ELSEVIER

Journal of Chromatography B, 661 (1994) 219–230

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs

Jens Hansen-Møller

Danish Meat Research Institute, Maglegårdsvej 2, DK-4000 Roskilde, Denmark

First received 24 March 1994; revised manuscript received 3 August 1994

Abstract

A rapid high-performance reversed-phase liquid chromatographic method for the simultaneous quantitative determination of the main boar taint compounds androstenone, skatole (3-methylindole) and indole, in back fat from pigs has been developed. The compounds are extracted by a simple homogenisation of adipose tissue in methanol; interfering lipids are removed by precipitation after cooling and centrifugation. Androstenone is derivatized pre-column with dansylhydrazine (5 min at ambient temperature) using BF_3 as catalyst. The compounds are separated on a 60 × 4.6 mm I.D., 3 μm Hypersil ODS column (Hewlett-Packard) using a step-gradient; total time for the separation is 15 min. Fluorescence is used for selective detection. The limit of quantitation for indole and skatole is 30 ng/g and for androstenone 200 ng/g back fat. The results for skatole obtained by the present method were compared with those of a colorimetric method, while androstenone determinations were compared with two GC–MS methods and a RIA method. The correlations observed were in the range of 0.946–0.993. The average contents of androstenone and skatole were 640 ± 700 and 78 ± 113 ng/g (n = 1162 male pigs), respectively.

1. Introduction

Castration of male pigs has been practised for centuries in order to avoid the occurrence of "male odour" in the meat from 5–10% of the animals. The male odour is only a problem when meat or meat products are heated by the consumer prior to consumption. However, the advantages of entire male (i.e. not castrated) pig production compared to hog production are large.

The major compounds responsible for the boar taint are known to be skatole (3-methylindole) [1,2], indole [3] and the pheromone androstenone (5-α-androst-16-en-3-one) [4]. Other steroids have also been associated with the boar taint: 5-α-androst-16-en-3α-ol and 5-α-androst-16-en-3β-ol [5,6].

In experiments describing the relationship between skatole/androstenone and odour/flavour in meat from entire male pigs, the correlation between skatole content and odour score was 0.76 thus explaining 58% of the variation of odour scores. If androstenone content was included, about 66% of the variation in odour score could be explained. Androstenone content alone explained only 24% of the variation [3].

Several immunochemical methods like RIA [7,8] or ELISA methods [9] as well as several

0378-4347/94/$07.00
SSDI 0378-4347(94)00364-5

chromatographic methods using HPTLC [10], GC–MS [4,6,11–13], GC-FID [14] or GC-ECD [15] for determination of androstenone in adipose tissue have been described. A variety of chromatographic methods for determining skatole in back fat using normal-phase [6] and reversed-phase HPLC [16–18] or GC [19] were also described. A routine UV-Vis method uses derivatization of skatole with 4-dimethylaminobenzaldehyde [20]. This method, which fully automated, has a capacity of 200 determinations per hour, is currently used for sorting male pig carcasses in slaughterhouses in Denmark.

A critical step is the sample preparation of the adipose tissue, due to the fact that skatole, indole and androstenone are very lipophilic. The sample preparation procedures previously used include liquid–liquid extraction [15,18], steam distillation [21], solid-phase extraction in the normal-phase [6,19] and reversed-phase mode [16]. Super-critical fluid extraction has also been tried [22].

In the present paper we report for the first time a simple method for the simultaneous determination of the three main compounds responsible for boar taint. The results for skatole are compared with the colorimetric method [20], and the results for androstenone are compared with two GC–MS methods [11,13] and a RIA method [23].

2. Experimental

2.1. Chemicals

Indole, 2-methylindole (2-MID), skatole (3-methylindole), androstenone (5α-androst-16-en-3-one), androstanone (5α-androstan-3-one), BHT, *n*-propylgallate, citric acid, $AlCl_3$, trichloroacetic acid and dansylhydrazine were obtained from Sigma (St. Louis, MO, USA). 4-Dimethylaminobenzaldehyde (DMAB) and a 20% solution of BF_3 in methanol were obtained from Merck (Darmstadt, Germany). Demineralised water was treated in a Milli-Q Plus water purification system from Millipore (Bedford, MA, USA). Methanol (MeOH), acetonitrile (MeCN), acetone and tetrahydrofuran (THF) were of HPLC grade obtained from Romil (Loughborough, Leics., UK). All other chemicals were of analytical grade.

2.2. Colorimetric method

A 5.00-ml volume of acetone–0.1 *M* Tris-HCl buffer (pH 7.6) (75:25, v/v) was added to approximately 500 mg of back fat. After homogenization and chilling to approximately 4°C, the homogenate was filtered through a sintered glass filter.

A 1.42-ml volume of DMAB reagent were added to 1.00 ml of the filtrate. (DMAB reagent was prepared by dissolving 10 g of DMAB in 1000 ml of a mixture of ethanol–75% sulphuric acid (60:40, v/v), previously degassed by applying a vacuum of 15 mmHg to the solution for 30 min).

After 180 s at ambient temperature 2 ml of the solution were transferred to the 30-mm flow-cell of a Lambda II spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), and the spectrum from 460 to 730 nm was recorded. The absorption at 580 nm was used for quantification. As the method is calibrated using skatole standards, results are expressed in so-called skatole equivalents. Experimental details may be found in Mortensen and Sørensen [20].

2.3. High-performance liquid chromatography

The HPLC systems were Hitachi systems from Merck. Each system consisted of an AS-4000 autosampler, a L-6200 gradient pump, a F-1080 fluorescence detector, a L-5025 column oven and a D-6000 HPLC-manager software installed on a Compaq Descpro 386s PC. An AS-4000 autosampler editor programme was used for modification of the derivatization procedure.

HPLC system 1

The column was a Hypersil ODS (3 μm, 60 × 4.6 mm I.D., Hewlett-Packard) operated at 40°C.

The mobile-phase buffers used were: (A)

THF–25 m*M* potassium phosphate buffer pH 6.0–acetic acid (31:67.6:1.4, v/v); (B) THF–acetonitrile–25 m*M* potassium phosphate buffer pH 6.0–acetic acid (34:23.8:41.4:0.8, v/v); and (C) THF–water (90:10, v/v). The following gradient profile was used: 0–4.5 min, 100% A; 4.5–5.0 min, 100% A–100% B; 5.0–10.5 min, 100% B; 10.5–10.6 min, 100% B–90% C + 10% A (2.0 ml/min); 12.0–12.5 min, 90% C + 10% A–100% A; 12.5–14.0 min, 100% A (2.0 ml/min–1.5 ml/min).

Fluorescence detection was employed with excitation at 285 nm and emission at 340 nm for the first 5.0 min (indole and skatole); then the wavelengths were changed to excitation at 346 nm and emission at 521 nm (dansylhydrazones of androstenone and androstanone).

HPLC system 2

The column was a Superspher 60 RP-8 (4 μm, 120 × 4 mm I.D.) operated at 40°C, fitted with a 4-mm RP-8 precolumn (LiChroCart system, Merck).

The composition of the mobile-phase buffers was: (A) acetonitrile–25 m*M* potassium phosphate buffer pH 6.0–acetic acid (30:69.6:1.4, v/v) and (B) methanol.

The following gradient profile was used: 0–3.5 min, 90% A (1.5 ml/min); 3.0–3.5 min, 90–45% A; 3.5–15.0, min 45–5% A; 15.0–16.1 min, 5% A (1.5–2.0 ml/min); 16.1–17.0 min, 5–90% A; 17.0–19.0, 90% A (2.0–1.5 ml/min).

Fluorescence detection was performed with excitation at 346 nm and emission at 521 nm.

In all assays 20 μl of the sample were injected. 2-MID was used as an internal standard for determination of indole and skatole, while androstanone was used for determination of androstenone. The linearity of the standard curves, based on peak height, was investigated in the range of 0.001 to 1.0 mg/l for indole and skatole, and 0.01 to 5.0 mg/l for androstenone.

2.4. Sample pretreatment

A 3.00-ml volume of methanol containing the internal standards (0.033 mg/l and 0.33 mg/l of 2-MID and androstanone, respectively) was added to 0.50 g of back fat. After homogenization by means of a Kinematica Polytron PT 3000 (Littau, Switzerland) fitted with a 12-mm aggregate, the samples were sonicated for 5 min and cooled on an ice bath for 15 min. After centrifugation for 5 min at 4000 *g* at 5°C, 250 μl of the extract was transferred to an autosampler vial and analyzed. All samples were analyzed in duplicate.

2.5. Standard solutions

The standard solutions were prepared in methanol–water (95:5, v/v).

2.6. Derivatization

The AS-4000 autosampler was programmed by the AS-4000 programme editor software (Merck) to perform the derivatization. A 30-μl aliquot of 2% dansylhydrazine dissolved in methanol, 4.4 μl of water, and 10 μl of 20% BF_3 were mixed with 140 μl of sample extract. After 5.0 min at ambient temperature 20 μl of the mixture were injected onto the HPLC system.

2.7. Back fat samples

Back fat samples for recovery and reproducibility studies were obtained from a local abattoir. Prior to use in recovery studies the samples were tested for skatole, indole and androstenone contents to ensure a low content of these compounds.

2.8. Validation

Recovery

The recovery of the sample preparation procedure was determined by spiking three different back fat samples containing low amounts of the compounds with known amounts of the compounds at four different levels (see Table 1).

Intra- and inter-assay variation

For intra- and inter-assay variation 10 different samples were selected on the basis of their content of the three compounds. The samples were analyzed in duplicate on five different days;

Table 1
Recovery of the method using HPLC system 1

Level	Indole/skatole					Androstenone		
	Added (ng/g)	Recovery indole (%)	*n*	Recovery skatole (%)	*n*	Added (ng/g)	Recovery (%)	*n*
1	40	92.0 ± 13	5	99.1 ± 20	3	100	102.2 ± 14	6
2	100	102.8 ± 4	5	95.2 ± 9	5	250	101.9 ± 3	6
3	200	110.1 ± 13	5	98.7 ± 5	5	500	98.3 ± 5	6
4	1000	106.6 ± 9	4	97.3 ± 4	4	2500	100.3 ± 7	5
Average recovery		104.8 ± 9	19	96.9 ± 6	17		100.7 ± 5	23

Back fat samples with a low content of indole, skatole and androstenone were spiked at the levels indicated; *n* is the number of experiments.

however, on one of these days the samples were analyzed 7 times each.

2.9. Preparation of "in house reference material"

Approximately 1 kg of back fat was taken from each of five carcasses having a content of skatole equivalents between 250 and 300 ng/g (using the colorimetric method). The samples were tested to ensure an androstenone content in the range of 1–3 μg/g. The final 5 kg of back fat were homogenised using a bowl chopper (Meissner Maschinen, Wallau, Germany). To the homogenate were added citric acid, *n*-propylgallate and BHT (500 mg of each) dissolved in 50 ml of methanol and the resulting mixture was mixed carefully. Part of it was divided into 25-g aliquots and stored at −25°C. The material was analyzed in duplicate every day.

2.10. Application

Samples (1162) of back fat from randomly selected male pigs were analyzed in duplicate by means of HPLC system 1. The same samples were also analyzed by the colorimetric method and the results were compared using linear regression (least squares method). To clarify if the skatole equivalents obtained by the colorimetric method are a combination of skatole and indole the results were fitted to the linear model:

$$a \cdot \text{Skatole}_{\text{HPLC}} + b \cdot \text{indole}_{\text{HPLC}} + c = \text{skatole equivalents}_{\text{colorimetric method}}$$

using the multivariate analysis programme UNSCRAMBLER [24].

The method was also compared with other methods for determination of androstenone including GC–MS [11,13] and RIA [23] using linear regression (least squares method).

3. Results and discussion

3.1. Principle of detection

Fluorescence was selected for detection because of the good fluorescent properties of indole and skatole and the possibility to easily derivatize androstenone with dansylhydrazine, thus giving a better selectivity and sensitivity.

3.2. Selectivity of the chromatographic system

The selectivity of the two HPLC systems is illustrated in Figs. 1 and 2. The run-times of the systems including the pre-column derivatization are 15 and 19 min for systems 1 and 2, respectively. The last 3 min are not shown; during this period a few large peaks eluted. During optimization of the second mobile phase in system 1, in which isoelutrope mixtures of acetonitrile, THF and buffer were tested, it was observed that the

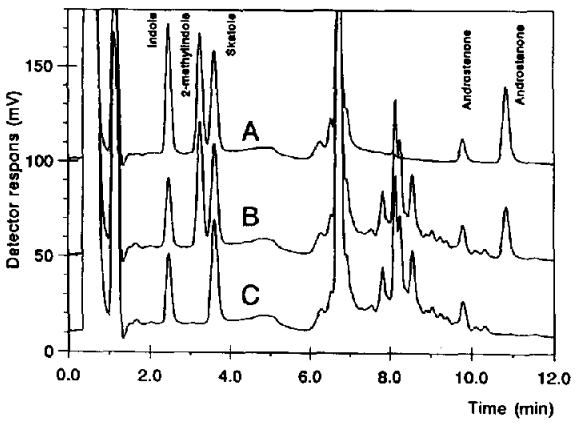

Fig. 1. Selectivity of chromatographic system 1. For chromatographic conditions see Experimental. (A) Standard solution containing: 33 ng/ml of indole, skatole and 2-methylindole, 400 ng/ml of androstenone and 330 ng/ml of androstanone. (B) Back fat sample spiked with internal standards (2-methylindole and androstanone, 200 and 2000 ng/g, respectively). Androstenone, skatole and indole content 1010, 190 and 110 ng/g, respectively. (C) Same as (B) but without internal standards.

peaks of androstenone and androstanone split into double peaks, when the THF content was less than 27%. This may be explained by the fact that the *syn*- and *anti*-forms of the dansylhydrazones are resolved because of the changed selectivity of the system. This is analogous to the separation of the *syn*- and *anti*-oxime derivatives observed by e.g. De Brabander and Verbeke [15]. For a routine analytical method only one peak for each compound is desirable. By extending the time of derivatization to more than 5 min it was observed that some of the interfering peaks around the androstenone peak increased.

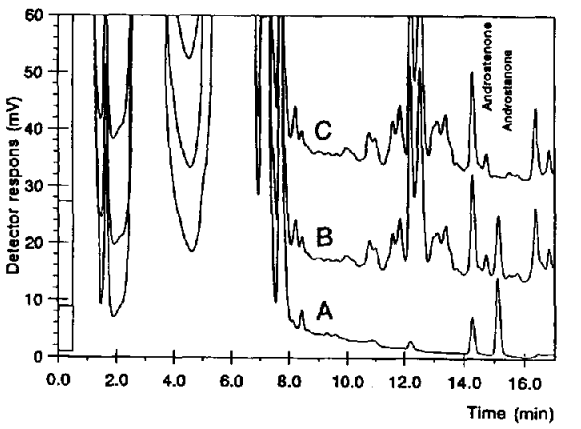

Fig. 2. Selectivity of chromatographic system 2. For chromatographic conditions see Experimental. (A) Standard solution containing 40 ng/ml of androstenone and 330 ng/ml of androstanone. (B) Back fat sample spiked with internal standard (androstanone, 2.0 μg/g). Androstenone content 3.33 μg/g. (C) Same as (B) but without internal standard.

HPLC system 2 was only designed for the determination of androstenone and has a selectivity different from that of system 1, in order to verify the specificity of the androstenone part of the method.

3.3. Principle of derivatization

Different methods to make the fluorescent derivatives and simultaneously determine the indolic compounds using fluorescence detection were investigated. Attempts to convert androstenone to a fluorescent compound by treatment with strong acid, according to the procedure described by Nozaki et al. [25] were abandoned because of the instability of the indolic compounds during derivatization. Another method to prepare a derivative at the 16-double bond using anisaldehyde [10] or resorcinylaldehyde [26] was abandoned for the same reason.

The procedure described by Kawasaki et al. [27] using dansylhydrazine and trichloroacetic acid as catalyst at 60°C was investigated. Following the reaction as a function of time it was seen that androstenone produced two peaks which during the first 60 min of derivatization merged to one peak coeluting with the peak of androstanone (the internal standard). Furthermore, the time for total derivatization of androstenone was more than 60 min at 60°C.

Alternatively different Lewis acids like $AlCl_3$ and BF_3 were investigated as catalysts. BF_3 appeared to give the best results. Initially it was discovered that, using back fat extracts and standard solutions prepared in 100% methanol, the reaction rate in standard solutions was significantly slower than that observed in a back fat extract. The difference was traced to differences in water content. It was observed that a water

content higher than 3% in a standard solution results in maximum yield.

Subsequently the derivatization procedure was optimized with respect to the amount of 2% dansylhydrazine and 20% BF_3 added to 140 μl of back fat extract as well as reaction time. Adding 10 μl of 20% BF_3, 30 μl of 2% dansylhydrazine and 4.4 μl of water to 140 μl of the extract, the derivatization is complete within 5 min at ambient temperature. Thus the final concentration of the reactants in the derivatization mixture is 1.1% of 20% BF_3 and 0.33% of dansylhydrazine. When calculating the amount of water in the reaction mixture one has to account for the amount of water in the back fat as well. This is normally >10% thus adding 4.4 μl of water to the water present in the extract, the water content will always be more than 3.7% which is enough to ensure maximum reaction rate. Afterwards the standard solutions were prepared in methanol–water (95:5, v/v) to ensure maximum yield.

Changing the temperature to either 5°C above or below ambient temperature only had a minor effect.

Exposure of the dansylhydrazine solution to light for a few hours produced a broad peak between 4 and 5 min; thus the dansylhydrazine solution has to be freshly prepared every day and kept in brown autosampler vials.

The indolic compounds were stable under the conditions investigated.

3.4. Linearity, detection and quantitation limits

The detection limits (Table 2; signal-to-noise ratio = 3) were determined in standard solutions. The quantitation limits (Table 2) were defined as 10 times the detection limit unless authentic samples showed less sensitivity. A quantitation limit of 30 ng/g (skatole and indole) and 200 ng/g (androstenone) is quite satisfactory when the determinations are used for elucidation of the relationships between smell/taste and content of the compounds, since the threshold for organoleptic sensing is 200 ng/g for skatole [28] and 1.0 μg/g for androstenone [29].

Table 2
Limits of detection and quantitation

Compound	Limit of detection (ng/ml)	Limit of quantitation (ng/g)
Indole	<3	30
Skatole	<3	30
Androstenone	20	200

The detection limits (signal-to-noise ratio = 3) were determined in standard solutions. The quantitation limits (in adipose tissue) were defined as ten times the detection limit unless authentic samples showed less sensitivity.

3.5. Recovery

Recovery of the sample preparation procedure was assessed by spiking samples with a low content of compounds (true blank samples do not exist) with known amounts of the same compounds. The levels used for the indolic compounds were chosen according to previous investigations [17,30]. The levels for androstenone were selected according to the literature. Only HPLC system 1 was used for the recovery studies.

The standard deviation on the lowest level used (level 1, Table 1) is generally high, which is due to the fact that all samples used contain 1/3 to 1/2 of the amount used for spiking; this and the fact that level 1 is close to the limit of quantitation explain the high standard deviation.

The average recoveries for androstenone, skatole and indole are 100.7 ± 5, 96.9 ± 6 and 104.8 ± 9%, respectively.

3.6. Intra- and inter-assay variability

The within-day ($n = 7$) and between-day ($n = 5$) C.V. for the compounds are given in Table 3. The samples used were selected on the basis of their content to ensure that the normal levels were included. It can be seen that the C.V. values are generally low, but they increase as the concentrations approach the limit of quantitation.

The indole content is generally low which

Table 3
Intra- and inter-assay variability

	Indole			Skatole			Androstenone		
	Concentration (mean ± S.D.) (ng/g)	C.V. (%)		Concentration (mean ± S.D.) (ng/g)	C.V. (%)		Concentration (mean ± S.D.) (ng/g)	C.V. (%)	
		Within day ($n = 7$)	Between day ($n = 5$)		Within day ($n = 7$)	Between day ($n = 5$)		Within day ($n = 7$)	Between day ($n = 5$)
1	74 ± 4	5.5	8.2	209 ± 8	3.9	7.0	2940 ± 88	3.0	2.8
2	90 ± 2	2.2	5.1	173 ± 2	1.4	4.0	870 ± 34	3.9	4.5
3	46 ± 3	5.4	9.6	228 ± 9	3.7	6.7	1970 ± 114	5.8	2.6
4	32 ± 1	3.9	6.2	46 ± 2	3.3	4.4	610 ± 24	3.9	4.9
5	44 ± 2	5.2	7.4	114 ± 2	1.1	5.6	1310 ± 61	4.7	4.4
6	36 ± 2	4.3	8.9	36 ± 2	4.9	7.9	250 ± 20	8.2	7.2
7	41 ± 2	4.2	12.6	126 ± 7	5.3	13.3	390 ± 9	2.2	8.6
8	103 ± 7	6.6	4.5	314 ± 20	6.2	5.3	490 ± 55	11.1	5.7
9	54 ± 4	7.2	7.3	171 ± 7	3.8	5.4	790 ± 57	7.2	4.1
10	69 ± 2	3.4	5.0	136 ± 4	2.6	5.9	670 ± 17	2.6	4.1

reflects the usually low level of indole in Danish pigs.

3.7. Application

From a large study including 1162 samples, 384 samples containing quantifiable amounts of indole and skatole were selected for comparison of the methods used for determination of skatole.

When comparing the content of skatole determined by HPLC with the skatole equivalents obtained by the colorimetric method (Fig. 3A) a good correlation ($r = 0.975$; $n = 384$) between the two methods is seen. The equation of the regression is $y = 1.097x - 0.037$. These results are identical to those reported earlier [17].

When fitting the results to the linear model (see equation in section 2.10) the best correlation ($r = 0.986$) is obtained by correlating 75.9% skatole (a) + 44.2% indole (b) + 0.027 (c) to the colorimetric results, line of regression: $y = 0.972x + 0.005$; $n = 384$ (Fig. 3B). This means that it is possible to predict the skatole equivalent from the content of indole and skatole obtained by HPLC, the reverse, however, is not possible. However, it is possible to make a new multicomponent algorithm for on-line determination of indole and skatole from the HPLC results and the spectra from the colorimetric method.

In an earlier investigation it was mentioned that the skatole equivalents may contain approximately 25% of the amount of indole determined by HPLC, but the results were not conclusive due to the relatively small number of samples ($n = 137$) [31].

Hawe et al. [32] found a correlation between a GC method and the colorimetric method of $r = 0.128$ ($n = 29$). They found an average content of skatole in back fat of 48 ng/g, which is very close to the limit of quantitation of the colorimetric method. This latter fact hampers the comparison of both methods.

Wirrer [33] found a correlation of 0.4495 ($n = 76$) between the colorimetric method and an HPLC method for the determination of skatole [16]. Unfortunately the indole content of the samples is not given which might explain the poor correlation between the methods.

The colorimetric method has never been designed for determination of skatole alone, but it was optimized for detection of tainted carcasses using a trained sensoric panel as criterion and skatole was only used as an adequate compound for calibration of the method. Therefore, the results are always given as skatole equivalents. Bejerholm and Barton-Gade [3] have, however, shown that indole contributes to the odour/

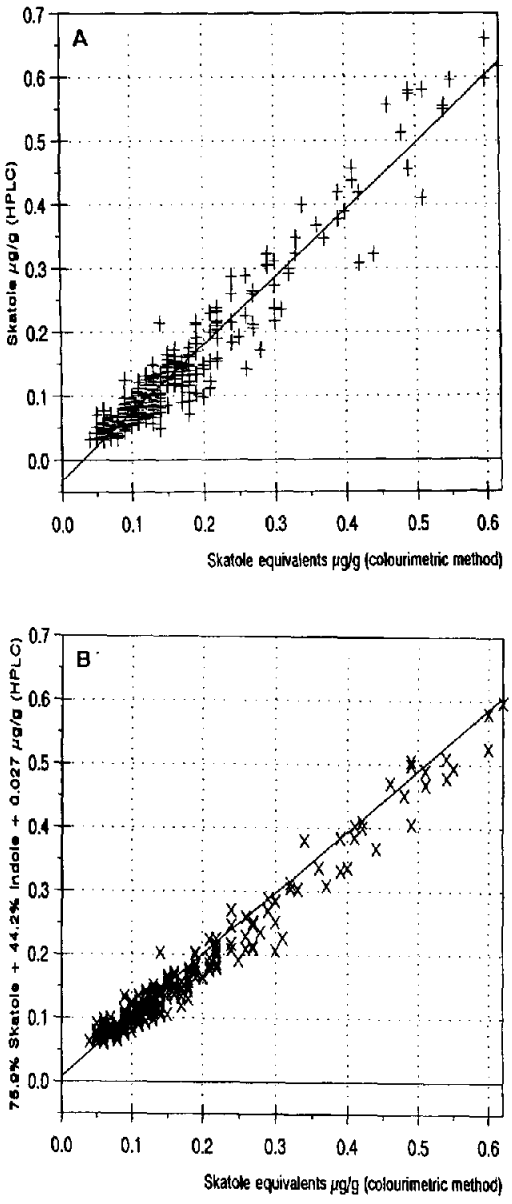

Fig. 3. (A) Comparison of the skatole equivalents determined in 384 pig back fat samples by means of the colorimetric method described by Mortensen and Sørensen [20] and the amount of skatole as determined by HPLC. The correlation between the methods is 0.975 while the equation of regression is $y = 1.097x - 0.037$. (B) Same samples as in A except that multicomponent analysis has been used to find a better correlation between the HPLC method and the colorimetric method. The best correlation ($r = 0.986$) is obtained by correlating 75.9% skatole (HPLC) + 44.2% indole (HPLC) + 0.027 to the colorimetric results, line of regression: $y = 0.972x + 0.005$.

flavour of meat from entire male pigs and this indicates that it is necessary to have a method for sorting of the carcasses which measures indole and skatole in the right proportions.

The androstenone part of the method was compared with two different GC–MS methods [11,13], a RIA method [23] and with the HPLC system 2 (Table 4). All samples used were from Danish male pigs except for one of the GC–MS methods [11] for which Swedish samples were used. The samples used for comparison of the RIA and the two HPLC methods are identical. The correlation with the Swedish GC–MS method [11] is illustrated in Fig. 4. From Table 4 it appears that the methods are almost identical. One of the GC–MS methods [13] slightly overestimates the content and it is slightly biased as well.

Tables 5 and 6 summarize the results along with results reported by other authors. The results are not directly comparable due to differences in the way the samples were selected. The Spanish samples [6] were selected on the basis of the presence of boar taint (subjective judgement). The samples used in this study were randomly selected from Danish pigs from the local abattoir. The highest content of skatole was found among the Danish samples, while the highest concentration of indole was reported in the German results [18]. High levels of indole may, however, be found in Danish pigs when they are kept heavily fouled in pens with high temperatures and a high stocking rate [30]. The highest androstenone content is found among the Danish samples, however, the average content is just as low as the results reported by Hansson et al. [21].

In Fig. 5 the concentration of skatole and androstenone in 796 samples containing quantitative amounts of the compounds is plotted. A correlation of $r = 0.365$ ($n = 1162$) can be calculated (for the calculation 0.1 and 0.015 were substituted for values below the limit of quantitation for androstenone and skatole, respectively). The correlation found is similar to that reported by Lundström et al. [34] of 0.32, but less than that reported by Hansson et al. [21] of 0.54. However, factors such as age and cross-breeding

Table 4
HPLC system 1 was compared with different methods for determination of androstenone

Method for androstenone determination	Line of regression	Correlation (r)	n
GC–MS (Mågård [11])	$y = 1.07x - 0.052$	0.977	16
GC–MS (Le Bizec et al. [13])	$y = 0.82x + 0.124$	0.949	31
RIA [23]	$y = 1.15x - 0.047$	0.946	37
HPLC system 2	$y = 1.03x + 0.086$	0.993	37

The least squares method was used for calculation of the line of regression. The samples used for comparison to the RIA and HPLC system 2 are identical.

are not known for the present samples, and thus the results are not conclusive.

4. Conclusions

A rapid, reproducible and simple method for the determination of androstenone, skatole and indole in adipose tissue from pigs was developed. For the first time a HPLC method for the simultaneous determination of the main boar taint compounds is reported. Compared with other methods the sample preparation is simple, its use of organic solvents is limited and there are no time-consuming and error-introducing liquid–liquid partition or solvent evaporation steps. Without any further clean up of the extract of back fat in methanol, androstenone is pre-col-

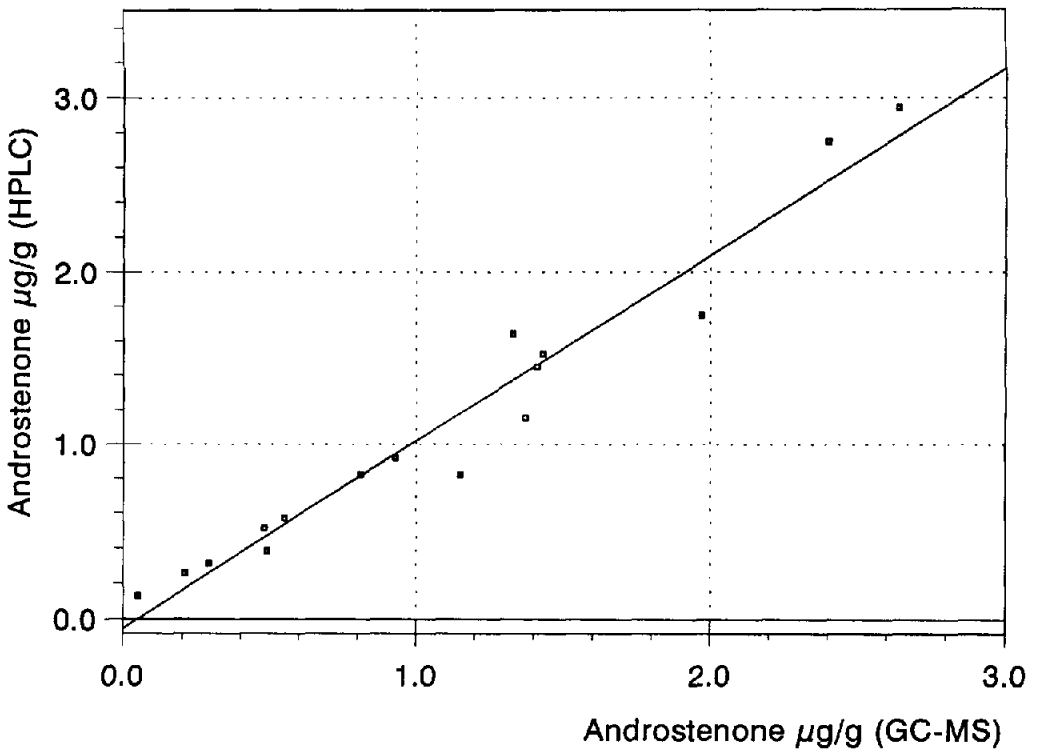

Fig. 4. Comparison of the results from androstenone determination in back fat samples, using HPLC system 1 and GC–MS [11]. The correlation between the methods is 0.977 while the equation of regression is $y = 1.07x - 0.052$ ($n = 16$).

Table 5
Comparison of the concentrations of indole and skatole obtained by the present method and other methods from the literature

	Results obtained by the present method			García-Requeiro and Diaz [6] (HPLC)			Gibis et al. [16] (HPLC)			Dehnhard et al. [18] (HPLC)		Porter et al. [19] (GC)	
	Indole (μg/g)	Skatole (μg/g)	Ratio	Indole (μg/g)	Skatole (μg/g)	Ratio	Indole (μg/g)	Skatole (μg/g)	Ratio	Indole (μg/g)	Skatole (μg/g)	Indole (μg/g)	Skatole (μg/g)
Highest	0.716	1.90	9.9	0.16	0.186	3.2	0.602	0.901	4.0	0.736	1.005	0.057	0.177
Lowest	<0.03	<0.03	0.3	0	0	0.4	0.013	0.023	0.4	0	0	0.012	0.019
Average	0.027[a]	0.078[a]	2.3	0.084	0.101	1.4	0.151	0.201	1.8	0.02	0.04	0.029	0.046
n	1162	1162	384	15	15	15	20	20	20	349	349	14	14

[a] Values below the limit of quantification were substituted by 0.015 μg/g.

umn derivatized to a dansylhydrazone within 5 min at ambient temperature using BF_3 as catalyst. The total time needed for one assay is 15 min which makes the method useful as a laboratory method for large scale investigations of the relations between boar taint compounds and the factors causing boar taint. Only 500 mg of sample is used, however, the sample preparation procedure can easily be scaled down which makes it useful for assay of small samples like biopsies.

The correlation between the present method and other methods used for the determination of skatole and androstenone is very high (in the range of 0.946 to 0.993) which documents the selectivity of the method.

The method is suitable as a reference method for the development of alternative rapid analytical methods for on-line determination of boar taint compounds in an abattoir or for evaluation of other methods. Investigations are currently in progress for evaluation of an ELISA kit for androstenone commercialized by Riedel-de Häen (Art. Nr. 54198).

The factors causing the occurrence of male odour are mainly unknown. In order to elucidate

Table 6
Comparison of the concentrations of androstenone in entire male pig back fat samples obtained by the present method and other methods from the literature

Authors	Method	Highest (μg/g)	Lowest (μg/g)	Average (μg/g)	*n*
Present method	HPLC	7.67	<0.2	0.64 ± 0.70[a]	1162
Garcia-Requeiro and Diaz [6]	GC–MS	4.7	nd	1.29	15
De Brabander and Verbeke (average weight 86 ± 12 kg) [15]	GC-ECD	1.4	0.08	0.6 ± 0.4	40
Bonneau [35]	RIA	2.0	0.5		150
Bonneau and Sellier [36]	RIA			1.65 ± 0.34	330
Andersen and Bakke [37]	RIA	7.31	0.78		6
Hansson et al. [21]	RIA			0.63 ± 0.68	83
Lundstrøm et al. [34]	RIA	4.8	0.01	1.26 ± 0.94	143

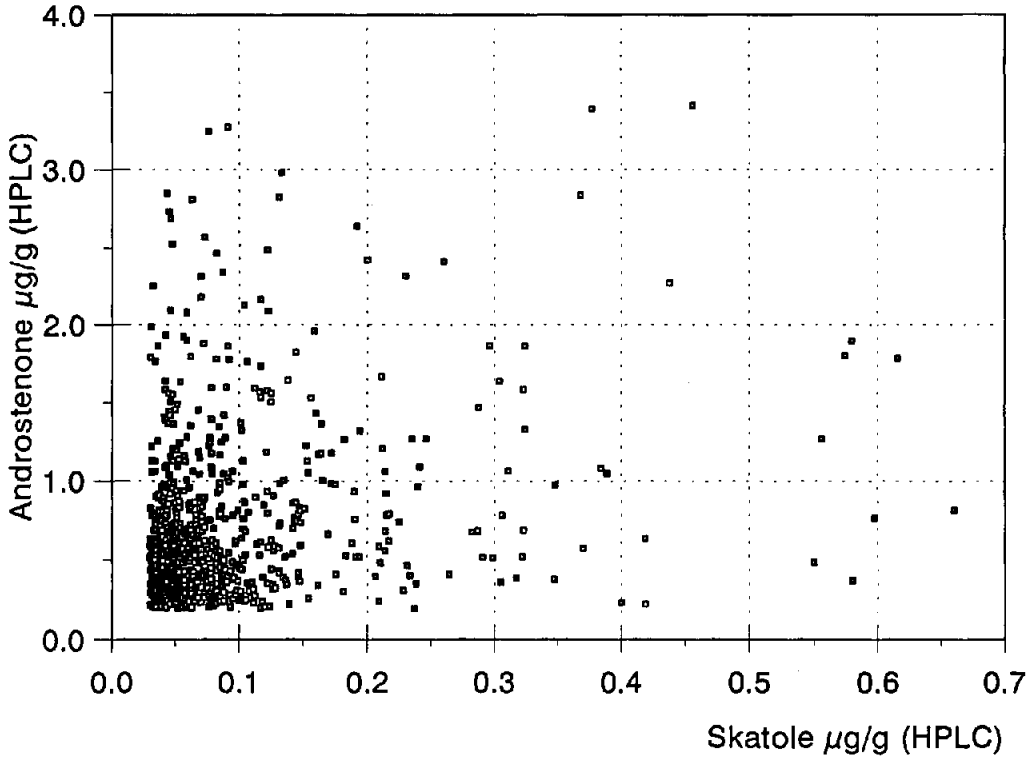

Fig. 5. Plot of the skatole content versus the androstenone content in 796 samples, with concentrations above the quantitation limit of the method. Samples with concentrations above 4.0 μg/g and 0.7 μg/g for androstenone and skatole, respectively are not shown (12 samples). The samples are from Danish domestic pigs randomly selected in an abattoir. A correlation of 0.365 ($n = 1162$) can be calculated (for the calculation 0.1 and 0.015 were substituted for values below the limit of quantitation). The age and cross-breeding are not known for the present samples, thus the results are difficult to interpret.

the mechanisms behind the boar taint problem, further investigations are in progress, such as studies on the physiological, microbiological, hereditary as well as the practical conditions which are considered important for development of boar taint.

Acknowledgements

Jan Rud Andersen, Danish Meat Research Institute, is acknowledged for critical reading of the manuscript. Hans Berg, Swedish Meat Research Institute and Bruno Le Bizec and François Andre, Laboratoire Des Dosages Hormonaux (Nantes, France) are acknowledged for performing the GC–MS determinations. Michel Bonneau (INRA, Saint-Gilles, France) is acknowledged for performing the RIA determinations.

References

[1] E. Vold, Report No. 238, Institute of Animal Genetics and Breeding, NLH, Volebekk, Norway, 1970.
[2] P. Walstra and H. Maarse, IVO-Rapport C-147 and Rapport No. 2, Research Groep Vlees en Vleeswaren T.N.O., Zeist, 1970.
[3] C. Bejerholm and P. Barton-Gade, in M. Bonneau (Editor), *Measurement and Prevention of Boar Taint in Entire Male Pigs*, INRA, Paris, 1993, p. 75.
[4] R.L.S. Patterson, *J. Sci. Food Agric.*, 19 (1968) 31.
[5] R.L.S. Patterson, *J. Sci. Food Agric.*, 19 (1968) 434.
[6] J.A. García-Regueiro and I. Diaz, *Meat Sci.*, 25 (1989) 307.
[7] Ø. Andresen, *Acta Vet. Scand.*, 20 (1979) 343.

[8] R. Claus, B. Hofmann and H. Krag, *J. Anim. Sci.*, 33 (1971) 1293.
[9] R. Claus, G. Mahler and E. Muenster, *Arch. Lebensm-Hyg.*, 39 (1988) 87.
[10] K. Gernhard and I. Kunde, German patent DD 293 432 A5, (1991) 1.
[11] M. Mågård, H. Berg, V. Tagesson, M. Jäeremo, L. Karlsson, L. Mathiasson, M. Bonneau and J. Hansen-Møller, *J. Agr. Food Chem.*, submitted.
[12] R. Schilt, W. Haasnoot, E.O. Van Bennekom, H. Hooijerink, J.M. Weseman, H.J. Korbee and W.A. Traag, *Arch. Lebensm-Hyg.*, 40 (1989) 51.
[13] B. Le Bizec, M.P. Montrade, F. Monteau and F. André, *Food Chemistry*, submitted.
[14] G. Kaufmann, F. Ritter and K. Schubert, *J. Steroid Biochem.*, 7 (1976) 593.
[15] H.F. De Brabander and R. Verbeke, *J. Chromatogr.*, 363 (1986) 293.
[16] M. Gibis, M. Dehnhard and A. Fischer, *Z. Lebensm. -Unters. Forsch.*, 193 (1991) 220.
[17] J. Hansen-Møller, *J. Chromatogr.*, 624 (1992) 479.
[18] M. Dehnhard, R. Claus, M. Hillenbrand and A. Herzog, *J. Chromatogr.*, 616 (1993) 205.
[19] M.G. Porter, S.M. Hawe and N. Walker, *J. Sci. Food Agric.*, 49 (1989) 203.
[20] A.B. Mortensen and S.E. Sørensen, in *Proceedings of the 30th European Meeting of Meat Research Workers*, Bristol, UK, Sept. 9–14, 1984, p. 394.
[21] K. Hansson, K. Lundström, S. Fjelkner-Modig and J. Persson, *Swed. J. Agric. Res.*, 10 (1980) 167.
[22] D.A. Zabolotsky, J.A. Patterson, J.C. Forrest, L.F. Chen and A.L. Grant, *Meat Focus*, (1994) 494.
[23] G. Uzu and M. Bonneau, *Ann. Zootech.*, 29 (1980) 23.
[24] Anon, UNSCRAMBLERII User's Guide, Version 3.0, 1990.
[25] O. Nozaki, T. Ohata, Y. Ohba, H. Moriyama and Y. Kato, *J. Chromatogr.*, 570 (1991) 1.
[26] E.J. Squires and H. Deng, *Can. J. Anim. Sci.*, 70 (1990) 1198.
[27] T. Kawasaki, M. Maeda and A. Tsuji, *J. Chromatogr.*, 272 (1983) 261.
[28] A.B. Mortensen, C. Bejerholm and J. Pedersen, in *Proceedings of the 32th European Meeting of Meat Research Workers*, Ghent, Belgium, Aug. 24–29, 1986, p. 23.
[29] R.I. Brooks and A.M. Pearson, *Meat Sci.*, 25 (1989) 11.
[30] L.L. Hansen, A.E. Larsen, B.B. Jensen, J. Hansen-Møller and P. Barton-Gade, *Livest. Prod. Sci.*, 59 (1994) 99.
[31] J. Hansen-Møller and J.R. Andersen, in *Proceedings of the 38th International Congress of Meat Science and Technology*, Clermont-Ferrand, France, August 23–28, 1992, p. 907.
[32] M. Hawe, B.W. Moss, N. Walker and M. Porter, in *Proceedings of the 35th International Conference of Meat Meat Science and Technology*, Copenhagen, Denmark, August 20–25, 1989, p. 1036.
[33] B. Wirrer, Dissertation, Ludwig-Maximilians-Universität Munich, 1993.
[34] K. Lundström, B. Malmfors, G. Malmfors, S. Stern, H. Petersson, A.B. Mortensen and S.E. S¢rensen, *Livest. Prod. Sci.*, 18 (1988) 55.
[35] M. Bonneau, *Reprod. Nutr. Dev.*, 27 (1987) 413.
[36] M. Bonneau and P. Sellier, *World Rev. Anim. Prod.*, 22 (1986) 27.
[37] Ø. Andresen and H. Bakke, *Acta Vet. Scand.*, 16 (1975) 492.