

Generic method approach for determination of residual solvents in active pharmaceutical ingredients by gas chromatography

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Introduction

Solvents are defined as organic chemicals that are used to dissolve components; they are used during the manufacture of drug substances. Solvents can also potentially be formed as by-products of the synthetic process. Depending on their structure, solvents have varying degrees of toxicity. Upon completion of the synthetic process, an objective is to remove completely or reduce the residual levels of these solvents to points at which they pose no risk to patient safety in the final formulated drug product. Some examples of commonly used residual solvents and their structures are presented in Figure 1.

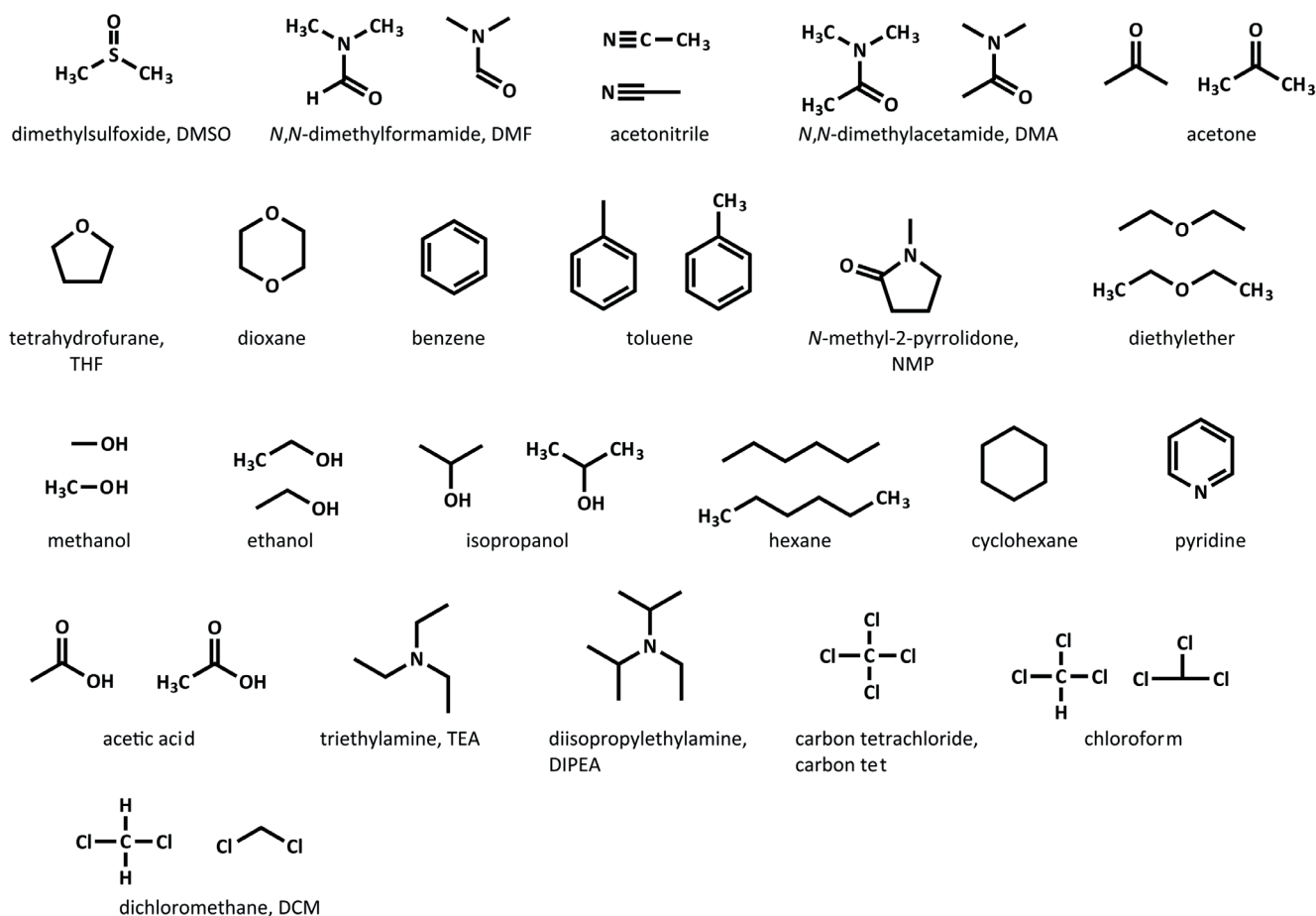


Figure 1: Chemical Structure of Residual Solvents

Typically, there is no technique via which solvents can be completely removed from pharmaceutical ingredients or products. Therefore, it is necessary to have a suitable analytical technique according to which materials can be tested to assure that the levels of solvents are below those that are classified as safe by regulatory guidelines. The International

Council for Harmonisation of Technical Requirements for Pharmaceuticals Use (ICH) Guidelines details the requirements for quality attributes for the manufacture of drug substances. The specific guideline that details the quality requirements for residual solvents is Q3C(R8).

Technique for determination of residue solvents

Gas Chromatography (GC) is often the technique of choice for determination of residual solvents in drug substances. Contract research organisations regularly work on the development and manufacture of several different drug substance products. Each drug substance material has a different route of manufacture and will include a set of solvents that are specific to that individual process.

Almac Sciences recognised the potential efficiency gains from establishing a single set of method conditions from which a robust and reliable quantification of residual solvents can be consistently obtained.

A diagram of a GC-HS set up is illustrated in Figure 2.



Figure 2: Typical Gas Chromatograph with Headspace Sampler (GC-HS)

Equipment for Quantitative Liquid Transfers

For all solvent transfers it is essential to use positive displacement pipettes, during the study it was determined that this design is more amenable for the accurate transfer of non-aqueous and volatile liquids.

The design of a positive displacement pipette is that the tips contain the piston. Refer to Figure 3 for design.

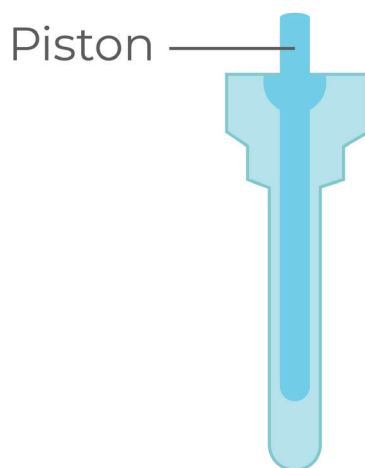


Figure 3: Diagram of Positive Displacement Pipette

Selection of residual solvents for study

The solvents selection of solvents for inclusion in the generic method were based upon a comprehensive review of the residual solvents that are required to be tested across a range of projects for small molecule and peptide manufacture. The rationale for a decision on which residual solvents to test is obtained from ICH quality guideline for residual solvents Q3C(R8) as follows:

Class 1 Solvents: Solvents to be avoided.

Known human carcinogens, strongly suspected human carcinogens and environmental hazards. This paper does not cover the scope of Class 1 Solvents. This class of solvent are always controlled to very low ppm levels; control to these levels typically requires development of specific high sensitivity methods.

Class 2 Solvents: Solvents to be limited.

Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.

Class 3 Solvents: Solvents with low toxic potential.

Solvents with low toxic potential to humans; no health-based exposure limit is needed. Class 3 solvents have permitted daily exposure (PDE limits) of 50mg or more per day.

In the unusual instance of where Class 1 Solvents are included in a process then typically a product specific

method would be developed to determine these solvents to the low ppm levels according to which the levels of this class of solvents would be restricted. The above procedure for selection of residual solvents to be tested is the default guideline but ultimately the list of residual solvents to be tested is decided based upon the risk of them being present in the final API product.

With consideration to the above, a final list of residual solvents to be included in the study for development of a generic GC residual solvents method was compiled as detailed in ICH Guideline Q3C(R8) and based upon Option 1 with a daily product dose of 10g.

Almac's decision policy for testing of residual solvents that are potentially present is based upon a risk-based approach. This risk-based approach considers the stage in the synthetic process at which the residual solvents are used and the likelihood of solvents being purged based upon the technical details of the process.

Generic method conditions

A DB624 60m x 0.32mm, 1.80µm GC column was chosen for the generic method. This is a methyl polysiloxane column with 6% cyanopropyl-phenyl groups which is a mid-polarity column. In general, one aspect for a successful separation is that the polarity of a column should be matched as closely as possible to the expected analytes.

The DB624 column polarity has a broad range of applicability for retention and separation of a range of solvent polarities and volatilities. This broad range of applicability is based upon its moderate polarity which is suitably matched to the polarity of most process solvents. The stationary phase structure is illustrated in Figure 4.

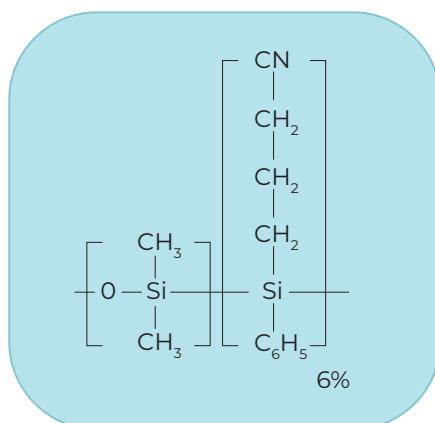


Figure 4: Structure of DB-624 column stationary phase

Several sample introduction methods are available for the technique of gas chromatography. These techniques include but are not limited to direct injection of a liquid into a heated injector, purge and trap injection, cold old column injection and headspace sampling.

The sample introduction technique of headspace sampling (GC-HS) was chosen for this generic method. Headspace sampling is a sample introduction technique where a drug substance is dissolved in a

suitable solvent matrix, this solvent matrix typically being a high boiling solvent in a sealed glass vial with a typical volume of 10mL. The solution is heated to a set temperature for a specified time to establish a partition of the relatively volatile analyte solvents between the solvent matrix and the 'headspace' gas in the 10mL glass vial. The vial headspace is sampled using a probe which transfers the sample into the gas chromatograph injector. An illustration of the headspace vial components is presented in Figure 5.

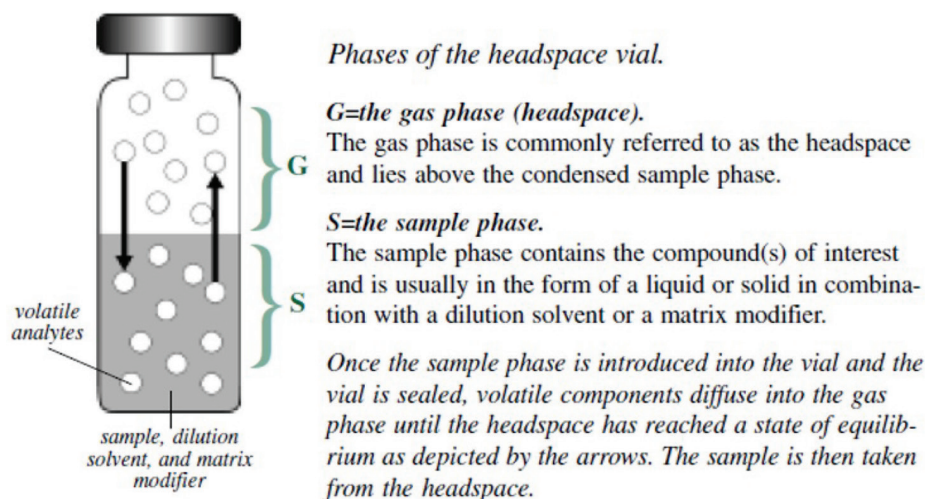


Figure 5: Illustration of GC Headspace Sampling

The reasons why GC-HS was chosen as the base of the sample introduction for this generic method were several-fold.

- The GC-HS sampling technique does not directly introduce solutions of API directly into the GC instrument injection port. The solutions of the analyte solvents are sampled in the gas phase before introduction into the GC instrument. The relatively involatile API in the solution matrix is not sampled or introduced into the GC instrument injection port. This avoids contamination of the GC instrument and column with API material. This is especially advantageous if the system is set up to perform GC testing for multiple API projects in which case there would be potential for multiple and additive effects of API contamination if liquid solutions of API were introduced into the GC injector port.
- GC-HS sampling provides an enhanced response for some more volatile solvents. The basis for the enhanced response for the more volatile solvents is that they partition more favourably into the gas phase.

A series of experiments were performed to establish the thermal gradient conditions to achieve the optimal resolution of all solvents. The study was performed on an Agilent model 7694 gas chromatograph equipped with a model 7697 headspace autosampler. This is a summary of the GC conditions.

- Carrier Gas: Hydrogen
- Thermal Gradient
- Headspace Sampling

The GC headspace autosampler conditions were devised based upon the boiling points of the solvents tested as part of the generic method. The range of the boiling points for the solvents included in the method is 39.6°C for Dichloromethane to 189°C for Dimethylsulphoxide. The GC-HS temperatures were selected based on keeping them as low as possible but whilst assuring sufficient sensitivity for the higher boiling solvents in the mixture. This is to reduce the likelihood of interfering peaks originating from degradation of the API material during headspace vial heating.

The diluent used for the Standard and Sample Diluent was 1,3-Dimethyl-2-imidazolidinone (DMI). The structure of 1,3-Dimethyl-2-imidazolidinone is shown in Figure 6.

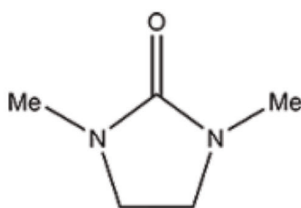


Figure 6: Structure of 1,3-Dimethyl-2-imidazolidinone (DMI)

Standard and Sample Solvent – 1,3-Dimethyl-2-imidazolidinone (DMI) was selected as the solvent. This selection was made firstly based on the following

- a) its high boiling of 225°C which facilitates a distinct separation from the residual solvent analytes.
- b) The solvent peak has a sharp profile which minimizes interferences and there is no tailing.

- c) The solvent blank profile is sufficiently free from interferences with the residual solvent peaks to be suitably specific to facilitate accurate quantification of residual solvents in test samples.

Mixed Stock Standard Preparation

The solvents were prepared at concentrations equivalent to the specification limits according to Equation 1. 50mg/mL was selected as the sample concentration which was prepared in the DMI solvent.

This sample concentration was selected on the bases of a) being suitably high to assure sufficient sensitivity for each of the residual solvents towards the limits of quantification (LOQ) and b) the sample amounts do not consume excessive quantities of API material.

$$\text{Residual solvents standard weight (mg)} = \frac{\text{ICH limit (ppm)} \times \text{sample conc } \left(\frac{\text{mg}}{\text{mL}}\right)}{400}$$

Equation 1: Calculation of Residual Solvents Standard Weight (mg) for Stock Standard

The factor of 400 factors the relative standard and sample dilutions to re-calculate the target standard weight for different ICH limits and sample preparation concentrations.

Adding weights of solvents by pipette to obtain a target weight for liquids and in particularly volatile liquids can be tricky.

The strategy taken was to calculate the volumes required that correspond to the target weights based upon solvent density at ambient temperature. These volumes can be added to the 100mL flask for the standard preparation and then the weights recorded. This procedure is amenable to a faster turnaround time for the standard preparation.

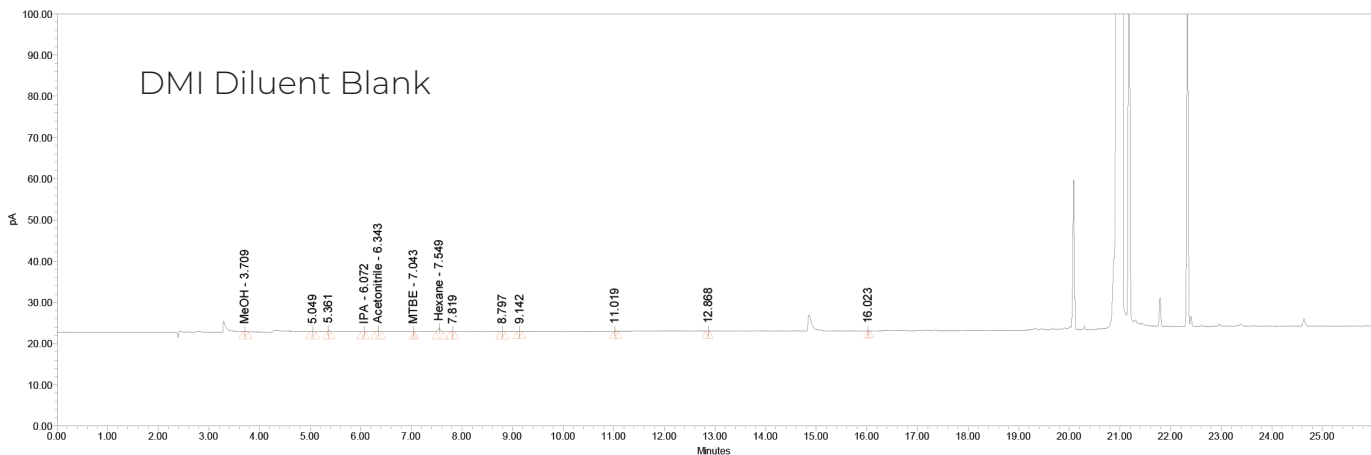
Solvent	Volume (μL)	Weight (mg)	Density
Methanol (MeOH)	473	375	0.792
Ethanol (EtOH)	792	625	0.789
Acetone	790	625	0.791
2-Propanol (IPA)	795	625	0.786
Acetonitrile (ACN)	65	51	0.786
Dichloromethane (DCM)	57	75.0	1.325
Tert-butanol	565	438	0.775
MTBE	845	625	0.740
Hexane	55	36	0.659
Ethyl Acetate	693	625	0.902
Tetrahydrofuran (THF)	717	90	0.889
Cyclohexane	732	485	0.778
Isopropyl Acetate	914	625	0.872
2-Methyl-tetrahydrofuran (2-Me-THF)	772	625	0.854
Heptane	46	625	0.684
1-Butanol	25	625	0.810
1,4-Dioxane	128	48	1.033
Pyridine	721	25	0.982
Toluene	116	111	0.870
Isobutyl Acetate	721	625	0.867
Dimethylformamide (DMF)	116	110	0.948

Table 1: Summary of Calculated Solvent Weights for Residual Solvents

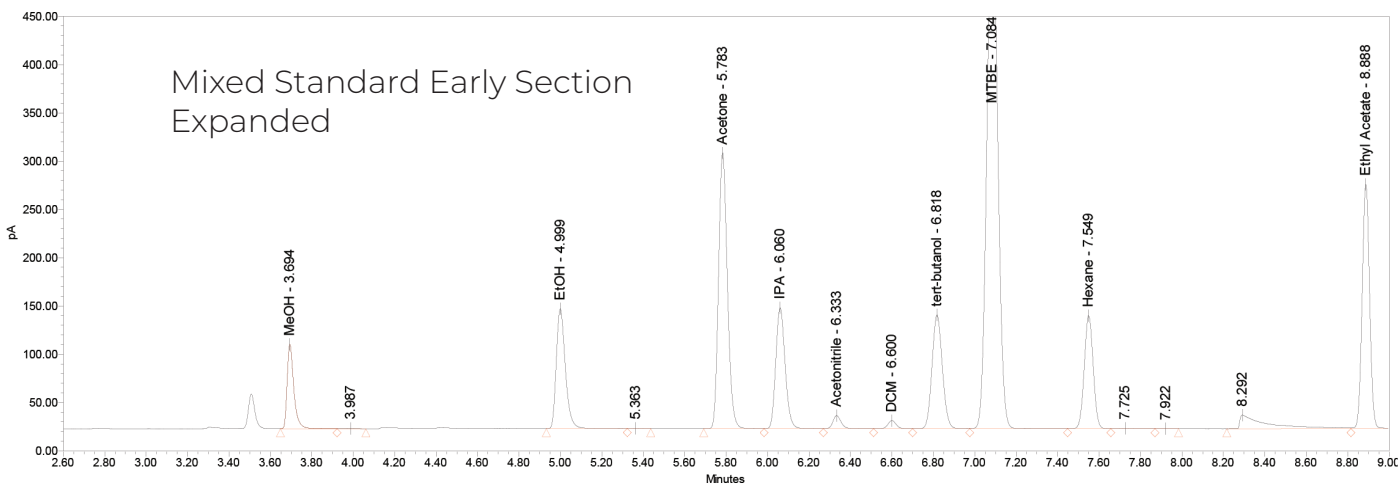
Mixed Stock Standard Preparation

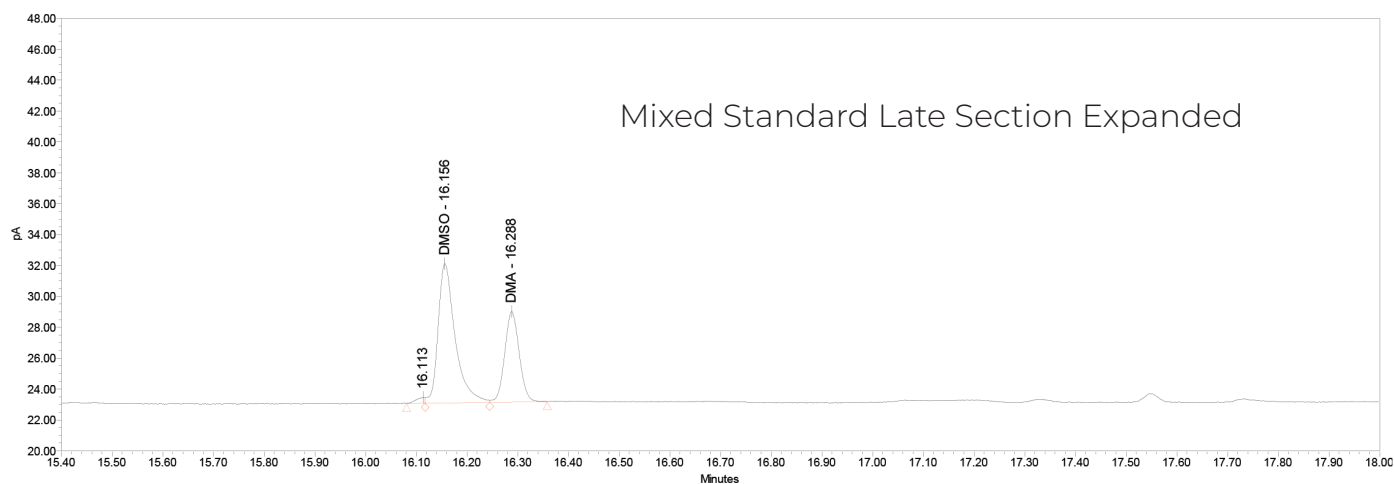
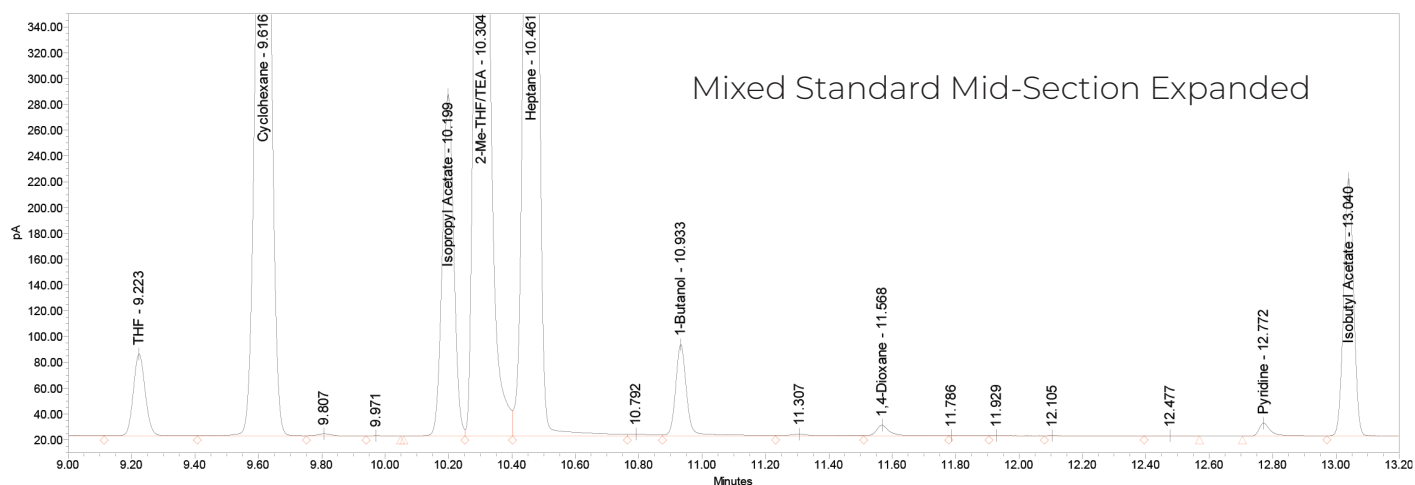
4.0mL of the mixed stock standard preparation is diluted to 100mL with DMI and mixed thoroughly.

Representative Chromatography



The diluent blank profile demonstrates that the level of interference from the DMI diluent is sufficiently low to not impact quantification of the solvents.





Summary

A review of the chromatography for the DMI blank concluded that it was sufficiently free of interference. The resolution of the solvents in the mixed standard was sufficient to be suitable for accurate quantification. This was evidenced by all solvents having a USP resolution of ≥ 1.5 . Based upon these aspects the diluent blank and mixed standard chromatogram profiles were considered suitable as a basis for the generic GC method conditions.

Method Qualification - Linearity

The linearity of the response for each residual solvent was evaluated across the range of 10% to 120% of the respective ICH limit for each residual solvent and was determined to be suitably linear across this range for each of the solvents with insignificant intercepts.

This is evidenced by all correlation coefficients ≥ 0.99 and the intercept being statistically or practically insignificant. A summary of the linearity studies is presented in Table 2.

Table 2: Summary of residual solvents linearity studies

Residual Solvent	Correlation Coefficient (r)	Intercept (p Value)	Random Residuals
	0.99990	0.114	Residuals confirmed as being random
Ethanol	0.99988	0.148	
Acetone	0.99982	0.087	
2-Propanol	0.99988	0.286	
Acetonitrile	0.99988	0.067	
Dichloromethane	0.99938	0.463	
Tert-Butanol	0.99984	0.273	
MTBE	0.99913	0.112	
Hexane	0.99685	0.135	
Ethyl Acetate	0.99990	0.604	
Tetrahydrofuran (THF)	0.99986	0.086	
Cyclohexane	0.99920	0.132	
Isopropyl Acetate	0.99988	0.174	
Heptane	0.99936	0.112	
1-Butanol	0.99947	<0.05*	
1,4-Dioxane	0.99982	0.755	
Pyridine	0.99927	0.820	
Isobutyl Acetate	0.99985	0.535	
Dimethylformamide (DMF)	0.99950	0.010	
Dimethylsulphoxide (DMSO)	0.99936	<0.05*	
Dimethylacetamide (DMA)	0.99974	0.570	

* 1-Butanol and DMSO had statistically significant intercepts, but they were practically insignificant with the intercepts being less than 5% of the theoretical response at the respective specification limits so these statistically significant intercepts were not thought to impact the method accuracy.

Method Qualification - Sensitivity

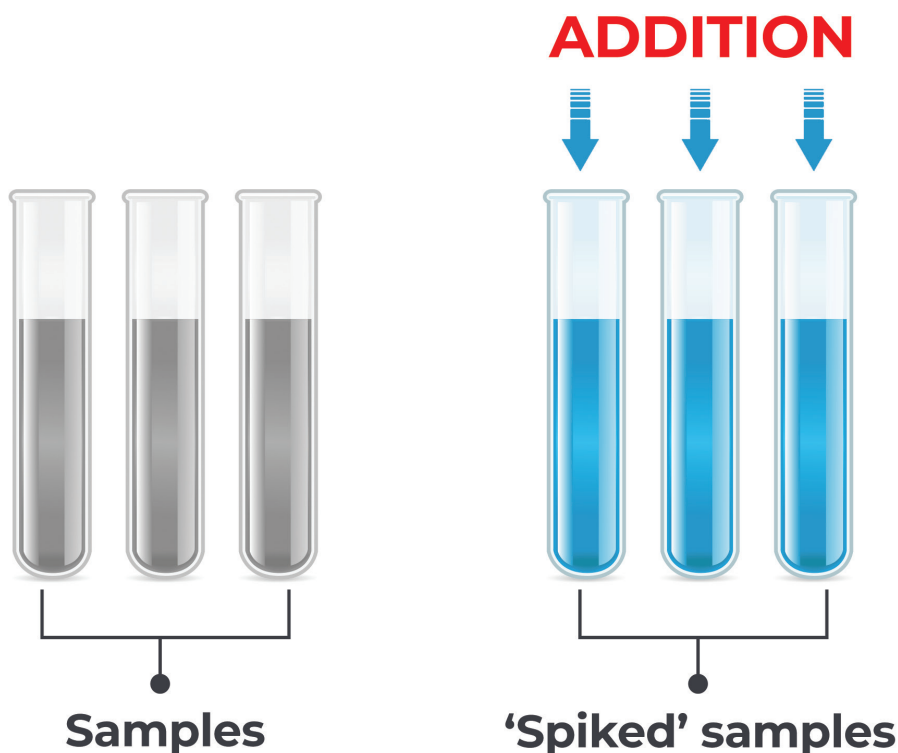
Residual solvents testing is regarded as a limit test. Almac Sciences considers 10% of the specification limit to be the level declared as the limit of quantitation (LOQ). For API release testing the testing requirement is that the levels of the residual solvents are below the specified limits. For some types of API samples, the actual values of the residual solvents levels are required to be used in further calculations, such as for reference standard potency. 5000ppm is the highest limit for an individual residual solvent.

Ability to quantify to 10% of this amount corresponds to 500ppm (0.05%) is sufficiently low to include in the calculation for material potency. This corresponds to the typically accepted LOQ for other organic impurities quantified on chromatographic methods of 0.05%, a level below which impurities are not included in calculation of standard potency.

Method Qualification - Accuracy

The generic method was designed to be considered as a generic and robust set of method conditions for which suitable linearity has been established. As the generic method was intended to be used for several different API products it is recommended that a check for method accuracy is performed when applying the method to new API materials. This was easily be accomplished by dissolving the API sample material in the mixed standard preparation at a level of 50mg/mL. Spiked Sample Preparation:

Select a sample that does not contain high intrinsic levels of the residual solvents of interest. If for example the sample contains 2000ppm of a solvent with a limit of 5000ppm, consider further drying of the sample or spike at 3000ppm to ensure recovery is performed at the specification limit. Accurately weigh 50 ± 5 mg of API sample material into a 10mL GC headspace vial. Add 1.0mL of the mixed standard preparation and seal the GC headspace vial.



Modification for Different Material Dosage Levels

The suggested standard and sample preparations are based upon ICH Q3C (R8) Option 1 for a daily dosage not exceeding 10g. If there are instances where there is

a requirement for a higher daily dose, then the sample preparation can be modified according to Equation 1 to assure that suitable sensitivity is obtained.

Conclusion

The generic GC method condition are a valuable starting point as they provide a readily available resource from which a high quality method can be developed for different API or drug product materials. In summary the following points are noted.

- The method conditions are available for users to readily set up and facilitates the scenario of maintaining an uninterrupted set up on a GC system, mitigating the need to repeatedly take down and set up the system which drives laboratory efficiency.
- There is assurance that a quality method will be delivered in terms of the separation and this scope includes all residual solvents that are likely to be included within the scope of API products.
- Linearity, sensitivity, and specificity for the solvent blank are demonstrated and consequently the scope of the method validation is reduced.

Acknowledgements

Ismail Pinar MSc
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Agilent Technologies
7890B GC System

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Actual Setpoint

Oven Ramp
Pre Run Initial Temp Rate Post Run
Stop Run Start
Oven Open Close Cal 1 Cal 2
Valve # Back Intro Cal 2
Aux Temp # Aux Temp # Aux Temp #
Status Mode Type Info Clear
Time On/Off 7 8 9
Post Run 4 5 6
Logs 1 2 3
Options Blank 0
Config Delete
Method Storage and Automation
Load Method Save Method
Store Save Save Save

GMP
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Prepared by: J. Smith
Reviewed by: J. Smith
Date: 10-10-10
Signature: J. Smith

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