl)-4,6-ditertpentyl phenol), Irganox 1010 (pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate), Irganox 1330 (3,3′,3′,5,5′,5′-hexa-*tert*-butyl-a,a′,a′-(mesitylene-2,4,6-triyl)tri-*p*-cresol), Irganox 1076 (octadecyl-3-(3,5di-*tert*-butyl-4-hydroxyphenyl)-propionate) and Irgafos 168 (tris(2,4-ditert-butylphenyl) phosphite)) were supplied by Ciba-Geigy, Additives Division (Barcelona, Spain). Polyethylene films were supplied by AMCOR flexibles TOBEPAL. The solvents from Merck (Darmstadt, Germany) and Scharlab (Barcelona, Spain) were HPLC grade. Milli-Q (Millipore, Molsheim, France) deionised water was used.

2.2. Sample treatment

The polyethylene films were treated in order to reduce the particle size before introduction in the extraction cell. Samples were cut approximately to 1 cm^2 using scissors, ground with an IKA A10 grinder (IKA Labortechnik, Staufen, Germany) that uses water as coolant for 15 min, and ground with a 6750 Freezer/mill (Spex CertiPrep, NJ, USA) that uses liquid nitrogen to keep the sample at cryogenic temperature for 4 min at a rate of 10 impacts s^{-1}. Ground samples were sieved to obtain a $\leq$1 mm particle size. Approximately 1 g of cut or ground polymer was dispersed in sand to prevent the particles from coalescing during extraction. The mixture was placed in the extraction cell and the cell was completely filled with sand and closed in order to reduce the dead volume and thus minimise the amount of solvent required.

2.3. Pressurized fluid extraction

A pressurised fluid extractor ASE 200 (Dionex, Sunnyvale, CA, USA) with a solvent controller was used in all the extractions. The extractions were performed at 10.3 MPa (1500 psi) using isopropanol as extraction solvent. Swelling solvents such as THF and CHx were tested at concentrations of between 0 and 7.5%. Temperature ranged from 80 to 110 °C where the upper limit was set in order to avoid polymer melting; extraction times ranging from 2 to 22 min and solvent volumes from 50 to 100% of the cell volume were tested. The use of several cycles of static extraction was also studied.

Extracts were made up to the same volume (25 ml) by evaporation under a nitrogen stream or by dilution. Solutions were filtered through a 0.45 μm Nylon syringe filter prior to HPLC analysis.

2.4. HPLC analysis

HPLC analysis were performed with an Agilent 1100 (Hewlett Packard, Palo Alto, CA, USA) series chromatograph equipped with a Variable Wavelength Detector (VWD) and a Quadrupole HP 5989B mass spectrometer with a HP59987A interface for electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI).

A *n*-hexane:dichloromethane:acetonitrile mobile phase at a flow rate of 1.5 ml min^{-1} was used for NP-HPLC. The mobile phase gradient started at 100% of *n*-hexane and was maintained for 3 min, then increased to 25% of dichloromethane in 27 min, and to 100% of dichloromethane in 10 min and maintained for 1 min, finally reaching 90% of dichloromethane and 10% of acetonitrile in 4 min. The temperature column was maintained at 30 °C. A 250 mm × 4.6 mm, 5 μm particle size Nucleosil 120-5 SIL column with a 15 mm × 4.6 mm and a 5 μm particle size Nucleosil 120-5 SIL (Scharlab, S.L., Barcelona, Spain) pre-column were used.

For reverse phase separations, a water:acetonitrile mobile phase was used. The flow rate started at 0.5 ml min^{-1} and was then increased to 1.5 ml min^{-1} in 5 min and maintained for the rest of the analysis. The mobile phase gradient started at 40% of acetonitrile and was maintained for 5 min, and then increased to 60% in 35 min and to 100% of acetonitrile in 35 min, and was finally maintained at 100% of acetonitrile for 5 min. The temperature column was maintained at 50 °C. A 150 mm × 3.9 mm, 4 μm particle size Nova-Pak C18 60 Å column with a 15 mm × 3.9 mm, 4 μm particle size Nova-Pak C18 60 Å pre-column (Waters Corporation, Milford, MA, USA) were used.

Extracts were injected into the HPLC column using a 200 μl sample loop and chromatograms were recorded at 275 nm in both cases.

Mass spectrometry identification was carried out by ESI and APCI in positive mode at a split ratio of 1:100. The dissecant gas was nitrogen at 210 °C and at a flow rate of 20 ml min^{-1}. The auxiliary solvent was methanol:water:acetic acid (80:18:2) with a small amount of Na^+ and Li^+ at a flow rate of 10 μl min^{-1} and capillary voltage was −4150 V in ESI. A source temperature of 300 °C and a capillary and corona voltaje of −4860 and 1540 V, respectively, were used for APCI.

2.5. Data processing

The Statistica 6.1 (2004, StatSoft, Tulsa, USA) program was used for construction of the experimental design matrix and evaluation of the results. A dummy variable was included to complete the 4N-1 variables of PB experimental design and to validate the results. An α-value of 1.5 and two centre points were used in the CCD.

3. Results and discussion

3.1. Separation of additives in liquid extracts

Preliminary experiments showed that Irgafos 168 and Irgafos 126 were oxidised very quickly in solutions. An oxidation study was carried out and results showed that complete oxidation of Irgafos 168 and 126 takes place in 7 days after extraction. Therefore, the extracts were stored 1 week before HPLC analysis and Irgafos 168 and 126 were determined as their oxidation products, as proposed by Dopico-García et al. [15].

A standard solution containing a mixture of the 11 additives was used to study their separation by NP and RP-HPLC. The best separation of the additives obtained by NP- and RP-HPLC is shown in Fig. 1.

The chromatographic peaks were identified by comparing retention times with those obtained by injection of the pure compounds and by mass spectrometry detection. Ionization was performed by ESI for Irganox 1076, Irganox 1010 and Irganox 3114 (the first two required the addition of a sodium salt and the latter required the addition of a lithium salt) and APCI for the rest of additives. HP 136, BMP and BHA could not be detected by MS. Table 1 lists the retention times in normal and RP-HPLC and mass fragment by MS.

The standard solution was processed by the two chromatographic methods ($n = 7$), and the repeatabilities of both methods proved to be statistically equal for most additives (RSD between 3.5 and 13.0%, depending on the compound). The peak area was significantly higher in the normal-phase system for all the compounds, except for MD 1024 and HP 136. The signal increase observed (between 1.1 to 6.1 times) could be due to a solvatochromic effect produced by the normal-phase solvents.

Table 1
Identification conditions for the additives studied

Compound	NP retention time (min)	RP retention time (min)	Mass fragments
Irgafos 168 oxidation product (I 168ox)	3.2	76.9	607.4, 647.6, 663.5 (APCI)
BMP	4.6	37.8	N.D.*
Tinuvin 328 (T 328)	8.2	70.7	352.3 (APCI)
Irgafos 126 oxidation product (I 126ox)	12.5	49.2	605.4, 621.4, 637.4 (APCI)
Irganox 1330 (I 1330)	14.7	73.5	768.7, 770.0 (APCI)
HP 136	20.1	63.0	N.D.*
Irganox 1076 (I 1076)	20.8	78.3	269.2, 351.1, 433.1, 515.2, 553.6 (ESI +Na)
Irganox 3114 (I 3114)	33.4	65.3	787.8 (ESI +Li)
Irganox 1010 (I 1010)	34.7	73.0	803.9, 843.3, 925.4, 1007.4, 1200.6 (ESI +Na)
BHA	36.2	7.0	N.D.*
Irganox MD 1024 (MD 1024)	47.1	33.5	441.3, 479.3, 497.4, 535.5, 553.5 (APCI)

* Not detected.

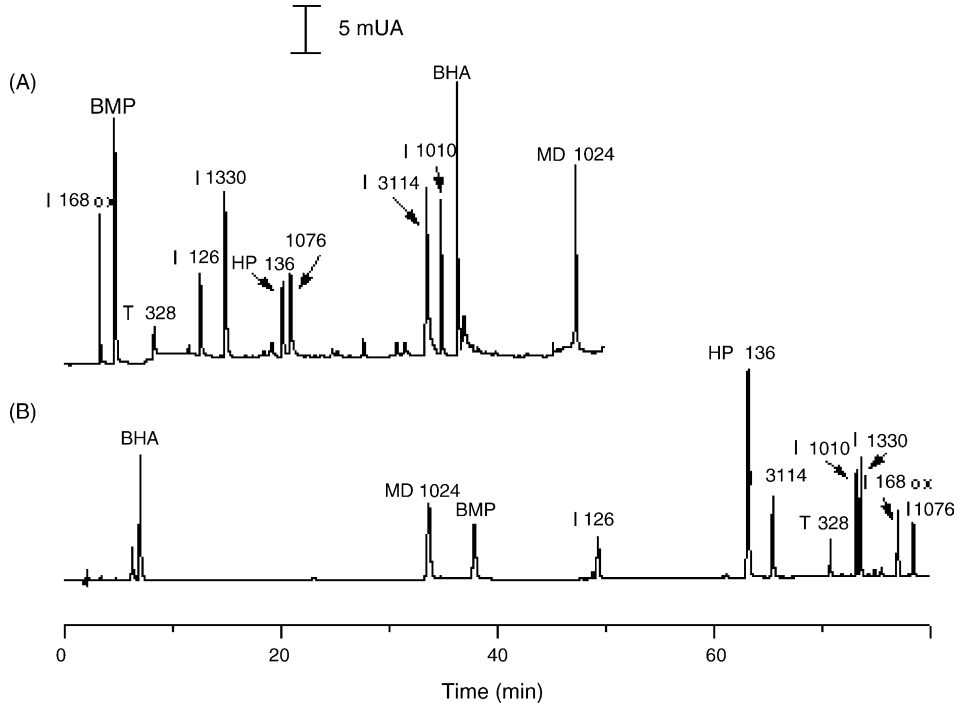

Fig. 1. Chromatograms of an additive mixture recorded at 275 nm: (A) normal and (B) reverse phase HPLC. For chromatographic conditions, see Section 2. Additive names and codes can be found in Table 1.

Although RP-HPLC is normally chosen for the determination of additives in polyethylene [5–9,12–16], the silica column was chosen instead of the C18 column because the separation of the additives was faster and no column temperature control was needed. In addition, the increased sensitivity of this particular NP-HPLC system allows to determine lower concentrations, and thus NP-HPLC was selected for the determination of additives in polyethylene films.

The features of the NP-HPLC method were established after a linearity study using standard solutions of the additives in isopropanol, and the results are listed in Table 2. The linear range was between the quantification limit (calculated by using the noise signal plus 10 times its standard deviation) and 25 μg ml^{-1} (concentration corresponding to the highest level of additives expected to be found in polyethylene film extracts). The correlation coefficient R was higher than 0.996 for all the compounds. The detection limits LOD (calculated by using the noise signal plus three times its standard deviation) found ranged from 0.3 μg ml^{-1} for BHA to 1.1 μg ml^{-1} for Irganox 1010, and the relative standard deviation (obtained at 10 μg ml^{-1} concentration level) was less than 10% in all cases. Additive LOD in polymer (calculated for 1 g of polymer and 25 ml of extract) ranged from 7.5 μg of BHA g^{-1} to 27.5 μg of Irganox 1010 g^{-1}.

Table 2
Features of the NP-HPLC method for additives in an isopropanol solution

Compound	Linear range (mg l^{-1})	R	LOD (mg l^{-1})	RSD[a] (%)
I 168ox	1.1–25	0.996	1.0	8
BMP	0.7–24	0.998	0.6	7
T 328	1.1–25	0.9998	0.7	9
I 126	1.3–25	0.997	0.9	7
I 1330	1.2–24	0.997	1.0	8
HP 136	1.5–25	0.998	0.9	7
I 1076	1.0–25	0.999	1.0	6
I 3114	0.6–25	0.998	0.5	5
I 1010	1.2–24	0.997	1.1	5
BHA	0.4–26	0.999	0.3	10
MD 1024	0.8–25	0.998	0.7	6

[a] $n = 3$.

3.2. Study of sample treatment prior to PFE

The particle size of film samples was reduced before PFE by scissors cutting and by grinding at room and cryogenic temperatures. Seven replicates of each sample treatment were done. Cells were filled with approximately 1 g of cut/ground sample. PFE conditions were 100 °C, 50% of flush volume, one static cycle and 10 min of static time. Swelling solvents were not used for this study. Hartley's, Cochran's and Bartlett's tests were applied in order to check variance homogeneity; p-values less than 0.05 were obtained in all cases (0.03, 0.04 and 0.04 for I 1076, I 1010 and I 168 ox, respectively), indicating statistically significant differences among the variances. According to these results, a multiple comparison test such as NK, used for determining significantly different means and dividing them into subsets, was carried out for each analyte; the results are listed in Table 3.

From the results of NK test, three or two subsets can be established depending on the analyte. In the case of I 1076, three subsets were observed, where the cryogenic grinding method was the best as it provided a mean signal value significantly higher than those yielded by the other methods. In contrast, for

Table 3
Homogeneous subsets of particle size reduction methods obtained by Newman–Keuls test

Method	I 1076; Subsets for $\alpha = 0.01$			I 168 ox; Subsets for $\alpha = 0.01$		I 1010; Subsets for $\alpha = 0.01$	
	1	2	3	1	2	1	2
Room temperature grinding	21581.3			10572.6		11526.9	
Scissors cutting		25721.6			12909.0		24852.2
Cryogenic grinding			29324.7		14076.9		26369.4

$n = 21$.

Table 4
Plackett–Burman design matrix and results of pressurised fluid extraction

Experiment	THF	CHx	Time	Temperature	Flush	Cycles	Dummy	I 168 ox (mAU s g^{-1})	I 1076 (mAU s g^{-1})	I 1010 (mAU s g^{-1})
1	0	0	2	100	50	2	1	13.3	166.7	60.6
2	5	0	2	80	100	1	1	12.4	95.0	54.5
3	0	5	2	80	100	2	−1	36.1	151.6	57.2
4	5	5	2	100	50	1	−1	35.1	57.4	60.1
5	0	0	10	100	100	1	−1	4.7	147.7	61.4
6	5	0	10	80	50	2	−1	31.9	152.2	63.0
7	0	5	10	80	50	1	1	23.7	129.9	63.3
8	5	5	10	100	100	2	1	40.2	182.2	86.7

$n = 2$. THF, percentage of tetrahydrofuran as swelling solvent (%); CHx, percentage of cyclohexane as swelling solvent (%); time, extraction time (min); temperature, extraction temperature (°C); flush, flush volume (percentage of extraction cell volume); cycles, number of static cycles.

I 168 ox and I 1010, sample treatments were grouped into two subsets, one of them including scissors cutting and cryogenic grinding, both providing higher mean values than room temperature grinding. Room temperature grinding gave rise to the lowest analytical signal in all cases. According to the previous study and the cost, equipment and time required, scissor cutting was selected to reduce particle size in the film samples.

3.3. Optimisation of pressurized fluid extraction variables

In order to find the best extraction conditions, two experimental designs were performed: the first was a PB design for determining the significant variables and the second was a CCD to obtain the response surfaces for the aforementioned significant variables and to calculate the optimal values.

The experiments were carried out using one of the polyethylene film samples. Only three of the additives studied (I 168 ox, I 1076 and I 1010) were found in the samples; hence, the optimisation study was limited to these additives.

The variables and the levels considered in the PB design used for screening their significance and the mean response (peak area divided by the sample amount) obtained in each run are indicated in Table 4. The experiments were carried out in duplicate and responses were expressed in mili Absorbance Units second per gram (mAU s g^{-1}). The 16 replicates were performed randomly to nullify the effect of extraneous variables.

An ANOVA was performed with results for testing model signification. R^2 values showed that the adjusted model accounted for 84–89% of the variability of the peak area for I 1010 and I 168 ox, respectively. Pareto charts are shown in Fig. 2. In these

Table 5
Matrix and results obtained with a central composite design

Experiment number	CHx (%)	Time (min)	Temperature (°C)	I 168 ox (mAU s g^{-1})	I 1076 (mAU s g^{-1})	I 1010 (mAU s g^{-1})
1	6	6	85	63.2	244.8	139.5
2 (C)	3.75	12	95	57.9	209.1	109.9
3	3.75	2	95	41.3	158.7	83.7
4	1.5	6	85	53.4	69.6	89.8
5	6	6	105	70.3	126.7	156.2
6	1.5	18	105	49.9	284.8	104.1
7	3.75	12	80	61.3	186.8	116.8
8	6	18	105	46.6	216.7	150.8
9	3.75	22	95	50.5	176.8	117.5
10	1.5	6	105	54.5	281.2	98.5
11	1.5	18	85	47.8	276.5	103.1
12	6	18	85	58.4	226.8	108.0
14	0	12	95	53.0	284.7	97.8
15	3.75	12	110	51.5	200.7	104.4
16	7.5	12	95	79.3	228.8	108.0

C, mean value of central point.

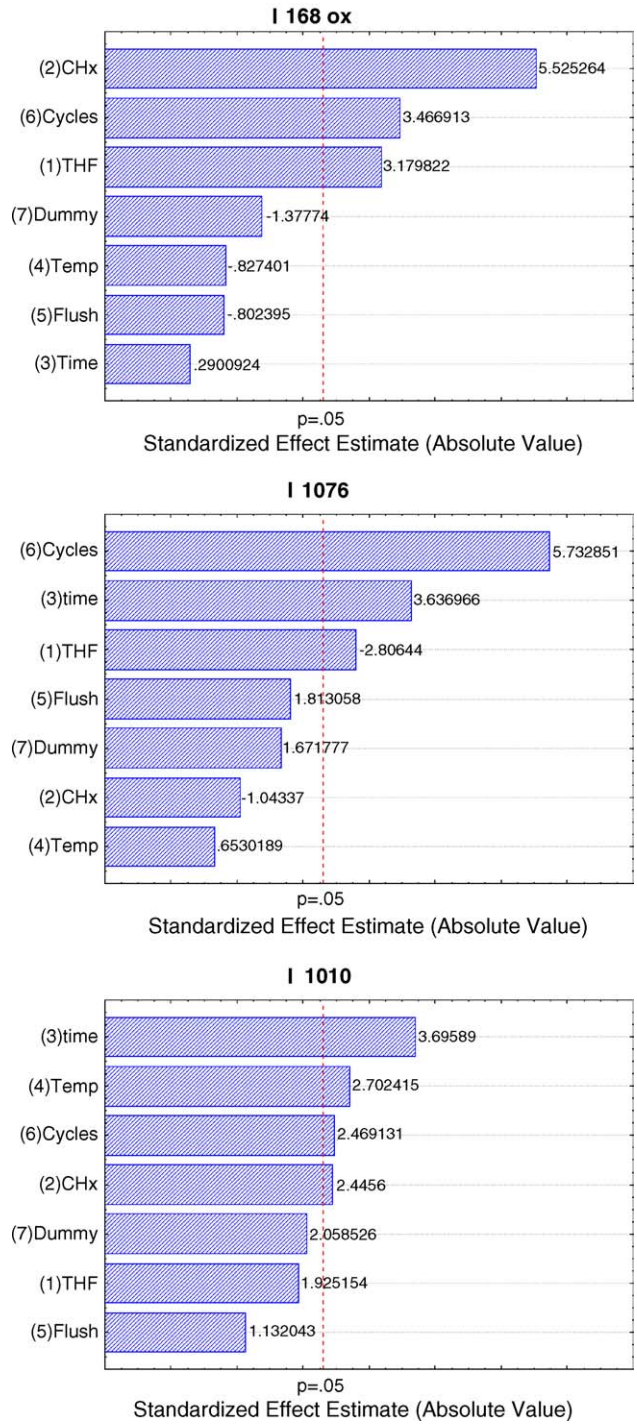

Fig. 2. Pareto charts of effects obtained from the Plackett–Burman design.

charts, the length of the bars is proportional to the absolute value of the estimated effects. The dashed line represents 95% of the confidence interval. Effects that cross this line are significant values with respect to the response.

Different results were obtained for the additives found in the samples. The use of two static extraction cycles with addition of fresh solvent improved the extraction of the three additives found in the polyethylene films analysed. The use of two or more cycles will be studied separately from CCD. The use of CHx as swelling solvent is a main parameter in the extraction of I 168 and I 1010. THF had the opposite effect on the extraction of I 168 and I 1076, whereas THF improved I 168 yield, low levels of THF increased the amount of I 1076 extracted. Therefore, THF was not used as an extraction modifier in further experiments. Flush volume was not significant in any case, so it was maintained at minimum (50%) in order to avoid a higher dilution of extracts. In contrast to what was expected, time and temperature were only significant in some cases: temperature and time increased I 1010 extraction, and I 1076 extraction was only improved significantly by increasing time. The dummy variable did not appear as a main parameter, so the method was validated.

According to the results and discussion of the Pareto charts, the variables and the experimental domain selected for the optimisation study were: the amount of CHx (between 0 and 7.5%), temperature (from 80 to 110 °C) and time (from 2 to 22 min). High temperature value and high amount of CHx were set in order to avoid polymer melting or dissolution in the extraction solvent and consequently problems of instrument pipeline blockage.

A CCD consisting of a factorial design (2^3) with six star points placed at $\pm\alpha$ from the central point of the experimental domain was applied. The axial size (α) value was 1.5, close to the value of 1.68 that establishes the rotatability condition. The CCD matrix consisted of 16 random experiments in which the central point value was measured twice. Values are listed in Table 5.

ANOVA was used to evaluate the main effects and interactions (data not shown). The p-values showed that the effect of the percentage of cyclohexane was only statistically significant ($p < 0.05$) for I 168 ox and almost statistically significant for I 1010 ($p = 0.07$).

The response surfaces were drawn to obtain the optimum of the variables studied in the CCD. Fig. 3 shows the most relevant fitted surface for each analyte. Fig. 3a and b show that a high amount of CHx increases yield, while temperature had no influence on the extraction of I 168 ox, which was completed in 12 min. The highest response was observed at 12 min with 7.5% CHx in isopropanol.

As shown in Fig. 3c, the influence of temperature on the extraction of I 1076 was only significant at a low extraction time. The equilibrium was achieved in 14 min for any temperature. Similar behaviour was observed in terms of the percentage of CHx (Fig. 3d). To summarise, I 1076 can be extracted in 14 min at any temperature and amount of CHx.

As shown in Fig. 3e and f, the use of high temperatures and percentages of CHx gave the best yields for the extraction of I 1010; only 2 min were required for its extraction. The use of a temperature of 110 °C and a 7.5% of CHx for 2 min was just enough for achieving the maximum extraction of I 1010.

As a result, the extraction conditions chosen were: 7.5% CHx as a swelling solvent, 15 min of static extraction and 105 °C (110 °C was no recommended because the polymer starts to melt and can obstruct the valves and tubes of the extractor).

Finally, the number of cycles was studied under the selected conditions and the mean values obtained for one, two, three

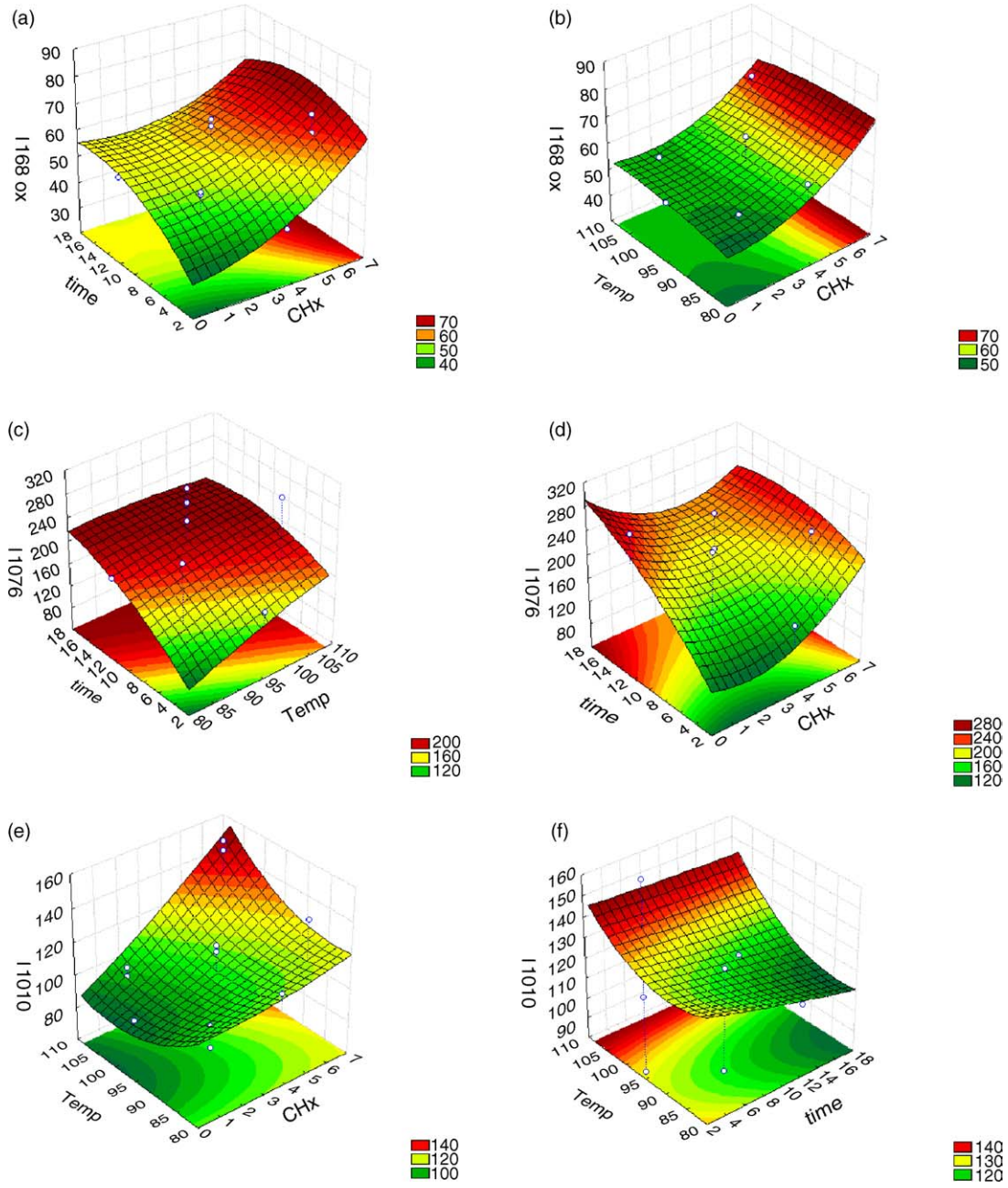

Fig. 3. Response surfaces estimated from the central composite design: (a) percent CHx vs. time for I 168 ox; (b) percent CHx vs. temperature for I 168 ox; (d) percent CHx vs. time for I 1076; (c) temperature vs. time for I 1076; (e) percent CHx vs. temperature for I 1010; and (f) time vs. temperature for I 1010.

and four extraction cycles were compared. First, Hartley's, Cochran's and Bartlett's tests were applied in order to check variance homogeneity; *p*-values above 0.05 were obtained in all cases (data not shown), indicating that there were no statistically significant differences among the variances. Second, an ANOVA test was performed according to the results obtained. *F*-values were higher than the critical value ($F_c = 4.077$) for I 1076 and I 168 ox (15.4 and 4.9, respectively) while for I 1010, the *F*-value ($F = 3.1$) showed that mean values were similar when one to four extractions were used. Third, the least significance difference (LSD) multicomparison test was used to determine the significantly different means and grouping into subsets. For all the additives, extraction cycles were grouped into two subsets. The use of two, three or four extraction cycles (included in the second subset) provided a significantly higher mean signal than with one extraction cycle. Therefore, two extraction cycles were chosen in order to reduce the total extraction time.

In summary, PFE was performed twice with a 92.5:7.5 isopropanol and cyclohexane mixture as extraction solvent, and at a temperature of 105 °C and a pressure of 10.3 MPa (1500 psi) for 15 min. The extracts were collected by flushing with 5.5 ml of fresh solvent (50% of cell volume) and then purging with N_2 for 60 s.

Table 6
Recoveries of additives from spiked sand

Additive	Level 1			Level 2		
	Added (mg l^{-1})	Found (mg l^{-1})	Recovery (%)	Added (mg l^{-1})	Found (mg l^{-1})	Recovery (%)
I 168ox	64.5	68 ± 5	105 ± 7	387	377 ± 30	98 ± 6
BMP	64.5	66 ± 5	103 ± 7	387	377 ± 30	98 ± 6
T 328	57.5	61 ± 6	105 ± 8	345	333 ± 27	97 ± 5
I 126	70.0	69 ± 5	98 ± 7	420	402 ± 32	96 ± 7
I 1330	62.0	64 ± 6	104 ± 8	372	373 ± 30	100 ± 5
HP 136	71.0	75 ± 6	105 ± 8	426	441 ± 35	104 ± 8
I 1076	69.5	72 ± 4	103 ± 6	417	439 ± 35	105 ± 8
I 3114	55.5	54 ± 5	97 ± 7	333	335 ± 27	101 ± 5
I 1010	61.0	60 ± 6	98 ± 8	366	383 ± 31	105 ± 6
BHA	75.0	74 ± 6	98 ± 8	450	434 ± 35	96 ± 8
MD 1024	73.0	77 ± 7	105 ± 8	438	429 ± 34	98 ± 8

$n = 3$.

Table 7
Analysis of polyethylene films

Sample	Concentration ± SD (μg g^{-1})					
	I 168 ox	HP 136	I 1076	I 1010	BHA	MD 1024
1	168 ± 12		343 ± 22	135 ± 12		
2	91 ± 6					
3		66 ± 6	58 ± 4		37 ± 1	70 ± 6
4			54 ± 6			
5	285 ± 25		37 ± 4	130 ± 13		
6	190 ± 20		71 ± 6	138 ± 13		

$n = 3$.

3.4. Study of recovery from spiked sand

In order to check the accuracy of the PFE/HPLC method, a recovery study was performed at two concentration levels. Five hundred microlitres of a standard solution were added to 7 g of sand placed in the extraction cell. Then, the cell was completely filled with sand and the mixture was processed in the same way as the samples. After the chromatographic analysis of the extracts, the recoveries were calculated and they are shown in Table 6. For all the analytes, the recoveries were around 100%.

3.5. Analysis of polyethylene films

The method was tested by using it to determine additives in six polyethylene film samples. The samples were analysed in triplicate by the PFE and NP-HPLC method under the conditions described above. The results (expressed as μg of additive per gram of polyethylene) obtained are given in Table 7. In order to check the completeness of PFE extraction, a second extraction of extracted samples was carried out, obtaining blank extracts in all cases. Additives HP 136, BHA and MD 1024 were found in sample three. Although the proposed method was not optimised for these additives, a quantitative extraction was achieved. In samples 2 and 4, only one additive was found (I 168 ox and I 1076, respectively). Samples 5 and 6 contained the same additives as sample 1, which was used in the optimisation of the analytical method.

4. Conclusions

A PFE and NP-HPLC method for the determination of additives in polyethylene films was optimised and applied.

Two chromatographic systems were compared and the NP-HPLC system proved to be more advantageous for additive separation than the RP-HPLC one in terms of sensitivity, analysis time and temperature control requirements.

Although cryogenic grinding yielded better results than room temperature grinding for particle size reduction of polyethylene films, scissors cutting is recommended for film samples because no significant differences were shown with respect to cryogenic grinding and it is also cheaper and requires less labour.

Pressurised fluid extraction variables were optimised by Placket–Burman and Central Composite Experimental designs and the final working conditions were selected as a compromise for the three analytes found in the polyethylene film sample studied.

Extraction was carried out twice using isopropanol with 7.5% cyclohexane as modifier at 105 °C and at 10.3 MPa (1500 psi) for 15 min. Under these conditions, PFE proved to be a suitable technique for the fast and complete extraction of additives from polyethylene films.

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