Analytical method for the determination of PDMN in mash and pelleted feed

Index

1 FOREWORD 3
2 INTRODUCTION 3
3 TITLE 3
4 WARNINGS 3
5 SCOPE 4
6 NORMATIVE REFERENCE 4
7 DEFINITIONS 4
8 PRINCIPLE 4
9 REACTIONS 5
10 REAGENTS AND MATERIALS 5
11 APPARATUS 6
12 SAMPLING 7
13 PROCEDURE 7
14 CALCULATION 8

1 Foreword

This test method has been developed for the determination of PDMN in mash and pelleted feed.

2 Introduction

N/A

3 Title

Analytical method for the determination of PDMN in mash and pelleted feed

4 Warnings

Warning: Persons using this analytical method should be familiar with normal laboratory practice. This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

Table 1: Safety aspects of chemicals

Compound	GHS word/Symbol	Hazard statements	Precautionary statements
Acetonitrile	Danger 	H225 Highly flammable liquid and vapor. H302 + H312 + H332 Harmful if swallowed, in contact with skin or if inhaled H319 Causes serious eye irritation.	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280 Wear protective gloves/ protective clothing. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
PDMN	Danger 	H302 Harmful if swallowed H315 Causes skin irritation. H319 Causes serious eye irritation.	P264 Wash skin thoroughly after handling. P270 Do not eat, drink or smoke when using this product. P280: Wear protective gloves/protective clothing/eye protection/face protection P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell. P337 + P313 If eye irritation persists: Get medical advice/ attention. P501 Dispose of contents/ container to an approved waste disposal plant.
Methanol	Danger 	H225: Highly flammable liquid and vapor. H331: Toxic if inhaled. H311: Toxic in contact with skin. H301: Toxic if	P210: Keep away from heat/sparks/open flames/hot surfaces. - No smoking. P233: Keep container tightly closed. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302+P352: IF ON SKIN: Wash with plenty of

		swallowed. H370: Causes damage to organs.	soap and water.
Formic Acid	Danger 	H226 Flammable liquid and vapour. H314 Causes severe skin burns and eye damage.	P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a POISON CENTER or doctor/ physician.
Methanesulfonic acid	Danger 	H290 May be corrosive to metals. H302 + H312 Harmful if swallowed or in contact with skin H314 Causes severe skin burns and eye damage.	P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a POISON CENTER or doctor/ physician.

5 Scope

This described method is applicable for mash and pelleted feed samples containing ≥ 10 mg/kg and ≤ 2.5 g/kg PDMN.

6 Normative reference

This analytical method is written according to the ISO 78-2 guideline.

7 Definitions

ISO	International Organization for Standardization
PDMN	Propanediol mononitrate, 3-(nitrooxy)propane-1-ol
MilliQ water	purified water

8 Principle

PDMN is extracted during treatment in the ultrasonic water bath with water/acetonitrile + formic acid solution. The centrifuged extract is analysed via a reversed phase HPLC column using UV-detection at 210 nm and PDMN as external standard.

Propanediol mononitrate $C_3H_7NO_4$

9 Reactions

n/a

10 Reagents and materials

10.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralised water or water of equivalent purity.

10.2 Products used in their commercially available form

10.2.1 Acetonitrile (HPLC grade, e.g. Merck, Germany, product number 1.00030, CAS 75-05-8)
10.2.2 Methanol (≥ 99.8 %, e.g. Merck, Germany, product number 1.06009, CAS 67-56-1)
10.2.3 Formic acid (≥ 95 %, Sigma-Aldrich, Switzerland, product number F0507, CAS 64-18-6)
10.2.4 Methanesulfonic acid (≥ 99 %, Fluka, Switzerland, product number 64280, CAS 75-75-2)
10.2.5 PDMN (Analytical standard of known purity, DSM Nutritional Products Ltd, CAS 100502-66-7)

10.3 Aqueous solutions

10.3.1 Mobile phase A
Mix 1000 mL of MilliQ water with 1 mL methanesulfonic acid. This solution is stable for at least one month at room temperature.

10.3.2 Mobile phase B
Mix 1000 mL of methanol with 1 mL methanesulfonic acid. This solution is stable for at least one month at room temperature.

10.3.3 Extraction solvent
Mix 900 mL of acetonitrile with 100 mL of water and 5 mL formic acid and shake well. This solution is stable for at least one month at room temperature. Use only after solution has reached room temperature.

10.4 Solutions of defined concentration

10.4.1 Standard reference solution

Accurately weigh approx. 33 mg of PDMN in a brown 100 mL volumetric flask, dissolve in extraction solvent (see 10.3.3).
The standard reference solution is stable for 4 days at 2 - 8°C.

10.4.2 Standard solution

Dilute the standard reference solution according to the following table (table 2) with extraction solvent (see 10.3.3).

Table 2: Standard solutions of PDMN

Solution ID	Use solution with PDMN concentration of	Pipette aliquot of solution [mL]	Dilute to [mL]	Obtain solution with a concentration of [µg/mL]
C01	S 330 µg/mL	5	50	33
C02		2.5	50	16.5
C03		1	50	6.63
C04	C01 33 µg/mL	5	50	3.33
C05	C02 16.5 µg/mL	5	50	1.65
C06	C03 6.63 µg/mL	5	50	0.663
C07	C04 3.3 µg/mL	5	50	0.333

The standard solutions are stable for 4 days at 2 - 8°C.

11 Apparatus

11.1 Equipment

11.1.1 Analytical balance (e.g. AT 261 Delta Range, Mettler-Toledo, Nänikon, Switzerland)

11.1.2 Glassware such as pipettes, volumetric flasks, graduated glass cylinders, test tubes, HPLC vials for the autosampler

11.1.3 Dispenser (e.g. Dispensette®, 5 - 50 mL, Brand, Wertheim, Germany)

11.1.4 Falcon® tubes (50 mL)

11.1.5 Grinder (e.g. IKA A11 basic, IKA Werke GmbH & CoKG, Staufen, Germany)

11.1.6 HPLC system: e.g. Agilent 1200, equipped with a pump capable of generating pressures of up to 200 bar, degasser, injector, column thermostat, DAD- or UV-detector, and integrator

11.1.7 Ultrasonic bath (e.g. USC300D, 80 W, 45 kHz, heatable, VWR, Dietikon, Switzerland)

11.1.8 Centrifuge (e.g. Eppendorf, mini Spin plus, Schönenbuch, Switzerland)

11.1.9 AQUASIL C18, 3 µm, 150x3 mm (Thermo, Product number 77503-153030) or equivalent

11.2 HPLC conditions

Column: AQUASIL C18, 3 µm, 150x3 mm (Thermo) or equivalent
Mobile phase: see 10.3.1 and 10.3.2
Gradient:

Time [min]	A [%]	B [%]	Flow rate [mL/min]
0	92	8	0.4
14	92	8	0.4
14.1	50	50	0.4
20	50	50	0.4
20.1	92	8	0.4

25	92	8	0.4

Pressure: approx. 150 bar
Column temperature: nominal 25°C
Injection volume: 5 µL
Autosampler temperature: nominal room temperature
Detection: 210 nm
Retention time: approx. 8.0 min
Run time: 25 min

12 Sampling

A preparation of the laboratory sample is not necessary. A representative sample of approx. 100 g can be sent as such to the laboratory for analysis. Use aluminium foil as packaging material and seal the package air tightly. Apply storage conditions of 5°C (± 3°C). When opening the aluminium bag for analyses, homogenize mash feed with a spatula if the sample aliquot is homogeneous. Inhomogeneous mash (e.g. also optically containing ingredients of different sizes) and pelleted feed homogenize with an appropriate grinder (see also 11) before weighing an aliquot.

13 Procedure

13.1 General

Determine each sample in duplicate.

13.2 Test portion

Into a 50 mL Falcon® tube accurately weigh the amount of test sample specified in the table below (table 3).

Table 3: Sample weights

Target concentration of PDMN [mg/kg]	Sample weight [g]
≥ 10 and ≤ 500	10 ± 1
> 500 and ≤ 2500	1 ± 0.2

13.3 Determination - extraction and preparation of test solution

Add exactly 30 mL of extraction solvent (see 10.3.3) with a calibrated dispenser and treat in the ultrasonic water bath for 30 min at 55°C (±5°C). Shake the tube from time to time. Cool down to room temperature and transfer an aliquot to a 2 mL Eppendorf tube and centrifuge for 3 min at 14000 rpm. Dilute if necessary to appropriate concentrations with extraction solution and fill the clear supernatant into an HPLC vial and analyse with HPLC.

13.4 Calibration

Analyse the standard solutions (see 10.4.2) using the HPLC system described in section 11.2. Prepare the calibration curve by plotting the corresponding PDMN concentrations of the seven standard solutions C01 to C07 in micrograms per millilitre and the areas of the integrated PDMN peaks (no-weighted linear regression curve, forced through zero).

Frequency of calibration:
Ideally, the calibration should be carried out before every sequence of analysis. Alternatively, it should be repeated at least after essential parts of the HPLC system were replaced (e.g. HPLC column, lamp, etc.). If the calibration is not carried out daily, it should be checked with a QC sample, e.g. a PDMN standard solution, daily.

14 Calculation

Calculate the concentration of the standard solutions C01-C07 in micrograms per millilitre:

$$c_{\text{standard solution}}\ [\mu g/mL] = \frac{m_{PDMN} * 1000 * P}{100 * d * 100}$$

where
m_{PDMN} is the weight of PDMN in milligrams.
1000 is the conversion factor from milligrams to micrograms.
P is the purity of the reference standard of PDMN in percent.
100 is the volume of the volumetric flask used to prepare the standard reference solution in millilitres.
d is the dilution factor used to prepare the standard solutions C01-C07.
100 is the conversion of percent purity.

Calculate the test sample concentration with the calibration equation in micrograms per millilitre according to the formula $y = m * c_{sample} + b$

$$c_{sample}\ [\mu g/mL] = \frac{y - b}{m}$$

where
y is the area of test sample peak in response units.
m is the slope of the equation.
b is the axis intercept.
c_{sample} is the unknown concentration of PDMN in test sample in micrograms per millilitre.

Calculate the concentration of PDMN in the test sample in milligram per kilogram according to the following formula:

$$\text{PDMN}\ [mg/kg] = \frac{c_{sample} * V * d}{w}$$

where

c_{sample} is the numerical value of the test sample concentration of PDMN in micrograms per millilitre.

V: is the volume of used extraction solvent in millilitre (30 mL).

d: is the dilution factor.

w: is the sample weight in gram.

Calculate the mean according to the following formula:

Mean:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where

x_i represents the test sample concentrations in milligram per kilogram.

n is the number of measurements.

Report the result with three significant figures.