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A generic static headspace gas chromatography method for determination of residual solvents in drug substance

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ABSTRACT

In order to increase productivity of drug analysis in the pharmaceutical industry, an efficient and sensitive generic static headspace gas chromatography (HSGC) method was successfully developed and validated for the determination of 44 classes 2 and 3 solvents of International Conference of Harmonization (ICH) guideline Q3C, as residual solvents in drug substance. In order to increase the method sensitivity and efficiency in sample equilibration, dimethylsulfoxide (DMSO) was selected as the sample diluent based on its high capacity of dissolving drug substance, stability and high boiling point. The HS sample equilibration temperature and equilibration time are assessed in ranges of 125–150 °C and 8–15 min, respectively. The results indicate that the residual solvents in 200 mg of drug substance can be equilibrated efficiently in HS sampler at 140 °C for 10 min. The GC parameters, e.g. sample split ratio, carrier flow rate and oven temperature gradient are manipulated to enhance the method sensitivity and separation efficiency. The two-stage gradient GC run from 35 to 240 °C, using an Agilent DB-624 capillary column (30 m long, 0.32 mm I.D., 1.8 μm film thickness), is suitable to determine 44 ICH classes 2 and 3 solvents in 30 min. The method validation results indicate that the method is accurate, precise, linear and sensitive for solvents assessed. The recoveries of most of these solvents from four drug substances are greater than 80% within the method determination ranges. However, this method is not suitable for the 10 remaining ICH classes 2 and 3 solvents, because they are too polar (e.g. formic acid and acidic acid), or have boiling points higher than 150 °C, (e.g. anisol and cumene). In comparison with the previous published methods, this method has a much shorter sample equilibration time, a better separation for many solvents, a higher sensitivity and a broader concentration range.

1. Introduction

Residual solvents are critical impurities in drug substances, drug products and excipients, because they may cause toxicity and safety issues, and affect physicochemical properties of drug substances and drug products. In order to control residual solvent contents in drug substances, products and excipients, ICH Q3C guideline provides specific criteria for class 1 solvents (5) – known or suspected human carcinogens or environmental hazards, class 2 solvents (26) – suspected of other significant but reversible toxicities, and class 3 (28) solvents – low toxic potential to man [1]. Therefore, determination of residual solvents becomes a necessary procedure for quality control of drug substances and drug products to meet regulatory expectations and ensure patient safety.

Developing and validating an efficient and sensitive generic analytical method for the determination of residual solvents may significantly increase productivity of an analytical laboratory in the pharmaceutical industry. Determination of residual solvents using GC with a flame ionization detector (FID) is the most common technique in the pharmaceutical industry, because of its high separation efficiency and sensitivity for volatile organic compounds. GC analysis may be performed by either direct injection or HS sampling [2]. The advantage of the direct injection mode is that all analytes in a sample solution are directly injected into the GC, leading to a lower sample load or sample requirement and a simpler analytical procedure. But, the high boiling/melting point or polar components of the sample may not be eluted through a GC column, and they may contaminate the GC injection port and/or column. In contrast, HS sampling can prevent this from occurring, but it limits the analysis to those solvents being evaporated from the HS only, and it requires a larger sample load. In addition, the analysis time can be longer due to sampler equilibration prior to injection on column.

There are two types of HS sampling techniques, static HS and dynamic HS sampling. The static HS sampling is more easily automated. Dynamic HS sampling with purge and trap is less suitable for automation but has a higher sensitivity [2,3]. Currently, static

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Table 1
HSGC parameters for the method development and validation.

Parameter	Evaluated settings for development	Optimized settings for validation
HS		
Equilibration temperature	125, 140, 150 °C	140 °C
Transfer line temperature	135, 140, 150 °C	140 °C
Loop temperature	125, 140, 150 °C	140 °C
Vial pressure	9 psi	9 psi
Vial equilibration time	8, 10, 15 mim	10 mim
Vial pressurization time	0.4 min	0.4 min
Loop size	1 mL	1 mL
Loop equilibration time	0.5 min	0.5 min
Loop fill time	1 min	1 min
GC		
Inlet temperature	200 °C	200 °C
Carrier (He) flow rate	1.5, 1.8, 2.0 mL/min (28–40 cm/s)	1.8 mL/min (constant flow, approximately 30–33 cm/s)
Inlet split ratio	1:0, 1:1, 1:2, 1:5	1:1 at the split flow of 1.9 mL/min
Oven temperature gradient	Hold 0, 1, 3, 5 min at 35 °C	0–3 min at 35 °C
	Ramping at 2, 3, 4, 5, 8, 10 °C/min	3–21.75 min, ramping to 110 °C at 4 °C/min,
	Ramping to 240–280 °C at 10, 20, 30, 40 °C/min	21.75–25 min, ramping to 240 °C at 40 °C/min
	Hold at 240–280 °C for 2–10	25–30 min at 240 °C
FID temperature	260, 280, 300 °C	260 °C
Detector gas flows	Hydrogen 30–40 mL/min, air 300–400 mL/min	Hydrogen, air, make up at 35, 350, 23.2 mL/min, respectively

HSGC with FID is more popular for analyzing residual solvents in drug substances [4–8] and drug products [9–11] in the industry. Static HS sampling is based on thermostatic partitioning of volatile compounds in a sealed vial between the sample diluent and the gas phase. Sample diluent is a critical factor affecting HSGC method sample load, sensitivity, HS equilibration temperature and time. A good sample diluent for analyzing residual solvents in drug substances or drug products should have a high capability for dissolving a large amount of samples, a high boiling point and a good stability. There are a number of commonly used sample diluents for HSGC analyses, such water, DMSO, N, N-dimethyformamide (DMF), N, N-dimethylacetamide (DMA), benzyl alcohol (BA), 1,3-dimethyl-2-imidazolidinone (DMI), and mixtures of water–DMF or water–DMSO [12].

Water is a good diluent for water soluble samples and analytes, because it is clean, stable and inexpensive. However, many organic synthetic drug substances and drug products have low water solubilities, which would limit the sample load. Meanwhile, using water as a diluent may also lead to a lower method precision than organic solvent, like DMF [5]. When a mixture of water–DMF or water–DMSO is used as a sample diluent, it may increase the solubility of many drug substances or drug products, and decrease the partition coefficient of the analytes, resulting in better transfer of analytes from the diluents to the gas phase, and improved method sensitivity [4,6,10]. If the sample diluent uses these aqueous mixtures, two other important factors, HS equilibration temperature and time, must be taken into consideration for obtaining HS equilibration efficiency. It is required that the HS equilibration temperature should be lower than the boiling point of the sample diluent. Otherwise, if the sample was equilibrated at or above the boiling point of the sample diluent, e.g. water at 100 °C, a large amount of sample diluent may be vaporized (at 100 °C), resulting in a dangerously high sample vial pressure, and a flood of the sample diluent and analytes to the GC system. This means that if water or water–organic mixture is chosen as the sample diluent, the HS equilibration temperature must be lower than 100 °C, i.e. 75–80 °C [4–6,12]. However, more than half of the organic solvents listed in ICH guideline Q3C may not be fully vaporized below 100 °C, because their boiling points are higher than 80 °C. In order to increase method sensitivity, equilibration at a low HS oven temperature requires a longer equilibration time, e.g. 30–90 min [4–6,12], to obtain a good phase distribution of the volatile compounds between the gas phase and the sample diluent.

In contrast, those organic solvents, e.g. DMSO (b.p. 189 °C), DMF (b.p. 153 °C), DMA (b.p. 166 °C), BA (b.p. 204 °C), and DMI (b.p. 105 °C), may provide better solubilization of sample, and they also have higher boiling points than water. When they are used as the sample diluents for HSGC, higher method sensitivity due to better solvent recoveries and improved method precision were observed [3,12,13]. However, DMF, DMA and BA are not very stable at high temperature and are susceptible to degradation when exposed to ultrasonic wave energy during sample preparation. The degradants from high HS equilibration temperature or sonication process during sample preparation may interfere with the analyses of the residual solvents [12]. Since DMSO is more stable at high temperature than the other solvents, e.g. DMF and BA, and has a higher capacity of dissolving drug substances and drug products, as well as a higher boiling point than water, it is a better sample diluent for HSGC analyses.

A number of parameters may affect GC method sensitivity and separation efficiency, such as sample injection split ratio, GC carrier gas linear velocity or flow rate and oven temperature program (isocratic or gradient). The typical GC parameters for a generic separation of residual solvents in previous publications are: split ratio 1:5–20; carrier gas linear velocity 20–36 cm/s; oven temperature at 40 °C isocratic, or with gradient programming from 40 to 90–160 °C at 5–10 °C/min [3–7,12,13]. These parameters may be optimized for separation efficiency and detection sensitivity for determining specific ICH Q3C solvents.

The objective of this study was to develop and validate generic HSGC method which has a shorter sample equilibration time, a better separation for most of the interested solvents, a higher sensitivity and a broader concentration range. We selected 4 mL of DMSO as the sample diluent for 200 mg of drug substance in order

Table 2
ICH classes 2 and 3 solvents unsuitable for this HSGC method.

Number	Solvent	FW (g/mol)	b.p. (°C)
1	Anisole	108	154
2	Cumene	120	152
3	Tetralin	132	206–208
4	Ethylene glycol	62	197
5	N, N-dimethylacetamide	87	164–166
6	Formamide	45	210
7	Sulfolane	120	285
8	N-methyl pyrrolidone	99	202–204
9	Formic acid	46	101
10	Acetic acid	60	118

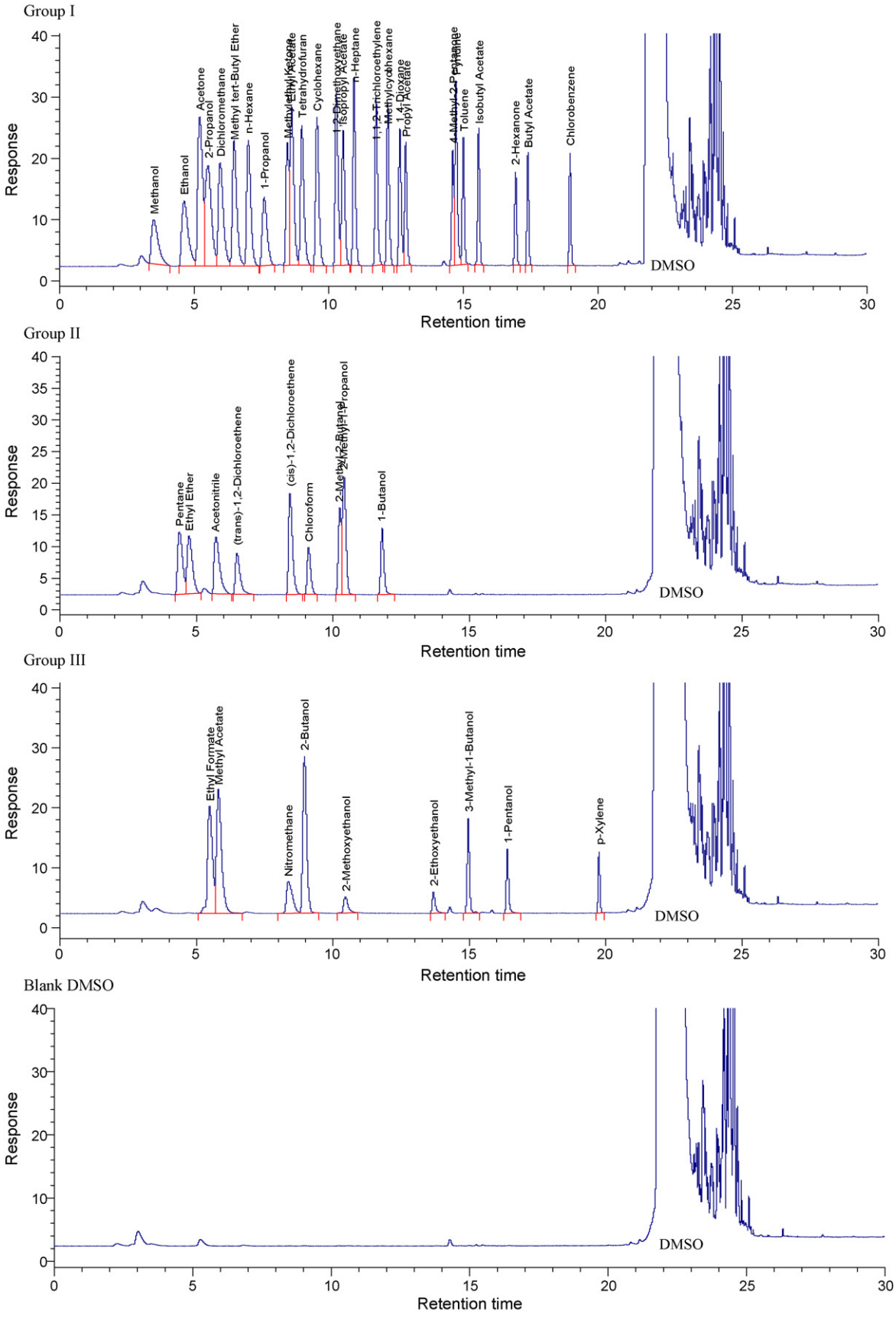

Fig. 1. Typical chromatograms of 44 ICH solvents at 20–900 ppm.

Table 3
Retention times and linearity of 44 ICH classes 2 and 3 solvents.

Organic solvents	b.p. (°C)	Retention time (min)	Range (ppm)	r^2
Methanol (I)	64.7	3.53	8.9–8868	0.9996
Pentane (II)	36.1	4.40	0.4–401	0.9996
Ethanol (I)	78.3	4.66	8.8–8837	0.9994
Ethyl ether (II)	34.6	4.75	0.6–571	0.9999
Acetone (I)	56.0	5.22	12.6–6320	0.9998
Ethyl formate (III)	54.0	5.50[a]	8.8–4402	0.9999
2-Propanol (I)	82.0	5.54	17.6–8803	0.9996
Acetanitrile (II)	81.0	5.74	4.4–4402	0.9997
Methyl acetate (III)	56.9	5.82[a]	1.8–4474	0.9999
Dichloromethane (I)	40.0	6.00	14.9–14,857	0.9997
1,2-Dichloroethene (II)	47.5	6.51[a]	7.1–7112	0.9999
Methyl tert-butyl ether (I)	55.2	6.52	1.2–1185	1.0000
n-Hexane (I)	69.0	7.04	0.5–524	0.9998
1-Propanol (I)	97.1	7.64	18–8998	0.9994
Nitromethane (III)	100.0–103.0	8.39[a]	7.3–18,194	0.9990
1,2-Dichloroethene (II)	60.0	8.45[a]	7.1–7112	0.9999
Methylethyl ketone (I)	79.6	8.50	3.9–3864	0.9996
Ethyl acetate (I)	76.5	8.65	4.3–5305	0.9999
2-Butanol (III)	99.0	8.98	4.1–10,342	0.9998
Tetrahydrofuran (I)	65.0–67.0	9.03	2.9–2845	0.9999
Chloroform (II)	60.5	9.13	11.8–11,840	0.9999
Cyclohexane (I)	80.7	9.60	0.6–623	1.0000
2-Methyl-2-butanol (II)	102.0	10.28[a]	4.6–4564	0.9995
1,2-Dimethoxyethane (I)	85.0	10.32	7.0–6946	0.9998
2-Methyl-1-propanol (II)	108.0	10.44[a]	6.4–6416	0.9999
2-Methoxyethanol (III)	124.0	10.48[a]	24.7–12,352	0.9990
Isopropyl acetate (I)	85.0–91.0	10.56	2.8–2816	1.0000
n-Heptane (I)	98.0	10.97	0.6–547	1.0000
1,1,2-Trichloroethylene (I)	87.2	11.79	9.3–9344	0.9998
1-Butanol (II)	117.0	11.83[a]	6.5–6478	0.9996
Methylcyclohexane (I)	101.0	12.23	0.6–616	1.0000
1,4-Dioxane (I)	101.1	12.68	8.3–8264	0.9997
Propyl acetate (I)	102.0	12.89	2.8–2841	0.9999
2-Ethoxyethanol (III)	135.0	13.71	23.8–11,904	0.9993
4-Methyl-2-pentanone (I)	115.0–117.0	14.64	2.6–2560	0.9996
Pyridine (I)	115.2	14.77	7.9–7855	0.9997
3-Methyl-1-butanol (III)	131.2	14.98[a]	4.1–10,355	0.9994
Toluene (I)	110.0–111.0	15.04	1.4–2774	0.9994
Isobutyl acetate (I)	118.0	15.60	2.8–2800	0.9999
1-Pentanol (III)	138.0	16.41	4.2–10,424	0.9997
2-Hexanone (I)	126.0–128.0	16.98	2.6–2596	0.9996
Butyl acetate (I)	126.0	17.43	2.8–2816	0.9997
Chlorobenzene (I)	131.0	19.00	3.6–3552	0.9994
p-Xylene (III)	138.4	19.77	0.3–689	0.9997

( ) indicates the corresponding groups.
[a] Overlapped or partially overlapped peaks.

to develop a generic HSGC method with efficient HS equilibration, GC separation and high detection sensitivity. We assessed a number of HSGC parameters, as listed in Table 1. Since class 1 solvents (5) are highly carcinogenic or toxic, they are generally avoided in pharmaceutical manufacturing. Only ICH class 2 (26) and class 3 (28) solvents were evaluated during this method development. The method validation was performed to demonstrate the method specificity, accuracy, precision, linearity and sensitivity. There are a number of calibration methods for the determination of residual solvents in drug substances by HSGC, such as calibrations using external standard, internal standard and standard addition, but there are no significant differences among these approaches with respect to accuracy and precision [4,14]. Therefore, we used an external standard approach in this method, and evaluated the drug substance matrix impacts on residual solvents recoveries using four synthetic small molecule drug substances during method validation.

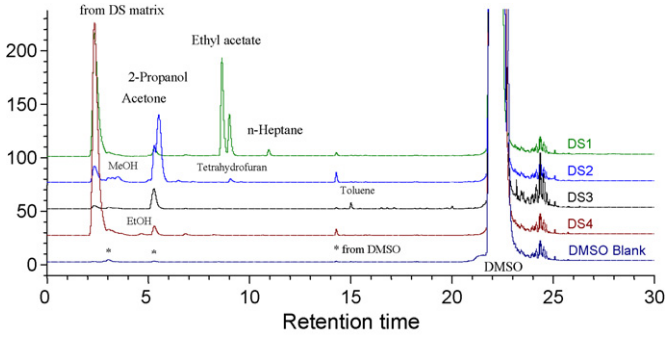

Fig. 2. Typical chromatograms of residual solvents in four drug substances.

2. Experimental

2.1. Reagents and chemicals

The drug substances were synthesized by Astrazeneca Pharmaceuticals LP (Wilmington, DE, USA). Solvents used were of ≥98% purity, and purchased from the following sources: acetone, acetonitrile, n-heptane and toluene from Fisher Scientific (Fairlawn, NJ, USA); formic acid and acetic acid from Acros Organics (Geel, Germany); ethyl ether from J.T. Baker (Phillipsburg, NJ, USA). The remaining organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 4
Accuracy and precision of 44 ICH solvents at working concentrations.

Peak no.	Compound	Accuracy bias%	Precision (RSD%, $n = 6$)	
			Intraday	Interday
Group I				
1	Methanol	1.54	1.76	1.53
2	Ethanol	−0.40	2.28	1.87
3	Acetone	−1.07	1.47	1.76
4	2-Propanol	0.94	1.82	1.90
5	Dichloromethane	−0.05	1.36	1.68
6	Methyl tert-butyl ether	−0.46	1.53	1.33
7	n-Hexane	−1.04	1.98	0.92
8	1-Propanol	0.23	1.79	1.58
9	Methylethyl ketone	−2.69	1.88	1.47
10	Ethyl acetate	1.65	1.56	1.91
11	Tetrahydrofuran	−0.74	1.64	1.42
12	Cyclohexane	−0.91	1.73	1.00
13	1,2-Dimethoxyethane	−0.10	1.63	1.19
14	Isopropyl acetate	−0.28	2.16	1.42
15	n-Heptane	−0.78	2.02	0.69
16	1,1,2-Trichloroethylene	−0.42	1.55	1.31
17	Methylcyclohexane	−0.61	1.70	1.11
18	1,4-Dioxane	0.23	2.09	1.45
19	Propyl acetate	−0.20	1.82	0.97
20	4-Methyl-2-pentanone	0.80	1.70	1.81
21	Pyridine	−0.40	1.62	1.27
22	Toluene	−0.43	2.21	1.52
23	Isobutyl acetate	−0.02	1.57	1.29
24	2-Hexanone	0.05	1.53	1.23
25	Butyl acetate	−0.73	1.62	1.29
26	Chlorobenzene	−0.60	1.53	1.23
Group II				
1	Pentane	2.49	1.50	1.86
2	Ethyl ether	−0.04	2.01	1.99
3	Acetonitrile	−0.95	1.68	1.55
4	(trans)-1,2-Dichloroethene	0.86	1.45	1.92
5	(cis)-1,2-Dichloroethene	0.49	1.37	1.67
6	Chloroform	−0.90	1.85	1.67
7	2-Methyl-2-butanol	−1.63	2.15	2.19
8	2-Methyl-1-propanol	2.34	1.96	1.87
9	1-Butanol	0.04	1.62	1.72
Group III				
1	Ethyl formate	−0.49	0.76	1.48
2	Methyl acetate	2.31	0.57	1.52
3	Nitromethane	1.42	1.59	1.69
4	2-Butanol	−1.28	1.30	2.02
5	2-Methoxyethanol	1.34	1.05	1.66
6	2-Ethoxyethanol	−2.50	1.14	1.50
7	3-Methyl-1-butanol	0.19	0.75	2.13
8	1-Pentanol	0.10	0.90	1.98
9	p-Xylene	0.42	1.28	1.14

2.2. Instrumentation

An Agilent 6890A GC equipped with an FID and a 7694 HS sampler was used for the experiments. The HSGC system was controlled using Agilent Chem32 software, and data acquisition and processing were accomplished using Thermo Atlas software. The GC column was an Agilent DB-624 (6% cyanopropylphenyl/94% dimtheyl polysiloxane) fused silica capillary column, 30 m long, 0.32 mm I.D., 1.8 μm film thickness (Part No. 123-1334, Serial No. US1613334H). The HSGC parameters assessed during the method development and validation are listed in Table 1.

2.3. Standard solutions

The ICH Q3C classes 2 and 3 solvents (54) were prepared at about 1000 ppm individually in DMSO, and injected to the HSGC system. Since 10 of these 54 solvents were not suitable for the current method due to their high boiling points or high polarities, only 44 of these solvents were used for the method validation experiments. In order to obtain good separations and proper signal strength, these 44 solvents were separated as three groups, I (26), II (9) and III (9), respectively. The concentrations of these solvents were prepared in ranges of 0.2–15,000 ppm (from the quantitation limit to full GC chromatogram scale) by sequential diluting high concentration stock mixture solutions with DMSO to 10 concentration levels, because the sensitivity of these solvents to FID varies significantly. Two identical samples were prepared for the accuracy test for each group mixture at 20–900 ppm levels. All the solvent concentrations were calculated based on 200 mg drug substance being dissolved in 4 mL of DMSO. For the HSGC analysis, 4 mL (sample load) of the standard solutions was pipetted into an Agilent 20 mL headspace sample vial and immediately sealed with a Teflon-lined septum and an aluminum crimp cap (Agilent, Wilmington, DE, USA).

2.4. Drug substance sample solution

The four drug substances were dissolved individually (200 mg each) in 4 mL of blank DMSO or in the three group mixtures at both working concentration (20–900 ppm) and low concentration (2–90 ppm) levels. For example, 200 mg for one drug substance was weighed in seven HS sample vials, 4 mL of blank DMSO was added into the first vial; then either 4 mL of groups I, II or III solvent mix-

Table 5
Quantitation limit (QL) and detection limit (DL) of 44 ICH solvents.

Peak no.	Peak name	QL (ppm)	Precision at QL (RSD%, $n = 6$)		DL (ppm)
			Intraday	Interday	
Group I					
1	Methanol	2.11	8.53	5.35	0.63
2	Ethanol	3.68	6.70	8.94	1.10
3	Acetone	0.65	4.31	3.39	0.20
4	2-Propanol	1.87	7.57	6.81	0.56
5	Dichloromethane	3.71	4.50	4.83	1.11
6	Methyl tert-butyl ether	0.24	2.31	4.17	0.07
7	n-Hexane	0.10	2.72	6.27	0.03
8	1-Propanol	3.21	8.45	3.59	0.96
9	Methylethyl ketone	0.79	4.82	7.76	0.24
10	Ethyl acetate	0.68	2.19	2.95	0.21
11	Tetrahydrofuran	0.48	3.44	2.31	0.14
12	Cyclohexane	0.11	2.77	5.23	0.03
13	1,2-Dimethoxyethane	0.91	1.56	5.07	0.27
14	Isopropyl acetate	0.44	2.08	1.78	0.13
15	n-Heptane	**0.07**	1.95	2.57	**0.02**
16	1,1,2-Trichloroethylene	1.35	2.06	2.80	0.41
17	Methylcyclohexane	0.09	2.68	3.19	0.03
18	1,4-Dioxane	1.50	2.14	2.02	0.45
19	Propyl acetate	0.57	4.18	2.43	0.17
20	4-Methyl-2-pentanone	0.51	2.34	3.95	0.15
21	Pyridine	1.27	2.56	1.90	0.38
22	Toluene	0.28	1.94	3.35	0.08
23	Isobutyl acetate	0.47	3.26	4.93	0.14
24	2-Hexanone	0.74	2.58	2.96	0.22
25	Butyl acetate	0.64	3.23	4.40	0.19
26	Chlorobenzene	0.81	4.61	4.64	0.24
Group II					
1	Pentane	0.24	3.35	9.75	0.07
2	Ethyl ether	0.38	9.12	8.69	0.11
3	Acetonitrile	2.59	4.23	4.55	0.78
4	(trans)-1,2-Dichloroethene	5.47	7.74	9.34	1.64
5	(cis)-1,2-Dichloroethene	2.16	7.92	5.47	0.65
6	Chloroform	7.40	6.56	8.36	2.22
7	2-Methyl-2-butanol	1.47	4.72	8.17	0.44
8	2-Methyl-1-propanol	1.60	3.07	7.67	0.48
9	1-Butanol	3.08	8.13	3.99	0.93
Group III					
1	Ethyl formate	0.96	2.81	1.44	0.29
2	Methyl acetate	0.85	8.67	2.36	0.26
3	Nitromethane	3.64	9.43	10.15	1.09
4	2-Butanol	1.53	2.94	4.25	0.46
5	2-Methoxyethanol	**24.70**	7.07	2.60	**7.41**
6	2-Ethoxyethanol	18.31	7.26	2.43	5.49
7	3-Methyl-1-butanol	2.59	3.49	5.59	0.78
8	1-Pentanol	2.61	7.04	9.71	0.78
9	p-Xylene	0.23	6.91	5.59	0.07

tures at working concentration levels (20–900 ppm) was added; or 4 mL of groups I, II or III solvent mixtures at low concentration levels (2–90 ppm) was added. All the solvent concentrations were calculated based on 200 mg drug substance being dissolved in 4 mL of DMSO. The sample solutions were vortexed using a Thermolyne mixer (Dubuque, IA, USA), and sonicated for 5 min using a Bransonic 3200 (Danbuty, CT, USA) to dissolve all samples completely. The samples for each drug substance were prepared in duplicate.

2.5. Procedure

During the HSGC method development, in order to select the most appropriate system parameters to obtain the best separation, sensitivity and time efficiency, 54 classes 2 and 3 solvents, and typical samples of the 3 groups of solvent mixtures were injected under a variety of conditions, e.g. at different HS oven temperatures (125–150 °C), equilibration time (8–15 min), GC gradients (35–280 °C, ramping speed 3–10 and 10–40 °C/min), carrier flow rate (28–40 cm/s), sample split ratio (1–5), etc. The final HSGC conditions used for method validation were obtained based on optimized HS and GC parameters.

The HSGC system was equilibrated under the experimental conditions by injecting 3 blank DMSO samples every day before sample sequence injections. Each of the ICH Q3C classes 2 and 3 solvents (54) was injected once separately to determine method specificity and signal response sensitivity. Since 10 of these 54 solvents are unsuitable for the current method due to their high boiling points or polarities, only 44 of these solvents were used for the method validation experiments, and they are separated as three groups according to their retention behavior and detectability by FID.

The method validation experiments of these 44 solvents were performed by injecting the 3 groups of solvents sequentially from low to high concentrations to determine the method specificity, sensitivity, linearity, accuracy and precision. Each of the 3 groups of solvent mixtures at working concentrations (20–900 ppm for accuracy and precision testing) and at low concentrations (2–90 ppm for sensitivity testing) were injected six times on the first day of experiment, and one time in each of the following five days. The samples of four drug substances in blank DMSO and in three spiked groups

Table 6
Residual solvents in four drug substances.

Drug substance (DS)	Residual solvent	RT (min)	Concentration (ppm)
DS1	Acetone	5.22	91.2
	Ethyl acetate	8.65	589.4
	Tetrahydrofuran	9.03	201.9
	n-Heptane	10.97	4.6
DS2	Methanol	3.53	246.4
	Acetone	5.22	270.8
	2-Propanol	5.54	1451.6
	Tetrahydrofuran	9.03	19.4
DS3	Acetone	5.22	254.8
	Toluene	15.04	9.7
DS4	Ethanol	4.66	109.6
	Acetone	5.22	112.3

of solvent mixtures at 2–90 and 20–900 ppm levels were injected once to evaluate the method feasibility to drug substance and the impact of drug substance on the recoveries of those solvents.

3. Results and discussion

3.1. Optimization of HS conditions

The HS sampler has a number of parameters affecting the method sensitivity, precision, and efficiency, including: temperature (oven, transfer line, and loop), time (vial equilibration and pressurization, loop fill, and injection), pressure (vial and carrier gas) and phase ratio (vial size and sample volume). Selecting a proper sample diluent for HSGC analysis is very critical for method sensitivity, precision and sample equilibration temperature and time, and it will affect the final optimized HS conditions. When we evaluated HS equilibration temperature at 125, 140 and 150 °C with equilibration times of 8, 10 and 15 min, many solvents with boiling point higher than 125 °C could not evaporate efficiently at 125 °C within 15 min, while a significant amount of DMSO evaporated at 150 °C even in 8 min, overloading the GC column, and interfering with the method separation efficiency. When the equilibration time at 140 °C was extended from 10 to 15 min, the recoveries of

Table 7
Recoveries of 44 ICH solvents spiked in four drug substances.

Solvent	Recovery (%)							
	DS1		DS2		DS3		DS4	
	WL	LL	WL	LL	WL	LL	WL	LL
Group I								
Methanol	97.0	17.9	102.7	108.1	100.2	110.1	68.2	ND
Ethanol	97.9	**118.0**	103.4	105.8	95.4	**122.8**	106.0	126.9
Acetone	94.2	57.0	92.2	94.3	**75.1**	**360.3**	95.4	89.0
2-Propanol	103.1	129.7	101.3	112.8	95.9	ND	103.0	121.6
Dichloromethane	94.3	**116.8**	105.1	ND	90.5	**119.2**	91.8	99.5
Methyl tert-butyl ether	83.8	99.6	95.1	94.8	70.2	86.4	79.6	90.0
n-Hexane	79.8	104.1	89.2	89.3	66.6	79.9	74.8	109.7
1-Propanol	98.0	99.7	100.2	98.9	96.7	102.6	100.1	96.0
Methylethyl ketone	81.7	**238.2**	98.0	90.9	92.6	94.4	99.7	106.6
Ethyl acetate	112.3	**140.2**	94.4	95.7	85.7	96.1	91.8	108.2
Tetrahydrofuran	96.0	85.4	94.5	98.4	84.3	95.7	90.3	109.4
Cyclohexane	82.9	95.0	91.2	93.6	69.4	73.9	89.1	95.7
1,2-Dimethoxyethane	95.7	95.7	97.0	93.5	89.3	89.4	100.2	99.3
Isopropyl acetate	92.6	106.1	95.8	100.8	85.2	92.5	98.8	102.6
n-Heptane	81.7	102.4	90.7	94.3	66.7	71.3	86.9	98.4
1,1,2-Trichloroethylene	92.7	97.9	96.2	96.2	88.2	92.5	99.0	103.8
Methylcyclohexane	84.4	91.6	91.6	93.9	71.2	78.2	90.2	97.4
1,4-Dioxane	96.6	99.1	97.5	101.2	91.7	95.0	100.1	99.7
Propyl acetate	94.8	99.0	97.7	100.9	88.5	96.3	100.7	101.1
4-Methyl-2-pentanone	96.6	102.5	97.4	96.1	91.8	94.6	104.1	110.5
Pyridine	97.8	98.7	96.7	98.1	93.9	113.8	100.1	100.3
Toluene	92.9	98.3	94.8	103.3	88.6	85.4	98.3	99.6
Isobutyl acetate	95.4	96.4	97.8	92.3	92.5	95.2	101.9	99.8
2-Hexanone	96.4	99.4	97.8	99.1	92.7	91.0	101.5	103.0
Butyl acetate	97.0	99.6	97.8	101.6	93.9	102.3	102.7	104.2
Chlorobenzene	97.0	101.2	98.1	99.4	92.5	98.5	101.5	104.0
Group II								
Pentane	85.6	87.2	94.7	89.1	79.2	73.6	93.4	77.5
Ethyl ether	88.6	95.3	96.1	89.8	77.1	74.6	98.9	**162.3**
Acetonitrile	96.5	**122.6**	91.6	**170.4**	103.3	**140.9**	97.4	**120.3**
(trans)-1,2-Dichloroethene	87.1	100.4	93.6	79.1	87.6	95.2	92.9	90.1
(cis)-1,2-Dichloroethene	75.8	64.7	97.1	89.7	92.3	84.8	97.3	89.0
Chloroform	115.0	76.3	100.9	99.3	96.5	94.1	100.6	105.7
2-Methyl-2-butanol	97.8	99.4	94.7	98.1	96.5	96.1	100.3	96.5
2-Methyl-1-propanol	103.6	99.7	104.7	95.6	101.4	98.9	105.0	104.7
1-Butanol	101.2	103.0	101.1	91.2	100.0	99.3	101.2	103.1
Group III								
Ethyl formate	95.0	ND	**271.2**	**1751.6**	97.1	88.0	110.7	ND
Methyl acetate	85.2	88.0	95.1	ND	84.0	69.4	92.5	103.0
Nitromethane	**278.2**	ND	109.8	**120.7**	98.4	110.8	91.4	93.2
2-Butanol	**124.0**	ND	108.3	**197.8**	88.6	95.5	97.2	105.4
2-Methoxyethanol	94.7	ND	105.6	110.0	92.3	48.4	101.8	94.9
2-Ethoxyethanol	94.7	98.0	103.8	88.6	81.2	96.5	105.0	107.0
3-Methyl-1-butanol	97.0	100.3	103.8	109.8	105.6	ND	108.1	111.2
1-Pentanol	98.7	99.5	106.8	103.1	91.3	99.9	107.8	108.4
p-Xylene	89.8	92.8	93.0	97.3	91.9	88.4	99.0	103.9

WL stands at working concentration level (20–900 ppm); LL stands at low concentration level (2–90 ppm).

the 44 classes 2 and 3 solvents remained constant. Therefore, we determined that equilibrating at an oven temperature of 140 °C for 10 min was optimal. It was observed that when the temperatures of the injection loop and the transfer line were 10 °C higher than the HS oven temperature of 140 °C or kept the same as that of the oven temperature, there was no significant change in solvent recoveries. However, when the HS sampler was equilibrated at 140 °C, those ICH Q3C solvents with a boiling point higher than 150 °C, as listed in Table 2, could not be analyzed by this HSGC method.

3.2. Optimization of GC separation

The choice of GC column is crucial for establishing an efficient and robust HSGC method. The Agilent DB-624 column (30 m × 0.32 mm I.D., 1.8 (m) is a commonly used column for residual solvents determination, because of its medium polarity. Most of the ICH Q3C classes 2 and 3 solvents can be resolved by the Agilent DB-624 column except formic acid and acetic acid, due to their high polarities. To obtain efficient separation and sample sensitivity, a number of GC parameters were evaluated when developing this method, such as the GC oven temperature gradient, carrier gas flow rate and sample split ratio: initial temperature 35 °C at different holding time (0, 1, 3, and 5 min), temperature ramping rate (2, 3, 4, 5, 8, 10 °C/min), carrier flow rate (1.5, 1.8, and 2.0 mL/min) and split ratio (splitless or 1 to1–5 ratio). Our data indicated that the GC parameters listed in Section 2.2. were the most efficient combination for separation and sensitivity of this method. Under these optimized conditions, 44 of classes 2 and 3 solvents were analyzed by this method. The separation efficiency of this method is better than previously reported methods, because more classes 2 and 3 solvents can be resolved by this method [3–8]. Another advantage of our generic HSGC method is its capability to separate most of the frequently used solvents in a considerably shorter time (total running time is 40 min, including 10 min for HS vial equilibration and 30 min for GC separation) compared to previously reported methods [4,5].

3.3. Method validation

3.3.1. Specificity

The typical HSGC chromatograms of 44 ICH Q3C classes 2 and 3 solvent standards are shown in Fig. 1. As indicated in the retentions of these solvents in Table 3, most of these solvents (33) are well separated from each other and DMSO, but some of the solvents in group II and group III are incompletely resolved with those in group I, such as ethyl formate (III) and 2-propanol (I). However, there are rare cases when a drug substance contains more than five residual solvents at or around meaningful detection limits. For example, each of the four drug substances evaluated in this study contain a mixture of two to four residual solvents at or above the quantitation limits of this HSGC method, as shown in Fig. 2. That means this HSGC method is a suitable approach in many pharmaceutical applications for screening and determining the 44 ICH Q3C solvents.

3.3.2. Linearity

The method linearity was investigated using 10 concentration levels ranging from 0.2 to 15,000 ppm, and the linearity of each solvent was assessed using linear regression. Since the sensitivity of each of the 44 solvents to FID varied significantly, the concentration ranges of each organic solvent were adjusted during the sample preparation procedures to obtain a relatively reasonable peak height for each organic solvent and to cover appropriate linear ranges. As shown in Table 3, the regression coefficients (r^2) of these 44 solvents are within the range of 0.9990–1.0000. The intercepts of these regression lines are less than 2% of the high calibration concentrations (20–900 ppm) for all 44 solvents. This means that the 44 solvents have linear responses within the calibration ranges studied, which are broader than ICH guideline detection range of 50–5000 ppm.

3.3.3. Accuracy

Accuracy of this method was determined by analyzing duplicate sample preparations of the three groups of the ICH Q3C solvents at working concentration levels (20–900 ppm level). As shown in Table 4, the bias values (the difference between the measured value and the theoretical value) of these 44 solvents are equal or less than ±2.69% of the theoretical values. The results indicate that the HSGC method has sufficient accuracy for screening and determining the 44 solvents studied at the working concentration level.

3.3.4. Precision

The precision of the HSGC method was assessed by evaluating both method precision (intraday precision) and system repeatability (interday precision). The method precision is presented by the relative standard deviation of the response (RSD%, $n = 6$) of six injections (six vials) of the same sample (groups I–III) at both a working concentration (20–900 ppm) and a lower concentration (2–90 ppm) on the same day. The relative standard deviations, RSD% of six injections of each solvent in the same day (intraday) were in the range of 0.57–2.28% at the 20–900 ppm level and of 1.56–9.43% at the 2–90 ppm level, respectively, as shown in Tables 4 and 5. Similarly the relative standard deviations, RSD%, of six injections of each solvent in six consecutive days (interday) were in the range of 0.69–2.19% at the 20–900 ppm level and of 1.44–10.15% at the 2–90 ppm level, respectively, as shown in Tables 4 and 5. These results indicated that this HSGC method has reasonable precision and system repeatability within the analytical range of determinations.

3.3.5. Method sensitivity

The sensitivity of this HSGC method is presented as the quantitation limit (QL) with a signal–noise ratio of 10–1, and detection limit (DL) with a signal–noise ratio of 3–1. As shown in Table 5, the QL values of the 44 solvents evaluated range from 0.07–24.70 ppm, and DL range from 0.02 to 7.41 ppm. The broad ranges of QL and DL are due to the differences of hydrocarbon content in different solvents. Since some elements, e.g. chlorine, oxygen and nitrogen, are incombustible, solvents containing these elements have lower molar combustion capacities than pure hydrocarbons, leading to lower detection sensitivities by FID. However, our results demonstrate that this HSGC method is sensitive enough for determination of the 44 solvents in drug substances, because the QL values (0.07–24.70 ppm) of these solvents are much lower than the requirements of ICH guideline for classes 2 and 3 solvents (50 ppm or higher in most cases).

3.3.6. Sample analyses and matrix impacts of drug substances

In order to demonstrate the suitability of the HSGC method for determination of residual solvents in real drug substances, and for evaluating the impact of the drug substance matrix on solvent analyses, we analyzed four synthesized small molecule organic drug substances from Astrazeneca Pharmaceuticals, Wilmington, Delaware. We also spiked the 44 solvents in 3 groups into the four drug substances at both the 20–900 and 2–90 ppm levels. As shown in Fig. 2 and Table 6, the eight solvents in the four drug substances are successfully determined by this HSGC method, and these results are consistent with those results from direct injection GC methods. When the 44 solvents were spiked into these four drug substances at both the 20–900 and 2–90 ppm levels, most of the spiked solvents could be recovered from 70% to 115% during the HSGC analysis, as shown in Table 7, especially at the higher concentrations. These results suggest that interferences from the drug

substance matrix or from the impurity peaks in DMSO, e.g. the peak at 3.1, 5.4 and 14.3 min, should not have a significant impact on this HSGC method at regular working concentration. However, attention should be paid to those solvents, e.g. methanol (for DS1and DS4) and 2-methoxyethanol (for DS1 and DS3), where obvious interferences were observed for particular drug substance at the low solvent concentration levels. A more specific method validation may be required when some solvents recoveries are extremely out of range due to drug substances interferences. For example, drug substance samples containing hydrocarbon residual solvents (e.g. hexane, pentane, etc.) should be analyzed with a smaller sample load, i.e. 20–30 mg, to cover the ICH determination range of 5–5000 ppm interest, because these hydrocarbons have lower QLs when using FID detection.

4. Conclusions

In this study, a generic HSGC method is successfully developed and validated for the determination of 44 ICH Q3C classes 2 and 3 residual solvents in drug substances. The method is specific, accurate, precise, linear, sensitive and efficient. DMSO was selected as the sample diluent due to its high capacity for dissolving organic drug substances, stability and high boiling point. The conditions of HS sampler and GC were optimized to make the HSGC method more sensitive, efficient and reproducible. This method has a much shorter sample equilibration time, a better separation for many solvents, a higher sensitivity and a broader concentration range comparing with the previously published methods. The examples of real drug substance analyses demonstrate the broad application potential of this HSGC method in the determination of residual solvents in drug substances. This method meets ICH guideline requirements, and may be suitable for residual solvent determinations in a variety of pharmaceutical application.

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