Application Note 1118

Determination of Sunscreen Agents in Sunscreen Cream

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Key Words
Acclaim 120 C18 Column, Cosmetic Quality, Cosmetic Safety, HPLC, UV-absorbent

Goal
To develop an efficient High-Performance Liquid Chromatography (HPLC) method for the determination of sunscreen agents in sunscreen cream due to the potential of developing a skin allergy to light and birth defects in humans. The sunscreen agents to be determined are *p*-aminobenzoic acid, phenyl salicylate, 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid, 2-ethylhexyl-4-(dimethylamino)benzoate, 2-ethylhexyl salicylate, 3,3,5-trimethylcyclohexyl salicylate, 2-hydroxy-4-methoxybenzophenone, 2-ethylhexyl-4-methoxycinnamate, 2,2',4,4'-tetrehydroxybenzophenon, avobenzone butylmethoxydibenzoylmethane, and 3-(4-methylbenzylidene) camphor.

Introduction
Long term exposure to strong sunlight may result in skin burns (i.e. "sunburn") and a potential future risk of developing skin cancer, due to the ultra violet (UV) portion of sunlight. Therefore, sunscreen agents are added to some cosmetics to deliver sunscreen function. However, long-term contact to the cosmetics that have excessive addition of sunscreen agents may increase the risk of developing a skin allergy to light as well as birth defects.[1] As a result, the types and amounts of sunscreen agents in sunscreen cosmetics have been strictly regulated. Table 1 lists the permitted active ingredients of sunscreen agents in sunscreen cosmetics in the European Union (EU), the United States (USA), China (CHN), and Japan (JP),[2-5] showing that the requirements of some sunscreen agents in these regulations vary greatly. For example, *p*-aminobenzoic acid is permitted in the USA and CHN, but is forbidden in the EU and JP; 3-(4-methylbenzylidene) camphor is permitted in the EU and CHN, but is forbidden in the USA and JP. In addition, some compounds with sunscreen function are not listed in these regulations (e.g., phenyl salicylate), yet they are still added to some sunscreen cosmetics and that may result in potential human health problems.[1] Therefore, effective methods for simultaneously detecting multiple sunscreen agents are necessary. HPLC has been applied extensively for this application.[6,7] Figure 1 shows structures of eleven sunscreen agents that will be determined in this work, including the first eight compounds in Table 1 and three unlisted compounds, phenyl salicylate, 2,2',4,4'-tetrehydroxybenzophenon, and 2-ethylhexyl-4-(dimethylamino)benzoate.

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Table 1. Active ingredients of sunscreen agents in sunscreen cosmetics and their permitted concentrations in different parts of the world.

Active Ingredients	Permitted Concentration			
	USA	EU	CHN	JP
***p*-Aminobenzoic acid**	15%	Forbidden	5%	Forbidden
2-Hydroxy-4-methoxybenzophenone	6%	10%	10%	5%
3,3,5-Trimethylcyclohexyl salicylate	15%	10%	10%	10%
2-Ethylhexyl-4-methoxycinnamate	7.5%	10%	10%	20%
2-Ethylhexyl salicylate	5%	5%	5%	10%
2-Hydroxy-4-methoxy-benzophenone-5-sulfonic acid	10%	5%	5%	10%
Avobenzone butylmethoxydibenzoylmethane	3%	5%	5%	10%
3-(4-Methylbenzylidene) camphor	Forbidden	4%	4%	Forbidden
Octocrylene	10%	10%	10%	10%
Menthyl anthranilate	5%	Forbidden	Forbidden	Forbidden
Trolamine salicylate	12%	Forbidden	Forbidden	Forbidden
Ecamsule	3%	10%	10%	Forbidden
Titanium dioxide	25%	25%	25%	No limit
Zinc oxide	25%	25%	25%	No limit
Tinosorb M	Forbidden	10%	10%	10%
Tinosorb S	Forbidden	10%	10%	3%
Neo heliopan AP	Forbidden	10%	Forbidden	Forbidden
Mexoryl XL	Forbidden	15%	15%	Forbidden
Benzophenone-9	Forbidden	Forbidden	Forbidden	10%
Uvinul T 150	Forbidden	5%	Forbidden	3%
Uvinul A plus	Forbidden	10%	Forbidden	10%
Uvasorb HEB	Forbidden	Forbidden	10%	5%
Parsol SLX	Forbidden	10%	10%	10%
Isopentenyl-4-methoxycinnamate	10%	10%	10%	Forbidden
Padimate-O	8%	Forbidden	8%	10%
Phenylbenzimidazole sulfonic acid	4%	8%	8%	3%
Cinoxate	3%	Forbidden	Forbidden	Forbidden
Dioxybenzone	3%	Forbidden	Forbidden	Forbidden

Note: USA (the United States of America), EU (European Union), CHN (China), and JP (Japan).

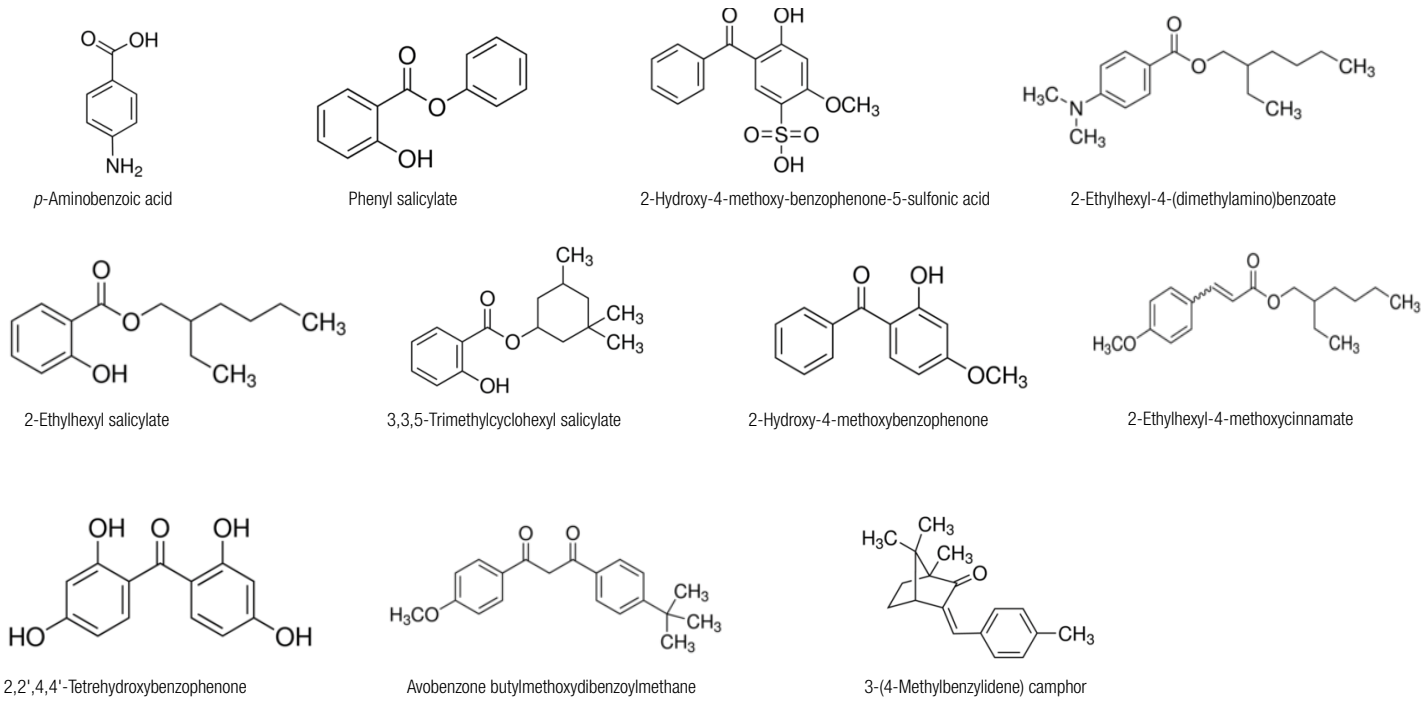

Figure 1. Structures of sunscreen agents.

Equipment and Software

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation Dual HPLC system, including:
  - DGP 3600RS Dual Ternary Pump System (P/N 5040.0066) with SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
  - WPS 3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020) with a 25 µL sample loop (P/N 6820.2415) and a 25 µL syringe (P/N 6822.0001)
  - TCC 3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - DAD-3000RS Diode Array Detector (P/N 5082.0020) with 5 µL flow cell (P/N 6082.0200)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2
- Thermo Scientific™ Sorvall ST16™ centrifuge (P/N 75004240)
- Fisher Scientific™ CPXH Series Digital Ultrasonic Cleaners (P/N 15-337-410)
- Thermo Scientific RT Stirring Hotplate (P/N SP136320-33Q)

Consumables

- Thermo Scientific™ Target2™ Polypropylene Syringe Filters (0.45 µm, 30 mm, P/N F2502-9)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity (generated from the Thermo Scientific™ GenPure Pro UV-TOC™, P/N 50131948)
- Methanol (CH_3OH), 99.8%, HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH_3CN), HPLC Grade (Fisher Scientific P/N AC610010040)
- Formic acid (HCOOH), Fisher Scientific™ Optima™ LC/MS Grade (Fisher Scientific P/N A117-50)
- *p*-Aminobenzoic acid (Fisher Scientific P/N 0520115510)
- 2-Hydroxy-4-methoxybenzophenone, 98% (Fisher Scientific P/N AC12136-0050)
- 3,3,5-Trimethylcyclohexyl salicylate (Fisher Scientific P/N 50-014-50082)
- 2-Ethylhexyl-4-methoxycinnamate (Fisher Scientific P/N 50-014-40745)
- 2-Ethylhexyl salicylate (Fisher Scientific P/N 50-014-46763)
- 2-Hydroxy-4-methoxy-benzophenone-5-sulfonic acid (Fisher Scientific P/N 50-014-36526)
- Avobenzone butylmethoxydibenzoylmethane (Fisher Scientific P/N 50-719-418)
- 3-(4-Methylbenzylidene) camphor (Fisher Scientific P/N 50-702-1295)
- Phenyl salicylate (Fisher Scientific P/N 50-014-46398)
- 2-Ethylhexyl-4-(dimethylamino) benzoate (Fisher Scientific P/N 50-014-30608)
- 2,2',4,4'-Tetrahydroxybenzophenone (Fisher Scientific P/N 50-700-1981)

Preparation of Standard Solutions

Stock Standard 1

Dissolve 0.1 g of each UV-absorbent standard in 10 mL of methanol, respectively. The concentration of each Stock Standard 1 is 10000 mg/L.

Stock Standard 2

Dilute 1.25 mL of Stock Standard Solution 1 to 25 mL with methanol. The concentration of each Stock Standard 2 is 500 mg/L.

Stock Standard 3

Dilute 0.5 mL of Stock Standard Solution 1 to 50 mL with methanol. The concentration of each Stock Standard 3 is 100 mg/mL.

Mixed Standard Solutions for Calibration

For calibration, prepare ten mixed working standard solutions with different concentrations by diluting the proper amount of the Stock Standard Solutions with methanol. The volumes of each solution needed to make the calibration standards are shown in Table 2.

Table 2. Preparation of Mixed Standards for Calibration (each Mixed Standard contains 11 sunscreen agents).

Stock Standard	Volume of Each Stock Standard (mL)	Volume of Methanol (mL)	Final Volume (mL)	Final Concentration of each Sunscreen Agent in the Mixed Calibration Standard (mg/L)
Stock Standard 1 (10000 mg/L)	0.5	4.5	10	500
	0.2	7.8		200
	0.1	8.9		100
Stock Standard 2 (500 mg/L)	0.8	1.2		40
	0.4	5.6		20
	0.2	7.8		10
	0.1	8.9		5
Stock Standard 3 (100 mg/L)	0.2	7.8		2
	0.1	8.9		1

Sample Preparation

The sunscreen cream sample was provided by a customer from Jiangsu, China.

Add 10 mL of methanol and 0.125 g of the sunscreen cream sample to a 20 mL centrifuge tube. Mix for 5 min and extract in an ultrasonic bath for 30 min, centrifuge the extract for 10 min at 10000 rpm, remove the supernatant, and add 8 mL of methanol to the residue and extract a second time in the same manner. Combine the two supernatants (total volume ~20 mL) in a 25 mL volumetric flask, and bring to the volume with methanol. Filter the sample solution through a 0.45 µm syringe filter prior to injection. Dilute the sample if necessary.

Add 10 mL of methanol, 0.125 g of the same sunscreen cream sample, and 5 mL of the Mixed Calibration Standard with concentration of 500 mg/L for each sunscreen agent to a 20 mL centrifuge tube. Sample preparation is completed using the procedure above. The spiked concentration of each sunscreen agent in the cream sample will be 100 mg/L.

Conditions	
Columns:	Thermo Scientific™ Acclaim™ 120 C18 Analytical, 3 µm, 3 × 150 mm (P/N 063691)
Mobile Phase:	Acetonitrile/0.1% formic acid solution (add 0.5 mL of formic acid to a 500 mL volumetric flask, and bring to the volume with DI water, without pH adjust)
In Gradient:	0–2 min, 15% acetonitrile; 3 min, 75% acetonitrile; 6 min, 84% acetonitrile; 6.1–9.0 min, 100% acetonitrile; 9.5–11.5 min, 15% acetonitrile
Injection Volume:	1 µL (partial loop injection)
Flow Rate:	0.425 mL/min
Temperature:	40 °C
Detection:	UV absorbance, 311 nm

Results and Discussion

Separation of Eleven Sunscreen Agents

The eleven compounds in this work are all ideal candidates for reversed-phase chromatography with UV detection. The Acclaim 120 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 determined by reversed-phase chromatography.[6] Figure 2 shows a chromatogram of the eleven sunscreen agents under the specified chromatographic conditions. Substituting formic acid for the tetrahydrofuran (THF) and perchloric acid which are used in mobile phase in method SN/T2032-2002 in China[8] provides symmetrical peaks (asymmetries all between 0.93-1.12) and baseline separation except for 2-hydroxy-4-methoxybenzophenone (peak 4) and phenyl salicylate (peak 5) with a peak resolution of 1.3. Moreover, compared to THF and perchloric acid being paired with acetonitrile, formic acid and acetonitrile is a simpler mobile phase system and is less damaging to the consumable parts (e.g., seals and check valves) of the HPLC system.

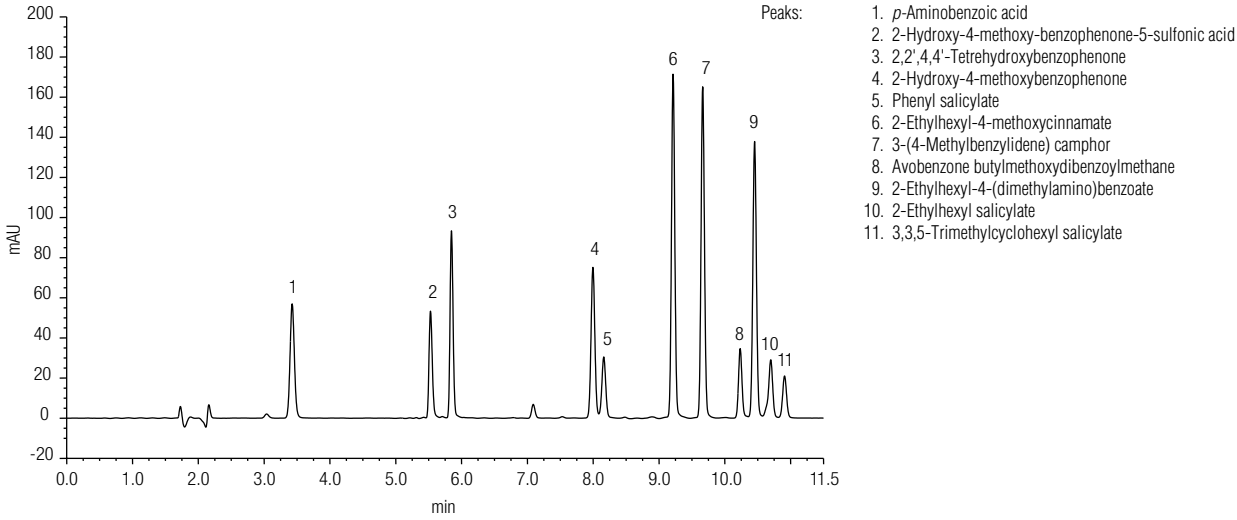

Figure 2. Chromatogram of eleven sunscreen agents (5 mg/L for each).

Method Reproducibility, Linearity, and Detection Limits

Short-term method reproducibility was estimated by making eight consecutive injections of the Mixed Calibration Standard with concentration of 5 mg/L for each sunscreen agent. Retention time and peak area reproducibilities (RSDs) were obtained for each analyte (Table 3). Retention time RSDs were all ≤0.05%, and peak area RSDs were all ≤1.20%, demonstrating good short-term precision for this method.

Calibration linearity for UV detection of the sunscreen agents was investigated by making three consecutive 1 µL injections of a standard prepared at nine different concentrations (i.e., 27 total injections). Each analyte exhibited a linear relationship in the specified concentration range when plotting concentration (c) versus peak area (A). The calibration data are listed in Table 4. Those calibrations will be used to quantify the sunscreen agents in the cosmetic samples. Seven replicate injections of a Mixed Calibration Standard with a concentration of 5 mg/L for each UV-absorbent were used for estimating the method detection limit (MDL) using a signal-to-noise ratio = 3. The measured MDLs are also list in Table 4.

Table 3. Method reproducibility data.

UV-absorbent	Retention Time RSD	Peak Area RSD
***p*-Aminobenzoic acid**	0.04	0.43
2-Hydroxy-4-methoxy-benzophenone-5-sulfonic acid	0.05	0.62
2,2',4,4'-Tetrehydroxybenzophenone	0.02	0.42
2-Hydroxy-4-methoxybenzophenone	0.01	0.87
Phenyl salicylate	0.01	0.88
2-Ethylhexyl-4-methoxycinnamate	0.03	0.56
3-(4-Methylbenzylidene) camphor	0.02	0.54
Avobenzone butylmethoxydibenzoylmethane	0.02	0.35
2-Ethylhexyl-4-(dimethylamino)benzoate	0.03	0.33
2-Ethylhexyl salicylate	0.03	1.20
3,3,5-Trimethylcyclohexyl salicylate	0.03	1.20

Table 4. Calibration data and MDLs.

Analyte	Regression Equation	r^2	Range (mg/L)	MDL (mg/L)
***p*-Aminobenzoic acid**	$A = 0.0832c + 0.2247$	0.9995	5–500	0.80
2-Hydroxy-4-methoxy-benzophenone-5-sulfonic acid	$A = 0.0604c + 0.0689$	0.9998	5–500	0.86
2,2',4,4'-Tetrehydroxybenzophenone	$A = 0.0959c + 0.2148$	0.9995	2–500	0.53
2-Hydroxy-4-methoxybenzophenone	$A = 0.0987c + 0.1608$	0.9997	5–500	0.61
Phenyl salicylate	$A = 0.0409c + 0.0387$	0.9998	5–500	1.52
2-Ethylhexyl-4-methoxycinnamate	$A = 0.1928c + 0.6309$	0.9990	2–500	0.22
3-(4-Methylbenzylidene) camphor	$A = 0.1858c + 0.7391$	0.9990	2–500	0.23
Avobenzone butylmethoxydibenzoylmethane	$A = 0.0433c - 0.0340$	1.0000	5–500	1.32
2-Ethylhexyl-4-(dimethylamino)benzoate	$A = 0.1654c + 0.4911$	0.9994	2–500	0.33
2-Ethylhexyl salicylate	$A = 0.0414c + 0.0745$	0.9996	10–500	1.60
3,3,5-Trimethylcyclohexyl salicylate	$A = 0.0280c + 0.0299$	0.9999	10–500	2.24

Table 5. Detected amounts of sunscreen agents in sunscreen cream samples.

Analyte	Detected (mg/g)	Added (mg/L)	Found (mg/L)	Recovery (%)
***p*-Aminobenzoic acid**	ND*	100	77	77
2-Hydroxy-4-methoxy-benzophenone-5-sulfonic acid	ND	100	77	77
2,2',4,4'-Tetrehydroxybenzophenone	ND	100	78	78
2-Hydroxy-4-methoxybenzophenone	18.3	100	196	104
Phenyl salicylate	ND	100	78	78
2-Ethylhexyl-4-methoxycinnamate	ND	100	78	78
3-(4-Methylbenzylidene) camphor	ND	100	78	78
Avobenzone butylmethoxydibenzoylmethane	34.4	100	263	91
2-Ethylhexyl-4-(dimethylamino)benzoate	ND	100	78	78
2-Ethylhexyl salicylate	8.4	100	148	106
3,3,5-Trimethylcyclohexyl salicylate	59.1	100	412	116

** Not detected*

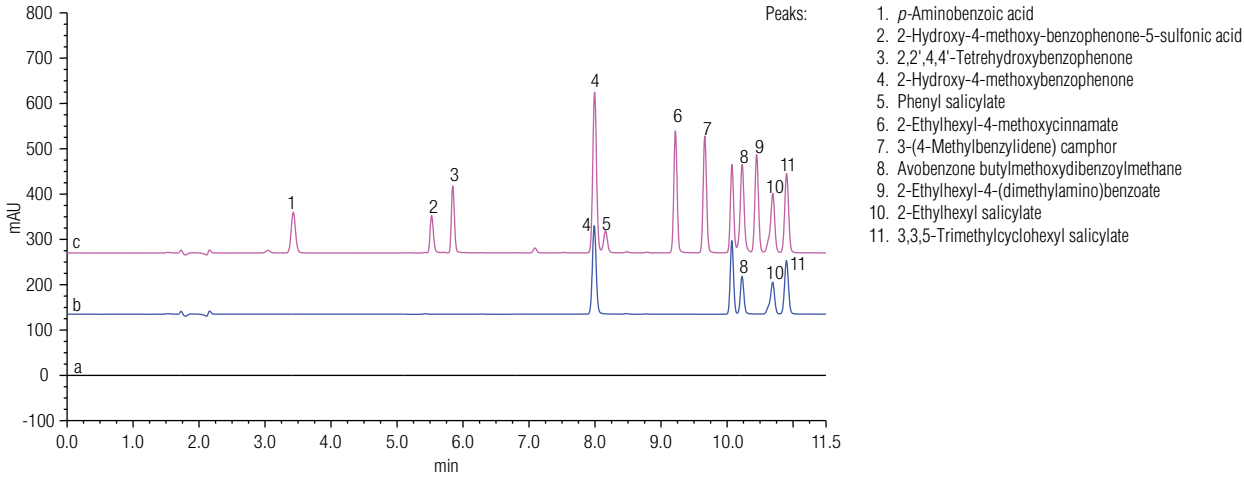

Figure 3. Chromatograms of (a) blank, (b) sunscreen cream sample 1#, and (c) the same sample spiked with UV-absorbent standards with concentration of 100 mg/L for each.

Sample Analysis

Using methanol as extractant in an ultrasonic bath efficiently extracts the sunscreen agents and removes oil components from cream sample.[6,8] The sunscreen cream sample was analyzed after such an extraction using the HPLC method described here, and the analysis results are summarized in Table 5. Figure 3 shows chromatograms of the sample and the same sample spiked with standards. Four sunscreen agents, 2-hydroxy-4-methoxybenzophenone (peak 4), avobenzone butylmethoxydibenzoylmethane (peak 8), 2-ethylhexyl salicylate (peak 10), and 3,3,5-trimethylcyclohexyl salicylate (peak 11) were found, while the other seven compounds were absent. To judge method accuracy, recoveries of the sunscreen cream sample spiked with a mixed standard were investigated. The recoveries ranged from 77 to 116% for the eleven sunscreen agents, demonstrating that this method is suitable for the determination of sunscreen agents in sunscreen creams.

Conclusion

This work describes an efficient HPLC method with UV detection for a simultaneous determination of eleven sunscreen agents in a sunscreen cream with the advantages of good method reproducibility, a wide linearity range, and that the mobile phase does not include THF and perchloric acid.

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