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Determination of UV filters in packaging by focused ultrasonic solid–liquid extraction and liquid chromatography

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ABSTRACT

A focused ultrasonic solid–liquid extraction (FUSLE) and high performance liquid chromatography (HPLC) with a diode array detector (DAD) is proposed for the determination of ten fat-soluble UV filters in packaging. FUSLE technique is relatively new and has been used for the extraction of a few analytes; such as polycyclic aromatic hydrocarbons and other organic pollutants. In this work, it has been demonstrated that FUSLE is a useful, fast and simple extraction methodology for UV filters because the complete extraction was carried out with just 6 ml of tetrahydrofuran and in only one cycle of 30 s. The developed method has been validated and applied to the analysis of polyethylene-based multilayer packaging samples. The FUSLE-based method allows the sensitive detection of most of the UV-filters in polyethylene, with limits of detection between 0.4 and 8.5 ng mg^{-1} (except for BDM). Intra and inter-day relative standard deviation values were below 5 and 14%, respectively, except for MBP. In addition, the proposed method was more efficient than tetrahydrofuran extraction under reflux for 2.5 h for all the analytes except for EMT and BDM. Therefore, the developed method can be used to establish the absorption capability of different types of packaging and this information will be very useful in packaging selection.

1. Introduction

Nowadays UV filters are commonly used in many cosmetic products in order to protect us from over-exposure to sunlight which promotes skin ageing as well as other harmful effects on human health, such as skin tumours [1]. UV filters are divided into two basic groups, chemical or organic and physical or inorganic protectors. The organic filters, which are used most commonly, absorb the ultraviolet light (UVA and/or UVB rays) and convert it into a small amount of heat, and inorganic filters can reflect and scatter the UV light [2]. In the European Union (EU), 26 organic compounds have been approved to be used as UV filters in personal care products with maximum individual concentrations of up to 10%, but for dometrizole trisiloxane with a maximum permissible concentration of 15% [3], and the usual concentrations in these products are between 0.1 and 10% [4–6]. The UV filters investigated in this paper (see Table 1 and Fig. 1) are fat-soluble compounds. The organic UV filters can be classified in two groups: the most fat-soluble, and the easily water-soluble, which are determined under different chromatographic conditions [7]. In this study, fat-soluble UV filters determination has been carried out because they are more common and numerous in creams available on the European market.

UV filter determinations have increased in recent years, not only in personal care products [4,5,7–15] but also in water [6,16], wastewater [17], seawater [18], sludge [19], dust [20], fish [21] urine [22] and semen [23]. This is because recent studies have indicated that some UV filters can accumulate in biota and act as endocrine disruptors which have estrogenic effects [24–27], hence many personal care product ingredients, such as UV filters, have been included in the so-called emerging contaminants.

Often new cosmetic formulations are promoted in multilayer packaging sachets which consist of several layers fixed together by extrusion or by an appropriate adhesive. The materials normally used in this packaging are polymers (polyethylene, polyester, polypropylene, etc.) and thin aluminium foils used to provide a hermetic barrier. The advantages of these multilayer packaging materials are their impermeability, good external appearance, flexibility and versatility. However, the main disadvantage of them is their interaction with the product. For instance, certain ingredients of personal care products or food are able to pass through the inner layer (a polymer), causing a loss of adhesion followed by delamination. Personal care products are in a constant evolution, with the development of new formulations and applications. Several investigations have been carried out to identify these aggressive compounds such as 2-phenoxyethanol, benzyl-3-hydroxypropanoate, dihydromyrcenol, menthol, 3,7-dimethyl-3-octanol and p-propenylanisole [28–30]

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Table 1
List of the target UV filters.

INCI name[a]	Abb.	λ_{max}[b] (nm)	MAC[c] (%)	Absorption
Benzophenone-3	BZ3	290	10	UVA + UVB
4-Methylbenzylidene camphor	MBC	303	4	UVB
Octocrylene	OCR	306	10	UVA + UVB
2-Ethylhexyl dimethyl PABA	EDP	315	8	UVB
Ethylhexyl methoxycinnamate	EMC	312	10	UVB
Butyl methoxydibenzoylmethane	BDM	360	5	UVA
Ethylhexyl salicylate	ES	306	5	UVB
Homosalate	HS	306	10	UVB
Methylene bis-benzotriazolyl tetramethylbutylphenol	MBP	305/347	10	UVA + UVB
Bis-ethylhexyloxyphenol methoxyphenyltriazine	EMT	343	10	UVA + UVB

[a] INCI (International Nomenclature of Cosmetic Ingredients).
[b] Wavelength of maximum absorption.
[c] MAC (maximum authorized concentration (%, w/w)) by EU Cosmetic Directive.

and in recent studies (under confidentiality contract) we have found that UV filters are some of the most active cosmetic ingredients involved in the deterioration of multilayer packaging.

Different methods have been used to determine this kind of UV filters in sunscreen products and other matrices. The most used technique to determine them has been HPLC-UV because they are polar and UV-absorbing compounds, however their chro-

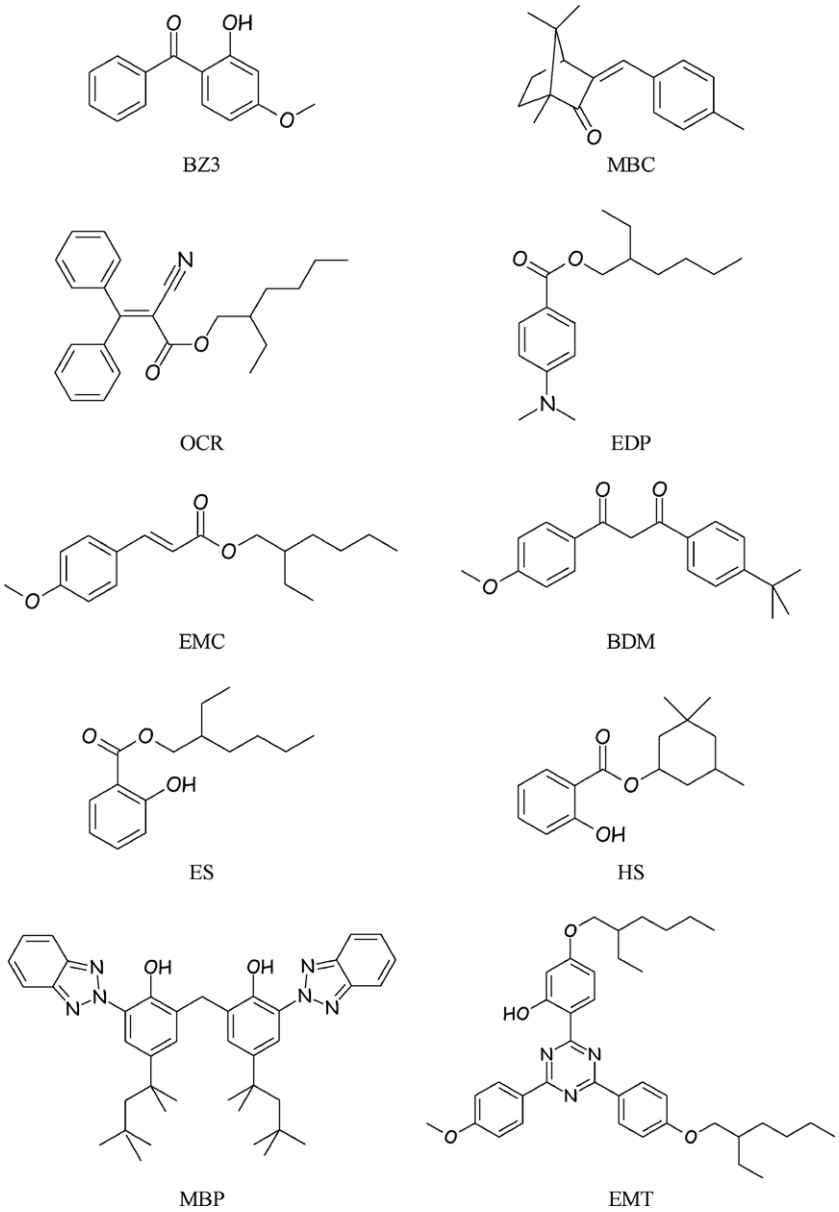

Fig. 1. Chemical structures of the ten target UV filters.

matographic separation by gas chromatography and detection by mass or tandem mass spectroscopy (MS or MS/MS) have also been reported [16,18,20,31].

In order to determine compounds absorbed in packaging coming from the migration from the product, HS-SPME-GC has been the most appropriate and chosen method when they are volatile and have low molecular weight. However, in the case of UV filters because of their polarity and low volatility, chromatographic separation by HPLC was selected.

In some of the reported methods to determine UV filters, the isolation and pre-concentration of sunscreen agents from matrices has been required prior to chromatographic analysis. For instance supercritical fluid extraction (SFE) [8] has been used for cosmetic samples; solid-phase extraction (SPE) [32], solid-phase microextraction (SPME) [16,33], dispersive liquid–liquid microextraction (DLLME) [18] and membrane-assisted liquid–liquid extraction (MALLE) [6] for liquid samples such as water. Traditionally, extraction from polymers has been carried out by Soxhlet extraction or by boiling under reflux [34], and more recently by microwave assisted extraction (MAE) [35], supercritical fluid extraction (SFE) [36], pressurized fluid extraction (PFE) [37], headspace solid-phase microextraction (HS-SPME) [38,39] and ultrasound assisted extraction using an ultrasonic bath [40–44]; but this is the first time that focused ultrasonic solid–liquid extraction (FUSLE) has been used to sample preparation of packaging samples.

FUSLE is a fast and low-cost technique, relatively new, that has shown similar results to other extractions, such as MAE in the determination of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters and nonylphenols from environmental matrices [45–47]. In addition, FUSLE is expected to be a more efficient extraction technique than others.

FUSLE is based on the cavitation phenomena: ultrasonic waves crossing a liquid cause the generation, growth, oscillation, splitting and implosion of numerous tiny gas bubbles (cavitation bubbles) [48]. As a result of cavitational bubble implosion, very high temperatures (up to 5000 K) and pressures (up to 2000 atm) are reached locally, and the implosion of the cavitation bubble also results in liquid jets of up to 280 m/s velocity [49]. These features favour extraction efficiency. Furthermore, the size of the bubbles is very small relative to the total liquid volume, so the heat they produce is rapidly dissipated with no appreciable change in the environmental conditions; this is why cavitation is also known as “cold boiling” [46]. It is worth mentioning, that the focused ultrasound microtip is immersed directly in the extracting solution and this, together with the higher ultrasound power, makes the power of the focused ultrasound technique 100 times higher than that of the traditional ultrasonic bath [45]. Therefore, the focused ultrasound approach is very useful for developing new solid–liquid extraction procedures.

In this work, a fast and simple method based on FUSLE has been developed for the determination of ten fat-soluble UV filters sorbed in different polyethylene-based flexible multilayer packaging. The extraction was carried out with only 6 ml of tetrahydrofuran in one cycle of 30 s and the extract analysis was performed by HPLC-UV. The method can be very useful to study the migration of UV filters to the layer of packaging contact; this information is important for packaging selection.

2. Experimental

2.1. Materials and reagents

Benzophenone-3 (oxybenzone) (BZ3) 98%, 4-methylbenzylidene camphor (enzacamene) (MBC) ≥98.0%, octocrylene (octocrilene) (OCR) 97%, 2-ethylhexyl dimethyl PABA (padimate O) (EDP) 98%, ethylhexyl methoxycinnamate (octinoxate) (EMC) 98%, butyl methoxydibenzoylmethane (avobenzone) (BDM) ≥99.0%, ethylhexyl salicylate (octisalate)(ES) 99% and methylene bis-benzotriazolyl tetramethylbutyl phenol (bisoctrizole) (MBP) 99% were supplied by Sigma–Aldrich (St. Louis, MO, USA). OCR, BDM, HS and EMT were also supplied by Beiersdorf (Eimsbüttel, Hamburg, Germany).

Polyethylene (PE) film and multilayer packaging samples were obtained from AMCOR Flexibles. Multilayer packaging consisted of several layers of different materials, including aluminium, polyethylene (PE) and polyester (PES) fixed together by extrusion or by different polyurethane adhesives.

Ethanol (HPLC grade) and tetrahydrofuran (THF) were provided by Scharlab (Barcelona, Spain).

A 1% (v/v) acetic acid aqueous solution was prepared from acetic acid supplied by Scharlab (Barcelona, Spain) in Milli-Q deionised water (Bedford, MA, USA).

Cream samples containing known concentration of analytes were prepared in a base cream containing 20% NeoPCL® Autoemulsionable O/W (oil in water) from Acofarma (Terrassa, Spain) and 80% Milli-Q deionised water (Bedford, MA, USA).

2.2. Solution and sample preparation

Individual standard solutions containing 10 mg/ml of the UV filter were prepared in ethanol for all UV filters but for MPB and EMT prepared in THF. A multicomponent standard solution was prepared containing 80 μg/ml in ethanol from individual standard solutions and subsequently diluted as necessary.

Base cream was prepared from Milli-Q deionised water and NeoPCL. They were heated separately to 90 °C. The water was added slowly to the oily mixture while stirring. It was necessary to continue stirring until the emulsion was cooled to room temperature to obtain a homogeneous mixture. Then, sunscreen agents were incorporated into this emulsion at different levels: 5% (w/w), for determining the absorption in the sachets, 7% (w/w), in order to study the influence of the number of cycles, and 10% (w/w), for the study of the rest of FUSLE variables.

In order to study the influence of FUSLE variables, PE film samples containing UV-filters were prepared. These treated PE samples were prepared by immersing 3 cm^2 of PE film in 1 g of cream formulation containing UV filters between 7 and 10% (w/w) in a NeoPCL base, for 15 days at 40 °C, to favour the absorption, and protected from the light. It is worth mentioning that two cream formulations were made to attain these concentrations for the ten UV filters. The first cream formulation contained OCR, BDM, HS, ES and EMT; the second contained the remaining UV filters. Therefore, 6 cm^2 of fortified PE film were used to the study FUSLE variables.

The determination of UV filter sorption in PE-based multilayer packaging was carried out using 6 cm × 8 cm and 10 cm × 10 cm sachets containing 1.5 and 3.0 g of 5% (w/w) UV filter cosmetic formulation, respectively. In all cases the cosmetic mass-packaging surface ratio was around 30 mg/cm^2. Sachets were thermosealed at 190 °C and were kept in an oven at 40 °C for 23 days to favour the sorption.

PE film and packaging samples were washed with water, dried with paper towel and stored at 4 °C protected from light before their analysis.

2.3. FUSLE procedure

All FUSLE processes were performed at 0 °C in an ice-water bath, using a SONOPLUS 2070 focused ultrasound system equipped with a 3 mm titanium microtip and sound proof box (Bandelin Sonoplus, GmbH & Co. KG). Samples were cut in small pieces of around 6 mm^2 before FUSLE. Around 42 mg (6 cm^2) of PE film were extracted with a volume of an organic solvent (THF, ethanol or

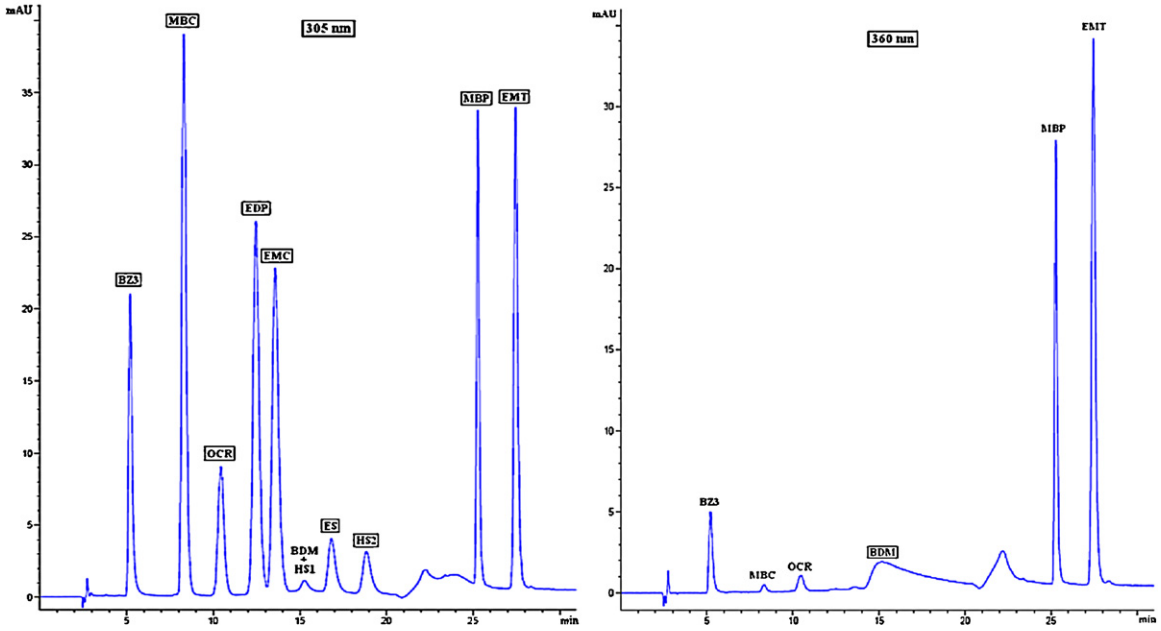

Fig. 2. Chromatograms corresponding to the separation of the ten UV filters in ethanol recorded at 305 and 360 nm. Chromatographic conditions are reported in Section 2.

acetone) ranging from 2 to 10 ml for a period of time between 30 and 300 s, at an ultrasound power from 20 to 90%, once to four times, at 50% pulsed cycle, depending on the experiment. Microtip was immersed into a cylindrical glass vessel with flat-bottom, about 5 mm above the bottom of the vessel. Extracts were evaporated up to ~0.5 ml under a nitrogen stream using a Turbo Vap II concentrator (Zymark, Hopkinton, MA, USA). The extracts were transferred to 5 ml volumetric flask, made up to 5 ml with ethanol and filtered through a 0.45 nylon filter before HPLC injection.

2.4. Chromatographic separation

HPLC analysis was performed with an Agilent modular 1100/1200 liquid chromatograph system (Agilent Technologies, Palo Alto, CA, USA) equipped with a G1379A degasser, a G1311A HPLC quaternary pump, a G1329A Automatic Liquid Sampler (ALS) and a G1315D diode array detector (DAD). A Scharlau Nucleosil 120-C18 (5 μm packing, 250 mm × 4 mm i.d.) column protected with a precolumn of the same material (Scharlab, Barcelona, Spain) was used. The temperature of the column was set at 45 °C with a Waters column heater module and a temperature control module (Milford, MA, USA). A 1% (v/v) acetic acid aqueous solution and ethanol mixture mobile phase at a flow rate of 1.0 ml/min was used for RP-HPLC. The mobile phase gradient started at 70% of ethanol and was maintained for 17 min, then increased to 100% in 1.5 min and maintained for 7.5 min. Finally, it was decreased to 70% of ethanol in 1 min and was maintained for 4 min in order to attain the initial gradient conditions for the next injection. The injection volume was 30 μl and the chromatogram was recorded at 305 nm for all analytes, except for BDM, which was detected at 360 nm, its absorption maximum, and because the interference by the coeluting HS isomer was avoided at this wavelength (Fig. 2).

2.5. Software for statistical analysis

Experimental designs and statistical analysis were performed using Statgraphics Centurion XV (Statpoint, Herndon, VA, USA), and Microsoft Excel was used for drawing response surfaces and plots.

3. Results and discussion

3.1. Chromatographic separation of UV filters

3.1.1. Preliminary experiments

In order to quantify the ten UV filters, any wavelength between 305 and 315 nm provided good sensitivity for all analytes, except for BDM. Therefore, BDM was measured at 360 nm the wavelength corresponding to its maximum of absorbance, while the rest of analytes were determined at 305 nm. The chromatographic method used to separate the ten UV filters of this study was a modification of that reported by Salvador and Chisvert [7].

Preliminary experiments on the chromatographic separation of the ten UV filters carried out by injecting the individual standard solutions showed that HS was a mixture of two isomers. This has been already reported [7]. In this work, the quantification of HS was carried out using the peak area of the most abundant isomer which represented 83.22% (RSD = 0.03%) of total HS. It is worth mentioning that although retention time of the minority isomer was very close to that of BDM, it did not pose a problem because the latter was detected at a wavelength at which HS does not absorb at all.

In order to select the chromatographic condition, different mobile phase compositions were tested: Methanol and ethanol as organic modifiers at different percentages, acetic acid and $AcOH/AcO^-$ buffer aqueous solutions, temperatures between 25 and 45 °C, and flow-rate values from 0.7 to 1.1 ml/min. However, no improvement of the BDM separation was achieved and this compound showed a significant peak tailing which spoils its determination.

3.1.2. Features of HPLC-UV method

The HPLC-UV method was characterized in terms of linearity, limit of detection (LOD) and quantification (LOQ), and repeatability (RSD, %). Results are shown in Table 2. The features of the HPLC-UV method were established using standard solutions of the UV filters in ethanol. The linear range of all compounds was studied between the limit of quantification, estimated as ten times the standard deviation of a blank divided by the slope, and an upper limit of 80 μg/ml. The limit of detection was estimated as three times the standard deviation of a blank divided by the slope. As can be

Table 2
Features of the HPLC-UV method.

Compound	Retention time (min)	Slope (ml/μg) ± SD	Intercept ± SD	R^2	LOD (ng/ml)[a]	LOQ (ng/ml)[b]	RSD (%)[c]
BZ3	5.188 ± 0.022	77.1 ± 0.3	10 ± 9	0.99990	21	68	0.28
MBC	8.27 ± 0.04	173.3 ± 0.6	22 ± 18	0.99990	3	11	0.17
OCR	10.40 ± 0.06	52.74 ± 0.19	9 ± 6	0.99990	6	19	0.17
EDP	12.40 ± 0.07	154.2 ± 0.5	24 ± 15	0.99990	3	9	0.24
EMC	13.51 ± 0.08	148.0 ± 0.5	14 ± 14	0.99992	10	33	0.25
BDM	14.63 ± 0.13	106.6 ± 1.2	−116 ± 43	0.9993	278	926	1.95
ES	16.72 ± 0.10	26.81 ± 0.11	6 ± 4	0.99990	36	119	0.60
HS	18.74 ± 0.11	23.71 ± 0.08	3 ± 3	0.99992	21	69	0.34
MBP	25.27 ± 0.04	89.0 ± 0.3	9 ± 9	0.99991	9	31	0.30
EMT	27.51 ± 0.06	129.2 ± 0.4	10 ± 10	0.99993	2	6	0.24

[a] Estimated as three times the standard deviation of a blank divided by the slope.
[b] Estimated as ten times the standard deviation of a blank divided by the slope.
[c] Relative standard deviation (n = 10) at 4 μg/ml.

seen, BDM offered worse features than the rest of UV filters, even the wavelength selected for its detection (360 nm) corresponds to its absorption maximum, and this could be because BDM showed a significant peak tailing which reduces sensibility and precision. Therefore, this HPLC-UV method is not suitable for quantifying BDM. The other UV filters showed good features. The correlation coefficients R^2 were higher than 0.99990. LOD and LOQ ranged from 2 to 36 ng/ml and from 6 to 119 ng/ml, respectively. The relative standard deviation (obtained at 4 μg/ml concentration level) was less than 0.60% therefore the results showed to be precise, even for BDM (1.95%).

3.2. Study of FUSLE variables

The main objective of this study was to select the best FUSLE conditions to extract UV filters from multilayer packaging.

3.2.1. Preliminary considerations

Variables affecting the FUSLE process include: ultrasonic irradiation power, extraction time, solvent volume, composition of the extraction solvent, number of cycles of extraction, sample mass, particle size, extraction temperature, pulse time and vessel shape.

The analyte amount extracted depends on the distribution constant, given by the analyte solubility in the solvent and sample matrix–analyte interaction, as well as the solvent–sample phase ratio. Therefore, solvent and sample volumes are correlated variables, and so they were studied as one, by testing different volumes while the sample amount was maintained constant at a value of 42 mg.

The influence of particle size on extraction of multilayer packaging was already studied elsewhere [30]. According to the results of this previous work, scissor cutting was selected to reduce particle size. Thus, 6 cm^2 of sample (to get 42 mg of sample mass) were cut in small pieces of around 6 mm^2 before FUSLE.

In order to select the extraction temperature, it should be taken into account that higher temperatures increase analyte solubility in the solvents and favour the disruption of analyte–matrix interactions, but also increased temperatures negatively affect the cavitation phenomena. As temperatures increase, the cavities immediately fill with liquid vapour which cushions the implosive action which extracts [49]. The optimal temperature of the extraction solvent was investigated elsewhere by Sanz-Landaluze et al. [47], who found that the compromise between temperature and cavitation was achieved at 0 °C so it was decided to keep the solvent temperature at 0 °C during all the extraction, immersing vessels into an ice bath.

In the case of the pulse time, it is worth mentioning that Henglein [50,51] proposed that during cavitation there are two different time periods: "activation time" which is the time required to produce chemically active bubbles with a sufficient size to allow the implosion to be effective, and "deactivation time" which is the interval between pulses; there is a compromise between the two. If the pulse time is too short, cavitation bubbles will not have enough time to grow to the suitable size to collapse; if the pulse interval is too long, growth and collapse of bubbles disappear slowly and the following pulse will have to reactivate a new extraction system. This compromise was studied by Sun and Weavers [52], who found that irradiation for 50% of the time offered the best results. Thus, in this work, it was decided to set the pulse time at a 50% pulsed cycle.

The vessel shape is quite important because "dead zones" where there is no cavitation, and therefore no implosion of the bubbles and no extraction, should be avoided during the extraction. The extraction vessel should be as narrow as possible to avoid this problem [48]. It is worth mentioning that the titanium microtip of the probe must be immersed into the vessel 1–2 cm from the upper surface of the slurry according to manufacturer's recommendations, and about 5 mm above the bottom of the vessel to minimize "dead zones". For this reason, it was decided to use a different vessel to be able to cover the whole volume range to optimize (2–10 ml) using at all times the narrower vessel. Then a 5 ml vessel (9 mm i.d.) was used for solvent volumes between 2 and 4 ml, while 10 ml (18 mm i.d.) and 20 ml (23 mm i.d.) vessels were employed for ranges 4–7 and 7–10 ml, respectively.

The rest of FUSLE variables, including the ultrasonic irradiation power, the extraction time, the solvent volume, the composition of the extraction solvent and the number of extraction cycles were the chosen parameters to study.

In addition, the UV filter stability under strong FUSLE conditions was studied. 6 ml of a UV filter solution containing 20 μg/ml of each in THF was subjected to FUSLE under extreme conditions (at 90% ultrasound power and 50% pulsed cycle for 300 s) in triplicate. The solutions were evaporated to ~0.5 ml under a nitrogen stream, reconstituted in 5 ml of ethanol and filtered before HPLC injection. Differences between analyte signal for solutions subjected to FUSLE and the control (untreated solution) were less than 1.5%. Therefore, it can be concluded that UV filters are stable during FUSLE.

3.2.2. Solvent selection

First, in order to find the best extraction conditions, the influence of extraction solvent was studied. Usual solvents reported in literature for dissolving UV filters were tested for FUSLE: ethanol, acetone and tetrahydrofuran (THF). 6 cm^2 of spiked PE film were extracted with 10 ml of each organic solvent for 300 s, at 50% of ultrasound power and at 50% pulsed cycle. Experiments were performed in triplicate. After FUSLE, extracts were evaporated to dryness under nitrogen and transferred with ethanol to a 5 ml volumetric flask and filtered through a 0.45 nylon filter before HPLC injection.

Results, presented in supplementary materials, showed that THF extracted the highest amounts of UV filters in all cases, followed

by acetone. BZ3 was also well extracted with acetone, while this solvent extracted the same OCR, MBP and EMT amounts as ethanol. Therefore, THF was selected for further extractions.

It is also worth mentioning that the injection of the UV filters dissolved in THF decreased the efficiency and resolution of the chromatographic separation. Therefore, a change of solvents was mandatory and extracts were evaporated and reconstituted in ethanol before HPLC injection. The final THF percentage in the extract was studied; the evaporation process was carried out to dryness, up to 0.5 ml and up to 1.0 ml. No significant differences were observed between evaporation to dryness and up to 0.5 ml in the resolution peaks. Therefore, the extracts were reconstituted containing 10% of THF in ethanol, and the evaporation time was 50% shorter and peaks showed the same resolution as that for ethanolic extracts.

3.2.3. Central composite design

Once THF was selected as extraction solvent, a composite central design (CCD) was carried out to study the influence of the ultrasonic irradiation power, extraction time and solvent volume.

The central composite design consisted of a 2^3 factorial design with six star points located at $\pm\alpha$ from the centre of the experimental domain. The axial distance α for this design was 1.68 in order to establish the rotatability condition. The design was also completed with nine replicates of the central point to obtain an orthogonal design. Therefore, the complete design consisted of 23 randomly performed experiments. All the experiments were carried out using 6 cm^2 of spiked PE film (prepared as described under Section 2). The pulsed cycle was set at 50%, the titanium microtip was immersed about 5 mm above the bottom of the vessel and the vessels were immersed into an ice bath. Ultrasonic irradiation power values ranged from 20 to 90%, including the following levels: 20, 34, 55 (central value), 76 and 90% of ultrasound power. Extraction time was studied between 30 and 300 s and the levels were 30, 85, 165 (central value), 245 and 300 s. THF volume used in extractions was between 2 and 10 ml with levels of 2, 3.62, 6 (central value), 8.38 and 10 ml. The ANOVA test of the results (data not presented) showed that only seven of the coefficients were significant (p-value < 0.05). No first order coefficients were statistically significant and only second order coefficients were statistically significant. Pareto-charts of the standardized effects for the six UV filters affected significantly by some of FUSLE variables are included in supplementary materials. However, in order to determine the optimal values for the variables, the coefficients which became significant by eliminating the non-significant ones, because they were close to 0.05 (p value < 0.08), have been also taken into account. Then, the effects considered were eleven: the time–ultrasound power interaction for MBC, the quadratic effects of ultrasound power and volume for OCR, BDM, ES and HS, the effect of volume for ES, and the effect of ultrasound power for MBP. Response plot/surfaces for these compounds (included in supplementary materials) showed that the highest responses for most of the compounds (OCR, BDM, ES and HS) were attained at 55% of ultrasonic power and at about 6 ml of THF. However, in the case of MBC and MBP, the maximum of the response surface was located at 90% and 20% of ultrasound power, respectively. Therefore, an ultrasound power value of 55% was selected as a compromise. Finally, the extraction time effect was only significant in the MBC response which attained its maximum at 30 s. According to these results, the optimal conditions selected for the FUSLE step were as follows: 30 s of extraction at 55% of ultrasound power with 6 ml of THF.

3.2.4. Study of number of extraction cycles

Once the best FUSLE conditions were established, the number of extraction cycles required for complete extraction was determined. The effect of a different number of FUSLE steps, from one to four, was studied. Extractions were performed in triplicate using the treated film (prepared as described under Section 2). No significant differences were observed using more than one cycle for all analytes. MBP seemed to be better extracted using three cycles but results obtained for three cycles were statistically equal to those for one or two cycles (F-value of 3.366 lower than the critical value 5.143). Therefore, it can be concluded that one extraction cycle was enough to extract all the UV filters from PE. Further experiments were performed using one extraction cycle.

3.3. Features of the FUSLE–HPLC-UV method

The whole analytical method including FUSLE and HPLC determination was characterized for the ten UV filters, in terms of limit of detection (LOD) and quantification (LOQ), repeatability (intra-day RSD, %), intermediate precision (inter-day RSD, %) and recovery. Results are listed in Table 3.

The limits of detection and limits of quantification were estimated as three and ten times the standard deviation of a blank (a FUSLE extract of a PE film free from UV filters) divided by the slope, respectively, and expressed as nanograms of analyte per milligram of film. The limits of detection and quantification were below 10 and 30 ng/mg of PE film, respectively, for all of the analytes except for BDM. The BDM detection was less sensitive than that of the other analytes even the wavelength selected for its detection (360 nm) corresponds to a maximum of its UV spectrum, but it shows a notable peak broadening as it was explained above.

The repeatability and intermediate precision of the method were calculated by processing 9 replicates of spiked PE film (three days × three replicates per day). ANOVA was used to obtain repeatability and intermediate precision. As can be seen in Table 3, repeatability and intermediate precision were satisfactory for all analytes (RSDs less than 5 and 14%, respectively), but for MBP. The RSD values for this compound were too high. Since the HPLC repeatability was good for this compound, there must be a problem during the extraction of MBP. Therefore, the proposed method cannot be used for quantifying MBP until this problem was solved.

In order to check the accuracy of the method, a treated PE sample was extracted by using the FUSLE method and with 20 ml of THF for 2.5 h under reflux. Recovery values, calculated using the concentrations found by extraction under reflux as reference values, were close or higher than 100% for most analytes except for EMT (58%) and BDM (74%). EMC recovery could not be calculated because it was poorly extracted in THF under reflux.

3.4. Analysis of samples

The method was applied to determine the UV filter sorption in different PE contact layer packaging. Packaging samples containing UV-filters were obtained from sachets of multilayer packaging filled with the same amount of a cream, containing the ten UV-filters, and stored for 23 days. UV-filter concentration in the cosmetic preparation was the same for the five samples. Sachets were made of different multilayer complexes, all of them with a polyethylene contact layer, but different number of layers and including both extrusion-coated and adhesive–joint packaging. In the case of adhesive–joint complexes different adhesives were used.

Although external layer was printed, the whole multilayer packaging sample can be processed without layer separation, because no coextracted compounds were found in the chromatograms. A typical chromatogram is shown in Fig. 3. The concentrations found, expressed as μg of compound per milligram of packaging, are given in Table 4.

Table 3
Features of the FUSLE-HPLC-UV method.

Compound	LOD[a] (ng UV filter/mg PE)	LOQ[b] (ng UV filter/mg PE)	Repeatability[c] (RSD, %)	Intermediate precision[d] (RSD, %)	Recovery ± SD (%)[e]
BZ3	4.9	16.3	3.3	3.9	113 ± 12
MBC	0.8	2.7	1.8	3.3	199 ± 42
OCR	1.4	4.6	4.4	5.4	179 ± 14
EDP	0.6	2.2	1.5	4.8	99 ± 3
EMC	2.3	7.8	1.7	1.5	-
BDM	66.1	220.4	2.5	2.2	74 ± 5
ES	8.5	28.2	2.3	3.9	100 ± 4
HS	4.9	16.4	2.2	3.2	100 ± 3
MBP	2.2	7.3	34.8	47.7	132 ± 4
EMT	0.4	1.4	4.7	13.4	58 ± 2

[a] Estimated as three times the standard deviation of a blank divided by the slope.
[b] Estimated as ten times the standard deviation of a blank divided by the slope.
[c] Intra-day relative standard deviation ($n = 3$ replicates × 3 days).
[d] Inter-day relative standard deviation ($n = 3$ replicates × 3 days).
[e] Recovery values have been calculated using the results obtained by THF extraction under reflux for 150 min.

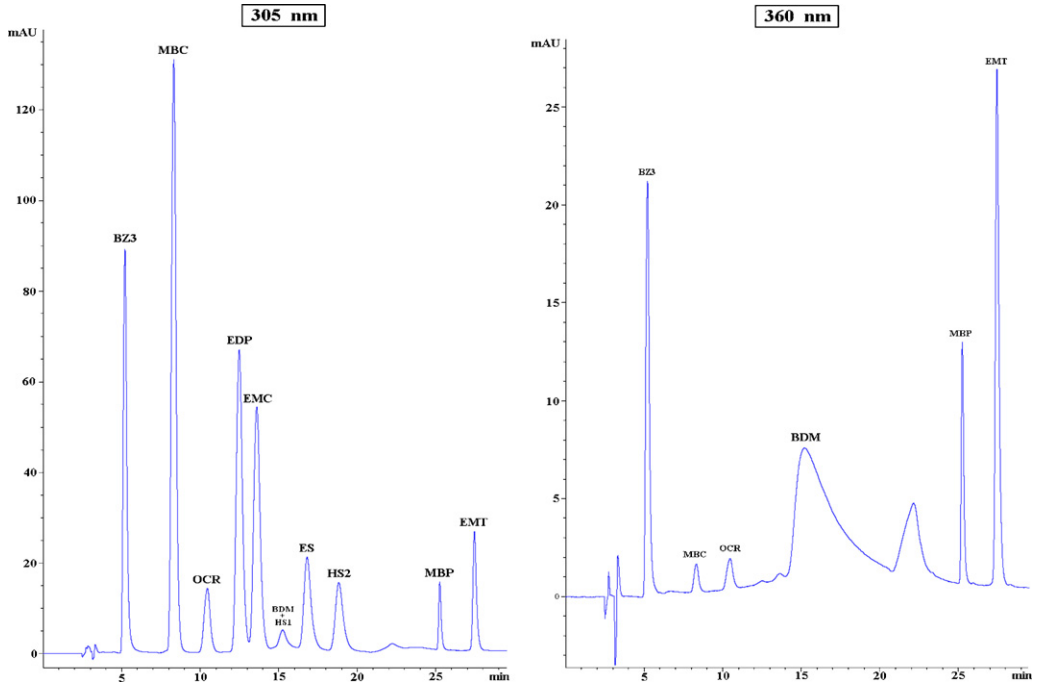

Fig. 3. Chromatograms of a multilayer packaging extract containing UV-filters (sample 5 in Table 4). Chromatographic conditions are reported in Section 2.

Table 4
Concentration of each UV filter found in PE-based multilayer packaging (μg UV filter/mg packaging).[a]

Sample	BZ3	MBC	OCR	EDP	EMC	BDM	ES	HS	MBP	EMT
1	2.74 ± 0.04	2.24 ± 0.03	1.05 ± 0.01	1.65 ± 0.02	1.52 ± 0.03	1.501 ± 0.006	3.47 ± 0.03	3.23 ± 0.05	0.099 ± 0.004	0.43 ± 0.07
2	3.12 ± 0.13	2.18 ± 0.05	1.13 ± 0.03	1.62 ± 0.03	1.51 ± 0.02	1.55 ± 0.05	3.05 ± 0.08	2.88 ± 0.07	0.17 ± 0.02	0.36 ± 0.05
3	1.87 ± 0.03	1.88 ± 0.06	0.72 ± 0.03	1.41 ± 0.04	1.26 ± 0.04	1.05 ± 0.04	3.01 ± 0.11	2.75 ± 0.11	0.08 ± 0.01	0.258 ± 0.004
4	3.39 ± 0.03	2.59 ± 0.05	1.74 ± 0.07	2.07 ± 0.06	2.00 ± 0.06	2.05 ± 0.04	3.63 ± 0.01	3.46 ± 0.02	0.13 ± 0.04	0.63 ± 0.02
5	2.57 ± 0.07	2.12 ± 0.03	0.99 ± 0.05	1.59 ± 0.04	1.47 ± 0.03	1.56 ± 0.05	3.26 ± 0.04	3.05 ± 0.04	0.23 ± 0.10	0.44 ± 0.04

[a] Concentration ± SD; $n = 3$. Samples 1 and 5: PE/Al/PES extrusion-coated complex, and 2–4: PE/PES/Al/PET adhesive–joint complex.

4. Conclusions

A fast and simple FUSLE method has been developed to determine the sorption in polyethylene-based multilayer packaging of seven of the main compounds authorized and used as UV filters in Europe nowadays to offer a sun protection factor (SPF).

FUSLE was carried out with just 6 ml of tetrahydrofuran in only one cycle of 30 s. The proposed method allows the sensitive detection of most of the UV-filters in polyethylene, with limits of detection between 0.4 and 8.5 ng mg^{-1} (except for BDM). Intra and inter-day relative standard deviation values were below 5 and 14%, respectively, except for MBP. In addition, the proposed method was more efficient than tetrahydrofuran extraction under reflux for 2.5 h for all the analytes except for EMT and BDM. Moreover, the whole packaging can be processed without layer separation, which simplifies the analysis. Therefore FUSLE has shown to be faster and easier to implement than other extraction techniques

such as microwave-assisted extraction (MAE) or pressurized liquid extraction (PLE). As well FUSLE is a low-cost technique versus other extraction techniques.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.11.087.

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