Application note | 90724

Liquid chromatography

Simultaneous determination of multiple sunscreen filters using Vanquish Flex System for efficient UHPLC-PDA sunscreen analysis

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Keywords

Vanquish Flex System, UHPLC, Accucore C18 column, sample preparation, Chromeleon CDS, sunscreen filter, photodiode array detector (PDA)

Application benefits:

- Workflow provides quantitative analysis of multiple sunscreen ultraviolet (UV) filters in a single application using the Thermo Scientific™ Vanquish™ Flex UHPLC system.
- Workflow enables efficient analysis of multiple UV filters irrespective of product formulation by utilizing a single application.

Goal

To develop a single workflow for the simultaneous quantitative analysis of UV filters in sunscreen formulations irrespective of formulation using the Vanquish Flex UHPLC System and the Thermo Scientific™ Accucore™ C18 column, with the potential to combine fifteen separate product formulation analysis methods into one application.

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Introduction

One in every three cancers diagnosed globally is a skin cancer[1], with melanoma being the deadliest form of skin cancer.

Australia has one of the highest rates of melanoma in the world, with 1 Australian diagnosed with melanoma every 30 minutes[2]. Overexposure to UV light causes 95% of melanoma. As with the majority of skin cancers, the best prevention strategy involves a combination of sun protection measures.

Since the usage of broad-spectrum sunscreen is vital in the prevention of melanoma and other skin cancers, it is important for the skincare industry to develop robust analytical methods to maintain compliance with safety regulations. The formulations developed may not all contain the same combination of UV filters.

High-performance liquid chromatography (HPLC) methods specific to each formulation add to the operating costs of the analytical laboratory and may significantly decrease output efficiency due to the system setup and shutdown required during analysis method change. Hence, a challenge was set to develop a single method to analyze UV filter content of multiple product formulations. In addition to being a universal method, the method was designed to reduce environmental impact as much as possible.

This application note details a single ultra high-performance liquid chromatography (UHPLC) analysis method that provides a robust, quantitative, reproducible and accurate analysis of multiple sunscreen UV filter ingredients when used to analyze different sunscreen formulations. The UHPLC method conditions and its performance are described in this application note. Precision, linearity and accuracy are evaluated to ensure that the method is fit for purpose.

Experimental

Chemicals for Chromatography

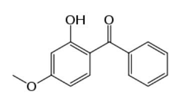
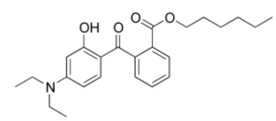
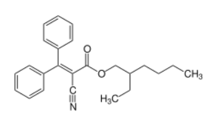
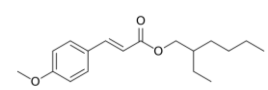
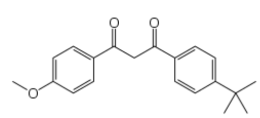
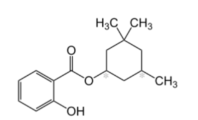
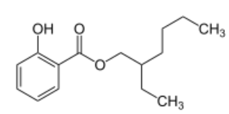
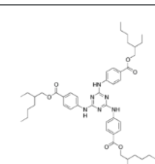
- Deionized (DI) water 18 MΩ·cm resistivity or higher
- Ethanol, HPLC grade (P/N 10542382)

Chemicals for Sample Preparation

- Methyl-Tetrahydrofuran (mTHF), HPLC Grade (P/N 10387820)
- Acetic acid, Optima™ LC/MS grade (P/N 10860701)
- Ethanol 95% (P/N 10315752)

Standards

- (2-Hydroxy-4-methoxyphenyl)(phenyl)methanone (OB)
- Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate (UAP)
- 2-Ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate (OC)
- (*RS*)-2-Ethylhexyl (2*E*)-3-(4-methoxyphenyl)prop-2-enoate (OMC)
- 3-(4-*tert*-Butylphenyl)-1-(4-methoxyphenyl)propane-1,3-dione (BMDM)
- 3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate (HS)
- 2-ethylhexyl 2-hydroxybenzoate (OS)
- 4-[[4,6-bis[[4-(2-ethylhexoxy-oxomethyl)phenyl]amino]-1,3,5-triazin-2-yl]amino]benzoic acid 2-ethylhexyl ester (OTA)
- 2,2′-Methylenebis[6-(2*H*-1,2,3-benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol] (MBTP)
- 2,2′-[6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl]bis{5-[(2-ethylhexyl)oxy]phenol} (BEMPT)

	
(2-Hydroxy-4-methoxyphenyl)(phenyl)methanone (OB)	Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate (UAP)
	
2-Ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate (OC)	(*RS*)-2-Ethylhexyl (2*E*)-3-(4-methoxyphenyl)prop-2-enoate (OMC)
	
3-(4-*tert*-Butylphenyl)-1-(4-methoxyphenyl)propane-1,3-dione (BMDM)	3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate (HS)
	
2-ethylhexyl 2-hydroxybenzoate (OS)	4-[[4,6-bis[[4-(2-ethylhexoxy-oxomethyl)phenyl]amino]-1,3,5-triazin-2-yl]amino]benzoic acid 2-ethylhexyl ester (OTA)

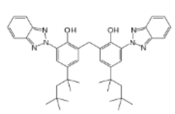
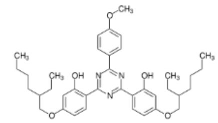
	
2,2′-Methylenebis[6-(2*H*-1,2,3-benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol] (MBTP)	2,2′-[6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl]bis{5-[(2-ethylhexyl)oxy]phenol} (BEMPT)

Figure 1. Chemical structures of sunscreen filters

Sample Handling

- Schott bottle 500 mL, 1000 mL
- 50 mL grade A volumetric flask
- Solvent filter system fitted with 0.2 µm membrane
- 0.2 µm nylon filters & 5mL Luer lock syringes
- Thermo Scientific™ Sorvall ST16™ centrifuge
- 0.2 µm Fisher Scientific™ Fisherbrand™ filter centrifuge Tubes, 2 mL (P/N 10477254)
- Fisher Scientific ™ CPXH Series Digital Ultrasonic Cleaners
- Thermo Scientific™ Finnpipette™ F1 Variable Volume Single-Channel Pipettes: 100–1000 µL, 10–100 µL, 1–10 µL (P/N 4641100N, 4641070N, 4641030N)
- 4-digit analytical balance
- 2 mL HPLC vials and screw caps (P/N 2-SVWGKST-CPK)

Instrumentation

Vanquish Flex UHPLC system consisting of:

- Vanquish System Base (P/N VF-S01-A-02)
- Vanquish Binary Pump F (P/N VF-P10-A-01)
- Vanquish Autosampler FT (P/N VF-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-03), active pre-heater was not enabled
- Vanquish Photodiode Array Detector (P/N VF-D11-A-01)

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3.1 (P/N CHROMELEON7)

Accucore C18 Column (P/N 11317471)

Preparation of Standard Solutions

Stock Standard

The stock standard was prepared as follows:

1. Accurately weigh each UV filter into a 50 mL volumetric flask to prepare a single solution with the concentrations given in Table 1
2. Add extraction solvent Acetic Acid:mTHF, 30:70 v/v
3. Sonicate to dissolve UV filters
4. Cool to ambient temperature
5. Make to 50 mL volume with extraction solvent Acetic Acid:mTHF, 30:70 v/v

Table 1. Concentration of UV filters in stock standard

UV filters (Abbreviation)	Concentration (w/V)
OB	0.1500 mg/mL
UAP	0.2500 mg/mL
OC	0.5000 mg/mL
OMC	0.5000 mg/mL
BMDM	0.2500 mg/mL
HS	0.5000 mg/mL
OS	0.5000 mg/mL
OTA	0.1000 mg/mL
MBTP	0.1500 mg/mL
BEMPT	0.1500 mg/mL

Standard Solutions

Standard solutions were prepared by diluting the stock standard solution using Ethanol 95%, according to volume stated in Table 2.

Table 2. Dilution for standard solution preparation

Standard Solution	Pipette Volume (mL)	Final Volume (mL)
STD 1	5.00	50
STD 2	10.00	50
STD 3	15.00	50
STD 4	20.00	50

After dilution, standards were filtered using either a 0.2 µL nylon syringe filter or 0.2 µL nylon centrifuge filter (5,000 rpm for 5 minutes) and immediately transferred into HPLC vials.

Sample Solutions

Sample solutions were prepared in duplicate as follows:

1. Accurately weigh 0.1000 g of sunscreen formulation sample into a 50 mL volumetric flask
2. Disperse sample using 5 mL water
3. Sonicate for 2-3 minutes to disperse sample
4. Add 10 mL Acetic Acid:mTHF, 30:70 v/v using Finnpipette F1 Variable Volume Single-Channel Pipette
5. Sonicate each sample for 10 minutes, inspect sample for dispersion, sonicate further if desired.
6. Add Ethanol 95% to bring volume to approximately 45 mL
7. Cool flasks to ambient temperature
8. Make to 50 mL volume with Ethanol 95%, shake well
9. Filter standard solutions using either a 0.2 µL syringe filter or 0.2 µL centrifuge filter (5,000 rpm for 5 minutes)
10. Immediately transfer into HPLC vial

Chromatographic Conditions

The chromatographic system was set up according to the conditions given in Table 3.

Table 3. Chromatographic conditions

Column	Accucore C18, 2.6 µm, 100 mm x 2.1 mm			
Mobile Phase	A: Deionized (DI) water B: Ethanol, HPLC grade, 100%			
Gradient Program	Time (min)	(%A)	(%B)	Flow rate (mL/min)
	0.00	25.0	75.0	0.12
	8.00	25.0	75.0	0.12
	8.10	0.0	100.0	0.12
	13.00	0.0	100.0	0.18
	19.00	0.0	100.0	0.18
	20.00	25.0	75.0	0.18
	22.00	25.0	75.0	0.18
	23.00	25.0	75.0	0.12
	25.00	25.0	75.0	0.12
Column Temperature	40 °C, forced air mode			
Detection, dual wavelength	UV Absorbance, 311 nm UV Absorbance, 350 nm, for OB detection			

Results and Discussion

Separation of Ten UV filters

The ten UV filters in this work are all ideal candidates for reversed-phase chromatography with UV detection. The Accucore C18 column was chosen because it contains small-pore, high-purity, low-metal content silica with high C18 surface coverage (i.e. high carbon load), that is ideal for developing high resolution separations of compounds typically analyzed by reversed-phase chromatography.

Figure 2 shows a chromatogram of the ten sunscreen UV filters under the specified chromatographic conditions, where HS-1 and HS-2 are the 2 stereoisomers of 3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate (HS).

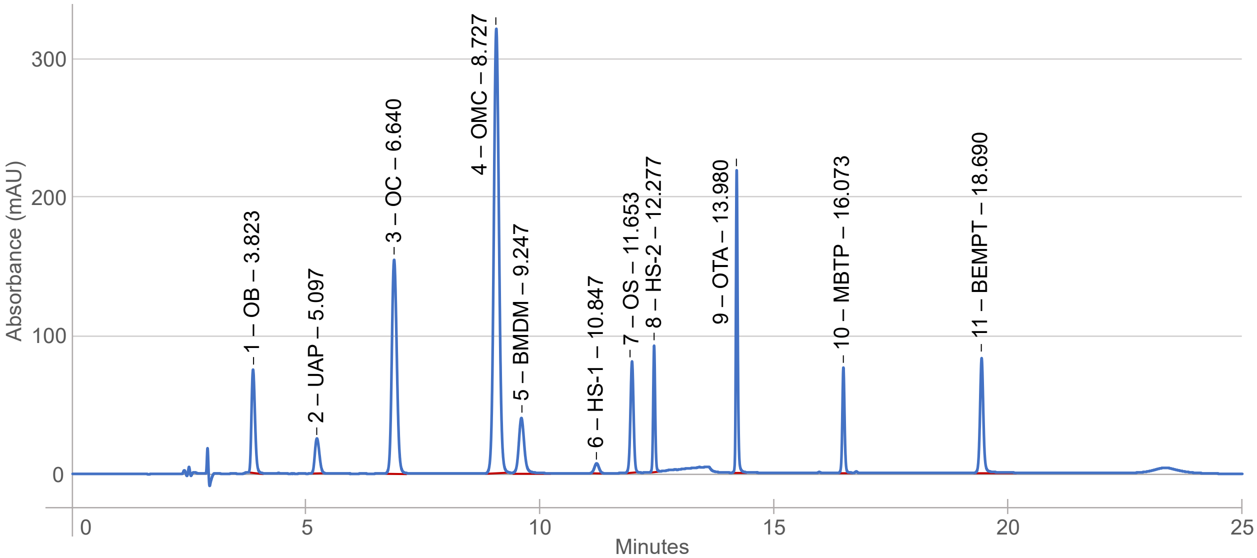


Figure 2. UV chromatograms obtained from Standard 1, where ten sunscreen UV filters are separated via a 25 min gradient.

Method Precision, Linearity, and Accuracy

Precision Data

Short-term method precision was evaluated by making six consecutive injections of the Calibration Standard 2. Retention time and peak area reproducibility (RSDs) were obtained for each analyte (Table 4). Retention time RSDs were all ≤0.50%, and peak area RSDs were all ≤1.10%, demonstrating good short-term precision for this method.

Linearity Data

Calibration linearity for UV detection of the UV filters was investigated by making duplicate consecutive 1 µL injections of a standard prepared at seven different concentrations. Each analyte exhibited a linear relationship in the specified concentration range when plotting peak area versus concentration. The linearity data is listed in Table 5. These calibrations will be used to quantify the UV filters in the sunscreen formulation samples.

Table 4. Method precision data

Analyte	Retention Time %RSD	Peak Area %RSD
OB	0.29	0.31
UAP	0.41	0.34
OC	0.50	0.08
OMC	0.38	0.27
BMDM	0.33	0.80
HS -1	0.24	1.07
HS -2	0.07	0.26
OS	0.21	0.22
OTA	0.02	0.64
MBTP	0.01	0.14
BEMPT	0.02	0.63

Table 5. Method linearity data

Analyte	Regression Equation	r^2	Range (mg/mL)
OB	y = 210.8x + 0.0320	0.9999	0.007-0.058
UAP	y = 55.1x + 0.0425	0.9998	0.012-0.097
OC	y = 182.6x + 0.1911	0.9998	0.026-0.204
OMC	y = 429.0x + 0.4652	0.9997	0.025-0.198
BMDM	y = 116.4x - 0.0801	0.9997	0.020-0.101
HS	y = 58.425x + 0.044	1.0000	0.025-0.201
OS	y = 61.8x + 0.0927	0.9998	0.025-0.204
OTA	y = 486.0 – 0.3327	0.9999	0.008-0.041
MBTP	y = 156.1x + 0.0406	0.9998	0.012-0.058
BEMPT	y = 249.5x – 0.3672	0.9997	0.012-0.058

Accuracy Data

Accuracy of the analysis method for analysis of the UV filters was investigated by making duplicate consecutive 1 µL injections of a standard prepared at seven different concentrations. The accuracy and recovery data are found in Table 6 and demonstrates the suitability of this method for accurate quantitation of UV filters.

Sample Analysis

Using the earlier described extraction solvent, Acetic Acid:mTHF, 30:70 v/v, UV filters were efficiently extracted from five sunscreen formulations in an ultrasonic bath. The sunscreen formulations were analysed after extraction, using the UHPLC method described. Figure 3 shows the chromatogram of a representative sample.

Table 6. Method accuracy data

Analyte	% recovered	%RSD
OB	99.92	0.64
UAP	100.04	0.74
OC	99.99	0.67
OMC	99.99	0.67
BMDM	101.35	0.97
HS	99.88	0.73
OS	100.39	1.01
OTA	100.21	0.81
MBTP	99.89	0.67
BEMPT	99.50	1.14

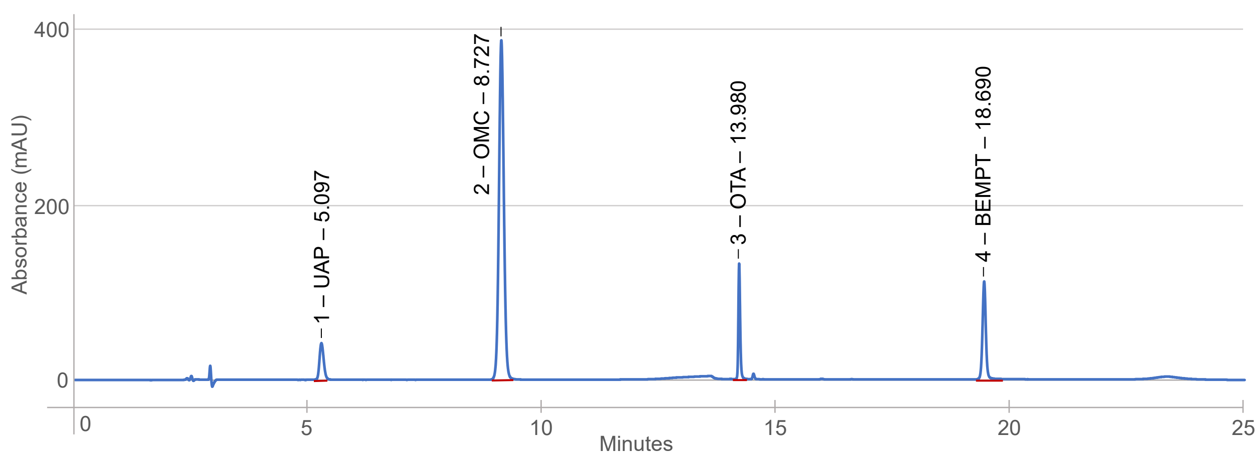


Figure 3. UV chromatogram of a representative sample, separated via a 25 min gradient, demonstrating efficiency and fit for use in sunscreen UV filter quantitation

Seven of the sunscreen UV filters were observed in the formulations assayed. The analysis results, % recovered and %RSD are given in Table 7.

Table 7. % Recovery and %RSD data of ten UV filters in five sunscreen formulations

Analyte	Formulation 1		Formulation 2		Formulation 3		Formulation 4		Formulation 5	
	% recovered	% RSD	% recovered	% RSD	% recovered	% RSD	% recovered	% RSD	% recovered	% RSD
OB										
UAP	101.9	1.10	104.0	0.63	100.5	0.90	103.3	0.08	101.3	0.11
OC			101.0	0.53	101.0	0.43			100.0	0.03
OMC	101.8	1.10			97.7	0.56	103.2	0.15	101.3	0.03
BMDM			99.5	0.53	99.3	0.87				
HS			100.5	0.50						
OS										
OTA	101.47	0.59					105.9	0.67	99.5	0.5
MBTP										
BEMPT	98.64	1.50	101.7	0.23	101.2	0.49	100.5	1.2	100.0	0.08

Conclusion

This work describes an efficient UHPLC method with UV detection for the simultaneous determination of ten sunscreen UV filters in five sunscreen formulations with the advantages of good method precision, accuracy and a wide linearity range. The method design has successfully minimised the environmental impact of sunscreen analysis in the following ways:

- Replacing tetrahydrofuran (THF) with the more environmentally friendly option, mTHF, which is derived from biomass and renewable, aligns with the "Safer Solvents and Auxiliaries" principle of green chemistry.
- Reduction of solvent consumption and waste generation by transitioning from traditional HPLC to UHPLC, allowing for a reduction in the flow rate.
- Continuous system operation reduces wear and tear on instrument components by minimizing the stress associated with startup and shutdown procedures.
- Reduced use of chemicals, through creation of a universal standard solution.

References

[1] www.skincancer.org/skin-cancer-information
[2] https://melanoma.org.au/about-melanoma/