Residual Solvent Impurities; USP <467>

Thanutchaporn Semathong
GCMS Product Specialist
Agilent Technologies (Thailand)

DE91722834

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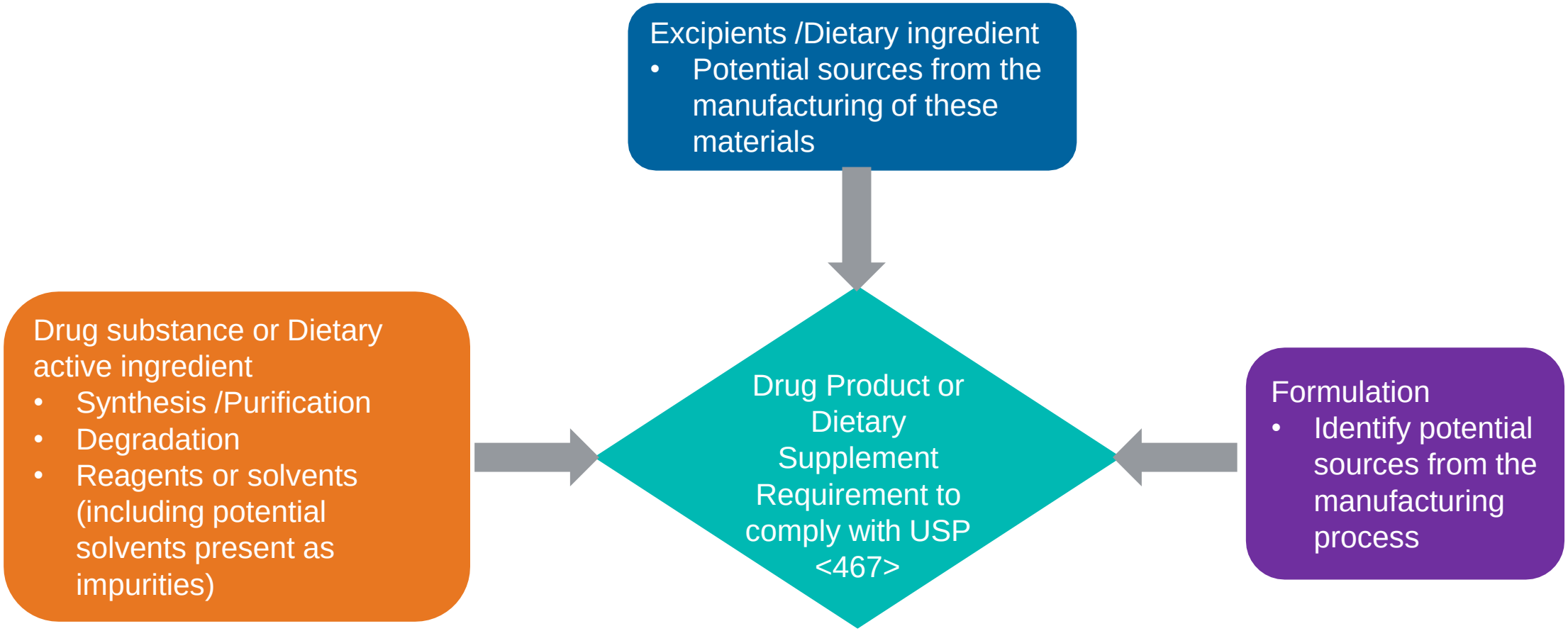
Trusted Answers

Potential Sources of Residual Solvents to be Considered

In pharmaceutical drug products and dietary supplements

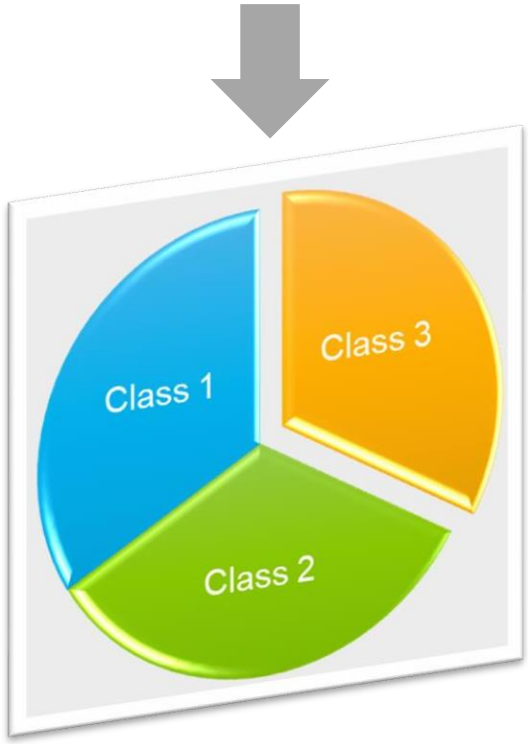


For more details: refer to 467 RESIDUAL SOLVENTS (uspnf.com)

Residual Solvent Analysis

USP <467> and ICH Q3C (R5 and R8) compliance for residual solvent analysis

Organic volatile chemicals used in the preparation of APIs, excipients & drug products



ICH

USP

Require Chromatographic Analysis

Class 1
- Solvents to be avoided
- Know or strongly perceived human carcinogens

Class2
- Solvents to be limited
- Non-genotoxic carcinogens or possible neurotoxic or teratogenic agents

Class 3
- Solvents with low toxic potential
- No health-based exposure limit is needed

USP 467 Limits of Residual Solvents

Table 2. Control Limits for Class 1 Residual Solvents in Official Products: Solvents to Be Avoided

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 3. Class 2 Residual Solvents in Official Products

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
▲Methylisobutylketone	45	4500▲ (Official 1-Dec-2020)
N-Methylpyrrolidone	5.3	530

Table 3. Class 2 Residual Solvents in Official Products *(continued)*

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
Trichloroethylene	0.8	80
Xylene[a]	21.7	2170

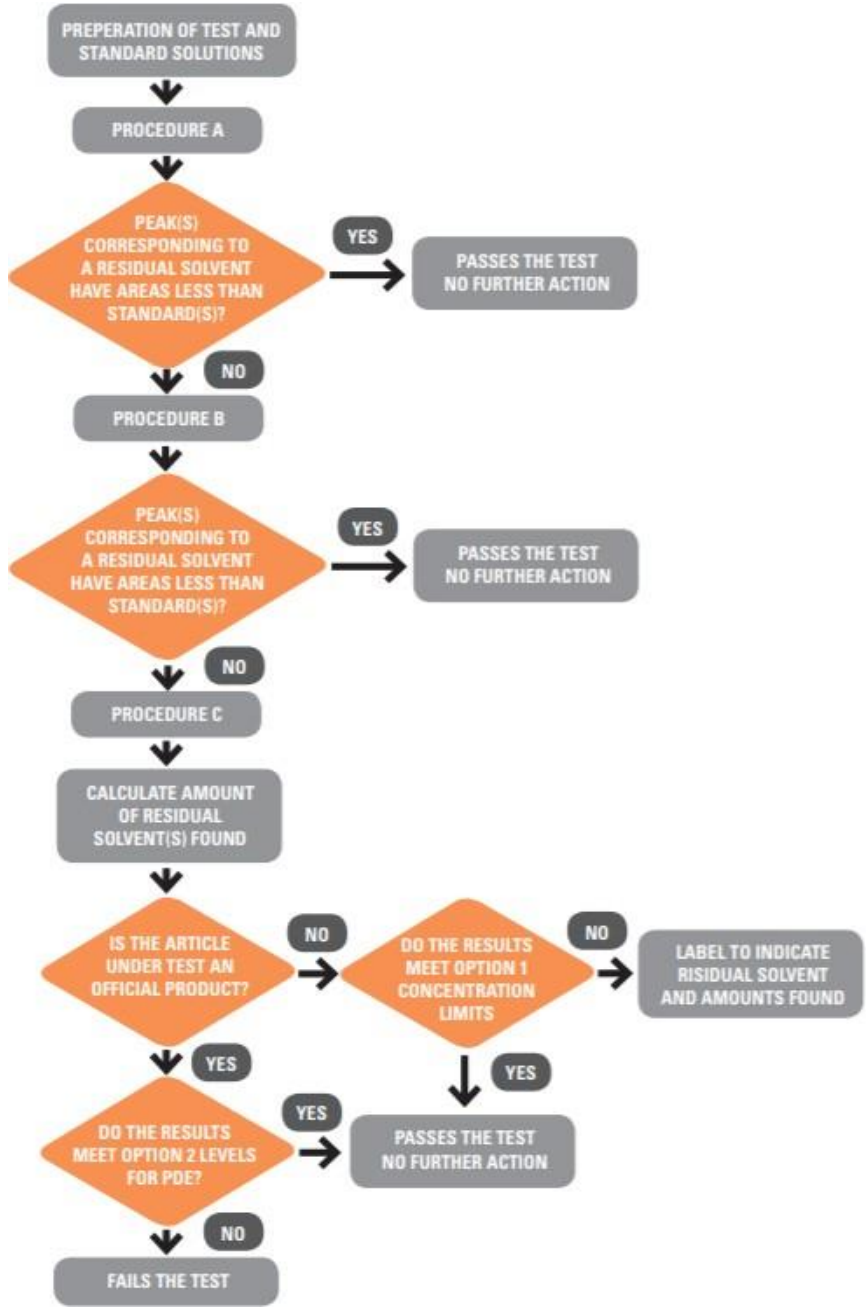
Residual solvents should be limited in drug substances, excipients, dietary ingredients, and official products because of the inherent toxicities of these residual solvents. - 467 RESIDUAL SOLVENTS (uspnf.com)

Compendia Testing Methodology

Determination of residual solvents and decision tree using Procedures A, B, and C

- The method is divided into two separate sections based upon sample solubility and referred to
  - Water-soluble articles
  - Water-insoluble articles

- The methodology for both types of articles is similar and consists of three procedures:
  - Procedure A for identification and limit test
  - Procedure B for confirmatory test
  - Procedure C for quantitative test



USP <467> Analytical flowchart for residual solvent analysis

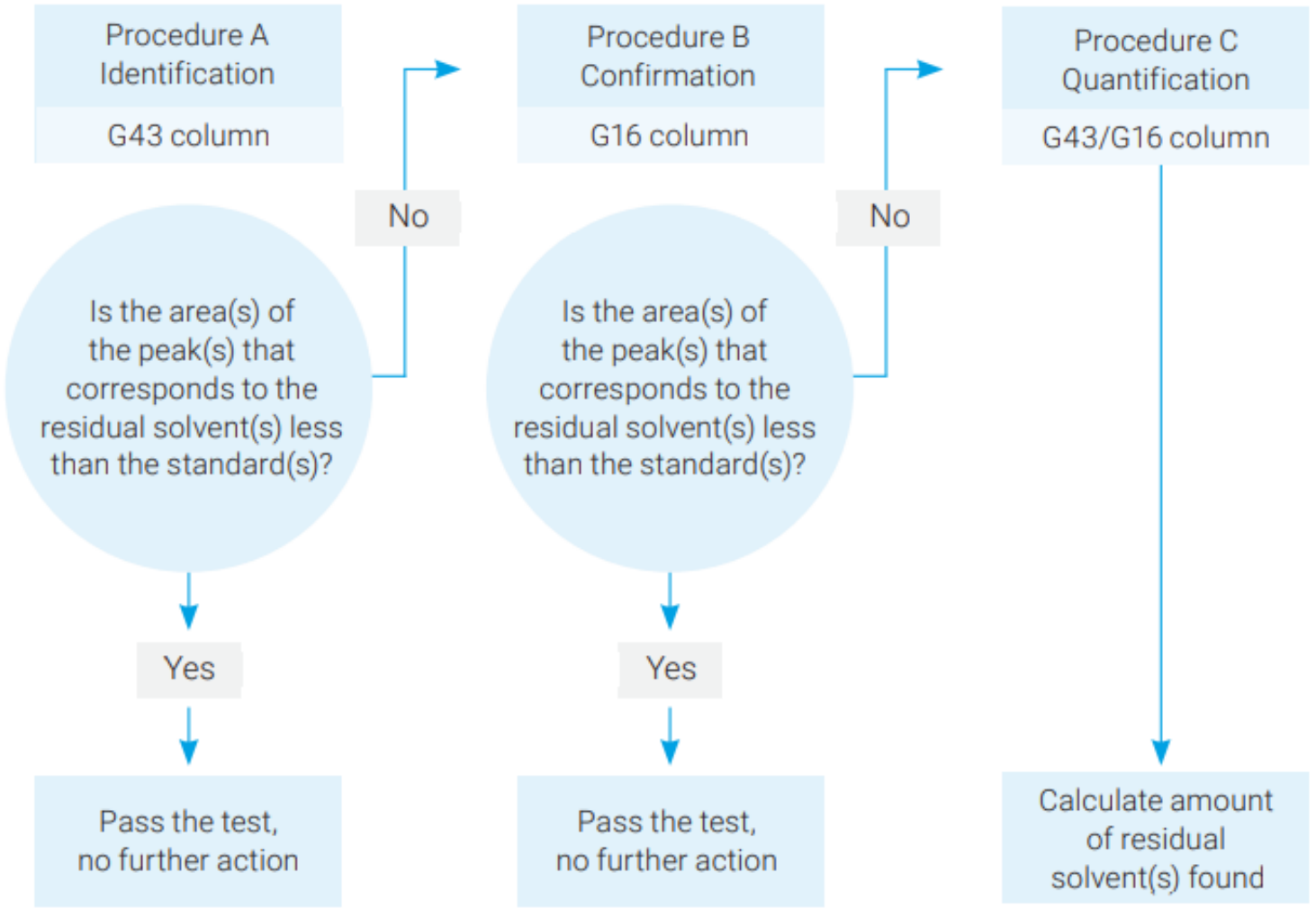
United States Pharmacopeia (USP) Method <467> is the QC method used worldwide and closely follows ICH Q3C guidelines.

The method is composed of 3 analytical procedures for identification & quantification

– **Procedure A**: Identification and limit testing
Uses a **G43** phase (624-type column)

– **Procedure B**: Confirms whether an identified solvent is above the regulated limits
Uses a **G16** phase (WAX-type column)

– **Procedure C**: Quantitative test using a G43 phase or G16 phase, depending on which produced fewer coelutions.

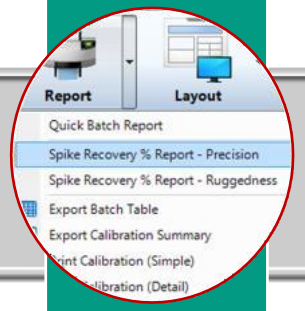


Agilent Innovative Solutions for Residual Solvent Analysis

Sample Preparation Guidance

Spares & solutions (USP/ICH calibration standards)

Easy-to-use, Robust & Reliable Instruments



Preset Methods pre-define settings & reporting tools

SOPs/start-up documentation for method set-up

Installation & Validation Services & Support

Compliant SW options, for any size and type of laboratory

Technical expertise, training & support

Agilent GC/FID and GC/MS Workflow for Residual Solvent Analysis

Confidently identify and quantify residual solvent in APIs and drug products

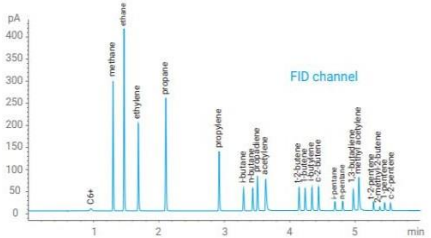
Calibrate

Ideal for routine & HT analysis

Separate

Identify

Analyze



Quantitative Analysis of USP residual solvents even in trace levels

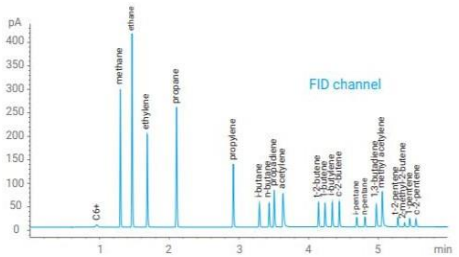
Calibrate

Ideal for unknown analysis

Separate

Identify

Analyze



Residual Solvents Analysis with the Agilent 8697 Headspace Sampler and Intuvo 9000 GC

Agilent

USP <467> Residual Solvent Requirements

Analysis of residual solvent is a critical application in the pharmaceutical industry.

- The choice of solvent during manufacturing can improve yield or affect chemical properties of the product synthesized.
- Solvents do not enhance the product's efficacy and **must be removed** as completely as possible to meet product specification and good manufacturing practices.

USP <467> specifies a single column analysis

- A secondary analysis is performed if the solvent is found above limit detection.
- An Intuvo9000 GC configured with an inlet split to two columns and two FIDs can perform both analysis in a single run.

USP 40, general chapter USP <467> Residual Solvents https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c467.pdf

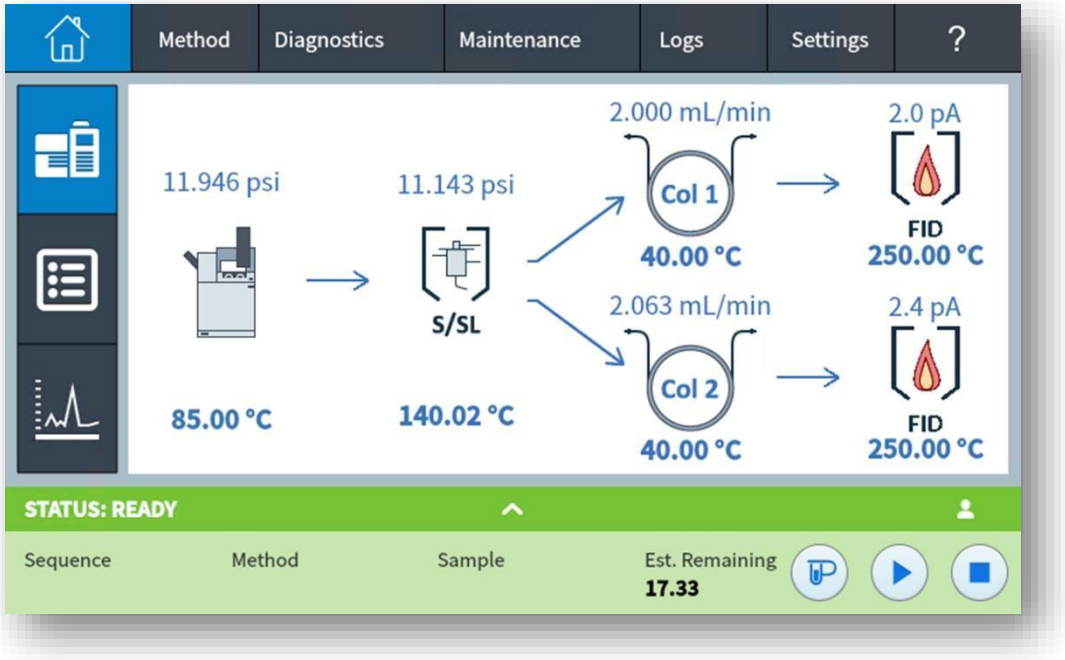
USP <467> Columns and Performance Requirements

Procedure A – Initial identification and limit test

- **DB-Select 624 UI (G43 phase)**
  - s/n of 1,1,1-trichloroethane > 5
  - s/n of all Class 1 solvents > 3
  - Resolution of acetonitrile and methylene chloride > 1

Procedure B – Secondary analysis for confirmation

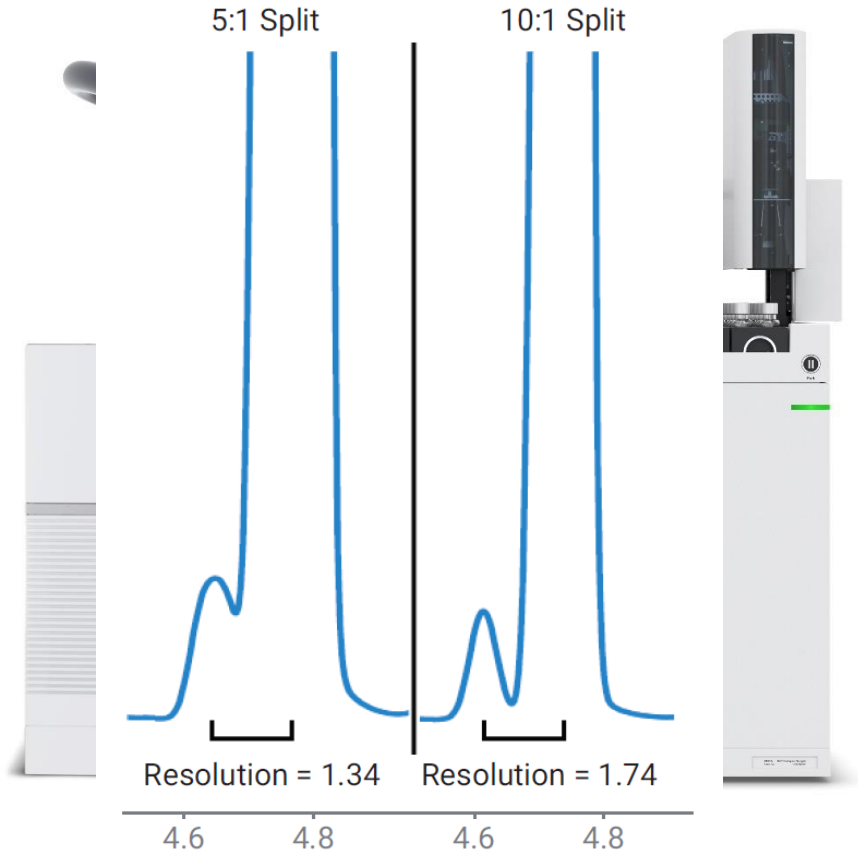
- **DB-Wax Ultra Inert (G16 phase)**
  - s/n of benzene > 5
  - s/n of all Class 1 solvents > 3
  - Resolution of **methylisobutylketone** and cis-dichloroethene > 1



USP <467> https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/generalChapter467Current.pdf

Method Transfer

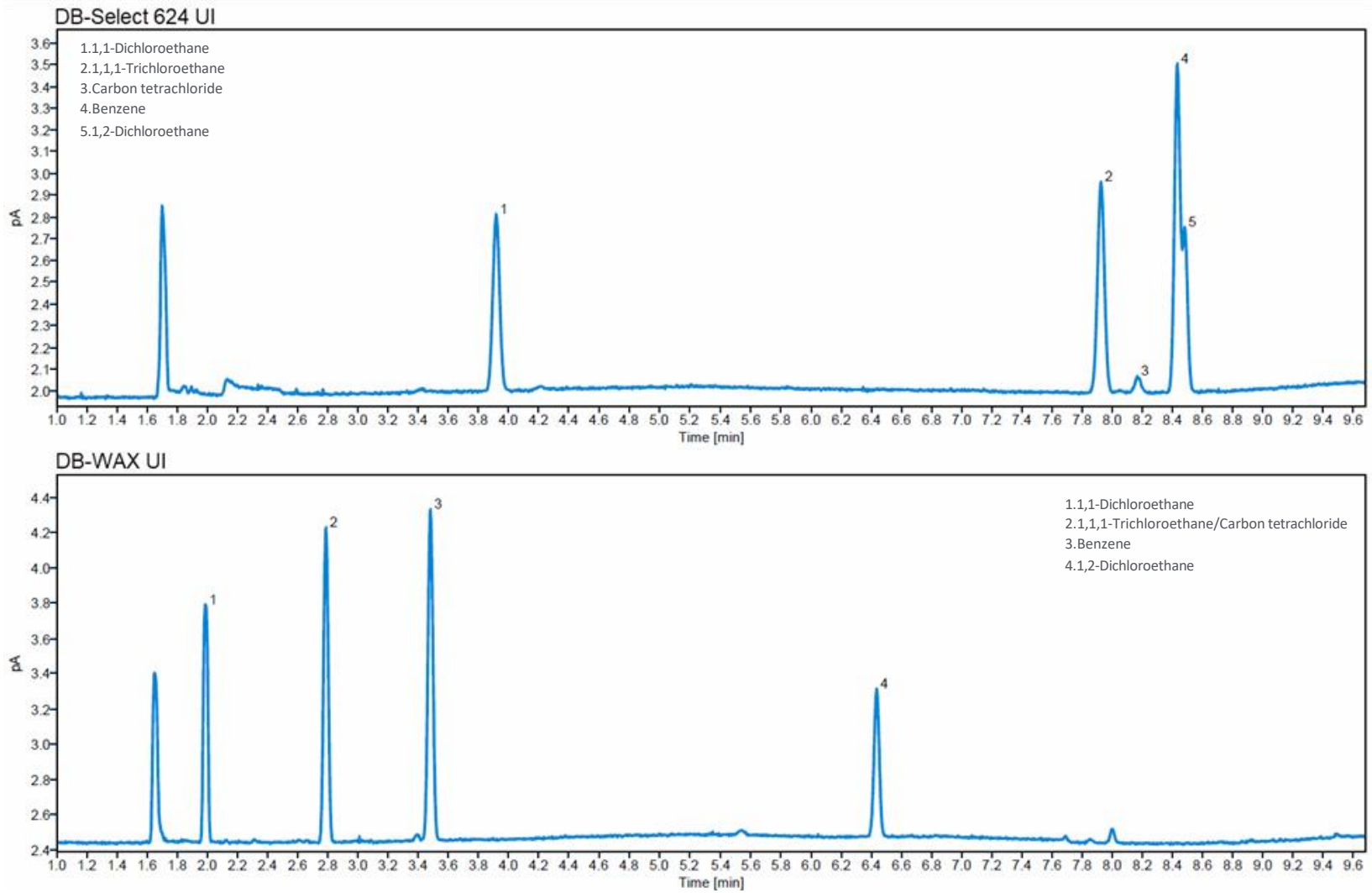
Parameter	Value
Headspace	
Oven	85 °C
Loop	85 °C
Transfer Line Temperature	100 °C
Transfer Line	Fused silica, 530 µm id
Vial Equilibration	40.00 min
Injection Duration	0.50 min
Vial Shaking	Level 2, 25 shakes/min
Vial Fill Mode	Default
Vial Fill Pressure	15 psi
Vial Pressurization Gas	Nitrogen
Loop Fill Mode	Custom, 20 psi/min
Loop Final Pressure	4 psi
Loop Equilibration Time	0.05 min
Loop Volume	1.0 mL
Inlet (SSL)	
Mode	Split
Heater	140 °C
Carrier	Helium
Split Ratio	10:1
Split Flow	20 mL/min
Septum Purge	3 mL/min
Liner	Ultra Inert, straight, 0.75 mm id (p/n 5190-4048)
Septum	9mm Headspace (p/n 5183-4801)
Jumper Chip	140 °C (p/n G4587-60575)
Columns	
Column 1 (Procedure A)	Agilent DB-Select 624 UI, 30 m × 320 µm, 1.8 µm (p/n 624 123-0334UI-INT); 2 mL/min, constant flow
Column 2 (Procedure B)	Agilent DB-WAX UI, 30 m × 320 µm, 0.25 µm (p/n 123-7032UI-INT); 2 mL/min, constant flow
Inlet Chip	Inlet splitter chip (p/n G4588-60601)
Bus Temperature	Default
Oven	40 °C hold for 5.5 min 15 °C/min to 180 °C, hold 2.5 min
Detector (FID)	
Heater	250 °C
Air	400 mL/min
H_2	30 mL/min
Makeup	N_2 at 25 mL/min



Class 1, DB-Select 624 UI & DB-WAX Ultra Inert

Signal to Noise requirements are met

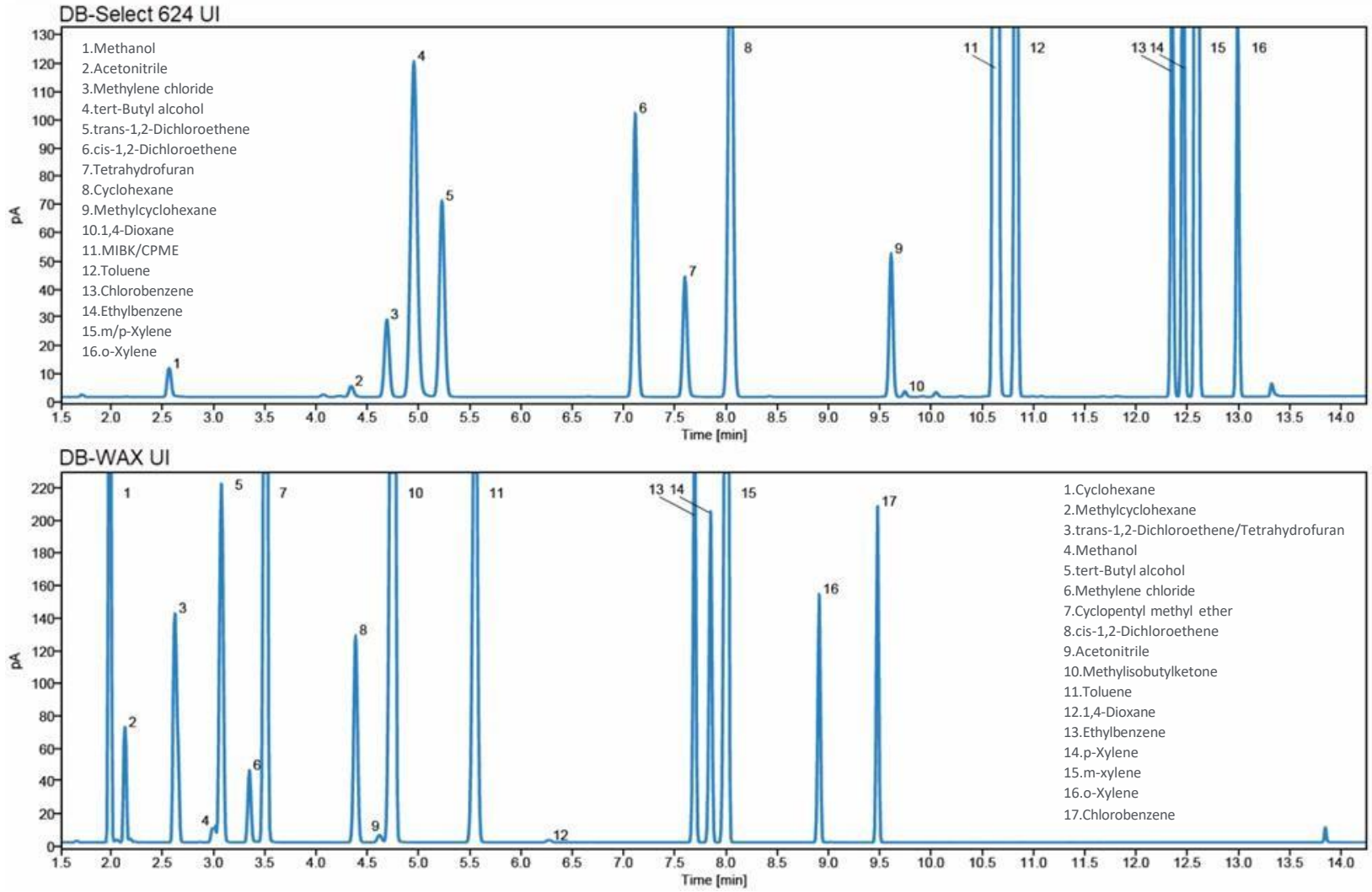
Signal to Noise requirements are met



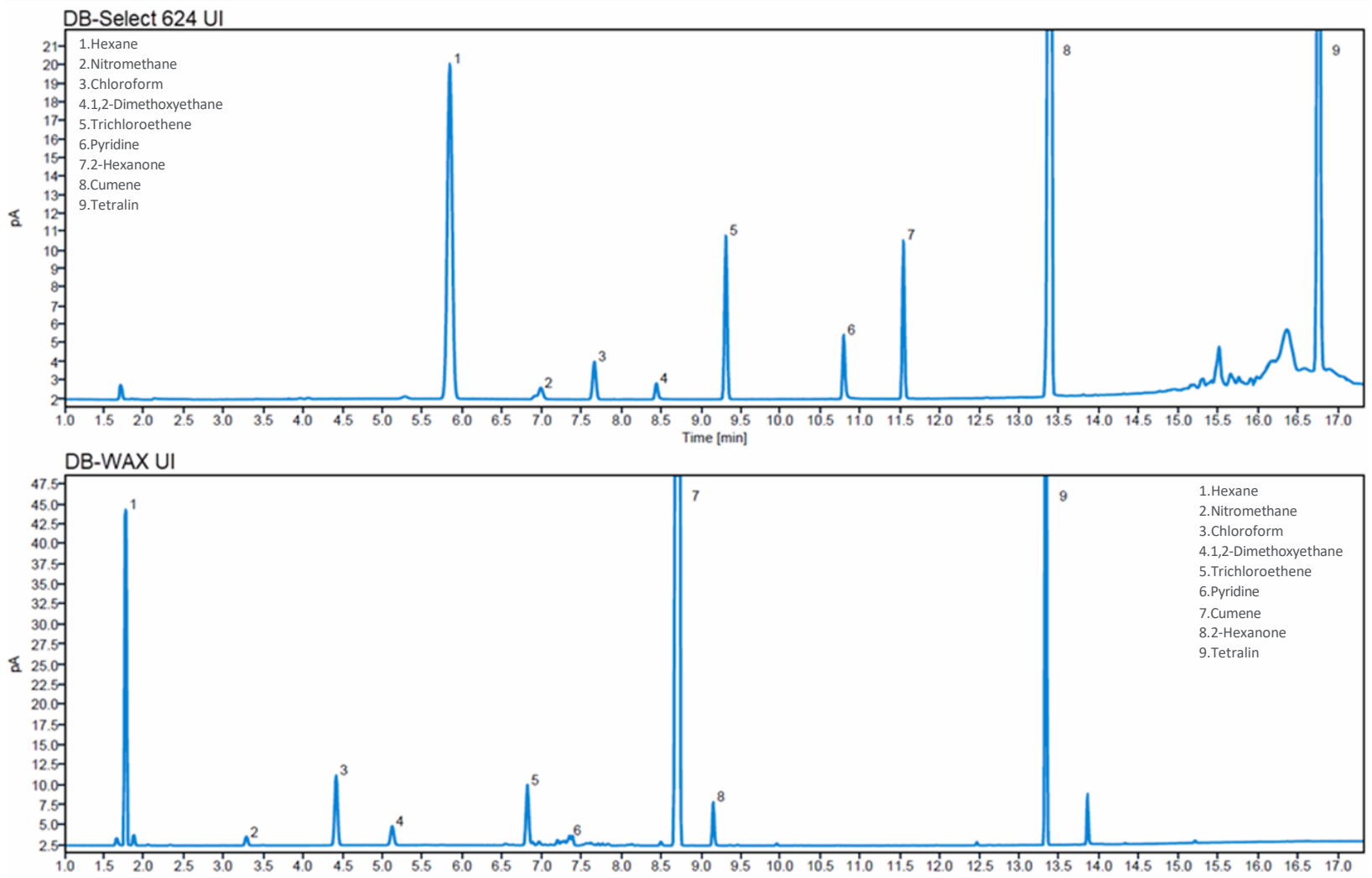
Class 2A, DB-Select 624 UI & DB-WAX Ultra Inert

Resolution > 1

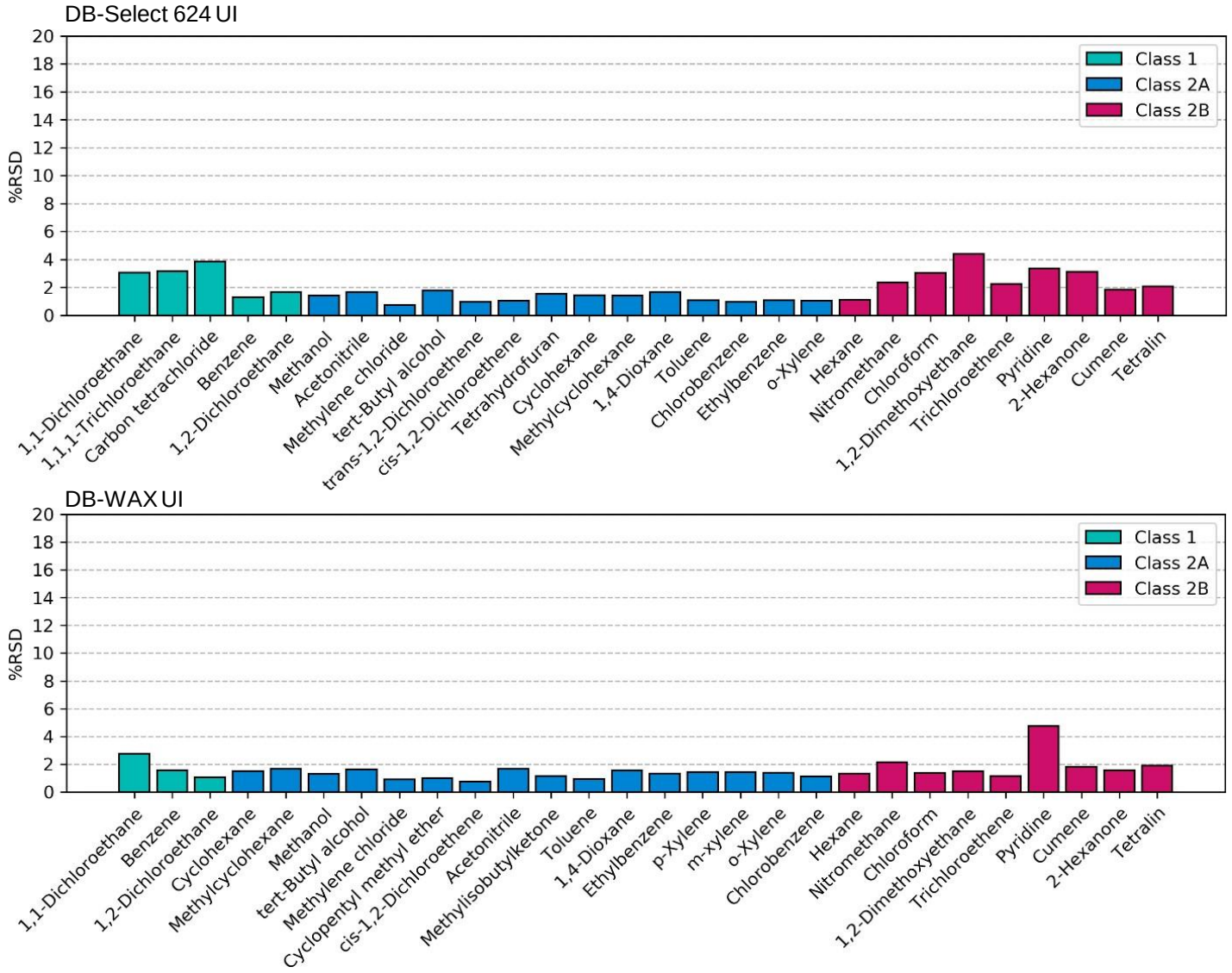
Resolution > 1



Class 2B, DB-Select 624 UI & DB-WAX Ultra Inert



Repeatability (n=10)



Analysis of Three Classes of Residual Solvents in USP <467> and Chinese Pharmacopoeia by using GC/FID/MSD System

Configuration Highlights

- This application covers three classes of solvents with a total of up to 62 compounds.
- A purged two-way CFT device was used to split the column effluent 1:1 to the MSD and FID.
- When unknown peaks or unknown solvents appear, this system is the best solution for solvent identification and quantification
- Both MSD and FID signals can be used for quantitative analysis, MSD is a good quantitative supplement for compounds with poor resolution, while FID can expand the linear range.

Compounds List

The list of compounds in USP <467> and Chinese pharmacopoeia is almost the same.

Class 1

Table 1. Class 1 Residual Solvents
(Solvents that should be avoided)

Solvent	Concentration Limit (ppm)
Benzene	2
Carbon tetrachloride	4
1,2-Dichloroethane	5
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1500

Class 3

Table 3. Class 3 Residual Solvents
(limited by GMP or other quality-based requirements in drug substances, excipients, and drug products)

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethylketone
tert-Butylmethyl ether	Methylisobutylketone
Cumene	2-Methyl-l-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	

Class 2

Table 2. Class 2 Residual Solvents

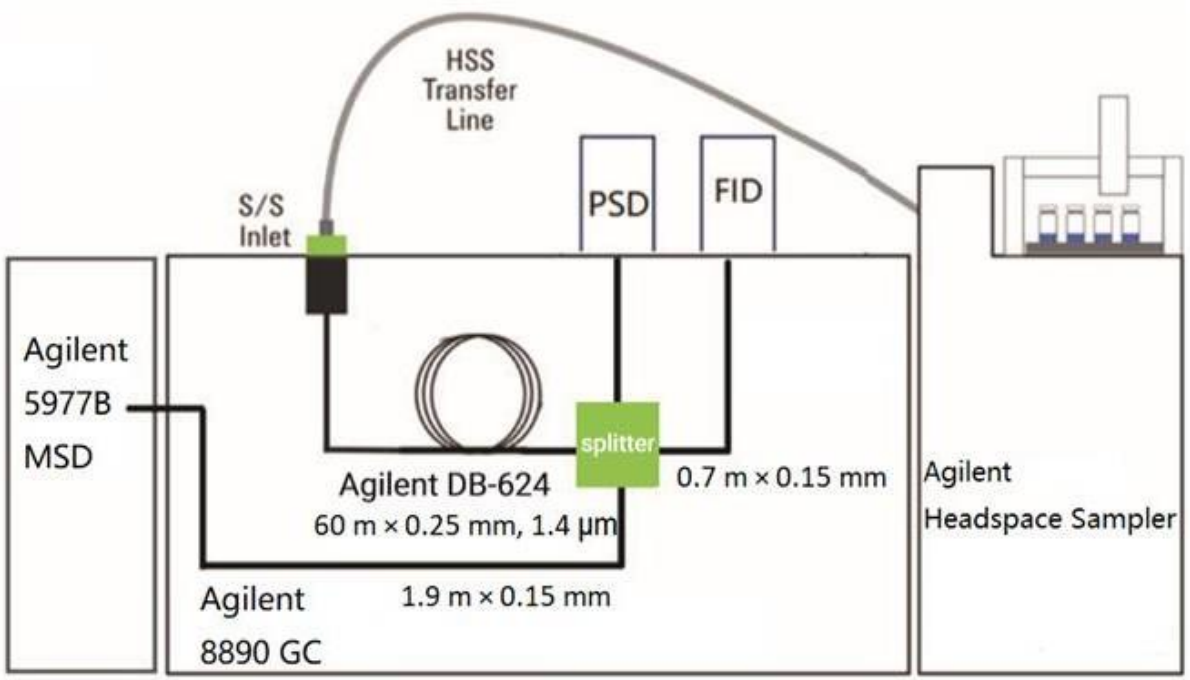
Solvent	PDE (mg/day)
Acetonitrile	4.1
Chlorobenzene	3.6
Chloroform	0.6
Cumene	0.7
Cyclohexane	38.8
1,2-Dichloroethene	18.7
1,2-Dimethoxyethane	1.0
N,N-Dimethylacetamide	10.9
N,N-Dimethylformamide	8.8
1,4-Dioxane	3.8
2-Ethoxyethanol	1.6
Ethylene glycol	6.2
Formamide	2.2
Hexane	2.9
Methanol	30.0
2-Methoxyethanol	0.5
Methylbutylketone	0.5
Methylcyclohexane	11.8
Methylene chloride	6.0
N-Methylpyrrolidone	5.3
Nitromethane	0.5
Pyridine	2.0
Sulfolane	1.6
Tetrahydrofuran	7.2
Tetralin	1.0
Toluene	8.9
Trichloroethylene	0.8
Xylene*	21.7

Red: liquid injection

Others: headspace injection

Headspace Injection

Instrument conditions



Agilent 8890 GC	
Parameter	Value
Inlet	SSL, 250 °C, split 10:1
Liner	Straight, deactivated, 2 mm ID (part number 5181-8818)
CFT Device	Purged 2-way splitter Split Ratio 1:1 MSD:FID
PSD	3.8 psi constant pressure
Column	Agilent DB-624 60 m × 0.25 mm, 1.4 µm (part number 122-1364)
Carrier	Helium, 1 mL/min, constant flow
FID Restrictor	0.7 m × 0.15 mm id deactivated fused silica tubing
MSD Restrictor	1.9 m × 0.15 mm id deactivated fused silica tubing
Oven	40 °C (10 min), then 5 °C/min to 80 °C, then 12 °C/min to 220 °C (10 min)
FID	Temperature: 250 °C Hydrogen: 30 mL/min Air: 300 mL/min Make -up gas (N2):25 mL/min
Transfer line temperature	250 °C
Agilent 5977B GC/MSD	
Parameter	Value
Ionization type	EI
Source temperature	230 °C
Quad temperature	150 °C
Drawout plate	3 mm
Tune file	Atune.u
Acquisition type	Scan
Solvent delay	6 min
Relative Voltage	0

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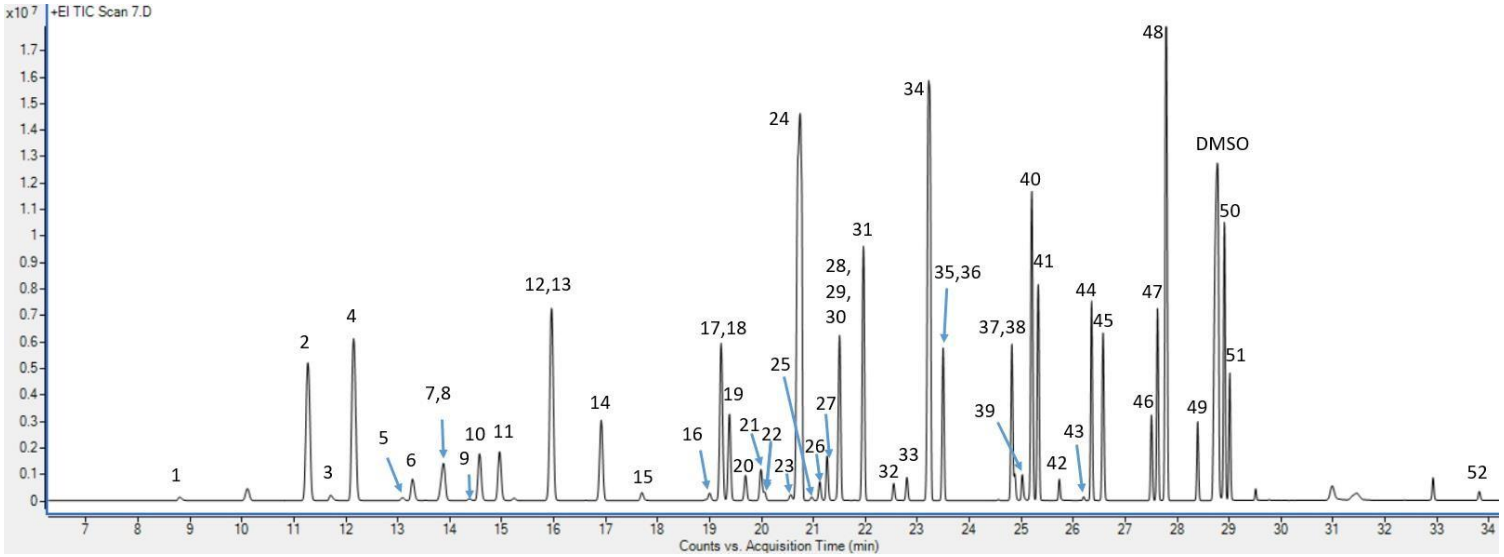
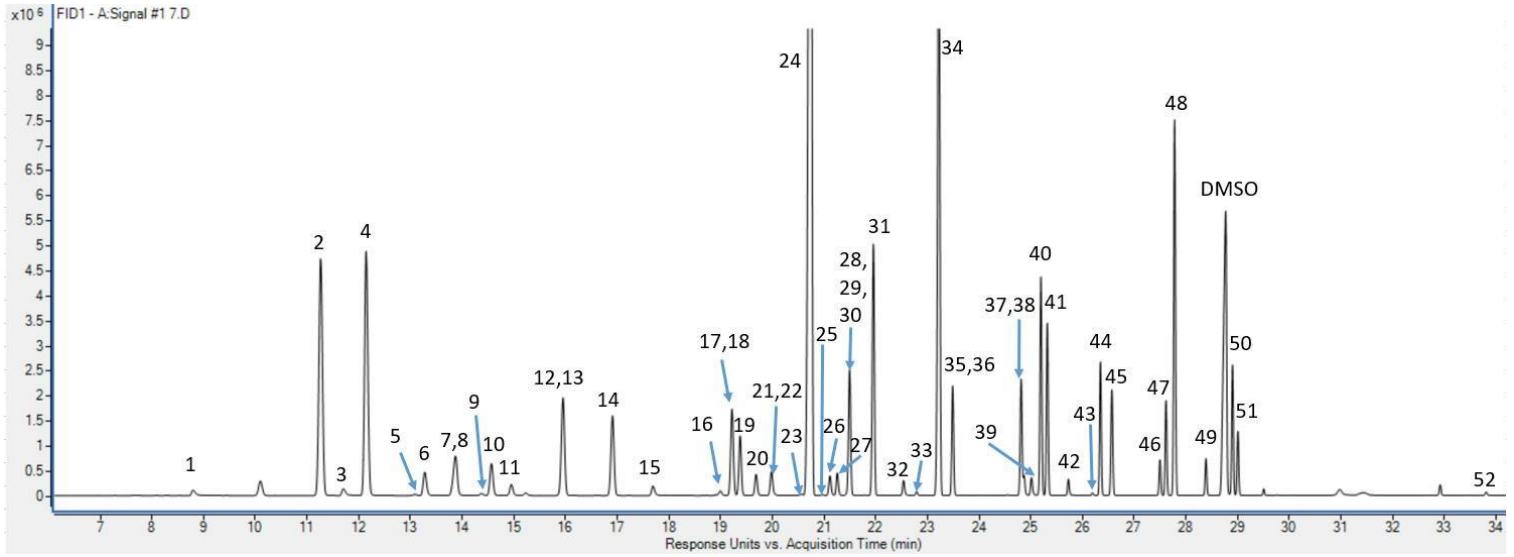
Headspace Injection



MSD scan



FID

Headspace Injection

Results of the **52 compounds**

No.	Name	RT	m/z	Linearity range(µg/mL)	MSD R2	FID R2	Area RSD% L4 (n=8)	MDL (MSD) µg/mL L2 (n=8)
1	Methanol	8.818	31	0.75-150	0.9998	0.9994	2.2	0.194
2	Pentane	11.251	43	0.5-100	0.9944	0.9997	2	0.1428
3	Ethanol	11.73	31	2-100	0.9999	0.9993	1.2	0.5137
4	Ethyl ether	12.142	74.1	0.5-100	0.9911	0.9993	4.3	0.1469
5	1,1-Dichloroethene	13.083	61	0.004-0.8	0.9997	0.9986	1.7	0.0028
6	Acetone	13.283	43	0.5-100	0.9999	0.9996	2.1	0.2265
7	Isopropanol	13.854	45	0.5-100	0.9997	0.9979	2.9	0.2446
8	Ethyl formate	13.873	45	0.5-100			4.3	0.2449
9	Acetonitrile	14.39	41	0.1-20	0.9996	0.9984	4.2	0.0319
10	Methyl acetate	14.564	43	0.5-100	0.9998	0.9993	2.7	0.4236
11	Methylene chloride	14.947	84	0.15-30	0.9997	0.9997	2.1	0.0326
12	2-Methoxy-2-methylpropane	15.938	73	0.1-20	0.9988	0.9993	2.1	0.0352
13	trans-1,2-Dichloroethene	15.979	95.9	0.235-47	0.9969	0.9993	1.7	0.065
14	Hexane	16.899	57	0.1-20	0.9995	0.9993	2.2	0.0739
15	1-Propanol	17.712	31	0.5-100	0.9995	0.9996	2	0.1799
16	Nitromethane	19	46	0.5-100	0.9999	0.9991	1.9	0.2521
17	cis-1,2-Dichloroethene	19.21	96	0.235-47	0.9988	0.9999	2.5	0.0447
18	2-Butanone	19.225	43	0.5-100	0.998	0.9999	2.3	0.1471
19	Ethyl acetate	19.375	43	0.5-100	0.9986	0.9997	1.4	0.3054
20	2-Butanol	19.688	45	0.5-100	0.9998	0.9999	2.4	0.2371
21	Tetrahydrofuran	19.985	42	0.18-36	0.9998	0.9993	2.1	0.0532
22	Chloroform	20.054	83	0.015-3	0.9997		1.6	0.0058
23	1,1,1-Trichloroethane	20.546	97	0.005-1	0.9999	0.9993	1.3	0.0025
24	Cyclohexane	20.707	84	1.0-49 (195)*	0.9908	0.9997	1.8	0.188
25	Carbon tetrachloride	20.962	117	0.002-0.4	0.9998	0.9992	2.8	0.002
27	1,2-Dimethoxyethane	21.265	45	0.5-100	0.9999	0.9995	1	0.2561
28	Benzene	21.442	78	0.001-0.2	0.9995	0.9998	5.8	0.0008
29	1,2-Dichloroethane	21.442	62	0.01-0.5	0.9989		1.5	0.0016
30	Isopropyl acetate	21.496	61	0.5-100	0.9985	0.9998	0.8	0.1636
31	Heptane	21.956	71	0.1-20	0.9974	0.9996	2.4	0.0343
32	1-Butanol	22.547	56	0.5-100	0.9994	0.9998	2.4	0.1717
33	Trichloroethylene	22.791	130	0.015-3	0.9999	0.9999	1.8	0.0065
34	Methyl cyclohexane	23.208	83	0.3-15 (59)*	0.9989	0.9997	2.3	0.0722
35	1,4-Dioxane	23.489	88	0.095-19	0.9999	0.9999	3.3	0.0549
36	Propyl acetate	23.491	43	0.5-100	0.9966		3	0.2675
37	4-Methyl-2-pentanone	24.815	43	0.5-100	0.9985	0.9999	2.2	0.1429
38	Isoamyl alcohol	24.879	55.1	0.5-100	0.9991	0.9996	2.4	0.2562
39	Pyridine	25.024	79	2-100	0.9992	0.9997	2.1	0.5016
40	Toluene	25.196	91	0.225-22 (44)*	0.9964	0.9998	2.1	0.0651
41	Isobutyl acetate	25.322	56	0.5-100	0.9958	0.9999	2.1	0.1784
42	1-Pentanol	25.735	42	0.5-100	0.9996	0.9998	2.1	0.3319
43	2-Hexanone	26.201	58	0.06-3	0.9995	0.9998	2.1	0.0107
44	Butyl acetate	26.351	43	0.5-100	0.9957	0.9999	2.3	0.2502
45	Tetrahydrothiophene	26.571	88	0.5-100	0.9996	0.9999	1.4	0.18
46	Chlorobenzene	27.503	112	0.09-18	0.9999	0.9997	2.5	0.0215
47	Ethylbenzene	27.618	91	0.09-18	0.9986	0.9997	4.1	0.0288
48	m,p-xylene	27.782	106	0.4-40 (80)*	0.9963	0.9997	3.3	0.1074
49	o-xylene	28.393	91	0.05-10	0.9999	0.9996	2.6	0.0173
50	Isopropylbenzene	28.904	105	0.1-20	0.9983	0.9996	2.4	0.0391
51	Anisole	29.011	108	0.5-100	0.9999	0.9997	2.8	0.1892
52	1,2,3,4-Tetrahydronaphthalene	33.814	104	0.015-3	0.9998	0.9993	2	0.0045

Liquid Injection

Compounds list in liquid injection

2-Methoxyethanol

2-Ethoxyethanol

N,N-dimethylformamide

N,N-dimethylacetamide

Acetic acid

Formic acid

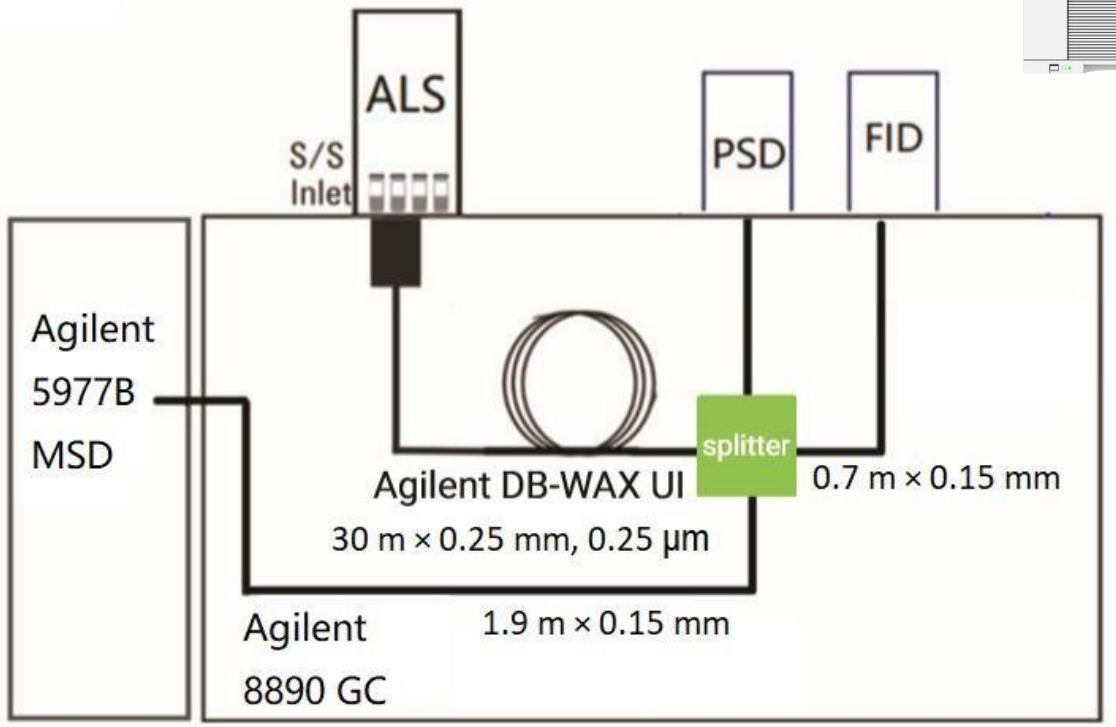
Ethylene glycol

N-methylpyrrolidone

Formamide

Sulfolane

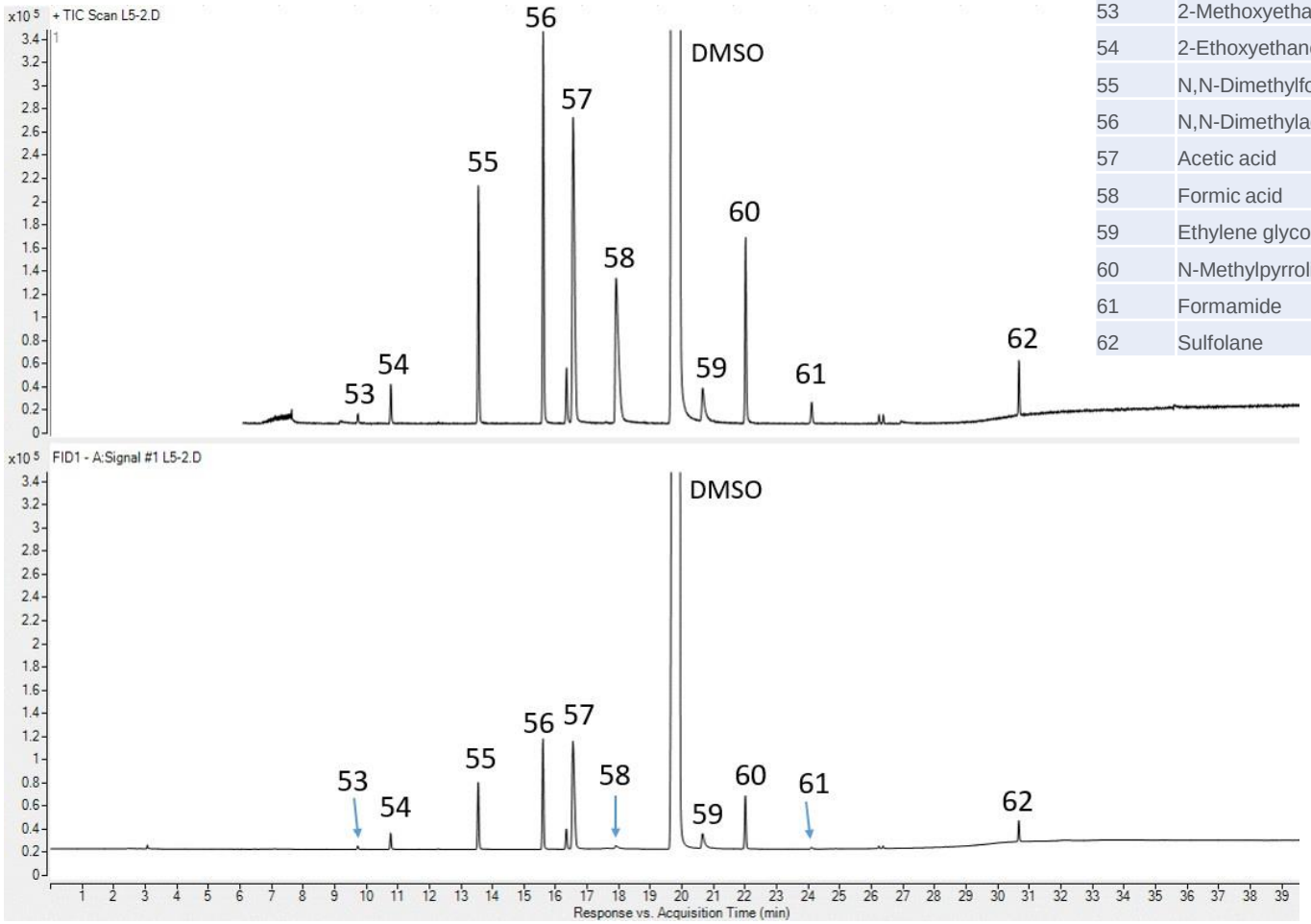
Instrument configuration



Standards: USP 467 Class 2C (Agilent p/n: 5190-0493)
acetic acid (99.8%, purity), formic acid (98%, purity)

Liquid Injection

Results of the 10 compounds



No.	Name
53	2-Methoxyethanol
54	2-Ethoxyethanol
55	N,N-Dimethylformamide
56	N,N-Dimethylacetamide
57	Acetic acid
58	Formic acid
59	Ethylene glycol
60	N-Methylpyrrolidone
61	Formamide
62	Sulfolane

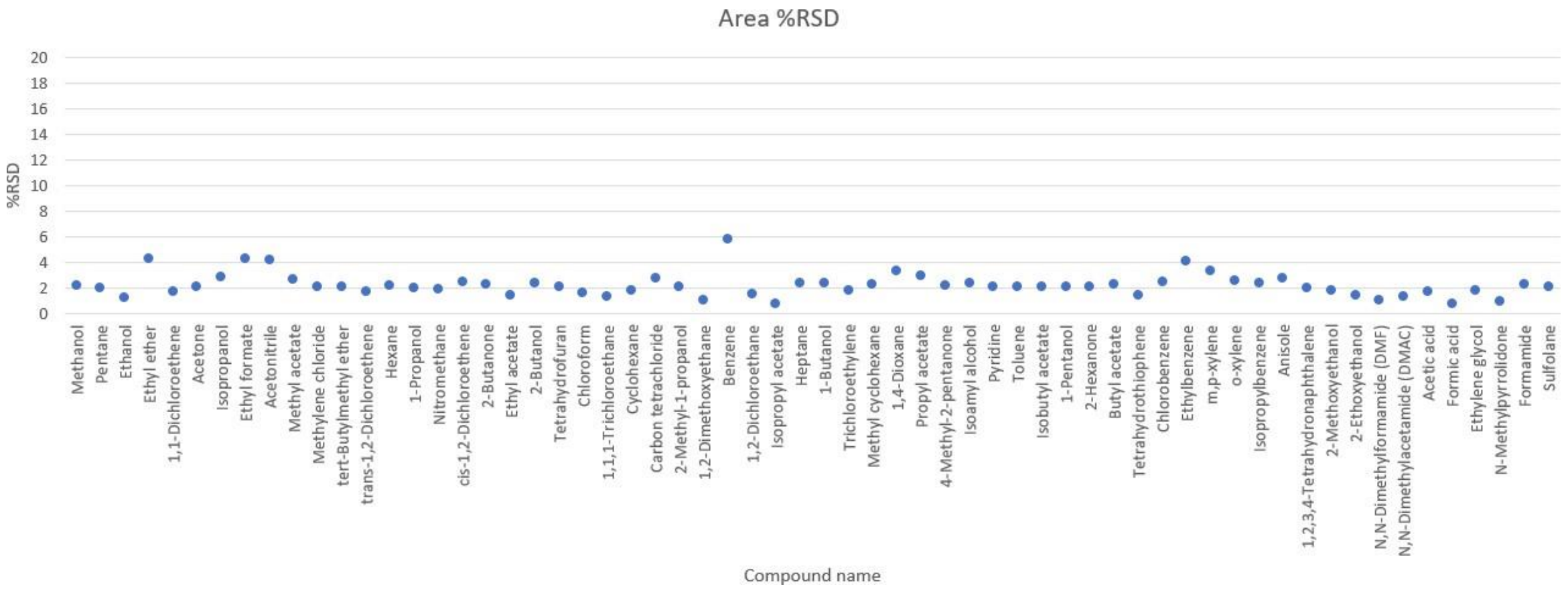
Agilent 8890 GC	
Parameter	Value
Inlet	SSL, 250 °C, split 30:1
Liner	Ultra Inert, split, low pressure drop, glass wool (p/n: 5190-2295)
Injection volume	0.5 uL
CFT Device	Purged 2-way splitter Split Ratio 1:1 MSD:FID
PSD	3.8 psi constant pressure
Column	Agilent DB-wax UI 30 m × 0.25 mm, 0.25 µm (part number 122-7032UI)
Carrier	Helium, 1 mL/min, constant flow
FID Restrictor	0.7 m × 0.15 mm id deactivated fused silica tubing
MSD Restrictor	1.9 m × 0.15 mm id deactivated fused silica tubing
Oven	40 °C, then 5 °C/min to 160 °C, then 10 °C/min to 220 °C (10 min)
FID	Temperature: 250 °C Hydrogen: 30 mL/min Air: 300 mL/min Make -up gas (N2):25 mL/min
Transfer line temperature	250 °C
Agilent 5977B GC/MSD	
Parameter	Value
Ionization type	EI
Source temperature	230 °C
Quad temperature	150 °C
Drawout plate	3 mm
Tune file	Atune.u
Acquisition type	Scan
Solvent delay	6 min
Relative Voltage	0

Liquid Injection

Results of the 10 compounds

No.	Name	RT	m/z	Linearity range µg/mL	R^2 MSD	R^2 FID	Area RSD% L4 (n=8)	MDL (MSD) µg/mL
53	2-Methoxyethanol	9.783	45	5-50	0.9984	0.9995	1.8	0.68
54	2-Ethoxyethanol	10.816	59	16-161	0.9973	0.9987	1.4	1.93
55	N,N-Dimethylformamide (DMF)	13.607	73	88.3-883	0.9997	0.9999	1	2.19
56	N,N-Dimethylacetamide (DMAC)	15.667	87	109.4-1094	0.9997	0.9996	1.3	2.58
57	Acetic acid	16.493	60	400-3000	0.9984	0.9997	1.7	90.12
58	Formic acid	17.774	46	400-3000	0.9995	0.9939	0.8	120
59	Ethylene glycol	20.652	31	62.2-622	0.9983	0.9982	1.8	4.44
60	N-Methylpyrrolidone	22.074	98	53-530	0.9995	0.9997	0.9	3.02
61	Formamide	24.157	45	22-221	0.9992	0.9986	2.3	2.11
62	Sulfolane	30.706	120	16-160	0.9994	0.9997	2.1	1.33

Repeatability (n=8) for 62 Compounds

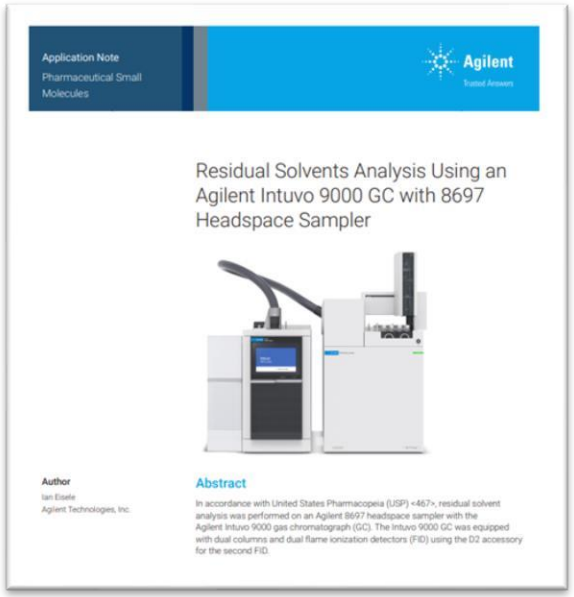


Summary

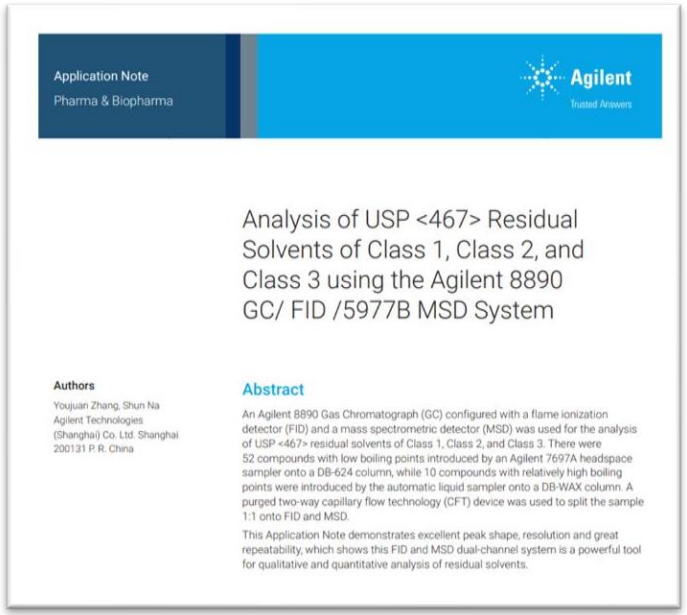
- Residual solvents of Class 1, 2, and 3 were tested using the Agilent 8890 GC/FID/MSD
  - system.
- For new drug development and quality control, FID and MSD dual-channel configurations can be powerful tools for solvent residue analysis.
- MSD analysis can avoid the uncertainty of more than 60 solvents involved in drug production.
- When unknown peaks or unknown solvents appear, this system is the best solution for
  - solvent identification and quantification

Learn More – @Agilent.com

Application Notes

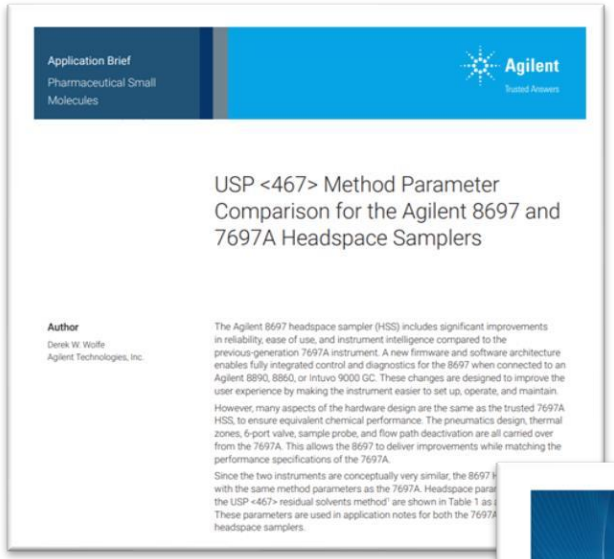


[Residual Solvents Analysis Using an Agilent Intuvo 9000 GC with 8697 Headspace Sampler]



[Analysis of USP <467> Residual Solvents of Class 1, Class 2, and Class 3 using the Agilent 8890 GC/ FID /5977B MSD System]

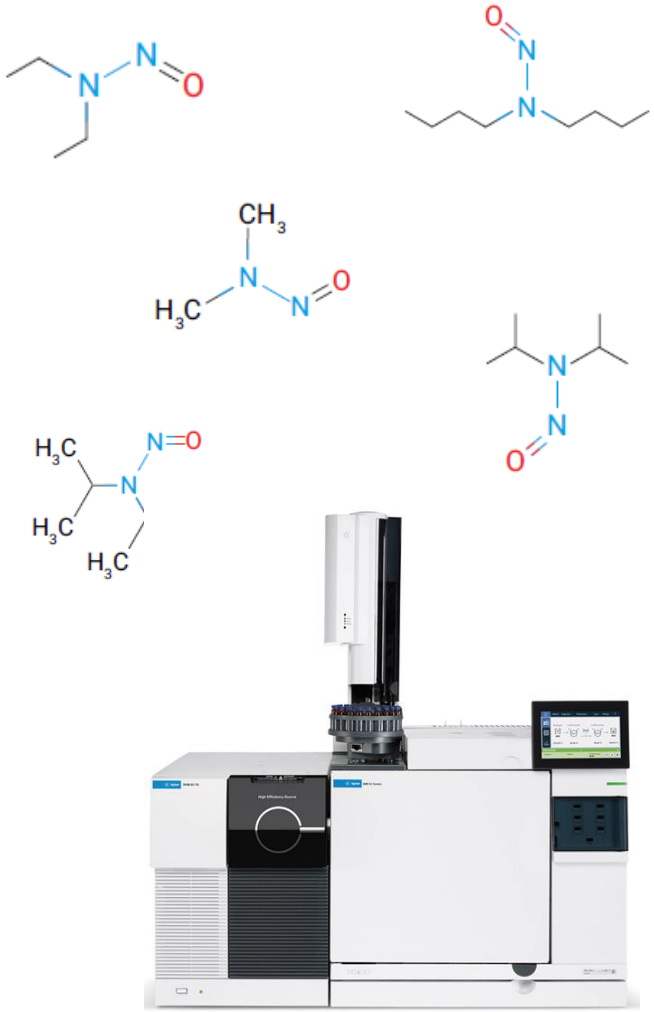
Application Brief



[USP <467> Method Parameter Comparison for the Agilent 8697 and 7697A Headspace Samplers]

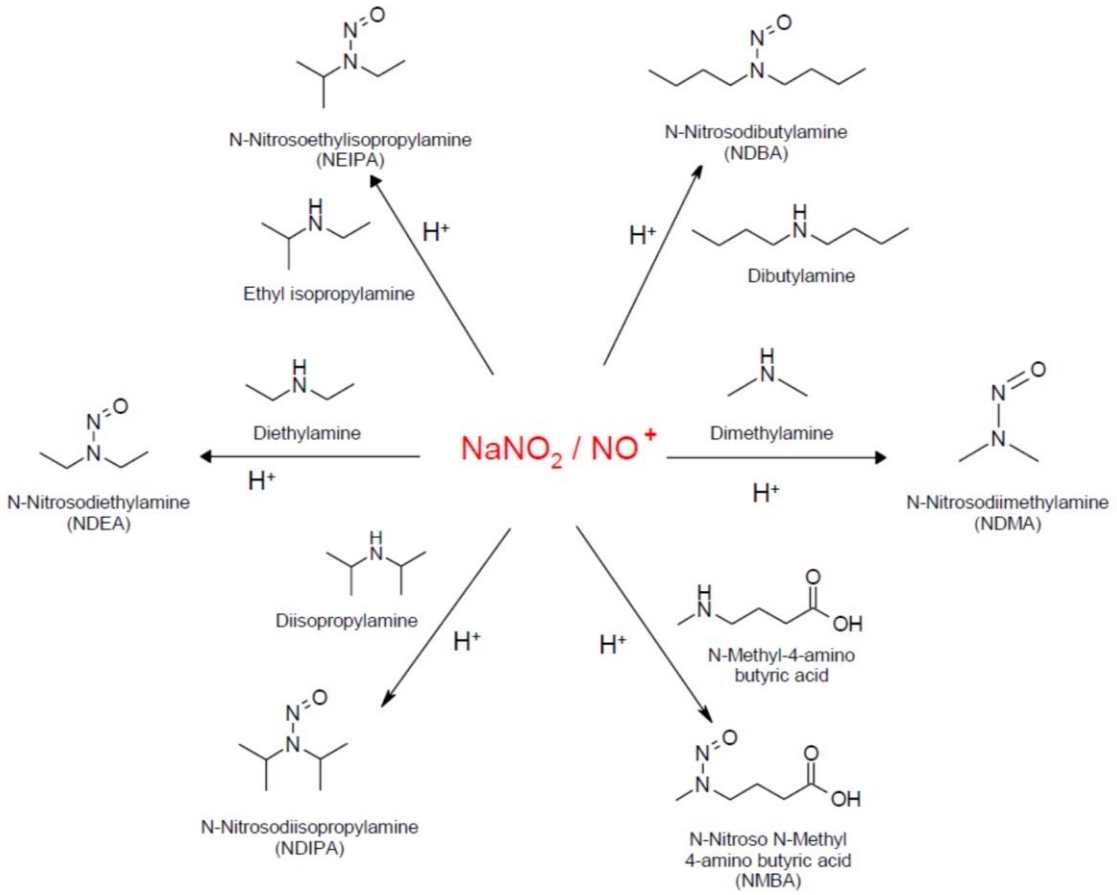
[https://www.agilent.com/cs/library/applications/5991-8032EN.pdf]

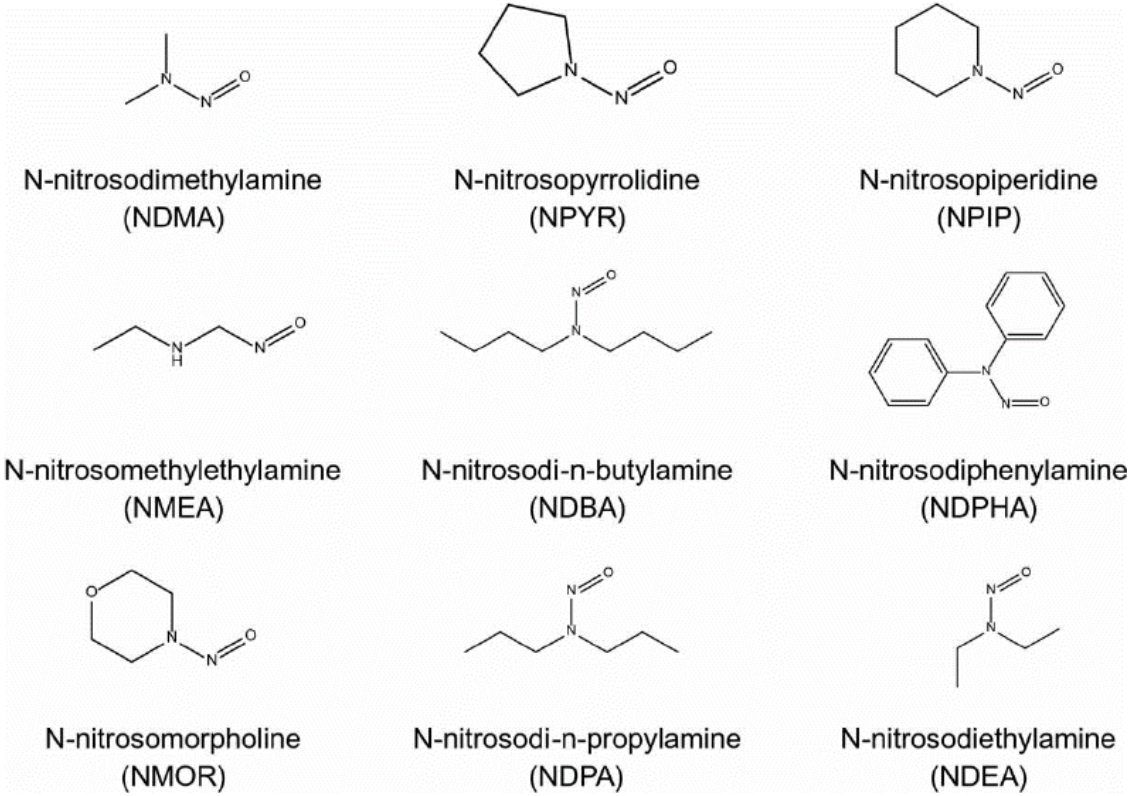
GC/MS Methods For The Accurate Determination of Nitrosamines Produced In The Manufacture Of APIs and Drugs



Agilent
Trusted Answers

What are Nitrosamines?





Nitrosamines are formed when **nitrites** react with a secondary or tertiary amine. The concentration of nitrosamines tends to increase over time, and their formation is enhanced by high temperatures or high acidity.

https://www.lhasalimited.org/Public/Library/2020/ICH%20M7%20-%20Regulatory%20Updates%20and%20Industry%20Practices.pdf

Nitrosamine Impurities Are Not Limited to Sartans & Ranitidine

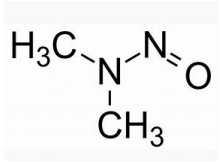
Possible root causes of nitrosamine formation

- Formation of nitrosamine during synthesis i.e. in the presence of raw materials, starting materials and intermediates and/or through incomplete depletion of nitrosamine in subsequent synthesis steps
- Use of sodium nitrite or other nitrites in the presence of secondary or tertiary amines in the course of the API synthesis
- Cross contamination with sodium nitrite despite intensive but inefficient cleaning of the manufacturing equipment
- When solvents such as DMF (dimethylformamide), DMA (dimethylacetamine), or DEA (diethylacetamide) are used in the manufacturing of a drug substance
- Recycled solvents, especially when performed by an external partner
- Raw materials, starting materials, excipients, reagents, etc. that do not come from adequately qualified suppliers

- **Regulatory agencies advises companies on steps to take to avoid nitrosamines in medicines**
- Risky preparations must be tested for nitrosamine contamination via **validated and appropriately sensitive analytical methods** and inform competent authorities of nitrosamine detection, irrespective of the amount detected.

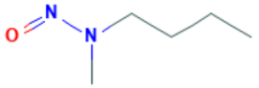
Why is Nitrosamines Analysis Important?

July 2018
FDA recalled Valsartan DP due to NDMA contaminant



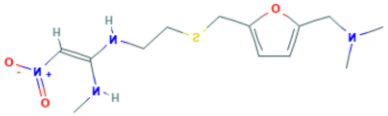
NDMA

Feb 2019
FDA expanded recall; new contaminant identified, NMBA

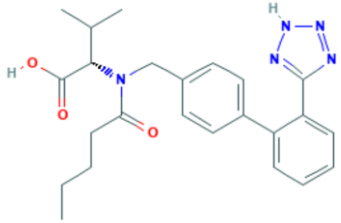


NMBA

April 2020
FDA recalled Ranitidine based drugs

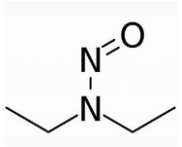


Ranitidine

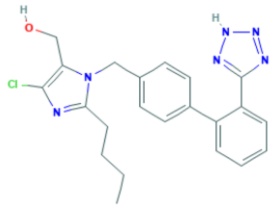


Valsartan

October 2018
FDA expanded recall to other Sartans due to NDMA and NDEA

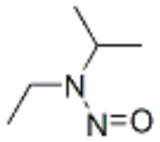


NDEA

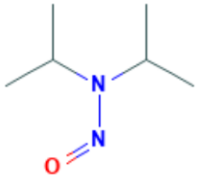


Losartan

April 2019
FDA identified additional contaminants, EIPNA & DIPNA



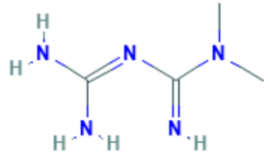
EIPNA



DIPNA

May 2020
FDA recalled Metformin based drugs

Metformin



For detailed info, refer to: FDA Press Releases; EMA Press Releases

Published FDA Testing Methods For The Detection of Nitrosamines

Date	Method	System	Analyte LOQ
1/28/2019	Combined headspace method	Agilent 7890B GC - 5977A MSD - 7697A HS	NDMA 0.10ppm NDEA 0.05ppm
04/19/2019 and later updated 4/21/2019	Combined direct injection method Direct injection GC-MS method	Agilent 7890 GC-7010 QQQ (not declared in the publication)	NDMA 0.013ppm NDEA 0.08ppm NDIPA 0.08ppm NEIPA 0.08ppm NDBA 0.040ppm
4/29/2019	Headspace GC-MS method	Agilent 7890B GC - 5977A MSD - 7697A HS	NDMA 0.05ppm NDEA 0.05ppm NDIPA 0.05ppm NEIPA 0.05ppm
10/17/2019	LC-Triple Quad	Agilent 6420 Triple Quad LC/MS system with APCI source or equivalent	NDMA 0.03ppm
5/21/2019	RapidFire-MS/MS method	Agilent RapidFire-6460C	NDIPA 0.1ppm NEIPA 0.1ppm NMBA 0.1ppm NDBA 0.1ppm Not Recommend for NMDA/NDEA

- The LC-HRMS and RapidFire-MS/MS methods are the first methods FDA has posted for detecting NMBA.

FDA-published testing methods to provide options for regulators and industry to detect NDMA and NDEA impurities

The links below are to FDA-published testing methods to provide options for regulators and industry to detect nitrosamine impurities in ARB drug substances and drug products. These methods should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- Combined headspace method: a GC/MS method that allows determination of both N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) simultaneously
- Combined direct injection method: a GC-MS/MS method that allows for determination of both NDMA and NDEA simultaneously
- Direct injection GC-MS method: a method that can detect NDMA, NDEA, N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA)
- Headspace GC-MS method: a method that can detect NDMA, NDEA, NDIPA, and NEIPA
- LC-HRMS method: a method that can detect NDMA, NDEA, NEIPA, NDIPA, NDBA, and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)
- RapidFire-MS/MS method: a method that can detect NEIPA, NDIPA, NDBA, and NMBA. We do not recommend using this method to detect NDMA or NDEA because it is less sensitive to those impurities.

The LC-HRMS and RapidFire-MS/MS methods are the first methods FDA has posted for detecting NMBA. The European Directorate for the Quality of Medicines (EDQM) has also published methods to detect NDMA and NDEA. FDA has not validated EDQM's methods.

USFDA Methods and Limits Using GC/MS

FDA U.S. FOOD & DRUG ADMINISTRATION

Updated 1/25/19

GC/MS Headspace Method for Detection of NDMA in Valsartan Drug Substance and Drug Products

Background:
Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of valsartan tablets because of the potential for certain products to contain an impurity, N-nitrosodimethylamine (NDMA). This impurity is classified as a probable human carcinogen and is believed to have been introduced into the finished products as a result of the manufacturing process of the drug substance. OTR has been asked to develop a gas chromatography-mass spectrometry (GC/MS) headspace method to detect the presence of NDMA in valsartan drug substance and drug products.

Conclusions:
The OTR method was developed on **drug substance** samples. The method details are reported below. A separate report including full method validation will follow.

Impurity	LOD (ppm)	LOQ (ppm)
N-nitrosodimethylamine (NDMA)	0.05	0.3

FDA U.S. FOOD & DRUG ADMINISTRATION

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Testing and Research
Division of Pharmaceutical Analysis

Updated 1/25/19

Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS-Headspace

Background:
Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of Valsartan tablets because of the potential for certain products to contain an impurity, N-Nitrosodimethylamine (NDMA). A second impurity was subsequently reported, N-Nitrosodiethylamine (NDEA). NDMA and NDEA are classified as probable human carcinogens and were believed to have been introduced into the finished products because of the manufacturing processes used to make the drug substance. OTR has developed a gas chromatography-mass spectrometry (GC/MS) headspace method to detect the presence of NDMA and NDEA in valsartan drug substance.

Conclusions:
The combined method has been validated to simultaneously quantify NDMA and NDEA.

Impurity	LOD (ppm)	LOQ (ppm)
N-Nitrosodimethylamine (NDMA)	0.005	0.10
N-Nitrosodiethylamine (NDEA)	0.02	0.05

SQ with HSS injection

FDA U.S. FOOD & DRUG ADMINISTRATION

Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Testing and Research
Division of Pharmaceutical Analysis

Combine d Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS

Background: Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of valsartan tablets because of the potential for certain products to contain an impurity, N-nitrosodimethylamine (NDMA). This impurity is classified as a probable human carcinogen and is believed to have been introduced into the finished products as a result of the manufacturing process of the drug substance. Subsequently, an additional nitrosamine, N-nitrosodiethylamine (NDEA), has also been detected in some valsartan products. OTR has been asked to develop a gas chromatography-tandem mass spectrometry (GC-MS/MS) method utilizing liquid injection.

Conclusions: The combined method has been validated to simultaneously quantify NDMA and NDEA.

Impurity	Drug Substance Limit of Quantitation (LOQ), ppm	Drug Product Limit of Quantitation (LOQ), ppm
N-nitrosodimethylamine (NDMA)	0.05	0.08
N-nitrosodiethylamine (NDEA)	0.03	0.04
Impurity	**Drug Substance Limit of Detection (LOD), ppm**	**Drug Product Limit of Detection (LOD), ppm**
N-nitrosodimethylamine (NDMA)	0.010	0.015
N-nitrosodiethylamine (NDEA)		

Note: LOQ's determined utilizing the ICH signal to noise approach. S/N = 10
LOD's determined based on the ICH's statistical formula: LOD = [3.3σ ÷ S]
where σ is the standard deviation of y-intercepts for the regression line and S is the slope of the regression line.

TQ with Liquid injection

Latest Methods on GC/MS Updated April 2019

FDA U.S. FOOD & DRUG ADMINISTRATION

4/11/2019

Combined Headspace N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), and N-Nitrosodiisopropylamine (NDIPA) Impurity Assay by GC-MS/MS

Background

Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of valsartan tablets because of the potential for certain products to contain nitrosamine impurities. These impurities: (N-nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-diisopropylnitrosoamine (NDIPA), and N-ethyl-N-isopropylnitrosoamine (NEIPA) are classified as probable human carcinogens and are believed to have been introduced into the finished products because of the manufacturing process. OTR has been asked to develop a gas chromatography-mass spectrometry (GC/MS) headspace method to comprehensively detect the presence of NDMA, NDEA, NDIPA, and NEIPA in angiotensin II receptor blockers (ARBs).

Conclusions

The combined OTR GC/MS headspace method was developed successfully for the simultaneous evaluation of four nitrosamine impurities in ARB drug substance and drug product. The specific sensitivity details of the validated method for each of the four nitrosamine impurities are reported below. The method was developed and validated on valsartan drug substance and drug product.

4 impurities
Single Quad
HSS injection

Impurity	Drug Substance LOQ, ppm	Drug Substance LOD, ppm	Drug Product LOQ, ppm	Drug Product LOD, ppm
NDMA	0.05	0.01	0.05	0.01
NDEA	0.05	0.01	0.05	0.01
NEIPA	0.05	0.025	0.05	0.025
NDIPA	0.05	0.025	0.05	0.025

FDA U.S. FOOD & DRUG ADMINISTRATION

4/11/2019

Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA) Impurity Assay by GC-MS/MS

Background: Valsartan products are used to treat high blood pressure and congestive heart failure. On July 13, 2018, FDA announced a recall of valsartan tablets because of the potential for certain products to contain an impurity, N-nitrosodimethylamine (NDMA). This impurity is classified as a probable human carcinogen and is believed to have been introduced into the finished products as a result of the manufacturing process. Subsequently, an additional nitrosamine, N-nitrosodiethylamine (NDEA), has also been detected in some valsartan products. N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA), and N-Nitrosomethyl-4-amino-butyric acid (NMBA) have also been flagged as potential nitrosamine impurities. OTR has been asked to develop a gas chromatography-tandem mass spectrometry (GC-MS/MS) method utilizing liquid injection to look for all these nitrosamine impurities.

Conclusions: The combined method has been validated to simultaneously quantify NDMA, NDEA, NEIPA, NDIPA, and NDBA in Valsartan API and verified for Valsartan drug products. It should be verified for other sartan API's and drug products.

Impurity	Drug Substance Limit of Quantitation (LOQ), ppm	Drug Product Limit of Quantitation (LOQ), ppm
N-nitrosodimethylamine (NDMA)	0.008	0.013
N-nitrosodiethylamine (NDEA)	0.005	0.008
N-nitrosoethylisopropylamine (NEIPA)	0.005	0.008
N-nitrosodiisopropylamine (NDIPA)	0.005	0.008
N-nitrosodibutylamine (NDBA)	0.025	0.040

5 impurities
Triple Quad
Liquid injection

Why GC/MS for Nitrosamines Analysis?

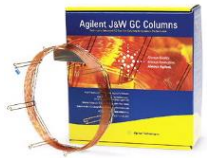
Highlights – GC/MS approaches

1. Cost effective, easy to use
2. Quick implementation in labs
3. More API can be used (100 mg/mL or more) for sample preparation
4. Most APIs are insoluble in Dichloromethane, so doesn't overload column
5. Easy sample preparation
6. All Sartans can be analyzed by a single GC-MS/MS method. No method modification because of API or formulation (tested for Valsartan, Irbesartan, Losartan, Telmisartan, Olmesartan) Lower detection limits can be achieved
7. NMDA has low molecular weight (74.04) and is volatile.

GC/MS Based Targeted Quantitation of Nitrosamines in APIs and Drug Products

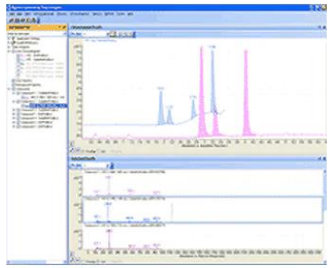
Quantitative Analysis of **4** Nitrosamines

8890 GC/7697A HSS/ 5977B MSD



PN: 122-7033
J&W DB-WAX GC Column
30 m, 0.25 mm, 0.50 µm, 7 inch cage

GC Columns & Supplies



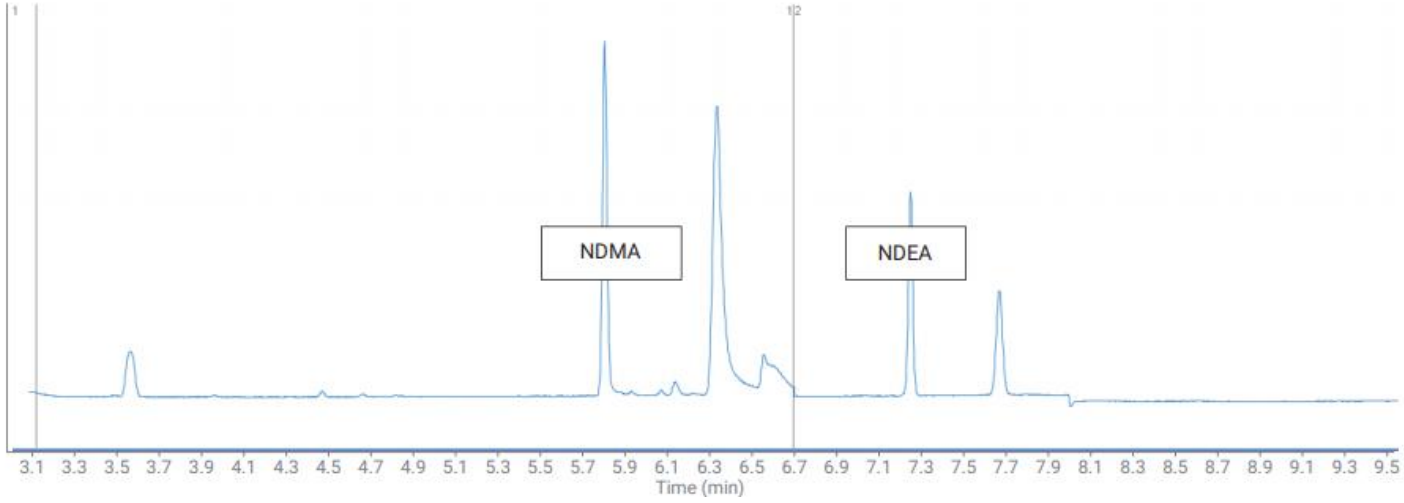
MassHunter

Quantitative Analysis of **5** Nitrosamines

8890 GC/7693 LS/ 7010B TQ

PN: CP9206
J&W VF-WAXms GC Column
30 m, 0.25 mm, 1.00 µm, 7 inch cage

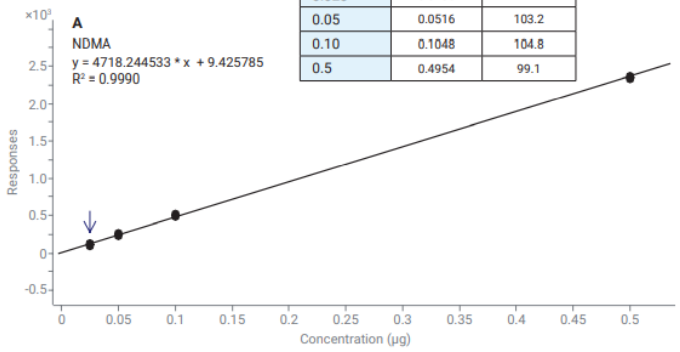
Analysis NDMA & NDEA Using the Agilent 7697A Headspace Sampler, 8890/5977 GC/MSD System



	Target LOQ	Average Recovery	Average Recovery
	1 ppm	0.05 ppm	0.10 ppm
NDMA	0.10	0.056	0.11
NDEA	0.05	0.057	0.11

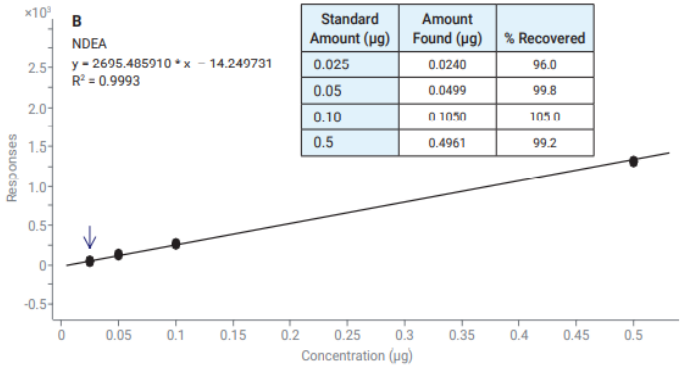
NDMA calibration stats

Standard Amount (µg)	Amount Found (µg)	% Recovered
0.025	0.0235	92.9
0.05	0.0516	103.2
0.10	0.1048	104.8
0.5	0.4954	99.1



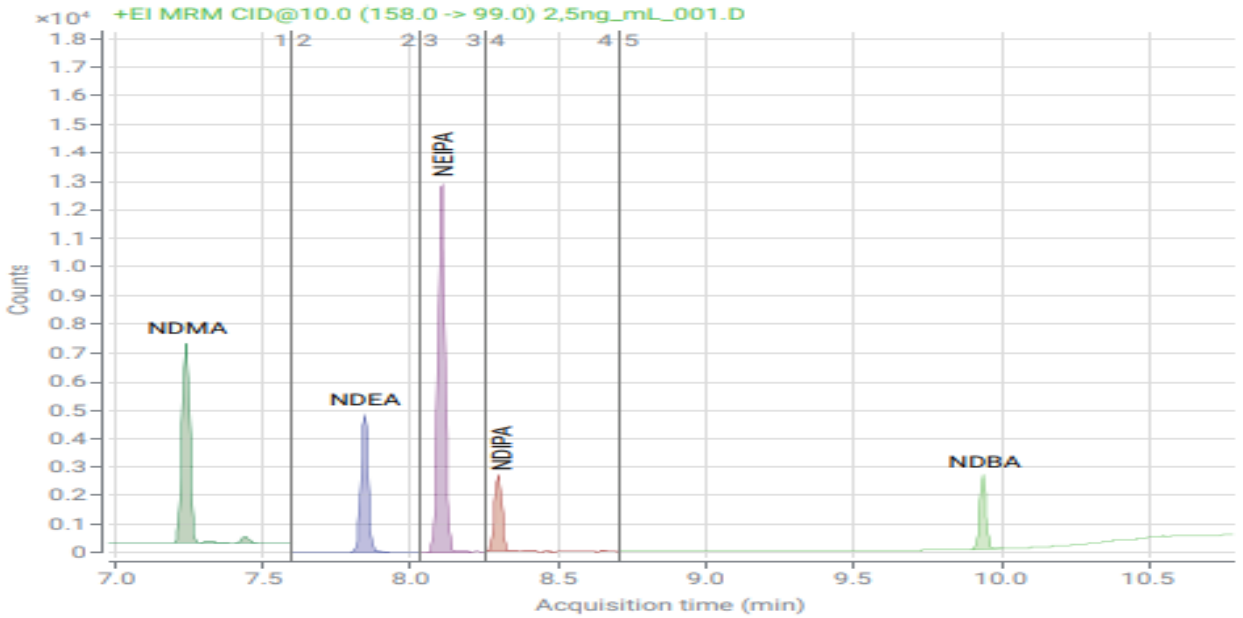
NDEA calibration stats

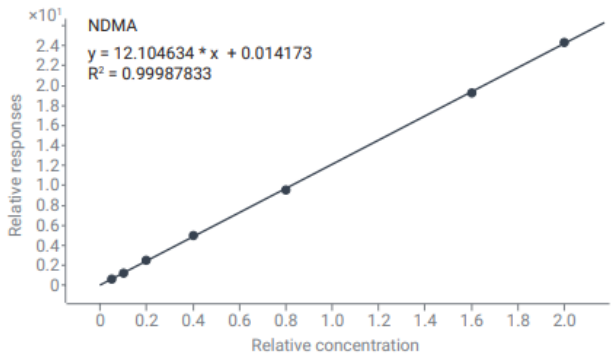
Standard Amount (µg)	Amount Found (µg)	% Recovered
0.025	0.0240	96.0
0.05	0.0499	99.8
0.10	0.1050	105.0
0.5	0.4961	99.2

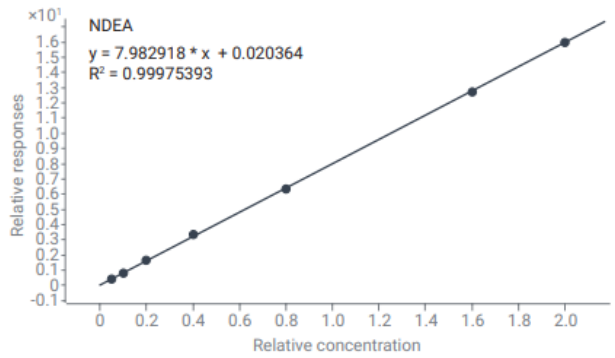


8890 GC/7697A HSS/ 5977B MSD

Analysis of Five Nitrosamine Impurities in Drug Products and Drug Substances Using Agilent GC/MS/MS



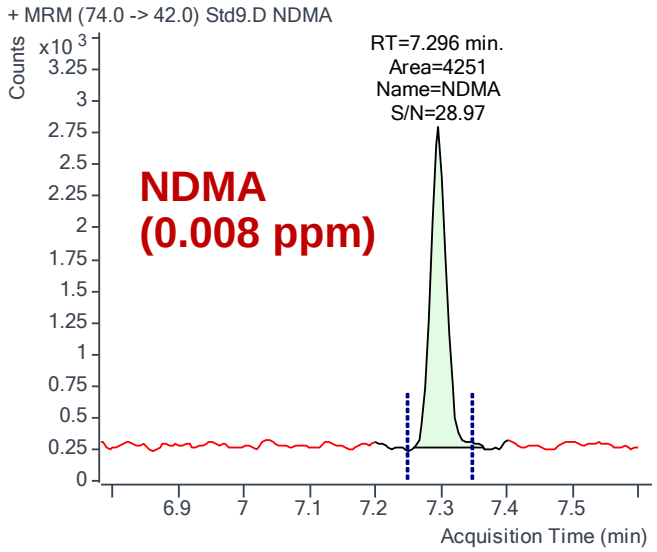


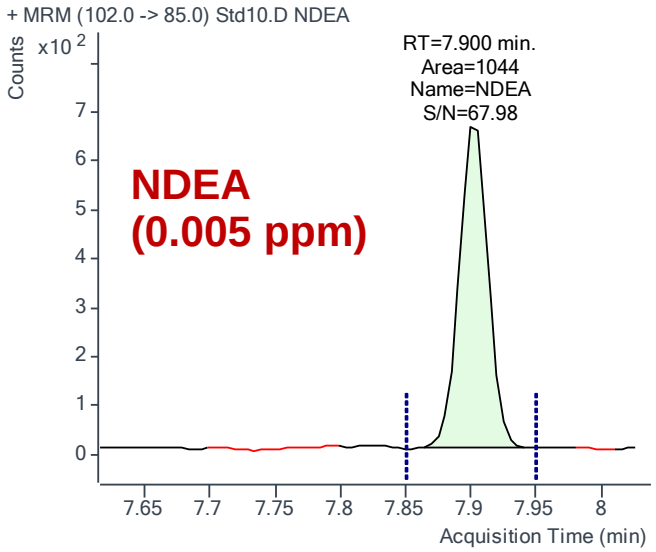


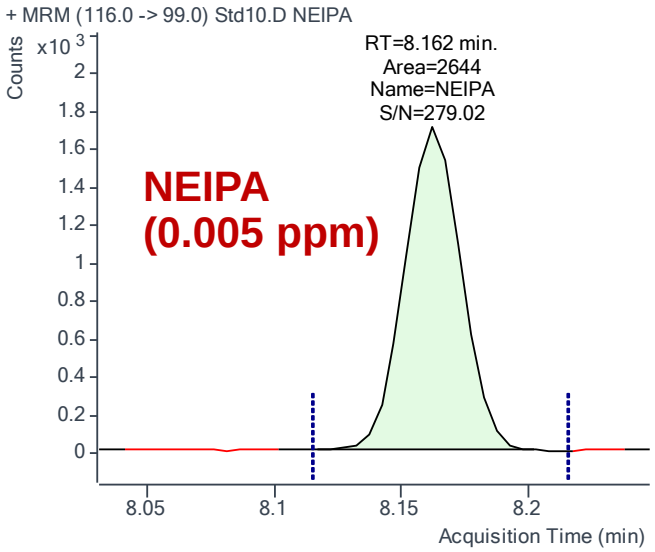
Impurity	FDA LOQ (ppm)	LOQ (Obtained, in ppm)	Improvement Factor
NDMA	0.008	0.0025	>3
NDEA	0.005	0.0005	10
NEIPA	0.005	0.00025	20
NDIPA	0.005	0.0025	2
NDBA	0.025	0.008	>3

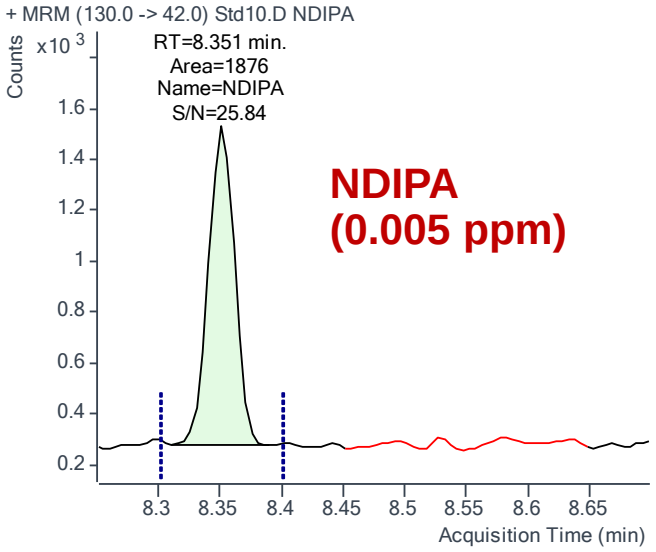
8890 GC/7693 LS/ 7010B TQ

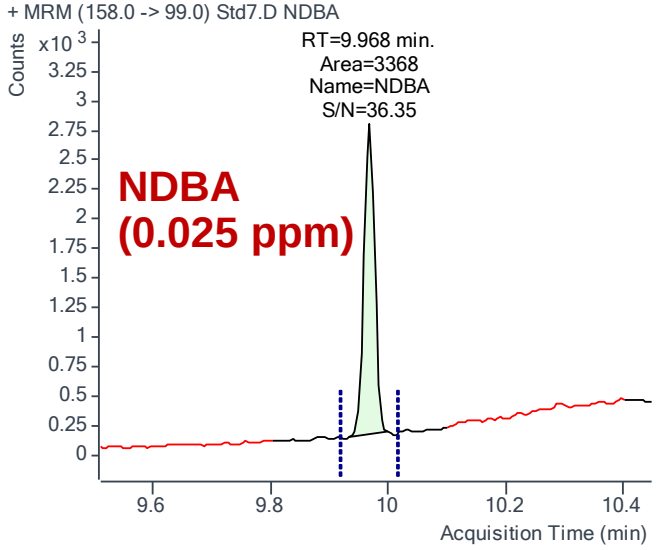
Response at FDA Specified LOQ







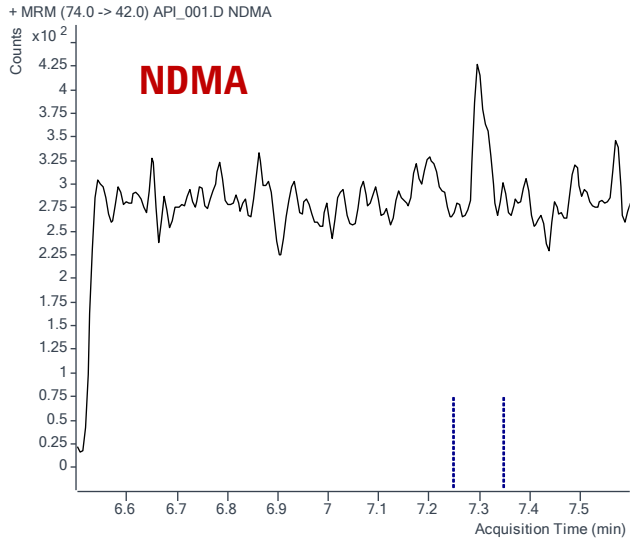


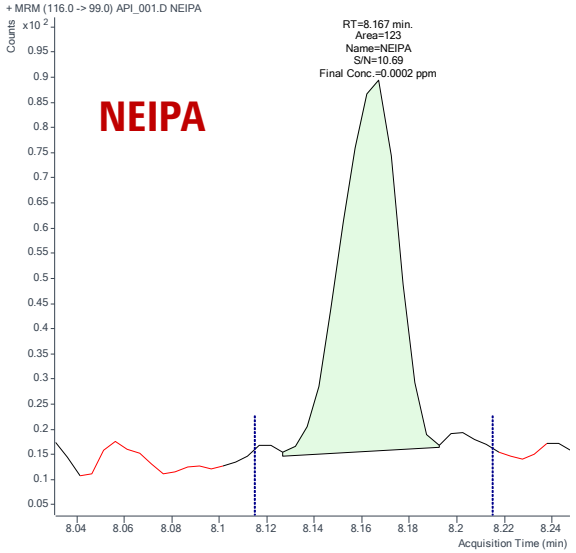


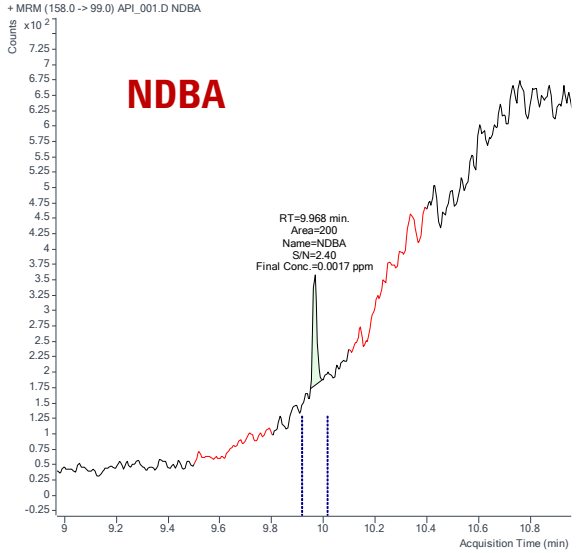
S/N at FDA LoQ

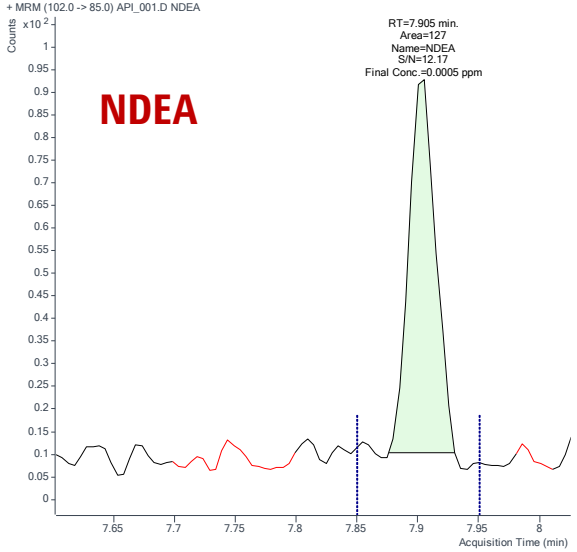
Name	S/N
NDMA	28.97
NDEA	67.98
NEIPA	279.02
NDIPA	25.84
NDBA	36.35

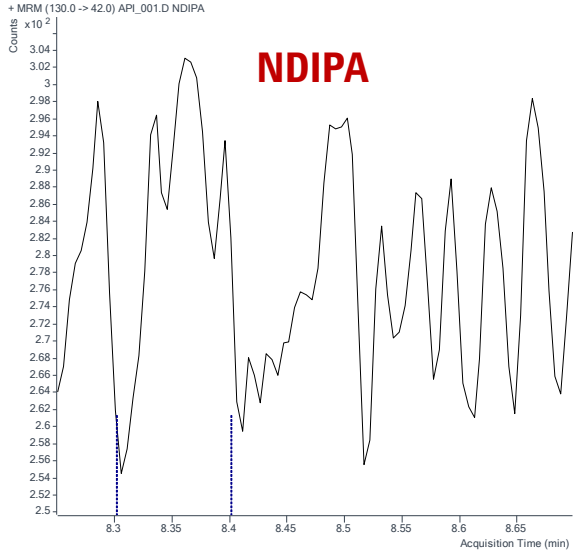
Sample Results (Valsartan API, Extraction 1)







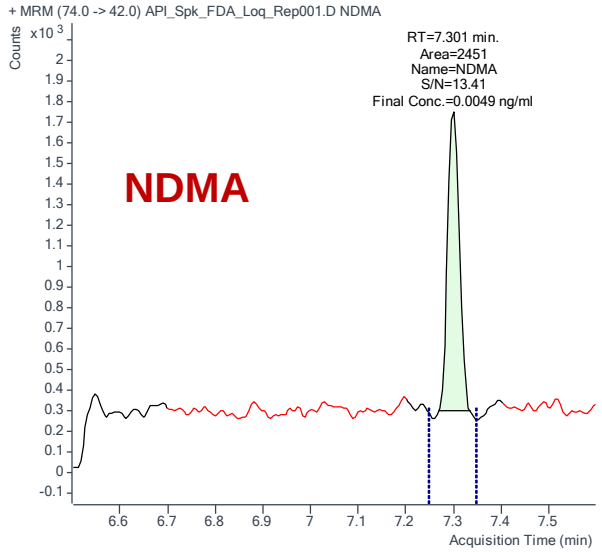


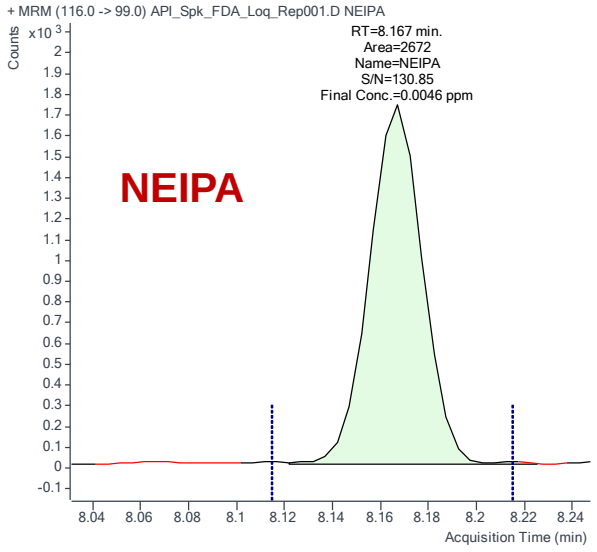


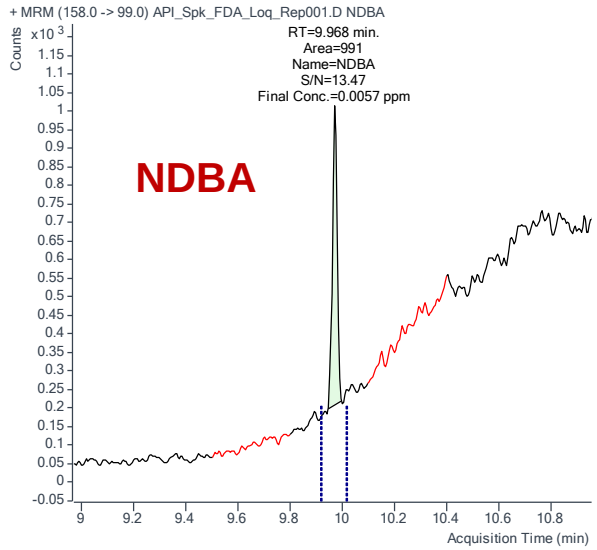
Sample Results

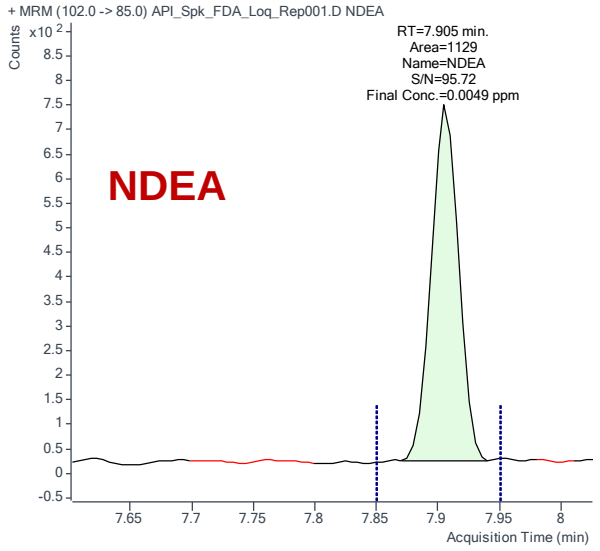
Name	FDA LoQs (ppm)	LoQ Obtained, (ppm)	Sample Results (ppm)
NDMA	0.008	0.0025	ND
NDEA	0.005	0.0005	0.0005
NEIPA	0.005	0.00025	0.0002
NDIPA	0.005	0.0025	ND
NDBA	0.025	0.008	BLQ

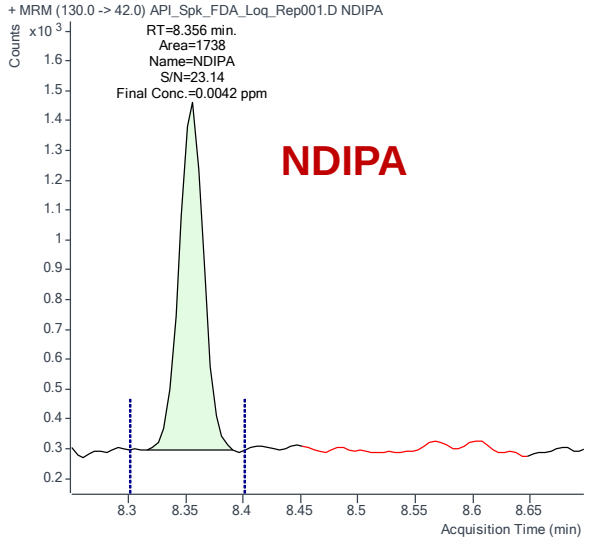
Spiking Studies At 0.005 ppm









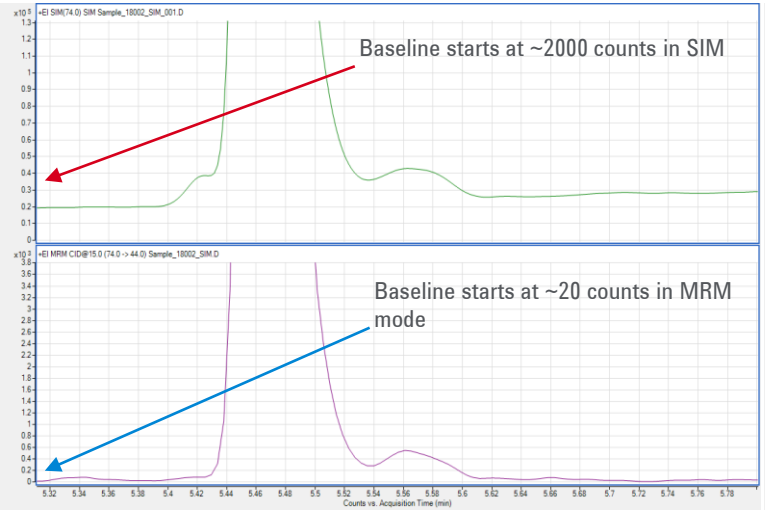


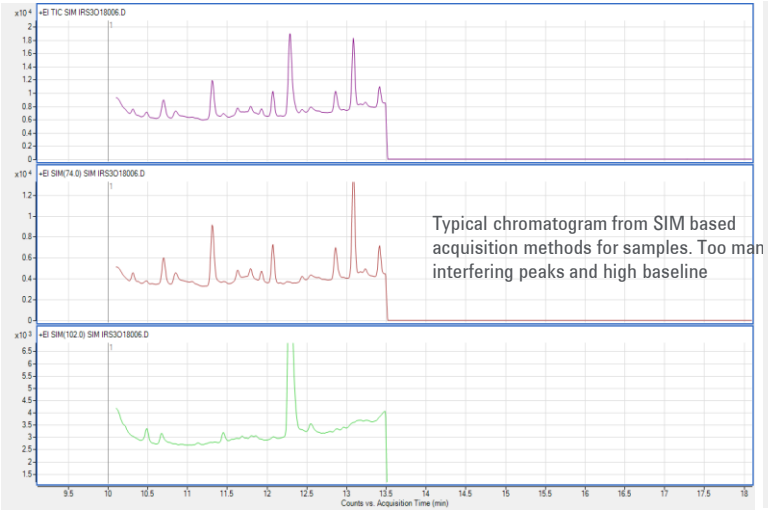
Sample Recovery (0.005 ppm)

Name	Spiking Level (ppm)	Sample Results (ppm)	Recovery (%)
NDMA	0.005	0.0049	98
NDEA	0.005	0.0049	98
NEIPA	0.005	0.0046	92
NDIPA	0.005	0.0042	84
NDBA	0.005	0.0057	114

Detection Enhancements with GC/TQ

1. Lower baseline ensures better S/N ratio hence better the method LOQs
2. Lower LOQs ensure a future proof system in the possibility of further stringent limits
3. MRM transitions reduce the interferences and thereby increase method selectivity and specificity
4. Additional Qualifier MRM transitions can be used to confirm the presence/absence of the impurities
5. Better linearities across the dynamic range due to method specificity
6. Removal of interferences using MRM acquisition ensures reliable quantification





Summary for GCMSD & GCMSMS

Regulatory agencies advise companies on steps to take to avoid nitrosamines in medicines

Risky preparations must be tested for nitrosamine contamination via validated and appropriately sensitive analytical methods

Agilent 8890 GC coupled with Agilent 5977 GC/MSD or Agilent 7010B GC/TQ comply with all regulatory directives as well as meet and exceed stringent detection limits for the trace-level Nitrosamine impurities analyses

Agilent GC/MS equipped with a high-efficiency source offers excellent sensitivity, repeatability and precision while outperforming regulatory limits. The GC/MS method is 8-10 times more sensitive than required by current regulations

What are the Challenges of NMDA Analysis using GC/MS?

Some concerns with GC/MS

1. GCMS may not suitable for detection of **NMBA**, which is non-volatile.

2. GCMS is not the right technique for detecting **NDMA in ranitidine**, due to high temperature degradation of ranitidine into NDMA.

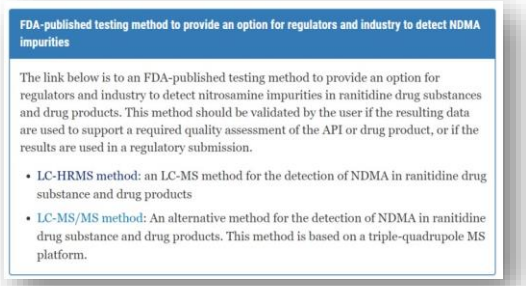
3. LOQ depends to a large extent on the purity of the solvent. There are several interferences from NMP, DMSO and DCM

4. Two headspace methods with GC/SQ (NMP and DMSO). DMSO reacts with **NDEA** at higher temperature. Higher HSS temperature affects response

Ranitidine Based Drugs

- Ranitidine is a histamine-2 receptor antagonist (acid inhibitor or H2 blocker) and is available as both prescription and over-the-counter drug to treat acid reflux. Examples of H2 receptor blockers include: Ranitidine (Zantac), Nizatidine (Axid), Famotidine (Pepcid, Pepcid AC) and Cimetidine (Tagamet, Tagamet HB).
- N-nitrosodimethylamine (NDMA) impurity was detected in some ranitidine products and the levels were found to increases with time and temperature, and thus ranitidine drugs were recently recalled from the U.S. market
- Regulatory agencies (for e.g. including US Food and Drug administration (US FDA)) provided guidance on the detection and quantification of NDMA impurity in ranitidine based drugs

US FDA

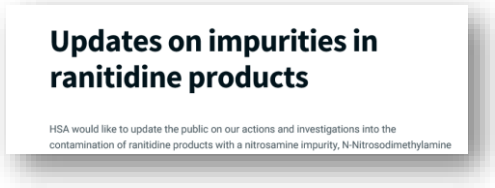

FDA-published testing method to provide an option for regulators and industry to detect NDMA impurities

The link below is to an FDA-published testing method to provide an option for regulators and industry to detect nitrosamine impurities in ranitidine drug substances and drug products. This method should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission.

- LC-HRMS method: an LC-MS method for the detection of NDMA in ranitidine drug substance and drug products
- LC-MS/MS method: An alternative method for the detection of NDMA in ranitidine drug substance and drug products. This method is based on a triple-quadrupole MS platform.

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine

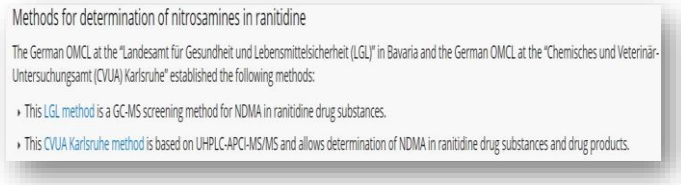
HAS, Singapore


Updates on impurities in ranitidine products

HSA would like to update the public on our actions and investigations into the contamination of ranitidine products with a nitrosamine impurity, N-Nitrosodimethylamine

https://www.hsa.gov.sg/announcements/safety-alert/updates-on-impurities-in-ranitidine-products

Council of Europe


Methods for determination of nitrosamines in ranitidine

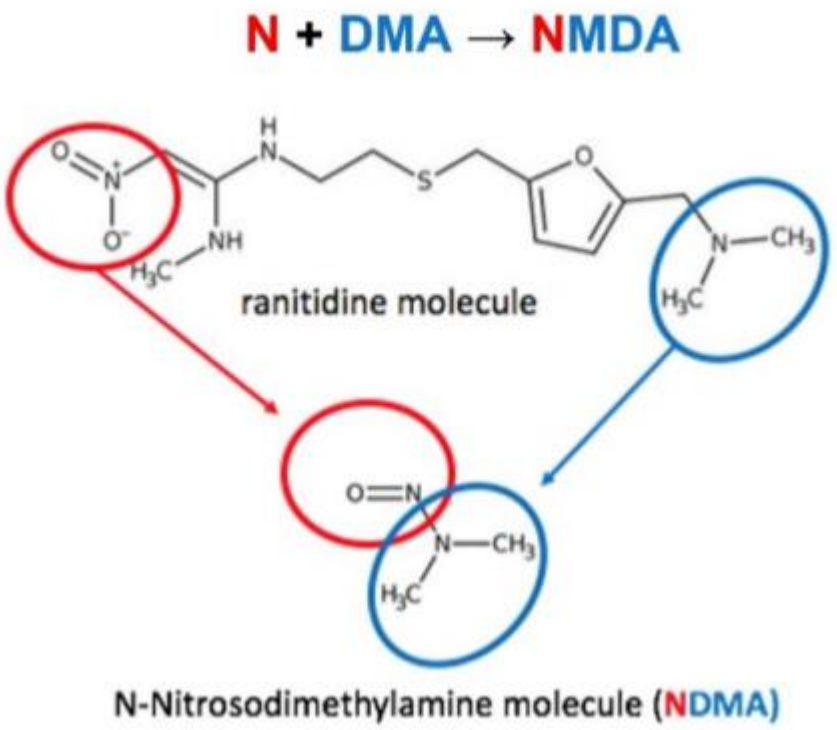
The German OMCL at the "Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)" in Bavaria and the German OMCL at the "Chemisches und Veterinär-Untersuchungsamt (CVUA) Karlsruhe" established the following methods:

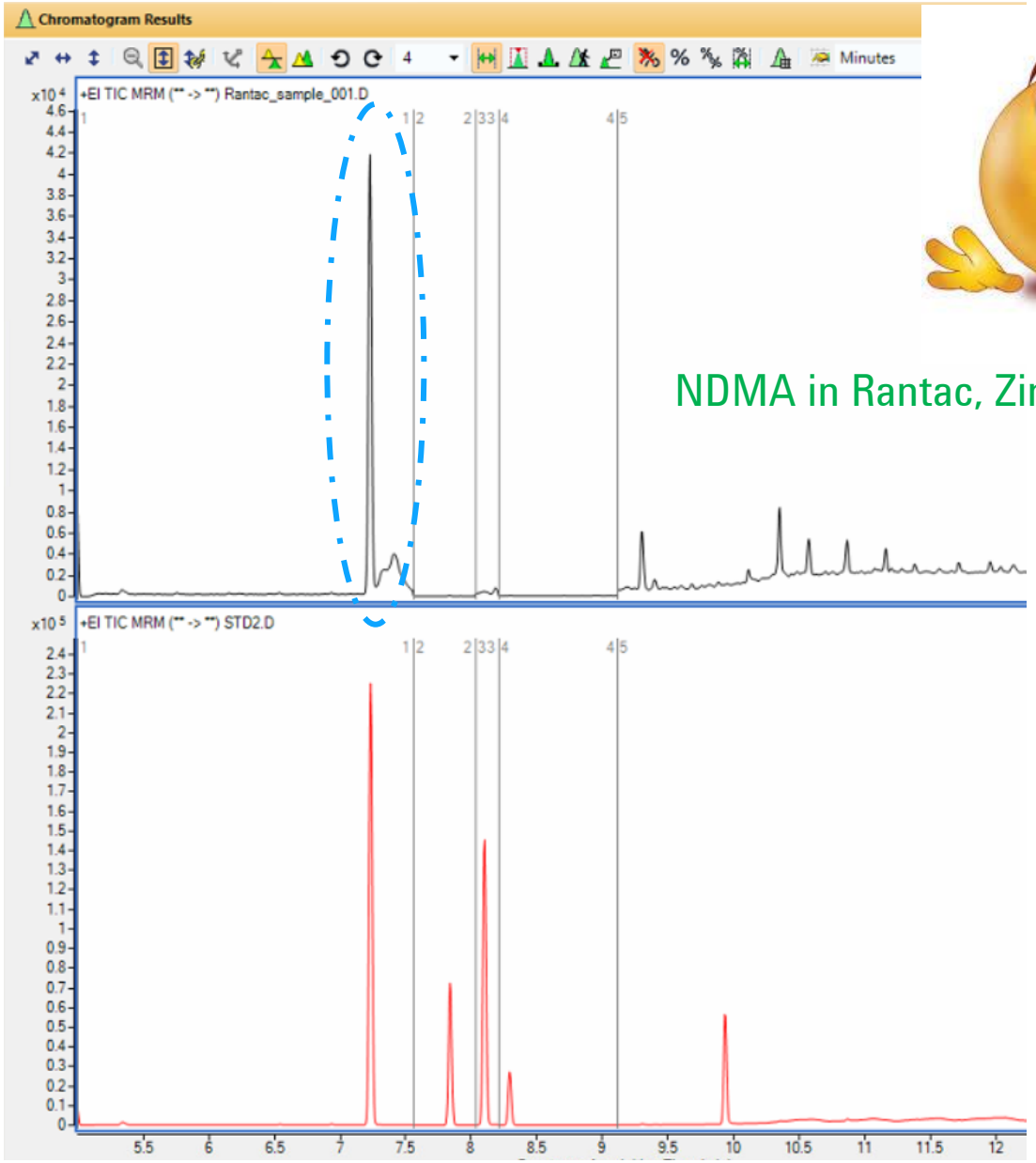
- This LGL method is a GC-MS screening method for NDMA in ranitidine drug substances.
- This CVUA Karlsruhe method is based on UHPLC-APCI-MS/MS and allows determination of NDMA in ranitidine drug substances and drug products.

https://www.edqm.eu/en/ad-hoc-projects-omcl-network

Ranitidine and NDMA

-Ranitidine by GCMS results in elevated levels of NDMA due to conversion at the injector port





NDMA in Rantac, Zintac>100 ppm

Variety of Analytical Solutions for Nitrosamines in Drug Substances and Products

Points to consider:

- Type of nitrosamines
- LOD, LOQ
- API and drug substance

GC-MS Based Screening and Targeted Quantitation

Columns

7890B GC System

5977B GC/MSD

7010B GC/QQQ

High Throughput Gerstel Anatune GC/MS/MS Screening

Mass Hunter Software

LC-MS Based Screening, Targeted and Untargeted Quantitation

Columns

Rapid Fire- MS/MS

Infinity II LC

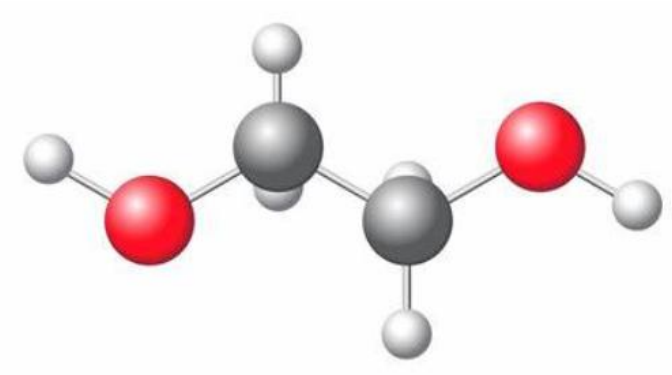
6470B LC-TQ

6495C LC-TQ

6546 QTOF

Agilent

Estimation of Ethylene Glycol and Diethylene Glycol in Propylene Glycol, Glycerin, and Syrup Samples



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Estimation of Ethylene Glycol and Diethylene Glycol in Propylene Glycol, Glycerin, and Syrup Samples

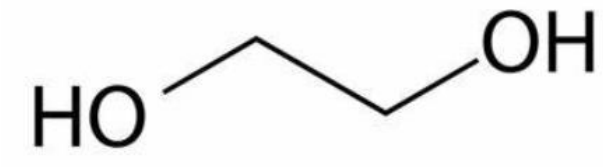
Propylene glycol and glycerin are commonly used in medicinal syrups as excipients.

The solubility of active ingredients is enhanced by their use during formulation.

These excipients should be tested for any contamination by ethylene glycol (EG) and diethylene glycol (DEG) as mentioned in regulations such as Indian Pharmacopeia and USP-NF monographs. Some USP-NF monographs include, as part of the applicable identity testing, a limit test for DEG and EG. The relevant safety limit for DEG and EG is not more than (NMT) 0.10%, as recognized by the applicable USP-NF monograph.

Ethylene Glycol and Diethylene Glycol in Syrup Samples

Ethylene glycol (EG)

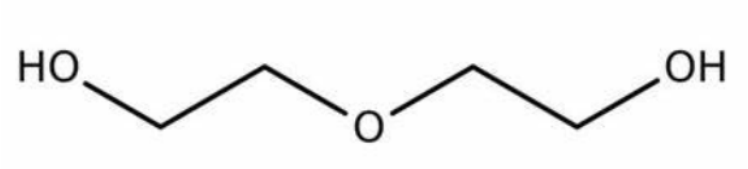


$C_2H_6O_2$

MW : 62.068

Bp : 197.3 °C

Diethylene glycol (DEG)



$C_4H_{10}O_3$

MW : 106.12

Bp : 245 °C

Instrument parameters

Parameter	Value
Inlet Temperature	250 °C
Inlet Liner	Ultra Inert, low pressure drop, split liner, 4 mm ID (p/n 5190-2295)
Inlet Septa	Inlet septa, long life, 11 mm (p/n 8010-0239)
Injection Volume	0.5 µL
Column	Agilent J&W DB-624 GC column, 30 m × 0.53 mm × 3.00 µm, 7 inch cage, (p/n: 125-1334
Column Flow	Helium, 2.5 mL/min
Split Ratio	10:1
Oven Program	70 °C for 1 min
	6 °C/min to 150 °C, hold 3 min
	25 °C /min to 245 °C, hold 12 min
FID Temperature	250 °C
FID H2 Flow	40 mL/min
FID Air Flow	300 mL/min
FID Make Up Gas	Nitrogen, 25 mL/min

Standard calibration & Sample preparation

Standard solution-1

weighing 100 mg each of EG and DEG in a 100 mL volumetric flask

making up the volume to 100 mL with methanol

with thorough mixing

This solution has a concentration of 1,000 µg/mL for EG and DEG

IS solution-1

weighing 100 mg of 1,3-propanediol in a 100 mL volumetric flask

making up the volume to 100 mL with methanol with thorough mixing.

This solution has a concentration of 1,000 µg/mL for 1,3-propanediol.

Sample

500 mg of sample was added to a 10 mL volumetric flask.

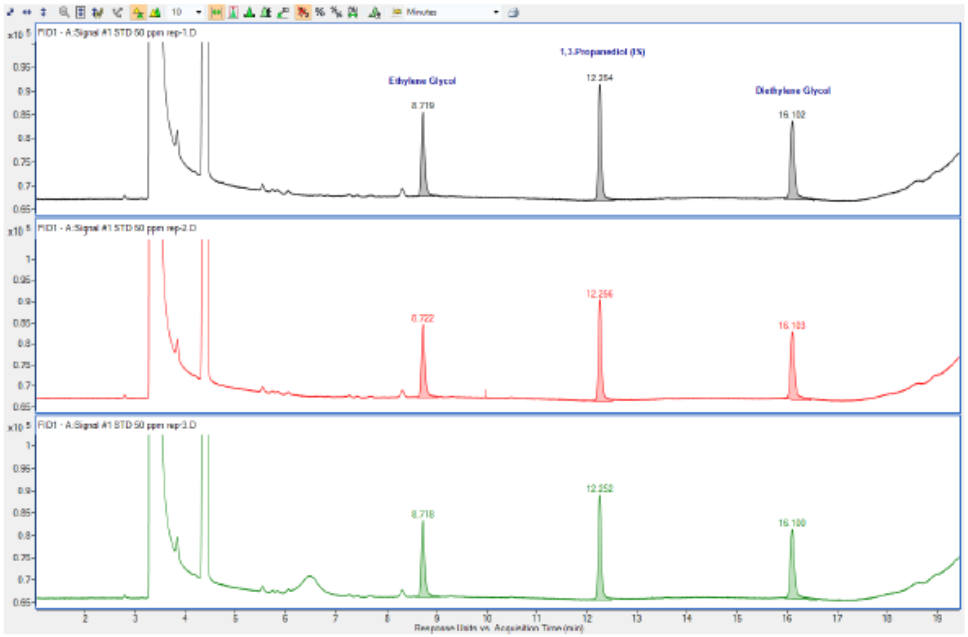
500 µL of IS solution was added

the volume was made up to 10 mL with methanol

with thorough shaking.

Three replicates of 50 µg/mL EG and DEG standards with IS using methanol as diluent.

	Standard Solution-1, 1,000 µg/mL (mL)	IS Working Solution, 1,000 µg/mL (mL)	Make Up Volume with Methanol (mL)	Final Concentration for EG and DEG (µg/mL)	Final Concentration for 1,3-propanediol (µg/mL)
Calibration Standard-6	5	0.5	10	500	50
Calibration Standard-5	2.5	0.5	10	250	50
Calibration Standard-4	1	0.5	10	100	50
Calibration Standard-3	0.5	0.5	10	50	50
Calibration Standard-2	0.25	0.5	10	25	50
Calibration Standard-1	0.1	0.5	10	10	50



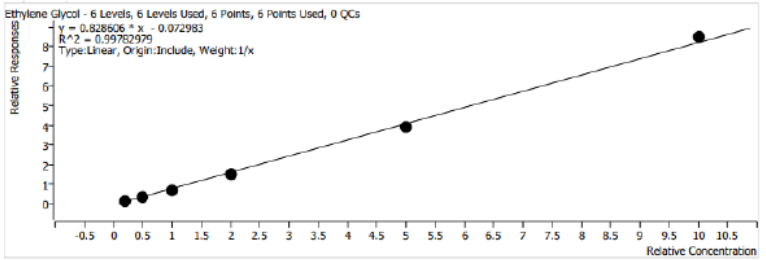
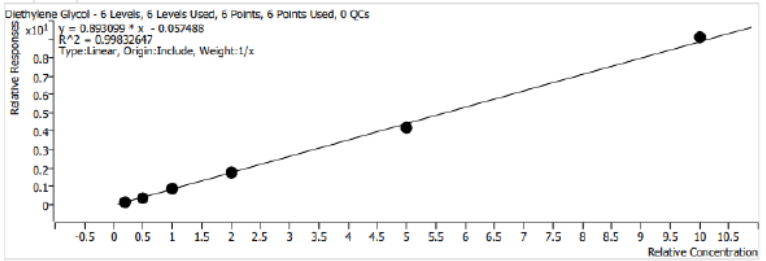


Figure 2. Six-point calibration of EG for 10, 25, 50, 100, 250, and 500 µg/mL, respectively.

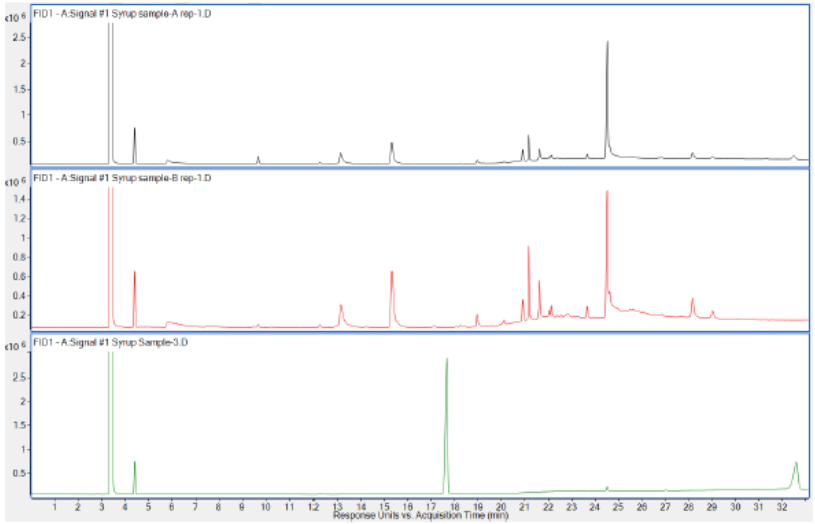


Results Standards and Spike in Syrup sample

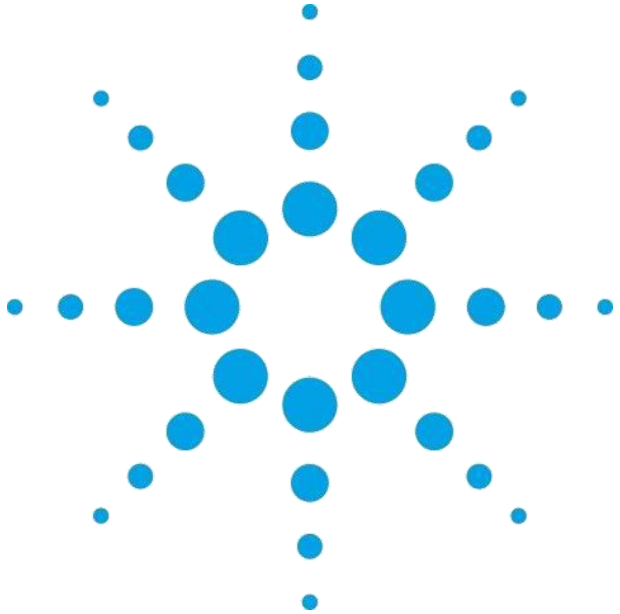
	IS RT (min)	IS Area (Counts)
STD-1_10 µg/mL	12.249	113107
STD-2_25 µg/mL	12.252	109688
STD-3_50 µg/mL	12.255	106848
STD-4_100 µg/mL	12.255	110376
STD-5_250 µg/mL	12.244	117225
STD-6_500 µg/mL	12.255	111914
Mean	12.252	111526.333
SD	0.0045	3513.21
%RSD	0.036	3.15

Spiked Sample	EG Results		DEG Results	
	Calculated Amount (µg/mL)	Recovery (%)	Calculated Amount (µg/mL)	Recovery (%)
200 µg/mL spike-1	208.04	104.02	185.17	92.585
200 µg/mL spike-2	214.14	107.07	187.7	93.85
200 µg/mL spike-3	210.9	105.45	181.68	90.84
Mean	211.03	105.51	184.85	92.43
SD	3.052		3.023	
%RSD	1.446		1.635	

Spiked Sample	EG Results		DEG Results	
	Calculated Amount (µg/mL)	Recovery (%)	Calculated Amount (µg/mL)	Recovery (%)
500 µg/mL spike-1	433.18	86.636	389.61	77.922
500 µg/mL spike-2	429.64	85.928	392.47	78.494
500 µg/mL spike-3	431.82	86.364	385.83	77.166
Mean	431.55	86.31	389.30	77.86
SD	1.786		3.331	
%RSD	0.414		0.856	



Chromatograms of three different brands of syrup samples



Thank you for your attention!

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