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Article

Simultaneous Liquid Chromatographic Determination of 10 Ultra-Violet Filters in Sunscreens

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A rapid HPLC method was developed for the simultaneous determination of 10 UV filters found in sunscreen. The following UV filters were analyzed in this method; 2-phenylbenzimidazole-5-sulfonic acid, benzophenone-3, isoamyl *p*-methoxycinnamate, 4-methylbenzylidene camphor, octocrylene, ethylhexyl dimethyl 4-aminobenzoic acid, ethylhexyl methoxycinnamate, butyl methoxydibenzoylmethane, ethylhexyl salicylate and homosalate. The method was developed on two columns; a Thermo Hypersil C_{18} BDS, 3 μm column (4.6 × 100 mm) and a Chromolith RP-18e Monolithic column (4.6 × 100 mm). The same mobile phase of ethanol and 1% acetic acid (70:30, v/v) was employed for both columns. The separation of the 10 UV filters was carried out successfully on both columns; the optimal resolution was obtained on the Thermo Scientific Hypersil column in a time frame of 7 min. An isocratic elution utilizing ethanol and acetic acid (70:30, v/v) at a temperature of 35°C was employed. The method was applied to a number of commercial samples of sunscreen and lotions and was validated according to International Conference on Harmonisation guidelines for selectivity, linearity, accuracy, precision and robustness. A comparison of the performances of both columns was also carried out.

Introduction

The harmful effect of UV radiation on the skin has led to the development of organic chemicals known as UV filters. The use of sunscreen products containing these UV filters may prevent or minimize the harmful effects of solar radiation. Chemical UV filters are organic compounds with high molar absorptivity in the UV range. The compounds have single or multiple aromatic structures, sometimes conjugated with carbon, carbon double bonds and/or carbonyl groups (1, 2). There are currently 27 UV filters permitted by the EU Regulation 1223/2009 for commercial use. There are 26 organic filters, some water soluble and some fat soluble and one inorganic filter, titanium dioxide. UV organic filters can be classified into different groups according to their chemical structures: benzophenone derivatives: benzophenone 3 (BZ3) and benzophenone 4 (BZ4), EDP and P25; salicylates: ethylhexyl salicylate (ES) and homosalate (HMS); methoxycinnamates: ethylhexyl methoxycinnamate (EMC) and isoamyl *p*-methoxycinnamate (IMC); camphor derivatives: benzophenone-3, 4-methylbenzylidene camphor (MBC), benzilidene camphor sulphonic acid, camphor benzalkonium methosulphate, terephthalylidene dicamphor sulphonic acid and polyacrylamidomethyl benzylidene camphor; triazine derivatives: ethylhexyl triazone, diethylhexyl butamido triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine; benzotriazole derivatives: drometrizole trisiloxane and 2-hydroxy-4-methoxybenzophenone and benzimidazole derivatives: phenylbenzimidazole sulphonic acid and disodium phenyl dibenzimidazole tetrasulfonate. Others include butyl methoxydibenzoylmethane (BDM) and octocrylene (OCR).

All of the above listed compounds can be used in sunscreen and cosmetic products in amounts ranging from 2 to 10% and 25% for the inorganic compound titanium dioxide (3, 4).

There are three main regulatory bodies that control the amount and types of UV filters used in sunscreen and cosmetic products. They are the EU Regulation 1223/2009, the US Food and Drug Administration (FDA) and other equivalent agencies in countries around the world. While legislation exists, there are few official methods listed in the directives to determine the concentration of these components. The only method listed in the EU directive is a thin layer chromatography method, followed by a quantitative HPLC method for the analysis of glycerol 4-aminobenzoic acid, which is now banned in the EU (4–6).

Many HPLC methods have been developed by researchers over the years for the determination of UV filters in sunscreen and cosmetic products. The comparison of the photostability of five UV filters in sunscreen agents was carried out by Vanquerp *et al.* using a C_8 column and a mobile phase consisting of methanol and water (7). Chisvert *et al.* determined seven UV filters simultaneously, employing a C_{18} stationary phase. This was combined with a mobile phase of ethanol, water and acetic acid, containing cyclodextrins as a mobile phase modifier (8). Schakel *et al.* determined 16 UV filters including BZ3, BDM, EMC and OCR in sunscreen formulations. They employed a C_{18} stationary phase and a gradient ethanol–aqueous acetate buffer mobile phase, containing 0.2 mM ethylenediamine tetra acetic acid and the analysis took 32 min (9). Smyrniotakis and Archontaki used a 5-μm Hypersil BDS column for the determination of OCR, EMC, Tinosorb M and ES employing a mobile phase of methanol–acetonitrile (90:10, v/v) (10). Salvador and Chisvert developed an environmentally friendly method for the determination of 18 UV filters in cosmetics. Their method employed a C_{18} stationary phase and a mobile phase of ethanol and acetic acid was used for the fat-soluble compounds. A mixture of ethanol and sodium acetate buffer was used for the water-soluble compounds. The analysis run time was <30 min for the 12 fat-soluble filters, while the water-soluble filters took <10 min (11). Gasper and Campos evaluated the photostability of different UV filter combinations in sunscreen employing an ODS 5 μm column in combination with a mobile phase consisting of methanol and water. The analysis time took 27 min (12). Simeoni *et al.* developed a rapid HPLC method for the simultaneous determination of eight of the most common sunscreen filters using a cyanopropyl-bonded silica column with a mobile phase consisting of methanol–acetonitrile–tetrahydrofuran–water (13). Imamovic *et al.* carried out the determination of UV filters employing a range of columns. The most successful method

employed a C_8 column coupled with an acetonitrile–0.5% phosphoric acid (70:30, v/v) mobile phase, and the analysis time took 20 min (14). Dencausse *et al.* carried out the separation of four UV filters on a RP C_{18} Nucleodur Gravity column (150 × 4.6 mm, 5 μm). The mobile phase used was a gradient mixture of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid. The analysis time took ~30 min (15). Thomas of Thermo Fisher Scientific has recently developed an ultra-high performance liquid chromatographic method determining six UV organic filters, combining a Hypersil Gold 1.9 μm column and a mobile phase of 40:60, water–acetonitrile (16). Wharton *et al.* carried out the simultaneous rapid determination of seven of the UV filters most commonly found in sunscreen and cosmetics. The analysis was carried out on a Thermo Scientific Hypersil column 4.6 × 100 mm, 3 μm at a temperature of 25°C. The mobile phase employed was a gradient elution of ethanol and water containing 1% acetic acid (17). Balaguer *et al.* developed a method for the simultaneous determination of 12 UV filters in cosmetic samples. They carried out their analysis using an EMD Millipore Chromolith RP-18e monolithic column (4.6 × 100 mm) and a mobile phase of ethanol and acetic acid by gradient elution. (18). Liu and Wu carried out the simultaneous determination of 11 UV filters employing an Agilent SB-C_{18} column (4.6 × 250 mm, 5 μm), and a gradient elution of methanol, tetrahydrofuran and perchloric acid (19). Peruchi and Rath conducted the separation of eight UV filters on an Ace C_{18} column (4.6 × 250 mm, 5 μm). The mobile phase employed was an isocratic elution of methanol and water (80:20, v/v). The total run time was 18 min (20). Kapalavavi *et al.* carried out the separation of UV filters in skincare creams employing greener high-temperature liquid chromatography and subcritical water chromatography (21).

Other studies involve the examination of UV filters in environmental samples (22, 23). Chisvert *et al.* have more recently presented work detailing a reliable and environmentally friendly liquid-chromatographic method for multi-class determination of fat-soluble UV filters in cosmetic products; this separates 15 compounds in <30 min (24).

Many of the methods cited above have employed the use of toxic solvents involving longer analysis times. The objective of this study is to develop a method for the simultaneous determination of 10 UV filters in sunscreen employing ethanol, an environmentally friendly less toxic solvent and to carry this out in the shortest time possible. Some of the UV filters chosen for analysis have been found to be more difficult to separate because of the similarities in their structure, for example the organic filters EDP, EMC, BDM and ES. Also researchers have frequently found it difficult to separate the UV filter HMS because it exists in two isomers and the HMS(I) isomer co-elutes with BDM. The UV filters IMC and MBC are another pair that can be difficult to resolve. The study involves the examination of two columns; a Thermo Scientific Hypersil BDS and an EMD Chromolith RP-e Monolithic column, at various temperatures for the purpose of determining which column yields the optimal performance (Figure 1).

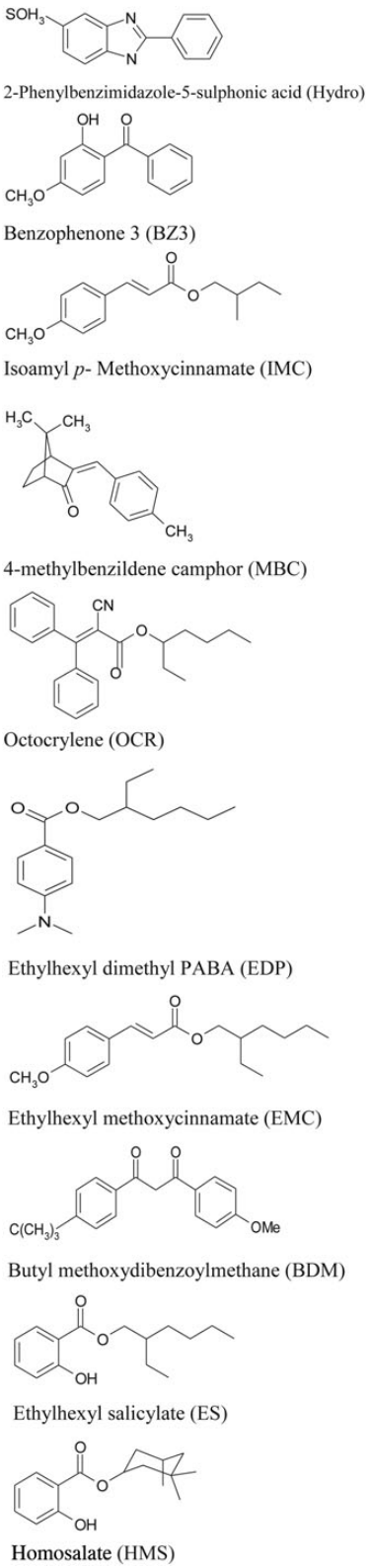

Figure 1. Structures of UV filters analyzed in this study.

Experimental

Apparatus

An Agilent HPLC 1100 Series equipped a G1312A binary pump, a G1313A auto-sampler, a G1315A DAD, a VWR Model 2003 Stand Alone Vacuum Degasser and a Shimadzu CTO-6A5 Stand Alone Column Oven. Data Acquisition was by Agilent Chemstation Rev. A.10.02. A Grant GD100 Water Bath was used for heating

the solvents. A Thermo Scientific Hypersil C_{18} BDS, 3 μm column (4.6 × 100 mm) and an EMD Millipore Chromolith RP-18e Monolithic column (4.6 × 100 mm) were employed for the method development and validation.

Reagents

BZ3, EDP 98%, OCR 97% and ES 99% were purchased from Sigma-Aldrich Ireland. BDM, MBC, HMS and EMC were obtained from S. Black & Co., UK. 2-Phenylbenzimidazole-5-sulfonic acid (Hydro) and IMC were obtained from Symrise Ltd., UK. Analytical grade acetic acid and sodium hydroxide were obtained from Sigma-Aldrich Ireland and HPLC gradient grade ethanol was obtained from VWR Ireland. Ultra-pure water was obtained using an Easypure LF ultra-pure water system. A number of commercial samples of sunscreen products were analyzed.

Method

A 500 μg/mL multicomponent stock solution was prepared by weighing 50 mg of each filter and dissolving in ethanol. Two drops of a 2% solution of sodium hydroxide was added to this solution to dissolve the UV filter Hydro, which is not soluble in ethanol. The solution was diluted to the mark in a 100-mL volumetric flask with ethanol. A working solution of 100 μg/mL was prepared by taking 20 mL of the 500 μg/mL solution and diluting to the mark in a 100-mL volumetric flask with ethanol. This 100 μg/mL standard was used to determine the selectivity of the method and for robustness studies.

A series of multicomponent standards of the 10 UV filters were prepared in the following range; 10, 20, 40, 60, 80 and 100 μg/mL for the purpose of carrying out linearity and accuracy studies. A series of 20 and 80 μg/mL multicomponent standards of the 10 UV filters were prepared for the determination of injection and analysis precision, whereas 10, 60 and 100 μg/mL standards were prepared for the intermediate precision. All solutions were prepared on a daily basis.

The commercial samples were prepared by dissolving 0.1 g of each sample in ethanol and diluting to the mark in a 25-mL volumetric flask. The solutions were then placed in a sonication bath to dissolve the samples. A 1 mL aliquot of the 25 mL solution was then diluted to 10 mL with ethanol; this solution was filtered prior to analysis.

Table I
Gradient Table for the Determination of Ten UV Filters Employing the EMD Millipore Chromolith Monolithic Column

Time (min)	% A	% B	Flow rate (mL/min)
0.00	40.0	60.0	2.0
4.80	30.0	70.0	2.0

Results and discussion

Development and optimization of the method employing the Chromolith RP-18e Monolithic column

The method was initially developed employing the Chromolith RP-18e monolithic column to evaluate its suitability to separate the 10 compounds in question. A 100-μg/mL multicomponent solution of all compounds was analyzed employing an isocratic elution of ethanol and 1% acetic acid (70:30, v/v) at a flow rate of 1.0 mL/min, the column temperature was maintained at 25°C and the overall analysis time took 8 min. The resolution between the UV filters IMC and MBC and the UV filters EMC and BDM was less than desired for the monolithic column under these conditions. Another problem also existed in that HMS elutes as two isomers; HMS (I) and HMS (II). The isomer HMS (I) usually co-elutes with BDM. The aforementioned peaks could not be resolved isocratically. A number of gradient separations were also attempted at various flow rates and temperatures ranging from 25 to 30°C in an attempt to successfully resolve all the compounds. The best resolution of these compounds was achieved employing the gradient elution seen in Table I, which was carried out at a temperature of 28°C and a flow rate of 2 mL/min.

The resolution achieved with the gradient employed for the separation of the IMC/MBC was $R_s = 1.63$ and EMC/BDM the $R_s = 2.2$. Unfortunately, the HMS (I) isomer could not be separated sufficiently without compromising the resolution of the other peaks of interest. The total run time was 7.0 min with 3.0 min calculated for column re-equilibration at the initial conditions, giving a total analysis time of 10 min for the elution of the 10 UV filters. The injection volume was 5 μL and detection was performed by using a diode array detector (DAD) at 313 nm with a reference wavelength of 700 nm. The chromatogram of the peaks generated employing this method is shown in Figure 2.

Development and optimization of method employing the Thermo Scientific Hypersil BDS column

The evaluation of the Thermo Scientific Hypersil (4.6 × 100 mm, 3 μm) column to determine its suitability in separating the 10 UV filters was carried out employing the same mobile phase of

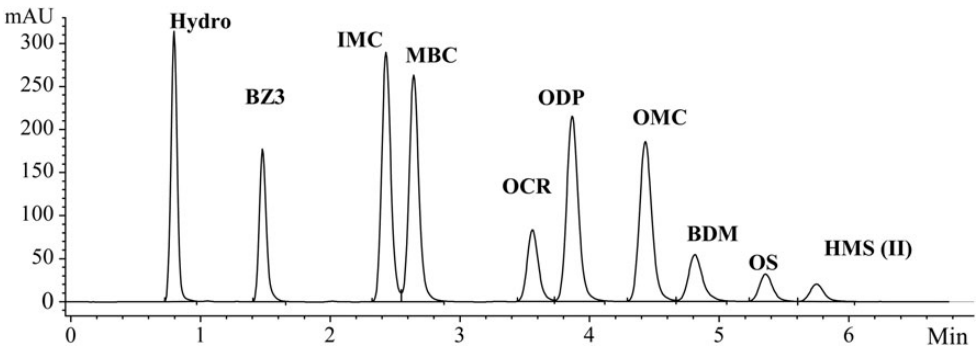

Figure 2. Chromatogram of peaks employing the Chromolith RP-18e Monolithic column.

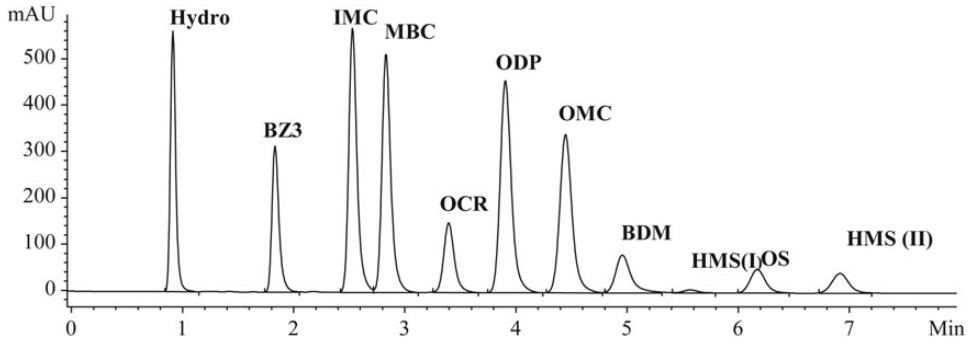

Figure 3. Chromatogram of peaks employing the Thermo Hypersil BDS column.

ethanol and acetic acid (70:30, v/v). A 100 μg/mL multicomponent standard was analyzed employing an injection volume of 10 μL, a flow rate of 1.0 mL/min, at a temperature of 25°C with detection at 313 nm. The total analysis time took ~12 min, with all of the compounds well resolved including the critical pairs IMC/MBC which had $R_s = 2.19$ and EMC/BDM with $R_s = 2.20$. It was also noted that the HMS (I) isomer was well resolved from the UV filter BDM, with $R_s = 2.26$. However, the overall analysis time was disappointing at 12 min. It was decided to try to further optimize the method to see if the analysis time could be improved on without compromising the resolution of the critical peaks. A number of isocratic and gradient runs were attempted at various flow rates and at temperatures varying from 25 to 40°C in an effort to resolve the critical peaks in the shortest time possible. The system was also optimized by installing shorter capillary connections for the purpose of reducing all dead volume.

The analysis which achieved the desired results was carried out in an isocratic mode of (70:30, v/v) ethanol and 1% acetic acid, at a flow rate of 1.0 mL/min, a temperature of 35°C, detection by DAD at 313 nm and by reducing the injection volume to 5 μL. The back pressure was found to be 250 bar, and the overall analysis time was <7.0 min, with the last peak eluting at 6.916 min. The resolution for the critical pair IMC/MBC was found to be 2.24 and the resolution of the critical pair EMC/BDM was 2.38. The HMS (I) isomer was completely resolved from the BDM peak with resolution of 2.60. The analysis was repeated over a number of days with replicates of six injections of 100 ppm multicomponent standards of the UV filters, to check the reproducibility of the method. A chromatogram of peaks generated with this method can be seen in Figure 3.

Validation of optimized method

The method employing the Thermo Scientific Hypersil column was found to generate consistent results and so it was decided to validate this method according to the acceptance criteria of the FDA and ICH guidelines for linearity, accuracy, limit of detection, limit of quantitation and precision. All criteria were met for each of the parameters tested and the results are shown in Tables II and III.

Robustness test

The robustness of the method was tested by making minor changes to the pH of the mobile phase and to the operating temperature of the method. The pH of the mobile phase was adjusted by increasing the concentration of the acetic acid in the mobile phase from 1 to 1.5% and 2%; this corresponded to pH 2.68, 2.72 and 2.80, respectively. Six injections of a 100 ppm multicomponent standard of the UV filters were carried out and the mean of the retention times and resolution of the peaks of interest were evaluated to determine if the pH adjustments had any significant impact on the results. For the evaluation of the impact of temperature change on retention times and resolution, six replicate injections of the 100 ppm multicomponent standard of the UV filters were carried out at temperatures of 34 and 36°C, respectively. The average resolution (R_s) of the peaks of interest was calculated for this test. It was concluded that the various pH adjustments and temperature changes had very little impact on the retention times or the resolution of the compounds with all peaks eluting within 7 min.

The conclusion for the robustness test is that this method has proven to be a robust and reliable method with consistently reproducible results, provided it is operated within the parameters established in the method developed. To further test this method

Table II
Regression Equations, Correlation Coefficient for Linearity, LOD and LOQ on the Thermo Scientific Hypersil BDS Column

Compound	Regression equation	r^2 values	LOD, μg/mL	LOQ, μg/mL
Hydro	$y = 20.02x + 9.705$	0.9991	0.10	0.50
BZ3	$y = 13.917x + 3.501$	0.9998	0.25	0.50
IMC	$y = 25.921x + 10.225$	0.9998	0.10	0.40
MBC	$y = 28.553x + 8.582$	0.9998	0.10	0.40
OCR	$y = 10.011x - 3.195$	0.9999	0.40	0.80
EDP	$y = 31.905x + 0.612$	0.9999	0.30	0.50
EMC	$y = 26.437x - 3.901$	0.9999	0.30	1.00
BDM	$y = 7.7847x - 13.520$	0.9986	0.50	1.00
ES	$y = 5.0031x - 2.7434$	0.9999	1.00	3.00
HMS	$y = 4.5166x - 0.9143$	0.9995	2.00	5.00

Table III
Percentage RSD Values for Accuracy for Ten UV Filters Employing the Thermo Scientific Hypersil BDS Column

UV filter	10 ppm	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm
Hydro	98.03	100.10	100.40	102.30	99.45	98.75
BZ3	98.10	100.25	100.70	101.30	99.99	99.40
IMC	98.20	100.35	100.90	101.50	99.80	99.16
MBC	98.30	100.60	100.75	101.38	99.79	99.30
OCR	98.26	98.75	99.73	101.78	100.76	100.50
ODP	99.60	99.35	100.47	101.05	99.88	99.38
OMC	98.66	99.60	100.17	101.20	100.30	100.70
BDM	99.50	98.75	98.50	98.46	101.80	102.31
OS	98.35	98.00	100.40	100.60	101.40	101.20
HMS	99.60	98.75	102.20	98.90	100.40	99.17

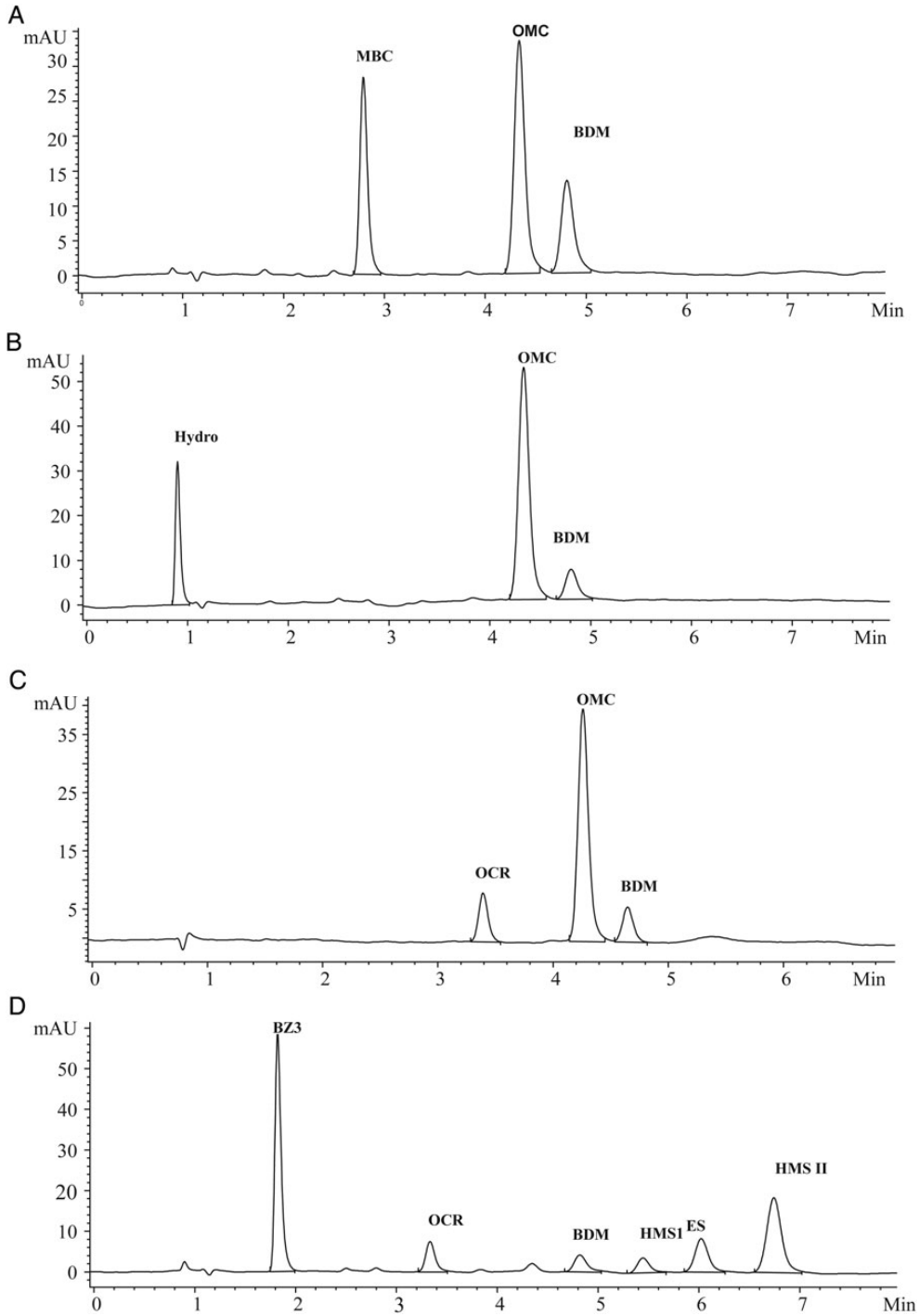

Figure 4. Chromatograms of commercial sun protection products: (A) Simple SPF 15 lotion, (B) L'Oreal SPF 30 spray, (C) Boots SPF 30 lotion and (D) Banana Boat SPF 50.

it was employed for the identification of UV filters in eight commercial samples. An example of some of the chromatograms of the commercial samples is shown in Figure 4.

Comparison of the Thermo Scientific Hypersil 3 μm column and the EMD Millipore Monolithic column

The EMD Millipore Chromolith RP-18e and the Thermo Scientific Hypersil columns used in this project for the determination of the 10 UV filters found in sunscreen and cosmetics have both been found suitable for their intended purpose. However after conducting a comparative study based on the separation of the 10 UV filters on both columns using a 100-ppm multicomponent standard, the Thermo Scientific Hypersil 3 μm column was found to offer more advantages in terms of faster analysis time, reduced solvent use and greater resolution of the critical pairs IMC/MBC and EMC/BDM. The HMS (I) isomer was also completely resolved from the BDM filter. In relation

Table IV
Comparison of Performance of the EMD Millipore Monolithic Column Versus the Thermo Scientific Hypersil BDS Column

UV filter	R_t BDS	R_t Mono	R_s BDS	R_s BDS	Peak width BDS	Peak width mono	Plates BDS	Plates mono
Hydro	0.92	0.79			0.053	0.053	1,693	1,248
BZ3	1.83	1.47	9.30	7.32	0.063	0.058	4,701	3,721
IMC	2.52	2.43	5.86	8.54	0.075	0.075	6,215	5,875
MBC	2.81	2.65	2.24	1.65	0.082	0.079	6,512	6,238
OCR	3.37	3.59	3.01	6.44	0.097	0.092	6,745	8,376
EDP	3.88	3.90	2.93	2.13	0.101	0.097	8,123	9,037
EMC	4.42	4.49	2.41	3.43	0.113	0.103	8,409	10,454
BDM	4.92	4.88	2.48	2.13	0.114	0.113	7,370	10,280
ES	6.13	5.45	2.39	2.96	0.142	0.113	10,387	13,112
HMS	6.87	5.86	2.89	2.11	0.158	0.117	10,436	14,127

to the analysis time, while the overall analysis time was reduced with the BDS column, technically the monolithic column provided the faster analysis times, with the last peak eluting in <6 min, whereas with the BDS column the last peak elutes in <7 min. However because the separation with the monolithic column was a gradient one, the column had to be re-equilibrated at the initial conditions for 3 min, hence giving it a longer run time. A point in favor of the monolithic column is the fact that it can be operated at higher flow rates without generating higher backpressures. Unfortunately in this case it presents a disadvantage because of the greater solvent usage. In terms of solvent use the BDS column offered the greater advantage. Taking as an example each time that linearity studies are conducted a series of six injections of six standards have to be carried out; this equals a total of 36 injections. With the method developed on the BDS column having a run time of 7 min and operating at a flow rate of 1.0 mL/min, this is equivalent to using 252 mL of mobile phase. Whereas the monolithic column having an overall run time of 10 min and a flow rate of 2.0 mL/min, resulting in a solvent consumption of 720 mL, more than double that of the BDS column. In terms of resolution the BDS column offers better separation of the critical pair IMC/MBC with $R_s = 2.24$, whereas with the monolithic column the resolution value is 1.65. With the BDS column the resolution for the critical pair OMC/BDM is 2.41 while the resolution for the same pair with the monolithic column is 2.13. The Thermo Scientific Hypersil BDS column also allows the separation of the HMS (I) isomer from the UV filter BDM; whereas the UV filter BDM co-elutes with the HMS (I) isomer and cannot be separated on the monolithic column without compromising the resolution of the critical pairs. This is important in terms of being able to quantify the amount of BDM that is contained in a sample which also contains the UV filter HMS. The successful separation of these two UV filters is shown in Figure 4, sample D, for the analysis of the sun protection lotion Banana Boat SPF 50, which contains both the UV filter BDM and HMS. The comparison between the two columns in terms of retention time, resolution, peak widths and theoretical plates is shown in Table IV.

The conclusion that may be taken from these results is that the Thermo Scientific Hypersil BDS column in combination with the mobile phase employed is the more suitable column for the simultaneous determination of the 10 UV filters employed in this research. This is a rapid and simple method and because of the isocratic separation employed with the BDS column it makes it a simpler method to transfer than a gradient method, from one HPLC system to another and from one laboratory to another, provided the conditions outlined in the method are adhered to.

Conclusion

The objective of the method is to develop a rapid HPLC method for the simultaneous determination of 10 UV filters found in sunscreen, employing an environmentally friendly solvent and carrying out the analysis in the shortest possible time. This objective was achieved using a Thermo Scientific Hypersil C_{18} column (4.6 × 100 mm, 3 μm), employing an isocratic mobile phase of ethanol and 1% acetic acid (70:30, v/v), at a temperature of 35°C, in a time frame of 7 min. The advantage of using the method that was developed on the Thermo Scientific Hypersil BDS column over the monolithic column is that it uses almost a third less of solvent therefore making it even more environmentally friendly. It also offers better resolution of all the compounds and completely resolves the HMS (I) isomer from the BDM UV filter. The fact that this is an isocratic elution makes it easier to transfer from one system to another and from one laboratory to another.

This method demonstrates that it is possible to develop a rapid method with the clever use of temperature on a conventional HPLC system. It also demonstrates the suitability of the Thermo Scientific Hypersil column for the analysis of UV filters, which gave the optimal performance.

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