

# At-line rapid instrumental method for measuring the boar taint components androstenone and skatole in pork fat

Claus Borggaard, Rune Birkler, Lene Meinert and Susanne Støier

## AIM

An increase in the number of slaughtered male pigs has led to demands for a rapid, reliable and inexpensive instrumental means of measuring androstenone and skatole. The aim was to develop an accurate method for measuring boar taint components in backfat from uncastrated male pigs, matching industrial demands for speed of operation and robustness.

## REQUIREMENTS FOR AN AT-LINE BOAR TAIN DETECTION SYSTEM

- The system must measure both skatole and androstenone simultaneously.
- Cost of operations must be below 1€ per carcass.
- The measuring system must run fully automated.
- Adequate capacity for keeping up with line-speeds at the largest Danish slaughterhouses.
- Samples for analysis are extracted from the male pig carcass on the slaughter line and results of the analysis must be available before the carcass enters the overnight cold storage room (typically 45 minutes).
- The system must be robust and run with long intervals between maintenance.

## THE DETECTION METHOD

A tandem mass spectrometer (Sciex 6500 or 4500 QTRAP) front-ended by a Laser Diode Thermal Desorption and APCI ionization (LDTD-MS-MS, Phytronix, Quebec City) was chosen as detection system. Advantages are:

- No need for slow and expensive chromatographic columns that need frequent replacement.
- Samples can be analysed at a rate of 360 samples/h on a single instrument.
- High selectivity, sensitivity and reproducibility.

In the LDTD, dried residues from an extraction process, deposited in a Lazwell™ microtiter plate, are heated and thereby desorbed by applying a short laser pulse. The analytes are then drawn by a flow of air past a corona discharge needle, where they are ionized and subsequently pass into the MS-MS for detection in SRM mode.

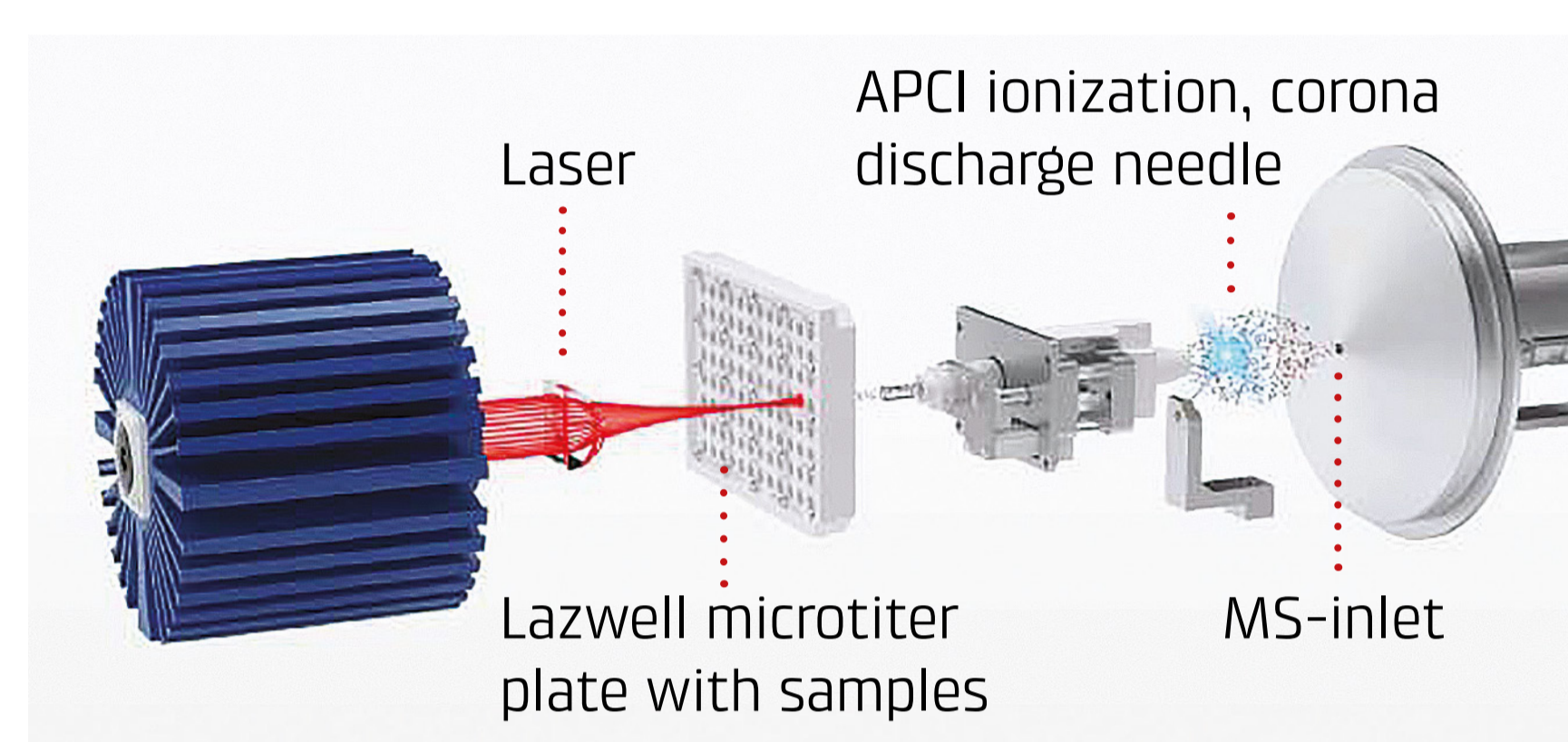


Figure 1. The LDTD work principle



Figure 2. LDTD in the Luxon version front-ending a Sciex MS-MS. The Luxon LDTD can be automatically loaded with Lazwell plates by a Scara robot.

## SAMPLE EXTRACTION FROM CARCASS AND PRETREATMENT

The fully automated steps in the analysis are as follows:

1. On the slaughter line, 0.3 – 0.8 gram backfat samples are extracted from each male carcass and placed in a 24 well deep well plate (DWP). Samples are weighed automatically during the filling process.
2. When filled with 24 samples the DWP is transported by a conveyor to an in-house laboratory.
3. To each sample in the DWP are added 1.5ml brine and 1.5ml acetonitrile with internal standards (androstanone (5 $\alpha$ -Androstan-3-one) and deuterated skatole) by a pipetting liquid handling robot.
4. DWP is moved to a homogenizer workstation, where the contents of each well are homogenized for 30 seconds.
5. Two DWPs with a total of 48 homogenized samples are now transferred to a centrifuge and exerted to 5000 x g for 5 minutes.
6. After centrifugation, 4 $\mu$ l supernatant is transferred from each sample on to separate wells of a Lazwell™ microtiter plate (Phytronix, Quebec City, Canada) and left to dry for 2 minutes.
7. The lazwell™ plate is now automatically inserted in the LDTD where sample residues are desorbed, ionized and measured by the MS-MS system running in SRM mode. Sample plates are moved through the above steps using SCARA laboratory robots.

## RESULTS

Limit of detection (LOD) and limit of quantification (LOQ)

	LOD	LOQ
Androstenone	0.05 $\mu$ g/g	0.1 $\mu$ g/g
Skatole	0.02 $\mu$ g/g	0.05 $\mu$ g/g

Proposed sorting thresholds:  
Skatole: 0.25  $\mu$ g/g  
Androstenone: 0.5 - 2.0  $\mu$ g/g  
(still being investigated)

Calibration curves for androstenone and skatole, were prepared by spiking a blank fat matrix with varying concentrations of the two analytes.

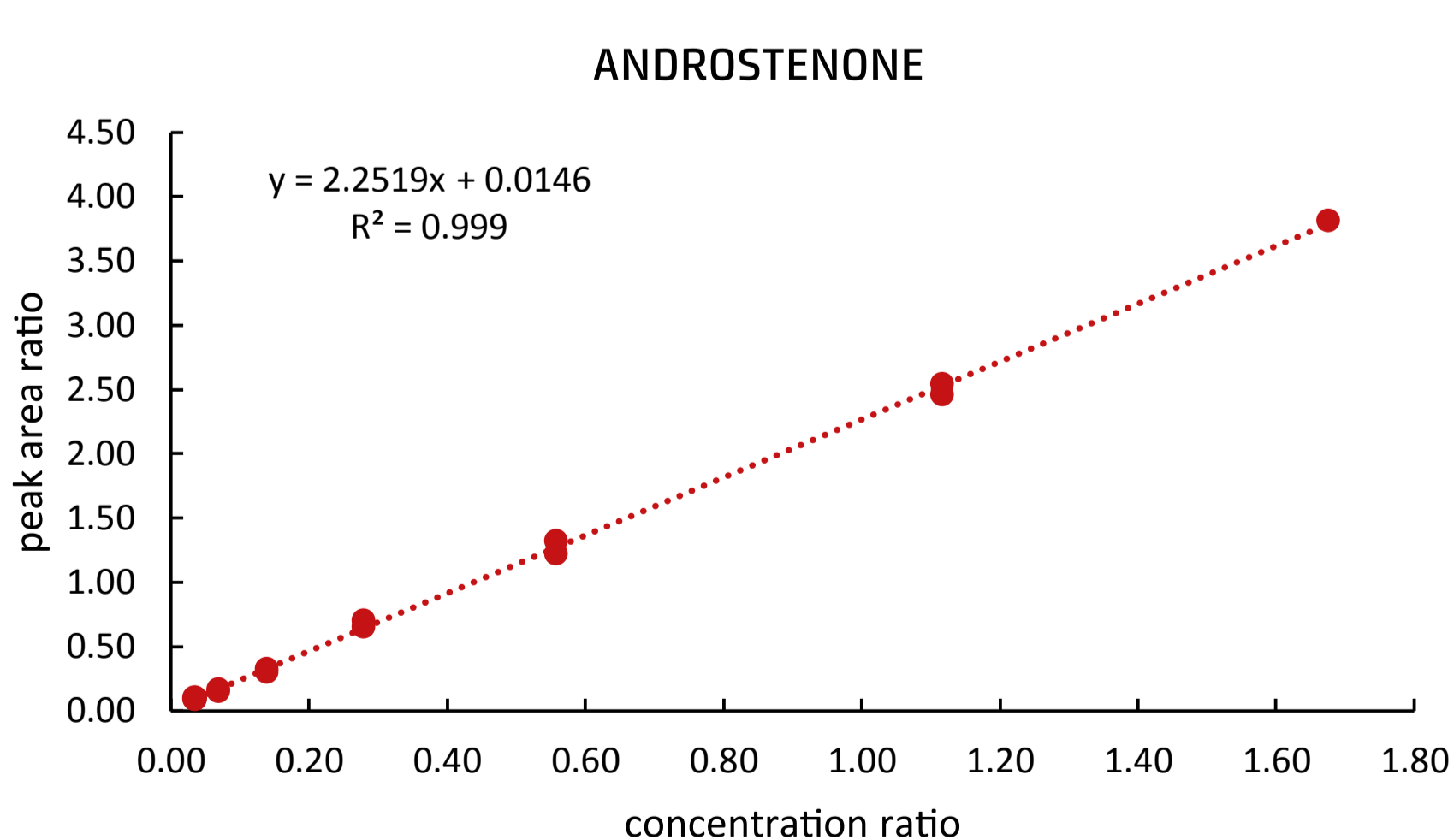


Figure 3: Calibration curves for androstenone. Measured peak area ratios vs. spiked concentration ratios (androstenone/androstanone)

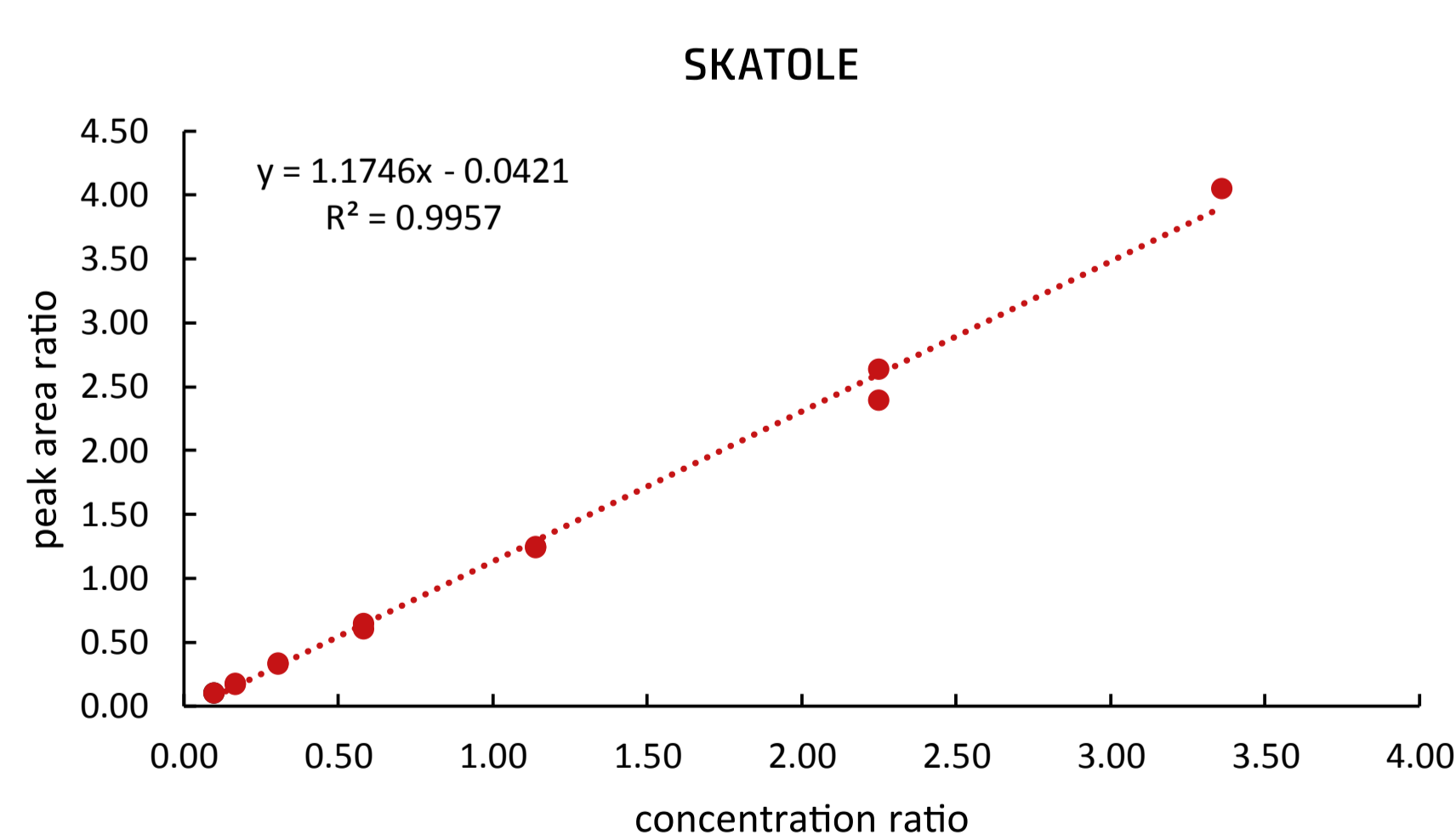


Figure 4: Calibration curve for skatole

## CONCLUSION

A rapid instrumental at-line method for simultaneous measurement of androstenone and skatole in back fat samples from entire male pigs has been developed. With an automated sample pre-treatment, it will be possible with a single LDTD-MS-MS system to keep up with a line speed of 360 male pig carcasses per hour and to run 16 hours per workday. Cost of operations is expected to be below 0.7€/carcass. Reproducibility on fully homogenized fat samples is better than 3% relative CV for androstenone and 5% relative CV for skatole.



## CONTACT

CLAUS BORGGAARD, SENIOR SPECIALIST  
+45 7220 2560, CBO@TEKNOLOGISK.DK