Original article

In-house validation method for quantification of formaldehyde in silage and feedingstuffs with the use HPLC-DAD technique

E. Patyra, K. Kwiatek

Department of Hygiene of Animal Feedingstuffs,
National Veterinary Research Institute, Partyzantów 57 Avenue, 24-100 Puławy, Poland

Abstract

An HPLC-DAD method was developed for the determination of formaldehyde in animal feed and silage. The method is based on the determination of the product of chemical reaction between formaldehyde and 2,4-dinitrophenylhydrazine. A 3 g of feed or silage were extracted with Milli-Q water with phosphoric acid and next formaldehyde was derivative with the use 2,4-dinitrophenyl-hydrazine in acetronitrile solution. The extract was purified with 0.45 μm syringe filters and separeted on Zorbax Eclipse XDB C18 column and detection was carried out at 360 nm. Formaldehyde was eluted with a mobile phase consisting of acetonitrile/water in isocratic elution. This method provided average recoveries of 90.6% to 102.2%, with CVs of 2.6% to 6.4% for feed and from 91.3% to 108.7% with CVs of 1.1% to 4.1% for silage in the ranged of 50 to 1000 mg/kg feeds and silage. The LOD and LOQ for formaldehyde in feed and silage ranged from 1.6 to 2.6 and 2.7 to 5.7 mg/kg, respectively. The methodology was applied for the analysis of feed and silage samples collected from poultry, pigs and cows farms.

Key words: formaldehyde, feed, silage, 2,4,-dinitrophenylhydrazine, validation, HPLC-DA

Introduction

Formaldehyde (FA) (CH_2O, CAS 50-0-0) is a colour-less, flammable gas that is commercially available as a 35 – 40% aqueous solution (formalin), as formol (a mixture of formaldehyde, formic acid and methanol in water) or as the precursor hexamethylenetetramine (a complex of formaldehyde with ammonium) (Claeys et al. 2009). FA is commercially produced from metha-nol and used as a preservative, reducing agent, and a sterilizing agent in food industry (Norliana et al.

2009). Formaldehyde is produced industrially for a large number of applications such as the production of resins that act as adhesive and binders for wood products, pulp, paper, glass wool and rock wool, plas-tics, paints, industrial chemicals and textile finishing. It is also used in packaging and as a disinfectant and preservative (Claeys et al. 2009). FA is authorised in European Union (EU) as a preservative in cosmetics (Environmental Protection Agency 2010). Formalde-hyde is also used in hospitals, research and teaching laboratories as a sterilizing and preserving agent (Luo et al. 2001). Furthermore it is also used in vaccines

as a biocidal agent and to prevent bacterial or fungal contamination. It is a naturally occuring product of normal metabolism in many foods including fruits, vegetable, meats, fish, crustacean and dried mushrooms (Norliana et al. 2009).

Formaldehyde is classified by International Agency for Research on Cancer (IARC) into Group 1, as being carcinogenic for humans. Within the European Union formaldehyde is currently classified as a category 1b carcinogen and category 2 mutagenic. Most studies regarding the toxicity of formaldehyde relate to the inhalation of formaldehyde, which is probably the most important route of exposure (IARC 2006).

Since formaldehyde is water soluble, high reactive with biological macromolecules (formaldehyde induces DNA-protein and protein-protein cross-link), and rapidly metabolised, the effects of exposure are mainly observed in those tissues or organs which come into first contact with formaldehyde, namely the respiratory and gastrointestinal tracts, oral and gastrointestinal mucosa included (erosion, ulceration, inflammation and hyperplasion of stomach were observed in rats) (IARC 2006, Schulte et al. 2006). There is no evidence that formaldehyde is carcinogenic by the oral route. Formaldehyde causes toxicity to the nasal epithelium of rats and mice upon inhalation. Epidemiological data have shown that formaldehyde is cancerogenic in human by the inhalation route (sinonasal and nasopharyngeal cancers) (EFSA 2006).

An oral reference dose (RfD) for formaldehyde of 0.2 mg/kg body weight per day has been set by the US Environmental Protection Agency (1999), based on chronic exposure to free formaldehyde in drinking water studies. A tolerable daily intake (TDI) of 0.15 mg/kg body weight per day has also been set for formaldehyde by the World Health Organization. Symptoms of acute toxicity after ingestion include systemic acidosis and gastrointestinal bleeding (WHO 2002).

For some time in the European Union there has been a debate on the extension of the scope of registration of formaldehyde as a feed additive to compound feed belonging to the category of "technological additives" and the functional group "preservatives". The use of formaldehyde as a feed additive was limited to silage in order to stop fermentation processes and skimmed milk for piglets (Xu et al. 2011). In skimmed milk, formaldehyde could be used to feed piglets up to 6 months at the maximum authorized dose of 600 mg/kg, for silage has not set maximum dose for formaldehyd. The proposal to authorize formaldehyde as a feed additive for all animal species has been widely discussed by EU member states. This was the result of the described side effects on humans as well as the dose

of formaldehyde proposed by the applicants in feed, which would be from a minimum of 200 to a maximum of 1000 mg/kg of complete feed. Scientific opinions issued by the European Food Safety Authority (EFSA) in 2014 indicated that doses of 470 mg formaldehyde/kg feed are safe for chicken broilers, laying hens and Japanese quails, and 630 mg formaldehyde/kg feed is safe for piglets but no safe level can be determined for all species and categories of animals, including all species of poultry and pigs. In addition, the concentration of formaldehyde, which would be safe for the reproduction of target species, can not be determined on the basis of available studies. When formaldehyde is administered as a feed additive, similar side effects are seen in animals as in people exposed to this substance. In a study conducted in poultry, it was found that the administration of formaldehyde to broilers did not affect feed intake, but focal necrosis and blood effusions in the gut were observed. Necrotic and ulcerative abomasum changes were also observed in calves fed with formaldehyde-containing milk. Moreover, the use of formaldehyde in cattle feed can move the release of formaldehyde with milk, which can be a risk to human health. In animals under chronic exposure, inflammatory and reactive lesions in the nasal cavity were found. The desire to market formaldehyde as a feed additive stems from its high biocidal efficacy compared to *Salmonella*. On 7th February 2018, Commission Implementing Regulation (EU) 2018/183 issued refusal to authorize the use of formaldehyde as a feed additive belonging to the functional groups 'preservatives' and 'hygiene improving substances'.

The official method for the determination of formaldehyde in foodstuffs is based on a colorimetric reaction where sample distilates are mixed with sulphuric acid yielding a purpre colour if formaldehyd is present. The intensity of the colour is proportional to formaldehyde concentration and can be measured by ultraviolet (UV) spectrophotomether (AOAC 1931). Titrations and acetyloholine have also been used to detect and quantify relatively the presence of formaldehyde in food (European Pharmacopoeia 6.0). For the detection and determination of formaldehyde in food, water and air spectrofluorimetry are also used (Yilma et al. 1992, EFSA 2006, Weng et al. 2009, European Pharmacopoeia 6.0), isotope dilution mass spectrometry (MS) (Sakuragawa et al. 1999), liquid chromatography-mass spectrometry (LC-MS) (Schulte et al. 2006, You et al. 2009), gas chromatography (GC) (Kim and Kim 2005), GC-MS (Bianchi et al. 2007) and high performance liquid chromatography (HPLC) (Żegota 1999, Heyden et al. 2002, Oliva-Teles et al. 2002, Iqbal and Novalin 2009). In recent years HPLC is broadly applied to the separation of formaldehyde from possible interferents

and improvement of the detection limit (Jones et al. 1999, Chen et al. 2008). Formaldehyde can react with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form the corresponding hydrazone before HPLC analysis (Liu et al. 2005, Chen et al. 2008). In this work, for the first time liquid chromatography with diode array detector after precolumn derivatization with 2,4-dinitrophenyl-hydrazine are used for detection and quantification of formaldehyde in feed and silage. This paper reports the development of a selective and sensitive method for analysing formaldehyde from feed and silage using a liquid-liquid extraction, 2,4-dinitrophenylhydrazine derivatization and high performance liquid chromatography with diode array detection (HPLC-DAD) analysis.

Materials and Methods

Chemicals and reagents

Formaldehyde solution in water (37% v/v) was obtained from Sigma Aldrich (St. Louis, USA), 2,4-dinitrophenylhydrazine was from Spectrum Chemical (New Brunswick, USA). HPLC-grade acetonitrile was from Baker (Deventer, The Netherlands) and phosphoric acid 85% was purchased from POCH (Gliwice, Poland). Water was deionised (>18 MΩ cm^{-1}) by a Milli-Q water purification system (MO, USA).

Instrumentation

For sample preparation, vortex mixer (Select Bio-Products, N.J, USA), magnetic stirrer (IKA, Germany), ultrasonic bath (Bandelin, Germany) and laboratory centrifuge (Sigma, Germany) were used. The chromatographic system consisted of HP 1100 series HPLC from Agilent Technologies (USA) equipped with automatic injector, degasser system, quaternary pump with four solvent channels, column thermostat, and diode array detector.

Chromatography

The separation of the formaldehyde was performed on an Agilent Eclipse XDB C18 (150 × 4.6 mm, 5 μm) column (Agilent Technologies, MO, USA) protected by a RP18 guard column (4.0 × 3.0 mm, 5 μm) from Phenomenex (USA), using a mobile phase consisting of HPLC-grade acetonitrile and water (70:30 v/v) mixture prepared in one glass bottle. The flow rate was 0.45 mL/min and the column thermostat was set at 30°C. The injection volume was 5 μL. The UV detection was monitored at 360 nm.

Standard solution

Stock standard solution of formaldehyde was prepared by dissolving 0.5 g formaldehyde in 50 mL Milli-Q water, next put in an ultrasonic bath for 1 min.

2,4-dinitrophenylhdrazine solution was prepared by weighting of 1,346 mg of 2,4-DNPH and dissolving in 200 mL of acetonitrile.

Sample preparation

A sample feed and silage of 3 ± 0.01 g previously minced was weighted into a 100 mL screw-cap bottles. The samples were fortified with formaldehyde and shaken on a vortex mixer for 30 s, then allowed to stand at room temperature for 12 hours to enable sufficient equilibration with the feed/silage matrix. Next, 30 mL of Milli-Q water and 1 mL of 85% phosphoric acid were added and samples place in heated magnetic stirrer set at 80°C for 20 minutes. Then the samples were removed from the magnetic stirrer and cooled to room temperature for 20 min. For the derivatisation reaction of formaldehyde 15 mL of 2,4-DNPH solution was added and the mixture was warmed to 80°C for 30 min in a magnetic stirred. After this time samples were transfered into a 50 mL polypropylene centrifuge tubes and centrifuged at 4000 rpm for 10 min at 20°C. The supernatants were filtered through 0.45 μm syringe filters and injected into the chromatographic system.

Validation procedure

The proposed HPLC-DAD method was in-house validated. Linearity and working range were calculated by preparing five series of matrix-matched calibration curves (for silage and feed). The matrix-matched calibration curve was prepared by spiking the samples (at the beginning of sample preparation) with different volumes of the standard solution to obtain an appropriate final concentration of formaldehyde (50, 200, 600, 1000 and 1500 mg/kg). The curve was prepared with every batch of samples and the concentrations of the analytes were calculated. Correlation coefficient values in this concentration range were >0.99 for formaldehyde in feed and silage.

Limit of detection (LOD) and limit of quantification (LOQ) values were the concentrations in a matrix-matched samples which gave a signal-to-noise ratio higher than three and LOQ concentration which gave a signal-to-noise ratio higher to ten of blank feed and silage samples spiked with formaldehyde. In the accuracy and precision study, feed and silage samples were spiked with formaldehyde at three different levels (50, 200, and 1000 mg/kg).

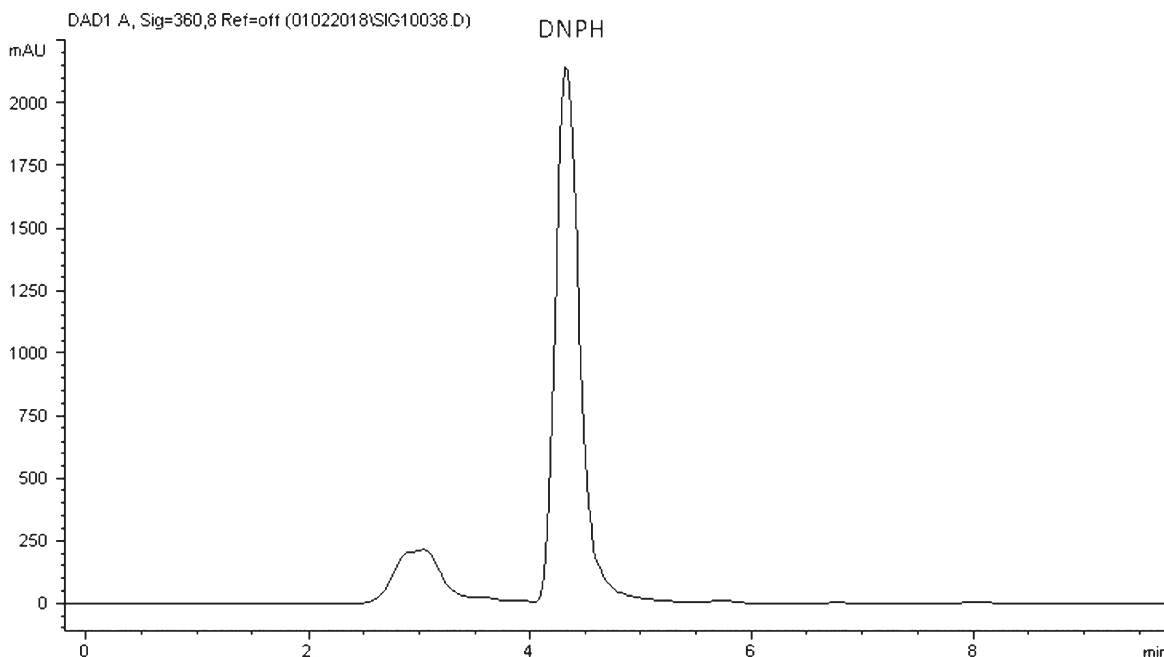In the selectivity/specificity study, 20 blank feed

Fig. 1. HPLC chromatogram of blank feed sample.

and 20 blank silage samples were analysed. For the repeatability study, three series of feed and silage samples were analysed under the identical conditions (six samples for each spiking level). Standard deviations (SD) and coeficents of variation (CV, %) were calculated for each level. The within-laboratory reproducibility was obtained by the analysis of two additional series (at three levels of concentration) in reproducibility conditions (another technician, on two different days) of feed and silage and overall SD and CV were calculated. The overall mean concentrations obtained in the reproducibility study were used to calculate accuracy (%). The recoveries were evaluated by comparing the measured concentrations with the fortified concentrations of the samples.

The uncertainty (U) was calculated as the ratio of coverage factor (k = 2) and standard deviation (SD) of within-laboratory reproducibility, and expressed in percent.

U = k × SD within-laboratory reproducibility

Sample collection

Feed and silage samples were provided to the laboratory by inspectors from pig and poultry farms and feed producers and were collected from all over Poland. The samples were taken as a part of the national feed monitoring programme. During 2018–2019, a total of 97 samples of feed and silage were analysed.

Results

The developed procedure was designed to elaborate a qualitative and quantitative method of determination of formaldehyde in feed and silage. In this work liquid chromatographic conditions were optimised to improve separation, sensitivity and selectivity of the formaldehyde. A two different mobile phases were investigated including methanol and water and acetonitrile and water. For the separation of formaldehyde the most popular are C18 chromatographic columns such as Hypersil ODS C18 (Rivero and Topiwala 2004), XDB C18, Luna C18 (Wahed et al. 2016), Supelcosil C18 (Claeys et al. 2009), Zorbax StableBond SB-C18 (Luo et al. 2001) or Zorbax Bonus-RP-C18 (Weng et al. 2009). In our work two different C18 chromatographic columns (Zorbax Eclipse XDB and Thermo BDS C18) were tested. The best results were achieved using acetonitrile and water 70:30 v/v, using an isocratic elution and Zorbax Eclipse XDB C18 chromatographic column. Under selected conditions, FA displayed high UV absorption at 360 nm, while no interference of the matrix was observed (Figs. 1, 3).

In the presented work different extraction methods for FA in feed and silage were tested. In a first approach method of extraction was tested described by Wahed et al. (2016). Formaldehyde was extracted with the use of acetonitrile and samples were put in an ultrasonic bath for 30 min. A second option was the use of a mixture of phosphoric acid in water and samples were placed in heated magnetic stirrer. In this work effect of extraction time was examined for a range from
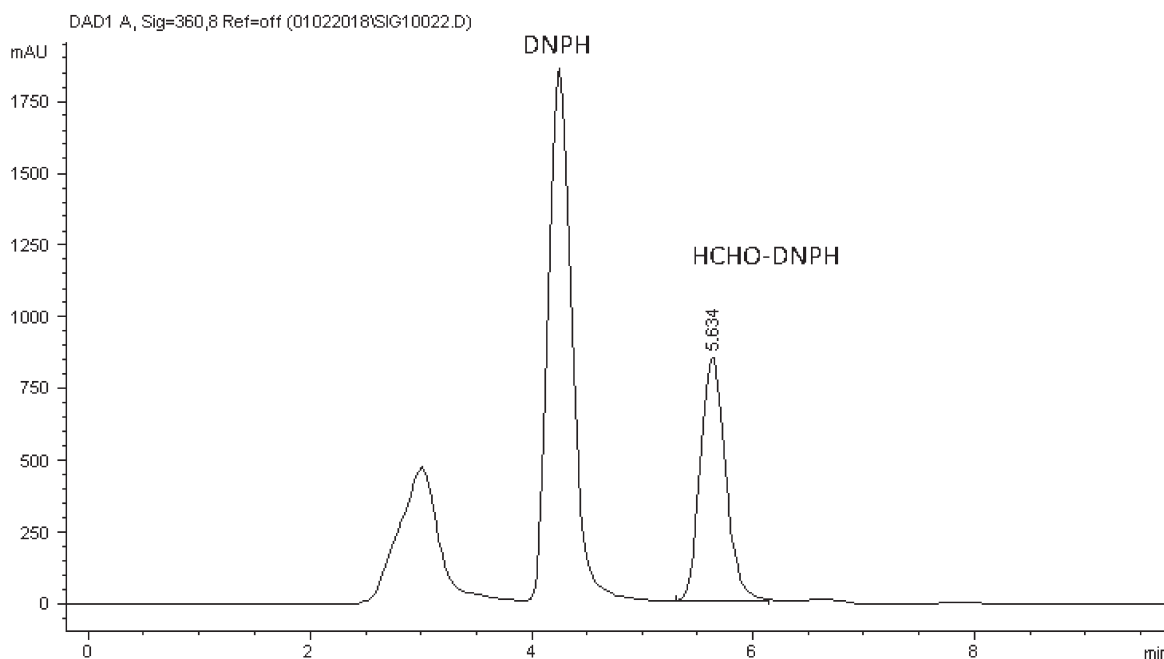
Fig. 2. HPLC chromatogram of feed sample spiked formaldehyde at the concentration level 200 mg/kg.
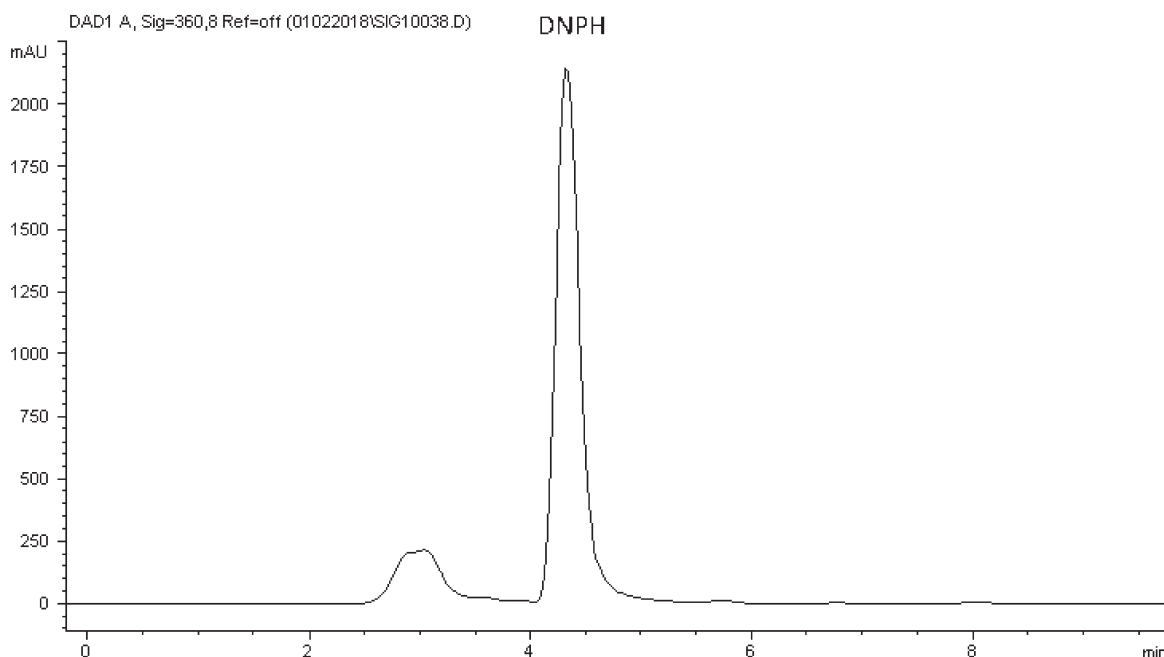

Fig. 3. HPLC chromatogram of blank maize silage.

10 to 40 min. The HCHO recovery increased from 65.0% to 100.0% with the increasing of the extraction time from 10 to 20 min. The recovery did not significant change between 20 to 40 min. So the extraction time of 20 min was chosen in the study.

Due to the lack of certified reference material for analyte under investigation, the accuracy and precision assay of the method in terms of repeatability (CV %, intra-day precision), reproducibility (CV % inter-day precision) and uncertainty were determined using blank feed and silage samples fortified with know amounts

of the analyte. Feed and silage were fortified with concentrations of 50 to 1500 mg/kg of FA. Correlation coefficient (R) values in this concentration range were 0.999 for FA in feed and silage. The accuracy of the method and repeatability were evaluated by analyzing formaldehyde spiked feeds at levels of 50, 200, and 1000 mg/kg feed (six replicates for each level). The results (Table 1) show that the assay recovery for feed and silage were in the range of 90.6% to 102.2% and 88.9% to 108.7%, respectively and CVs were less than 9.0% for both matrices. The reproducibility was
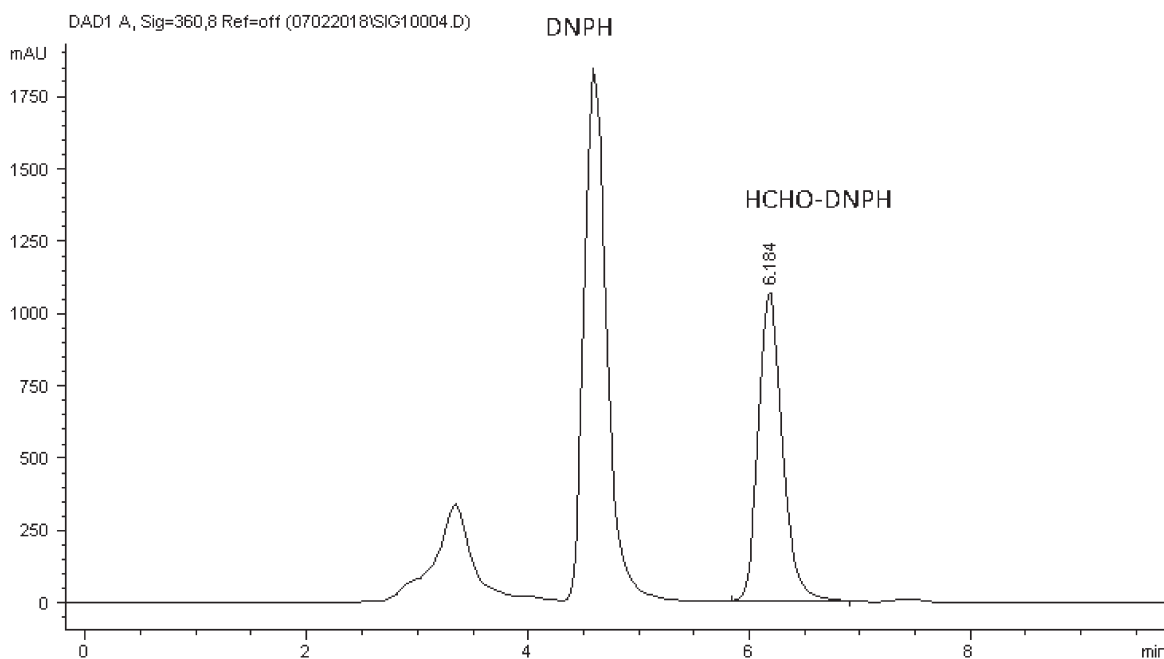
Fig. 4. HPLC chromatogram of maize silage spiked formaldehyde at concentration level 200 mg/kg.

Table 1. Validation parameters of the HPLC-DAD method.

Analyte Formaldehyde	Recovery (%)			Repeatability (%)			Whitin-laboratory reproducibility (%)			LOD (mg/kg)	LOQ (mg/kg)	U (%)
	Concentration levels (mg/kg)			Concentration levels (mg/kg)			Concentration levels (mg/kg)					
	50	200	1000	50	200	1000	50	200	1000			
Compound feeds	102.2	96.0	90.6	3.2	6.4	2.6	6.8	4.3	8.7	2.6	5.2	17.4
Silage	108.7	88.9	91.3	4.1	1.1	2.2	2.2	4.0	9.2	1.6	2.7	18.4

established by analyzing another sets of samples spiked at the same concentration levels as for the repeatability. Specificity is the ability of a method to distinguish between the analyte and other substances that may be present in a sample. In the evaluation of the specificity, blank feed and silage was analyzed by the established protocol. The results obtained with blank samples were compared with fortified samples. No interfering peaks were observed.

The limits of detection and quantification for the formaldehyde in feed and silage were from 1.6 to 2.7 and 2.6 to 5.2 mg/kg, respectively. The expanded uncertainty, obtained by multiplying the combined uncertainty by the coverage factor of k=2, was found to be 17.4% for feed and 18.4%vfor silage. Parameters obtained during the validation of the method are presented in Table 1. The method was shown to be appropriate for the identification and quantification of formaldehyde with acceptable accuracy and precision.

Discussion

This work was aimed to develop a reversed-phase HPLC method for the detection of FA from feed and silage. To ensure the safety of food and feed, efficient methods are required for the simultaneous monitoring of presence of prohibited substances in feed and silage. In the literature there are not described methods of detection of formaldehyde in the feed and silage although formaldehyde was authorized for use in silage and skim milk for food producing animals as a preservative.

Chromatographic techniques such as LC or GC are used for the detection and determination of formaldehyde in various matrices such as: water, air, fish tissue, shrimp, animal tissue, blood and beer, fruit and vegetables (Luo et al. 2001, Rivero and Topiwala 2004, Weng et al. 2009, Afkhami and Begheri 2012, Wahed et al. 2016, Borges de Freitas Rezende et al. 2017). For chromatographic detection, the researchers used gas chromatography with mass spectrometry (Bianchiet et al. 2007), mass spectrometry with isotope dilution

(Sakuragawa et al. 1999) and liquid chromatography-
-mass spectrometry (Rivero and Topiwala 2004), but
the most often use liquid chromatography with an ultra-
violet detector (Żegota 1999, Claeys et al. 2009, Wahed
et al. 2016, Borges de Freitas Rezende et al. 2017).
The most popular for formaldehyde separation are C18
chromatographic columns, such as Hypersil ODS C18
(Rivero and Topiwala 2004), XDB C18, Luna C18
(Wahed et al. 2016), Supelcosil C18 (Claeys et al.
2009), Zorbax StableBond SB-C18 (Luo et al. 2004)
or Zorbax Bonus-RP-C18 (Weng et al. 2009).
As a mobile phases for the analysis of formaldehyde
from various matrices, researchers typically use water
or acetic acid in water in combination with methanol
or acetonitrile. Weng et al. (2009) used acetonitrile and
0.5% acetic acid in water (60:40, v/v) and isocratic elu-
tion to separate the formaldehyde from beer samples.
Wahed et al. (2016) developed a method for analyzing
FA in mango, milk and fish. They used acetonitrile for
extraction and 2,4-DNPH for derivatization, next, sam-
ples were incubated at 40°C for 60 min. They used
HPLC with a diode array detector. The wavelength
was set to 355 nm and the separation was achieved us-
ing an isocratic elution with water/methanol mixture
(35:65, v/v). In our work, the experimental results
showed that a mixture of acetonitrile and water
(70:30, v/v), and isocratic elution with the use Zorbax
Eclipse C18 chromatographic column gave the best
results.

Sample preparation is a critical step in determining
the active ingredient in feed and silage. To develop
an appropriate method for formaldehyde, it is important
to isolate the analyte and to minimize co-extracted in-
terfering compounds. There are many analytical meth-
ods available in the literature for the extraction of this
compound from biological matrices, e.g. in milk, fish,
shrimp, fruit, vegetable, water or air samples (Rivero
and Topiwala 2004, Wahed et al. 2006, Afkhami and
Begheri 2012, Borges de Freitas Rezende et al. 2017).
Researchers use various solutions to extract formalde-
hyde, such as methanol, acetonitrile, acidified matha-
nol, or acidified acetonitrile. Xu et al. (2011) used
acetonitrile and microwave extraction for the extraction
of formaldehyde from beer, cola, apple, orange and
peach juices. Borgas de Freitas Rezende et al. used 1M
sulfuric acid for analyzing formaldehyde in milk
sample (Borges de Freitas Rezende et al. 2017). Wahed
et al. used acetonitrile and ultrasonic extraction for
extraction of FA from fruit and fish samples (Wahed
et al. 2016). On the other hand, Bianchi et al. used solid
phase micro-extraction and in situ derivatization with
pentafluorobenzyl-hydroxyamine hydrochloride for
extraction of formaldehyde from fish samples (Bianchi
et al. 2007).

For the determination of formaldehyde 2,4-DNPH
derivative reagent before the analysis is often used.
2,4-DNPH reacting with formaldehyde to form the cor-
responding hydrazone (Chen et al. 2008). The 2,4-DNPH
technique has been used in food analysis as well
as to determine the aldehyde content in polluted air.
We chose 2,4-DNPH for the derivatization of formalde-
hyde because its high selectivity in combination with
HPLC indicates that it could be a promising method
for determining low concentrations of formaldehyde
in feed and silage. In addition, heating of the samples
was used during the formaldehyde derivatization step
with DNPH. In the developed method, the temperature
of 80°C was assumed as the optimal temperature for
the derivatization of formaldehyde to hydrazone and
the derivatization time was set at 30 minutes. The use
of phosphoric acid in water and 2,4-DNPH in acetoni-
trile allows to obtain high recoveries of FA without
an additional purification step. The combination of the
extraction method and the use of a magnetic stirrer
heated at 80°C allows obtaining clear chromatographic
images indicating the effectiveness of the procedure.
The greatest advantage of the proposed method is a simple
and robust procedure that saves time and is economical
for the analysis of feed and silage samples. Figures 1, 2,
3, 4 show chromatograms of blank and spiked feed
and silage samples at the concentration 200 mg/kg.

In 2018 and 2019, a total of 97 samples were ana-
lyzed (9 samples of alfalfa, corn and grass silage,
wet beet root pulp and 88 samples of feed for broiler
chickens, laying hens, chickens for fattening, turkeys,
geese, pigs and dairy cows). Our study showed that
only 1 feed sample for dairy cows was contaminated
with formaldehyde. The formaldehyde concentration
in this sample was 59.8 mg/kg. Our research showed
that feed and silage produced in Poland did not contain
formaldehyde. Good production practice and good
hygiene practice allow for a low level of contamination
of feed and silage with *Salmonella*, therefore formalde-
hyde is not used as a preservative in feed and silage
in Poland.

The developed procedure is simple, fast and inex-
pensive, and the obtained validation results indicate that
it can be used for the quantification of formaldehyde in
feed and silage for control purposes. The presented
HPLC-DAD method is efficient, precise and suitable
for routine analyzes. The presented results confirmed
the suitability of the method for testing formaldehyde
in samples of feed and silage.

References

Afkhami A, Bgheri H (**2012**) Preconcentration of trace amounts of formaldehyde from water, biological and food samples using an efficient nanosized solid phase, and its determination by a novel kinetic method. Microchim Acta 176: 217-227.

AOAC Official Method. 1931.08. Formaldehyde in Food [Internet]. Available from:http://files.foodmate.com/2013//files_2990.html

Bianchi F, Careri M, Musci M, Mangia A (**2007**) Fish and food safety: Determination of formaldehyde in 12 fish species by SPME extraction and GC-MS analysis. Food Chem 100: 1049-1053.

Borges de Freitas Rezende F, de Souza Santos Cheibub AM, Duarte Pereira Netto A, Ferreira de Carvalho Marques F (**2017**) Determination of formaldehyde in bovine milk using a high sensitivity HPLC-UV method. Microchem J 134: 383-389.

Chen LG, Jin HY, Wang LG, Sun L, Xu HY, Ding L, Yu AM, Zhang HQ (**2008**) Dynamic ultrasound-assisted extraction coupled on-line with solid support derivation and high-performance liquid chromatography for the determintion of formaldehyde in textiles. J Chromatogr A 1192: 89-94.

Claeys W, Vlamnicx C, Dubois A, Huyghebaert A, Hofte M, Daenens P, Schiffers B (**2009**) Formaldehyde in cultivated mushrooms: a negligble risk for the consumer. Food Additiv Contam A 26: 1265- 1272.

Commission Implementing Regulation (EU) 2018/183 of 7 February **2018** concerning the denial of authorisation of formaldehyde as a feed additive belonging to the functional groups of preservatives and hygiene condition enhancers. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32018R0183

Cui XJ, Fang GZ, Jiang LQ, Wang S (**2007**) Kinetic spectrophotometric method for rapid determination of trace formaldehyde in foods. Anal Chim Acta 590: 253-259.

EPA (U.S. Environmental Protection Agency). **2010**. Toxicological Review of Formaldehyde (CAS No. 50-00-0) – Inhalation Assessment: In Support of Summary Information on the Integrated Risk Information System (IRIS). External Review Draft. EPA/635/R-10/002A. U.S. Environmental Protection Agency, Washington, DC. Avaliable from: http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=223614.

European Food Safety Authority (EFSA) (**2006**) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to the use of formaldehyde as a preservative during the manufacture and preparation of food additives (Question No EFSA Q-2005-032). EFSA Journal 415. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/415

European Food Safety Aurhority Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (**2014**) Scientific Opinion on the safety and efficacy of formaldehyde for all animal species based on a dossier submitted by Adiveter S.L. EFSA Journal 12: 3562. Available from: https://www.efsa.europa.eu/en/efsajournal//pub/3562

European Pharmacopoeia 6.0, method 01/2008:0826.

Heyden YV, Nguyet AN, Detaevernier MR, Massart DL, Plaizier-Vercammen J (**2002**) Simultaneous determination of ketoconazole and formaldehyde in a shampoo: Liquid chromatography method development and validation. J Chromatogr A 958: 191-201.

International Agency for Research on Cancer (IARC) (**2006**). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 88 Formaldehyde, 2-butoxy-ethanol and 1-tert-butox-2-propanol. Lyon, France.

Iqbal MZ, Novalin S (**2009**) Analysis of formose sugar and formaldehyde by high-performance liquid chromatography. J Chromatogr A 1216: 5116-5121.

Jones SB, Terry CM, Lister TE, Johnson DC (**1999**) Determination of submicromolar concentrations of formaldehyde by liquid chromatography. Anal Chem 71: 4030-4033.

Kim S, Kim HJ (**2005**) Comparison of standard methods and gas chromatography method in determination of formaldehyde emission from MDF bonded with formaldehyde-based resins. Bioresour Technol 96: 1457-1464.

Liu J, Peng J, Chi Y, Jiang G (**2005**) Determination of formaldehyde in shiitake mushroom by ionic liquid-based liquid-phase microextraction coupled with liquid chromatography. Talanta 65: 705-709.

Luo W, Li H, Zhang Y, Ang CYW (**2001**) Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B 753: 253-257.

Norliana S, Abdulamir AS, Abu Bakar F, Salleh AB (**2009**) The health risk of formaldehyde to human beings. Am J Parmacol Toxicol 4: 98-106.

Oliva-Teles MT, Paíga P, Delerue-Matos CM, Alvim--Ferraz MC (**2002**) Determination of free formaldehyde in foundry resins as its 2,4- dinitrophenylhydrazone by liquid chromatography. Anal Chim Acta 467: 97-103.

Rivero RT, Topiwala V (**2004**) Quantitative determination of formaldehyde in cosmetics using a combined solid--phase microextraction-isotope dilution mass spectrometry method. J Chromatogr A 1029: 217-222.

Sakuragawa A, Yoneno T, Inoue K, Okutani T (**1999**) Trace analysis of carbonyl compounds by liquid chromatography-mass spectrometry after collection as 2,4-dinitrophenylhydrazine derivatives. J Chromatogr A 844: 403-408.

Schulte A, Bernauer U, Madle S, Mielke H, Herbst U, Richter-Reichhelm HB, Appel KE,Gundert-Remy U (**2006**) Assessment of the Carcinogenicity of Formaldehyde [CAS No.50-00-0], BfR (Bundesinstitut für Risikobewertung), Berlin, Germany. Wahed P, Razzaq MA, Dharmpuri S, Corrales M (2016) Determination of formaldehyde in food and feed by an in-house validated HPLC method. Food Chem 202: 476-483.

Weng X, Chon CH, Jiang H, Li D (**2009**) Rapid detection of formaldehyde concentration in food on a polydimethylsiloxane (PDMS) microfluidic chip. Food Chem 114: 1079-1082.

World Health Organisation (WHO) (**2002**) Formaldehyde. Geneva

Xu X, Su R, Zhao X, Liu Z, Li X, Zhang H, Wang Z (**2011**) Determination of formaldehyde in beverages using microwave-assisted derivatization and ionic liquid--based dispersive liquid-liquid microextraction followed by high-performande liquid chromatography. Talanta 85: 2632- 2638.

Yilmaz VT, Kindness A, Glasser FP (**1992**) Determination of sulphonated naphthalene formaldehyde superplasticizer in cement: A new spectrofluorimetric method and assessment of the UV method. Cem Concr Res 22: 663-670.

You J, Yan T, Zhao H, Sun Z, Xia L, Suo Y, Li Y (**2009**) A sensitive fluorescence reagent for the determination of aldehydes from alcoholic beverage using high-performance liquid chromatography with fluorescence detection and mass spectrometric identification. Anal Chim Acta 636: 95-104.

Żegota H (**1999**) High-performance liquid chromatography of methanol released from pectins after its oxidation to formaldehyde and condensation with 2, 4-dinitrophenylhydrazine. J Chromatogr A 863: 227-233.