SCF 534

Recovery of Nitrosamines from Frankfurters using Supercritical CO_2

Introduction

Since some *N*-nitrosamines are carcinogenic compounds, the USDA monitors nitrite-cured meat products for high levels of volatile nitrosamines. Typically, nitrosamines are removed from the meat sample by vacuum distillation in a mixture of mineral oil and base. Next, a dichloromethane extraction is performed followed by GC-TEA. If the sample is found to have high levels of nitrosamines, a more lengthy and specific procedure is employed involving additional solvent washes and a column chromatographic clean-up step before GC/MS is performed. As these methods are time consuming, solvent intensive, and require the use of expensive equipment, better methods of extraction are needed.

SFE is an alternative extraction technology that uses supercritical CO_2 to recover trace analytes from a variety of matrices. When placed above the critical point (31.1°C and 73 atm) CO_2 becomes a remarkable solvent for many analytes. In addition, supercritical CO_2 is harmless ecologically, readily available, non-toxic and non-explosive. SFE significantly reduces the use, exposure to, and disposal of hazardous solvents, while providing comparable extraction results to standard methods in less time

There have been some problems identified with using typical SFE methods to isolate analyte residue from tissue matrices. One of the main difficulties is that when trace levels of residues are isolated from fat tissue by SFE using CO_2, various amounts of lipids can be co-extracted. If a modifier is used with CO_2, the resultant extract becomes more complex and the desired analyte is more difficult to recover from the mixture.

A solution to these problems is to use an SFE instrument and method that simplifies the separation and recovery of trace residues from an analyte/fat matrix. This application describes a procedure for coupling SFE technology with an offline trapping technique for the rapid extraction of nitrosamines from frankfurters without co-extracting lipids.

Equipment

- ✓ Applied Separations' *Spe-ed*™SFE-2 or Helix Supercritical Extraction System
- ✓ Analytical Balance
- ✓ GC-TEA (Gas Chromatographic-Thermal Energy Analyzer)

Materials

- ✓ Carbon Dioxide – Supercritical grade
- ✓ 6 mL silica gel SPE columns, Applied Separations – Cat. #2107, 1gm/6ml
- ✓ *Spe-ed* Matrix – Cat . #7950
- ✓ Hexane
- ✓ Dichloromethane

- ✓ Ethyl Ether
- ✓ Propyl Gallate (Aldrich Chem Co.)
- ✓ Pentane
- ✓ NDPA (*N*-nitrosodipropylamine)

Method

Prepare the sample by weighing out 2.5 g of comminuted frankfurter, and placing in a 100 mL beaker. Add 250 mg of propyl gallate and 0.5 mL of NDPA internal standard solution (0.10 g/mL of NDPA in dichloromethane) to the beaker. Weigh 5.0 g of *Spe-ed* Matrix, and place into the beaker. Stir this mixture until it appears uniform in appearance (approximately 1 minute). Transfer the dry, free-flowing mixture to extraction vessel, and then seal the vessel. Install the vessel into the *Spe-ed* SFE. Place a 6 mL SPE column containing 1.0 sieved silica gel on the offline micrometering valve. Extract sample according to the specified extraction conditions.

Extraction Conditions

Extraction vessel:	24 mL
Sample:	2.5 g
Pressure:	680 BAR
Temperature:	40 ºC
Valve temperature:	110 ºC
Static time:	2 minutes
CO_2 Flow Rate:	3.0 L/min
Dynamic time:	20 minutes
Collection:	silica SPE column

Analyte Recovery

Wash trace residue of fat remaining on the discharge tube into the SPE column with 0.25 mL of hexane using a syringe and stainless steel needle. Next, wash SPE column with 2 x 4-mL portions of 25% dichloromethane in pentane. Discard washings containing the recovered fat, and elute nitrosamines from the column by passing 2 x 4 mL of 30% ethyl ether in DCM through the sorbent bed. Concentrate these washings to a 1.0 mL volume at 70 °C.

Analysis

GC-TEA

Results

SFE Recovery of 10 Nitrosamines from Fortified All-Meat Frankfurters

N-Nitroso Compound	Range (%)	Mean (%)	RSD (%)	CV (%)
NDMA	94.0-100.9	97.50	2.85	2.92
NMEA	87.0-92.6	89.45	2.34	2.62
NDEA	84.3-92.6	92.37	6.13	6.34
NDPA	84.8-98.6	92.37	6.13	6.34
NAZET	94.3-104.8	100.57	3.55	3.53
NDBA	92.2-101.8	95.22	3.54	3.72
NPIP	91.1-101.8	97.80	5.89	6.02
NPYR	91.0-103.2	95.17	4.41	4.63
NMOR	97.1-100.9	100.86	3.87	3.84
NHMI	91.5-102.0	97.62	4.06	4.16

Conclusion

SFE technology offers a viable alternative to traditional solvent based extraction methods. The accuracy and precision of the results were comparable to the standard method while extraction times were reduced.

References

Maxwell, R.J.; Pensabene, J.W.; Fiddler, W. "Multiresidue Recovery at PPB Levels of 10 Nitrosamines from Frankfurters by Supercritical Fluid Extraction." *Journal of Chromatographic Science*, Vol. 31, (1993) 212-215.