NCL Method PCC-23

Quantitation of Residual DMSO in Nanoformulations Using Gas Chromatography with Direct Injection and Flame Ionization Detection

Nanotechnology Characterization Laboratory
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
Frederick, MD 21702
(301) 846-6939
ncl@mail.nih.gov
https://www.cancer.gov/nano/research/ncl

https://ncl.cancer.gov

This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

Method written by:
Krishna Kattel
Jeffrey D. Clogston

Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, MD 21702

Please cite this protocol as:
Kattel K, Clogston JD, NCL Method PCC-23: Quantitation of Residual DMSO in Nanoformulations Using Gas Chromatography with Direct Injection and Flame Ionization Detection. https://www.cancer.gov/nano/research/ncl/protocols-capabilities DOI: 10.17917/C75P4P

1. Introduction

Residual solvents are volatile organic chemicals that are used in the synthesis of complex drug products such as nanomedicines and manufacturing/purification of active pharmaceutical ingredients (APIs) and excipients [1]. The key methodology for analysis of residual solvents is gas chromatography (GC) with various sample introduction techniques such as static/dynamic headspace analysis, solid phase microextraction, or direct injection of the analyte into the GC [2].

Dimethyl sulfoxide (DMSO) is a polar aprotic solvent having low vapor pressure and high solubility for organic compounds and hence commonly used as a solvent in headspace-GC. DMSO is known to coelute with other organic solvents such as N-N'-dimethylformamide (DMF), N-N-Dimethylacetamide and benzyl alcohol in HS-GC. However, the headspace technique is not suitable for less volatile analytes such as DMSO as the analyte may not reach the injector and column due to lack of static equilibrium between liquid and gaseous phases. Consequently, the quantitation of high boiling/semi-volatile solvents becomes challenging. In addition, low volatility impacts the method sensitivity. Therefore, direct injection gas chromatography is the preferred method for quantitation of DMSO [3].

This protocol describes the procedure for quantitation of DMSO using direct injection gas chromatography with flame ionization detection (FID). Herein we used a PerkinElmer Clarus® 690 GC. Methanol was used as the diluent and an Elite 624 (Crossbond 6% cyanopropyl phenyl 94% dimethylpolysiloxane) 0.32 mm ID x 30 m with 1.8 µm layer capillary column was used for the separation.

2. Reagents and Equipment

Note: The NCL does not endorse any of the suppliers listed below; their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be used. CAUTION: PERSONAL PROTECTION EQUIPMENT SUCH AS SAFETY GOGGLES, LAB COAT, AND GLOVES MUST BE USED.

2.1 Reagents

2.1.1 Dimethyl sulfoxide (DMSO) reference standard

2.1.2 Test sample solution

2.1.3 Ultra-pure helium (research grade, purity>99.999%)

2.1.4 Zero grade air

2.1.5 Ultrapure hydrogen (research grade, purity>99.999%)

2.2 Equipment

2.2.1 Clarus 690 GC system or equivalent

2.2.2 TotalChrom Workstation (TCNAv) chromatographic data software

2.2.3 Column: Elite 624 (crossbond 6% cyanopropylphenyl 94% dimethylpolysiloxane) 0.32 mm ID x 30 m capillary column with 1.8 µm layer

2.2.4 Analytical balance with 0.001 g accuracy

2.2.5 Parker hydrogen generator

2.2.6 2 mL GC vial, 11 mm crimp and 11 mm crimp seal with rubber/PTFE

2.2.7 11 mm hand crimper

2.2.8 Volumetric flasks (A-grade)

2.2.9 Glass pipettes/pasteur pipettes

2.2.10 Vortex mixer

3. Instrumentation

Turn on the instrument/gas flow and allow 10-15 minutes for warm-up. Adjust the gas pressure (helium carrier gas, zero grade air and hydrogen) to 70-80 psi. See manufacturer's recommendation for start-up procedures.

See Table 1 for recommended analytical conditions for the instrument described herein.

Table 1. Analytical Conditions

Instrument	Clarus® 690, PerkinElmer
Column	Elite, 624 (Crossbond 6% cyanopropylphenyl 94% dimethylpolysiloxane) 30 m, 0.32 mm ID, 1.8 µm df
Carrier gas/ Pressure	Helium/20 psi
Detector, Temperature	FID, 250°C
Detector Gas Flow	Hydrogen: 45 mL/min Air: 450 mL/min
Ramp	Initial Temp: 70°C and hold for 1 min, ramp to 130°C @ 50°C/min, hold for 1 min, ramp to 220°C @ 50°C/min and hold for 3 min
Injector Temperature	220°C
Injection Volume	1.0 µL
Split Ratio	None (Spitless)
Split Flow	20 mL/min
Number of pre-injection solvent washes	2
Number of post-injection solvent washes	2
Run Time	8 min

4. Evaluation of Method

4.1 Linearity and Sensitivity: Prepare a set of calibration standards of DMSO from the limit of quantitation (LOQ) to 155% of nominal concentration (USP limit, 5000 ppm) in methanol. A minimum of six standards are recommended. DMSO was evaluated for sensitivity of the method and the corresponding limit of quantification (LOQ) was determined to be 0.026 mg/mL. The overlaid chromatogram of DMSO at various linearity levels is presented in Figure 1. The summary of linearity data is presented in Table 2.

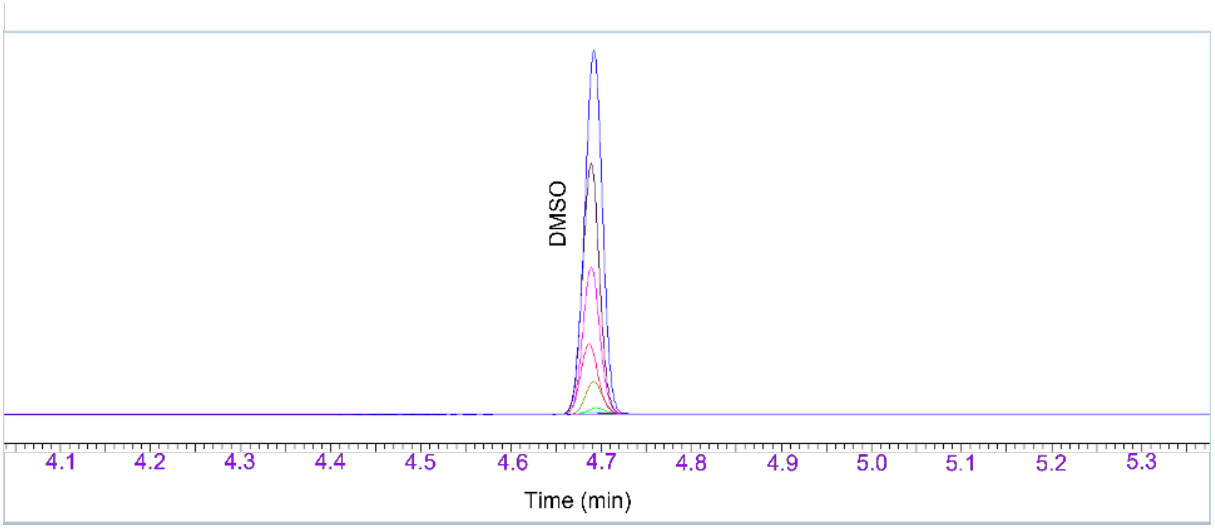

Figure1. Overlaid chromatogram of DMSO at various linearity levels.

Table 2. Linearity and LOQ.

Analyte	ICH Limit (ppm)	Retention Time (min)	LOQ Concentration (mg/mL)	Linearity Range	Regression Coefficient (R^2)
DMSO	5000	4.7	0.026	0.026-1.551 mg/mL (No of Std, n =7)	1

4.2 Accuracy and Spiked Recovery The spiked recovery of DMSO was performed at two different levels: at the practical limit of quantitation (PLOQ, 129 ppm) and USP limit (5169 ppm). Prepare samples in triplicates for each level including negative control and spike control at 100% of limit. The data are summarized in Table 3.

Table 3. Accuracy and spike recovery.

Analyte	Level, n=3 (%)	mg/mL added (μ_o)	mg/mL recovered ($\bar{X}$)	Bias ($\bar{X}$-μ_o)	%Bias [($\bar{X}$-μ_o/μ_o) × 100]	% Recovery [$\bar{X}$/μ_o×100]
DMSO	LOQ	0.0258	0.0243	-0.001	-5.72	94.3
DMSO	100 (Limit)	1.0338	1.1170	0.0832	8.0450	98.4 (Normalized)

4.3 Specificity. No interference was observed from the diluent (methanol) and lipid nanoparticles (used as a matrix for spiked recovery) at the retention time of DMSO. Chromatogram of diluent along with standard are shown in Figure 2.

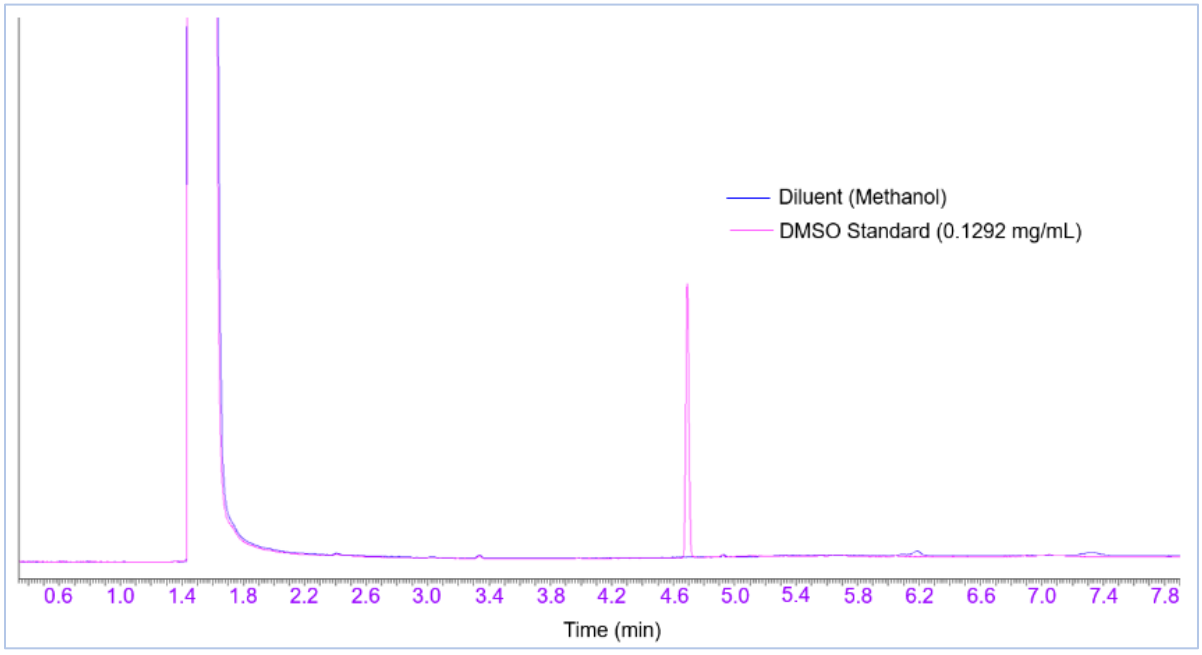

Figure 2. Diluent (Blue) and DMSO Standard chromatogram (red).

4.4 Stability. Standard stability is evaluated at three different concentrations at 4 days and compared with the initial standard concentration. DMSO is stable in methanol up to 4 days. The data are presented in Table 4.

Table 4. Stability information.

Analyte	Level	Amount added (Initial, mg/mL)	Amount observed (Initial, mg/mL)	Amount (Day 4, mg/mL)	% Difference with Initial
DMSO	LOQ	0.0258	0.0240	0.0211	12.9
DMSO	PLOQ	0.1292	0.1252	0.1247	0.40
DMSO	USP Limit	1.0338	0.9781	0.990	1.2

5. Standard Preparation

Prepare two sets of standard solutions in methanol using the certified analytical DMSO reference standard. Use one standard for analysis and the second set as a check to verify the standard preparation. Diluted to volume with methanol to prepare working standards.

Note: Gas-tight glass syringes and A grade volumetric flasks with caps should be used for standard preparation.

6. Sample Preparation

Transfer a known amount of sample into a 2 mL GC vial using a calibrated analytical balance. Dilute to volume up to 1 mL with methanol. Crimp immediately, vortex for 30 seconds, and submit for analysis.

Table 5. Suggested injection sequence.

Injection	Number of Injections	Acceptance Criteria
Blank (Diluent)	1*	No interference at the RT of Analyte
System Suitability	5	The % RSD of 5 consecutive injections of the standard solution chromatograms is not more than 15%.
Check Standard	1	The recovery of the check standard solution is between 85-115%
Sample	1**	N/A
Bracketing Standard	1	The recovery of the bracketing standard solution is between 85-115%

*Multiple injections may be performed to ensure no carryover of DMSO.

**Not more than 6 samples in between two bracketing standards. One diluent injection should be performed before and after each sample injection.

7. Calculations

Report residual DMSO content in sample in terms of %(w/w) or ppm according to the equations below.

$$residual\ solvent\ (\%) = \frac{sample\ peak\ area}{standard\ peak\ area} * standard\ concnetration\ (mg/mL) * \frac{dilution}{sample\ weight\ (mg)} * 100\%$$

$$residual\ solvent\ (ppm) = \frac{sample\ peak\ area}{standard\ peak\ area} * standard\ concnetration\ (mg/mL) * \frac{dilution}{sample\ weight\ (mg)} * 10^6$$

8. Example, Quantitative Analysis of Residual DMSO in Nanoformulation

8.1 **Working Standard Preparation**: Prepare a working standard of DMSO with a concentration of 1.0 mg/mL. For example, transfer ~25 mg DMSO reference standard into a 25 mL class A volumetric flask containing ~20 mL methanol. Dilute to volume with methanol, vortex for a while and shake well.

8.2 **Check Standard Preparation (For new preparation only)**: Prepare a second set of working DMSO standards as per Step 8.1.

8.3 **Blank Solution**: Transfer 1.0 mL DMSO into 2 mL GC vial.

8.4 **Sample Preparation**: Accurately weigh and transfer the nanoformulation sample by glass pipette directly into a 2 mL GC vial using an analytical balance. Dilute to volume to 1 mL. Crimp immediately and vortex for 30 seconds and submit for analysis.

The amount of DMSO in the sample was determined to be 0.025 % or 253 ppm per the equations in Section 7. The sample chromatogram is presented in Figure 3.

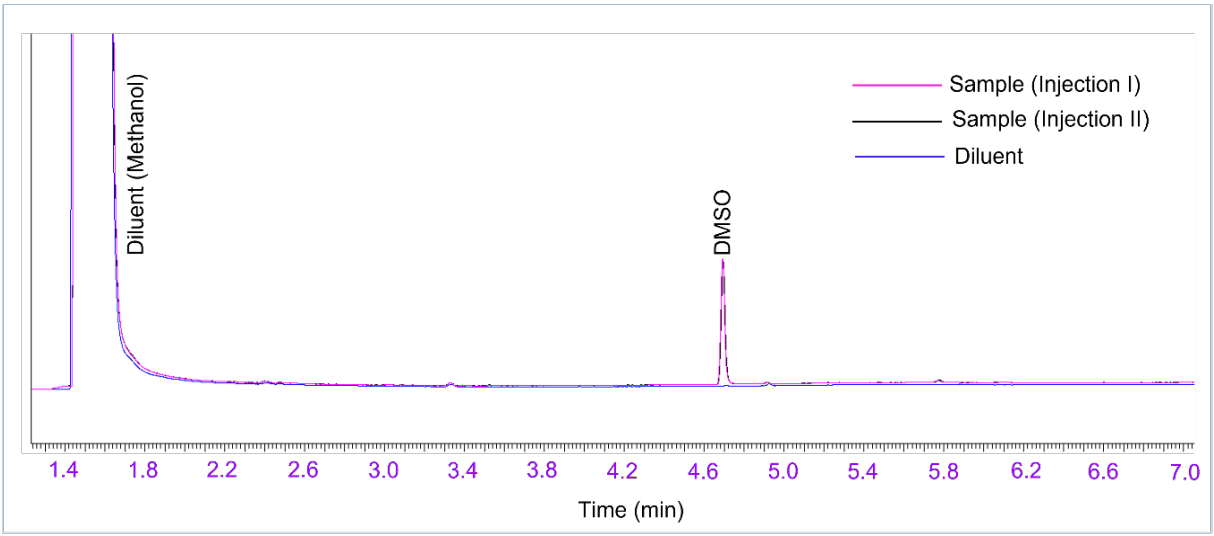

Figure 3. Sample Chromatogram (Black/red). Diluent (Blue) chromatogram is also shown for comparison.

9. References

1. Sitaramaraju, Y; Riadi, A.; D'Autry, W.; Wolfs, K. Evaluation of the European pharmacopeia method for control of residual solvents in some antibiotics. J. Pharm. Biomed. Anal. 2008; 48; 113-119.
2. B'Hymer, C. Residual solvent testing: a review of gas chromatographic and alternative techniques. Pharm Res. 2003; 20; 337-344.
3. Stolarczyk, E.U.; Groman A.; Luniewski, W. Development and validation of the GC method for the quantitative determination of semi-volatile solvents in the pharmaceutical substance bosentan. Acta Pol Pharm-Drug Res. 2014; 71(6); 1107-1113.