NCL Method PCC-22

Residual Organic Solvent Analysis in Nanoformulations Using Headspace Gas Chromatography

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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.

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1. Introduction

Various organic solvents are used in the synthesis of complex drug products (for example, nanomedicines) and in the manufacturing/purification of drug substances and other excipients. Residual organic solvents may originate from the purification of drug materials and cleaning/maintenance of equipment used to manufacture the drug products. These residual organic solvents are considered drug product impurities having no therapeutic benefit. High or inconsistent concentrations of these volatile residual impurities not only pose health risks for patients but also affect the product's quality. In addition, residual organic solvents can affect the physicochemical properties of therapeutics, such as particle size, dissolution and wettability [1].

The amount of residual organic solvent tolerated in final drug products is well-described in pharmacopeias such as United States Pharmacopeia (USP) and European Pharmacopeia (EP), and is closely monitored by regulatory agencies. The International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the US Food and Drug Administration (FDA) have released a guideline for Residual Solvents Q3C approach for their classification [2]. The residual solvents were evaluated for their possible risk to human health and were placed into one of three classes based on their toxicity data and environmental impact.

Class 1 solvents such as benzene, carbon tetrachloride, dichloroethane, and trichloroethane are highly suspected human carcinogens and must be completely avoided. Class 2 solvents such as acetonitrile, chlorobenzene, chloroform, cyclohexane, hexane, and methanol produce non-genotoxic carcinogens and induce neurotoxicity. Class 3 solvents such as acetone, ethanol, ethyl acetate, formic acid, heptane, and propanol have low toxic potential. Class 3 solvents are typically limited to 5000 ppm or 0.5% (w/w). Class 2 solvents have their own individual limits; the acceptable levels of residual solvents are specified by ICH Q3C [2].

Headspace-Gas Chromatography (HS-GC) is the preferred technique for analysis of residual organic solvents in nanoformulations/drug products because it offers several advantages over direct injection GC. In using HS, only volatile components are introduced into the GC system, resulting in extended column lifetime and reduced instrument maintenance, providing superior sensitivity and reproducibility. Headspace sampling is

conducted by placing a liquid or solid sample in a sealed vial until a thermodynamic equilibrium between the sample and gas phase is reached. A known aliquot of the gas phase analyte is then transferred to the gas chromatograph for analysis.

This protocol outlines procedures for quantitative determination of various residual organic solvents using headspace-gas chromatography.

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 RUBBER GLOVES (LATEX OR NITRILE) MUST BE USED.

2.1 Reagents

- 2.1.1 Residual organic solvent reference standards
- 2.1.2 Dimethyl sulfoxide (DMSO), GC grade
- 2.1.3 Test sample solution
- 2.1.4 Ultra-pure helium (research grade, purity>99.999%)
- 2.1.5 Zero grade air
- 2.1.6 Ultrapure hydrogen (research grade, purity>99.999%)

2.2 Equipment

- 2.2.1 Glarus 690 GC system or equivalent
- 2.2.2 Turbo 40 HeadSpace autosampler or equivalent
- 2.2.3 TotalChrom Workstation (TCNAv) chromatographic data software
- 2.2.4 Column: Elite 624 (crossbond 6% cyanopropylphenyl 94% dimethylpolysiloxane) 0.32-mm ID x 30-m capillary column with 1.8-µm layer
- 2.2.5 Analytical balance
- 2.2.6 Parker hydrogen generator
- 2.2.7 Headspace PTFE/SIL liner/vials/hand crimper
- 2.2.8 Glass pipettes/pasteur pipettes
- 2.2.9 Gas tight syringes

2.2.10 Volumetric flasks (25 and 50 mL, A-grade)

2.2.11 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

Headspace Conditions		**Gas Chromatography Conditions**	
Instrument	Turbomax 40 Trap, PerkinElmer	Instrument	Clarus® 690, PerkinElmer
Oven Temperature	90°C	Column	Elite, 624 (Crossbond 6% Cyanopropylphenyl 94% Dimethylpolysiloxane) 30 m, 0.32 mm ID, 1.8 µm df
Needle Temperature	150°C	Carrier gas/ Pressure	Helium/10 psi
Carrier Gas Pressure	Helium, 15 psi	Detector, Temperature	FID, 250°C
Thermostatting Time	5 min	Detector Gas Flow	Hydrogen: 35 mL/min Air: 400 mL/min
Pressurization Time	1 min	Ramp	Initial Temp: 60°C and hold for 2 min, ramp to 200°C @ 25°C/min, hold for 2.4 min
Withdraw Time	0.1 min	Injector Temperature	200°C
Operating Mode	Constant	Split Ratio	None (Splitless)
Shaking / Hi PSI Inject	Disabled	Detector Temperature, FID	250°C Flow (H_2): 35 mL/min Flow (Air): 400 mL/min
Transfer line Temperature	110°C	Carrier gas/ Pressure	Helium/10 psi
Injection Time	0.04 min	Run Time	10 min

4. Evaluation of Method Sensitivity and Linearity

Six analytes (methanol, ethanol, acetone, acetonitrile, chloroform and tetrahydrofuran) were evaluated for sensitivity of the method and establishment of their corresponding limit of quantification (LOQ). The LOQ and linearity data of each analyte are summarized in Table 2.

The spiked recovery of each analyte was performed at three different levels starting from their respective LOQ. The recoveries of all six analytes obtained were between 90-115%.

There is no interference of the dimethyl sulfoxide diluent at the retention time of analytes. Dimethyl sulfoxide is used as the diluent due to its low vapor pressure, its high solubility of organic compounds, and boiling point (189°C).

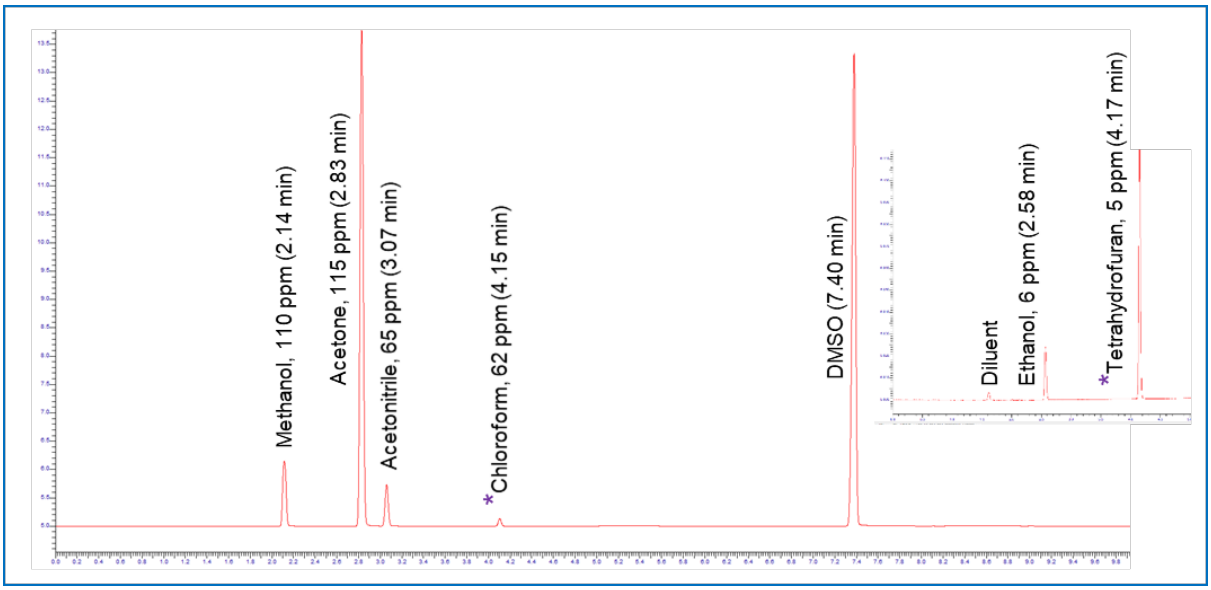
The chromatogram of each analyte at different concentrations and diluent chromatogram are presented in Figures 1A Figure 1B, respectively.

Table 2. LOQ and Linearity Information

Analytes	ICH Limit (ppm)	Retention Time (min)	LOQ Concentration (ppm)	Linearity Range	Regression Coefficient (R^2)
Methanol	3000	2.14	3	3-921 ppm	1.000
Ethanol	5000	2.58	6	6-474 ppm	1.000
Acetone	5000	2.83	3	3-244 ppm	1.000
Acetonitrile	410	3.07	3	3-268 ppm	0.9999
Chloroform	60	4.15*	2	2-1168 ppm	0.9999
Tetrahydrofuran	720	4.17*	4	4-321 ppm	1.000

*Chloroform and Tetrahydrofuran coelute at 4.2 minutes

A.



*Chloroform and Tetrahydrofuran coelute at 4.2 minutes

B.

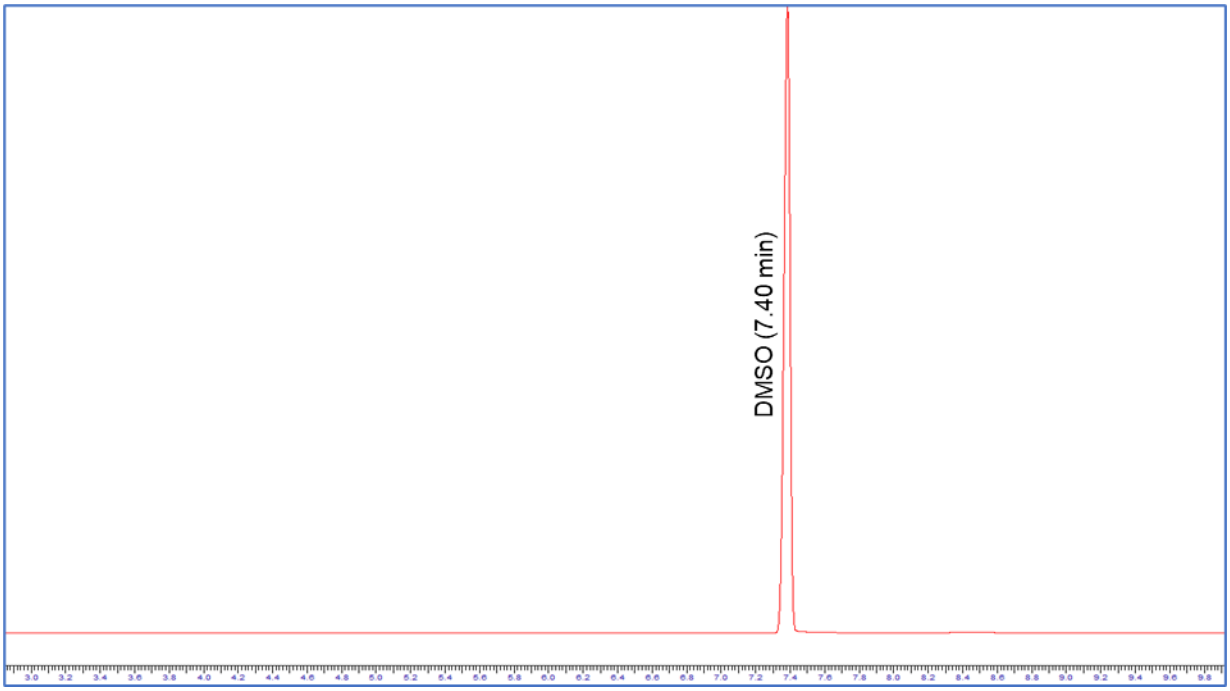


Figure 1. (A) Representative analyte chromatograms at different concentrations. **(B)** Diluent (DMSO) chromatogram.

5. Standard Preparation

Prepare two set of standards of residual organic solvent using their certified analytical reference standard. Use one set of standards for analysis and the second set as check standards to verify the standard preparation. Dilute to volume with DMSO 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 by glass pipette directly into GC vial using an analytical balance. Dilute volume up to 1 mL. Crimp immediately, vortex for 30 seconds, and submit for analysis. See Table 3 for a suggested injection sequence.

Table 3. 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 if required.

**Not more than 12 samples in between two bracketing standards.

7. Calculations

Report residual solvent 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 Ethanol in Doxil (Lot# JHZUA01)

8.1 **Working Standard Preparation**: Prepare a working standard of ethanol with a concentration of 0.04 mg/mL. For example, weigh 17 mg ethanol reference standard and transfer to a 50 mL class A volumetric flask containing ~ 40 mL DMSO. Dilute to volume with DMSO and shake well (concentration: 0.3399 mg/mL). Next, transfer 3.0 mL of this stock ethanol standard to a 25 mL volumetric flask containing 15 mL DMSO. Dilute to volume with DMSO and shake well (working concentration: ~0.04 mg/mL).

8.2 **Check Standard Preparation (For new preparation only)**: Prepare a second set of working ethanol standards as per Step 8.1.
Note: The slope differences at LOQ level (0.006% ethanol) and higher level (0.1%) is ~1% in linearity of ethanol.

8.3 **Blank Solution**: Transfer 1.0 mL DMSO into 20-ml GC vial.

8.4 **Sample Preparation and Analysis**: Transfer a known amount of Doxil into a GC vial using a calibrated analytical balance. Dilute to volume to 1 mL. Crimp immediately, vortex for 30 seconds, and submit for analysis. An example chromatogram is shown in Figure 2. Report ethanol content in the sample in terms of %(w/w) or ppm using the formulas shown in step 7. The amount of ethanol in this lot of Doxil was determined to be 0.004 % or 37 ppm.

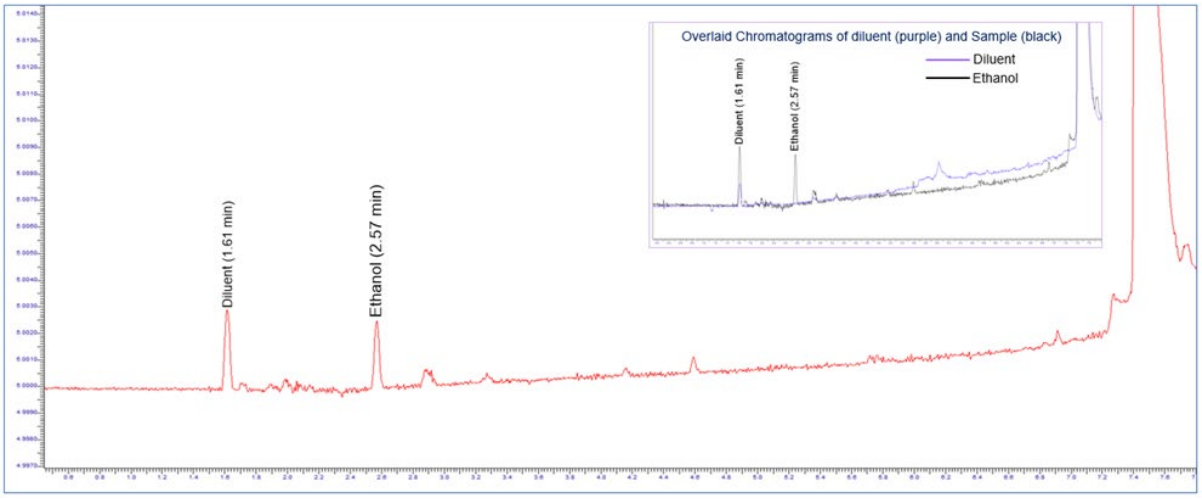


Figure 2. Representative chromatogram of Doxil.

9. References

1 Witschi C, Doelker E. Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. Eur. J. Pharm. Biopharm. 1997: 43; 215-242.

2 FDA Guidance for Industry: Q3C(R8) Impurities: Guidance for Residual Solvents. December 2021. https://www.fda.gov/media/138334/download