# HPLC Application

ID No.: **21872**

## Extraction of Phenylbutazone from Beef Using Strata-X-A on Kinetex 2.6u XB-C18 50x2.1 by LC/MS/MS

**Column:** Kinetex® 2.6 µm XB-C18 100 Å, LC Column 50 x 2.1 mm, Ea
**Dimensions:** 50 x 2.1 mm ID
**Order No:** 00B-4496-AN
**Elution Type:** Gradient
**Eluent A:** 0.1% Formic Acid in DI Water
**Eluent B:** 0.1% Formic Acid in Methanol

**Gradient Profile:**

| Step No. | Time (min) | Pct A | Pct B |
|---|---|---|---|
| 1 | 0 | 50 | 50 |
| 2 | 0.5 | 50 | 50 |
| 3 | 2 | 5 | 95 |
| 4 | 3.5 | 5 | 95 |
| 5 | 3.51 | 50 | 50 |
| 6 | 5 | 50 | 50 |

**Flow Rate:** 0.45 mL/min
**Col. Temp.:** 45 °C
**Detection:** Mass Spectrometer (MS) @ amu (ambient)
**Detector Info:** <a target="_blank" href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<,

**Analyst Note:** Mass spec conditions:

API 4000, ESI TurboIon Spray, + Ionization;

| | |
|---|---|
| CAD: | 6.00 |
| CUR: | 20.00 |
| GS1: | 50.00 |
| GS2: | 50.00 |
| IS: | 5500.00 |
| TEM: | 600.00 |
| ihe: | ON |
| DP | 50.00 |
| EP | 10.00 |

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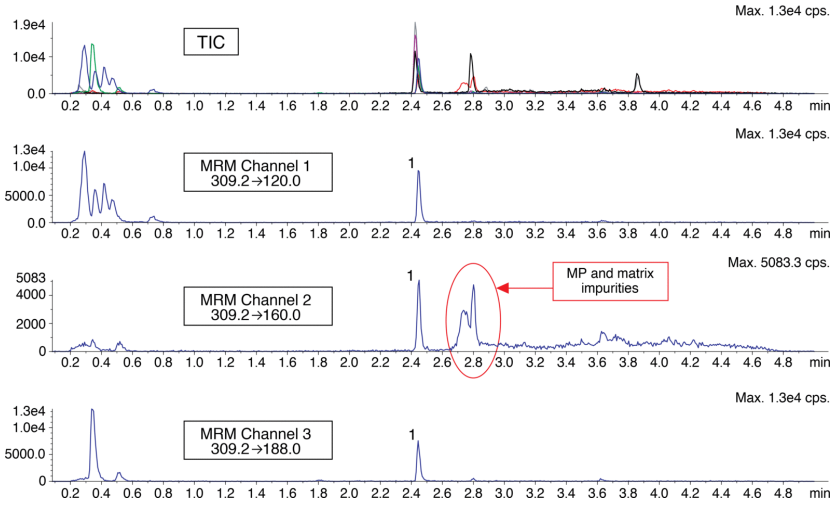


**Products used in this application:**

## Extraction of Phenylbutazone from Beef Using Strata-X-A on Kinetex 2.6u XB-C18 50x2.1 by LC/MS/MS

### ANALYTES:

**1** Phenylbutazone

# Sample Preparation Details

for **HPLC** Application ID No.: **21872**

## Extraction of Phenylbutazone from Beef Using Strata-X-A on Kinetex 2.6u XB-C18 50x2.1 by LC/MS/MS

### PRODUCT DESCRIPTION:

Strata™-X-A 33 µm Polymeric Strong Anion, 100 mg / 3 mL, Tubes , 50/Pk

Order No.: 8B-S123-EBJ

### SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

**Note:** The solvent volumes shown below are for a 100 mg bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

**Condition:**

**Load:**

Sample Pretreatment:

1. In an Erleneyer flask (or similar), combine 2 ±0.1 g sample (well-homogenized ground beef or pork sausage) with 2 mL 0.1N NaOH and 50 uL of 4 ug/mL Int Std (phenylbutazone-D10) solution
2. Cap the flasks and mix vigorously for at least 10 sec
3. Add 8 mL 100% MeOH to the flask and cap the vessel
4. Place the flasks securely on a lab shaker and shake the vessels for 55-60 min at medium-high to high setting
5. Transfer the contents of the flask into a 15 mL conical tube and centrifuge them for 10 min @ 4000-4500 rpm
6. Remove 2 mL of supernatant and combine with 2 mL DI water into a glass tube and mix
7. Proceed to SPE method

**Wash:**

**Dry:**

5 mins at 10" of Hg

**Elute:**

**Final Prep and Analysis:**

Extraction Procedure:
1. Condition a Strata-X-A, 100 mg/6 mL with 3 mL 100% MeOH followed by 3 mL DI water

Inject: 5 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

| ANALYTES: | Spiked Conc. (ng/mL) | Log P | pKa | % Rec | %RSC (n=0) |
|---|---|---|---|---|---|
| **1** Phenylbutazone | 25 | | | 101 | |

**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.*