



## PURPOSE

The determination of residual solvents by USP <467> in Biopharmaceuticals including all drug substance, excipients and products is performed by utilizing Gas Chromatogram with Flame Ionization Detector (GC/FID). However, the detection of limits by GC/FID for most Class 2c compounds, including formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, N-methylpyrrolidone, and sulfolane are greater than the USP guideline. Also the limit of detection by GC/FID for Class 1 compound: benzene is very close to LOD of ICH regulation. In addition, GC/FID has limited application when unknown peaks or co-elutions were obtained.

This poster summarizes a screening test method using Gas Chromatograph with Mass Spectrometer (GC/MS) that meets the limitation test requirement for all USP Class 1, Class 2 and Class 3 residual solvents regulated by USP <467> and provides the additional flexibility of NIST library search based identification.

## METHODS

The developed and optimized GC/MS method combines two mass detection modes, SIM and Scan. USP Class 1 residual solvents are analyzed by Headspace GC/MS SIM mode; USP Class 2a & 2b are analyzed by Headspace GC/MS Scan mode. USP Class 2c are analyzed by Direct Injection GC/MS Scan mode. USP Class 3 solvents are identified by a NIST library search.

The details about instrument conditions were listed as follows,

**GC/MS System:** Agilent, 6890N GC with MS 5975 Inert XL Mass Selective Detector with EI Mode.

**Mass Mode:** SIM and Scan Mode

**Data Analysis:** Software: G1701DA ChemStation, Revision Code: D.02.00.

**GC Column:** Phenomenex, 30 m x 0.25 mm ID, 1.4 µm film thickness.

**Oven Temp Program:** 40°C hold 6 min, 40°C to 120°C at 10°C/min, 120°C to 210°C at 30°C/min hold 3 min.

**Total Run Time:** 20 min.

**Injection Temp:** 140°C.

**MS Source Temp:** 230°C.

**Flow Rate:** 1.0 mL/min.

**Split Ratio:** 5:1

**Autosampler:** CTC, Combi PAL with Incubator.

**Headspace Conditions:** Oven Temp: 80°C, Syringe Temp: 105°C, Transfer Line Temp: 105°C.

## RESULTS

For the analysis of residual solvents, sample preparation and introduction are done by static headspace, but for Class 3 residual solvents, the directly injection is chosen since Class 3 residual solvents have higher boiling points with higher water solubility. The mass ions of all USP Class residual solvents with other information were listed in Table 1 and Table 2.

Table 1: USP Class 1 and Class 2 Residual Solvents Information

No.	Item	Solvent	Class	Formula	Risk Assessment	Limit Conc. (ppm)	CAS #	Mass Ions
1	1	Benzene	1	C <sub>6</sub> H <sub>6</sub>	Avoid Use, See Limits	2	7143-2	78, 77, 50, 51, 52
2	2	Carbon tetrachloride		CCl <sub>4</sub>		4	56-23-5	117, 119, 121, 82, 47
3	3	1,2-Dichloroethane		C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>		5	107-06-2	62, 64, 49, 63, 98
4	4	1,1-Dichloroethane		C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>		8	75-35-4	61, 96, 98, 63, 60
5	5	1,1,1-Trichloroethane		C <sub>2</sub> H <sub>3</sub> Cl <sub>3</sub>		10	71-55-6	97, 99, 61, 117, 119
6	1	Acetonitrile	2a	CH <sub>3</sub> CN	Use Ok, See Limit Levels	410	75-05-8	41, 40, 39, 38
7	2	Chlorobenzene		C <sub>6</sub> H <sub>5</sub> Cl		360	108-90-7	123, 77, 114, 51, 50
8	3	Cyclohexane		C <sub>6</sub> H <sub>12</sub>		3880	110-82-7	56, 94, 41, 55, 42
9	4	cis-1,2-Dichloroethane		C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>		935	156-29-2	61, 96, 98, 63, 60
10	5	trans-1,2-Dichloroethane		C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>		935	156-60-5	61, 96, 98, 63, 60
11	6	1,4-Dioxane		C <sub>6</sub> H <sub>10</sub> O		380	128-91-1	88, 58, 29, 31, 43
12	7	Methanol		C <sub>1</sub> H <sub>4</sub> O		3000	67-56-1	31, 32, 29, 30
13	8	Methylcyclohexane		C <sub>7</sub> H <sub>14</sub>		1180	108-87-2	83, 55, 41, 98, 56
14	9	Methylene chloride		C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub>		600	75-09-2	49, 84, 86, 51, 47
15	10	Tetrahydrofuran		C <sub>4</sub> H <sub>8</sub> O		720	109-99-9	42, 72, 71, 41, 39
16	11	Toluene	C <sub>7</sub> H <sub>8</sub>	890	108-88-3	91, 92, 65, 63, 98		
17	12	Ethylbenzene	C <sub>8</sub> H <sub>10</sub>	369	100-41-4	91, 106, 77, 65, 51		
18	13	m-Xylene	C <sub>8</sub> H <sub>10</sub>	1302	108-38-3	91, 106, 105, 77, 51		
19	14	p-Xylene	C <sub>8</sub> H <sub>10</sub>	304	106-42-3	91, 106, 105, 77, 51		
20	15	o-Xylene	C <sub>8</sub> H <sub>10</sub>	195	95-47-6	91, 106, 105, 77, 51		
21	16	Chloroform	CHCl <sub>3</sub>	60	67-66-3	83, 85, 47, 87, 48		
22	17	1,2-Dimethoxyethane	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	100	110-71-4	45, 60, 29, 90, 58		
23	18	Hexane	C <sub>6</sub> H <sub>14</sub>	290	110-54-3	57, 43, 41, 56, 86		
24	19	Methylbutyl stearate	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	50	591-78-6	43, 58, 57, 101, 85		
25	20	Nitromethane	CH <sub>3</sub> NO <sub>2</sub>	50	75-52-5	30, 61, 46, 60, 45		
26	21	Pyridine	C <sub>5</sub> H <sub>5</sub> N	200	110-86-1	79, 52, 51, 50, 78		
27	22	Tetrahydrofuran	C <sub>4</sub> H <sub>8</sub> O	100	109-99-9	104, 132, 91, 117, 115		
28	23	Trichloroethylene	C <sub>2</sub> HCl <sub>3</sub>	80	79-01-6	130, 132, 95, 97, 60		
29	24	N,N-Dimethylacetamide	C <sub>4</sub> H <sub>9</sub> NO	1090	127-19-5	44, 87, 43, 42, 45		
30	25	N,N-Dimethylformamide	C <sub>3</sub> H <sub>7</sub> NO	880	68-12-2	73, 44, 42, 73, 43		
31	26	2-Ethoxyethanol	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	160	110-90-5	31, 39, 45, 72, 29		
32	27	Ethylene glycol	C <sub>2</sub> H <sub>6</sub> O <sub>2</sub>	620	107-21-1	31, 33, 43, 29, 62		
33	28	Formamide	CH <sub>3</sub> NO	220	75-12-7	45, 29, 44, 43		
34	29	2-Methoxyethanol	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>	50	109-86-4	45, 31, 76, 47, 38		
35	30	N-Methylpyrrolidone	C <sub>5</sub> H <sub>9</sub> NO	530	872-20-4	99, 44, 98, 42, 41		
36	31	Sulfolane	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub> S	160	126-33-0	41, 56, 55, 120, 39		

The results shown in Figure 1 illustrate the Headspace GC/MS SIM mode for Class 1 test is very sensitive, for example, the signal/noise ratio of benzene at 0.02 ppm is 310, as shown in Figure 2. The Scan mode by the Direct Injection GC/MS was used for USP Class 2c, as shown in Figure 3, the signal/noise ratio of 2-methoxyethanol at 0.5 ppm is 34, as shown in Figure 4. Therefore, using this method, a total of 62 residual solvents can be determined at the limitation test requirement for USP <467> residual solvents. A single Class 3 solvent, formic acid is not detected by this method, is quantitated by HPLC or IC methods.

Table 2: USP Class 3 Residual Solvents Information

No.	Item	Solvent	Class	Formula	Risk Assessment	Limit Conc. (ppm)	CAS #	Mass Ions
37	1	Acetic acid	3	CH <sub>3</sub> COOH	Use Ok, See Limit Levels	5000	64-19-7	60, 43, 43, 42
38	2	Acetone		CH <sub>3</sub> COCH <sub>3</sub>		5000	67-64-1	58, 43, 42
39	3	Anisole		C <sub>7</sub> H <sub>8</sub> O		5000	100-66-3	108, 78, 63, 95
40	4	1-Butanol		C <sub>4</sub> H <sub>10</sub> O		5000	71-36-3	56, 41, 43, 42, 35
41	5	2-Butanol		C <sub>4</sub> H <sub>10</sub> O		5000	78-92-2	45, 29, 45, 41
42	6	Butyl acetate		C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>		5000	123-86-4	41, 56, 61, 73
43	7	tert-Butyl methyl ether		C <sub>6</sub> H <sub>14</sub> O		5000	1634-04-4	73, 57, 41, 43
44	8	Cumene		C <sub>9</sub> H <sub>10</sub>		5000	98-82-8	105, 130, 77, 79, 103
45	9	Dimethyl sulfoxide		C <sub>2</sub> H <sub>6</sub> OS		5000	67-68-5	63, 78, 45, 61
46	10	Ethanol		C <sub>2</sub> H <sub>6</sub> O		5000	64-17-5	31, 45, 46, 29, 43
47	11	Ethyl acetate		C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>		5000	141-78-6	43, 61, 70, 88, 45
48	12	Ethyl ether		C <sub>4</sub> H <sub>10</sub> O		5000	60-29-7	31, 59, 74, 45, 29
49	13	Ethylformate		C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>		5000	109-94-4	31, 29, 45, 27, 74, 36
50	14	Formic acid		CH <sub>2</sub> O <sub>2</sub>		5000	64-18-6	29, 46, 45, 41
51	15	Heptane		C <sub>7</sub> H <sub>16</sub>		5000	142-82-5	43, 41, 57, 71, 100
52	16	Isobutyl acetate		C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>		5000	110-39-0	43, 56, 73, 41
53	17	Isopropyl acetate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	5000	108-21-4	43, 67, 87, 39		
54	18	Methyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	5000	79-20-9	43, 74, 59, 42		
55	19	3-Methyl-1-butanol	C <sub>5</sub> H <sub>12</sub> O	5000	123-51-3	55, 42, 70, 41, 43		
56	20	Methyl tert-butyl ether	C <sub>5</sub> H <sub>12</sub> O	5000	78-38-3	43, 72, 57, 29		
57	21	Methylisobutyl ether	C <sub>5</sub> H <sub>12</sub> O	5000	108-10-1	43, 58, 57, 85, 100		
58	22	2-Methyl-1-propanol	C <sub>4</sub> H <sub>10</sub> O	5000	78-83-1	43, 41, 42, 31, 23		
59	23	Pentane	C <sub>5</sub> H <sub>12</sub>	5000	109-66-0	43, 42, 41, 39, 57		
60	24	1-Pentanol	C <sub>5</sub> H <sub>12</sub> O	5000	71-41-0	42, 55, 70, 41, 21		
61	25	1-Propanol	C <sub>3</sub> H <sub>8</sub> O	5000	71-23-8	31, 29, 42, 39		
62	26	2-Propanol	C <sub>3</sub> H <sub>8</sub> O	5000	67-63-0	54, 43, 41, 39, 29		
63	27	Propyl acetate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	5000	109-60-4	43, 61, 73, 42		

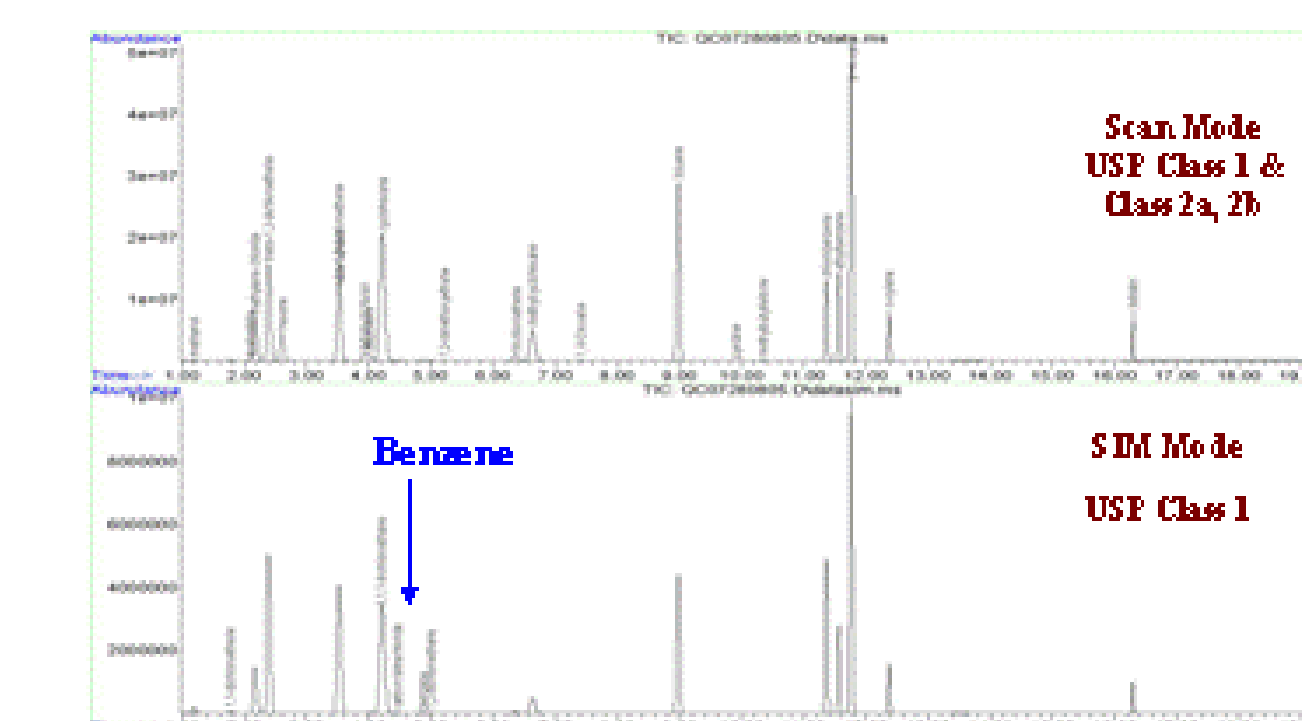


Figure 2: GC/MS Ion Chromatogram of USP Class 1, 2a, and 2b Solvents

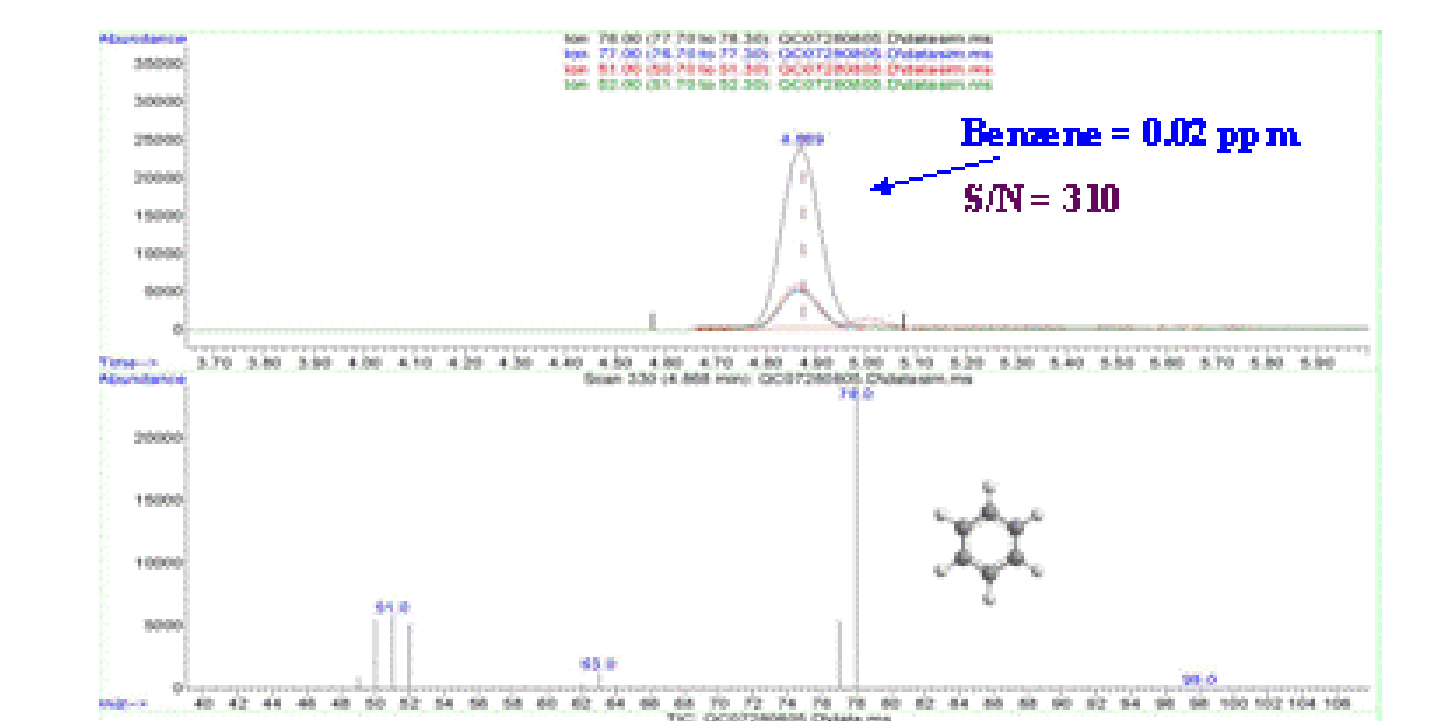


Figure 2: GC/MS Ion Chromatogram and Mass Spectrum of Benzene at 0.02 ppm

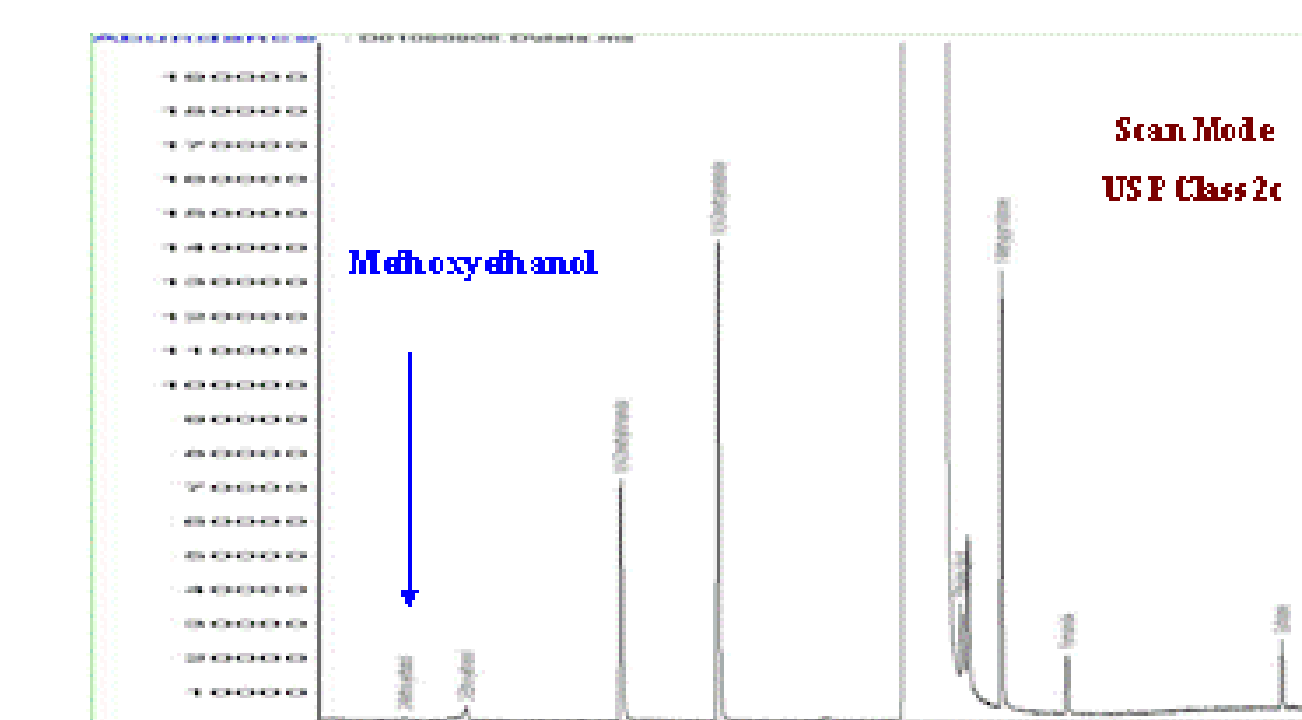


Figure 3: GC/MS Chromatogram of USP Class 2c Solvents

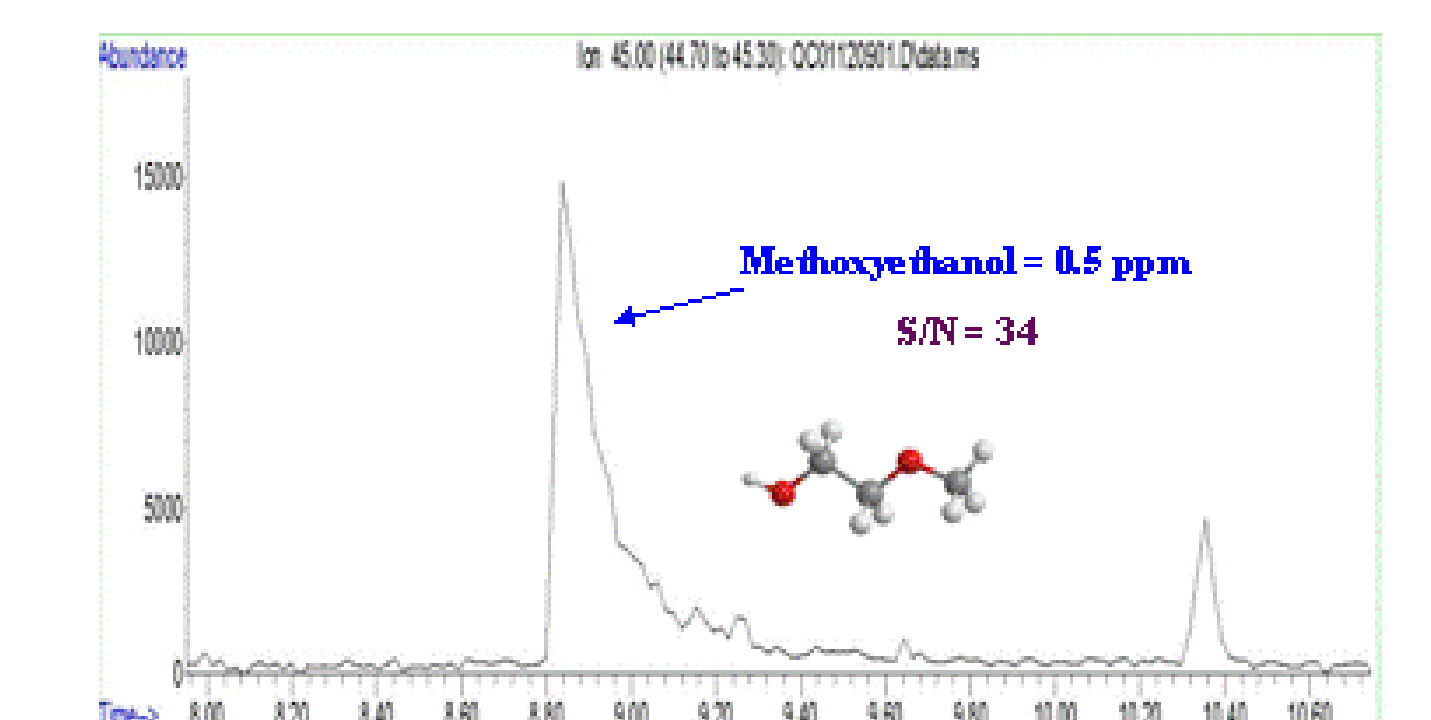


Figure 4: Mass Chromatogram of Methoxyethanol (m/z=45), Signal-to-Noise = 34 in DMSO/Methanol by Direct Injection

## CONCLUSIONS

This study summarizes a sensitive and selective GC/MS screening method for the detection of 62 residual solvents. Biopharmaceuticals are tested for residual solvents using both Headspace and Direct Injection GC/MS. The method was optimized to allow identification and quantification of all three USP classes of residual solvents at USP limit concentration levels and has the advantage of unknown peak identification by NIST library search methods.

## REFERENCE

- USP31-NF26 Second Supplement, Official Dec. 1, 2008 – May 1, 2009: General Chapters <467> - Organic Volatile Impurities.