Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1038 (2004) 11–18
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development of a rapid and accurate method for the determination of key compounds of pig odor

Stephanie Willig, Markus Lacorn, Rolf Claus*

FG Tierhaltung und Leistungsphysiologie, Institut für Tierhaltung und Tierzüchtung, Universität Hohenheim, Garbenstrasse 17, 70599 Stuttgart, Germany

Received 15 December 2003; received in revised form 2 March 2004; accepted 3 March 2004

Available online 9 April 2004

Abstract

Sampling of odor substances in the emissions from swine production facilities is still the limiting step for routine odor quantification. Solid sorption techniques based on cartridges were developed for three categories of substances (indoles, volatile fatty acids and methylthiol) and were standardized to a sampling time of 15 min. These cartridges also trap dust particles which transport odor substances. Quantification was performed by RP-HPLC or GC. Reliability criteria revealed excellent values for sensitivity (lower ppb level) and repeatability (R.S.D. $<$ 10%), thus they are comparable to fiber solid-phase micro extraction sorption techniques. Parallel determinations in feces and air revealed high correlations ($r = 0.99$, $P < 0.01$), so that microbial processes during digestion determine odor quality and hence provide a possibility to reduce odor via feeding.

Keywords: Odor compounds; Carboxylic acids; Volatile organic compounds; Volatile sulfur compounds; Organosulfur compounds

1. Introduction

Odor emissions from livestock facilities, particularly from swine housing, are a major environmental problem in animal production because they are regularly the source of conflicts with the neighborhood. Thus, many attempts have been undertaken to develop objective methods for the characterization of odor quality and intensity [1,2]. So far, more than 160 compounds were detected [3,4] which contribute to a varying degree to the malodor of swine slurry, but it was also described that comparatively few substances explain most of the offensive odor [5–7]. Methods using a low number of key substances were applied to evaluate the applicability of technical equipments which decrease concentrations of odor in exhaust of ventilation [8,9]. Similarly, attempts to influence the formation of secondary products during slurry storage by either microbial activity or due to chemical reactions were supported by analytical evaluation [4,10–15].

The predominant formation of malodorous substances occurs in the large intestine due to microbial degradation of several substrates. Volatile substances which are excreted already with the feces and remain unmetabolized during slurry formation and storage are candidate substances to judge the effectiveness of strategies for decreasing odor emission by feeding [16]. Such applications should allow an even more focused selection of odor compounds.

They are either formed out of feed components such as carbohydrates with a low prececal digestibility, so that they are metabolized by microbes in the colon. The resulting substances predominantly belong to the category of short aliphatic carboxylic acids (VFAs) with a chain length varying between C_2 and C_7. In contrast, branched chain carboxylic acids (isobytyric and isovaleric acid), which also belong chemically to the category of VFAs, result from microbial fermentation of the branched chain amino acids valine and leucine [17,18] which, however, represent mainly cell debris from shedded intestinal mucosa cells [19]. The same substrate is also fermented to other malodorous substances which again are formed out of amino acids. Thus, tryptophan is degraded to skatole and indole [20] and thyroxine to *p*-cresol [17]. These three substances are subsequently termed as the category of “indoles”. Fermentation of methionine leads to methylthiol [21,22] belonging to the category of volatile sulfur compounds (VSCs).

* Corresponding author. Tel.: +49-711-459-2455; fax: +49-711-459-2498.
E-mail address: thsekret@uni-hohenheim.de (R. Claus).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.03.007

For determination of these substances in vapor-phases methods using both GC or HPLC were reported [2,23–25]. They are based on adsorption techniques using different stationary phases, such as Tenax or carbon molecular sieves, combined with thermal desorption [26–28].

Such techniques require high amounts of sorbent material and long sampling times, so that their applicability under field conditions is limited. More recently solid-phase micro extraction (SPME) methods [1,2,8,29] were developed providing short sampling times combined with dynamic air sampling, but complex technical procedures (passage of air through filter systems and special sample vials or storage in Tedlar bags) limit the practicability [2,8]. Furthermore, rapid deterioration of the coating and additionally the low capacity of the fiber [30] reduce the overall efficiency. The adsorption on the fibers depends on a careful exclusion of dust particles by filter systems to protect the fibers [8]. These dust particles, however, bind a considerable amount of odorous compounds [31–33]. Consequently, analysis only represents a varying part of the actual odor presence [34,35].

Therefore, it was our aim to develop methods for odor determination which are based on published methods using both GC and HPLC, but to combine them with new principles of odor collection. The resulting methods should fulfill the following requirements:

(1) Simplified system for dynamic air sampling on solid sorption material in cartridges which allow sampling under field conditions.
(2) Selection of sorbent materials which have a high capacity for key substances of odor and can be eluted by organic solvents in the laboratory.
(3) Avoiding chemical modification or loss during transport to the laboratory.

2. Experimental

2.1. Chemicals

Cartridges (volume: 3 ml) were purchased from Phenomenex (Aschaffenburg, Germany). The different sorbent materials were as follows: 500 mg Strata C_{18}-E, 50 μm, end-capped reversed-phase material (used for indoles), 60 mg Strata X, 33 μm, polymeric sorption material (used for VFAs), 500 mg Strata FL-PR, 140 μm, Florisil (used for VSCs).

Skatole (3-methylindole) and 2-methylindole were supplied by Serva (Heidelberg, Germany) with a purity >97%. *p*-Cresol, formic, acetic (C_2), propionic (C_3), butyric (C_4), isobutyric (C_{4i}), valeric (C_5), iso-valeric (C_{5i}) and caproic (C_6) acids were purchased from Merck (Darmstadt, Germany), all in analytical-grade purity (>97%). Indole, enanthic acid (C_7) and 4-chloro-7-nitrobenzofurazan (NBD-Cl), used as a specific fluorescent labelling agent, were from Sigma (Munich, Germany) all in analytical-grade purity (>97%). Methanethiol and isobutylthiol were from Fluka (Munich, Germany) with a purity >90%. Methanol, ethanol, acetonitrile and 2-propanol (gradient grade) were purchased from Roth (Karlsruhe, Germany).

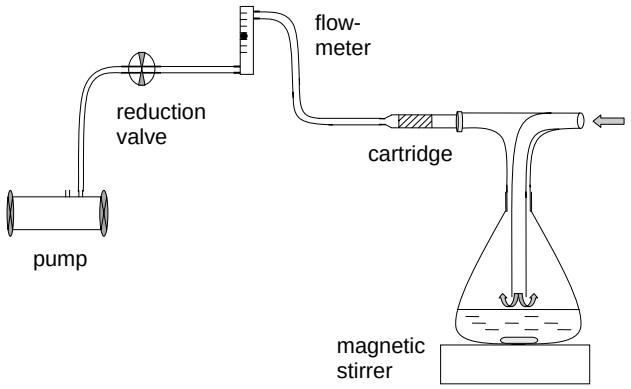

Fig. 1. Schematic diagram of the apparatus for odor generation under laboratory conditions.

2.2. Sampling apparatus

The sampling procedure was optimized in the laboratory using an odor generator (Fig. 1). The apparatus consists of a vacuum pump (SP 750 EC/50 Hz from Schwarzer Präzision, Essen, Germany) with two separate suction inlets each with a maximum flow rate of 12 l min^{-1}. One of them was connected to the insert of a washing flask by tubes to allow fresh air to enter at the open end, while the headspace of the liquid phase is pumped through the cartridge. It was ensured that the connection was absolutely air-tight. The pump capacity was fine tuned by reduction valves to a constant flow of 20 l h^{-1} per cartridge. The liquid phase consisted of about 30 g of fresh feces homogenized in 100–150 ml distilled water. This simulated slurry was renewed after each change of cartridges. The range of linearity of concentrations was determined by different combinations of sampling time and flow rate for future field studies.

The equipment for odor sampling under field conditions is schematically shown in Fig. 2. It consists out of a vac-

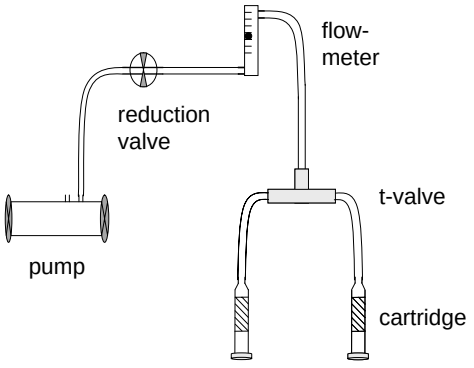

Fig. 2. Schematic diagram of the odor sampling apparatus for field determinations in duplicate.

uum pump as described above. For each suction inlet, the air stream was directed in parallel through two cartridges with the same sorbent. In consequence, odor components were determined in duplicate. The flow rate through each individual cartridge was regularly checked by inserting a flow meter between the t-valve and the cartridge.

2.3. Instrumentation for chromatographic analysis

For GC analysis, the system consists out of a GC 8000 (Fisons, Milano, Italy) connected to a flame ionization detection (FID) system EL 980 (Fisons) and an A200 S automatic liquid sampler (CTC Analytics, Zwingen, Switzerland) with an injection volume of 1 μl. The HPLC analyses were carried out on a system consisting of a Sil-9A auto injector, equipped with a Rheodyne injection valve connected to a Model L-6000 HPLC pump (Merck). The sample loop had a volume of 25 μl (Shimadzu, Munich, Germany). The column eluate was monitored by a Model F-1050 fluorescence spectrophotometer (Merck).

2.4. Development of the method

Key compounds used in this study are characterized in Table 1. *p*-Cresol is basically a phenolic substance which was allocated to the category of indoles due to its similar physical/adsorption characteristics and the possibility of a simultaneous chromatographic determination together with indole and skatole. Corresponding to the number of substance categories three different methods for sample collection and measurement were applied.

VFAs were determined by GC–FID, whereas indoles were determined by RP-HPLC with fluorescence detection. For measurement of VSCs, a pre-column fluorescent derivatization was performed prior to RP-HPLC.

2.4.1. Determination of indoles

For air collection, Strata C_{18}-E cartridges were pre-conditioned with 1.0 ml methanol. Dryness was reached again by suction of air through a vacuum chamber.

Dried cartridges were attached to the sampling apparatus for air collection and were run for the sampling time determined in the following at a constant flow rate of 20 l h^{-1}. Before elution, the cartridges were spiked with 50 μl I.S. solution (1.14 μmol ml^{-1} of 2-methylindole) and then first eluted with 0.950 ml acetonitrile, followed by another 1.0 ml. Elution was accelerated by a slightly reduced air pressure in a vacuum chamber. Aliquot portions of the eluted liquid phase were directly used for determination by RP-HPLC.

Skatole, indole and *p*-cresol determination was based on the method of skatole evaluation in blood plasma as described earlier [36]. The reversed-phase HPLC conditions were as follows: column, 250 mm × 4 mm Chrom-Sil ODS (particle size: 5 μm, Chromatographic Service, Düren, Germany); gradient elution: solvent A contained 0.02 M acetic acid–acetonitrile–2-propanole (55:30:15 (v/v/v)), and solvent B consisted of acetonitrile containing 0.02 M acetic acid. The flow rate was 1.0 ml min^{-1}. A gradient program was used as follows (min/% solvent B): 0/0, 15/0, 15.1/100, 20/100, 20.1/0, 24.9/0. The excitation wavelength was adjusted to 275 nm while emission was set to 345 nm.

The standard solutions for calibration were prepared in acetonitrile. The concentrations ranged between 2.6 and 366 pmol ml^{-1} (indole), between 0.066 and 6.9 nmol ml^{-1} (*p*-cresol), between 2.5 and 343 pmol ml^{-1} (skatole). The

Table 1
Key compounds for the characterization of odor offensiveness and I.S. used for quantification

Substance category	Substance	b.p. (°C)	Vapor pressure, p_o (hPa)[a]	Solubility in water[a] (g l^{-1})	M_r (g mol^{-1})
Indoles	Indole	254	0.016	20	108.1
	Skatole	266	0.020*	Sparingly soluble	131.2
	p-Cresol	203	0.060	3.5	117.2
	2-Methylindole[b]	272	0.013*	Sparingly soluble	131.2
VFAs	Acetic acid	117	15.4	Highly soluble	60.1
	Propionic acid	141	2.9	Highly soluble	74.1
	Isobutyric acid	154	12.0	210	88.1
	Butyric acid	164	0.9	Soluble	88.1
	Isovaleric acid	177	0.6	25	102.1
	Valeric acid	186	0.3	40	102.1
	2-Methyl-pentanoic acid[b]	195	0.03	13	116.2
	Caproic acid	206	0.3	Sparingly soluble	116.2
	Enanthic acid	223	0.1	Sparingly soluble	130.2
VSCs	Methylthiol	6	1700	0.024	48.1
	Isobutylthiol[b]	88	99*	Sparingly soluble	90.2

Data for b.p., vapor pressure, solubility in water and molecular mass (M_r) are given to characterize physical and chemical properties (database: chemical abstracts).

[a] Vapor pressure and water solubility at 20 °C.

[b] Internal standard substances, not detected in air samples.

* Vapor pressure at 25 °C.

same volume of the I.S. solution was added to the reference samples and the biological samples.

2.4.2. Determination of VFAs

For air collection of VFAs, preconditioning of cartridges was required: for each cartridge 0.5 ml bidistilled water, 0.5 ml ethanol and 1.0 ml of methanol were dropped consecutively through the sorbent material. Cartridges were then dried as described above for indoles.

For air collection, dried cartridges were connected to the sampling apparatus and were run for the sampling time determined in the following at a constant flow rate of 20 l h^{-1}. For elution, 100 μl I.S. solution (19.6 μmol ml^{-1} of 2-methylvaleric acid) and 500 μl bidistilled water were added. Thereafter, the liquid phase was gently pressed through the sorbent material with a pipette ball followed by another 400 μl of ethanol. Reduced air pressure should be avoided due to high vapor pressures of VFAs. Aliquot portions of the eluted liquid phase were directly used for GC analysis.

VFAs were determined by GC as described earlier [37] but minor modifications were introduced:

The glass liner of the injector was only plugged with glass wool and the filling with small glass beads was omitted. Priming of the injector needs about 20 injections of samples. Exhausting of the injector occurred after about 300 injections. Prevention of peak ghosting requires the injection of 10% aqueous formic acid after every sample run. For calibration, aqueous standard solutions were used. The concentrations ranged between 0.34 and 84 μmol ml^{-1} (C_2), between 0.17 and 42 μmol ml^{-1} (C_3), between 0.019 and 4.8 μmol ml^{-1} (C_{4i}), between 0.22 and 54 μmol ml^{-1} (C_4), between 0.017 and 4.3 μmol ml^{-1} (C_{5i}), between 0.017 and 4.2 μmol ml^{-1} (C_5), between 0.015 and 3.7 μmol ml^{-1} (C_6), between 0.032 and 7.9 μmol ml^{-1} (C_7). The same volume of the I.S. solution was added to the reference samples and the biological samples.

2.4.3. Determination of VSCs

For VSCs, a preconditioning of the cartridges was performed using fluorescent labeling with NBD-Cl following a method described elsewhere [25]. Preliminar results showed that NBD-Cl amount per cartridge was reduced from 12 to 6 mg in 2 ml diethylether. The cartridges were dried as described for VFAs for about 5 min and then stored in a plastic bag at 4 °C in the dark until use within 4 days.

To prevent light alterations of the fluorescent agent, preconditioned cartridges were wrapped with aluminum foil before use for air collection.

Cartridges were connected to the sampling apparatus and were run for the sampling time determined in the following at a constant flow rate of 20 l h^{-1}. After adding 50 μl I.S. (isobutylthiol, 0.53 μmol ml^{-1}), the elution was performed first with 0.950 ml methanol, followed by another 1.0 ml. Aliquot portions of the eluted liquid phase were directly used for HPLC analysis.

The chromatographic conditions were as follows: column, 125 mm × 4.6 mm Multohyp BDS (particle size: 3 μm, Chromatographic Service); gradient elution, solvent A is made of 0.02 M acetic acid in bidistilled water, and solvent B consists of 0.02 M acetic acid in acetonitrile. The flow rate was 1.3 ml min^{-1}. A gradient program was used as follows (min/% solvent B): 0/30, 5/40, 8/50, 17/50, 17.1/30, 20/30. The excitation wavelength was adjusted to 425 nm while emission was set to 510 nm.

For calibration, standard solutions were prepared in methanol. To each standard solution an equal amount of I.S. was added as described for sample solutions. Their derivatization with NBD-Cl was performed using 50 mg florisil per ml standard solution and 1.25 μmol ml^{-1} NBD-Cl in accordance to the method published earlier [25]. Florisil therefore acts as a catalyst. The concentration of methylthiol ranged between 0.52 and 17 nmol ml^{-1}.

2.5. Quality criteria

2.5.1. Sensitivity and range of linearity

The lower limit of sensitivity depends on the adsorption and elution characteristics, and the sensitivity of the detector. The instrument sensitivity was defined as the three-fold value of the noise of the detector.

Different combinations of air flow rate and total volumes were established under controlled laboratory conditions (Fig. 1). Linearity between sampling time and the retained analyte amount on the cartridge was determined at a flow rate of 20 l h^{-1}.

2.5.2. Repeatability

It is known that slurry contains extreme high amounts of microbes. Because we found that odor substance composition changes continuously during storage, repeatability determination within 1 day was performed on pooled slurry. In addition, day to day repeatability was based on an artificial mixture of analytes (“synthetic solution”), which was obtained by adding reference substances to water (Table 3), so that relationships between analyte concentrations are similar to slurry sample. These samples were evaluated under laboratory conditions in the apparatus described in Fig. 1. All determinations were performed in duplicate and repeated five times.

2.5.3. Precision

The precision cannot be determined because the preparation of headspace reference samples is not possible due to different volatilities of the substances involved and the unpredictable effect of matrix on the volatility [1,38,39].

2.6. Validation in the field

Air samples were taken in a pig house, containing 44 growing pigs on an area of 80 m^2 and a room height of 2.9 m, consequently the air volume was 5.3 m^3 per pig. This vol-

Table 2
Linearity determination for the three groups of odor substances depending on the sampling time

Substance category	Substance	Linear equation[a]	r
Indoles	Indole	$y = 2.78x - 1.25$	0.9998
	Skatole	$y = 6.11x + 15.6$	0.9949
	p-Cresol	$y = 2.49x - 4.15$	0.9996
VFAs	Acetic acid	$y = 7.84x - 160$	0.9854
	Propionic acid	$y = 7.28x - 130$	0.9876
	Isobutyric acid	$y = 3.32x - 43.8$	0.9873
	Butyric acid	$y = 6.19x - 77.2$	0.9943
	Isovaleric acid	$y = 3.89x - 45.6$	0.9958
	Valeric acid	$y = 3.02x - 39.7$	0.9955
	Caproic acid	$y = 4.54x - 63.7$	0.9901
	Enanthic acid	$y = 3.63x - 51.0$	0.9901
VSCs	Methylthiol	$y = 129x + 97.0$	0.9830

[a] x: sampling time in min; y: adsorbed amount of analyte in nanograms in case of methylthiol and indoles; and micrograms in case of VFAs.

ume is therefor in the normal range for pigs under fattening conditions [40]. Fresh air was provided by forced ventilation. The resulting exhaust was guided through chimneys. Air samples were taken at three different collection sites, namely, in the headspace of the slurry duct, 1.5 m above the floor and at the inlet of the exhaust duct. Under these conditions, parallel determinations give an indication on the performance of the method. In consequence, the R.S.D. values between duplicates were calculated as suggested by Kromidas [41]. For all other R.S.D., a conventional calculation was used.

A liquid slurry sample was taken at the end of the fattening period and kept deep frozen till assayed. Additionally, fresh feces were taken from three to four animals at the same day. They were pooled and also kept deep frozen. Parallel determinations of the key substances in fresh feces, liquid manure and air within the pig house were performed expecting a high correlation between these matrices.

The contents in slurry and feces were determined by validated methods which had been published earlier [36,37,42].

3. Results

Sensitivities for the investigated substances are given in Table 4. For VFAs, an average value of 4.5 ng l^{-1} air was reached for carboxylic acids from isobutyric up to caproic acid. Sensitivity was remarkably higher for acetic and propionic acid whereas enanthic acid revealed a much lower sensitivity.

Within the category of indoles, indole and skatole showed very high sensitivities whereas *p*-cresol determinations were less sensitive so that a 30-fold concentration was necessary to reach the detection limit. The lower limit of detection for methylthiol was 1.4 ng l^{-1} and thus satisfying in regard to the detection demand of fluorescent labeling.

Table 3
Composition of the synthetic substrate solution (prepared in bidistilled water) for determination of day to day repeatability

Substance	Concentration (mg l^{-1})
Skatole	20.2
Indole	10.1
p-Cresol	95.8
Acetic acid	26.6
Propionic acid	15.3
Isobutyric acid	4.1
Butyric acid	16.8
Isovaleric acid	3.0
Valeric acid	3.0
Caproic acid	3.2
Enanthic acid	2.8
Methylthiol	0.0088

The adsorption of substances depending on the sampling time revealed high linearities as demonstrated by the corresponding linear equations and correlation coefficients in Table 2. In case of indoles, air collection from 5 up to 20 min led to linear relation. For methylthiol, linearity was obtained between 2 and 25 min sampling times. VFAs determination, in contrast, required sampling times ranging between 30 and 60 min. Especially, determinations of acetic and propionic acid were only linear above collection times of 37 min (data not shown).

Basing on these data and the sensitivity demands, air collection times in the pig house were adjusted to 15 min for indoles, 45 min for VFAs and 20 min for VSCs.

The repeatabilities are given in Table 5 both for interassay and intraassay variation.

Generally, the inter- and intraassay R.S.D. were below 10%. The only exceptions were the R.S.D. values of the intraassay determination for acetic and propionic acid and the elevated R.S.D. value for the interassay variation of methylthiol. R.S.D. determined for duplicates from field measurement are given in Table 6. They demonstrate for all

Table 4
Lower limits of detection (defined as the three-fold noise of the respective detector referred to an air volume of 6, 21, and 9.3 l for indoles, VFAs, and methylthiol, respectively) for the substances measured

Substance	Lower limit of detection (ng l^{-1})
Skatole	0.01
Indole	0.01
p-Cresol	0.3
Acetic acid	0.5
Propionic acid	0.6
Isobutyric acid	4.2
Butyric acid	4.8
Isovaleric acid	4.4
Valeric acid	4.3
Caproic acid	5.4
Enanthic acid	51.6
Methylthiol	1.4

Table 5
Repeatability data for each substance

	Intraassay variation		Interassay variation	
	Mean value ($\mu g\,l^{-1}$)	R.S.D. (%)	Mean value ($\mu g\,l^{-1}$)	R.S.D. (%)
Acetic acid	0.8	38	27.3	9.6
Propionic acid	0.3	20	20.3	7.6
Isobutyric acid	0.06	12	12.4	4.8
Butyric acid	0.6	6.7	26.5	1.7
Isovaleric acid	0.1	11	7.3	2.6
Valeric acid	0.3	5.3	26.5	1.7
Caproic acid	0.2	5.2	7.4	5.8
Enanthic acid	n.d.		6.1	4.3
	($ng\,l^{-1}$)	(%)	($ng\,l^{-1}$)	(%)
p-Cresol	125	6.5	138	8.3
Indole	7.7	3.5	18.6	5.8
Skatole	18.8	3.4	56.6	4.6
Methylthiol	373	7.7	856	20

For intraassays variation, a pooled slurry was used ($n = 10$) whereas interassay variation was determined using a synthetic substrate solution ($n = 10$).

substances low values, which do not exceed an R.S.D. of 8% even for low concentrations.

To give an impression on the relationships for the individual odor substances in feces, slurry and air, data are presented in Table 7. They show that those substances which reveal a low concentration already in feces also lead to low concentrations in the air and vice versa. Calculation of the correlation between the three different matrices revealed high significance, resulting in $r = 0.987$ ($P < 0.01$) and $r = 0.949$ ($P < 0.01$) for correlation between air and feces, and air and slurry, respectively (Table 7).

Concentrations in slurry are intermediate compared to those in feces due to a dilution with urine and water. The data also demonstrate that the air concentrations are primarily determined by the concentrations in feces and slurry. Even if the volatility characteristics of the individual substances may modify the relationships between feces/slurry and air.

Table 7
Parallel determinations ($n = 2$) of each substance in feces, slurry and in the air

	Feces ($mg\,g^{-1}$)	Slurry ($mg\,g^{-1}$)	Air ($ng\,l^{-1}$)
p-Cresol	360	226	150
Indole	38	24	4.2
Skatole	92	119	14.6
Methylthiol	n.m.*	3139	376
			($\mu g\,l^{-1}$)
Acetic acid	19.2	2.0	3.4
Propionic acid	7.7	1.1	2.3
Isobutyric acid	0.9	0.2	0.1
Butyric acid	7.0	0.7	0.8
Isovaleric acid	1.7	0.3	0.2
Valeric acid	2.0	0.2	0.2
Caproic acid	0.2	0.1	0.1

Concentrations of each compound in feces and slurry are referred to dry matter. Air samples were taken from the middle of the room.
* n.m.: not measured.

4. Discussion

Compared to existing methods for odor quantification, mainly the sampling procedure in our study was changed. It was based on cartridges, which allow a simplified sampling procedure and yet the performance data of the whole procedure are improved. For odor sampling, only calibrated pumps are essential to obtain a defined volume of air and revealed great robustness under field conditions. The chromatographic analyses by HPLC or GC require no specialized laboratory equipment. The adsorption capacity of the cartridges is high compared, e.g. to the SPME technique so that considerable variation in the sampling time and thus a 10-fold rise in the total volume led to a linear rise of the con-

Table 6
Mean value of each substance in the air determined in duplicate ($n = 2$) at three different sampling sites (slurry duct, middle of the room, exhaust duct) in the pig house

	Slurry duct		Middle of room		Exhaust duct	
	Mean value ($\mu g\,l^{-1}$)	R.S.D. (%)	Mean value ($\mu g\,l^{-1}$)	R.S.D. (%)	Mean value ($\mu g\,l^{-1}$)	R.S.D. (%)
Acetic acid	2.8	2.0	2.0	7.6	1.5	6.8
Propionic acid	1.4	1.7	1.3	6.2	0.9	5.1
Isobutyric acid	0.13	2.1	0.12	2.5	0.09	2.9
Butyric acid	1.1	0.5	0.9	1.8	0.7	1.8
Isovaleric acid	0.2	2.7	0.1	3.1	0.1	4.1
Valeric acid	0.6	0.6	0.4	1.9	0.3	2.6
Caproic acid	0.07	2.4	0.01	3.0	0.01	3.7
	($ng\,l^{-1}$)	(%)	($ng\,l^{-1}$)	(%)	($ng\,l^{-1}$)	(%)
p-Cresol	191	2.9	126	5.7	113	6.3
Indole	7.3	1.3	4.9	6.9	4.1	5.8
Skatole	19.9	4.8	6.3	6.9	5.7	6.4
Methylthiol	104	2.9	42	5.0	30	4.3

centrations. The resulting range of volumes was sufficient because all data were within the sensitivity of the method, as shown by our tentative determinations in the pig housing. In consequence, there was no need to extend the sampling time beyond the range of linearity.

Other sampling procedures described in the literature [1,2,8] require a protection of sorbents against dust particles, so that the resulting concentrations may be underestimated. This also probably is the main explanation for the higher concentrations of the individual substances found in this study compared to other results of determination at comparable collection sites [1,9,43,44].

Another advantage of the cartridge technique is the elution with solvents so that the analytes in the solvent phase can be quantified by different analytical methods. The fiber technique, in contrast, depends on thermal desorption combined with GC equipped with special injector devices. After solvent extractions, the cartridges can be reused for adsorption twice in case of VSCs and three times for indoles and VFAs (results not shown).

The quality criteria for the determinations were found to be fully satisfying. The decrease of sensitivity for VFAs with increasing chain length is explained by discrimination phenomena occurring in the injector of the GC under a split ratio of 1:10.

Among indoles, *p*-cresol revealed the lowest sensitivity due to the known lower response to fluorescent detection when compared to skatole and indole. Nevertheless, the sensitivity is fully sufficient due to the abundant amounts occurring in the air [45]. Alternative determination by UV detection would require a separate analysis or detection in series. Moreover, it was found that UV detection is not sensitive enough for determination of *p*-cresol in air (results not shown).

With only a few exceptions the R.S.D. values for both the intra- and interassay variations were below 10% and thus remarkably low for determinations of volatile substances. The increased R.S.D. values for acetic and propionic acid are explained by their low interaction with the polymeric sorbent material Strata X. Additionally, their chromatographic properties support variable repeatabilities. Because these two VFAs do not contribute much to malodor [4,46], these higher variabilities can be neglected under field conditions.

The high R.S.D. for the interassay variation for methylthiol is consistent with its extreme volatility but also its high tendency to dimerize to the less volatile disulfides (e.g. dimethylsulfide, dimethyldisulfide). This tendency together with its high oxidative susceptibility also explains why this substance disappears rapidly after emission from slurry [16,47].

The parallel determinations of selected substances in feces or slurry with those in air led to a remarkable correlation. Feces represent the end-product of digestive processes, predominantly those due to microbial activity in the hind gut. Thus, these parallel determinations justify the use of the selected compounds as indicators for the validation of future feeding strategies to reduce odor emissions from pig facilities.

Acknowledgements

The authors are grateful to Dorothea Lösel for the determination of analyte substances in feces and slurry samples as well as William Dunne for the construction of the sampling apparatus. Furthermore, we thank the MLR (Ministerium für Ernährung und ländlichen Raum, Baden-Württemberg) for the financial support of this work.

References

[1] J.A. Zahn, J.L. Hatfield, Y.S. Do, A.A. DiSpirito, D.A. Laird, R.L.J. Pfeiffer, J. Environ. Qual. 26 (1997) 1687.
[2] K.M. Keener, J. Zhang, R.W. Bottcher, R.D. Munilla, Trans. ASAE 45 (2002) 1579.
[3] J.R. Miner, M.D. Kelly, A.W. Anderson, in: Proceedings of the Third International Symposium on Livestock Wastes, American Society of Agricultural Engineers, St. Joseph, MI, 1975, p. 351.
[4] D.H. O'Neill, V.R.J. Phillips, J. Agric. Eng. Res. 53 (1992) 23.
[5] J. Schaefer, Agric. Environ. 3 (1977) 121.
[6] T.A. Travis, L.F. Elliot, J. Environ. Qual. 6 (1977) 407.
[7] R.T. Williams, Agric. Wastes 10 (1984) 15.
[8] E. Razote, I. Jeon, R. Maghirang, W. Chobpattana, J. Environ. Sci. Health B 37 (2002) 365.
[9] J.A. Zahn, A.A. DiSpirito, Y.S. Do, B.E. Brooks, E.E. Cooper, J.L. Hatfield, J. Environ. Qual. 30 (2001) 624.
[10] D.H. O'Neill, V.R.J. Phillips, J. Agric. Eng. Res. 50 (1991) 1.
[11] R.W. Melse, D.A.J. Starmans, N. Verdoes, in: J. Van Ham (Ed.), Proceedings of the Third International Symposium on Non-CO_2-Greenhouse Gases: Scientific Understanding, Control Options and Policy Aspects, Millpress Science Publishers, Rotterdam, 2002, p. 51.
[12] T.N. Whitmore, S.P. Etheridge, D.A. Stafford, U.E.A. Leroff, D. Hughes, Biomass 9 (1986) 29.
[13] W.F. Ritter, J. Agric. Eng. Res. 42 (1989) 51.
[14] A.L. Sutton, L.B. Kephart, M.W.A. Verstegen, T.T. Canh, P.J. Hobbs, J. Anim. Sci. 77 (1999) 430.
[15] V.H. Varel, Curr. Microbiol. 44 (2002) 38.
[16] S.F. Spoelstra, Agric. Environ. 5 (1980) 241.
[17] J. Macfarlane, G.T. Macfarlane, in: G.R. Gibson, G.T. Macfarlane (Eds.), Human Colonic Bacteria, CRC Press, New York, 1995, p. 75.
[18] M.J. Allison, Appl. Environ. Microbiol. 35 (1978) 872.
[19] R. Claus, S. Raab, S. Röckle, J. Anim. Physiol. Anim. Nutr. 76 (1996) 170.
[20] M.T. Yokohama, J.R. Carlson, L.V. Holdeman, Appl. Environ. Microbiol. 34 (1977) 837.
[21] J.R. Freney, in: A.D. McLaren, G.H. Petersen (Eds.), Soil Biochemistry, Marcel Dekker, New York, 1967, p. 229.
[22] H. Kadota, Y. Ishida, Ann. Rev. Microbiol. 26 (1972) 127.
[23] H.M. Chen, C.H. Lifschitz, Clin. Chem. 35 (1989) 74.
[24] J. Stein, J. Kulemeier, B. Lembcke, W.F. Caspary, J. Chromatogr. 576 (1992) 53.
[25] Y. Nishikawa, K. Kuwata, Anal. Chem. 57 (1985) 1864.
[26] J.C. Yu, C.E. Isaac, R.N. Coleman, J.J.R. Feddes, B.S. West, Can. Agric. Eng. 33 (1991) 131.
[27] H. Kim-Yang, S. Davies, R.D. von Bernuth, E.A. Kline, ASAE Meeting Paper No. 01-4037, St. Joseph, MI, 2001.

[28] Manual of Analytical Methods, National Institute for Occupational Safety and Health, fourth ed., 1996.
[29] A.T. Nielsen, S. Jonsson, J. Chromatogr. A 963 (2002) 57.
[30] L. Pionell, J.O. Bosset, R. Tabacchi, Lebensm. Wiss. Technol. 35 (2002) 1.
[31] J. Hartung, Environ. Technol. Lett. 6 (1985) 21.
[32] L.L. Oehrl, K.M. Keener, R.W. Bottcher, R.D. Munilla, K.M. Conelly, Appl. Eng. Agric. 17 (2002) 659.
[33] C.L. Barth, L.F. Elliot, S.W. Melvin, Trans. Am. Soc. Agric. Eng. 27 (1984) 859.
[34] R.I. Mackie, P.G. Stroot, V.H. Varel, J. Anim. Sci. 76 (1998) 1331.
[35] R.W. Bottcher, Chem. Senses 26 (2001) 327.
[36] R. Claus, M. Dehnhard, A. Herzog, H. Bernal-Barragan, T. Gimenez, Livestock Prod. Sci. 34 (1993) 115.
[37] R. Claus, D. Lösel, M. Lacorn, J. Mentschel, H. Schenkel, J. Anim. Sci. 81 (2003) 239.
[38] P.J. Hobbs, T.H. Misselbrook, B.F. Pain, J. Agric. Eng. Res. 60 (1995) 137.
[39] S. MacIntyre, R. Wanninkhof, J.P. Chanton, in: P.S. Matson, R.C. Harriss (Eds.), Biogenic Trace Gases: Measuring Emissions from Soil and Water, Blackwell, Cambridge, 1995, p. 52.
[40] S. Kromidas (Ed.), Validierung in der Analytik, Wiley–VCH, New York, 1999, p. 61.
[41] J. Hartung, Zent. Bl. Hyg. Umweltmed. 192 (1992) 389.
[42] M. Dehnhard, H. Bernal-Barragan, R. Claus, J. Chromatogr. 566 (1991) 101.
[43] J. Schaefer, J.M.H. Bemelmans, M.C. ten Noever de Brauw, Landbouwkd. Tijdschr. 86 (1974) 228.
[44] M. van Geelen, K.W. van der Hoek, Publikatie 167, IMAG, Wageningen, 1982.
[45] S.F. Spoelstra, J. Sci. Food Agric. 28 (1977) 415.
[46] P.J. Hobbs, T.H. Misselbrook, M.S. Dhanoa, K.C. Persaud, J. Sci. Food Agric. 81 (2000) 188.
[47] J. Hartung, H.G. Hilliger, in: J.K.R. Gasser (Ed.), Effluents from Livestock, Applied Science Publisher, London, 1980, p. 561.