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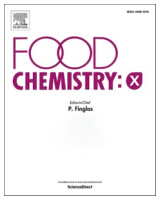

Development of a highly reproducible GC-HRMS method for determination of Skatole in pig tissues

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ABSTRACT

This study presents a sensitive and reproducible mass spectrometry method for quantifying skatole in porcine adipose tissue, muscle, and serum samples applicable for abattoirs and laboratories. Leveraging gas chromatography-high-resolution Orbitrap microscopy and microwave-assisted liquefication of the adipose tissue, the method demonstrates robust performance across key parameters. Impressive linearity (R^2) values of 0.9999 and 0.9996 for adipose tissue and serum, respectively. Notably, the method exhibits a low Limit of Detection (LoD) of 0.5 ppb for adipose tissue and 0.9 ppb for serum, with corresponding Limits of Quantification (LoQ) at 1.65 ppb and 3.04 ppb, respectively. The method showed significant reproducibility of 5.9% and repeatability (RSD%) of 8.78% for adipose tissue and 4.08% for serum, with recovery rates of 90% and 87%, respectively. This streamlined method offers promising, effective quantification of boar taint compounds, emphasizing its sensitivity and reproducibility.

1. Introduction

It is widely acknowledged that surgical castration of piglets is painful, coupled with a stressful process (Miller et al., 2023), and discomfort is a clinically relevant condition that negatively impacts animal welfare (Park et al., 2020). Therefore, animal welfare issues have raised questions regarding the widespread use of surgical castration (Sødring, Nafstad, & Håseth, 2020). Many EU stakeholders in the pig meat industry have signed the Brussels Declaration, which calls for a ban on the practice of surgical castration of pigs without anesthesia throughout the EU as of 2018 (Lund, Borggaard, Birkler, Jensen, & Støier, 2021; Mo et al., 2016).

In addition to prioritizing animal welfare, the aim is to enhance meat quality, given that pork represents one-third of the worldwide meat production with a high economic contribution to the livestock industry (Afe, Shen, Guo, Zhou, & Li, 2023; Tong et al., 2023). There is a growing interest in rearing complete boars instead of barrows to obtain carcasses with more muscle to improve meat quality. However, the presence of boar taint, an offensive odor emanating from pork, has proven to be a significant challenge as it discourages consumer demand. The prohibition of castration has led to an elevation in unpleasant scents in pork because when male pigs reach sexual maturity, their metabolism results in the accumulation of androstenone and skatole (3-methylindole), especially in fatty tissues due to taint compounds affinity for lipids (Mörlein et al., 2016).

Skatole is produced by the metabolic process of bacterial breakdown of the amino acid tryptophan in the large intestine (James Squires, Bone, & Cameron, 2020; Squires & Bonneau, 2022). A portion of skatole, which is produced in the intestines, is eliminated by feces, whereas the remainder is absorbed into the bloodstream through the intestinal wall (Brunius et al., 2016; Han et al., 2019). As pigs mature sexually, there is a gradual buildup of skatole in their fatty tissue (Squires & Bonneau,

Abbreviation: EU, European Union; LC, Liquid Chromatography-Mass Spectrometry; MS, Mass Spectrometry; LDTD, Laser Diode Thermal Desorption; GC-IMS, Gas Chromatography-ion Mobility Spectrometry; RI, Retention index; NIST, National Institute of Standards and Technology; ES, External Standard; IS, Internal standards; CAS, Chemical Abstracts Service; HPLC, High-Performance Liquid Chromatography; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; RSD, Relative standard deviations; CV, Coefficient of variation; MTBE, methyl tert-butyl ether; SERS, Surface-Enhanced Raman Scattering; ECL, Electrochemiluminescence or Electrogenerated Chemiluminescence; UHPLC-HRMS, Ultra-High-Performance Liquid Chromatography with High-Resolution Mass Spectrometry.; BDD, Boron-Doped Diamond.

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2022; Zadinová et al., 2016). The extent to which skatole accumulates in adipose tissue is contingent on the equilibrium between its production and clearance processes (James Squires, Bone, & Cameron, 2020). Adipose tissue and blood play pivotal roles in the manifestation of boar taint, where excess skatole accumulates in adipose tissue, while blood serves as a distribution channel. Screening carcasses for taint (skatole) has been challenging; therefore, we emphasize that our study is directed toward the validation methodology employed for precisely detecting and quantifying skatole in adipose tissue, muscle, and serum.

Many methods have been proposed for quantification(Burgeon et al., 2021). However, there is a lack of viable technological equipment capable of detecting this offensive odor in the slaughter line while also accommodating the speed limitations inherent in modern abattoirs, which process a significant number of pigs on a daily basis.

Boar taint compounds have been detected using various chromatographic methods such as Liquid Chromatography-Mass Spectrometry (LC–MS/MS)(Buttinger & Wenzl, 2020) and Laser Diode Thermal Desorption -Mass Spectrometry (LDTD-MS/MS)(Lund et al., 2021). Skatole is a flavor compound and a concern in boar taint contributing to the pork aroma. Gas chromatography-ion mobility spectrometry (GC-IMS) (Liu et al., 2023) and others listed above are commonly employed for its separation. Mass spectrometry (MS) is an effective approach in flavor research because of its capacity to provide detailed structural information and accurate identification capabilities(Diez, Roland, & Robert, 2019; Diez-simon et al., 2020; Wei, Dan, Zhao, & Wang, 2023). As a result, MS has become the predominant method used in the field of flavor and aroma research(Yang et al., 2022). The implementation of high-resolution mass spectrometry enhances the precision of fragment masses and significantly enhances the accuracy of identification(Rois et al., 2019). In our analysis, the identification of aroma compounds relied on the examination of mass spectrometry patterns and the retention index (RI) using databases such as NIST and Wiley. We employed an Orbitrap mass analyzer, known for its exceptional mass accuracy and resolving power. RI may exhibit slight variations depending on the specifications of the different column types. Additionally, diverse MS detectors, including quadrupoles, with distinct detection principles can significantly impact MS patterns. This variability has the potential to compromise identification accuracy and lead to false-positive or false-negative results when libraries are not appropriately matched.

The aversion caused by the off-flavor aroma originating from non-castrated pig meat poses a significant challenge in the realms of meat quality and livestock production. Nonetheless, the objective of this study was to create and validate a novel, streamlined approach for the determination and quantification of skatole in pig adipose tissue and serum samples.

2. Materials and methods

2.1. Reagents and chemicals

An external standard (ES) 3-Methylindole (CAS: 83–34-1 Solarbio, China) was used to prepare the calibration standard. The internal standard (IS) used was 3-methyl-d3-indole (CAS 111399–60-1), obtained from Toronto Research Chemicals Inc., Canada.

This study used acetonitrile as the extraction solvent, an HPLC grade with a purity >99.9%.

2.2. Samples

Adipose tissue, *Longissimus thoracis* (LT), and serum were utilized in this validation study. These samples were collected from the animals immediately post-slaughter on 25 May 2023 at 26 weeks of age. After the collection, the adipose tissue and the LT were swiftly frozen in liquid nitrogen and stored at −80 °C until the experiment. The blood samples were transported on ice to the laboratory. Upon arrival at the laboratory, the tubes were centrifuged at 3000*g* for 10 min at 4 °C. The collected serum was then stored at −80 °C until analysis.

2.3. Samples pretreatment

Adipose tissue samples were retrieved from the −80 fridge and allowed to defrost. The adipose tissue was liquefied using the microwave using a method from Brunius et al. (2016) with modifications. Adipose tissue samples were microwaved for 2 min at 280 W, with careful attention given to melting without reaching a boiling point to protect the analyte integrity. The decision to employ microwave liquefaction stems from its thermal uniformity, which enhances the breakdown of lipid matrices, thereby facilitating the efficient extraction of analytes in little time.

0.5 g of adipose tissue samples were accurately weighed into 5 mL centrifuge tubes spiked with internal standard and subjected to microwave liquefaction. 0.5 g of LT samples were minced in 5 mL centrifuge tubes after retrieval from the −80 °C freezer. The serum samples were prepared by weighing 0.25 *g* into 5 mL centrifuge tubes.

Each sample was spiked with 5 μL of a 10 ppm internal standard, specifically 3-methyl-d3-indole, prior to the extraction process. This isotope-labeled compound is a known reference for normalization and compensates for variations in sample preparation and instrument response, ensuring precision and accuracy in quantification. Extraction was carried out by adding 2 mL of acetonitrile to each sample, followed by vortexing for 1 min and centrifugation at 4000 rpm for 20 min at 4 °C. Subsequently, 1 mL of the supernatant from the samples was pipetted into vials for GC/MS analysis. The blanks also undergo the same process as the samples to examine the method's specificity and ensure that no interfering substances present could affect the analysis of the target analyte. Acetonitrile was selected for the extraction due to its minimal co-extractive extraction(Galindo, Da Oliveira, & Godoy, 2021).

2.4. Validation of Analytical Method

2.4.1. Calibration Standards Preparation

A solution with a concentration of 1 mg/mL of ES was prepared using acetonitrile. Calibration standards spanning concentrations of 250, 100, 50, 25, 10, 5, 1, 0.5, 0.25, and 0.1 ppb were derived from this stock solution, with each standard fortified with 50 ppb of the IS. These standards were stored in vials and placed in a freezer at −20 °C.

A unified calibration standard was employed for both adipose tissue and LT samples, which were analyzed on the same day. This approach ensured consistent analytical conditions and utilized a singular calibration curve. Conversely, a separate calibration standard was prepared for serum samples in a subsequent week to validate the method's applicability to different sample matrices.

2.4.2. Instrumentation (GC-HRMS)

The study used a Q-Exactive Orbitrap mass analyzer paired with a TriPlus RSH autosampler and a Trace 1310 GC (Thermo Fisher Scientific, Bremen, Germany). The analytical column employed was a VF-WAX ms column (60 m × 0.25 mm i.d. x 0.25 um film thickness, Agilent, Santa Clara, CA, USA). Helium (99.9999%) served as the carrier gas with a consistent flow rate of 1 mL/min. The temperature program for the column oven was initiated at 150 °C for 1 min, then ramped up to 230 °C at a rate of 20 °C/min and held at 230 °C for 15 min. Transfer lines 1 and 2 were set to 250 °C. Mass spectrometry (MS) was performed using electron impact ionization (EI) at 70 eV, operating in full-scan mode with a resolving power of 60,000 full width at half maximum (FWHM). The scan range was extended from 30 to 400 *m/z* with an automatic gain control target value of 1E6. The ion source and transfer line temperatures for MS were set to 280 °C and 250 °C, respectively.

GC–MS data were obtained and processed using the Xcalibur 4.1 and TraceFinder 4.0 software packages from Thermo Scientific. Volatile compounds were identified based on mass spectra and linear retention

indices (LRIs) sourced from NIST17 (v2.3) and an in-house library named the home flavor library. This domestic library was constructed using authentic reference standards, complemented by high-resolution mass spectra and linear retention indices. Additionally, the high-resolution filtering (HRF) tool in the TraceFinder software was employed to annotate each measured m/z peak and assess the mass accuracy of these ions when utilizing the NIST library. To determine LRIs, a set of standard alkanes (C7-C40: Sigma-Aldrich, St. Louis, MO, USA) was analyzed under identical chromatographic conditions.

2.5. Process efficiency

The research plan considered essential validation parameters, including linearity, repeatability, recovery, and sensitivity, such as Limits of Detection (LoD) and Limits of Quantification (LoQ) during the validation process.

2.5.1. Linearity

Linearity was assessed by applying the external standard calibration approach. The stock solution was prepared according to the instructions provided in the "Calibration Standards Preparation" section. The stock solution was diluted with acetonitrile to prepare 10 calibration standards ranging from 250 to 0.1 ppb. The ICH guidelines stipulate that a minimum of five concentrations should be analyzed to establish linearity (Alinafiah, Azlan, Ismail, & Rashid, 2021). Adipose tissue and LT samples were analyzed using a shared calibration standard and experimented on the same day. However, serum samples were experimented and analyzed separately, utilizing distinct calibration standards in a subsequent week.

2.5.2. Limits of Detection (LoD)/Limits of Quantification (LoQ)

The LoD and LoQ were determined through the analysis of 10-point calibration points within a concentration range of 0.1–250 ppb. LoD and LoQ were established based on the concentrations corresponding to chromatographic peaks with signal-to-noise ratios of 3:1 and 10:1, respectively. This methodology adheres to the guidelines for analytical procedures outlined in ISO 17025 (ICH, 2005).

2.5.3. Repeatability

The repeatability and reproducibility of the method were examined. The method's repeatability was validated by following the guideline of ISO 5725-2:1994 by analyzing relative standard deviations (RSD%) from the mean concentration values derived from each replicate. The method's repeatability was assessed using 10 samples from each tissue type. Each sample consisted of multiple replicates to ensure robustness in our analysis. For example, LT samples numbered from 1 to 10 were analyzed, with each sample comprising two replicates. The same approach was applied to adipose tissue and serum samples, ensuring consistency across all tissue types. The concentration of the replicates of each sample was calculated based on calibration with standards of known concentration.

$$RSD\% = \frac{s}{x} X\,100$$

s is the standard deviation of the replicates, while x is the mean of the concentration.

The method's reproducibility was assessed by analyzing all the replicates of the adipose tissue and LT, which were experimented on the same day and that of the serum samples, which were experimented on another week. Reproducibility was evaluated by calculating the coefficient of variation (CV) for all replicates across the samples(Ghosh, Philtron, Zhang, Kechris, & Ghosh, 2021).

$$CV = \frac{SD}{Mean} X\,100$$

SD is the standard deviation of replicates, and Mean of the concentrations.

2.5.4. Recovery

The recovery experiment was conducted in adipose tissue and serum. The initial experiment focused on assessing recovery in adipose tissue due to its distinct composition and lipid content, which pose challenges to extraction efficiency and matrix effects compared to muscle tissue (Stroh et al., 2021). The recovery was evaluated to determine the efficiency of the sample preparation and extraction process. This was assessed by calculating the mean ratio response of samples spiked before extraction to those spiked after extraction. Each sample were spiked with 5 μL of a 10 ppm internal standard (3-methyl-d3-indole), an isotope-labeled compound, to evaluate recovery. This addition serves to ensure precision and accuracy in the quantification process.

$$R(\%) = \frac{Cb}{Ca} X\,100$$

Ca is the concentration of the spiked sample after extraction, and Cb is the concentration of the spiked sample before extraction.

2.6. Statistical analysis

Mass signal alignment (signal/noise ratio $\geq$ 3) was executed using the Xcalibur 4.1 and TraceFinder 4.0 software packages from Thermo Scientific, respectively, incorporating the deconvolution plugin. Mass signals observed in less than or equal to four replicates were excluded. The means, standard deviations, coefficient of variation (CV), RSD%, and Table were calculated using Microsoft Excel, version 2310. The graph was constructed using GraphPad Prism 10.

3. Results and discussion

The following analytical parameters were examined using GC-HRMS to assess the method's effectiveness in detecting and quantifying 3-methylindole (Skatode) in adipose tissue, LT, and serum from pigs.

3.1. Process efficiency

3.1.1. Linearity

Linearity is an important parameter in analytical methods, as it can generate results that exhibit a direct proportionality to the concentration of an analyte within a specified range(López-Fernández et al., 2022). Our study assessed linearity using a set of 10 calibration standards derived from spanning concentrations from 250 to 0.1 ppb. The relationship between external standard concentrations and corresponding peak areas, illustrated in Figs. 1a and b, demonstrates the strong linearity of the method. The adipose tissue and the LT samples were analyzed using the same calibration standard, as the experiment was conducted on the same day to ensure consistency in analytical conditions. However, a new calibration standard was prepared for serum analysis on another week of the experiment. This custom calibration standard was tailored to optimize analytical accuracy. This methodological approach maintains rigor in our analytical procedure and ensures precise quantification of target analytes across different sample types. The R^2 values obtained from the 10 calibration curves for adipose tissue/LT and serum consistently indicated a high level of linearity, with R^2 of 0.9999 and 0.9996, respectively. The R^2 aligns with findings from other researchers who reported comparable R^2 values, such as 0.9954 by Lund et al. (2021) and 0.99 by Wauters et al. (2015), for boar taint compound detection using LDTD-MS/MS and HPLC, respectively. The values of R^2 in our study indicate a solid linear relationship between the analyte concentration and the instrument response, providing confidence in the accuracy and precision of the analytical method.

Relative retention time and other factors, such as mass spectral data and retention indices, are also used to confirm analyte identity. 3-methylindole was identified at 13.38–13.49 min retention times in adipose

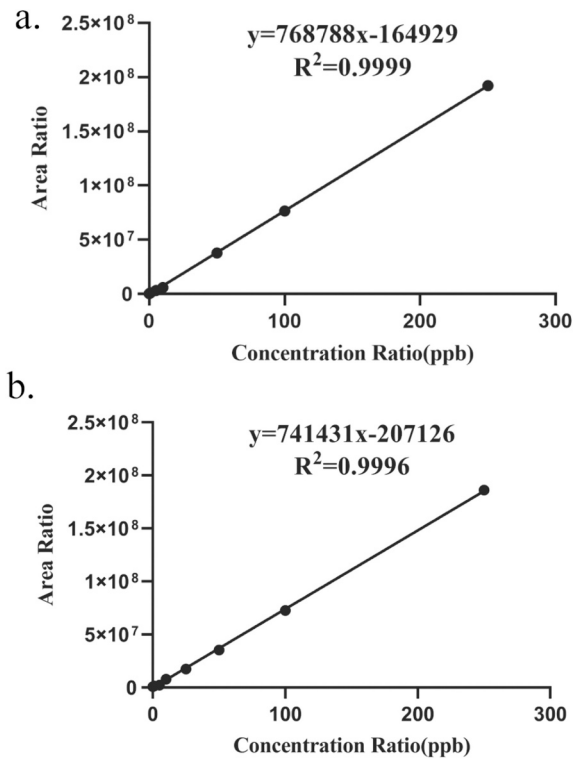

Fig. 1. Skatole Linear calibration curve (a). Adipose Tissue and *Longissimus thoracis* (LT); (b). Serum.

tissue and 13.41–13.50 for the LT sample. The retention time was slightly different in the serum samples in the 14.06–14.16 min range, as presented in Figs. 2a, b, and c. The outcomes of this methodology demonstrated its effectiveness in accurately determining skatole levels in pork samples, affirming its capability to deliver precise results without interference.

3.1.2. Limits of detection (LoD)/limits of quantification (LoQ)

The limits of detection (LoD) and limit of quantification (LoQ) are crucial parameters in quantitative analysis(Umit, 2015). LoD refers to the minimum analyte concentration that can be detected under the given experimental conditions(Umit, 2015), while LoQ targets low analyte levels within sample matrices. The LoD and LoQ were calculated as signal-to-noise (S/N) ratios of 3 and 10, respectively. These values were established through a comprehensive analysis of ten-point calibration curves within a matrix spanning a range of 250–0.1 ppb for each sample analyzed on different days. The derived LoD and LoQ (presented in Table 1) were 0.5 and 1.65 ppb, as reported for the adipose tissue/LT sample, with which a unified calibration standard was used. In comparison, this method for serum samples has a high value of 0.9 and 3.04 ppb, respectively, signifying the sensitivity of the analytical method within the specified concentration range. Jin, Jin, Yu, Lee, and Chen (2017) reported higher LoDs ranging from 0.82 to 3.69 ppm and LoQs ranging from 2.47 to 11.2 ppm for the classification of medical cannabis cultivars in Canada, utilizing cannabinoid and terpene quantification through HPLC-DAD and GC–MS.

3.1.3. Repeatability

The method's repeatability was evaluated by analyzing the mean concentration values derived from each sample. To ensure the precision and reliability of our findings, we conducted repeatability analyses using 10 samples from each tissue type, with each sample subjected to replicates for analysis. The resultant relative standard deviation (RSD%) values for repeatability and reproducibility are listed in Table 1. The results from the adipose tissue samples investigated demonstrate a commendable repeatability of 8.78% across adipose tissue samples, 9.4% for the LT samples, while the serum samples showed a better repeatability value of 4.08%; the lower the percentage of RSD repeatability, the better the repeatability of the method. Both repeatability and reproducibility exhibited commendable values of below 10. Notably, RSD values below 10% signify satisfactory repeatability (Bobo-garcía, Davidov-pardo, Arroqui, & Marín-arroyo, 2014); however, Hermabessiere et al. (2018) reported a method's repeatability RSD that falls below the threshold of 20%.

The reproducibility analysis of our method involved scrutinizing the outcomes obtained from experiments conducted on separate occasions, one within a single day and the other in a distinct week. Our method reproducibility is 5.9%, although some authors have reported various coefficient variances for the reproducibility, such a method for the determination of boar taint marker compounds in pork tissue through collaborative trials using GC–MS reported reproducibility of 10–30% (Buttinger & Wenzl, 2020), Wauters et al. (2015) reported reproducibility of <10.5% from the quantitative method for the assessment of boar taint compounds via UHPLC-MS. Our method shows acceptable repeatability and reproducibility, which shows the dependability of the analytical technique in producing consistent outcomes.

3.1.4. Recovery

Recovery values within the 80–120% range were deemed acceptable, suggesting that the method remained unaffected by the sample matrix (Alinafiah et al., 2021). The recovery of our study indicated a high percentage recovery of 90% for adipose tissue and 87% for serum samples, and recovery percentages falling within the range of 80–120% were deemed satisfactory(Rutkowska & Kaczy, 2018), suggesting that the analytical method remained unaffected by the matrix(Alinafiah et al., 2021). Our study concentrates solely on conducting recovery experiments on adipose tissue to assess the accuracy and reliability of the analytical method within the context of fat tissue analysis. Adipose tissue, with its unique composition and lipid content, presents specific challenges in extraction efficiency (Stroh et al., 2021) and matrix effects compared to muscle tissue. Focusing recovery experiments on adipose tissue enables a thorough evaluation of extraction and analysis processes under conditions directly relevant to the sample matrix. This targeted approach enhances the robustness and applicability of the validation strategy, ensuring a comprehensive assessment of the analytical method's performance for adipose tissue analysis.

3.2. Comparison with previous research findings

Our study represents a novel approach by employing gas chromatography in conjunction with a high-resolution mass spectrometer, specifically the Q-Exactive Orbitrap mass analyzer, for analyzing boar taint compounds. Table 2 summarizes the results of diverse studies on the quantification of boar taint compounds using different analytical techniques. It highlights vital parameters, such as linearity, repeatability, reproducibility, LoD, LoQ, and the extraction methods applied. Linearity plays a crucial role in ensuring analytical methods' accuracy, reliability, and regulatory compliance. Our method distinguished itself with a substantial R^2 value of 0.9999, indicating its reliability and accuracy. This high linearity reinforces the credibility of our approach, making it well-suited for precise quantitative analyses and meeting stringent regulatory standards for method validation.

Leveraging the Orbitrap mass analyzer in our study enhanced the dependability of the lower detection limit, signaling the robustness of our method when juxtaposed with other methodologies detailed in Table 2 from the literature. Furthermore, our analytical approach, featuring a LoD at 0.9 ppb and a LoQ at 1.65 ppb, demonstrates remarkable sensitivity, enabling the identification and quantification of skatole at concentrations significantly below the European Commission's stipulated threshold of 0.25 ppm in fat(Maribo, Jensen, & Nielsen, 2017), demonstrating applicability of our method. Likewise, the method

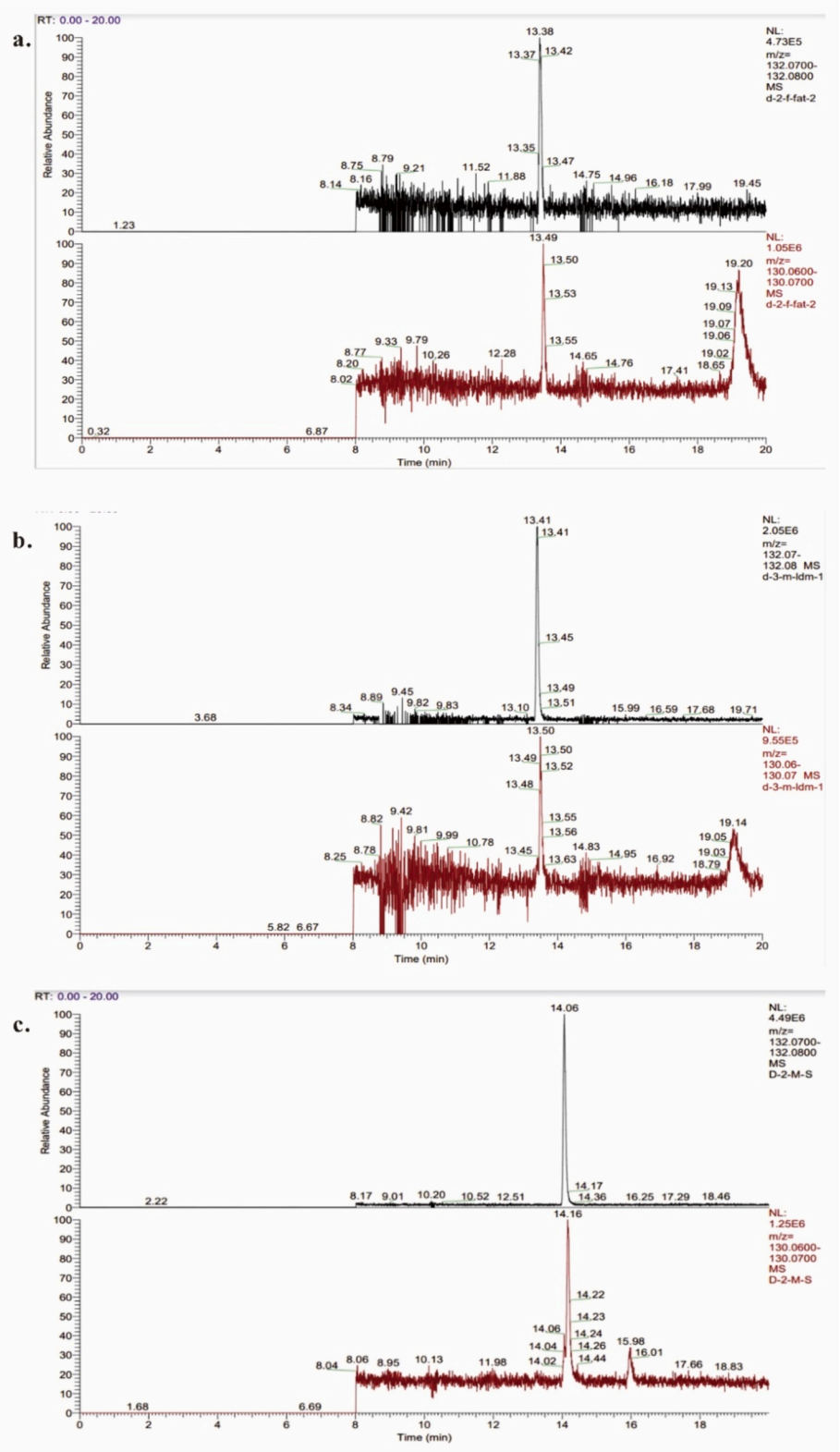

Fig. 2. Chromatograms of the Retention Time of IS and ES (a). Adipose Tissue; (b). *Longissimus thoracis* (LT); (c). Serum.

meets the regulatory requirements and can provide precise measurements within a specified range.

In a study by Stewart, Scorsone, Prunier, and Hamel (2022), skatole quantification in adipose tissue using electroanalytical methods ECL and HPLC resulted in a notably lower recovery rate of 67.60%. Their extraction process involved multiple steps, including melting adipose tissue in a microwave, adding a hygroscopic salt (sodium sulfate), and introducing a strong base (NaH). This lengthy procedure may have compromised analyte integrity, contributing to the reduced recovery observed. In contrast, our approach focuses solely on adipose tissue liquefaction during extraction, aiming to streamline the process and potentially enhance recovery efficiency. Belghiti, Scorsone, de Sanoit,

Table 1
Analytical parameters for GC–MS Method Developed validation.

Parameters	Adipose tissue	LT	Serum
Linearity R^2 ($n = 10$)	0.9999		0.9996
Slobe	768,788		741,431
LoD (ppb, n = 10)	0.5		0.9
LoQ (ppb, n = 10)	1.65		3.04
Retention Time (min)	13.49 ± 0.05	13.45 ± 0.05	14.175 ± 0.015
Repeatability (RSD%, n = 10)	8.78	9.4	4.08
Reproducibility (%CV)		5.9	
Recovery (%)	90		87

and Bergonzo (2016) also reported a recovery rate of approximately 50% using their electroanalytical method. They suggested that this lower recovery could be due to challenges in effectively removing a thicker layer of contaminants during the analysis. Selecting an extraction method that minimizes analyte loss or degradation from the sample matrix is essential for maintaining analyte integrity and achieving higher recovery rates.

Verplanken, Wauters, Vercruysse, Aluwé, and Vanhaecke (2016) reported moderate repeatability and reproducibility, with values of ≤14.9% and ≤ 17.2%, respectively, using UHPLC-HRMS; however, Buttinger and Wenzl (2020), employing GC–MS and LC-MS/MS, presented higher values for both repeatability (3−10) and reproducibility (10–30%) compared to our study (Fig. 3). However, various factors may influence method reproducibility, such as handling procedures and instrumentation; our study utilized GC-HRMS, offering high sensitivity, specificity(Lübeck, Alexandrino, & Christensen, 2020), and comprehensive analysis of complex sample matrices. The reproducibility of our study, at 5.9%, surpasses that of other methods in the field. It is crucial to note that lower values of repeatability and reproducibility indicate better performance.

Studying the reports of other authors detailing methodologies for quantifying boar taint compounds shows that our proposed method emerges as noteworthy, showcasing distinct advantages across various engaged parameters. The observed high recovery value for the adipose tissue sample in our study may be attributed to specific sample handling practices employed during the extraction process. For example, melting the adipose tissue in the microwave before extraction could potentially enhance skatole extraction efficiency by facilitating tissue structure disruption and releasing skatole molecules(Costa, 2016), leading to high recovery and showing the efficiency of the process.

The linearity and other parameters of our GC-HRMS method were in order. Notably, the GC–MS method retains its status as one of the most convenient analytical techniques, underscored by its superior selectivity and precision, as previously highlighted(López-Fernández et al., 2022).

4. Conclusions

Validation of this GC-HRMS method for skatole (3-methylindole) determination demonstrated compliance with linearity, repeatability, and reproducibility requirements. Both LoQ and LoD exhibited sufficient sensitivity for detecting skatole at levels relevant to sensory perception. This method demonstrated notable sensitivity with a lower LoD. A lower LoD indicates the capability of our analytical approach to detect skatoles at concentrations below the proposed threshold. These quality control tools are valuable for assessing skatole content in pig tissue.

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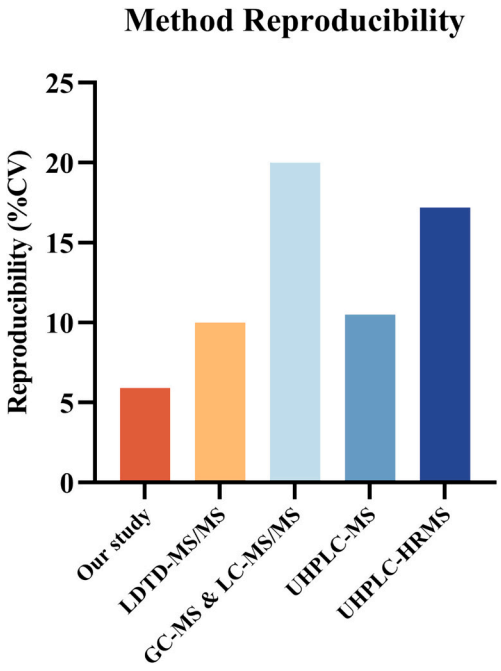

Fig. 3. Bar chat showing Reproducibility comparison from different authors.

Table 2
Validation methods for quantifying boar taint compounds from pork from other authors.

Technique	Sample (pork)	Repeatability (%)	Reproducibility (%)	Recovery (%)	LoD (ppb)	LoQ (ppb)	Linearity (R^2)	Reference
GC-HRMS	Fat/LT	8.78/9.4		90	0.5	1.65	0.9999	Our study
GC-HRMS	Serum	4.08	5.9	87	0.9	3.04	0.9996	Our study
LDTD-MS/MS	Fat	3–7	10	108–110	0.03–0.1	0.05–10	0.99960–0.99771	(Lund et al., 2021)
GC–MS & LC-MS/MS	Fat	3–10	10–30	–	–	–	–	(Buttinger & Wenzl, 2020)
UHPLC-MS	Serum, Plasma	< 7.6	< 10.5	87–97	0.5–1	2–3	0.99	(Wauters et al., 2015)
SERS	Fat	–	–	–	2.1×10^{-11} 1.8×10^{-10}			(Sørensen, Westley, Goodacre, & Engelsen, 2015)
ECL	Fat	–	–	67.60%	0.7	24.2	0.911	(Stewart et al., 2022)
UHPLC-HRMS	Pork	≤14.9	≤17.2	89–110	1–5	2.5–25	0.99	(Verplanken et al., 2016)
BDD	Fat	–	–	50	3×10^{-2}, 5×10^{-2}		–	(Belghiti et al., 2016)

CRediT authorship contribution statement

Ayoola Ebenezer Afe: Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zhao-ji Shen:** Formal analysis, Data curation. **Xiaorong Guo:** Formal analysis, Data curation. **Weihai Xing:** Methodology, Formal analysis, Data curation. **Kui Li:** Writing – review & editing, Supervision, Formal analysis. **Rong Zhou:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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