SHIMADZU
Excellence in Science

Phytronix

Application Note : 1804

High-throughput analysis of Indole, Skatole and Androstenone in pork fat by LDTD-MS/MS

Serge Auger, Jean Lacoursière and Pierre Picard
Phytronix Technologies, Québec, Canada
Keywords: High-throughput, boar taint, LDTD-MS/MS

Introduction

The European Union has decided to ban boar castration by 2018. As hundreds of millions of boars are slaughtered every year for meat consumption, there is a need for methods of detection of boar taint. Compounds responsible for boar taint include androstenone, indole and skatole.

We propose to perform a fast, cheap and simple sample preparation method followed by a quantification using Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS), an ultra-fast quantification technique.

LDTD-LC-MS/MS System

Figure 1 - LDTD-LC-MS/MS system

Sample Preparation Method

0.3 g of back fat sample in tube*.
3000 µL NaOH (1N in water)
3000 µL internal standard mixture in methyl-ter-butyl ether (MTBE)
- 200 ng/mL Androstenone-d_4
- 5.4 ng/mL Skatole-d_3

Vortex (1 minute at 3000 rpm) until complete fat dissolution.
Let stand at least 2 minutes for phase separation**.
Spot 4 µL of the upper layer in a LazWell™ plate
LDTD-MS/MS analysis after complete solvent evaporation (1 minute at room temperature).

* Note 1: Volume of NaOH and MTBE are adjusted against the back-fat's weight.
**Note 2. Minimum time needed according Figure 2 results.

LDTD-MS/MS Parameters

LDTD
Model: Phytronix, SH-960
Carrier gas: 6 L/min (air)
Laser pattern: 3 second ramp to 65% power

MS/MS
Model: Shimadzu LCMS-8060
Ionization: APCI
Positive MRM transition

MS/MS transition

Positive MRM transition for LDTD-MS/MS.

	LDTD	CE
Indole	118.0 → 91.0	40
Skatole	132.2 → 117.2	25
Skatole-d_3	135.2 → 117.2	25
Androstenone	273.3 → 215.3	18
Androstenone-d_4	277.3 → 215.3	18

Note: Skatole-d_3 is used as an internal standard for Indole.

Results and Discussion

Phase separation evaluation

After the mixing step, different times (1 to 10 minutes) for the phase separation are evaluated. The sample is mixed and left alone for different time points. The upper layer is spotted in triplicate. After a complete evaporation, sample concentrations are evaluated against a calibration curve. **Figure 2** shows the results of %Nominal against the separation time.

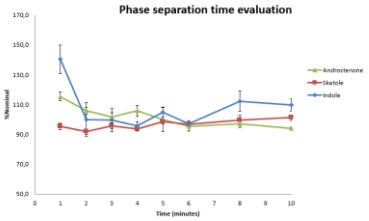
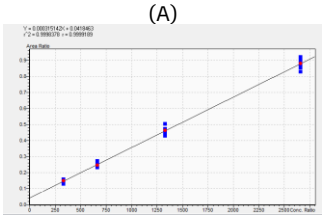


Figure 2 – Phase separation time evaluation curve

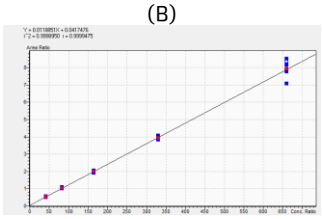
Linearity

The negative back fat sample extract is spiked to get the following calibration range around the proposed sorting thresholds: 332.5 to 2660 ng/g for Androstenone, 41.3 ng/g to 660 ng/g for Skatole and 16.5 ng/g to 132 ng/g for Indole. Correlation coefficients are equal or greater than 0.99 for the quantification curve of each molecule. The LLOQ is greater than the required concentration of boar taint analysis.

(A)



(B)



(C)

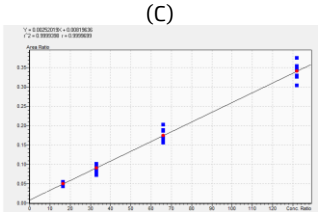
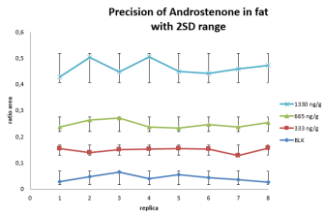


Figure 3 - Standard curve for Androstenone (A), Skatole (B) and Indole (C)

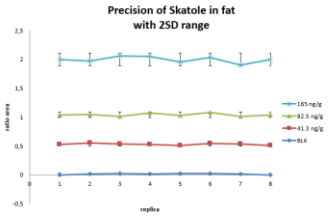
Precision

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against IS ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell™ plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15%. Results using the ± 2 STD overlay are plotted. **Figure 4** shows the result.

(A)



(B)



(C)

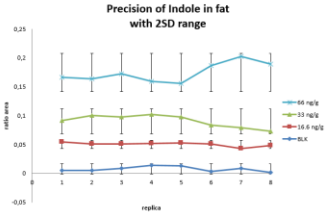


Figure 4 - Precision curve for Androstenone (A), Skatole (B) and Indole (C)

Wet stability of sample extracts

Following the extraction, samples are kept at room temperature in closed containers. After 2 hours, sample extracts were spotted on a LazWell™ plate and analyzed. The reproducibility and accuracy are reported in **Table 1, 2 and 3** for LLOQ samples. All the results are within the acceptable range (criteria %RSD ≤20% and %Nom 100 ± 20%) with less than 20% blank interference for 2 hours at room temperature.

Dry Stability of Samples Spotted in LazWell™

Androstenone, Skatole and Indole are volatile compounds and their dry stability in a LazWell™ kept at room temperature is evaluated. Extracted samples are spotted onto a LazWell™ plate and kept 1 hour at room temperature before analysis. The reproducibility and accuracy are reported in **Table 1, 2 and 3** for LLOQ samples. All the results are within the acceptable range (criteria %RSD ≤20% and %Nom 100 ± 20%) with less than 20% blank interference for 1 hour at room temperature.

Table 1 - Wet and dry stability Androstenone

Parameters	Dry stability	Wet stability
Time (h)	1	2
Temp. (°C)	22	22
Conc. (ng/g)	332.5	332.5
N	6	6
Mean (ng/g)	307.4	321.1
%RSD	12.6	15.7
%NOM	92.5	96.6

Table 2 - Wet and dry stability Skatole

Parameters	Dry stability	Wet stability
Time (h)	1	2
Temp. (°C)	22	22
Conc. (ng/g)	41.3	41.3
N	6	6
Mean (ng/g)	42.3	41.5
%RSD	6.8	8.5
%NOM	102.4	100.4

Table 3 - Wet and dry stability Indole

Parameters	Dry stability	Wet stability
Time (h)	1	2
Temp. (°C)	22	22
Conc. (ng/g)	16.5	16.5
N	6	6
Mean (ng/g)	16.0	15.5
%RSD	13.0	16.7
%NOM	97.2	93.9

Conclusion

LDTD technology combined with a LCMS-8060 system allows ultra-fast (**8 seconds per sample**) and accurate quantification of Androstenone, Skatole and Indole in back fat sample using a cheap and easily automated sample preparation.

For more information about your specific application, visit www.phytronix.com

Phytronix Technologies
Parc technologique du Québec métropolitain
4535, boulevard Wilfrid-Hamel, suite 120, Québec (Qc) Canada G1P 2J7
www.phytronix.com

For Research Use Only. Not for use in diagnostic procedures