

Fully Automated Process for Analysis of Indole, 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 full automated sample preparation method followed by a quantification using Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS/MS), an ultra-fast quantification technique.

Luxon Ionization Source

The Luxon Ion Source® (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (Figure 2) to obtain unmatched thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high intensity molecular ion signal in less than 1 second sample to sample and allows working with very small volumes.



Figure 1 - Luxon Ion Source®



Figure 2 - Schematic of the Luxon ionization source

Sample Preparation Method Automated System

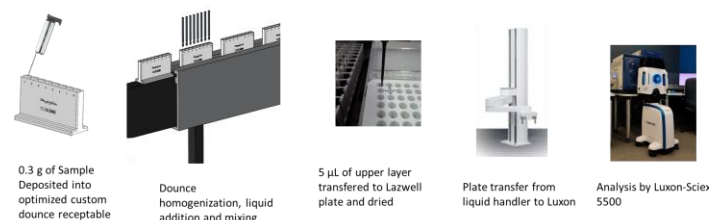


Figure 3 – Automated sample preparation and analysis of Indole, Skatole and Androstenone

Extraction method

- 0.3 g of back fat sample in 8 positions custom dounce receptacle. Transfer to homogenization section.
- Dounce homogenization and solvent addition:
 - 4 mL NaOH (1N) containing NaCl.
 - 3 mL internal standard mixture (200 ng/mL Androstenone-d₄ and 5.4 ng/mL Skatole-d₃ in MTBE).
- Mixing by Up and Down.
- Wait 1 minute for phase separation.
- Transfer 5 µL to Lazwell plate.
- Wait 1 minute for drying completely.
- Plate transfer to Luxon Ion Source and analyze by LDTD-MS/MS.
- Automatic data processing to give a GO or NO-GO sample analysis based on cut-off levels.

LDTD-MS/MS Parameters

LDTD

Model: Phytronix, LDTD® S-960, with LazWell Infeed System

Carrier gas: 6 L/min (air)

Laser pattern:

- 1 second start delay
- 3 seconds ramp to 65% power

MS/MS

Model: Sciex 5500 Qtrap system®

Ionization: APCI

Ionization mode: Positive

Curtain: 20

Time: 4 seconds

Table 1 - Positive MRM transition for LDTD-MS/MS.

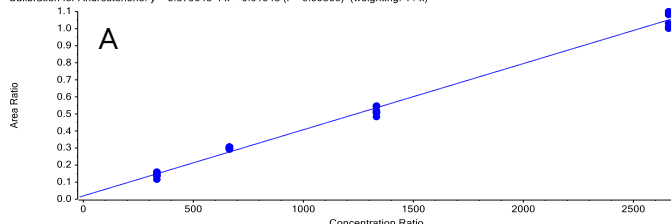
	LDTD	CE
Indole	118.0 → 91.0	30
Skatole	132.0 → 117.0	25
Skatole-d ₃	135.0 → 117.0	25
Androstenone	273.2 → 215.3	23
Androstenone-d ₄	277.2 → 215.3	23

Results and Discussion

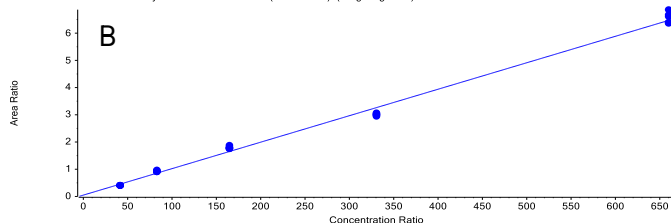
Linearity

Negative back fat sample extracts are 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.

Calibration for Androstenone: $y = 3.87964e-4 x + 0.01943$ ($r = 0.99566$) (weighting: $1/x$)



Calibration for Me.Indole-117: $y = 0.00973 x + 0.04882$ ($r = 0.99631$) (weighting: $1/x$)



Calibration for Indole-91: $y = 0.00316 x + 0.03162$ ($r = 0.99482$) (weighting: $1/x$)

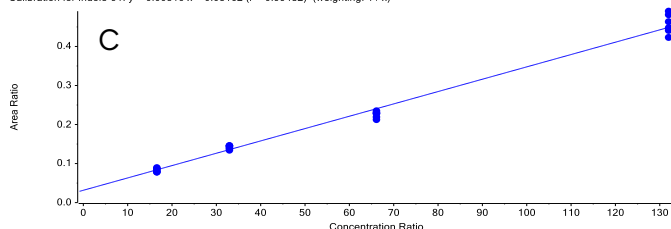


Figure 4 - Standard curve for Androstenone (A), Skatole (B) and Indole (C)

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 (± 2 SD) 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 SD overlay are plotted. **Figure 5** shows the results.

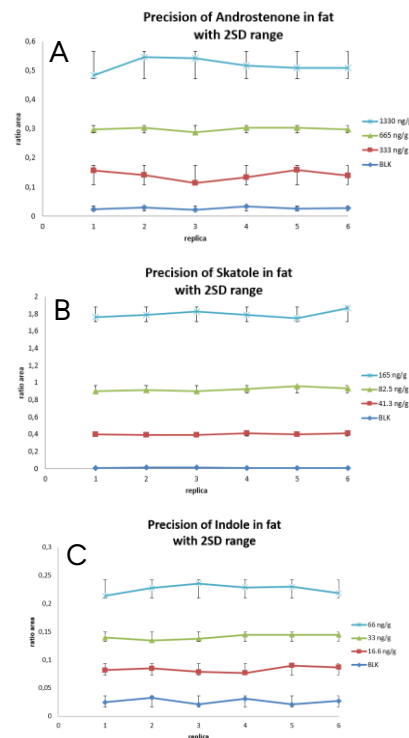


Figure 5 - Precision curve for Androstenone (A), Skatole (B) and Indole (C)

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 2** for LLOQ samples. All the results are within the acceptable range (criteria %CV $\leq 20\%$ and %Nominal $100 \pm 20\%$) with less than 20% blank interference for 1 hour at room temperature.

Table 2 - Dry stability results

Parameters	Androstenone	Skatole	Indole
Time (h)	1	1	1
Temp. (°C)	22	22	22
Conc. (ng/g)	332.5	41.3	16.5
N	6	6	6
Mean (ng/g)	307.4	42.3	16.0
%CV	12.6	6.8	13.0
%NOM	92.5	102.4	97.2

Conclusion

Luxon Ion Source® based on the LDTD technology combined with a Sciex 5500 Qtrap system allows ultra-fast (**8 seconds per sample**) and accurate quantification of Androstenone, Skatole and Indole in back fat sample using a full automated sample preparation process.