

HPLC Application

ID No.: 25782

Chiral Separation of Dextromethorphan/Levomethorphan and Dextrorphan/Levorphanol on Lux 3u AMP, 150x4.6

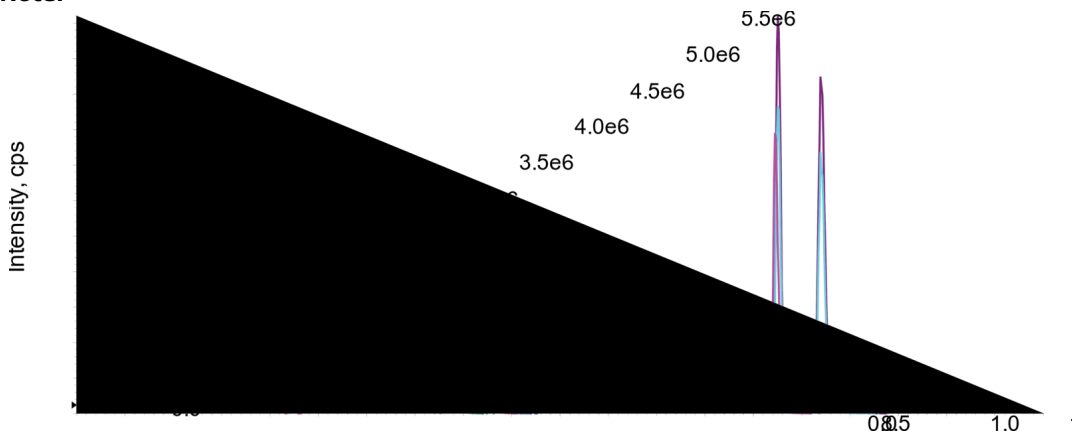
Column: Lux® 3 µm AMP, LC Column 150 x 4.6 mm, Ea
Dimensions: 150 x 4.6 mm ID
Order No: 00F-4751-E0
Elution Type: Gradient
Eluent A: 5mM ammonium bicarbonate, adjusted to pH 11 with ammonium hydroxide
Eluent B: Acetonitrile

| Gradient Profile: | Step No. | Time (min) | Pct A | Pct B |
|-------------------|----------|------------|-------|-------|
| | 1 | 0 | 55 | 45 |
| | 2 | 4.5 | 55 | 45 |
| | 3 | 4.6 | 20 | 80 |
| | 4 | 8 | 20 | 80 |
| | 5 | 8.1 | 55 | 45 |
| | 6 | 10 | 55 | 45 |

Flow Rate: 1 mL/min
Col. Temp.: 40 °C
Detection: LC/MS/MS @ (ambient)
Analyst Note: Urine hydrolyzed with beta-glucuronidase and extracted with Strata-X-C



Products used in this application:



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ANALYTES:

- 1** Dextrorphan-D3
- 2** Dextrorphan 1
- 3** Dextrorphan 2
- 4** Levorphanol 1
- 5** Levorphanol 2
- 6** Dextromethorphan-D3
- 7** Dextromethorphan 1
- 8** Dextromethorphan 2
- 9** Levomethorphan 1
- 10** Levomethorphan 2



Sample Preparation Details

for HPLC Application ID No.: 25782

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PRODUCT DESCRIPTION:

Strata™-X-C 33 µm Polymeric Strong Cation, 30 mg / well, 96-Well Plates , 2/Pk

Order No.: 8E-S029-TGB

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Wash:

Dry:

Elute:

Final Prep and Analysis:

Urine sample pretreatment: Combine 200µL urine sample, 200µL 100mM ammonium acetate buffer (pH 4.0), 20µL internal standard solution (250ng/mL), and 20µL of beta-glucuronidase

Inject: 10 µL on HPLC LC/MS/MS @ (ambient)

| ANALYTES: | Spiked Conc. (ng/mL) | Log P | pKa | % Rec | %RSC (n=0) |
|-----------------------|-------------------------|-------|-----|-------|---------------|
| 1 Dextrorphan-D3 | 0 | | | | |
| 2 Dextrorphan 1 | 0 | | | | |
| 3 Dextrorphan 2 | 0 | | | | |
| 4 Levorphanol 1 | 0 | | | | |
| 5 Levorphanol 2 | 0 | | | | |
| 6 Dextromethorphan-D3 | 0 | | | | |
| 7 Dextromethorphan 1 | 0 | | | | |
| 8 Dextromethorphan 2 | 0 | | | | |
| 9 Levomethorphan 1 | 0 | | | | |
| 10 Levomethorphan 2 | 0 | | | | |

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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