

# 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"

**Analyst Note:** href="https://sciex.com/products/mass-spectrometers?utm\_campaign=2019%20application%20search&utm\_source=phenomenex&utm\_medium=referral">SCIEX<

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

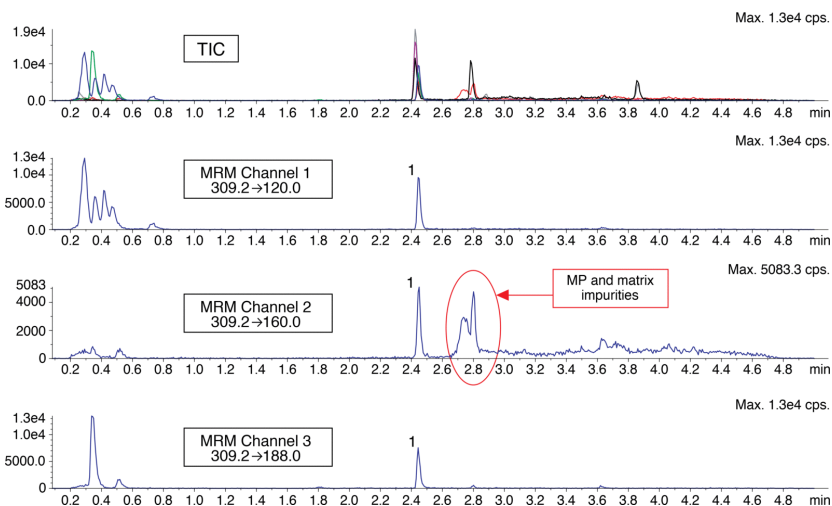
DP: 50.00

EP: 10.00

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Products used in this application:



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### ANALYTES:

- 1 Phenylbutazone



# Sample Preparation Details

for HPLC Application ID No.: 21872

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### 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) PROCEDURE:

**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:

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#### Load:

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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 µL of 4 µg/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:

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#### Dry:

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5 mins at 10" of Hg

#### Elute:

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### Final Prep and Analysis:

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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.

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For more information contact your Phenomenex Representative at support@phenomenex.com



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