Considerations for The Analysis of Organic Volatile Impurities in Pharmaceutical Products

by Mitch Hastings

Agilent Technologies, Inc.

Analyzing OVIs: What is at Stake?

- Ease of Development
- Equipment Availability
- Robustness
- Cost

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Analyzing OVIs: Two options

- Headspace
- Direct Injection
- Not Covered:
  - SPME
  - Manual Headspace
  - Ambient Headspace (5966-1473E)

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Questions to ask and answer before you start

- Can I use USP <467> to “escape” validation?
    - If yes, you can save yourself a lot of time up front, but it may cost more down the road.
- Is my sample “volatile”?
    - If yes, peaks may interfere with analytes of interest.
- Is my sample “non-volatile”?
    - If yes, residues could cause problems. Favor Headspace.

Questions to ask and answer before you start

- Is my sample thermally labile?
    - If yes, breakdown peaks may interfere with analytes of interest. Favor headspace.
- Is solvent potentially trapped in the sample matrix?
    - If yes, sample must be dissolved or melted.
- What does my method sensitivity need to be?
    - If 1 ng on-column or less, favor headspace

“Volatile” Samples

- Example: non-ionic organic APIs, b.p. < than 200°C.
- Column choice very important
    - sample and impurities should elute far from analytes
- If compound elutes after analytes, don’t forget a ramp to elute it
- If sample is not thermally labile, direct injection might be easiest option.

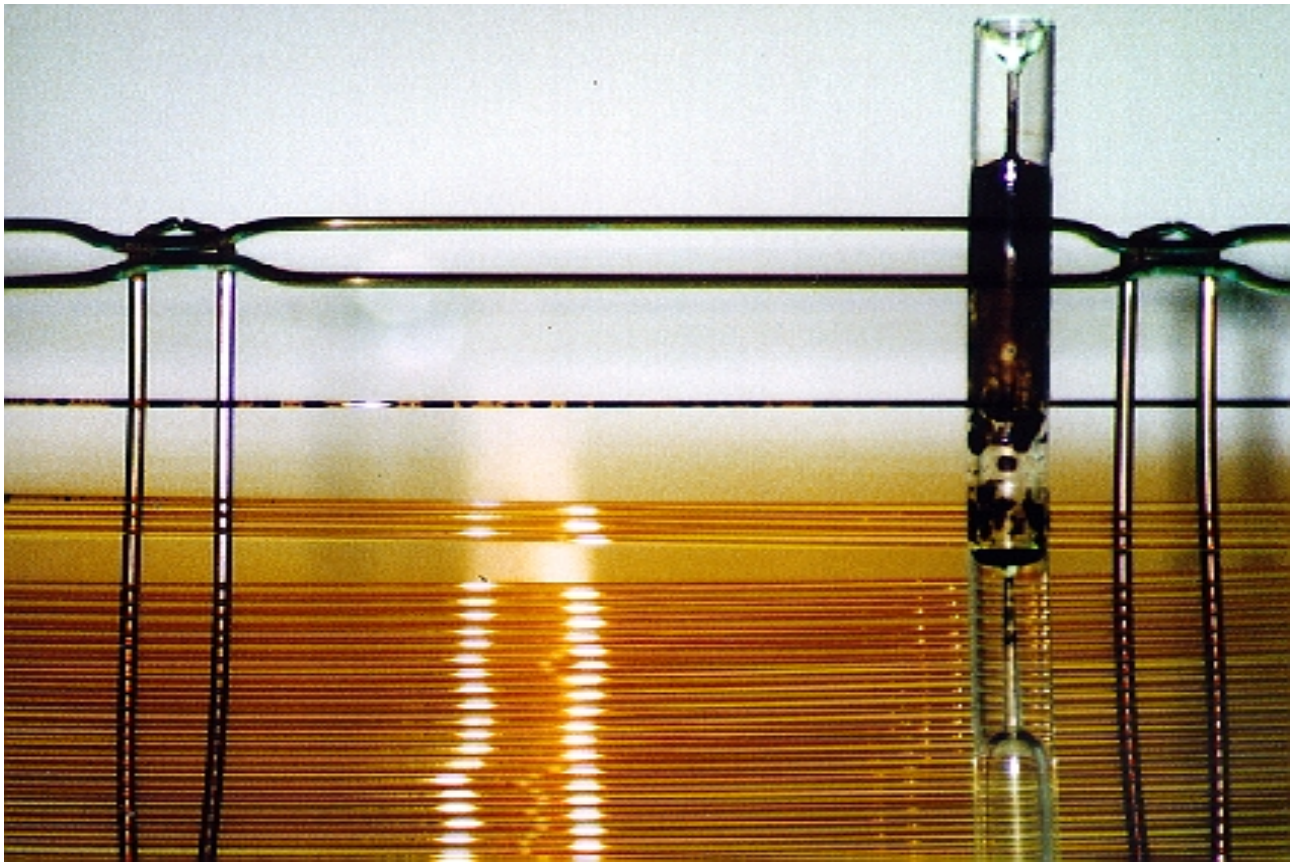
“Non-volatile” Samples

- Example: Organic Salts, excipients, b.p. > 200°C
- Residue problems with direct inject result in:
    - poor peak shape, especially for hydrogen bonding analytes
    - column damage
    - require guard column
    - requires routine maintenance of inlet and column
    - ghost peaks in blanks
- Headspace preferred technique

Thermally Labile Samples

- Examples: some epoxides, carbohydrates, peptides, multi -NO_3 many more
- With Direct Inject, breakdown products may result in peaks, or accumulate in the inlet (“caramelize”)
- Same problems as non-volatile residues
- Headspace is preferred

Sample Residues



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Solvents trapped in matrix or ionic?

- Examples: APIs with crystallization step
- Solvents can be trapped in crystal matrix
- Sample must be dissolved or melted to measure total OVIs with either Direct Inject or Headspace
- pH must be adjusted for ionic solvents for quantitative recovery

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USP <467> for OVIs

- Method 1: Direct Inject
  - Pluses:
    - Don’t have to validate
    - Easily developed
    - Instrumentation usually readily available
    - Initial instrumentation costs are less

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USP <467> for OVIs

- Method 1: Direct Inject
  - Minuses:
    - Chloroform hard to detect at 1 ppm
    - % RSD/flame-out problems due to water
    - Compound/matrix may not be suitable for method
    - Residues can cause problems, require increased maintenance
    - Limited to specified conditions
    - External standard method--greater %RSDs

USP <467> for OVIs

- Method IV: Automated Headspace
  - Pluses:
    - Don’t have to validate
    - Greater method sensitivity possible
    - Avoid problems inherent with Direct Inject
  - Minuses:
    - Questionable results if sample doesn’t dissolve or melt
    - Limited to specified conditions
    - External standard method--greater %RSDs

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In-house Methods for OVIs

- Disadvantages
  - Higher development costs
  - Longer time to finished method
  - Cost of validation
  - Risk of improperly developed method
  - Fast GC mostly applicable to Headspace

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In-house Methods for OVIs

- Advantages--Especially with Fast HS-GC
  - shorter analysis time (6.5 min, see pub 5968-4586E)
  - quicker turn around time (important for in-process checks)
  - lower long term cost of analysis via higher through-put
  - better resolution possible with smaller I.D. columns.

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In-house Methods for OVIs

- Advantages
    - better column selection for shorter run times
    - better reproducibility with use of Internal Standard
        - mitigate matrix effects and handling errors
    - greater method sensitivity
    - more robust methods

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Overview of Considerations for Methods Development

Areas of Optimization

- Sample Preparation
- Sample Introduction
- Chromatography
- **Detection**

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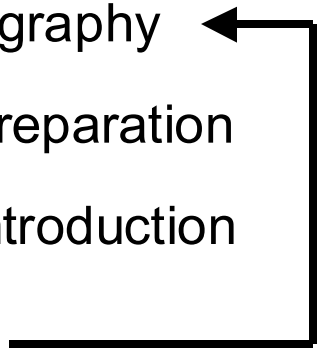
Order of Considerations for Methods Development

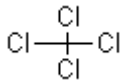
Areas of Optimization

- **Detection**
- Chromatography
- Sample Preparation
- Sample Introduction
- Repeat



Detection

Areas of Optimization

- MS for qualitative methods development, but mainly FID for Routine QC
- FID limitations
  - Oxidizable bond:
    - C-H good, example: Hexane 
    - C-Cl poor, example: Carbon Tetrachloride 
  - **Target = min 0.1-2 ng on column for robust (10:1) s/n**
  - Stable, linear to 6-7 orders of magnitude

AMD: Approximating On-column Amount

$$\text{OVI On-column (ug)} = \frac{S \times C \times V_l \times V_{ds} \times \%HS \times F_c}{V_d \times V_{hs} \times F_s}$$

Numerator

S = Sample size, g
C = Minimum concentration of the OVI that needs to be detected in the sample, ug/g
V_l = Sample loop volume, mL, or volume injected in mL (1 µL = 0.001 mL)
V_{ds} = Volume of diluted sample added to headspace vial, mL
%HS = Percent of OVI partitioned into the headspace*
F_c = Flow rate of the column, mL/min

Denominator

V_d = Volume of the diluent used to dilute the sample, mL
V_{hs} = Volume of headspace remaining in the vial after diluted sample is added, mL
F_s = Flow rate of the split vent, mL/min

*** If Headspace sample oven temp is greater than 10°C above B.P. of solvent, assume minimum 50% is in headspace.**

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AMD: Direct Inject v. Headspace

- Extra Sensitivity is money in the bank.

Direct Inject			Headspace
Sample Size (g)	0.1	0.1	Sample Size (g)
Solute Conc.(ug/g)	10	10	Solute Conc.(ug/g)
Amount Inj (mL)	0.001	1	*Sample Loop Size (mL)*
		1	*Volume of Sample in vial (mL)*
		5	*Column Flow*
		50	Percent into Headspace
Diluent Volume (mL)	1	1	Diluent Volume (mL)
		19	*Volume of Headspace (mL)*
		50	*Split Vent Flow*
Amount on Column (ug)	**0.0010**	**0.0026**	Amount on Column (ug)

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Chromatography

- Choices
  - Column Dimensions
    - on-column amount desired (larger diameter = more for headspace)
    - resolution (smaller I.D. + longer = more resolution and/or faster runs)
    - robustness (larger diameter = more)
  - Stationary Phase Type
    - selectivity (refer to mfgr's solvent tables)
    - durability (less polar = more robust)
    - main component (usually diluent) polarity match

Chromatography

- Choices
  - Guard Column?
    - Yes.
    - Wet-able to water, prevents FID flameouts
    - Focus analytes if using splitless and headspace with water present or polarity mismatch between solvent and stationary phase
    - Traps non-volatile residues if using splitless
- Favor 30 m x 0.53 or 0.32 mm ID column with G-43 (DB-624) stationary phase for most comprehensive separation, broadest range of solvents, with a guard column

Chromatography

- Elution Data Available in Catalogs:
  - DB-1
  - DB-Wax
  - DB-624 “best selectivity”

DB-624 - Retention

Column:	DB-624 30 m x 0.53 mm I.D., 3 µm
J&W P/N:	125-1334
Oven:	40°C for 5 min 40-260°C at 10°/min 260°C for 3 min
Carrier:	Helium at 30 cm/sec (40°C)
Injector:	Split 1:10, 250°C
Detector:	FID, 300°C

Retention Time	Compound
2.46	acetaldehyde
2.59	methanol
2.80	methyl formate
3.24	fluorotrichloromethane (freon 11)
3.37	pentane
3.47	ethanol
3.72	ethyl ether
3.72	ethyl vinyl ether
3.72	furan
3.81	acrolein
3.91	propionaldehyde
4.00	1,1-dichloroethylene (vinylidine chloride)
4.00	1,1,2-trichlorotrifluoroethane (Freon 113)
4.05	acetone
4.27	acetonitrile
4.27	bromoethane
4.27	carbon disulfide
4.27	ethyl formate
4.27	iodomethane
4.27	iso-propanol
4.57	3-chloropropene (allyl chloride)
4.60	methyl acetate
4.80	methylene chloride
5.01	tert-butanol
5.22	acrylonitrile
5.30	methyl tert-butyl ether (MTBE)
5.33	trans-1,2-dichloroethylene
5.66	iso-butyraldehyde
5.82	hexane
6.01	methacrolein
6.11	1,1-dichloroethane
6.21	2-propen-1-ol (allyl alcohol)
6.23	iso-propyl ether
6.34	1-propanol
6.37	vinyl acetate
6.41	allyl ethyl ether
6.84	butyraldehyde
6.90	tert-butyl ethyl ether
6.93	3-penten-2-one (methyl vinyl ketone)
6.99	2,3-butanedione (diacetal)
7.10	butyl methyl ether
7.16	cis-1,2-dichloroethylene
7.16	2,2-dichloropropane
7.19	2-butanone (MEK)
7.25	propionitrile
7.30	1,3-dioxolane
7.34	ethyl acetate
7.53	methacrylonitrile
7.55	2-propyn-1-ol (propargyl alcohol)
7.55	sec-butanol
7.59	bromochloromethane
7.64	THF (tetrahydrofuran)
7.66	propyl formate
7.75	chloroform
7.88	methyl propionate
7.91	2-methyl-3-buten-2-ol
8.05	1,1,1-trichloroethane
8.10	cyclohexane
8.25	1-chlorobutane
8.34	carbon tetrachloride
8.34	1,1-dichloropropene
8.60	iso-butanol
8.64	ethylene glycol monomethyl ether
8.65	glyme (ethylene glycol dimethyl ether)
8.67	2-methoxyethanol (methyl cellosolve)
8.69	benzene
8.72	1,1-dichloropropane
8.73	2-methyl-2-butanol (tert-amyl alcohol)
8.81	iso-octane
8.87	iso-propyl acetate
8.89	tert-amyl methyl ether
8.91	triethylamine
9.02	3-buten-1-ol
9.05	propyl ether
9.10	acetic acid
9.15	heptane
9.18	crotonaldehyde
9.18	fluorobenzene
9.21	3-methyl-2-butanone
9.34	butyl ethyl ether
9.62	1,2-dichloroethane (ethylene dichloride)
9.65	allyl ether
9.73	1-butanol
9.80	trichloroethylene
9.87	2-buten-1-ol (crotyl alcohol)
9.92	diglyme (diethylene glycol dimethyl ether)
10.02	ethyl acrylate
10.02	tert-butyl acetate
10.03	1-penten-3-one (ethyl vinyl ketone)
10.06	tetrahydropyran
10.11	2-pentanone
10.17	1,2-dichloropropane
10.20	1-penten-3-ol
10.25	pentanal (valeraldehyde)
10.34	2,3-pentanedione
10.34	3-pentanone
10.37	dibromomethane
10.37	ethyl propionate
10.38	1,4-dioxane
10.51	propyl acetate
10.58	acetal (acetaldehyde diethyl acetal)
10.60	2-pentanol
10.60	3-pentanol
10.64	bromodichloromethane
10.95	ethylene glycol monoethyl ether
10.98	2-ethoxyethanol (cellosolve)
11.22	epichlorhydrin
11.38	cis-1,3-dichloropropene
11.64	4-methyl-2-pentanone (MIBK; methyl isobutyl ketone)
11.70	pyridine
11.76	sec-butyl acetate
11.78	3-methyl-1-butanol (iso-amyl alcohol)
11.85	2-methyl-3-pentanone
11.87	2-methyl-1-butanol (active amyl alcohol)
11.89	propionic acid
11.93	glycidol
11.93	toluene
12.11	octane
12.15	ethylene glycol
12.19	iso-butyl acetate
12.21	acrylic acid
12.28	4-methyl-2-pentanol
12.30	trans-1,3-dichloropropene
12.60	1,1,2-trichloroethane
12.63	1-pentanol
12.63	2-penten-1-ol
12.80	3-hexanone
12.85	3-methyl-2-buten-1-ol
12.86	1,3-dichloropropane
12.86	tetrachloroethylene
12.94	2,4-dimethyl-3-pentanone (diisopropyl ketone)
12.98	morpholine
13.01	2-hexanone
13.07	propyl propionate
13.13	1,3-propanediol
13.14	hexanal
13.16	cyclopentanol
13.16	3-hexanol
13.16	propylene glycol (1,2-propanediol)
13.20	4-methyl-3-penten-3-one
13.24	butyl acetate
13.25	chlorodibromomethane
13.31	2-hexanol
13.39	cyclopentanone
13.43	1,2-dibromoethane (EDB)
13.73	DMF (dimethylformamide)
14.14	2,3-butanediol
14.21	1-chlorohexane
14.24	4-hexen-3-one
14.25	chlorobenzene
14.38	1,1,1,2-tetrachloroethane
14.41	butyl ether
14.42	ethylbenzene
14.62	m-xylene
14.62	p-xylene
14.63	furfural
14.63	nonane
14.73	5-methyl-2-hexanone
14.75	iso-amyl acetate
14.82	2-methylbutyl acetate
14.88	cis-3-hexen-1-ol
14.95	4-heptanone
14.98	4-hydroxy-4-methyl-2-pentanone
15.06	1-hexanol
15.19	cis-2-hexen-1-ol
15.28	styrene
15.28	o-xylene
15.29	3-heptanone
15.32	furfuryl alcohol
15.46	2-heptanone
15.53	3-heptanol
15.57	amyl acetate
15.57	2-ethoxyethyl acetate
15.58	DMSO (methyl sulfoxide)
15.60	heptanal
15.61	bromoform
15.63	cyclohexanol
15.63	2-heptanol
15.84	ethylene glycol monobutyl ether
15.88	iso-propylbenzene (cumene)
15.89	2-butoxyethanol (butyl cellosolve)
16.04	cyclohexanone
16.10	1,3-butanediol
16.38	1,1,2,2-tetrachloroethane
16.39	bromobenzene
16.46	trans-1,4-dichloro-2-butene
16.46	1,2,3-trichloropropane
16.52	5-methyl-3-heptanone
16.56	propylbenzene
16.71	2-chlorotoluene
16.78	diethylene glycol monomethyl ether
16.82	3-chlorotoluene
16.82	4-chlorotoluene
16.82	decane
16.82	1,3,5-trimethylbenzene (mesitylene)
17.06	2,6-dimethyl-4-heptanone (diisobutyl ketone)
17.22	1-heptanol
17.22	trans-2-hepten-1-ol
17.22	cis-4-hepten-1-ol
17.22	a-methylstyrene
17.39	tert-butylbenzene
17.45	benzaldehyde
17.45	3-octanone
17.47	1,2,4-trimethylbenzene (pseudocumene)
17.63	2-octanone
17.68	iso-butylbenzene
17.68	4-methylstyrene
17.68	sec-butylbenzene
17.70	1,4-butanediol
17.76	octanal
17.96	1,3-dichlorobenzene
18.04	diethylene glycol monoethyl ether
18.10	iodobenzene
18.18	1,2,3-trimethylbenzene (hemimellitene)
18.21	benzonitrile
18.24	diethylene glycol
18.40	2-ethyl-1-hexanol
18.53	pentyl ether
18.69	butylbenzene
18.69	1,2-dichlorobenzene
18.78	undecane
18.79	1,4-dichlorobenzene
19.17	1-octanol
19.27	benzyl alcohol
19.35	4-chlorostyrene
19.35	4-tert-butyltoluene
19.46	styrene oxide
19.52	1,5-pentanediol
19.60	a-methylphenyl alcohol
19.63	o-tolualdehyde
19.63	m-tolualdehyde
19.66	1-methyl-pyrrolidinone
19.69	acetophenone
19.71	nonanal
19.90	methyl benzoate
19.96	p-tolualdehyde
20.02	1,2-dibromo-3-chloropropane (DBCP)
20.35	1,3-diisopropylbenzene
20.35	nitrobenzene
20.35	1,3,5-trichlorobenzene
20.58	dodecane
20.73	1,4-diisopropylbenzene
20.97	1-nonanol
21.04	isophorone
21.07	benzyl acetate
21.22	ethyl benzoate
21.30	1,6-hexanediol
21.41	1,2,4-trichlorobenzene
21.46	diethylene glycol monobutyl ether
21.60	2-nitrotoluene
21.69	hexachloro-1,3-butadiene
21.95	triglyme (triethylene glycol dimethyl ether)
22.23	1,2,3-trichlorobenzene
22.24	tridecane
22.40	3-nitrotoluene
22.65	1-decanol
22.65	a-ethylphenethyl alcohol
22.65	2-phenoxyethanol
22.79	4-nitrotoluene
22.91	4-phenyl-2-butanone (benzyl acetone)
22.92	propyl benzoate
22.97	1,7-heptanediol
23.03	b-ethylphenethyl alcohol
23.06	1-chloro-4-nitrobenzene
23.80	tetradecane
24.02	1,2,4,5-tetrachlorobenzene
24.54	1,8-octanediol
25.26	pentadecane
26.02	1,9-nonanediol
26.63	hexadecane
27.10	pentachlorobenzene
27.43	1,10-decanediol
29.08	benzyl ether

Sample Preparation

- Types of Samples Include
  - drugs/intermediates (organic and organic/ionic)
  - excipients (carbohydrates, inorganics, polymers, gums, gels)
  - tableted forms
  - packaging (inks, foams, plastics)
  - etc…

Sample Preparation Considerations-- Dissolve Your Sample if Possible

- Non-volatile, thermally labile--*headspace preferred*.
- Many samples are polar or organic salts--*water solvent preferred*.
- Many samples are organic--*organic solvent preferred.*
- Many organic solutes are difficult to prepare as standards in water--*organic solvent preferred*.

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Tips for Methods Development

- Direct Inject
  - Avoiding Inlet Related Problems
  - Avoiding Interfering Peaks
  - Avoiding Detector Problems
- Headspace
  - Times, Temps, Flows

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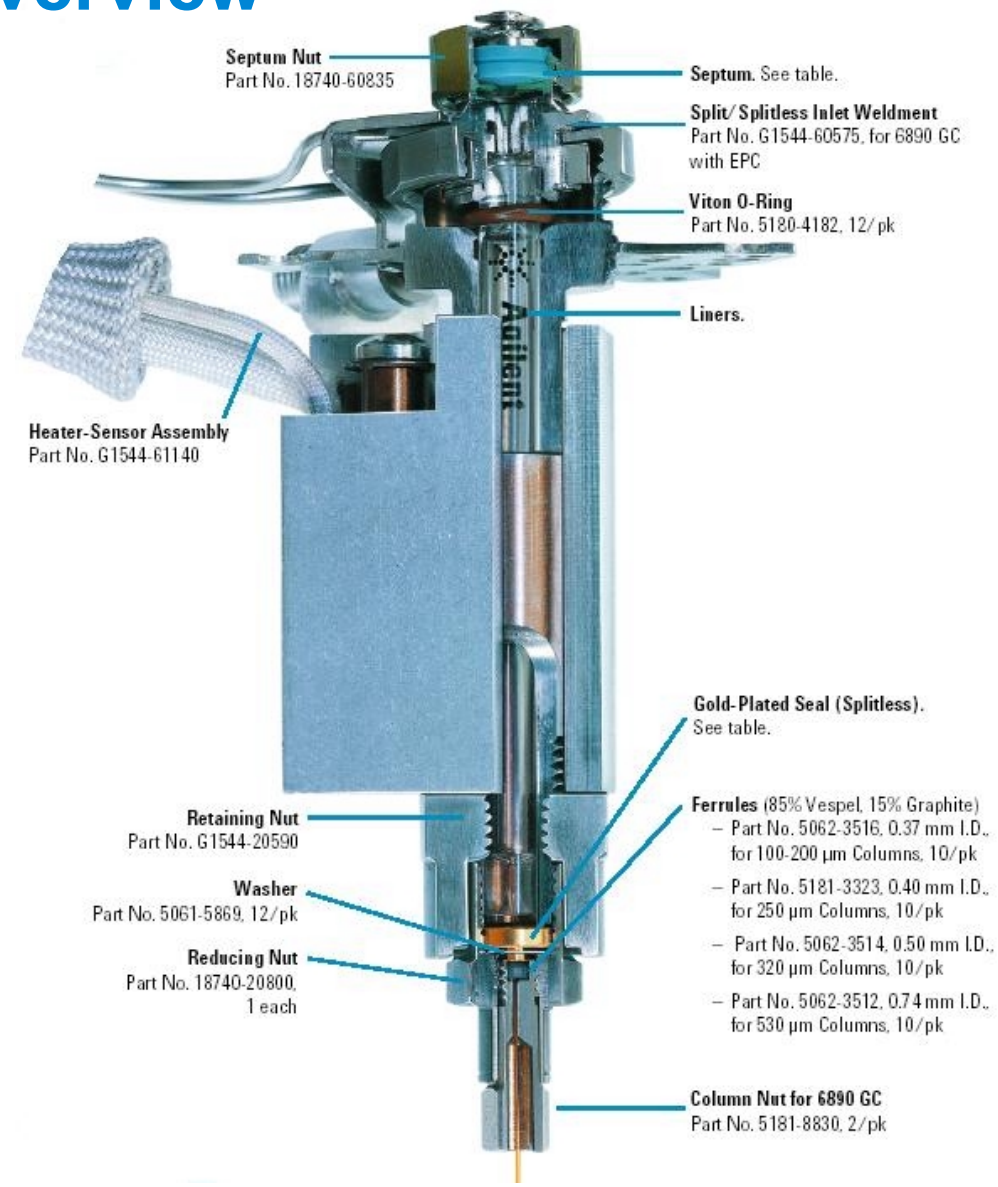
Split Splitless Inlet Overview

- Split/Splitless Operation (video)
- Common Problems/Solutions
- Benchmark Settings



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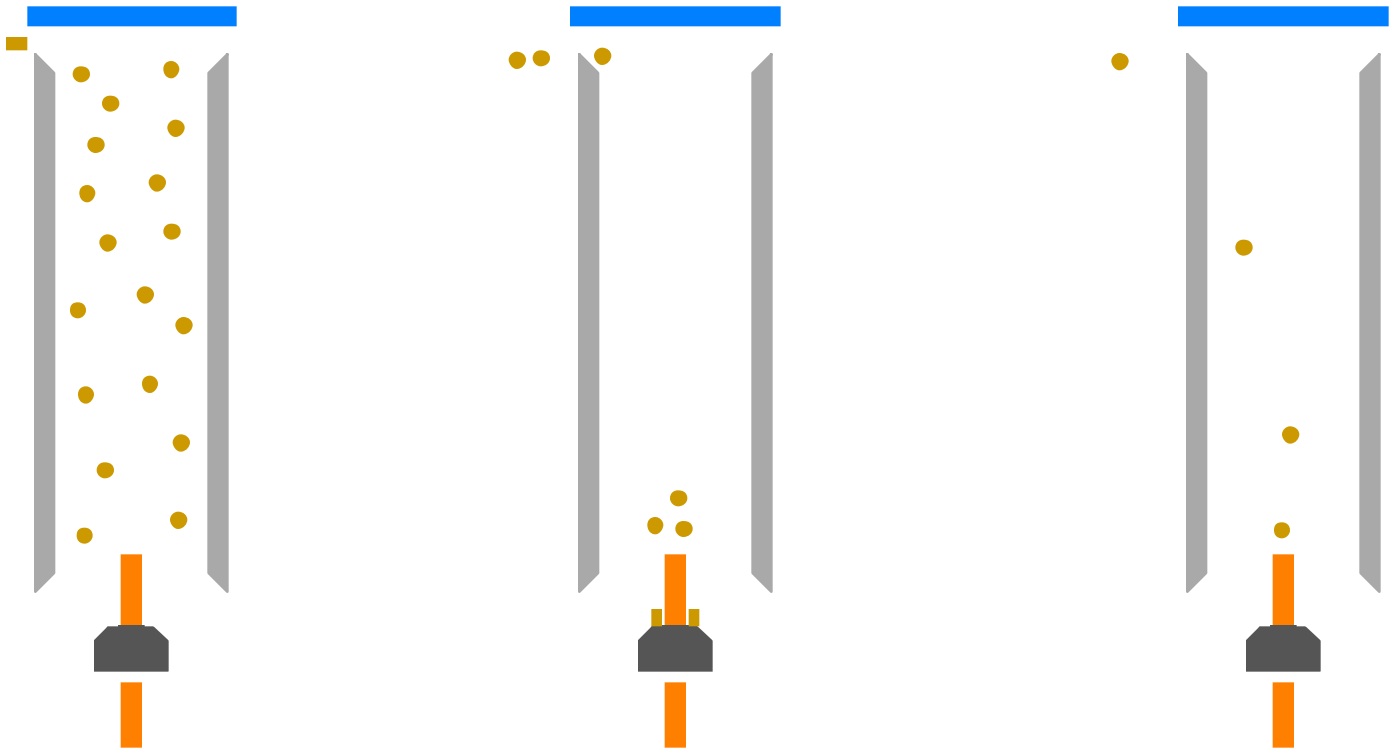
Direct Inject Tips

- Avoiding Inlet Related Problems
    - Use Deactivated glass wool for residues ONLY if OVIs are non-polar (hexane, $CHCl_3$ okay, methanol not okay)
    - Especially if you use water, use a guard column
        - minimizes split peaks, poor peak shape
    - Don’t use water as solvent if possible--Backflash
    - If water is required, use low injection temp (140°C)

Direct Inject Tips--Backflash



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Direct Inject Tips

- Avoiding Inlet Related Problems
    - Keep inlet temperature low enough to avoid sample degradation. Start at 200°C and decrease if necessary.
    - Use 0.53 mm ID columns (more forgiving)
    - Use 4 mm ID liner, single or dual taper (preferred)
    - Replace split vent line trap when RTs and %RSDs become erratic

Direct Inject Tips

- Avoiding Interfering Peaks
    - Column temperature at a minimum of 10°C below solvent boiling point
    - Use GC grade solvents (some LC grade may have interferences)
    - Purge on at 0.25-0.5 min, split flow 50 ml/min minimum

Direct Inject Tips

- Avoiding Interfering Peaks
    - Use solvents that elute well before (MeOH, Pentane, Diethyl Ether) or well after analytes of interest (DMF, DMA, DMSO)
    - DMSO harder to work with, but lower boiling solvents may have more interfering peaks than higher boiling solvents
    - Verify peak retention time with 0.05-0.1% solution

Direct Inject Tips

- Avoiding Interfering Peaks
    - Keep inlet temperature low enough to avoid sample degradation. Start at 200°C and decrease if necessary.
    - Stay ahead of the problem. Routinely clean inlet, clip 1/4-1/2 m from guard column after each full sequence.
    - Use chambered liner and 1 ul or less injection

Direct Inject Tips

- Avoiding Detector Problems
    - FID is very “forgiving,” but...
    - Flameout (common with water injections)
    - Use guard column to “spread out” water peak
    - Use proper detector flows on FID
        - 5890: Air 300 ml/min, H_2 = 35 ml/min, N_2 + Column = 30 mL/min
        - 6890: Air 400 ml/min, H_2 = 30 ml/min, N_2 + Column = 30 mL/min (auto light function helpful)
    - Clean/replace jet when flame-out problems persist

Headspace Primer

- The amount of solute partitioning into the gas phase over liquid for trace solutes is approximated by Henry's Law: $c = kP$
    - c = concentration (mol/L) of solute above liquid
    - k = Henry's Law constant for solute (mol/L•atm)
    - P = Pressure (atm) over the solution
  - k is temperature dependent, increase temperature, increase k, increase concentration of solute in headspace above liquid

Headspace Primer

- In practical terms…
  - %RSD can be decreased by increasing HS sample oven temperature above solute b.p., 5-10°C
  - diluent temperature, decomposition temperature, or vial septa defines upper temperature limit
  - choose a diluent with a boiling point well above the solute of interest (DMSO)
  - if necessary, modify solution to change k (salt in H_2O for organics)

Diluent Choice

- Water B.P. 100°C
- DMSO B.P. 189°C

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Headspace Times & Temps

- HP7694 tips -- Start with Defaults
  - Valve and transfer line temps 5°C above b.p. of diluent
  - HS Sample oven temperature
    - 5-10°C below diluent b.p. max, better 20-40°C
    - melt or dissolve sample if solid

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Headspace Times & Temps

- HP7694 tips -- Start with Defaults
  - Vial equilibration
    - 15 min to start, 1 mL sample, 10 mL vial
      - more mass = more time required
      - use parameter increment function
    - use shaking

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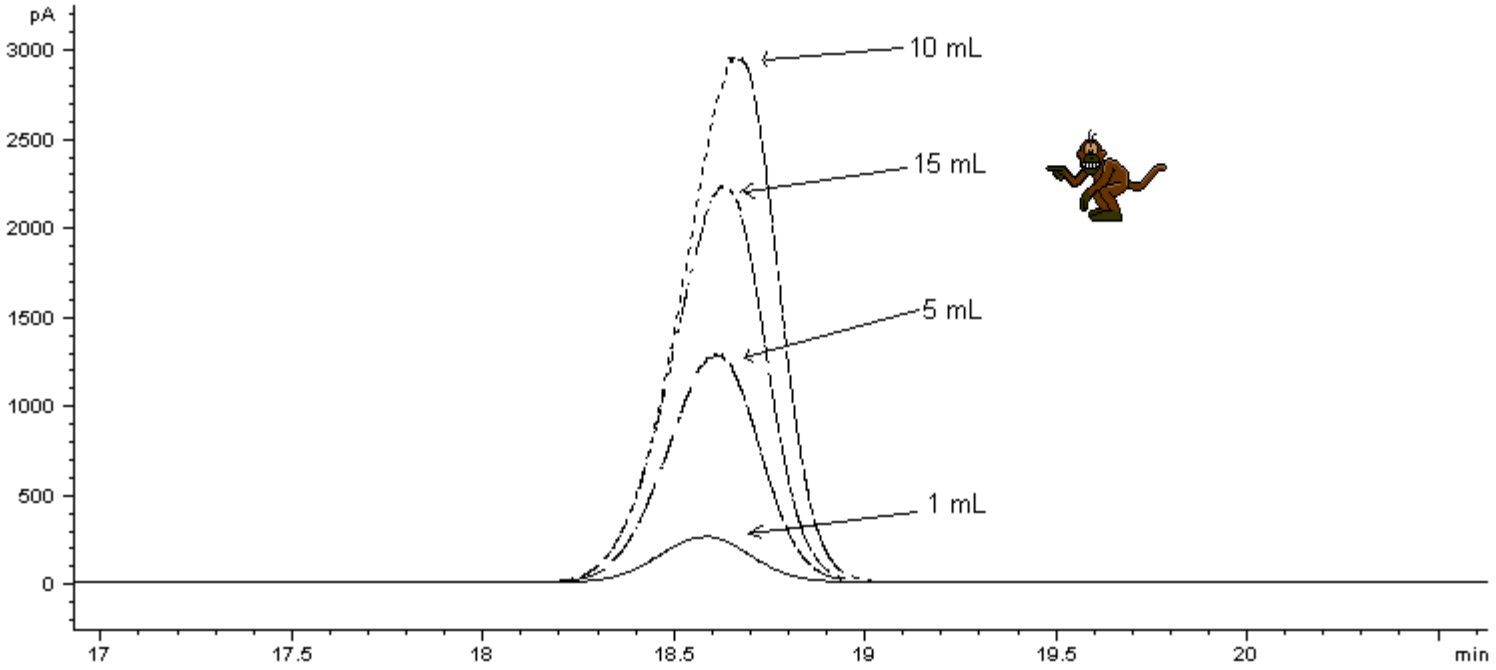
Headspace Times & Temps & Volumes

- Sample Oven Temp, 80°C
- Time, 15 min

Time	Volume	Toluene Area
15 min	1	4439
15 min	5	22332
15 min	10	52608
15 min	15	38981



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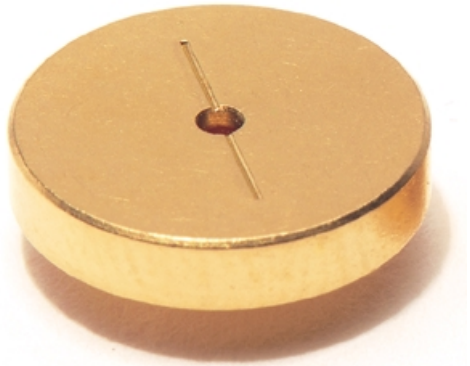
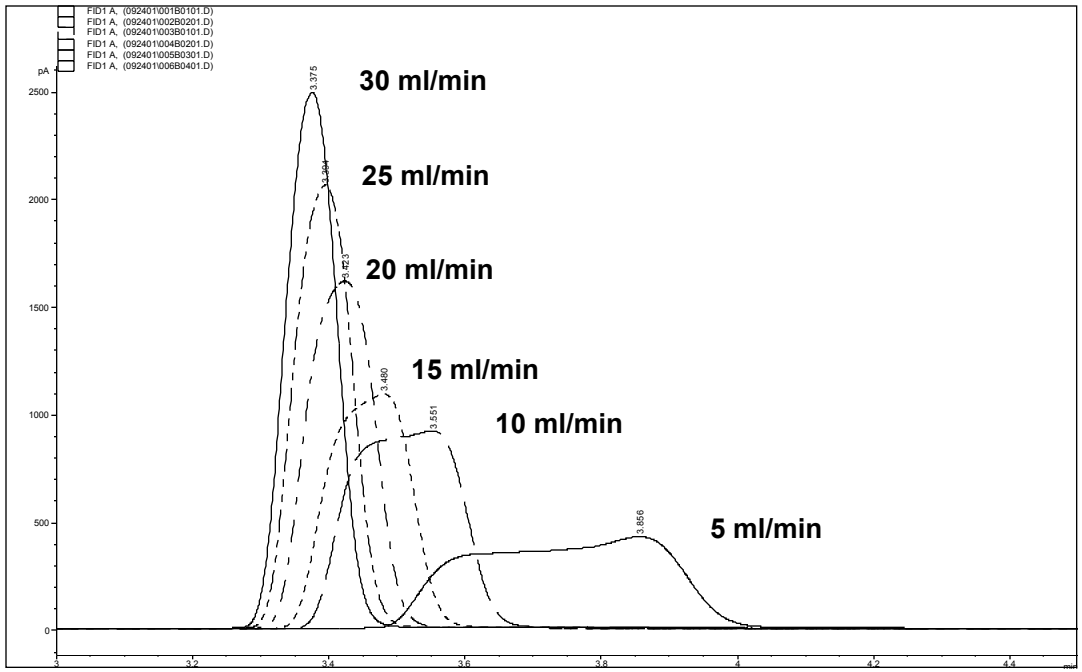
Headspace Times & Temps & Flows

- Vial pressurization, loop fill, 0.2 min @
  - 1 mL loop, 20 mL/min transfer line flow
  - 3 mL loop, 60 mL/min transfer line flow
  - 40 mL/min minimum total flow through inlet to avoid problems
  - Use single channel gold seal
- Vial pressure--about 2 psi above carrier pressure.



Sample Introduction--Conditions

- Conditions--Agilent 7694 Headspace Autosampler
  - Transfer line flow, peak shape of 500 ppm Acetone, DMSO/H20
  - Minimum 25 mL/min, actual 30 mL/min



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Headspace Times & Temps

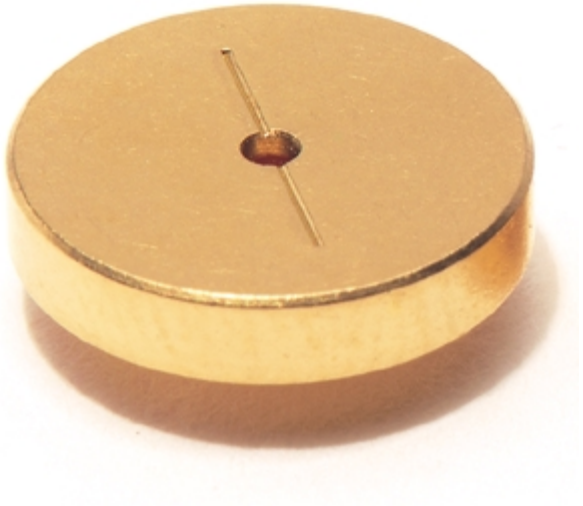
- HP7694 tips -- Start with Defaults
    - Loop equilibration, 0.05 min
    - Sample inject, 1 min (at aforementioned flow minimums)

Headspace & GC Flows

- Avoiding Inlet Related Problems
    - Use 2.0 mm ID straight tube liner (decreases expansion volume). No glass wool. No cup liners.
    - Keep temp at or slightly above temp of transfer line
    - Flow from transfer line adds to total flow in inlet. Chemstation flow will not reflect actual split vent flow on non-EPC controlled units.
    - Measure actual split flow

Headspace & GC Flows

- Avoiding Inlet Related Problems
    - 1 mL loop, minimum 20 mL/min transfer line flow
    - 3 mL loop, minimum 60 mL/min transfer line flow
    - 40 mL/min minimum total flow through inlet to avoid problems
    - Use single channel gold seal



Headspace Misc. Tips

- Keep spares of transfer line needle
- When inserting transfer line into inlet prevent coring/particles:
    - slightly loosen septa nut
    - insert needle (use strain relief fitting)
    - tighten
    - leave transfer line installed

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Headspace Misc. Tips

- When sample oven temp is
    - below 125°C, use Gray PTFE/black septa (easy seal

    - between 125-180°C, use Tan PTFE/white septa
- Use caps with safety feature

- 10 mL vials fine for most work
- Multiple injections, use single puncture option (not advisable in most cases).
- If option, use sample shaking

MS Development Tips

- Use same length, but smaller ID column with same Beta ($B = r/2d_f$).
    - Example: DB-624
        - 0.53 mm ID, 3.0 film
        - 0.32 mm ID, 1.8 film
        - 0.25 mm ID, 1.4 film
- Program transfers easily (keep same linear velocity, temperature program)

Active Analyte Tips

- Organic acids or amines
  - use a 0.1-1% acid or amine which is more volatile (acetic/ethylamine) if the drug is stable in this solution.
- Derivatize in situ.
    - Organic acids, try BF_3 in MeOH

Internal Standard Tips

- Use an IS when possible to mitigate effects of sample handling and matrix.
- Pick IS with similar chemical characteristics (functional group and volatility).
- Remember boiling points!

AMD: Sample Prep Tips

- Use Class A volumetric pipettes for the best accuracy
- Weigh standards instead of using volumetric when temperature changes are possible
- Minimize matrix effects by using 10:1 rule
    - 10X more diluent than sample.
        - 100 ± 10 mg sample, 1000 mg diluent
        - ±10 mg/1000 mg = ± 1%

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Wrapping it up

- Start with what is at stake
    - time available
    - equipment availability
    - desired robustness
    - cost (initial and long term)
- Determine sample attributes
- Determine sensitivity required
- Choose your method, USP or In-house.

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302-993-5304 (phone)*

** Select option 4, then option 1.*

916-608-1964 (fax)

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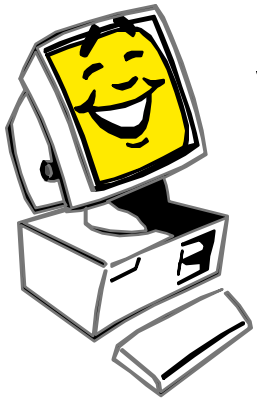

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