Determination of PDMN 10 % on silica - analytical method

Index

1 Summary

After a first ultrasonic extraction with acetonitrile and a second extraction with water, PDMN is quantified by a reversed-phase HPLC-UV method using PDMN as external standard.

This method is applicable for adsorbates on silica containing 2.5 % ands; 15 % PDMN.

2 Analytical method

2.1 Title

Determination of PDMN 10 % on silica

2.2 Warnings/safety notes

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 2: 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 swallowed. H370: Causes damage to organs.	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 soap and water.
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.

2.3 Scope

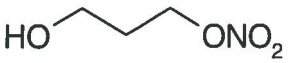
This method is applicable for adsorbates on silica containing ≥ 2.5 % and ≤ 15 % PDMN.

2.4 Definitions

PDMN Propanediol mononitrate, 3-(nitrooxy)propane-1-ol

2.5 Principle of method

PDMN is extracted after ultrasonic extraction with acetonitrile and water. The analysis is carried out by HPLC-UV using PDMN as external standard.



Propanediol mononitrate

2.6 Reagents

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

2.6.1 Products used in their commercially available form

2.6.1.1 Acetonitrile (HPLC grade, e.g. Merck, Germany, product number 1.00030, CAS 75-05-8)

2.6.1.2 Methanol (≥ 99.8 %, e.g. Merck, Germany, product number 1.06009, CAS 67-56-1)

2.6.1.3 Methanesulfonic acid (≥ 99 %, Fluka, Switzerland, product number 64280, CAS 75-75-2)

2.6.1.4 PDMN (Analytical standard, DSM Nutritional Products Ltd, batch number 15.02.07, CAS 100502-66-7)

2.6.2 Solutions of defined concentration

2.6.2.1 Standard reference solution

Accurately weigh approx. 50 mg of PDMN in a brown 50 mL volumetric flask, dissolve in 10 mL acetonitrile and fill to volume with water.
The standard reference solution is stable for at least 2 weeks at +4 °C.

2.6.2.2Standard solutions

Dilute the standard reference solution according to the following table (table 3) with water/acetonitrile solution (80:20, v/v).

Table 3: Calibration 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 1000 µg/mL	10	50	200
C02		5	50	100
C03		2.5	50	50
C04	C01 200 µg/mL	5	50	20
C05	C02 100 µg/mL	5	50	10
C06	C03 50 µg/mL	5	50	5
C07	C04 20 µg/mL	5	50	2
C08	C05 10 µg/mL	5	50	1

The standard solutions are stable for approx. 2 weeks at 4°C.

2.6.2.3Other solutions

2.6.2.3.1 Mobile phase A

Mix 940 mL of MilliQ water with 60 mL acetonitrile and 1 mL methanesulfonic acid. This solution is stable for one month at room temperature.

2.6.2.3.2 Mobile phase B

Mix 800 mL of MilliQ water with 200 mL acetonitrile and 1 mL methanesulfonic acid. This solution is stable for one month at room temperature.

2.6.2.3.3 Water/acetonitrile solution (80/20, v/v)

Mix 800 mL of water with 200 mL of acetonitrile and shake well. This solution is stable for at least one month at room temperature. Use only after solution has reached room temperature.

2.7 Apparatus

2.7.1 Equipment

2.7.1.1Analytical balance (e.g. AT 261 Delta Range, Mettler-Toledo, Nänikon, Switzerland)

2.7.1.2Glassware such as pipettes, volumetric flasks, graduated glass cylinders, test tubes, HPLC vials for the autosampler

2.7.1.3HPLC 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

2.7.1.4Ultrasonic bath (e.g. USC300D, 80 W, 45 kHz, heatable, VWR, Dietikon, Switzerland)

2.7.1.5Centrifuge (e.g. Eppendorf, mini Spin plus, Schönenbuch, Switzerland)

2.7.1.6AQUASIL C18, 3 µm, 150x3 mm (Thermo, Product number 77503-153030) or equivalent

2.7.2 HPLC conditions

Column: AQUASIL C18, 3 µm, 150x3 mm (Thermo) or equivalent
Mobile phase: see 2.6.2.3.1 and 2.6.2.3.2
Gradient:

Time [min]	A [%]	B [%]	Flow rate [mL/min]
0	100	0	0.4
15	100	0	0.4
15.5	0	100	0.4
21	0	100	0.4
21.5	100	0	0.4
25	100	0	0.4

Pressure: approx. 140 bar
Column temperature: nominal 25°C
Injection volume: 10 µL
Autosampler temperature: nominal room temperature
Detection: 210 nm
Retention time: approx. 8.6 min
Run time: 25 min

2.8 Sampling - preparation of the test sample

Homogenise the PDMN 10 % on silica with e.g. a spatula.

2.9 Procedure

2.9.1 General

Determine each sample in duplicate.

2.9.2 Test portion

Weigh the following amounts into 100 mL volumetric flasks (table 4):

Table 4: Sample weights

Target concentration of PDMN [%]	Sample weight [mg]
≥ 2.5 and ≤ 15	100 ± 50

2.9.3 Determination - extraction and preparation of test solution

Add 20 mL of acetonitrile and treat in the ultrasonic water bath for 10 min at 40°C. Swirl the volumetric flask from time to time. Directly afterwards, add approx. 20 mL of water and treat in the ultrasonic water bath for 10 min at 40°C. Cool down to room temperature and fill to mark with water. Transfer an aliquot to a 2 mL Eppendorf tube and centrifuge for 3 min at 14000 rpm. Fill the clear supernatant into an HPLC vial and analyse with HPLC.

2.9.4 Calibration

Analyse the standard solutions (see 2.6.2.2) using the HPLC system described in section 2.7.2. Prepare the calibration curve by plotting the corresponding PDMN concentrations of the eight standard solutions C01 to C08 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.

2.10 Calculation

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 PDMN 10 % on silica according to the following formula:

$$PDMN\ [\%] = \frac{c_{sample} * V}{w * 10}$$

where

c_{sample}	is the numerical value of the test sample concentration of PDMN in micrograms per millilitre.
V:	is the volume of volumetric flask used for extraction in millilitres (100 mL).
10	is the conversion factor to percent.
w:	is the sample weight in milligrams.

Calculate the mean according to the following formula:

Mean:

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

where

x_i represents the test sample concentrations in percent.
n is the number of measurements.

Report the result with three significant figures.