

Determination of glycerol triheptanoate (GTH) in animal feed : UHPLC-MSMS analysis preceded by microwave extraction

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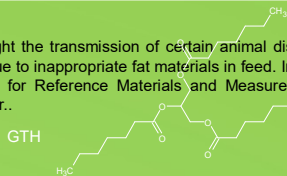
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INTRODUCTION

The mad cow disease crisis in the 1990s brought to light the transmission of certain animal diseases through contaminated animal by-products, particularly due to inappropriate fat materials in feed. In order to prevent further contaminations, the IRMM (Institute for Reference Materials and Measurements) identified GTH (glycerol triheptanoate) as an ideal marker.



To ensure that marketed food products do not contain inappropriate fat materials, the IRMM has validated an analytical method based on GC-MS to verify the absence of GTH.

The current method employed



Time-consuming
Tedious manipulations
large volumes of solvent

Therefore, in order to streamline these manipulations, the determination of GTH in food requires an analytical method that is faster, more sensitive, more selective, and requires fewer preparation steps.

The developed method



Fast
Effective
Reduced volumes of solvent

The envisioned method to detect GTH in a faster, more sensitive, selective, and less preparation-intensive manner would involve the use of UPLC®-MS/MS - Microwave-Assisted Fat Extraction.

SAMPLES/METHOD

Microwave-Assisted Extraction - Single Station

1 g of sample is placed in a CEM microwave extraction tube

6 ml of extraction solvent is added MeOH

The tube is placed in the microwave oven Extraction

Parameters : Temperature of 100 °C for 4 minutes.



CEM Discover 2.0



Temperature 50 °C

Gradient	Time (min)	Flow (ml/min)	% A	% B
1	-	0,400	60,0	40,0
2	3,00	0,400	46,0	54,0
3	3,10	0,400	70,0	70,0
4	18,00	0,400	99,0	1,0
5	18,10	0,400	60,0	40,0
6	20,00	0,400	60,0	40,0

UHPLC

Mobile phase A : Acetonitrile / Water (60/40), 10 mM Ammonium Formate, 0.1% Formic Acid

Mobile Phase B : Isopropanol / Acetonitrile (90/10), 10 mM Ammonium Formate, 0.1% Formic Acid

Stationary Phase : ACQUITY CSH C18 COLUMN, 1.7 µm

EXPERIMENT

Identification of the parent ion in MS

A standard GTH of concentration 10 mg/100 ml, diluted in MeOH solvent, was infused in the MS.



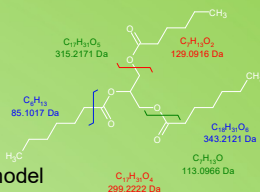
- **Analyzer Ionization Source** : Electrospray Ionization (ESI)
- **MS/MS** : Triple Quadrupole (QQQ)
- **Detection Mode** : Single Ion Monitoring (SIM)

GTH (glycerol triheptanoate)

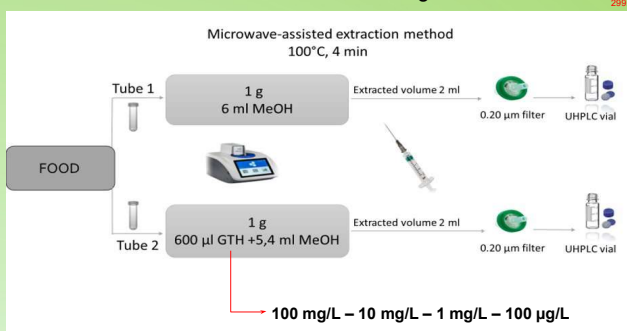
SIM mode	Capillary	cône	Adduit	Formule	MW
451.45	4,50 (KV)	50 (V)	22.52	C ₂₄ H ₄₄ O ₆	428.60

MSMS method : fragmentation and optimization

MRM mode	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (V)
1	451.45	85.1	0.01	50	26
2	415.45	113.1	0.01	50	26



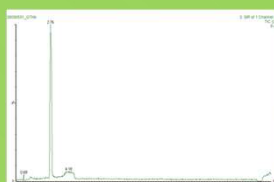
Microwave-assisted extraction method using CEM model



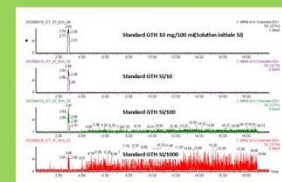
RESULTS

Identification of the GTH peak in the chromatogram : standard in MeOH and after microwave extraction

GTH Standard

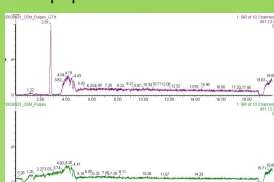


GTH Standard : some dilutions

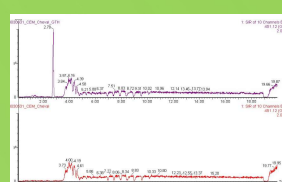


Analysis of spiked and non-spiked food samples.

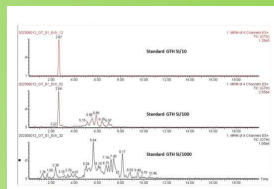
Beet pulp



Horse feed

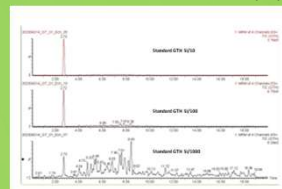


Pork feed



Sensitivity (GTH/g of sample)

Beet pulp



CONCLUSIONS

With the developed UPLC-MS/MS method, we successfully detected GTH in food samples containing inappropriate fat materials in a short and efficient timeframe, confirming the suitability of the developed method. Therefore, we can conclude that this method is capable of verifying the presence or absence of GTH in animal feed.

The next steps will be to evaluate the detection limit and determine the recovery rate at different concentrations of GTH. The robustness of the method will be determined on different feeds. To reduce the analysis time and thus the volumes of solvents used, the chromatographic method will be optimized to arrive at a run time of about 5 minutes.

PERSPECTIVES

