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

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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

Add 0.3 g of back fat sample in a tube.
Dounce homogenize the sample.
Add 3000 µL of NaOH (1N in water).
Add 1000 µL of Saturate solution of NaCl.
Add 3000 µL of internal standard mixture in methyl-tert-butyl ether (MTBE)
- 200 ng/mL of Androstenone-d_4
- 5.4 ng/mL of Skatole-d_3

Mix and wait 1 minute for phase separation.
Spot 5 µL of the upper layer in a LazWell™96 plate
LDTD®-MS/MS analysis after complete solvent evaporation (1 minute at room temperature).

LDTD-MS/MS Parameters

LDTD
Model: Phytronix, LDTD® 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.

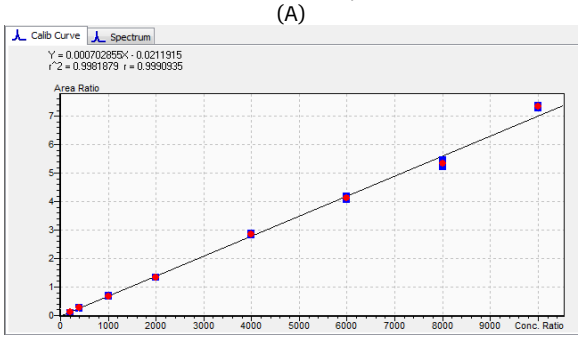
	LDTD	CE
Skatole	132.2 → 117.2	25
Skatole-d_3	135.2 → 117.2	25
Androstenone	273.3 → 215.3	18
Androstenone-d_4	277.3 → 215.3	18

Results and Discussion

Linearity

The negative back fat sample extract is spiked to get the following calibration range around the proposed sorting thresholds: 200 to 10000 ng/g for Androstenone and 25 ng/g to 1250 ng/g for Skatole. 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)

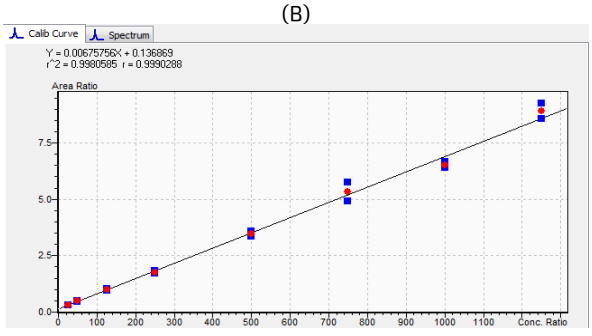


Figure 2 - Standard curves for Androstenone (A) and Skatole (B)

Sampling evaluation

Fat sample extracts of 0.3 g are compared to 0.9 g of a positive sample. Keeping a proportional extraction volume, samples are extracted, and the concentration is evaluated against the calibration curve. **Table 1** shows the comparison results. A percentage of difference of 3.5% is obtained.

Table 1 – Sampling extraction comparison of Androstenone

Sample	Androsterone Concentration (ng/g)
Sample 1 (0.3g)	7768
Sample 1 (0.9g)	7499
%Difference:	3.5%

Precision

Six different preparations of positive pork fat are extracted and quantified against the calibration curve. The reproducibility of the extraction of 0.3 g of fat is evaluated. **Table 2 and 3** show the results. All the results are below 15% RSD.

Table 2 – Extraction reproducibility of Androstenone

Sample	Concentration (ng/g)
R1	1347
R2	1502
R3	1394
R4	1580
R5	1571
R6	1503
Mean	**1483**
SD	**94**
%RSD	**6.4**

Table 3 – Extraction reproducibility of Skatole

Sample	Concentration (ng/g)
R1	94
R2	121
R3	128
R4	125
R5	141
R6	129
Mean	**123**
SD	**16**
%RSD	**12.7**

Wet stability of sample extracts

Following the extraction, samples are kept at room temperature in open containers in a fume hood. After 2 hours, sample extracts were spotted on a LazWell™96 plate and analyzed. The reproducibility and accuracy are reported in **Table 4 and 5** for samples at the cut-off level suggested by the industry. All the results are within the acceptable range (criteria %RSD ≤15% and %Nom 100 ± 15%) with less than 20% blank interference for 2 hours at room temperature.

Dry Stability of Samples Spotted in LazWell™

Androstenone and Skatole 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 at room temperature for 2 hours before the analysis. The reproducibility and accuracy are reported in **Table 4 and 5** for samples at the cut-off level suggested by the industry. All the results are within the acceptable range (criteria %RSD ≤15% and %Nom 100 ± 15%) with less than 20% blank interference for 2 hours at room temperature.

Table 4 - Wet and dry stability Androstenone

Parameters	Dry stability	Wet stability
Time (h)	2	2
Temp. (°C)	22	22
Conc. (ng/g)	2000	2000
N	3	3
Mean (ng/g)	2082.0	2049.5
%RSD	3.4	1.5
%NOM	104.1	102.5
%Blk interf.	1.1	0.7

Table 5 - Wet and dry stability Skatole

Parameters	Dry stability	Wet stability
Time (h)	2	2
Temp. (°C)	22	22
Conc. (ng/g)	125	125
N	3	3
Mean (ng/g)	128.0	128.2
%RSD	2.2	2.3
%NOM	102.4	102.6
%Blk interf.	13.2	14.2

Conclusion

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

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