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Measuring residual solvents in pharmaceutical samples using fast gas chromatography techniques

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Abstract

Residual process solvents in pharmaceutical samples are monitored using gas chromatography (GC) with either flame ionization detection (FID) or mass spectrometry. Based on good manufacturing practices, measuring residual solvents is mandatory for the release testing of all active pharmaceutical ingredients and is routinely performed on samples of process intermediates. General GC methods have been developed to monitor solvents routinely used in the drug synthesis process. It is now possible to take advantage of GC equipment with faster temperature ramping capabilities, in combination with shorter capillary GC columns, to achieve a considerable gain in efficiency and a reduction in analysis turnaround time. In this paper, the development and implementation of fast GC methods for residual solvents testing will be discussed. Continued efforts to improve the efficiency of gas chromatography using existing technologies such as, the ThermoOrion Flash GC will also be discussed.

Keywords: Residual solvents; Fast gas chromatography; Pharmaceutical samples

1. Introduction

Solvents are routinely used in the synthesis and process chemistry of drug substances. These process solvents cannot be completely removed by practical manufacturing practices such as freeze–drying and drying at high temperature under vacuum. Therefore, some residual solvents may remain in drug substance material. Since there is no therapeutic benefit from the presence of residual solvents in pharmaceuticals, their presence is strictly limited. In fact, some solvents are well known to cause unacceptable toxicity, emphasizing the importance of limiting the presence of residual solvents to a minimum.

Based on a United States Pharmacopea monograph ⟨4 6 7⟩ on organic volatile impurities in pharmaceuticals, solvents such as benzene, chloroform, 1,4-dioxane, methylene chloride and trichloroethylene are placed under surveillance [1]. There is also an existing ICH guideline for residual solvents in pharmaceuticals [2]. This guideline groups residual solvents in three separate classes: class 1 includes all solvents, which should be avoided due to their high level of toxicity. As an example, class 1 includes benzene the use of which has been banned in many laboratories across industrialized countries, due to its high carcinogenic potential. Class 2 includes solvents to be limited, while class 3 includes solvents with low toxic potential. The limits for class 2 solvents range from 50 ppm for methylbutyl ketone to 4840 ppm for *N*-methylpyrrolidinone based on therapeutic doses not exceeding 10 g per day [2].

The content of residual organic solvents in pharmaceuticals is routinely measured by gas chromatography (GC). Routine GC applications include the analysis of samples of active pharmaceutical ingredients and their intermediates to comply to good laboratory and good manufacturing practices, as well as *in-process* testing for residual solvents to optimize the drying procedures [3]. Over the last decade, several GC methods to monitor residual solvents in pharmaceutical samples have been reported in the literature [4–10]. Several of these GC methods tend to have long run times and to be very specific for a limited number of residual solvents and sample matrices. A general GC method with a short run time, which is suitable for rapid screening but

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unsuitable for release testing of pharmaceutical compounds due to several co-elution of test solvents, has also been reported [8].

Past experience at Bristol-Myers Squibb laboratories with GC methods involved the use of long capillary columns with slow temperature gradients, to achieve the separation of 25 process solvents with a wide range of boiling points. This initial method used static headspace (HS) sampling and a 105 m RTX502.2 column with a 0.32 mm i.d. Each analysis involved a slow 60 min temperature gradient, excluding cool-down time and oven temperature equilibration, which could take up to an additional 10 min. Other such general methods with slow temperature gradient have also been reported [4].

In an attempt to improve the efficiency of analyses, several alternatives can be employed to speed up GC separations. These alternatives include the use of shorter capillary columns, with narrower bores, and fast temperature programming [11]. However, this is not without consequences, such as loss in resolution and limited sample capacity. The use of hydrogen as an alternative carrier gas can also be considered to reduce the analysis time, since optimal separations are achieved at higher linear velocity [12]. The first three alternatives were used in combination to achieve faster GC analysis of residual solvents. The use of hydrogen as an alternative carrier gas to helium was not considered in this study due to safety concerns. The resulting combination of a shorter RTX502.2 column with a narrower bore and faster temperature gradient was used to increase the efficiency of GC analyses.

In this paper, the development and implementation of a more rapid general method for testing residual solvents in drug substances, suitable for release testing will be described. This general method can achieve the analysis of more than 20 common residual organic process solvents in less than 15 min. A significant gain in productivity can be obtained from the use of a general GC method for testing residual solvents, as compared to developing a specific GC method for each new drug substance submitted for analysis. The development of general methods using either direct liquid injection or static headspace sampling is described. Results from the validation of the general GC methods, including establishing the linearity, precision, specificity and sensitivity, are presented. Continued efforts to improve the efficiency of gas chromatography using existing technologies such as Flash GC, will also be discussed.

2. Experimental section

2.1. Gas chromatography instrumentation and supplies

Gas chromatographs from Agilent Technologies (Palo Alto, CA), models 5890 series II and 6890, were used in the development and validation of GC methods. Gas chromatographs were equipped with the standard oven option for temperature ramping, split/splitless injection ports and flame ionization detection (FID). For the GC experiments involving mass detection, a quadruple mass analyzer model 5973 from Agilent Technologies was used in the electron impact ionization mode. Liquid sample injection was achieved via the 6890 auto-sampling unit from Agilent Technologies, using a 10 μl syringe (part number 9301-0713) compatible with the Merlin Microseal™ (part number 5182-3444). Split 1 μl injections were achieved in the injection port maintained at high temperature. A tapered-liner with deactivated glass wool (Agilent Technologies, part number 5183-4711) was used as the port liner.

Static headspace (HS) sample injection was performed via a model 7694 headspace sampler from Agilent Technologies. The headspace sampler unit was installed on the split/splitless injection port of a GC model 6890. The headspace sampling unit was equipped with a 1 ml injection loop. To avoid carryover from the previous injection, a common problem especially with amines, the headspace sampler unit was equipped with a sample loop, needle assembly and transfer line made of Silcosteel®. A 4 mm i.d., open borosilicate glass tube packed with glass wool (Agilent Technologies, part number 19251-60540) was used as the injection port liner.

A RTX502.2 column (Restek, Bellafonte, PA, part number RK10915) with a 1.4 μm film of diphenyl/dimethyl polysiloxane stationary phase, 30 m × 0.25 mm i.d., was used in the development of GC methods for testing residual solvents. This 0.25 mm i.d. column with a low polarity phase offers the combined advantages of high resolution of analyte peaks and acceptable sample capacity. High purity grade helium was used as the carrier gas. All data was acquired via the Waters Millenium 32 software, validated under the GLP/GMP and CFR part 11 requirements.

2.2. Preparation of standard and sample solutions

Dimethylacetamide (DMA) was selected as the standard and sample diluent, based on its ability to dissolve a wide variety of drug substances. Also, DMA is a solvent with a high-boiling point that does not interfere with the more volatile solvents tested by GC. For the method involving the analysis of high-boiling point solvents, methanol was selected as the diluent. Methanol is a volatile solvent, which elutes first and does not interfere with the analysis of high-boiling point solvents that elute later in the chromatogram, at much higher temperatures. All the solvents used in this study were high-quality grade solvents.

Stock standard solutions at 1.0% (v/v) were prepared by pipetting 1 ml of each test solvent in a 100 ml volumetric flask and diluted to volume with the appropriate diluent. Working standard solutions were prepared by serial dilution. Working standard solutions ranging in concentration from 0.0004 to 0.04% (v/v) were used to validate the general

methods, involving conventional GC instrumentation. For the methods involving Flash GC technology, the working standard concentrations ranged from 0.001 to 0.1% (v/v).

For methods using liquid injection, a portion of each standard solution was transferred to a 2 ml vial and closed with a Teflon-rubber lined crimp cap (Agilent Technologies, part numbers 5181-3375 and 5183-4498). For headspace sampling, 1 ml volume of standard solution was transferred to a 10 ml vial, which was tightly closed with a crimp cap lined with a PTFE-butyl septum (Agilent Technologies, part numbers 5182-0838, 9301-0721, and 9301-0976). Appropriate diluent was used for blank injections. Samples of new drug substance intermediates were obtained from the Process Research Development Group of the Bristol-Myers Squibb Pharmaceutical Research Institute located in Candiac, Québec, Canada. Sample solutions were prepared by transferring appropriate weight of sample, ranging from 25 to 100 mg depending on its solubility in diluent, in a vial. Each sample was dissolved in 1 ml of diluent in a capped vial, which was swirled to achieve dissolution.

2.3. General GC method for residual solvents testing using liquid injection

The following experimental conditions were used: a 1 μl volume of either a standard or sample solution was injected in the GC split injection port, which was maintained at a temperature of 190 °C. A split ratio of 35:1 was used. The helium carrier gas pressure was set at 11.65 psi for an expected flow of 1.0 ml/min. The temperature of the flame ionization detector was set at 250 °C. The temperature gradient increased from 35 to 90 °C at a rate of 6 °C/min, then increased at 15 °C/min to reach a final temperature of 200 °C. This method was also applied successfully to a GC with mass detection.

2.4. General method for residual solvents testing using headspace injection

For the method involving headspace sample injection, the following experimental conditions were used: Each solution of either standard or sample was equilibrated at 80 °C for 10 min, before injection of 1 ml of the headspace gas phase in the split GC injection port maintained at a temperature of 225 °C. While the headspace sampler oven was set at 80 °C, the sample loop and transfer line were set at a higher temperature of 150 °C to avoid headspace sample condensation. After the standard or sample solution was equilibrated for 10 min, the vial was pressurized for 6 s. The sample loop was then filled with the headspace gas phase for 36 s and equilibrated for 9 s, before the actual injection that required 12 s. The entire headspace sample injection routine took about 1 min. A split injection ratio of 20:1 was used. The helium carrier gas pressure was set at 11.65 psi for a flow of 1 ml/min. The temperature of the flame ionization detector was set at 250 °C. For this general GC method involving headspace sample injection, the temperature gradient was slightly faster than for the general GC method using liquid injection, since there was less interference from poorly volatile impurities present in the diluent. In this method, the temperature gradient increased from 35 to 90 °C at a rate of 6 °C/min, then increased at 20 °C/min to reach a final temperature of 200 °C.

2.5. EZ Flash™ gas chromatography

The Flash GC technology uses resistive heating of a capillary column to achieve rapid separation of analytes with a wide boiling point range. Temperature programmable gas chromatography with resistive-heating technique was first reported in the early 1990s [13,14]. Resistive heating is based on the principle that the temperature of a metal increases when an electrical current is passed through it, and the metal resistance increases consequently, in a manner that can be predicted. The metal temperature can be determined by resistance measurements and can be adjusted by controlling the amount of power applied to it to reach a defined temperature set point.

With ThermoOrion's EZ Flash™ technology, the column assembly is made by inserting a standard capillary column into a metal sheath being used as the column heater [15,16]. Since the thermal mass of the heater is minimized, the heat-up and cool-down times of an EZ Flash™ capillary column are short, as opposed to conventional GC, which requires that the entire GC oven heats up and cools down. The capillary column assembly can be installed in the host GC injector and detector, through interface heaters. The column assembly is powered by a 96 V computer-controlled power source. A variety of 5 m as well as 10 m capillary column assemblies, with the same stationary phases as conventional GC, are available from ThermoOrion. The use of Flash GC™ is simple and does not require much additional operator training, with the exception of the column assembly installation requiring some know-how.

The EZ Flash™ Gas Chromatography accessories (ThermoOrion, Beverly, MA), including the injector and detector adapters, were installed on a conventional GC 5890 series II from Agilent Technologies (Palo Alto, CA). Corresponding 10 m EZ Flash™ capillary column assemblies were obtained from ATS Scientific Inc. (Burlington, Ontario, Canada), the Canadian distributor of ThermoOrion products.

2.6. Flash GC methods for residual solvents testing

An EZ Flash™ column assembly with RTX-624 column 10 m × 0.18 mm i.d. (ATS Scientific, part number C420011550) was used. The general Flash GC method involves liquid injections of 1 μl in the split port of the GC instrument. The temperature of the injection port was maintained at 250 °C, and split injections with a ratio of 100:1 were performed due to the limited sample capacity of the

narrow bore column. The helium carrier gas pressure was set at 9.5 psi for an expected flow of 0.6 ml/min. The temperature of the flame ionization detector was set at 280 °C. The EZ Flash™ thermal gradient maintained the initial temperature to 38 °C for 30 s, which was then increased at a rate of 48 °C/min to 110 °C, then at 130 °C/min to reach a final temperature of 240 °C, held for 1 min. The total thermal gradient was achieved in 4 min.

For the Flash GC method involving the analysis of high-boiling point solvents, the following experimental conditions were used: 1 μl of either standard or sample solution was injected in the split injection port of the GC, which was maintained at 250 °C. Split injections with a ratio of 100:1 were performed. The helium carrier gas pressure was set at 16 psi for an expected flow of 1.0 ml/min. The temperature of the flame ionization detector was set at 280 °C. The EZ Flash™ temperature gradient involved maintaining the initial temperature to 100 °C for 45 s. The EZ Flash™ temperature was then increased to 180 °C in 30 s, to reach a final temperature of 230 °C in 90 s, which was held for 15 s for a total run time of 3 min.

3. Results and discussion

3.1. Rapid GC methods using conventional technology

The initial general GC method in use at BMS laboratories achieved the separation and analysis of 25 organic process solvents in 60 min. Changing only few parameters, e.g. reducing the column length and diameter, as well as using a faster temperature ramp, allowed a significant reduction in the GC analysis time. Using a RTX502.2, 30 m × 0.25 mm i.d. column with a 1.4 μm film, the separation and analysis of 24 process solvents can be achieved in less than 15 min. This represents a four-fold reduction in analysis time as compared to the initial general GC method previously used at BMS laboratories. The experimental conditions were adapted to allow headspace injection as well as liquid injection. Example chromatograms for each method are presented in Fig. 1. When liquid injection is used, accumulation of non-volatile drug substances on the glass wool necessitates frequent replacement of the injection port liner. Since only the volatile portion of the analytical sample is injected into the GC,

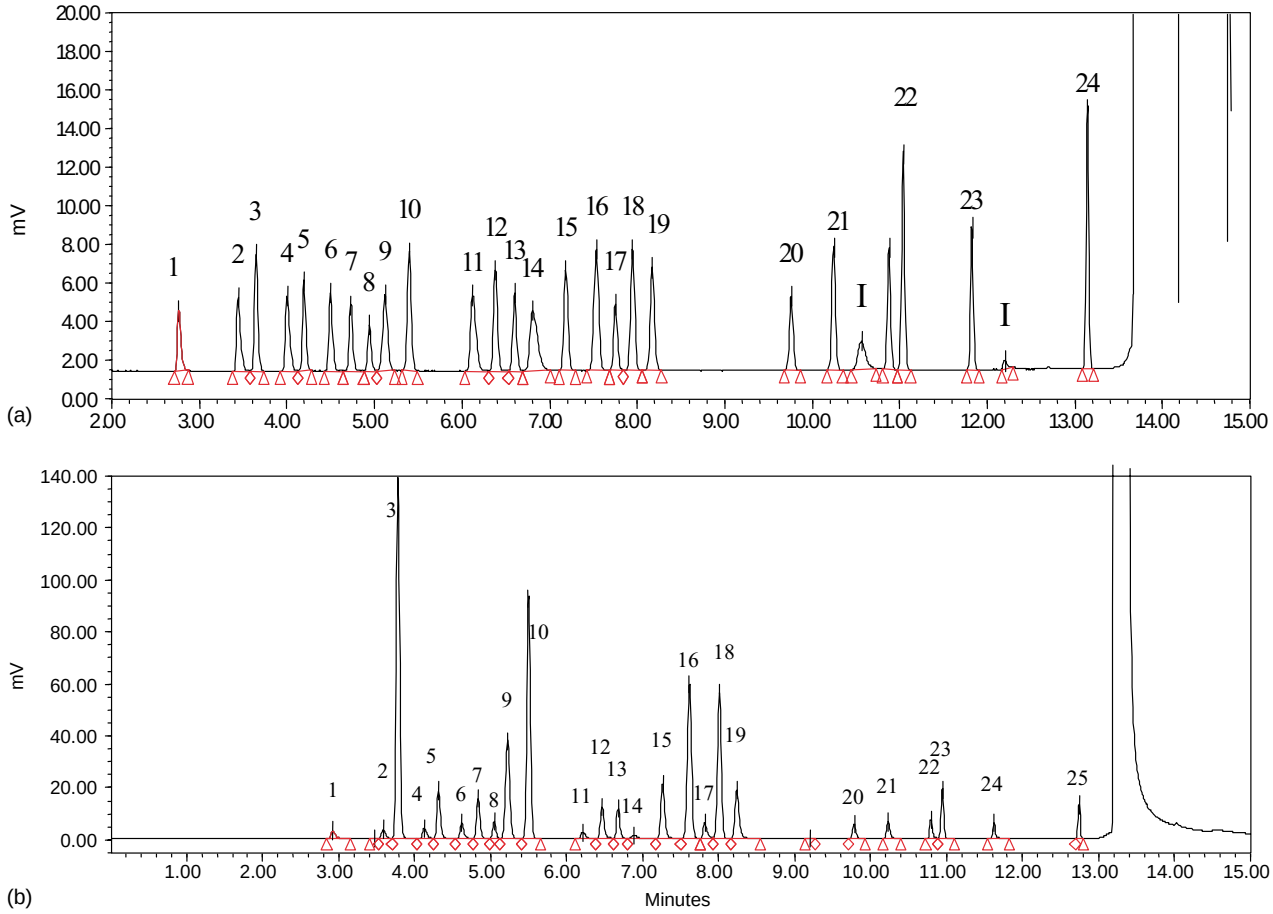

Fig. 1. GC chromatograms of common process solvents at a concentration of 0.004% (v/v). (a) GC method using liquid injection. The dissolution solvent, *N*,*N*-dimethylacetamide, elutes last after 13.5 min. The dissolution solvent impurities are indicated by an "I" on the chromatogram. *iso*-Butylacetate (RT, 10.85 min) was not included in the list of validated solvents due to the presence of dissolution solvent impurities, which tend to interfere. (b) GC method using headspace injection. The dissolution solvent, *N*,*N*-dimethylacetamide, elutes last after 13 min.

Table 1
Validation results for the general GC method for residual solvents testing

Component	Retention time (min)	Standard deviation[a] (area counts)	Density (g/ml)	Slope (area counts per % (v/v))	MQL[b] (ppm)
Methanol	2.76	65	0.79	2.00×10^6	26
Ethanol	3.44	91	0.79	2.78×10^6	26
Pentane	3.65	111	0.63	3.99×10^6	18
iso-Propanol	4.00	95	0.79	3.09×10^6	24
Acetone	4.19	87	0.79	2.95×10^6	23
Acetonitrile	4.50	100	0.78	2.74×10^6	28
Methyl acetate	4.73	70	0.93	2.23×10^6	29
Dichloromethane	4.94	47	1.33	1.51×10^6	41
Methyl-*t*-butylether	5.12	113	0.74	3.62×10^6	23
n-Hexane	5.39	144	0.66	4.57×10^6	21
sec-Butanol	6.12	159	0.81	3.87×10^6	33
Methylethylketone	6.38	144	0.81	3.71×10^6	31
Ethyl acetate	6.60	90	0.90	2.85×10^6	28
iso-Butanol	6.81	173	0.80	4.39×10^6	32
Tetrahydrofuran	7.18	119	0.89	3.86×10^6	27
Cyclohexane	7.53	171	0.78	5.62×10^6	24
1,2-Dimethoxyethane	7.75	69	0.87	2.42×10^6	25
Heptane	7.95	149	0.68	4.83×10^6	21
2-Methyl tetrahydrofuran	8.17	135	0.86	4.21×10^6	28
1,4-Dioxane	9.77	75	1.03	2.76×10^6	28
Methyl *iso*butylketone	10.24	121	0.80	4.30×10^6	23
Toluene	11.04	241	0.87	6.70×10^6	31
n-Butylacetate	11.82	114	0.88	3.86×10^6	26
p-Xylene	13.14	197	0.87	6.58×10^6	26

[a] From average standard deviation of the three most diluted standard solutions.
[b] Based on a sample concentration of 0.1 g/ml.

headspace sampling provides the additional advantage of reducing the frequency of injection port liner replacement.

The organic process solvents listed in Table 1 were selected based on a survey of the most common process solvents used by the local Process Research and Development Group. The chromatograms in Fig. 1 demonstrate baseline resolution of all the components of interest. Both the liquid injection and headspace methods test for the same solvents, to the exception of *iso*-butylacetate, which can best be determined by HS–GC. With the liquid injection method, presence of impurities in the diluent blank interferes with the determination of *iso*-butylacetate. Partial co-elution of 1,2-dimethoxyethane and heptane can occur when the methods are used with a Mass detector. When GC–MS is used, the vacuum at the detector end tends to speed up the separation by a factor of about 20%. When operated at a carrier gas flow of 1.0 ml/min, the detector end is exposed to a vacuum of 9×10^{-6} Torr. This leads to a partial loss in resolution between 1,2-dimethoxyethane and heptane. However, this critical pair can still be resolved based on the mass of characteristic ions. The identity of each individual peak was confirmed by mass detection.

Both GC–FID methods were successfully validated for linearity, precision, and sensitivity. Validation results for the general GC methods using liquid injection and headspace sampling are presented in Tables 1 and 2, respectively. The linearity for each solvent tested has been established at concentrations ranging from 0.0004 to 0.04% (v/v). Linear regression analysis was applied and a correlation coefficient $\geq$0.999 was obtained for each individual solvent. The relative standard deviations obtained from the results of six consecutive injections of working standard solutions at 0.0004% (v/v) and 0.004% (v/v) were all <10%, which is the self-established maximum tolerated limit. Both general GC methods, involving liquid sample injection as well as static headspace sampling, have been successfully used in the analysis of several active pharmaceutical ingredients. The reproducibility of results conducted on separate days on different instruments was found to be well within an expected variability of 10%. For an investigative new drug application, evaluation of the accuracy of the general GC method is required. The accuracy of the method can be obtained from a recovery study of spiked samples of the drug substance tested with a suitable standard solution.

The minimum quantifiable limit was estimated as a function of the standard deviation of the baseline noise. The baseline noise was estimated from the average standard deviation of the peak area of replicate injections of the most diluted standard solutions for each solvent of interest. The MQL is defined as 10 times the standard deviation of the baseline noise divided by the slope of the response versus concentration.

The MQL was established at 0.0003% (v/v) or better for all tested solvents. Based on a sample target concentration of 0.1 g/ml, this represents a MQL of about 25 ppm. The limit for *iso*-butanol by headspace GC was slightly higher

Table 2
Validation results for the general HS–GC method for residual solvents testing

Component	Retention time (min)	Standard deviation[a] (area counts)	Density (g/ml)	Slope (area counts per % (v/v))	MQL[b] (ppm)
Methanol	2.93	95	0.79	3.53×10^6	21
Ethanol	3.58	123	0.79	3.69×10^6	26
Pentane	3.78	2125	0.63	1.01×10^8	13
iso-Propanol	4.13	143	0.79	4.13×10^6	27
Acetone	4.31	278	0.79	1.47×10^7	15
Acetonitrile	4.62	166	0.78	5.30×10^6	24
Methyl acetate	4.84	205	0.93	1.22×10^7	16
Dichloromethane	5.05	114	1.33	4.79×10^6	32
Methyl-*t*-butylether	5.22	388	0.74	3.73×10^7	8
n-Hexane	5.50	824	0.66	7.60×10^7	7
sec-Butanol	6.21	142	0.81	2.78×10^6	41
Methylethylketone	6.46	258	0.81	1.05×10^7	20
Ethyl acetate	6.68	216	0.90	9.99×10^6	19
iso-Butanol	6.89	188	0.80	1.99×10^6	76
Tetrahydrofuran	7.26	347	0.89	1.88×10^7	16
Cyclohexane	7.61	604	0.78	5.71×10^7	8
1,2-Dimethoxyethane	7.82	151	0.87	6.23×10^6	21
Heptane	8.01	507	0.68	4.60×10^7	7
2-Methyl tetrahydrofuran	8.24	397	0.86	1.73×10^7	20
1,4-Dioxane	9.79	135	1.03	4.30×10^6	32
Methyl *iso*butylketone	10.23	205	0.80	5.07×10^6	32
iso-Butylacetate	10.80	202	0.87	5.08×10^6	35
Toluene	10.95	381	0.87	1.09×10^7	30
n-Butylacetate	11.62	139	0.88	3.74×10^6	33
p-Xylene	12.75	241	0.87	5.90×10^6	36

[a] From average standard deviation of the three most diluted standard solutions.
[b] Based on a sample concentration of 0.1 g/ml.

at 76 ppm. However, this solvent belongs to the class 3 category of the ICH guidelines, which can be tolerated up to 5000 ppm in pharmaceutical preparations [2]. Therefore, the sensitivity of the general method is sufficient to monitor residual solvents tested within the limits set by the ICH guideline. If the sample matrix interferes with the analysis of residual solvents or if a limited amount of sample is available, it is possible to reduce the concentration of tested drug substance from 100 to 25 mg per ml.

3.2. Rapid methods using Flash GC

A general Flash GC method for testing residual solvents in pharmaceuticals was developed. This method was derived from a ThermoOrion application note [17], which suggested the use of a short narrow bore column, 10 m × 0.1 mm i.d., with a 0.4 μm film of 6% cyanopropyl/94% polydimethyl siloxane. However due to limited sample capacity and difficulties in obtaining reliable and reproducible

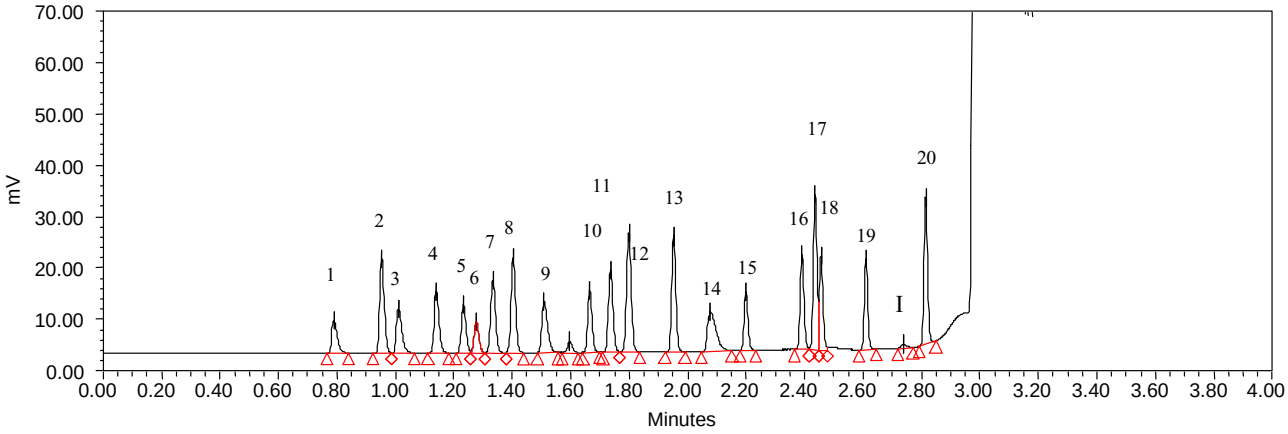

Fig. 2. Chromatogram of 20 solvents of pharmaceutical interest by Flash GC. The working standard concentration is 0.01% (v/v). The dissolution solvent employed, *N*,*N*-dimethylacetamide, elutes last after 3 min. The dissolution solvent impurities are indicated by an "I" on the chromatogram.

Table 3
Validation results for the Flash GC method for residual solvents testing

Component	Retention time (min)	Standard deviation[a] (area counts)	Density (g/ml)	Slope (area counts per % (v/v))	MQL[b] (ppm)
Methanol	0.79	202	0.79	7.54×10^5	212
Pentane	0.95	403	0.63	1.77×10^6	143
Ethanol	1.01	309	0.79	1.02×10^6	239
Acetone	1.14	314	0.79	1.18×10^6	210
Methylacetate	1.23	230	0.93	8.94×10^5	239
Dichloromethane	1.27	155	1.33	5.99×10^5	344
Methyl-*t*-butylether	1.33	381	0.74	1.49×10^6	189
Hexane	1.40	413	0.66	1.75×10^6	156
1-Propanol	1.51	389	0.80	1.25×10^6	249
Ethylacetate	1.66	307	0.90	1.11×10^6	249
Tetrahydrofuran	1.73	395	0.89	1.54×10^6	228
Cyclohexane	1.79	584	0.78	2.33×10^6	196
Heptane	1.95	510	0.68	1.98×10^6	175
1-Butanol	2.07	431	0.81	1.35×10^6	259
1,4-Dioxane	2.19	289	1.03	9.78×10^5	304
Methyl *iso*butylketone	2.39	422	0.80	1.48×10^6	228
Toluene	2.43	612	0.87	2.37×10^6	225
iso-Butylacetate	2.45	442	0.87	1.39×10^6	277
n-Butylacetate	2.61	422	0.88	1.31×10^6	283
p-Xylene	2.81	593	0.87	2.21×10^6	233

[a] From average standard deviation of the three most diluted standard solutions.
[b] Based on a sample concentration of 0.1 g/ml.

chromatography, the use of the narrow bore column with the 0.4 μm film of 6% cyanopropyl and 94% polydimethyl siloxane was soon abandoned.

A 10 m × 0.18 mm i.d. column, with a 1 μm film of 6% cyanopropyl/94% polydimethyl siloxane stationary phase was more successful in achieving the analysis of residual solvents in pharmaceuticals. An example GC chromatogram of volatile solvents using the EZ Flash™ technology is presented in Fig. 2. This method takes advantage of the use of a short narrow bore column and the fast temperature ramping to achieve the separation of 20 volatile solvents in 4 min. This represents a significant improvement in productivity in residual process solvents testing, since each analysis requires less than 7 min, including the injection routine, analysis and cool-down time. In an effort to reduce the time required for each analysis and optimize the sample throughput, the Flash GC method was restricted to liquid sample introduction. Static headspace sampling requires time-consuming incubation of each sample at high temperature, which is much less compatible with the principle of performing fast GC analyses.

Tested solvents by the Flash GC method are listed in Table 3. Toluene and *iso*-butylacetate are not baseline resolved in the Flash GC method. A few solvents tend to co-elute on the short RTX-624 column. For example, acetonitrile and dichloromethane co-elute, thus only dichloromethane was included in the list of test solvents by the Flash GC method. If both solvents are used in the synthetic process of a drug substance or its intermediates, the conventional GC method, which can achieve the separation of acetonitrile and dichloromethane, should be used rather than the Flash GC method.

Heat dissipation in the GC oven arising from the EZ Flash™ temperature gradient may create a temperature error message on the GC unit, if the oven is set at 35 °C for the duration of the chromatographic run. To avoid this difficulty, a slow temperature gradient was programmed with the GC oven. We have used a gradient from 35 °C, maintained for 30 s, ramped to 85 °C in 2 min that was held for 1.5 min. This slow gradient approximates the temperature rise due to the heat dissipation created by the EZ Flash™ temperature gradient in the GC oven. However, the draw back in using this slow gradient is an increased cool-down time to return to the initial conditions. The EZ Flash™ GC unit was initially installed on a single-port 6850 GC instrument from Agilent Technologies. Extended cool-down times attributed to the limited capacity of the 6850 GC oven, with its smaller dimension and apparently less efficient cooling fan, to dissipate the temperature generated by the EZ Flash™ column assembly and adapters, required the relocation of the EZ Flash™ GC unit on a 5890 GC instrument.

With the EZ Flash™ injection interface, the selection of an appropriate inlet liner appears to be critical. For example, the use of a borosilicate low-pressure drop, tapered liner with glass wool, such as the Agilent Technologies #5183-4647, affects the peak shape of low-boiling point solvents. With a tapered liner, the peaks corresponding to methanol, ethanol and dichloromethane appeared rather broad. The use of straight liners without glass wool also causes serious peak tailing, especially for low-boiling point solvents. A straight borosilicate liner with glass wool, such as the Agilent Technologies #19251-60540, was selected for this application. The presence of the glass wool inside the inlet liner provides the additional advantage of retaining

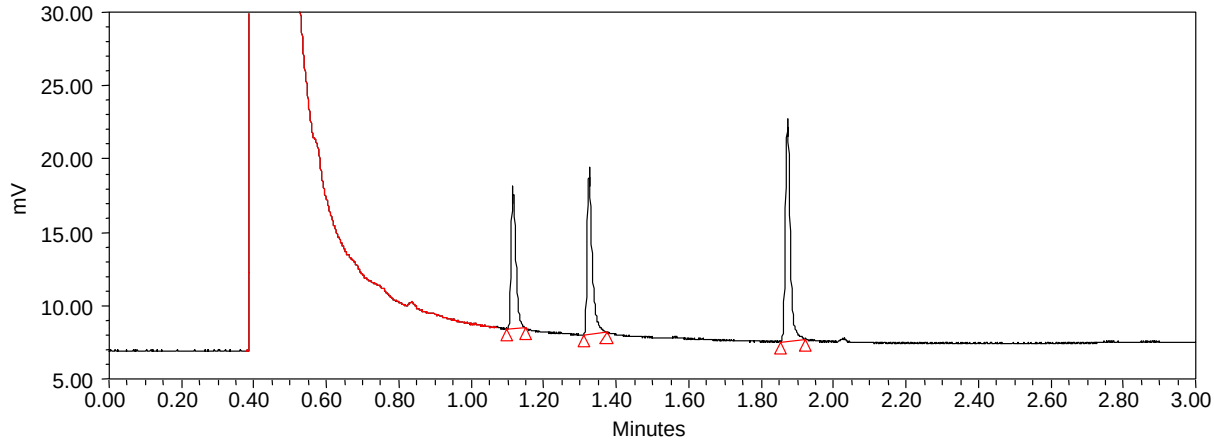

Fig. 3. Chromatogram of high-boiling point solvents by Flash GC. The working standard concentration is 0.01% (v/v). The dissolution solvent employed, methanol, elutes first in the first 60 s of the temperature gradient. The elution of methanol is followed by DMF, DMSO and NMP.

the non-volatile drug substances, which could otherwise accumulate on the gold seal of the instrument and contaminate the injection end of the GC capillary column. The glass wool can successfully retain the non-volatile component of the sample matrix, namely the drug substance, and the inlet liner can be easily replaced when the glass wool becomes contaminated with a significant amount of drug substances. With the EZ Flash^{TM} column assembly, a combination of straight liners with glass wool and higher split ratios appears to be ideal to avoid column overloading and to optimize the chromatography. The use of split injections at lower ratios than 50:1 causes peak tailing and loss in resolution. More so, splitless injections appear very difficult with the EZ Flash^{TM} technology for the same reasons.

Dimethylacetamide, a high-boiling point solvent, which elutes at the very end of the chromatographic run, was selected as the ideal solvent for the analysis of pharmaceutical samples. An attempt to use other high-boiling point solvents as dissolution solvents for pharmaceutical preparations was not successful. Dimethylsulfoxide and dimethylformamide have several impurities that interfere with the analysis of several commonly used organic process solvents, while the use of *N*-methylpyrrolidinone as the dissolution solvent causes significant peak tailing of the low-boiling point solvents. DMA gives rise to an acceptable chromatography of volatile solvents, for which pharmaceutical preparations are tested. As an additional advantage, many drug substances are soluble in DMA.

The linearity, precision and sensitivity of the Flash GC method were evaluated. The linearity for each solvent tested has been established at concentrations ranging from 0.001 to 0.1% (v/v). Linear regression analysis was applied and a correlation coefficient $\geq$0.999 was obtained for each individual solvent. The relative standard deviations obtained from the results of six consecutive injections of working standard solutions at 0.005% (v/v) and 0.01% (v/v) were all <10%. Since the retention time is the primary mean of peak identification in the Flash GC separations, a high precision of the retention time is of the utmost importance. Adjusting the data acquisition to the maximum rate of 20 Hz allowed by the GC 5890, the deviations in the peak retention times varied from 0.1 to 0.15 s in the same chromatographic run. Therefore, the reproducibility of the retention times is sufficient to identify analytes with a peak-to-peak resolution of 2–3 s or better.

The minimum quantifiable limit was established at 350 ppm or better, based on a sample target concentration of 0.1 g/ml. Results are presented in Table 3. The use of a higher split ratio explains the lower sensitivity of Flash GC, as compared to conventional GC. Despite the lower sensitivity of the Flash GC method, the minimum quantifiable limits are still sufficient for all the ICH class 2 solvents, which can be tested by this method. Several class 3 solvents, which can be tolerated at higher concentrations in drug substances, can also be successfully tested by this method.

The rapid temperature ramping capacity of the Flash GC technology can be advantageously used in the determination

Table 4
Validation results for Flash GC method for high-boiling point solvents

Component	Retention time (min)	Standard deviation[a] (area counts)	Density (g/ml)	Slope (area counts per % (v/v))	MQL[b] (ppm)
DMF	1.11	165	0.94	1.05×10^6	146
DMSO	1.31	274	1.10	1.33×10^6	227
NMP	1.85	391	1.03	1.72×10^6	234

[a] From average standard deviation of the three most diluted standard solutions.
[b] Based on a sample concentration of 0.1 g/ml.

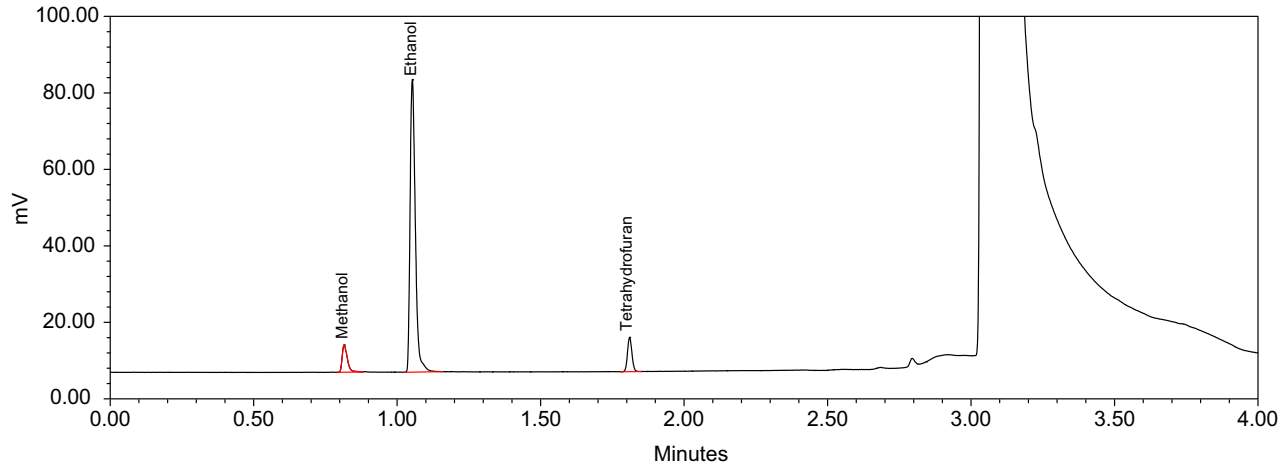

Fig. 4. Chromatogram of a pharmaceutical sample tested for residual process solvents using the Flash GC method. The pharmaceutical sample A was dissolved at a concentration of 0.025 g/ml in DMA. Methanol, ethanol and tetrahydrofuran have been identified and quantitated in Sample A.

of solvents with a high-boiling point. Non-volatile solvents are occasionally used as process solvents. Using a 10 m EZ Flash^{TM} RTX-624 column and a rapid ramping rate, *N*,*N*-dimethylformamide (DMF), methyl sulfoxide (DMSO) and 1-methyl-2-pyrrolidinone (NMP) can be analyzed in less than 3 min. An example chromatogram is illustrated in Fig. 3. Methanol, a volatile solvent, which elutes first, was used as the sample dissolution solvent. As with the other Flash GC method to monitor volatile residual solvents, the linearity, precision, and limits of sensitivity were successfully established with this method. Validation results are presented in Table 4. Based on a working sample concentration of 0.1 g/ml, the limits of quantitation established were 146, 227, and 234 ppm for DMF, DMSO, and NMP, respectively. All of these are sufficient to meet the limits established by the ICH guidelines.

3.3. Testing residual process solvents using Flash GC

Samples of new drug substance intermediates, from different research programs, were tested for residual solvents using the Flash GC method. The local Process and Research Development Group provided the samples of synthetic intermediates. Fig. 4 shows an example chromatogram of pharmaceutical sample A tested for residual organic process solvents using the Flash GC method. In Table 5, results obtained with the Flash GC method are compared to the conventional GC method. The results for the four tested samples are reported to the nearest hundredth of a percent. As listed in Table 5, the level of residual solvents obtained using the EZ Flash GC technology were found to compare favorably well with those obtained using conventional GC. If the tested substances were to be used in pharmaceutical preparations, the results in Table 5 clearly identify the necessity to optimize the drying process for samples A and D. Both GC methods indicate that the level of methanol, ethanol and xylene exceeds the ICH guideline limits set at 3000, 5000, and 2170 ppm, respectively.

Table 5
Results from residual solvent testing in samples of drug substance intermediates using conventional and Flash GC methods

Sample	Solvents found	Rapid GC method results (% (w/w))	EZ Flash GC method results (% (w/w))
Sample A	Methanol	0.34	0.35
	Ethanol	2.64	2.59
	Tetrahydrofuran	0.21	0.26
Sample B	Ethanol	0.04	0.04
Sample C	Dichloromethane	0.05	0.04
	Toluene	0.06	0.07
Sample D	Toluene	0.03	<0.03[a]
	p-Xylene	0.26	0.27

[a] Result below the estimated detection limit for toluene for sample D, which was prepared at 0.025 g/ml.

A recovery study was performed by spiking sample A with a mixture of residual solvents, at the lower and higher-end of the tested range of concentrations. The average recoveries varied from 85 to 93% for the sample spiked with 0.005% (v/v) of tested solvents, while the average recoveries were all superior to 92% for the same sample spiked with 0.05% (v/v) of tested solvents. These results certainly contribute to establish the validity of the Flash GC technology to monitor residual solvents in-process samples.

4. Conclusion

It is now possible to take advantage of improved GC technology to achieve fast separations and analyses of residual solvents in pharmaceutical samples. Using conventional gas chromatography and a shorter column with a narrower bore, more rapid separations and analyses of residual organic process solvents in pharmaceutical samples can be achieved. General GC methods with liquid sample injection as well as headspace sampling can separate 25 process solvents within

15 min, as reported in this paper. Both general GC methods were validated for specificity, linearity, precision, and sensitivity. General GC methods can routinely be applied for testing residual process solvents in drug substance material such as, synthetic intermediates and investigative new drug substances with acceptable accuracy. The sensitivity of the general methods using flame ionization detection is sufficient to monitor residual solvents within the limits set by ICH guidelines.

The use of fast GC techniques, such as the EZ Flash™ resistive-heating technology, can further speed up the analysis of residual solvents. Separations of 20 process solvents was achieved in less than 4 min using the EZ Flash™ technology. The Flash GC technology offers the additional advantage of a rapid cool-down time. Although less sensitive, the Flash GC method can still be advantageously applied to *in-process* solvent monitoring when a more rapid analytical response and sample turnaround time is required. A complete analysis including a blank injection, followed by calibration with a standard and *in-process* testing of a pharmaceutical sample solution can be achieved within about 20 min. Also, the Flash GC technology can economically transform an obsolete GC in an instrument that can achieve very rapid gas chromatographic separations.

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