

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

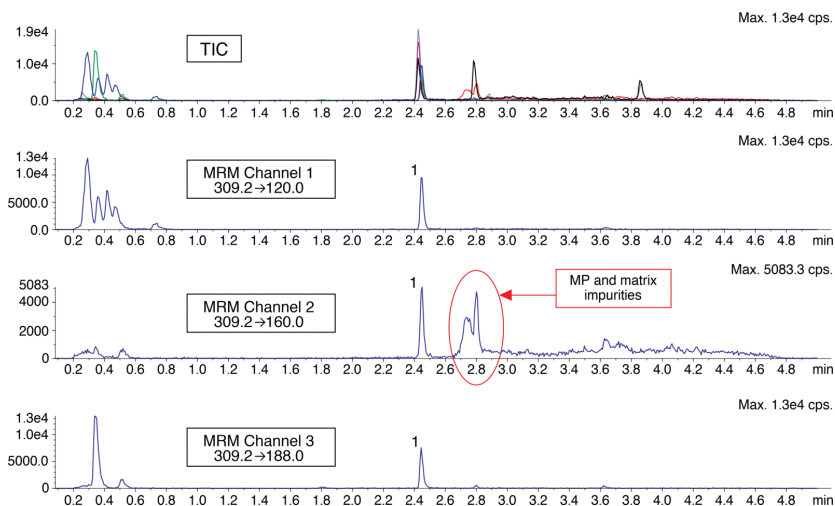
TEM: 600.00

ihe: ON

DP: 50.00

EP: 10.00

21872



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ANALYTES:

1 Phenylbutazone



Sample Preparation Details

for HPLC Application ID No.: 21872

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PRODUCT DESCRIPTION:

StrataTM-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:

Load:

Sample Pretreatment:

1. In an Erlenmeyer 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:

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.

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



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