Application Note 20688

# High-Throughput Analysis of Dextromethorphan and Dextrorphan

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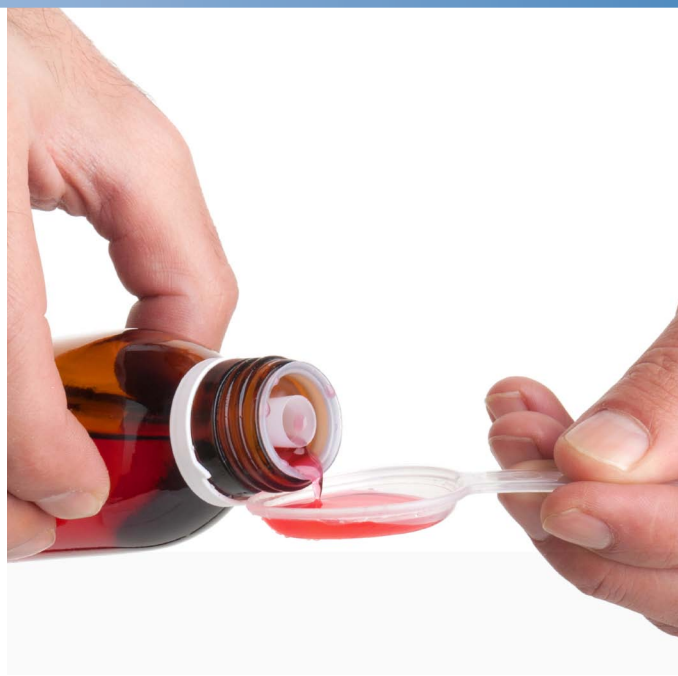
**Key Words**
Accucore C18, dextromethorphan, dextrorphan

**Abstract**
A liquid chromatography method for the analysis of dextromethorphan and dextrorphan was developed on a Thermo Scientific™ Accucore™ C18 2.6 µm, 50 x 2.1 mm HPLC column for a fast separation with a cycle time of 2 minutes and excellent peak shape and resolution.



## Introduction

Dextromethorphan (Figure 1) is used as a cough suppressant and can be found in many over-the-counter cough and cold remedies. Dextromethorphan is quickly metabolized to dextrorphan (Figure 2) in the liver. This process can be used to assess the speed of metabolism. The retention, peak shape, and resolution of dextromethorphan and dextrorphan are demonstrated in this application.

Thermo Scientific Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The carbon loading of the Accucore C18 HPLC column provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

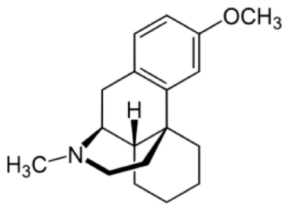



Figure 1. Dextromethorphan

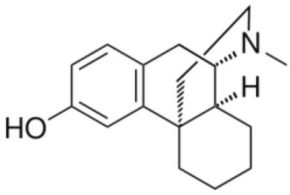



Figure 2. Dextrorphan

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## Experimental Details

| Sample Handling | Part Number |
|---|---|
| Fisher Scientific™ HPLC grade water | W/0106/17 |
| Fisher Scientific HPLC grade acetonitrile | A/0626/17 |
| Fisher Scientific HPLC grade ortho phosphoric acid (OPA) | J/5655/PB08 |
| Thermo Scientific™ National™ Mass Spec Target DP Certified 2 mL clear vial with ID patch and blue bonded cap with PTFE/Silicone septum | MSCERT4000-34W |

| Sample Preparation |
|---|
| A primary standard of dextromethorphan and dextrorphan was prepared in methanol at 1 mg/mL |
| The working standard contained 1 µg/mL dextromethorphan and dextrorphan in 80:20 (v/v) water / acetonitrile |

| Separation Conditions | | | Part Number |
|---|---|---|---|
| Instrumentation: | Thermo Scientific Dionex™ UltiMate™ 3000 HPLC system | | |
| Column: | Accucore C18 2.6 µm, 50 x 2.1 mm | | 17126-052130 |
| Mobile phase A: | Water + 0.1% OPA | | |
| Mobile phase B: | Acetonitrile + 0.1% OPA | | |
| Gradient: | Time (min) | %B | |
| | 0 | 5 | |
| | 1 | 95 | |
| | 1.01 | 5 | |
| | 2 | 5 | |
| Flow rate: | 1.4 mL/min | | |
| Column temperature: | 40 °C | | |
| Pressure: | 360 Bar | | |
| Injection details: | 1 µL | | |
| Injection wash solvent: | Water / acetonitrile (60:40 v/v) | | |
| Wavelength: | 194 nm | | |

## Results

This application was run at a high flow rate to provide a fast separation suitable for high-throughput laboratories. The Thermo Scientific Accucore C18 HPLC column gave excellent peak shape and resolution, as demonstrated in Figure 3. The calculated time of $T_0$ for this method is 7.43 seconds or 0.12 minutes. This can be clearly seen on the chromatogram and provides dextrorphan with a k' of 6.8, proving the peaks are well retained even with a short runtime.

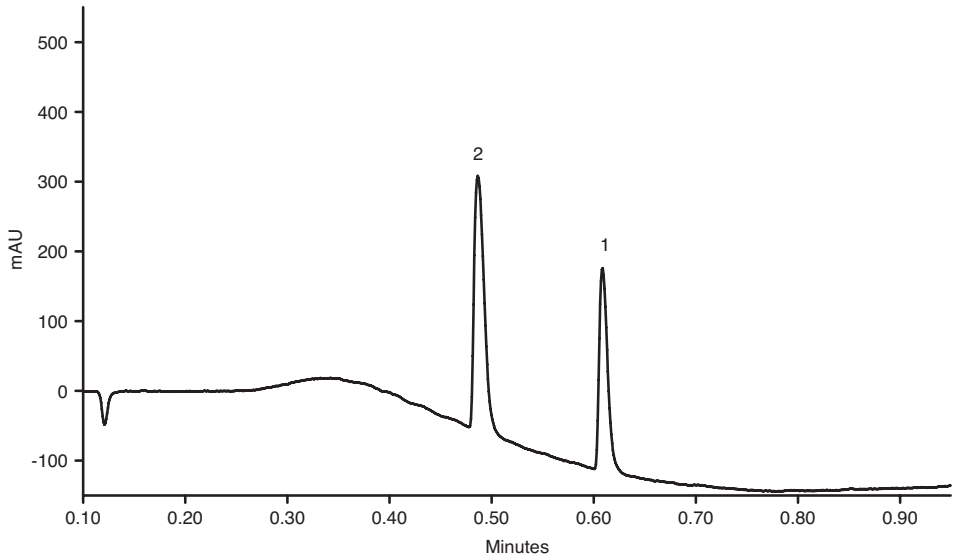


Figure 3: Chromatogram for dextromethorphan (1) and dextrorphan (2)

Replicate injections of the standard containing dextromethorphan and dextrorphan showed that the Accucore C18 HPLC column produced stable and reproducible results (Table 1).

| | Dextromethorphan | Dextrorphan |
|---|---|---|
| **Retention time (minutes)** | 0.60 | 0.48 |
| **% RSD on retention time** | 1.05 | 1.57 |
| **Asymmetry** | 1.43 | 1.46 |
| **Resolution** | - | 7.58 |

Table 1: Results obtained from an Accucore C18 column, based upon 6 replicate injections

## Conclusion

- Analysis of dextromethorphan and dextrorphan can be achieved using an Accucore C18 HPLC column.
- The Accucore C18 HPLC column provides good resolution for dextromethorphan and dextrorphan in less than 2 min.
- The Accucore C18 HPLC column gives excellent peak shape for dextromethorphan and dextrorphan.
- The Accucore C18 HPLC column performs reproducibly for the analysis of dextromethorphan and dextrorphan.

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