Application Note 1125

Rapid and Sensitive Determination of Dextromethorphan in Cosmetics

Liu Lvye,[1] Xu Qun,[1] Jeffrey Rohrer[2]
[1]Thermo Fisher Scientific, Shanghai, People's Republic of China;
[2]Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words
Acclaim 120 C18 Column, Cosmetic Quality, Cosmetic Safety, Fluorescence Detection, HPLC

Goal
To develop an efficient HPLC method without an ion-pairing reagent for the rapid and sensitive determination of dextromethorphan in cosmetics due to the potential threat of nausea, dizziness, blurred vision, body itching, and rash

Introduction
Dextromethorphan (structure shown in Figure 1) is a cough suppressant that may temporally relieve the cough caused by minor throat and bronchial irritation, as well as those resulting from inhaled particle irritants.[1,2] Besides its medical use, dextromethorphan has also been found in some cosmetic products, though its function in cosmetics has not been reported. Therefore, China and Europe have prohibited the use of dextromethorphan and its salts, e.g. dextromethorphan hydrobromide, as additives to cosmetic products due to the potential threat of nausea, dizziness, blurred vision, body itching, and rash.[3,4] It is necessary to establish effective methods for the determination of dextromethorphan in cosmetics. A high performance liquid chromatography (HPLC) method using a C8 stationary phase and fluorescence detection is recommended in China.[5] However, the use of triethylamine in the mobile phase, which is added to reduce the tailing problem of dextromethorphan on the C8 silica gel-based stationary phase, could reduce column lifetime.[6] The work shown here proposes a solution that uses a C18 stationary phase and acetic acid/acetonitrile mobile phase to fulfill rapid and sensitive determination of dextromethorphan hydrobromide in cosmetics.

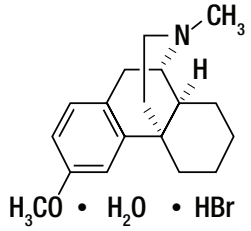

Figure 1. Structure of dextromethorphan hydrobromide.

Equipment and Software
Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system, including:

- LPG-3400RS Quaternary Pump (P/N 5040.0036)
- SRD-3400 Integrated Solvent and Degasser Rack (P/N5035.9245)
- WPS-3000TRS Well Plate Sampler, Thermostatted (P/N 5840.0020), with 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)
- FLD-3400RS Fluorescence Detector with Dual-PMT (without Flow Cell, P/N 5078.0025)
- Micro Flow Cell for FLD-3000 Series, SST, 2 µL Volume (P/N 6078.4330)

• Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2

• Fisher Scientific™ CPXH Series Digital Ultrasonic Cleaner (P/N 15-337-410)

• Thermo Scientific™ Sorvall™ ST16 Centrifuge (P/N 75004240)

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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)
- Acetic Acid (CH_3COOH), 99.5% (Fisher Scientific P/N AC12404-0010)
- Dextromethorphan hydrobromide (Fisher Scientific P/N 50-329-742)

Preparation of Standard Solutions

Stock Standard 1

Dissolve 0.10 g of dextromethorphan hydrobromide standard in 100 mL of methanol. The concentration of Stock Standard 1 is 1000 mg/L.

Stock Standard 2

Dilute 250 µL of Stock Standard Solution 1 to 25 mL with methanol. The concentration of Stock Standard 2 is 10 mg/L.

Standard Solutions for Calibration

For calibration, prepare nine working standard solutions with different concentrations by diluting the proper amount of the Stock Standard Solution 2 with methanol. The volumes of each solution needed to make the calibration standards are shown in Table 1.

Sample Preparation

Two cosmetic samples, one cream and one lotion, were purchased in a beauty shop and a supermarket in Shanghai, China. The procedure of sample preparation is based on what is specified in method SN/T2291-2009.[5]

Add 1 g of a cosmetic sample and 4 mL of methanol to a 10-mL centrifuge tube. Extract in an ultrasonic bath for 30 min, cool to room temperature, and centrifuge the extract for 20 min at 8000 rpm. Remove the supernatant, add 4 mL of methanol to the residue, and extract a second time in the same manner. Combine the two supernatants (total volume <10 mL) in a 10-mL volumetric flask, and bring to the volume with methanol. Store the solution at -18 °C or below for 10 min, and centrifuge for 5 min at 3500 rpm. Warm to room temperature, and filter the supernatant through a 0.45 µm syringe filter prior to injection.

Add 1 g of a cream cosmetic sample, 0.5 mL of the Mixed Calibration Standard 2 with concentration of 10 mg/L dextromethorphan hydrobromide, and 4 mL of methanol to a 10-mL centrifuge tube. Sample preparation is completed using the procedure above. The spiked concentration of dextromethorphan hydrobromide in the cream sample will be 0.5 mg/L.

Table 1. Preparation of calibration standards.

Stock Standard of Dextromethorphan Hydrobromide for Calibration	Volume of Stock Standard of Dextromethorphan Hydrobromide for Calibration (mL)	Volume of DI Water (mL)	Final Volume of Calibration Standard (mL)	Final Concentration of Calibration Standard (mg/L)
Stock Standard 2 (10 mg/L)	5	5	10	5.0
	2	8		2.0
	1	9		1.0
	0.5	9.5		0.50
	0.2	9.8		0.20
	0.1	9.9		0.10
	0.05	9.95		0.05
	0.02	9.98		0.02

Chromatographic Conditions	
Column:	Thermo Scientific™ Acclaim™ RSLC 120 C18 column, 2.2 µm, 3.0 × 50 mm (P/N 071605)
Mobile Phase:	3% Acetic acid (add 30 mL of acetic acid to 970 mL of DI water, without pH adjustment)/ Acetonitrile, 30 : 70 (v/v)
Injection Volume:	2 µL (partial loop injection)
Flow Rate:	0.6 mL/min
Temperature:	30 °C
Detection:	Fluorescence, excitation at 280 nm, emission at 310 nm

Results and Discussion

Optimization of Chromatographic Conditions

Different mobile phases, acetonitrile/0.1% acetic acid, acetonitrile/3% acetic acid, methanol/0.1% acetic acid, and methanol/3% acetic acid, were evaluted for the separation of dextromethorphan hydrobromide using the Acclaim 120 C18 column. Increasing aqueous phase acidity can improve dextromethorphan hydrobromide peak symmetry. The peak asymmetry was 1.53 when 0.1% acetic acid was paired with acetonitrile in the mobile phases; and it was improved to 1.24 by substituting 3% for 0.1% acetic acid. Compared to methanol, acetonitrile yielded better peak symmetry and faster separation of dextromethorphan hydrobromide. Therefore, an acetonitrile/3% acetic acid phase was used in this work. Moreover, compared to triethylamine being paired with acetic acid and acetonitrile in the method SN/T2291-2009[5], acetic acid and acetonitrile is a simpler mobile phase system and is less damaging to the silica gel-based C18 column.[6] Like the SN/T2291-2009, fluorescence detection was used due to its high selectivity and response for dextromethorphan hydrobromide.[5]

Reproducibility, Linearity, and Detection Limit

Short-term method reproducibility was estimated by making five consecutive injections of a calibration standard with a concentration of 5 mg/L dextromethorphan hydrobromide. The retention time reproducibility RSD was 0.10, and peak area reproducibility RSD was 1.21, demonstrating good short-term precision for this HPLC method.

Calibration linearity for fluorescence detection of dextromethorphan hydrobromide was investigated by making three consecutive 2 µL injections of a standard prepared at eight different concentrations (i.e., 24 total injections). Linearity was observed from 0.05 to 5 mg/L when plotting the concentration versus the peak area (Figure 2). The linear regression equation is A = 42239c - 22409, where, A represents peak area (counts*min), and c represents concentration of analyte (mg/L); and the coefficient of determination was 0.9990. This calibration curve was used to quantify dextromethorphan hydrobromide in cosmetic samples. Figure 3 shows a chromatogram of a dextromethorphan hydrobromide standard solution with concentration of 0.05 mg/L.

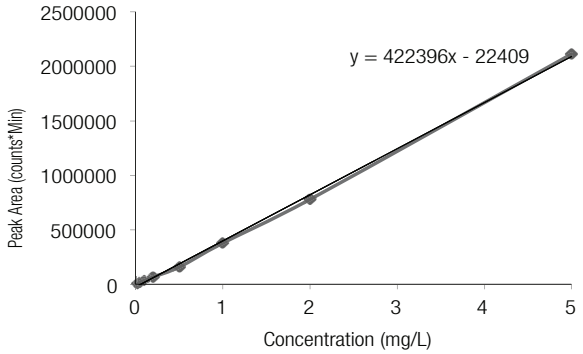

Figure 2. Calibration curve for dextromethorphan.

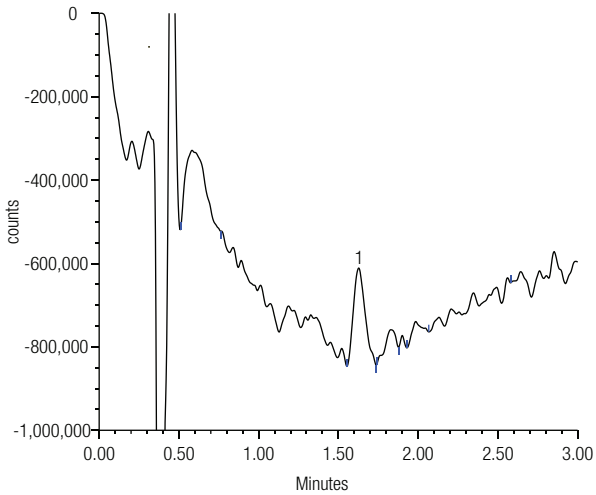

Figure 3. Chromatogram of a dextromethorphan hydrobromide standard solution. Peak, 1- dextromethorphan hydrobromide (0.05 mg/L).

Three replicate injections of a dextromethorphan hydrobromide standard with concentration 0.05 mg/L were used for estimating the method detection limit (MDL) using a signal-to-noise ratio (S/N) = 3. The measured MDL of dextromethorphan hydrobromide was 11 µg/L.

Analysis of Cosmetic Samples

Using methanol as an extractant in an ultrasonic bath efficiently extracts the cosmetic samples, and storing the extract at -18 °C removes oil components from cream sample.[5,7] Dextromethorphan hydrobromide was not found in the tested cosmetic samples. Figure 4 shows chromatograms of the cream sample and the same sample spiked with dextromethorphan hydrobromide. To judge method accuracy, three injections of the cream sample spiked with a 0.5 mg/L of dextromethorphan hydrobromide standard were made. The average recovery was 93%, demonstrating that this method is suitable for the determination of dextromethorphan in cosmetics.

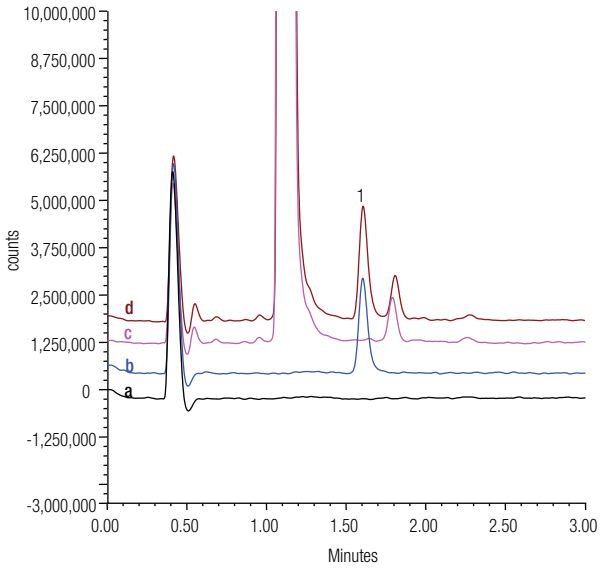

Figure 4. Chromatograms of (a) blank, (b) a dextromethorphan hydrobromide standard (0.5 mg/L), (c) a cream sample, (d) the same sample spiked with dextromethorphan hydrobromide standard (0.5 mg/L). Peak, 1- dextromethorphan hydrobromide.

Conclusion

This work describes an efficient HPLC method with fluorescence detection that does not require an ion-pairing reagent for a rapid and sensitive determination of dextromethorphan hydrobromide in cosmetics. This method exhibits good reproducibility and a wide linear calibration range.

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