APPLICATIONS

Evaluation of an Alternative Ion-pairing Chromatography Technique for the LC-MS/MS Analysis of Underivatized Biogenic Amines in Ground Beef

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Introduction

Previously, we reported that addition of ion-pairing reagent into a sample can produce sufficient retention of highly hydrophilic compounds on a reversed phase column[1]. This concept was further evaluated by Bergman et al[2] and Lahotay et al[3].

This approach eliminates the traditional method of fortifying the mobile phase with ion-pairing reagent and thus exposing every portion of a LC system and the detector to the undesirable properties of a detergent (ion-pairing agent) compound.

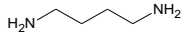
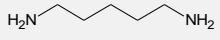
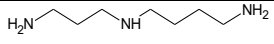
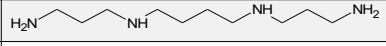
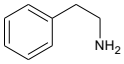
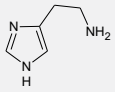
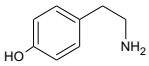
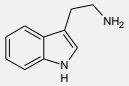
As a beneficial part of this technique, the ion-pairing agent can be removed from the column during the wash step and with the use of a diverter valve prevented the ion-pairing agent from entering a sensitive detector.

The aforementioned benefit is uniquely suited for LC-MS and LC-MS/MS methodologies. A reversed phase, MS friendly mobile phase could be employed in place of HILIC or normal phase under MS or MS/MS detection.

A group of eight biogenic amines, 2-phenylethylamine, cadaverine, histamine, putrescine, spermine, spermidine, tryptamine, and tyramine are considered for this analysis, see **Table 1** for structures and estimated logP. These compounds are basic analytes with one or more amine group(s) which require an acidic ion-pairing agent. In this case, sodium n-octane-1-sulfonate was added into the final sample extract prior to injection on column.

This group of compounds represents a tough challenge to food industry and their concentration in various food and animal feed is monitored to evaluate the food (or feed) acceptability.

TABLE 1.
Chemical Structures of Biogenic Amines and Estimated logP Values

Compound ID	Structure	logP (Est)
Putrescine		-0.79
Cadaverine		-0.49
Spermidine		-0.84
Spermine		-0.96
2-Phenylethylamine		1.46
Histamine		-0.96
Tyramine		0.72
Tryptamine		1.38

Materials and Methods

Sample Preparation

Solid Liquid Extraction

- Frozen ground beef was pulverized with a small amount of dry ice in a seed/nut grinder
- 0.5 ± 0.05 g of powdered frozen ground beef was placed in a 20 mL glass scintillation vial
- 10 mL 5% (w/v) trichloroacetic acid was added into each vial and capped
- The vials were placed on a benchtop mixer and vigorously shaken for 15 min at room temperature (15-25 °C)
- After centrifugation, 1 mL was removed for solid phase extraction procedure.

Solid-Phase Extraction (SPE)

SPE Cartridge: Strata®-XL-CW, 100 mg/6 mL
Part No.: 8B-S052-HCH
Condition: 2 mL 100% Methanol
Equilibrate: 2 mL DI water
Load: Sample (Gravity Flow)
Wash 1: 2 mL DI water
Wash 2: 2 mL 100% Methanol
Dry: Maximum Vacuum for 3-5 min
Elution: 2x 1.5 mL 95:5 Methanol/Ammonium Hydroxide

Collect eluate and evaporate to dryness under N_2 stream @ 45-50 °C
Reconstitute the dry residue in 200 µL of 70 mM n-Octane-1-sulfonate acidified with 1% formic acid to pH ~3

LC-MS/MS Conditions

Column: Kinetex® 5 µm C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4601-AN
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol

Gradient:

Time (min)	%B
0	10
0.5	10
5.5	50
5.51	95
7	95
7.01	10
8	10

Flow Rate: 0.6 mL/min
Col. Temp.: Ambient
Detector: SCIEX 4000 QTRAP®

Sample:

Analyte	RT (min)
1. Tyramine	3.67
2. Putrescine	4.34
3. Cadaverine	4.36
4. Histamine	4.40
5. 2-Pheylethylamine	4.57
6. Tryptamine	4.78
7. Spermidine	4.90
8. Spermine	5.18

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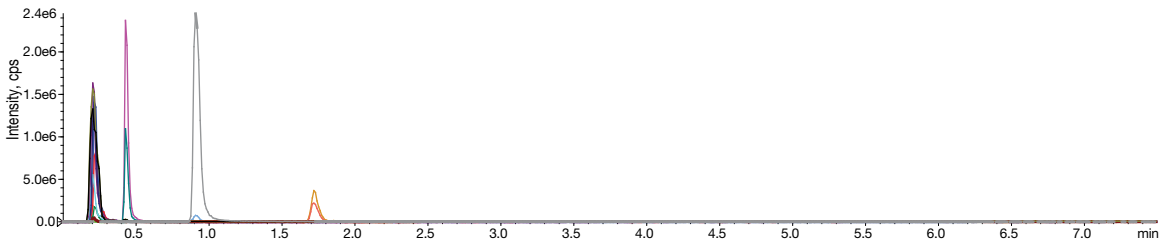
MS Parameters

- SCIEX 4000 QTRAP
- ESI in Pos. Polarity
- 2 MRM Transitions per compound

Compound	Q1, Th	Q3, Th	Coll E., V
2-Phenylethylamine	122	105	26
2-Phenylethylamine	122	77	20
Cadaverine	103	86	13
Cadaverine	103	41	30
Histamine	112	95	27
Histamine	112	68	33
Putrescine	89	30	30
Putrescine	89	72	13
Spermidine	146	72	30
Spermidine	146	112	20
Spermine	203	129	16
Spermine	203	112	24
Tryptamine	161	115	42
Tryptamine	161	127	34
Tyramine	138	121	25
Tyramine	138	93	25

Figure 1.

a. Retention of biogenic amines on a reversed phase column



b. Retention of Biogenic Amines with the Aid of In-sample Addition of n-octane-1-sulphonate

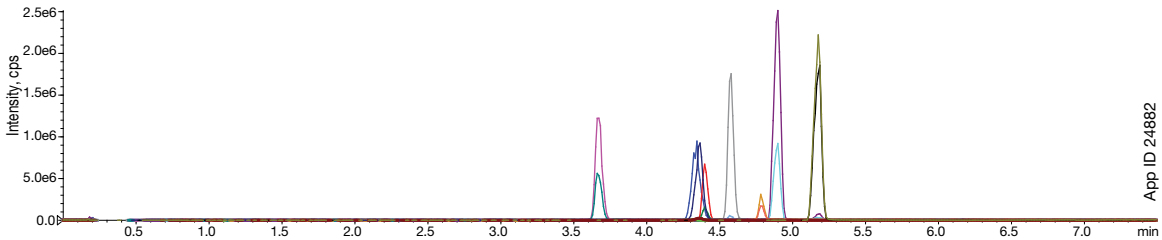


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Figure 2.
Unspiked 10% Fat Ground Beef Extract Showing Endogenous Levels of Biogenic Amines

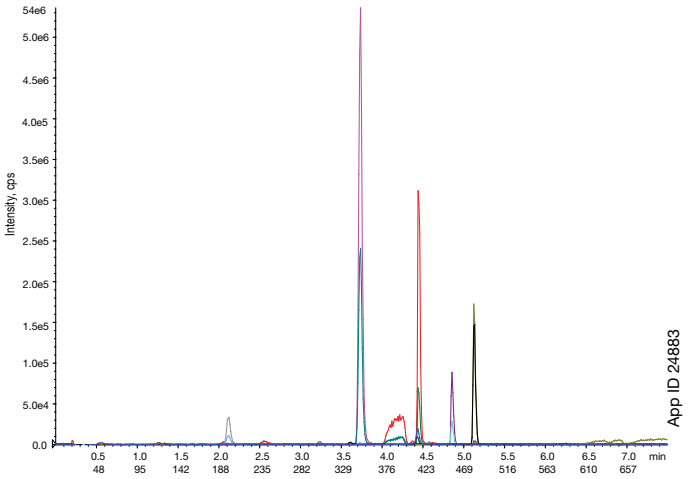


Figure 3.
Low Level Spiked 10% Fat Ground Beef Extract

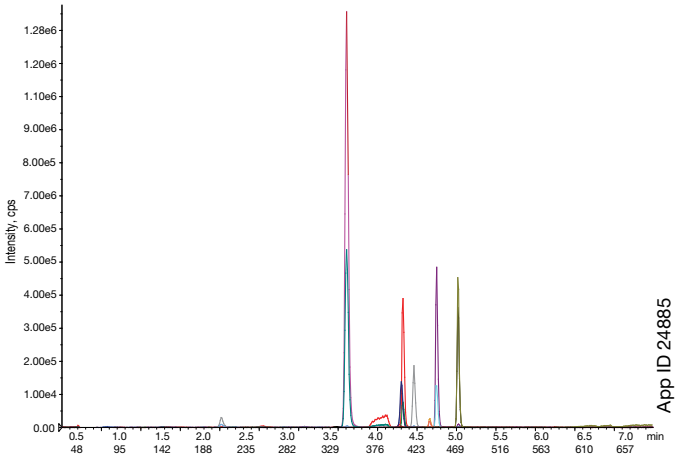
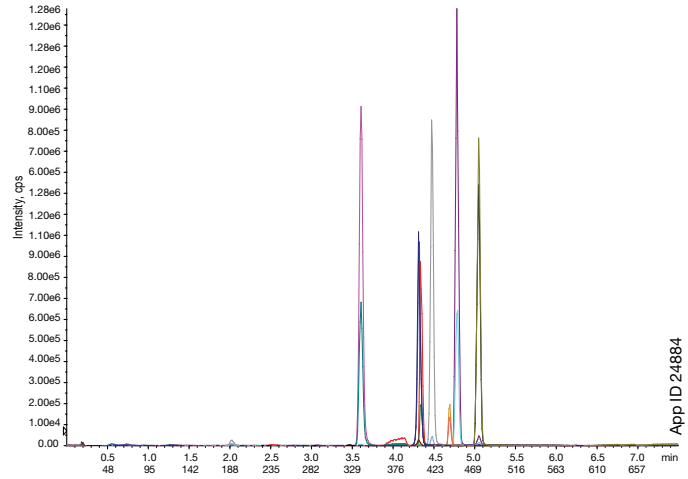


Figure 4.
Low Level Spiked 10% Fat Ground Beef Extract



Results and Discussions

I. The poor retention of the biogenic amines on a reversed phase column is best demonstrated in **Figure 1a**. Tyramine and 2-phenylethylamine show better retention than the rest of the group due to their slightly more hydrophobic nature. The addition of the n-octane-1-sulfonate into the sample shows increased retention and better over all resolution, **Figure 1b**.

II. Ground beef were purchased from local grocery stores and extracted for endogenous levels of biogenic amines. Essentially all samples contained varying levels of the target compounds. It was presumed that the samples with higher concentrations of these compounds are probably older specimens. A sample of ground beef containing 10 % fat produced the lowest signal for most of the target amines except for Tyramine. Extracts from this sample were spiked with reference standard to contain low to high level of the target analytes. Refer to **Figures 2-4** for unspiked, low, and high spiked chromatogram.

The sodium cations introduced into the final extract as counter ion to sulfonate will elute from the column virtually unimpeded at approximately 0.5 min. Their presence in the ion source suppresses the background signal. The sulfonate anion elutes from the column during the wash step. These two sections, from 0.0 to1.0 min and 5.5 to 7.5 min, are the main area of ion suppression and contaminants eluting in these segments will also foul the MS ion source and interface

III. The analytes spiked in meat extract showed small variations in retention times when compared against the analytes in neat sample, ± <1.9 %. However, subsequent injections of the beef extracts produced very little fluctuations in retention times. In general, large shifts in analyte retention times are commonly observed with ion-pairing chromatography. It can be argued that such minor variations in retention times are perfectly acceptable. As a future improvement, addition of a surrogate or internal standard compounds during the extraction or post extraction can effectively correct any changes in analytes retention time shifts that may occur.

Conclusion

It is possible to successfully use in-sample ion-pairing reagent for retention and resolution of biogenic amines from ground beef.
Stable retention time variation were observed for this analysis. Addition of a surrogate compound(s) in this analysis can improve this analysis.

References

1. Sadjadi, S., Preston, J, Layne, J., A New Twist to Ion-Pairing Chromatography: In-Sample Addition of Ion-Pairing Reagent, *LCGC North America,* Nov 01, 2017, Volume 35, Issue 11, pg 824–831
2. Bergmann, M.L.,Sadjadi, S., Schmedes, A., Analysis of catecholamines in urine by unique LC/MS suitable ion-pairing chromatography, *Journal of Chromatography* B (2017), Vol. 1057, 1 July 2017, pp. 118-123
2. Lehotay, S.J., Lightfield, A.R., Simultaneous analysis of aminoglycosides with many other classes of drug residues in bovine tissues by ultra high-performance liquid chromatography–tandem mass spectrometry using an ion-pairing reagent added to final extracts, *Analytical and Bioanalytical Chemistry,* January 2018, Vol 410, Issue 3, pp 1095–1109

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Kinetex® Core-Shell LC Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4601-AN	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJ0-8782 for 2.1 mm ID

5 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00B-4601-Y0	00D-4601-Y0	00F-4601-Y0	AJ0-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768 for 4.6 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768 for 4.6 mm ID

Strata®-XL-C

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S044-TAK	1 mL (100/box)
	60 mg	8B-S044-UBJ	3 mL (50/box)
	100 mg	8B-S044-EBJ	3 mL (50/box)
	100 mg	8B-S044-ECH	6 mL (30/box)
	200 mg	8B-S044-FBJ	3 mL (50/box)
	200 mg	8B-S044-FCH**	6 mL (30/box)
	500 mg	8B-S044-HCH	6 mL (30/box)
Giga™ Tube			
	2 g	8B-S044-KEG	20 mL (20/box)
	5 g	8B-S044-LEG	20 mL (20/box)
	5 g	8B-S044-LFF	60 mL (16/box)
	10 g	8B-S044-MFF	60 mL (16/box)

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