Better precision, sensitivity, and higher sample throughput for the analysis of residual solvents in pharmaceuticals

Using the Agilent 7890A GC system with G1888 headspace sampler in drug quality control

Application Note

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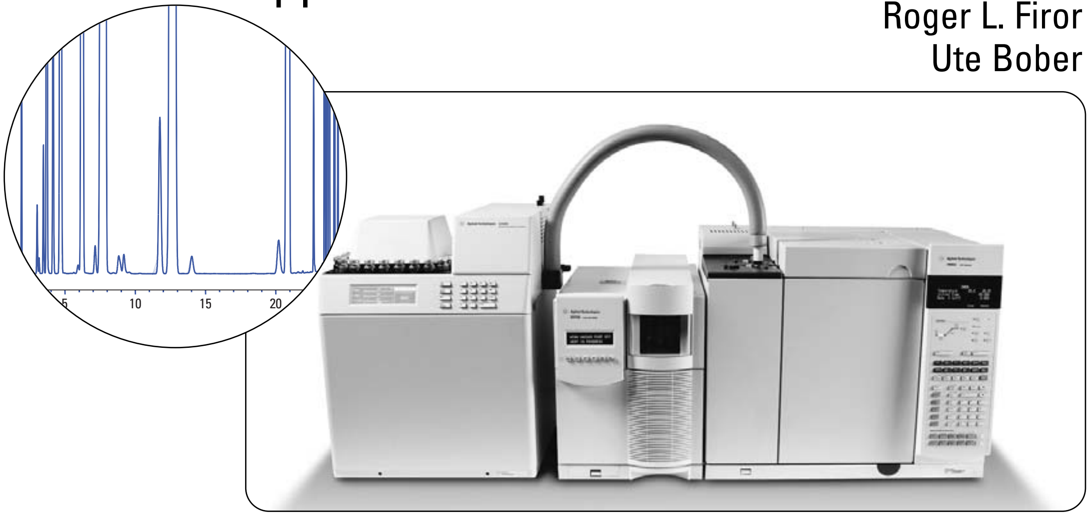


Abstract

Laboratories using headspace GC for the analysis of pharmaceutical impurities face a number of instrument-related issues:

- Area precision is negatively impacted by atmospheric pressure changes.
- Sensitivity is poor for some low-concentration analytes.
- Presence of high-boiling impurities noticeably extends the analysis time per sample and may even damage the analytical column.

In this Application Note an established Agilent 6890 GC method for residual solvents is transferred to the Agilent 7890A GC without any major changes. The results on both systems are compared.
Overall, the Agilent 7890A GC system delivers at least the same or better performance than the Agilent 6890N GC system:

- The new technology of the Agilent 7890A GC can significantly improve area and retention time repeatability and sensitivity.
- It can drastically reduce the overall analysis time, hence increasing sample throughput and productivity.

Agilent Equipment
7890A GC system,
G1888 headspace sampler

Application Area
Pharmaceutical quality control
Impurity analysis

Introduction

Because many solvents pose a major risk to human health, national and international regulatory bodies such as the United States Food and Drug Administration (U.S. FDA), the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), and the International Conference on Harmonization (ICH) require analysis for residual solvents in pharmaceutical drug substances, excipients and final products. Solvents are divided into three classes on the basis of possible risk. Class 1 solvents should be avoided. Class 2 solvents should be limited. Class 3 solvents are considered to have low toxic risk. The ongoing trend toward lower contaminant levels designated as safe requires more sensitive and accurate methods of analysis. New USP <467> regulations for residual solvents begin in July 2007. The goal of this initiative is the final alignment with the ICH Q3C(R3) guideline, which has also been adopted by the EP.

The analysis for residual solvents in pharmaceutical products and for solvents considered extractables/leachables in pharmaceutical packaging materials is typically done using headspace (HS) GC with a flame-ionization detector (FID) or, for identification and confirmation, with mass-selective detection (MSD). This has been covered in references 1-3. Residual solvents is the most common application for headspace GC in pharmaceutical quality control. Laboratories using HS GC currently face a number issues related to the analysis of volatiles and semivolatiles:

- The area precision in HS analysis can be compromised primarily due to atmospheric pressure variations influencing the amount of analytes injected from the sampling loop in the HS gas sampling valve (GSV).
- The sensitivity is poor for some low-concentration analytes, e.g., benzene.
- Sample turn-around time can be excessive, caused by late-eluting impurities and high-boiling solvents/diluents, e.g., 1,3-dimethyl-2-imidazolidinone (DMI) with boiling point of 225 ºC.

Further, when the need for new analytical equipment arises, the first question is whether an established validated method can be easily transferred to the next generation of instruments without any additional method development and resulting in no or minimal revalidation effort. The purpose of this study was to at the least demonstrate equivalence of the HS/7890A GC/FID and HS/6890N GC/FID systems when both are operated without pressure regulation of the sampling loop content of the HS GSV. But more importantly, to also show how new capillary flow technology, fifth generation pneumatics, and state-of-art electronics implemented in the 7890A GC have effectively addressed the above issues with significant improvements in area and retention time precision, sensitivity and productivity with increased sample throughput for residual solvent analysis.

Note: A list of acronyms and short-hand terms used in the text, figures and descriptions of experiments and calculation formulas are included in the appendix on page 8.

Experimental

Both 6890N and 7890A GC were equipped with an Agilent headspace sampler, volatiles interface (VI) and FID. Table 1 gives the experimental conditions used with the HS/VI/6890N/FID and HS/VI/7890A/FID systems. The 7890A system is operated with and without backpressue regulation on the HS sampling loop, whereas the 6890N does not have backpressure regulation. To calculate the repeatability expressed as %RSD values in area and retention time and to determine statistical Method Detection Limit (MDL) for each analyte, 20 identical samples were prepared. A standard solution in water was first prepared in a 100 mL volumetric flask by adding Restek class 1 and class 2 standards with an Eppendorf pipette. 5 mL of the aqueous standard was subsequently transferred quickly to 10 mL headspace vials containing 3 g sodium sulfate and immediately sealed with Teflon-seal caps. Each vial was then vortex mixed for half a minute. Ten of these samples were subsequently used with the 6890N system and ten with the 7890A system – both systems were operated without pressure control on the HS sampling loop. At the end of the HS equilibration, the HS vials were pressurized to 14.000 psi by an auxiliary (AUX) Electronic Pneumatic Control (EPC) module and injected in either the 6890N GC or the 7890A GC system. The same DB 624 column was used in the two GCs for the sequence of injections in order to eliminate the influence of batch-to-batch variations in column quality.

Another set of 20 samples was prepared in the same way as described before for use with the 7890A system, but now implementing backpressure regulation on the HS sampling loop. Figures 1, 2 and 3 show diagrams of the 7890A system where new pneumatic features of pressure regulation of the HS sampling loop, HS vial pressurization and backflush can be applied. At the end of the HS equilibration, the HS vials were pressurized to 20.000 psi by an AUX EPC channel and the loop was regulated at 5.000 psi with the backpressure regulator channel of the Pneumatic Control Module (PCM).

6890N or 7890A GC		G1888A Headspace Sampler	
Injection port	Volatiles interface	Loop size	1 mL
Temperature	160 °C	Vial pressure	14.0 psig
Split ratio	2 : 1	Headspace oven	85 °C
Carrier gas	Helium	Loop temp	100 °C
Carrier flow	9 mL/min	Transfer line temp	120 °C
GC oven program		Equilibration time	30 min, low shake
Initial temperature	35 °C	GC cycle time	50 min
Initial time	20 min	Pressurization	0.15 min
Rate	25 °C/min	Vent (loop fill)	0.5 min
Final temp	250 °C	Inject	0.5 min
Final time	15 min		
Column:	30 m x 0.45 mm x 2.55 µm DB-624 Agilent part number 124-1334		

Standards	
ICH class 1 and 2	Restek #36228 (Class 1) #36229 (class 2A) #36230 (class 2B)

Table 1
Instrument conditions for residual solvents analysis.

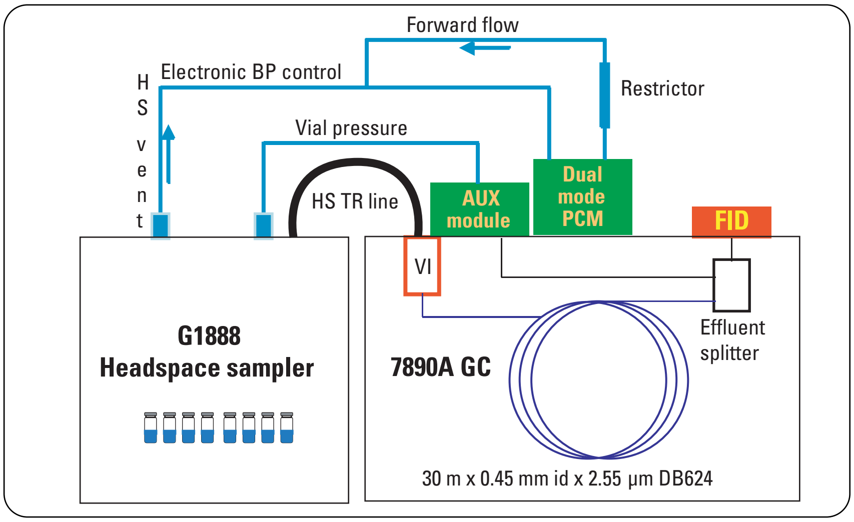
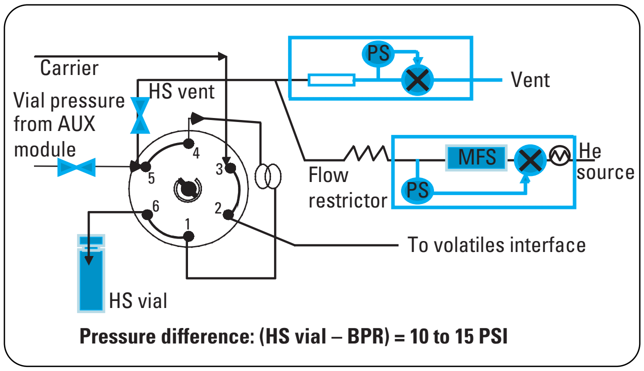
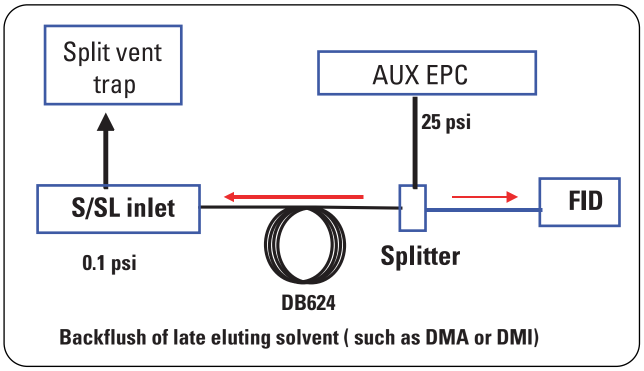


Figure 1
Block diagram of the 7890A GC configuration with backflush capability used in the backflush experiments.



Pressure difference: (HS vial – BPR) = 10 to 15 PSI



Backflush of late eluting solvent (such as DMA or DMI)

Figure 2
Headspace (HS) sampling scheme with backpressure regulation (BPR).

Figure 3
Schematic diagram of the reversed column flow used for backflushing of late eluting solvents.

Residual solvents	ICH Class	Excipient limit concentration [ppm]	7890A GC with BPR*			7890A GC at Atm P (no BPR)			6890N GC at Atm P (HS valve)		
			Repeatability [%RSD] N=8		Excipient MDL# [ppm]	Repeatability [%RSD] N=8		Excipient MDL# [ppm]	Repeatability [%RSD] N=8		Excipient MDL# [ppm]
			t_R	Area		t_R	Area		t_R	Area	
benzene	1	2	0.014	2.43	0.1	0.012	5.62	0.2	0.010	9.52	0.2
1,2-dichloroethane	1	5	0.005	4.47	0.7	0.02	8.03	0.5	0.016	8.63	0.5
1,1-dichloroethene	1	8	0.013	3.24	0.8	0.011	16.63	3.3	0.022	9.82	1.1
methylene chloride	2	600	0.009	2.85	54.9	0.016	7.15	61.4	0.018	8.20	62.7
hexane	2	290	0.014	4.18	23.1	0.027	7.15	33.2	0.020	10.61	39.1
cyclohexane	2	3880	0.042	3.59	341.0	0.012	4.29	299.9	0.018	9.79	501.1
trichloroethylene	2	80	0.012	2.69	5.8	0.016	5.29	7.9	0.013	7.91	7.9
toluene	2	890	0.025	2.11	46.3	0.024	5.41	85.9	0.031	7.90	90.3
ethylbenzene	2	369	0.002	2.27	24.4	0.002	4.90	35.3	0.003	7.40	35.5
ortho xylene	2	195	0.001	1.86	9.8	0.001	5.12	19.0	0.002	7.00	18.4
Average for 29 solvents			**0.013**	**2.83**		**0.017**	**8.77**		**0.021**	**9.34**	

* Backpressure regulation (HS-valve outlet pressure is regulated)   # Method detection limit

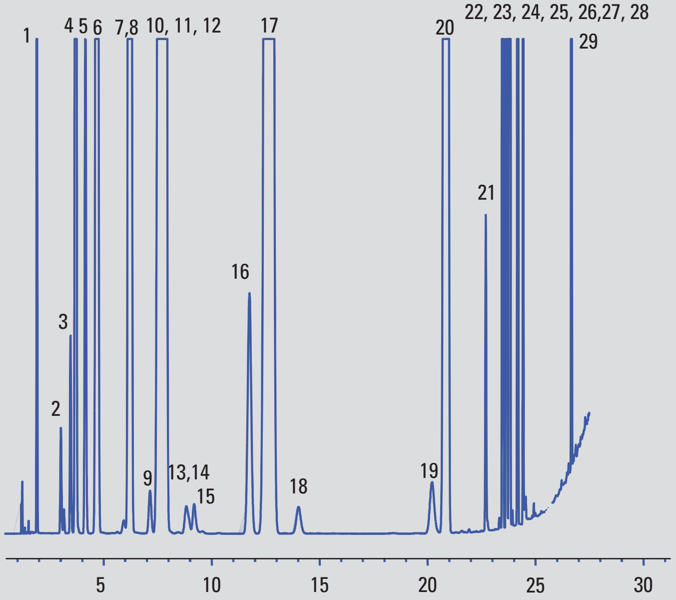
Table 2
Retention time and area repeatability and calculated MDLs of representative residual solvents for the 7890A and 6890N HS/VI/GC/FID systems.

Results from the experiments are summarized in table 2. From the 29 solvents some representative class 1 and 2 solvents were selected and the results summarized in this table. Further, the average values for all 29 solvents are shown. A series of experiments was also performed to demonstrate potential sensitivity gains realized from pressurizing the HS loop. This time, however, instead of preparing 5 mL aqueous standards in HS vials containing the 3 g of sodium sulfate, a Restek class 2B standard was used as is. A 5 µL capillary tube was filled by capillary flow action with the standard. The outside of the tube was carefully wiped with tissue paper, quickly transferred to an empty 10 mL headspace vial and immediately capped. This procedure ensured accurate and reproducible sample preparation by eliminating user bias to the extent possible in preparing identical samples. Results from this series of experiments are shown in figure 7. The error bars in the figure represent a 95 % confidence level (±2 times standard deviation or sigma).

Finally, the system was reconfigured to facilitate a column backflush to quickly remove late eluting impurities or high boiling solvent or diluents. This configuration is shown in figure 3. Instead of using the volatiles inlet (VI), a split/splitless (S/SL) inlet was interfaced to the headspace transfer line. The VI configuration is anticipated to have a limited backflush flow rate with the 0.45 mm diameter column, a limitation not observed with the S/SL inlet. The VI configuration was not tested for backflush operation.

Results and discussion

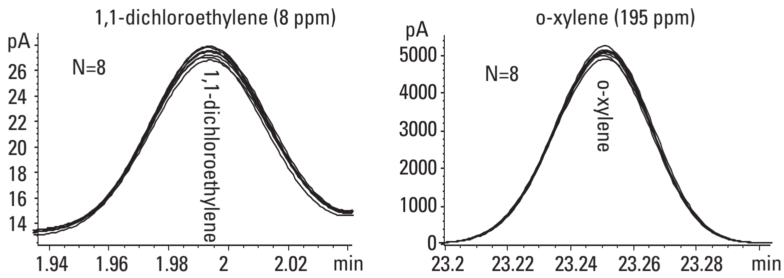
A typical chromatogram of residual solvents is shown in figure 4



1 Methanol
2 1,1 Dichloroethylene
3 Acetonitrile
4 Methylene chloride
5 Trans 1,2 dichloroethene
6 Hexane
7 Cis 1,2 dichloroethene
8 Nitrobenzene (co-elute with 7)
9 Trichloromethane
10 Carbon tetrachloride
11 Cyclohexane (co-elute with 10 & 12)
12 1,1,1 Trichloroethane (co-elute with 10 & 11)
13 Benzene
14 1,2 dimethoxyethane
15 1,2 dichloroethane
16 Trichloroethylene
17 Methyl cyclohexane
18 1,4 dioxane
19 Pyridine
20 Toluene
21 2 hexanone
22 Chlorobenzene
23 Ethylbenzene
24 DMF
25 M-xylene
26 P-xylene
27 O-xylene
28 N,N dimethylacetamide
29 Tetralin

Figure 4
Gas chromatogram of class 1 and 2 residual solvents.

and repeatability data for area and retention time of an early- and late-eluting peak are shown in figure 5. An improvement in area precision by a factor of 3 was typically observed, as in this example for 1,1-dichloroethylene. However, in some cases up to a factor of 4 was determined, for example, for o-xylene. Overall, the performance of the 7890A system is better than the 6890N system. A summary of performance characteristics for some representative class 1 and 2 analytes is given in table 2.



Repeatability (%RSD)	6890 GC	7890 GC
Retention time	0.022 %	0.013 %
Area	9.8 %	3.2 %

Improvement by a factor of ~ 3

Repeatability (%RSD)	6890 GC	7890 GC
Retention time	0.002 %	0.001 %
Area	7.0 %	1.9 %

Improvement by a factor of ~ 4

Figure 5
Examples of improved area and retention time precision by applying backpressure regulation (backpressure regulation: 5.000 PSI, headspace vial pressure: 20.000 PSI).

Backpressure regulation	Headspace vial pressurization	Averaged area Repeatability (%RSD)
No	14.000 PSI	15.8% (N=8)
10.000 PSI	25.000 PSI	4.4% (N=7)
5.000 PSI	20.000 PSI	2.8% (N=8)

Improvement by a factor of 5

Figure 6
Backpressure regulation – Effect of reducing atmospheric pressure variation at vent. Variability in loading the gas sampling valve can occur based on atmospheric pressure differences.

Improving peak area precision

From the results presented in table 2 and figure 6 the following conclusions can be drawn:

- Overall, the 7890A and 6890N show the same area repeatability when no backpressure regulation of the HS sampling loop is applied. Under the same conditions the results don't exhibit significant differences. The average area precision [%RSD] for 29 residual solvents (shown on the bottom line of table 2) for both systems is 9 %.
- The 7890A GC with backpressure regulation of the HS sampling loop, is at least 3 times better than the 7890A GC (or 6890N GC) operated without pressure regulation of the HS sampling loop. For individual analytes an improvement by a factor of 4 was observed in some cases, as shown in figure 5 for o-xylene.
- The observed differences are even more apparent when considering a very turbulent day with large variations in atmospheric pressure. This is when a series of measurement are performed. The data presented in figure 6 was obtained on such a stormy day. Under extreme weather conditions the averaged area repeatability can increase to 16 % when no backpressure regulation is applied. However, a method with optimized backpressure regulation fully compensates for the atmospheric pressure instabilities and the measured averaged repeatability returns to the same value of 3 % that was obtained under stable weather conditions in the previous experiments. The reason is that variability in loading the gas sampling valve can occur based on atmospheric pressure differences. This can happen when running the same method in different geographic locations at different altitudes or, as shown here, during a turbulent stormy day with large variations in atmospheric pressure. With backpressure regulation the gas sampling valve can operate under a constant set of conditions and precision and sensitivity improve.

Improving retention time precision

Similar to the results obtained for peak area precision a positive impact on the repeatability of retention time was observed:

- In comparison to the 6890N GC the averaged retention time repeatability for all 29 residual solvents measured on the 7890A GC was generally better, no matter whether backpressure regulation was applied or not.
- With backpressure regulation of the HS sampling loop the averaged retention time repeatability measured on the 7890A GC was best. It improved by a factor of two relative to the 6890N GC without any pressure regulation.

Optimizing sensitivity with backpressure regulation

Overall sensitivity could be doubled by applying backpressure regulation compared to the 6890N or the 7890A GC without backpressure regulation. Figure 7 shows the area changes for 1,4-dioxane with HS-vial pressurization and HS sampling loop pressurization. The more we pressurize the vial, the more we dilute the HS sample. This is clearly shown when the x-axis is zero (where the backpressure regulator is not used and the loop is exposed to atmospheric pressure). Pressurizing the HS vial to 14, 35 and 60 psi gives the highest peak area at 14 psi. When regulating the pressure in the loop with the BPR, we see an increasing area count that reaches a maximum and then decreases and eventually would give zero area counts. Once the top of the curve is reached, the depressurization of the HS vial through the HS loop (the venting cycle) is opposed by the excessive high backpressure and the sample flow through loop will diminish and may even reverse. After we reach the top of the curve, we no longer trap a representative sample in the loop. The pressure difference ($P_{HS\text{-}Vial}$ – BPR) should be 10 to 15 psi in order to collect and inject a proper HS sample.

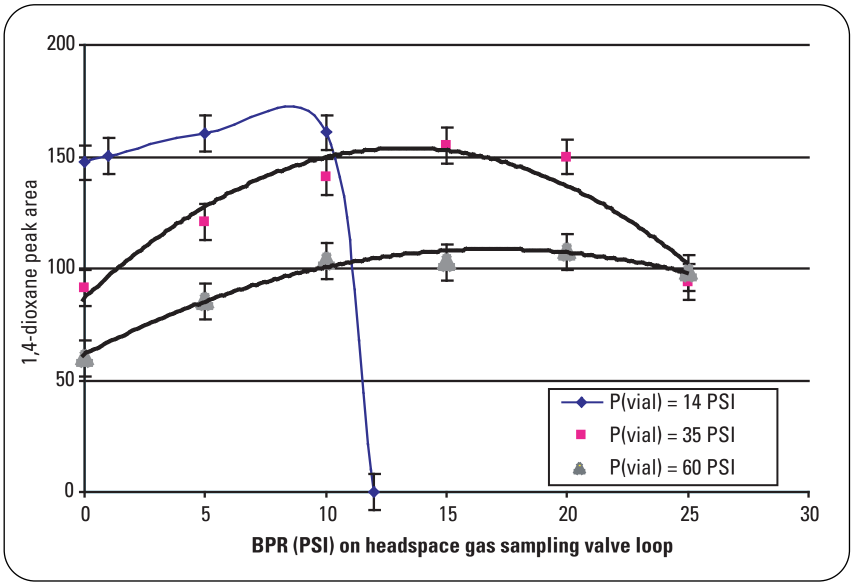


Figure 7
Improving sensitivity with the 7890A GC. Variation in peak area with headspace vial and sampling loop pressure.

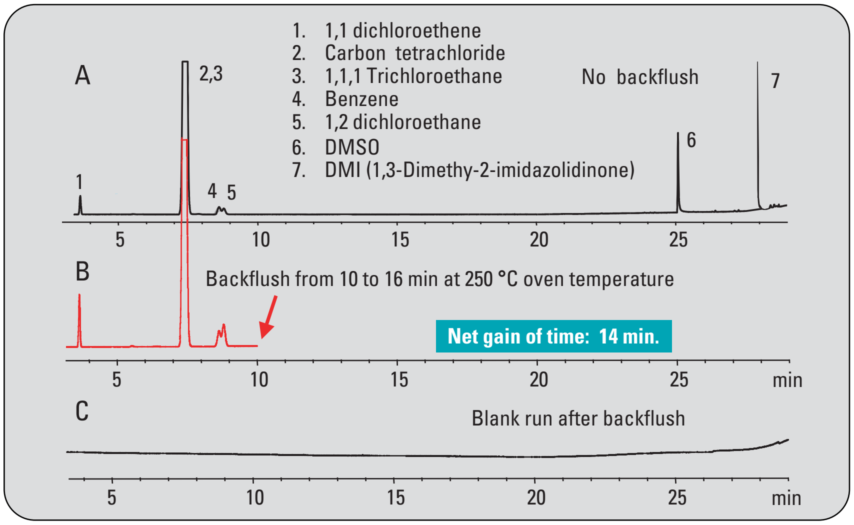


Figure 8
Example of how backflushing helps to decrease analysis time and increase workload efficiency (chromatogram of a sample containing all ICH class 1 residual solvents): A. Initial situation (no backflush) B. Reduced analysis time using backflush C. Blank run after backflush.

Increase efficiency with backflush

The backflush capability of the 7890A GC allows to remove late eluting compounds by reversing the flow. The benefits are:

- Shorter analysis time
- Extended capillary column life time.

Because this system has EPC, as soon as the last analyte of interest has eluted from the column, the AUX module can be pressure-programmed to a higher pressure (25 psi in this example) at the same time that the split/splitless inlet is programmed to a lower pressure (0.1 psi in this example). Now the flow in the column is reversed, backflushing the remaining eluents out through the split vent of the inlet. Figure 3 shows a schematic overview of the functionality. For the backflush experiment samples containing only class 1 residual solvents were prepared in DMSO and DMI, both high boiling diluents (figure 8). A typical chromatogram of such a sample lasting more than 30 minutes is shown in figure 8A. Since all the class 1 solvents elute in 10 minutes at 35 °C isothermal, a backflush was initiated from 10 to 16 minutes with an elevated column temperature of 250 °C. As a result the net gain of time per run was 14 minutes (figure 8B). The high-boiling compounds were successfully removed as can be seen from the chromatogram of the blank run that was executed afterwards (figure 8C). In this example backflush improves efficiency by almost doubling sample throughput. The fast oven cooldown of the 7890A GC further contributes to those time savings.

Equal in linearity

To measure linearity five dilutions were prepared ranging from 1/10th to two times the limit concentration. Based on the USP <467> method where 100 mg of the excipient/drug product is dissolved in 5 mL of water with 3 grams of Na_2SO_4, the solution concentration in 5 mL of water was converted to the concentration of the residual solvent in the 100 mg of excipient with the formula: c_e [ppm] = 50 · c_v. In the following text this concentration is described as excipient equivalent concentration c_e while c_v is the vial solution concentration. The linearity results for some representative residual solvents for the 7890A GC are compared to similar experiments for the 6890N GC[1] system in figures 9 and 10, respectively. The corresponding calculated values for linearity, slope and intercept are summarized in tables 3a and 3b.

Overall, the 7890A and 6890N GC systems compare well. All calibration curves are linear over a range from 1/10th to 2 times the limit concentration. The signal-to-noise (S/N) data, limit of detection (LOD) and limit of quantitation (LOQ) indicate that the systems are similar in performance (LOD and LOQ data are only shown for the 7890A in table 3a). Results from the MDL calculations (see table 2 and the appendix for the MDL equation) and a comparison of S/N ratios calculated for samples at the limit concentration indicate that the 7890A GC system is at least two times better in sensitivity than the 6890N GC system.

	Methylene Chloride	Benzene	1,4-Dioxane	Chloroform	Trichloroethylene
Linearity	0.99945	0.99859	0.99606	0.99362	0.99967
Slope	6.0935	50.2950	0.4214	2.7160	14.3362
Intercept	228.3704	4.6045	11.4008	5.2606	106.9926
LOD	10.2	0.02	2.5	0.25	0.07
LOQ	10.4	0.06	8.4	0.82	0.23

Table 3a
Linearity, LOD and LOQ results for the 7890A Headspace GC/FID system.

	Methylene Chloride	Benzene	1,4-Dioxane	Chloroform	Trichloroethylene
Linearity	0.9988	0.9995	0.9996	0.9991	0.9991
Slope	252.82	2106.2	15.268	192.41	535.39
Intercept	19.987	0.0015	0.3239	0.1851	3.7229

Table 3b
Linearity results for the 6890N Headspace GC/FID system.

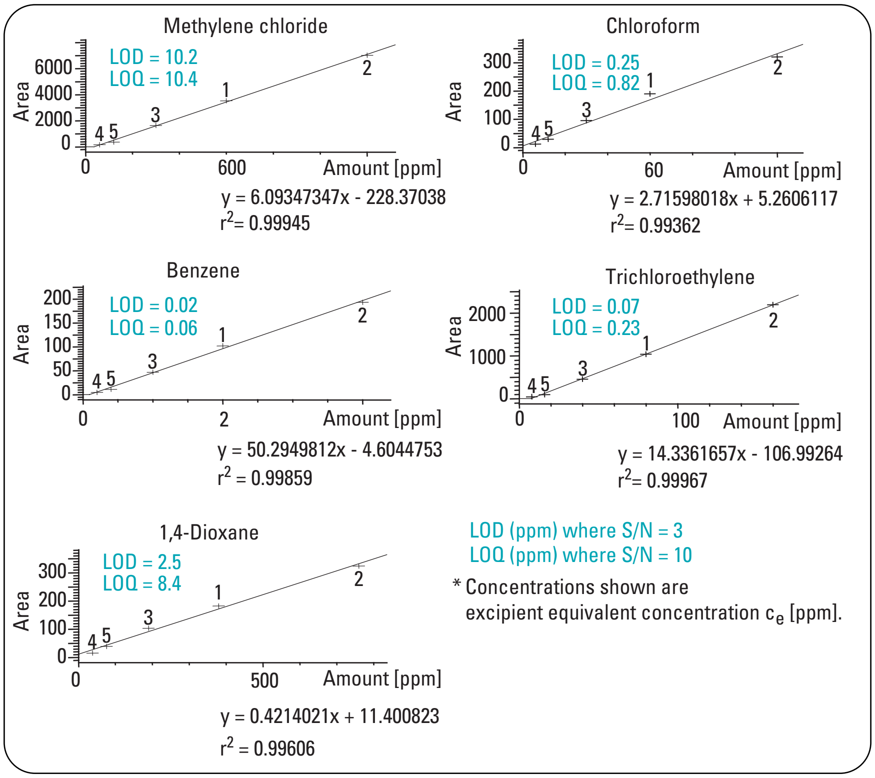


Figure 9
Linearity plots for some residual solvents determined for the 7890A Headspace GC/FID system*.

Conclusion

It was demonstrated that the 7890A GC delivers better results than the 6890 GC. In summary:

- It was possible to directly transfer an established method from the 6890 to the 7890A GC without any method development or altering the performance.
- Without backpressure regulation the 7890A GC shows the same or better performance.
- The backpressure regulation from the 7890A GC eliminates the influence of atmospheric pressure variations.
- With optimized backpressure regulation of the headspace sampling loop from the 7890A GC area precision (%RSD) could be improved by a factor of 3 to 5
- Under the same conditions retention time stability increased to ±0.001 min. (improved by a factor of 2)

- Sensitivity was doubled by pressurizing the headspace sampling loop of the 7890A GC.
- The backflush capability of the 7890A GC significantly reduces overall analysis time (in the example by 50 %).
- Both systems are equal in performance when evaluating linearity data.

The experimental setup in this application is suitable for routine analysis of residual solvents. However, it does not provide any further information when unknowns are present. The solution is to couple the GC to the Agilent 5975C Series MSD, where you can achieve superior results for both identification of unknowns and quantitation of target compounds.

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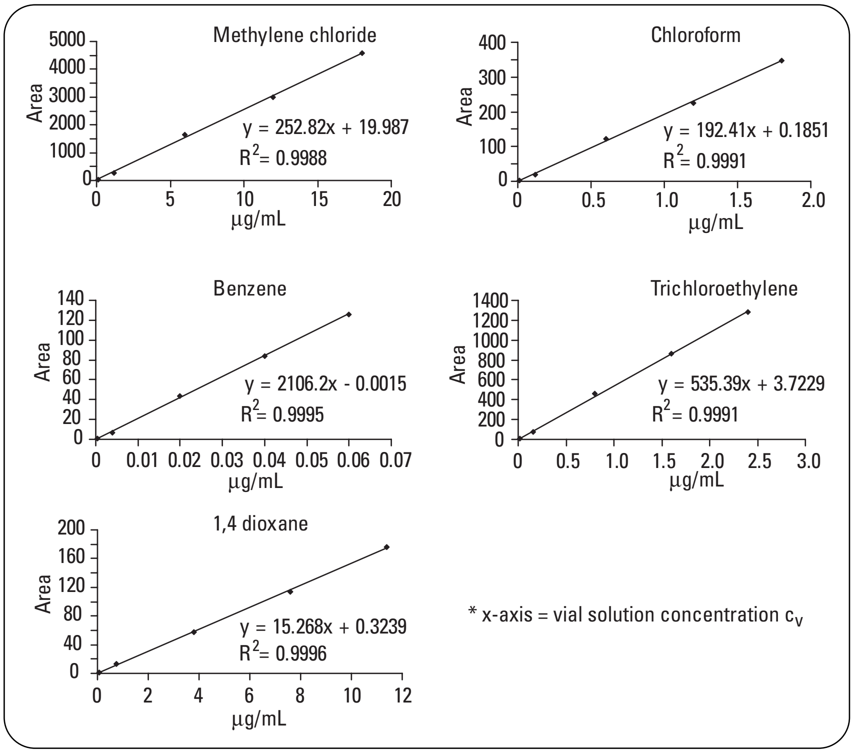

Figure 10
Linearity plots for some residual solvents determined for the 6890N Headspace GC/FID system*.

Appendix

List of acronyms

%RSD	– percent relative standard deviation	LOD	– limit of detection (S/N = 3)
[limit]	– limit concentration	LOQ	– limit of quantitation (S/N = 10)
Atm P	– atmospheric pressure	MDL	– method detection limit (statistical)
AUX	– auxiliary	MFS	– mass flow sensor
BP	– back pressure	min	– minutes
BPR	– backpressure regulation	MSD	– mass selective detector
C_v	– vial solution concentration	P(Vial)	– headspace vial pressure
C_e	– excipient equivalent concentration	PCM	– pneumatic control module
Cal	– calibration	PS	– pressure sensor
DMA	– dimethyl acetamide	PSI	– pounds per square inch
DMI	– 1,2-dimethyl-2-imidazolidinone	RT	– retention time
DMSO	– dimethyl sulfoxide	S/N	– signal-to-noise ratio
EP	– European Pharmacopoeia	S/SL	– capillary split/splitless inlet
EPC	– electronic pneumatic control	TR	– transfer
FID	– flame ionization detector	U.S. FDA	– United States Food and Drug Administration
GC	– gas chromatograph	USP	– United States Pharmacopoeia
GSV	– gas sampling valve	VI	– volatiles inlet
HS	– headspace	X	– proportional valve
ICH	– International Conference on Harmonization		

Statistical Method Detection Limit (MDL)

$$\mathbf{MDL = s \cdot t_{(n-1,\, 1-alpha=99)} = s \cdot 3.143}$$

Where

$t_{(n-1,\, 1-alpha)}$ = Student’s t value for the 99% confidence level with n-1 degrees of freedom

n = number of trials

s = standard deviation of the 7 trials

USEPA Method 524.2 (Revision 4, August 1992)

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Published February 15, 2007
Publication Number 5989-6023EN

Agilent Technologies