Determination of Residual Isobutylene Oxide—A Genotoxic Starting Material in a Drug Substance by Static Headspace Gas Chromatography

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Abstract

A sensitive static headspace gas chromatography (sHS-GC) method is developed and validated for the determination of residual isobutylene oxide (IBO)—a genotoxic starting material in a drug substance for Phase I clinical trial studies. The experimental parameters, such as headspace vial pressure, headspace oven temperature, vial equilibration time, column flow rate, and GC oven temperature programs are optimized. Under the optimal conditions, the recovery is between 89.3% and 102.4% for spiked samples at three levels of IBO concentration in triplicates in sample preparations. Limits of quantitation (LOQ) and detection (LOD) of the standard solutions are 0.048 and 0.018 µg/mL, respectively. Linear range from 0.018 to 6 µg/mL is obtained with a correlation coefficient of 0.9999. The method is applied to determine residual IBO in drug substance samples from different batches.

Introduction

Genotoxic impurities in the production of drug substances and products must be controlled to ensure the safety of pharmaceutical products (1,2). Epoxides, such as ethylene oxide (EO) and isobutylene oxide (IBO), belong to a class of compounds that are considered to be genotoxic based on structure-activity relationships (1–4). EO has been widely used as a sterilizer in medical devices and an alkylation reagent in the pharmaceutical industry. Because of its known genotoxicity, it has been regulated by the U.S. Food and Drug Administration (FDA), and the International Organization for Standardization (ISO) in the medical device industry (5,6). IBO has also been considered as a genotoxic material in *in vitro* studies (7–9).

Historically, static headspace-gas chromatography (sHS-GC) has been widely used in the pharmaceutical industry to detect residual solvents in drug substances and products (10,11). The advantages of using HS-GC is in the analysis of drug substances and/or products that have low solubility, or create non-volatiles and/or degradation products on GC columns, or thermally decompose on columns and/or interfere with analyte peaks. sHS-GC is also commonly used to detect residual solvents (e.g., benzene) at part-per million (ppm) levels in drug substances and products. Cyclic epoxides have been analyzed by sHS-GC (12–15), dynamic HS-GC (5), solid-phase microextraction (SMPE) HS-GC (6,16), and GC-mass spectrometry (GC–MS) (17). In August 2007, EO was officially listed as a residual solvent by US Pharmacopeia with a limit of 10 µg/g (or ppm) (18). The limit of quantitation (LOQ) of EO at low µg/g level in drug substances and products requires the application of sHS-GC methods. IBO is a colorless liquid at room temperature with a boiling point of 51–52°C. In our current project, IBO is a starting material used as a direct alkylation reagent for the synthesis of the drug substance. According to the recent staged threshold of toxicological concern (TTC) approach proposed by Pharmaceutical Research and Manufacturing Association's (PhRMA) White Paper (2), the specification for the residual IBO in the drug substance for Phase-I clinical trials was set at 5 µg/g based on the daily dosage of the drug substance (targeted dosage: 4 g/day) and duration of exposure [clinical trial duration: 3–6 months; allowable daily intake = 20 µg/day; specification = 20 [(µg/day)/4 (g/day) = 5 (µg/g)]. This specification was established to ensure the safety of the clinical trial subjects. Therefore, a sensitive analytical method was required to detect low concentrations of the residual IBO in the drug substance. To our knowledge, no analytical method has been reported for the determination of residual IBO in drug substances. Only one paper reported the GC retention data of C4–C5 epoxides using a glass capillary column (12) (without detection limit data). From our study, we discovered that the solvents used to dissolve the drug substance played an important role in the sensitivity of the method. Although water is an ideal solvent for both EO and IBO because it provides better sensitivity than other solvents, the drug substance was not soluble in water. Other commonly used solvents to dissolve drug substances in GC residual solvent methods, such as dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and dimethylacetamide (DMAC), have interfer-

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ences that affect the detection of low levels of IBO. Therefore, the choice of solvents to avoid the interferences from the solvent background became a key parameter of this method.

The objective of our study was to develop and validate [via a phase appropriate approach (20)] a specific, sensitive and accurate method for the determination of residual IBO in the drug substance by sHS-GC in order to ensure the safety of the Phase-I clinical trial studies.

Experimental

Reagents and materials

IBO (98%, CAS number: 558-30-5; molecular weight: 72.11; vapor pressure: 313 mbar; water solubility: 30 g/L at 20°C) and 2-butanol were purchased from Acros Organics (Geel, Belgium). *n*-Methylpyrrolidone (NMP), dimethyl formamide (DMF), dimethylacetamide (DMAC), dimethyl sulfoxide (DMSO), and isopropyl alcohol (IPA) were high purity solvents and purchased from Burdick & Jackson (Muskegon, MI). Methylene chloride (DCM) was purchased from J.T. Baker (Phillipsburg, NJ). Toluene was purchased from Sigma-Aldrich (Saint Louis, MO). The purity of all solvents is ≥ 99%, and they were used without further purification. The drug substance samples were obtained from the Department of Chemical Process Research and Development, Amgen Inc. (Thousand Oaks, CA). The preliminary solubility of the drug substance in different solvents was measured by the addition of the solid drug substance into vials. 1 mL of the solvent was added into each vial and the samples were sonicated to dissolve. The concentration of the supernatant of the saturated solutions was determined by HPLC.

Preparation of standard and test solutions

Standard: 0.4 mg/mL IBO stock standard was prepared by dilution of the neat IBO solution with NMP. Working standard solutions were prepared by serial diluting with NMP. Serial dilutions of IBO standard solutions in water were prepared in the concentration range from 0.006 to 6 µg/mL. 2 mL of solution was placed in a 10-mL headspace vial and sealed with an aluminum cap with a Teflon septum.

Test sample: 0.5 g of the drug substances sample was accurately weighted into a 10-mL headspace vials, and 2 mL of NMP was added into each vial. The vials were sealed with aluminum caps with Teflon septa. The samples were sonicated for 1 min to dissolve.

Instrumentation

All experiments were performed on an Agilent static G1888 headspace sampler with a 3-mL sample loop and a 6890N GC with a flame ionization detector (FID) (Santa Clara, CA). RTX-1, RTX-5, and Stabilwax capillary columns with a dimension of 60 m × 0.53 mm i.d. × 1 µm film thickness, and RTX-624 column with a dimension of 60 m × 0.32 mm i.d. × 1.8 µm film thickness were purchased from Restek (Bellefonte, PA). DB-624 with a dimension of 60 m × 0.32 mm i.d. × 1.8 µm film thickness was purchased from Agilent (Santa Clara, CA). SPB-624 with a dimension of 60 m × 0.32 mm i.d. × 1.8 µm film thickness was purchased from Supelco (Bellefonte, PA). The initial headspace sampler conditions were set up as follows: oven temperature, 100°C; loop temperature, 110°C; transfer line temperature, 120°C; vial equilibration time, 15 min; vial pressure, 15 psi; pressurization time, 0.2 min; injection time, 1.0 min. For the aqueous standards, the headspace oven temperature was set at 90°C, and other parameters remained the same.

During the preliminary studies, the GC conditions were as follows: GC oven temperature program was 40°C isothermal for 5 min and 35°C/min to 220°C and hold 4 min. The carrier gas was helium with a constant flow rate of 6.0 mL/min and split ratio of 2:1. The injection temperature was maintained at 180°C with a split ratio of 2:1 and FID detector temperature of 250°C. Chromatographic data were collected by Chemstation software from Agilent Technologies.

Results and Discussion

Method development

Preliminary study

Because the drug substance sample was not water-soluble, the initial solubility studies on the drug substance sample were carried out using acidified water to increase the solubility of the drug substance in order to achieve the desired sensitivity. The drug substance can be dissolved in 0.2 N sulfuric acid solution at 40 mg/mL (targeted concentration for most drug substances). However, no IBO peak was detected in the standard solution prepared in this diluent because IBO decomposed under such acidic conditions. Therefore, several commonly used organic solvents, such as NMP, DMF, DMSO, and DMAC, were used to dissolve the drug substance. From the preliminary results, NMP was selected because it did not have interference peaks that co-eluted with

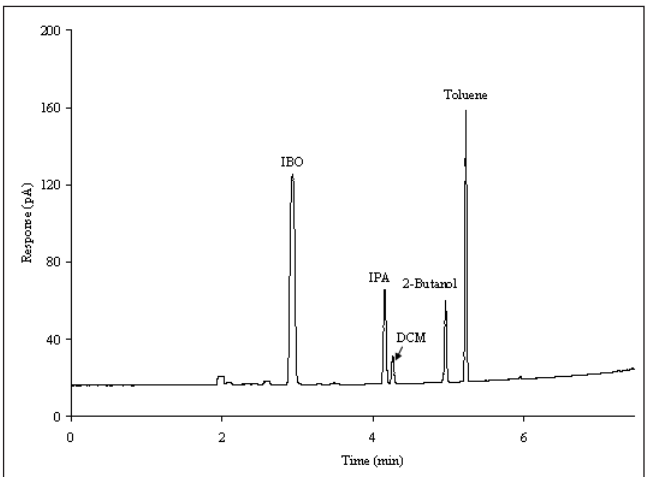

Figure 1. Chromatogram of a mixture standard solution containing IBO, IPA, DCM, 2-butanol, and toluene. 4 µg/mL each under optimal conditions: column flow rate: 10.0 mL/min; GC oven temperature program of 40°C isothermal for 1 min and 15°C/min to 80°C, then 35°C/min to 220°C and hold 4 min; 2:1 split ratio; FID detector temperature of 250°C; injector temperature of 180°C; headspace parameters: headspace oven temperature 100°C; vial equilibration time 10 min; vial pressure 6 psi; pressurization time 0.2 min; injection time 1.0 min, and sample volume 3 mL.

IBO peak. In addition, the drug substance has the highest solubility in NMP (≥ 250 mg/mL in NMP vs. 194 mg/mL in DMAC, 122 mg/mL in DMF and 67 mg/mL in DMSO).

Different types of GC columns, such as DB-624, SPB-624, RTX-624, RTX-1, RTX-5, and Stabilwax, were screened in order to separate IBO from other residual solvents (such as IPA, 2-butanol, DCM, and toluene in the drug substance). Stabilwax column was selected because of its peak efficiency and selectivity. Figure 1 shows the separation of IBO and other residual solvents in a standard solution on the Stabilwax column under the optimal conditions.

Optimization of HS-GC method conditions

In HS-GC analysis, the instrument parameters of the headspace and GC units need to be optimized in order to achieve high sensitivity. In this method development study, the following key parameters were selected to be optimized: headspace vial pressure, headspace oven temperature, vial equilibration time, column flow rate, and the GC oven temperature program. Headspace vial pressure and oven temperature are the key parameters contributing to the overall sensitivity of the method. All experiments were carried out with 0.4 µg/mL IBO standard solution.

Headspace vial pressure

The vial pressure range that was investigated was from 2 to 23 psi. From 2 to 6 psi, the peak area counts of IBO increased (ca. 5-7%) with the increase of vial pressure. From 6 to 23 psi vial pressure range the peak area counts of IBO decreased ca. 30%, therefore the vial pressure was set at 6 psi for the further optimization.

Headspace oven temperature

The headspace oven temperature has a profound impact on the concentration of analyte in the headspace. In general, the increase in temperature leads to increase IBO headspace concentrations by ca. 6 fold. The equilibration temperature was determined by varying the temperature from 60°C to 140°C. Figure 2 shows that the area counts increase with the increase in the headspace oven temperature. No optimum was found from this study. Logically, the highest oven temperature provides the best sensitivity. However, there was some concern about the decomposition of the drug substance and/or the solvent at higher temperatures. At 140°C with initial conditions (see Experimental section), 5.9 µg/g of residual IBO was detected in a sample at the concentration of 40 mg/mL. However, when we reduced the headspace oven temperature to 100°C, the residual IBO was undetectable (< 1 µg/g with signal/noise (S/N) ≥ 2). From the analytical data of IBO in the drug substance generated by sHS-GC, there were two sources of IBO coming from the drug substance: (*i*) the residual IBO in the drug substance; and (*ii*) the IBO (and/or interference peaks) generated from thermal decomposition of the drug substance and/or the solvent in the HS vials during the thermal extraction process. The residual IBO concentration in the drug substance should be a constant (against the standard solutions) with the change of HS oven temperature while the IBO (and/or degradant) concentration from thermal decomposition will change with the change of the headspace oven temperature. Obviously, the potential of the decomposition of the drug substance at 140°C became a major concern when the change of the oven temperature caused the drastic change of IBO concentration in the drug substance (5.9 µg/g at 140°C vs. < 1 µg/g at 100 °C). Because the preliminary experimental conditions could not detect IBO lower than 1 µg/g, the sensitivity of the method needed to be improved. With the increase in the sample concentration from 40 to 250 mg/mL and the optimization of GC parameters, the sensitivity of the method increased. The headspace oven temperature of 100°C was determined to be acceptable for sensitivity and for avoiding the potential decomposition of the drug substance.

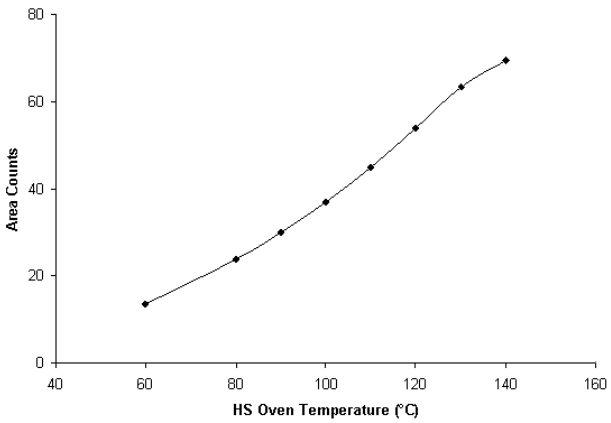

Figure 2. Effect of headspace oven temperature on peak area counts. Method conditions: column flow rate: 6.0 mL/min; GC oven temperature program of 40°C isothermal for 5 min and 35°C/min to 220°C and hold 4 min; 2:1 split ratio; FID detector temperature of 250°C; injector temperature of 180°C; headspace parameters: headspace oven temperature 100°C; vial pressure 6 psi; equilibration time 10 min; pressurization time 0.2 min; injection time 1.0 min and sample volume 3 mL.

Vial equilibration time

Vial equilibration time is another important parameter to be optimized. The equilibration time was varied from 5 to 60 min while holding the headspace oven temperature at 100°C with vial pressure at 6 psi. As shown in Figure 3, the area counts of IBO standard solution increased slightly from 5 to 15 min. The max-

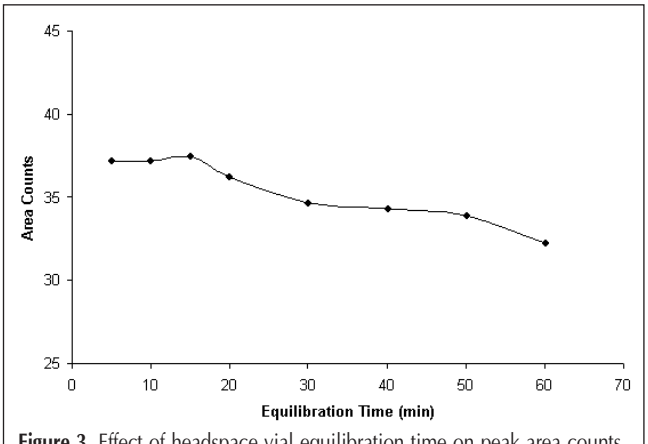

Figure 3. Effect of headspace vial equilibration time on peak area counts. Method conditions: the same as Figure 2 except vial equilibration time.

imum number of area counts was reached at 15 min. From 15 to 60 min the area counts decreased ca. 10%. The equilibration time of 10 min was selected because the increase in area counts from 10 to 15 min was less than 1% while the run-time could be reduced by 50%.

Column flow rate

Carrier gas flow rate is another GC parameter that needs to be optimized to achieve better sensitivity and to reduce overall analysis time. Figure 4 shows that the area counts increase (ca. 50%) with the increase of the carrier gas flow rate from 4 to 12 mL/min. Because there was adequate resolution between IBO and other residual solvents, theoretically, the higher the flow rate, the more sensitive and shorter the method could be. However, at the higher flow rate (> 10.0 mL/min), the resolution between IBO and the interference peaks from the solvent impurities became an issue. Therefore, the optimal condition for the column flow rate was set at 10.0 mL/min.

GC oven temperatures

GC oven temperature program is another important factor to be optimized in order to increase the efficiency and shorten the run time. Considering the lowest operating temperature of the Stabilwax column is 40°C, IBO standard solutions were injected under oven temperature program at different initial temperatures from 40°C to 80°C with 4 min holding time. The efficiency of IBO peak was the highest when the initial temperature was set at 40°C. The optimization of the initial holding time and temperature ramping programs were performed. Based on efficiency, sensitivity and the total run-time, the optimal conditions for the GC oven temperature program were as follows: 40°C with 1 minute hold, 40–80°C at 15°C/min, 80–220°C at 35°C/min, and with 4 min hold at 220°C. The total chromatographic run time was 11.7 min (Figure 1).

Method validation

Specificity

The residual solvents in the drug substance synthesis were IPA, 2-butanol, DCM, and toluene. Under optimal conditions, IBO was eluted first without any interference from the other solvents (Figure 1). From the spiked chromatogram (Figure 5D), it also demonstrated that no interference from the sample matrix was observed. As stated in the Experimental Section, only the high purity NMP from Burdick & Jackson had no interference on IBO peak.

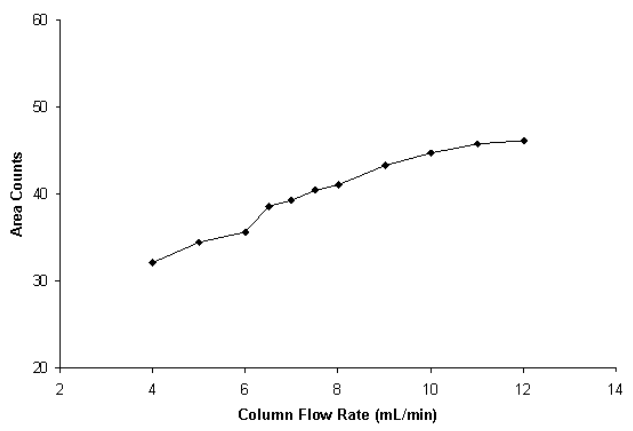

Figure 4. Effect of column flow rate on peak area counts. Method conditions: the same as Figure 2 except column flow rate.

Linearity

The linearity of the method was evaluated by IBO standard solutions at six levels from 0.018 to 6 µg/mL in NMP with a correlation coefficient (R^2) of 0.9999. It is worthwhile to point out that the linearity range could be extended to a lower concentration level if IBO standard solutions were prepared in water. In aqueous solutions, the linearity range of IBO was from 0.006 to 6 µg/mL with R^2 of 0.9997.

Limits of quantitation and detection (LOQ and LOD)

The LOD and LOQ were determined by making triplicate injections of low level of IBO standards with serial dilutions. The LOQ of IBO standard solution was 0.018 µg/mL with S/N ≥ 10

Table I. Conversion of IBS Standard Concentration to Solid Drug Substance Concentration

IBO C (µg/mL)	40mg/mL (µg/g)*	250mg/mL (µg/g)*
0.0048	0.12	0.019
0.018	0.45	0.072
0.04	1	0.16
0.1	2.5	0.40
0.2	5	0.80
0.4	10	1.60
1.2	30	4.80
2	50	8.0
4	100	16.0
6	150	24.0

* Relative to solid sample concentration (mass fraction).

Table II. Recovery Data from Spiking Experiment

	Spiking Level		
IBO standard concentration*	0.072 µg/g Recovery (%)	1.6 µg/g Recovery (%)	4.8 µg/g Recovery (%)
Column Batch I			
Sample 1	90.2	98.6	102.3
Sample 2	90.1	97.2	102.7
Sample 3	93	96.9	102.0
Average	91.1	97.5	102.4
% RSD	1.8	0.9	0.3
Column Batch II			
Sample 1	93.3	97.3	99.1
Sample 2	86.8	96.7	100.1
Sample 3	87.7	96.3	98.8
Average	89.3	96.8	99.3
% RSD	4.0	0.5	0.7

* Converted to sample concentration.

(19). The LOD of IBO standard solution was 0.0048 µg/mL with S/N ≥ 3 (19). As expected, the sensitivity was much better if IBO standard solutions were prepared in water. The LOQ and LOD of IBO aqueous solutions were 0.006 and 0.0016 µg/mL, respectively. The conversion of the standard concentration to the solid sample concentration (mass fraction) is listed in Table I.

Accuracy

Accuracy of the method was evaluated by spiking known amounts of IBO standard solutions to the drug substance samples at three levels with triplicates in sample preparations. A different batch of column was also used to demonstrate ruggedness of the method. The recovery results are listed in Table II. For the triplicate preparations, the average percent recovery was 91.1% for spiking IBO standard solution at LOQ level in the drug substance.

Precision

Injection repeatability of the method was determined by five injections of 0.04 µg/mL standard solution. The relative standard deviation (RSD) of the area counts from the injections was 1.3%. Method repeatability was assessed by evaluating the triplicate sample preparations. The RSD of the recovery was 1.8% at the spiking IBO concentration at LOQ level.

Table III. Batch Data of Residual IBO in Different Batches of the Drug SUbstance

Drug substance sample	Conc. (µg/g)*
I	0.326
II	0.317
III	0.116
IV	0.162

$$\text{* Residual IBO in µg/g (ppm)} = \frac{A_{ts}}{A_s} \times \frac{DF_{ts} \times d \times V_s}{DF_s \times W_{ts}} \times 10^6$$

where Ats is the peak area of IBO from a sample; As is the average peak area from three injections of the IBO standard solution; d is the density of IBO neat solution (d = 0.812 mg/µL); DFs is the dilution factor of the IBO standard solution (DFs = 50000); DFts is the dilution factor of sample solutions (DFts = 2); Wts is the weight of samples; Vs is the volume of the neat standard solution used in the standard solution preparations (Vs = 12.3 µL).

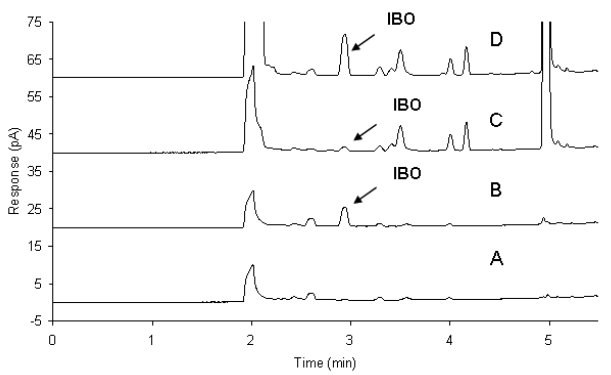

Figure 5. Overlay chromatograms for NMP blank (A); 0.2 µg/mL IBO standard solution (B); unspiked drug substance sample (C); and spiked sample with 0.4 µg/mL IBO standard (D). Method conditions: the same as Figure 1.

Analysis of IBO in the drug substance

The method was used to analyze the residual IBO in four drug substance samples from different batch productions. The batch data are listed in Table I. Levels of IBO in all four samples were well below the specification of the residual IBO in the drug substance (5 µg/g). Figure 5 shows the overlay of the chromatograms of NMP blank, 0.2 µg/mL IBO standard solution, drug substance sample, and spiked sample.

Conclusions

A simply, sensitive, and accurate sHS-GC method was developed and validated to determinate of the residual IBO in the drug substance. Several key parameters, such as headspace vial pressure and oven temperature, column flow rate and GC oven temperature programs, were optimized to increase the sensitivity and reduce the run-time of the method. Different batches of the drug substance were analyzed to demonstrate the suitability of the methodology for the quantitative analysis of the residual IBO in the drug substance. The method was applicable for the routine analysis of trace IBO in the drug substance.

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