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A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds

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ABSTRACT

Boar taint is an off-odour that can occur when meat or fat from entire male pigs is heated. Most of the currently available analytical methods are not capable of detecting the three known boar taint compounds (indole, skatole and androstenone) simultaneously, which renders their analysis often labour-intensive and time-consuming as separate analyses are required. In this study a validated U-HPLC–HR-Orbitrap-MS analysis method is described for the quantitative determination of the three boar taint compounds in fat. The sample pre-treatment involves a melting step followed by extraction with methanol and clean-up consisting of a freezing step and solid phase extraction (HLB cartridges). The analytes are then chromatographically separated and detected with an Exactive™ high-resolution mass spectrometer. Due to the absence of guidelines for the analysis of boar taint in fat, the Commission Decision 2002/657/EC [18] and ISO 17025 [19] guidelines were used as guideline for validation of the developed detection method. This resulted in limits of detection and limits of quantification between 2.5 and 7 $\mu g\,kg^{-1}$ and between 5 and 10 $\mu g\,kg^{-1}$ for the three compounds, respectively, which is far below the threshold values set at 100 $\mu g\,L^{-1}$ for indole, 200 $\mu g\,L^{-1}$ for skatole and 1000 $\mu g\,L^{-1}$ for androstenone in pig fat samples. The method obtained for the three compounds a repeatability (RSD) lower then 12.7% and a within-laboratory reproducibility (RSD) lower than 16.9%. The recovery of the three compounds ranged between 99 and 112 and an excellent linearity ($R^2 \geq 0.99$) was found. In the future, this method may be extended with other compounds that turn out to be correlated with boar taint.

1. Introduction

The castration of male animals intended for meat production has been widely practised for centuries. The main reasons were easier handling of the castrated animals and the fact that these castrated animals deposit more fat, which was at that time requested by consumers. In recent years, a trend has been observed towards consumers demanding a diet consisting of more lean meat. This trend, combined with the lower production costs of entire males, has led to cessation of castration of cattle and sheep in most countries [1]. The castration of male pigs, however, remains a common practice in most countries because 4–25% [2] of entire males produce moderate to high amounts of boar taint, an off-odour released when heating the meat of some boars.

Castration of male piglets is generally performed without anaesthesia or analgesia. This practice has led to ethical constraints [1] and several alternatives are now being explored (castration with anaesthesia, immunocastration, use of analgesia, or production of entire males in combination with management procedures) [3]. At this time European legislation does not forbid castration without anaesthesia or analgesia. Nevertheless, several countries have either already forbade these types of castration or have the intention to stop all kinds of castration completely within several years (i.e. Norway, the Netherlands, Switzerland). A European Declaration describes taking the first step to stop castration without anaesthesia or analgesia by January 2012 and in the longer term, to abandon castration by 2018. Adequate detection methods are essential to determine whether the alternatives to castration are successful in decreasing boar taint.

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The main compounds attributed to the boar taint off-odour are skatole and androstenone; indole only contributes to a lesser degree [4,5]. Because of their lipophilic characteristics, the compounds tend to accumulate in the fatty areas of the animal. The extraction and clean-up of the fat matrix is therefore considered to be the most critical and challenging step for their analytical determination [6]. Several authors have stated that the following sample preparations of this fat matrix lead to a positive influence on the sensitivity of the detection method: liquid–liquid extraction [7], saponification [8], solid phase extraction [9] and liquification of the fat [10].

In recent years, several methods have been developed to determine the boar taint compounds. However, only a limited number of authors describe analysis methods for the indolic compounds (indole, skatole) and the steroid compound (androstenone) simultaneously. Androstenone is often determined by ELISA [11] or gas chromatography coupled to electron-capture detection [8], flame ionisation detection [12] or mass spectrometry [13], while the indolic compounds skatole and indole are determined by colorimetric methods [14] or liquid chromatography coupled to fluorescence detection [7]. Analysis of all three boar taint compounds may therefore be considered to be labour-intensive and time-consuming, as separate analyses must be performed.

Hansen-Møller [15] was the first to describe a simple method for the simultaneous determination of the three compounds with HPLC coupled to fluorescence detection. The obtained limits of detection (LODs) for the indolic compounds and androstenone were <3 $\mu g\,kg^{-1}$ and 20 $\mu g\,kg^{-1}$, respectively. Androstenone was detected using derivation with dansylhydrazine. However, the use of a derivation step may lead to possible false-positive results and is labour-intensive [16]. In addition, the determination of the limits of detection and quantification are determined in standard solutions instead of in a matrix. The possibility therefore exists that the LODs and LOQs would be higher in a matrix. More recently, a similar method was developed which relys on HPLC coupled to mass spectrometry [17]. It has a limit of quantification for the indolic compounds of 50 $\mu g\,kg^{-1}$ and for androstenone of 125 $\mu g\,kg^{-1}$. For routine purposes, however, this method was not robust enough (mainly for androstenone). The extraction procedure in particular needed improvement. Fischer et al. [18] developed a method with limits of quantification of 0.5 and 1 $\mu g\,kg^{-1}$ for skatole and indole, respectively, and 60 $\mu g\,kg^{-1}$ for androstenone using HS-SPME-GC–MS. Prior to the extraction, the fat was melted by a microwave step, after which methanol was added. This was followed by a freezing and evaporation step. Afterwards HS-SPME was used for extraction, while separation and detection occurred by GC–MS.

The objective of this study was to develop a quantitative, accurate, robust and fast U-HPLC–MS-based method that is capable of quantifying the three known boar taint compounds simultaneously in fat. The method was validated according to the guidelines of 2002/657/EC [19] and/or ISO 17025 [20] which for makes the method suitable for use when comparing boar taint alternatives.

2. Materials and methods

2.1. Reagents and chemicals

The reference standards indole (2,3-benzopyrrole), skatole (3-methylindole) and androstenone (5α-androst-16-ene-3-one) and the internal standards 2-methylindole (2MID) and androstadienedione (1,4-androstadiene-3,17-dione, ADD) were obtained from Sigma–Aldrich (St. Louis, MO, USA). For each compound a stock solution was prepared in methanol at a concentration of 1 $mg\,mL^{-1}$. Working solutions were made for each compound in methanol at a range of 5–100 $ng\,\mu L^{-1}$. Solutions were stored in dark glass bottles at −20 °C.

Reagents were of analytical grade when used for extraction purposes and of MS-grade for U-HPLC–MS applications. They were obtained from VWR International (Merck, Darmstadt, Germany) and Fisher Scientific (Leichestershire, VS), respectively.

Table 1
Linear gradient for the separation of the boar taint compounds on a U-HPLC system.

Time	% 0.05 formic acid	% methanol
0.00	50	50
0.01	53	47
2.57	50	50
3.00	5	95
6.00	0	100
8.00	0	100
8.01	50	50
10.00	50	50

2.2. Samples

Barrows are normally slaughtered at a mean commercial slaughter weight of 110 kg. For the control samples, neck fat of barrows was collected 24 h after slaughter. Skin and muscle were separated from the fat before packaging and the samples were stored at −20 °C until analysis.

2.3. Extraction and clean-up

Two grams of fat were sliced into pieces and spiked with a mixture of internal standards (500 $\mu g\,L^{-1}$ 2-MID and 1000 $\mu g\,L^{-1}$ ADD). The fat was melted in a microwave oven for 3 min at 220 Watt and allowed to rest for another 3 min. This was repeated until at least 300 μL of liquid fat was obtained. An aliquot of 150 μL was taken and mixed with 750 μL of methanol by vortexing. The eppendorfs were put into a hot water bath (60 °C) for 60 min to enhance the liquid–liquid extraction. Next, the samples were frozen (−20 °C) for 60 min to clarify the supernatant. Afterwards the eppendorfs were centrifuged at 14,000 rpm for 5 min and 500 μL of the extract was diluted with 9500 μL water prior to solid phase extraction. Solid phase extraction (Oasis HLB 3 cm^3 (60 mg), (Waters)) was performed for further clean-up. The cartridge was conditioned and equilibrated with 2 mL of 100% and 5% methanol, respectively. After loading the sample, the cartridge was washed with 2 mL of 20% methanol and eluted with 1 mL of 100% methanol. Of the obtained extract, 500 μL was diluted with 500 μL of 0.05% formic acid, and 10 μL was injected directly onto the column.

2.4. Instrumentation

The U-HPLC system consisted of a Thermo Fisher Scientific (San José, CA, USA) Accela U-HPLC pumping system coupled to an Accela Autosampler and Degasser. Chromatographic separation was achieved using reversed phase chromatography with gradient elution. Separation of the compounds was carried out on a Hypersil Gold column (1.9 μm, 50 mm × 2.1 mm ID) (Thermo Fisher Scientific). The mobile phase consisted of a mixture of methanol and 0.05% formic acid, pumped at a flow rate of 0.3 $mL\,min^{-1}$. Optimized separation of the compounds was obtained using a linear gradient (Table 1).

Mass spectrometric analysis was carried out using an Exactive™ benchtop mass spectrometer (Thermo Fisher Scientific) fitted with an atmospheric-pressure chemical ionisation source (APCI) operated in the positive ion mode. The optimal ionisation source working parameters are given in Table 2. A scan range of m/z 100–500 was chosen and the resolution was set at 50,000 full width

Table 2
APCI working parameters for ionisation of the boar taint compounds.

Spray voltage (kV)	5.0
Sheath gas flow rate (arbitrary units, au)	40
Sweep gas flow rate (au)	2
Auxiliary gas flow rate (au)	10
Capillary temperature (°C)	270
Heater temperature (°C)	250
Capillary voltage (V)	70
Tube lens voltage (V)	90
Skimmer voltage (V)	20

half maximum (FWHM) at 2 Hz (2 scans per second). The automatic gain control (AGC) target was set at high dynamic range (3×10^6) and the maximum injection time was 500 ms. Initial instrument calibration was done by infusing calibration mixtures for the positive and negative ion modes (Thermo Fisher Scientific). The positive calibration mixture included caffeine, Met-Arg-Phe-Ala acetate salt (MRFA) and Ultramark® 1621, while the negative calibration solution comprised of sodium dodecyl sulfate, sodium taurocholate and Ultramark® 1621. These compounds were dissolved in a mixture of acetonitrile, water and methanol, and both mixtures were infused using a Chemyx Fusion 100 syringe pump (Thermo Fisher Scientific). The option of "all-ion fragmentation" using the High Energy Collision Dissociation (HCD) cell was turned off. The fore vacuum, high vacuum and ultra high vacuum were maintained at approximately 2 mbar, from 10^{-5} to 3×10^{-5} and below 8×10^{-10} mbar, respectively. Instrument control and data processing were carried out by Xcalibur 2.1 software (Thermo Fisher Scientific).

2.5. Quality assurance

Prior to the sample analysis, a standard mixture (2 ng on column) of the target compounds was injected to the operational conditions of the chromatographic devices. To every sample, a mixture of internal standards (2-MID and ADD) was added at a concentration of 500 $\mu g\,L^{-1}$ and 1000 $\mu g\,L^{-1}$, respectively, prior to extraction. Analytes were identified based on their retention time relative to that of the internal standard and their accurate mass (Table 3). For quantification purposes, eight-point-based matrix-matched calibration curves were prepared by spiking fat samples with a standard mixture of indole, skatole and androstenone obtaining seven concentrations in the range of 100–2000 $\mu g\,L^{-1}$ (100, 250, 500, 750, 1000, 1500 and 2000 $\mu g\,L^{-1}$). The internal standards 2-MID and ADD were added at a concentration of 500 $\mu g\,L^{-1}$ and 1000 $\mu g\,L^{-1}$, respectively.

Commission Decision 2002/657/EC [19] and the ISO 17025 [20] were used as guidelines to systematically cover the analytical performances of the method.

2.6. Analysis of fat samples

Neck and loin fat samples of 92 boars were collected from the project "On farm comparison of different alternatives for surgical castration without anaesthesia" (Agriculture and Fisheries Policy Area, Belporc, Flemish Centre for Agricultural and Fisheries Marketing (VLAM)). The neckfat samples were analysed by CCL Nutricontrol, Veghel, The Netherlands, while the loin fat samples were analysed at our laboratory. On these samples, our newly developed and validated U-HPLC–Orbitrap-MS method was applied. The method used by CCL consisted of the following procedure. Briefly, 0.15 g of liquid fat was transferred to centrifuge tubes (2 mL) and 1.75 mL of extraction solvent methanol:hexane (9:1) was added. After ultrasonic treatment and centrifugation, approximately 1.5 mL of the extract was then placed into an injection vial and allowed to get to room temperature. Finally, 2 μL of the extract was injected into a GC–MS, with the detection performed in SIM mode with m/z 257 (target), 258 and 259 and a dwell time of 100 ms.

3. Results and discussion

3.1. Development of sample pre-treatment

Reports from literature indicate that a critical step in the analysis of boar taint is the sample preparation of the adipose tissue [6] because the compounds of interest are lipophilic. Several extraction and clean-up steps were therefore tested. With respect to the extraction, saponification with potassium hydroxide and extraction with ether [8,22,23], melting of the fat and extraction with methanol [15,24] and only extraction with methanol [17] were tested. At this phase, only a freezing step was included as clean-up. Preliminary experiments showed that melting the fat followed by extraction with methanol provided the best results, but a more extensive clean-up was necessary to eliminate matrix interferences, especially for the detection of androstenone. Also internal standards were added before melting the fat to include the variability of the microwave.

Several clean-up and concentration steps were then tested and compared: solid phase extraction (3 cm^3, 60 mg), ultrafiltration (Amicon 10 kDa and 30 kDa), (partial) nitrogen evaporation (with redissolving) and the combination of SPE with nitrogen evaporation. The best results were obtained after solid phase extraction and solid phase extraction combined with evaporation. The peak areas and signal-to-noise ratios of the combination method were significantly higher. For both methods linearity was evaluated as well and the use of only one SPE elution step obtained a correlation coefficient of 0.90 for androstenone, whereas the combination method led to a regression coefficient of 0.61. For the clean-up with Amicon filters, the area was more than factor 8 lower than after solid phase extraction. Further optimisation of the clean-up with solid phase extraction was obtained by careful optimisation of the washing and eluting steps. The best results were reached with a washing step of 20% methanol and an elution step of 100% methanol (1 mL).

3.2. U-HPLC and MS parameters

Initially, the analytical method was developed on a HPLC system (Finnigan Surveyor, Thermo Fisher Scientific) coupled to a LTQ linear ion trap mass analyser (Thermo Fisher Scientific). The HPLC and MS conditions were taken from Verheyden et al. [17]. Because this HPLC–MS/MS application was subject to substantial matrix interferences, the method was transferred to a triple quadrupole analyser (TSQ Vantage, Thermo Fisher Scientific) coupled to a U-HPLC system. This transition did not decrease the problematic matrix effects, thus we selected an Orbitrap mass analyser (Exactive™, Thermo Fisher Scientific) coupled to an U-HPLC system, which did solve the problem of matrix effects. U-HPLC

Table 3
Compound specific MS parameters for the different boar taint compounds.

Analyte	Ion mode	Internal standard used	tR (min)	Accurate mass (m/z)	Mean mass error (ppm)
Indole	+	2-Methylindole	2.19	118.06500	1.01
Skatole	+	2-Methylindole	3.92	132.08043	0.45
Androstenone	+	Androstadienedione	5.68	273.22032	0.73

offers the advantage of a significantly shorter analysis time (10 min) than other methods that simultaneously detect the three boar taint compounds, e.g. Verheyden et al. [17] (30 min).

For chromatographic separation, different columns were tested, i.e. Nucleodur Sphinx RP column (1.8 μm, 100 mm × 2.1 mm ID, Macherey-Nagel), Hypersil Gold column (1.9 μm, 50 mm × 2.1 mm ID, Thermo Fisher Scientific) and (1.9 μm, 100 mm × 2.1 mm ID, Thermo Fisher Scientific) and Nucleodur C_{18} Isis column (1.8 μm, 50 mm × 2 mm ID, Maeherey-Nagel). The choice of column was mainly determined by the baseline separation and the retention time of the first and last eluting analytes. Good baseline separation between skatole and 2-methylindole was achieved with the Hypersil Gold column (1.9 μm, 50 mm × 2.1 mm ID, Thermo Fisher Scientific). Additional separation and optimal retention times were obtained by careful selection of the gradient programme.

Before determining the optimal MS conditions, the compounds were infused into the atmospheric-pressure chemical ionisation source (APCI) and the observed masses were compared with the theoretical masses. Every compound has a unique theoretical mass due to its specific elemental composition. The theoretical mass was calculated using Xcaliber 2.1 software (Thermo Fisher Scientific). The mass deviation was expressed in parts per million (ppm) and defined as: $10^6 \times$ [(measured mass – theoretical mass)/theoretical mass] (Table 3). The obtained mass deviations were below 5 ppm, indicating a high mass accuracy, which allowed identification of the compounds.

The MS conditions (Table 2) were optimised based on the peak intensities, peak areas and signal-to-noise ratios of the individual analytes. Because the different boar taint compounds belong to two classes, namely indolic and steroidal compounds, very often different values were obtained for both classes. In general, those conditions were selected which resulted in the most optimal detection of androstenone, because previous data had shown that this compound was subjected most to matrix interferences. The most optimal AGC value was determined by analysing spiked fat samples based on area, peak shape and signal-to-noise ratio. The best AGC target was a high dynamic scan range (3×10^6). The mean mass resolution was also varied by analysing spiked fat samples at 50,000 and 100,000 FWHM. The best results were obtained with a mass resolution of 50,000 at 2 Hz (2 scans per second) when looking to peak shape and peak area, among others.

3.3. Method validation

Due to the absence of guidelines for the analysis of boar taint compounds in fat, the Commission Directive 2002/657/EC [19] and ISO 17025 [20] were used as a guideline for the validation of the developed detection method for indole, skatole and androstenone. In literature, threshold values for boar taint compounds in pig fat samples are set at 100 μg L^{-1} for indole, 200 μg L^{-1} for skatole and 1000 μg L^{-1} for androstenone [2]. Appropriate internal standards were selected, capable of anticipating fluctuations in signal intensity upon extraction of boar taint compounds from fat samples. Verheyden et al. [17] used 2-methylindole as internal standard and this compound was found satisfactory in this study for the indolic compounds as well. Androstadienedione was added as an additional internal standard for the steroid compound androstenone.

3.3.1. Specificity

The specificity of the method could be demonstrated by analysis of blank fat samples and samples fortified with each analyte separately at 100 μg L^{-1}. Since true blank fat samples are not available [15], fat from barrows, which contains very low background concentrations of the analytes of interest, was selected to validate the method. For each analyte spiked, the chromatograms showed a

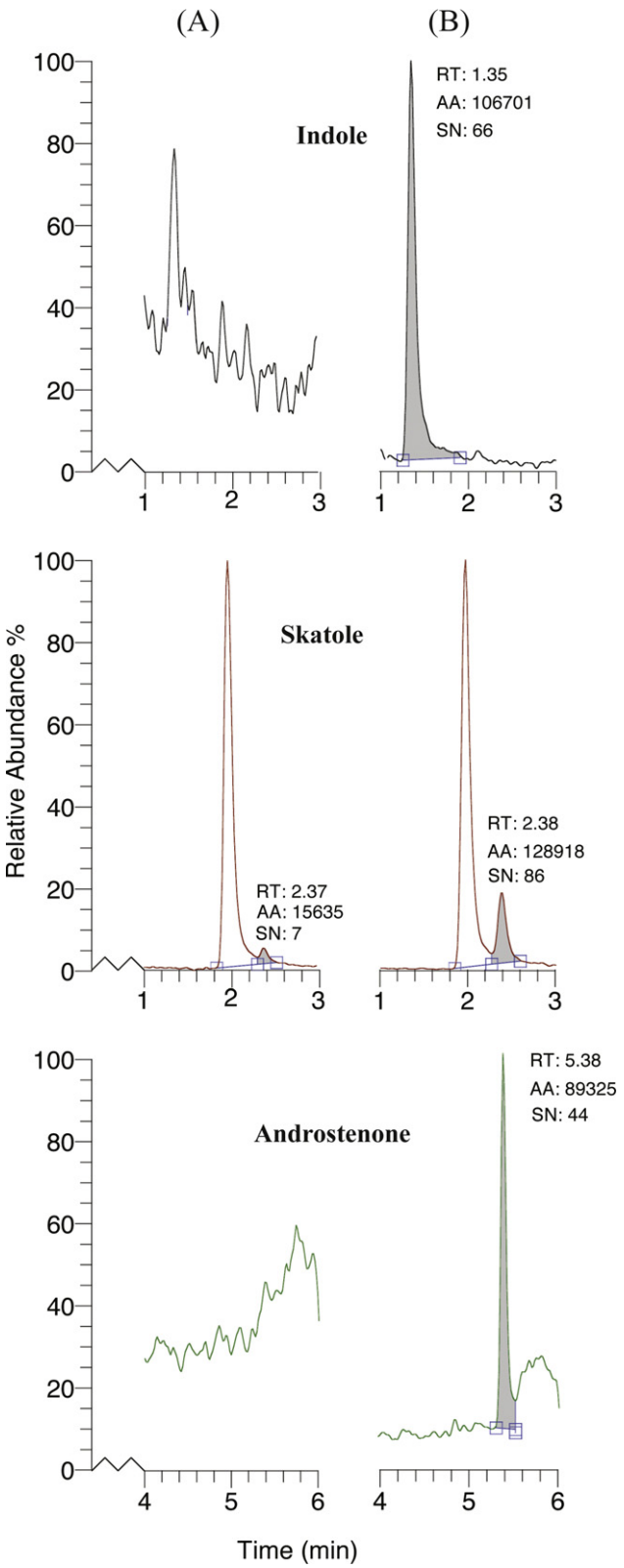

Fig. 1. Chromatogram of a blank fat sample (A) and a sample fortified with 100 μg kg^{-1} indole, skatole and androstenone (B) analysed on the U-HPLC-Exactive™ (RT: retention time; AA: area; SN: signal-to-noise ratio).

significant increase in peak area intensity at the specific retention time of the compounds, taking a signal-to-noise ratio of at least 3 into account. No other matrix substances interfered at this retention time (Fig. 1). As a result, the developed method was found to

Table 4
Method recovery and precision of the developed method for the three boar taint compounds, in fat.

Analyte	Nominal concentration ($\mu g\,kg^{-1}$)	Mean recovery	Precision		
		Mean ± SD (n = 18)	Repeatability		Within laboratory reproducibility
			Mean ± SD (n = 54)	RSD (%) (n = 54)	RSD (%) (n = 72)
Indole	200	105 ± 11	211 ± 23	11.2	16.4
	500	109 ± 13	546 ± 65	11.8	16.0
	1000	112 ± 13	1120 ± 133	11.9	13.5
Skatole	200	108 ± 13	216 ± 27	12.5	16.2
	500	102 ± 11	513 ± 56	10.9	12.4
	1000	105 ± 10	1054 ± 108	10.2	11.7
Androstenone	200	111 ± 14	222 ± 28	12.6	16.8
	500	100 ± 9	503 ± 45	9.0	12.4
	1000	99 ± 7	985 ± 76	7.7	12.9

be specific for indole, skatole and androstenone in the presence of matrix compounds.

3.3.2. Selectivity

Analytes were identified on the basis of their relative retention time, which is the ratio of the retention time of the analyte to that of the internal standard. In addition, the accurate mass of the ions was taken into account when the chromatographic peak of interest had a signal-to-noise ratio of at least 3. A maximum mass deviation of 3 ppm was allowed within this study.

3.3.3. Linearity

The linearity of the developed method was evaluated by preparing eight-point calibration curves in matrix for the different compounds. The blank samples were fortified with concentrations ranging from 100 to 2000 $\mu g\,L^{-1}$. Linear regression analyses were carried out by plotting the peak area ratios of the analyte against the internal standard versus the analyte concentration. Correlation coefficients (R^2) obtained for the compounds were ≥0.99.

3.3.4. Accuracy

As no certified reference material was available, the recovery was assessed by spiking fat of barrows, containing low background concentrations of the analytes of interest. Samples of fat were spiked at a concentration of 200, 500 and 1000 $\mu g\,L^{-1}$ for all compounds in six replicates. The recoveries obtained with this analytical method were satisfactory (Table 4). To the best of our knowledge, little [15,17] has yet been reported in literature about recoveries of boar taint compounds in fat.

3.3.5. Precision

To evaluate the precision of the method, repeatability and within-laboratory reproducibility were determined. Both validation parameters were evaluated by calculating the relative standard deviations (%RSD). For evaluating the repeatability, three series of six replicates of samples were analysed, this at a concentration of 200, 500 and 1000 $\mu g\,L^{-1}$. These analyses were carried out on different occasions by the same operator under repeatable conditions. As presented in Table 4, the calculated RSD values were below 15%, indicating a good repeatability according to European Criteria 2002/657 [19].

The within laboratory reproducibility was evaluated with four series of six replicates of samples analysed at a concentration of 200, 500 and 1000 $\mu g\,L^{-1}$. These series were analysed on different days by different operators. As presented in Table 4, the calculated RSD values were below 20%, indicating good precision of the method according to European Criteria 2002/657 [19].

3.3.6. Limit of detection and limit of quantification

Limits of detection and quantification were determined based on the outcome of seven-point calibration curves in matrix in a range of 1–100 $\mu g\,L^{-1}$. Table 5 summarises the calculated limits of detection and quantification for the compounds (Fig. 2).

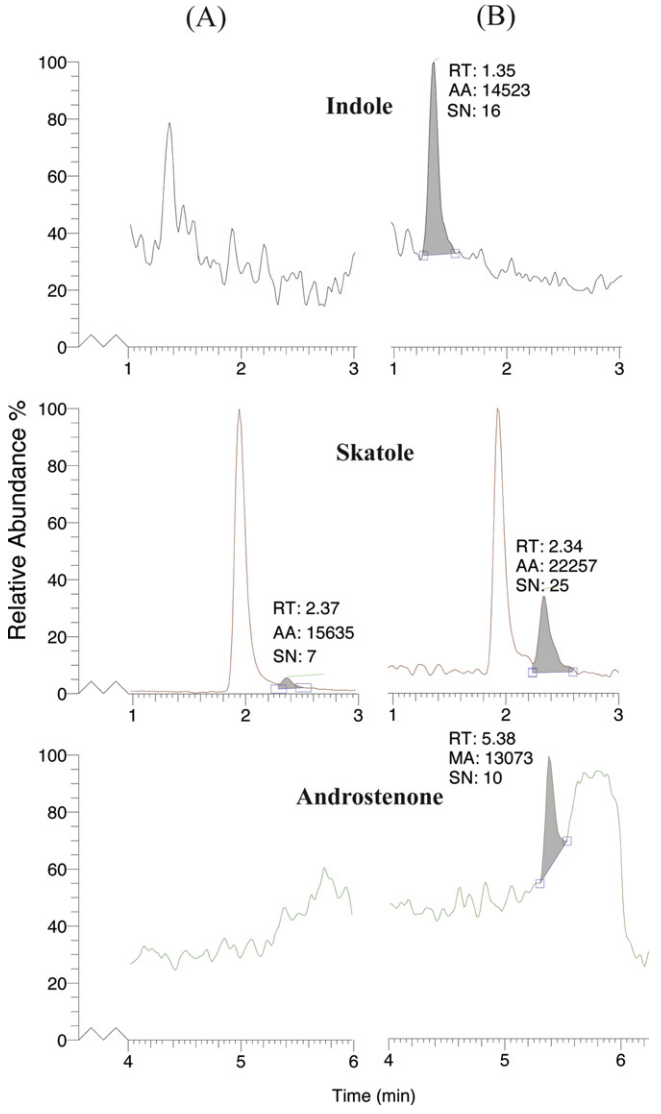

Fig. 2. Chromatogram of a blank fat sample (A) and a sample spiked at the limit of quantification (indole and skatole: 5 $\mu g\,kg^{-1}$; androstenone: 10 $\mu g\,kg^{-1}$ (B)) analysed on the U-HPLC-Exactive™ (RT: retention time; AA: area; SN: signal-to-noise ratio).

Table 5
Limits of detection (LOD) and limits of quantification (LOQ) for the three boar taint compounds, in fat.

Analyte	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)
Indole	2.5	5
Skatole	2.5	5
Androstenone	7	10

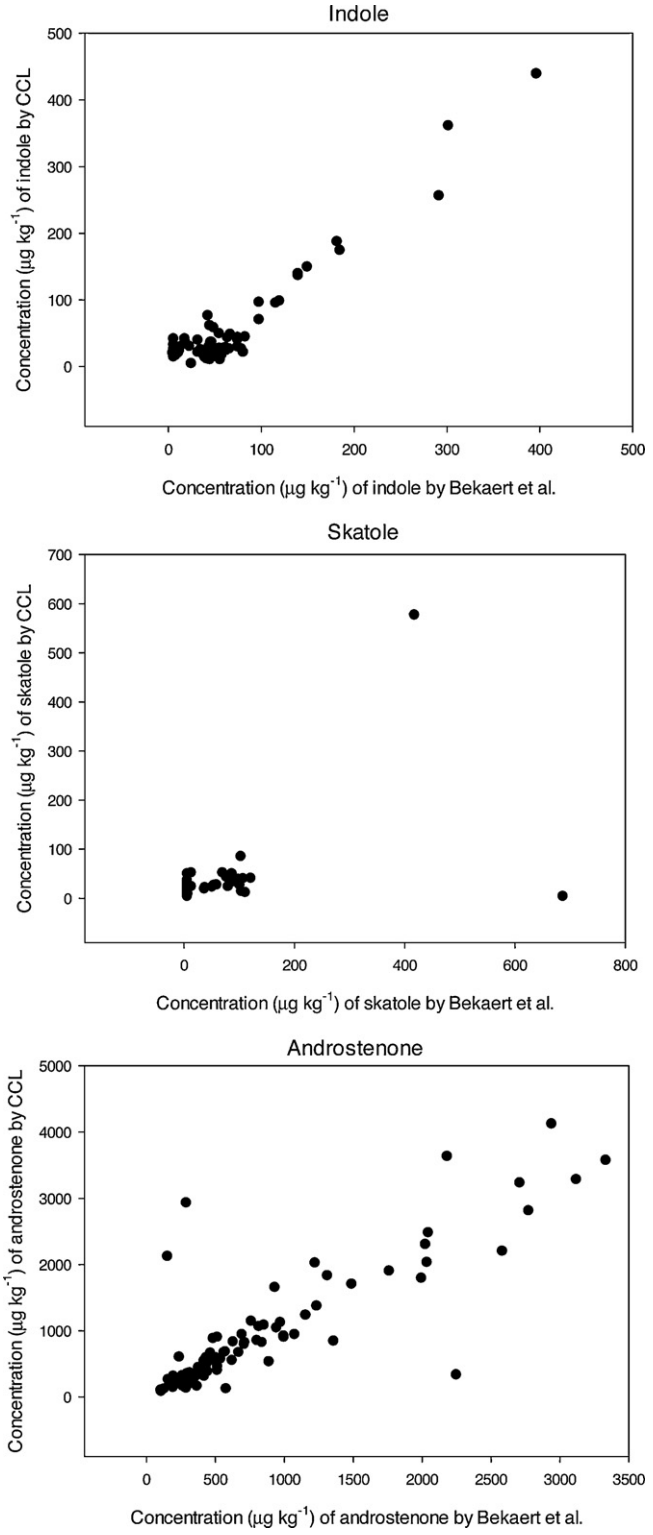

Fig. 3. Scatterplot of 92 samples analysed with both the newly developed method Bekaert et al. (loin fat, *X*-axis) and the CCl method (neck fat, *Y*-axis) for the three boar taint compounds.

3.3.7. Stability of the compounds

The stability of standard stock solutions was evaluated by Verheyden et al. [17]. RSDs of repetitive injections over 10 weeks were generally the lowest when storage occurred at −20 °C.

The stability of the three boar taint compounds in matrix was evaluated at −20 °C and −80 °C. Successive injections ($n = 12$) over 5 weeks revealed no decrease in concentration of the compounds between the different storage temperatures.

3.4. Analysis of fat samples

For the comparison of the samples between the two laboratories of interest a Spearman's rank correlation was used. This is a non-parametric statistic correlation that measures the strength of the association between two data sets. For the compounds indole, skatole and androstenone, significant correlations of 0.48, 0.75 and 0.92 were found, respectively (Fig. 3). For 21 samples, the concentration of skatole were below the limit of detection for both analysis; these samples were not included in the correlation. When the samples were divided into two groups with a cut-off level of 100 $\mu g\,kg^{-1}$ for indole and 500 $\mu g\,kg^{-1}$ for androstenone, lower correlations were found for the values below the cut-off level (0.31 and 0.77, respectively) and higher correlations (0.97 and 0.89, respectively) were found above the cut-off level. For skatole, only one value above 200 $\mu g\,kg^{-1}$ was found (Fig. 3).

The rather low correlations for indole and skatole may be explained by the samples having been taken from different parts of the carcass. Several authors have found that the concentration of skatole and indole varies significantly throughout the carcass [25,26]. Our results confirm these findings, emphasizing the need to develop and implement harmonised guidelines on the kind of fat to be considered for sampling. Further, the number of freeze-thaw cycles that the samples have undergone may explain the rather low correlations obtained for these compounds.

4. Conclusion

Most laboratory analyses reported so far detect either the indolic compounds or the steroid compound. Combining different detection techniques to analyse all known compounds has resulted in laborious and time-consuming practices which are most often not characterised by the same accuracy, specificity and sensitivity as analytical U-HPLC–HR-MS methods.

Additionally, the currently available methods to detect the three boar taint compounds simultaneously show substantial matrix interference. This either hampers the robustness and repeatability of the data, or ignores proper validation data. Therefore, we opted for the use of an extensively validated and reliable U-HPLC–HR-MS analysis method. This analysis, when preceded by minimal sample preparation, led to the validation of an accurate and robust method that can detect the three known compounds simultaneously. With this method, the selected thresholds found in literature, 100 $\mu g\,L^{-1}$ for indole, 200 $\mu g\,L^{-1}$ for skatole and 1000 $\mu g\,L^{-1}$ for androstenone, are easily reached as the limits of quantification were set at 5 $\mu g\,kg^{-1}$ for the indolic compounds and 10 $\mu g\,kg^{-1}$ for the steroid compound. This method will enable relatively fast screening of the prevalence of boar taint in fat samples and may be used as an objective countermeasure for consumer perception and sensory evaluation practices.

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