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Residual solvents determination in the antibiotic L-749,345 by static headspace gas chromatography

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Abstract

An automated static headspace gas chromatographic method for the determination of residual solvents in the antibiotic L-749,345 was developed and validated. Headspace analysis was used when direct injection of the compound was found to significantly degrade the performance of the column stationary phase. Quantitation was performed by external standard analyses and the method was found to be precise, linear, sensitive, accurate and rugged. The chromatographic conditions and headspace parameters were optimized in a separate experiment to provide a limit test for trace levels of methylene chloride in the presence of significant levels of ethanol. © 1998 Elsevier Science B.V.

Keywords: Solvents, residual; Headspace analysis; Antibiotics; L-749,345

1. Introduction

L-749,345, structure shown in Fig. 1, (4*R*,5*S*,6*S*, 8*R*,2′*S*,4′*S*)-3-[[2-[[(3-carboxyphenyl)amino]carbonyl]-pyrrolidin-4-yl]thio]-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt, is a broad spectrum β-methyl carbapenem antibiotic currently under development. Methanol, ethanol and *n*-propanol are used in the final isolation step. Methylene chloride, a solvent specifically limited by the OVI, organic volatile impurity, designated by the U.S. Pharmacopeia test, is used in an early step of the synthesis. Therefore, it was necessary to quantitatively determine the levels of residual methanol, ethanol, *n*-propanol and develop a sensitive limit test for methylene chloride in the drug substance. Direct injection of L-749,345, a non-volatile compound, was found to significantly degrade the column stationary phase producing poor peak shapes and a reduced response of the analytes. In addition, chromatographic resolution of methylene chloride in the presence of significant levels of ethanol was difficult to obtain. Due to these observations, it was necessary to develop alternate methods for residual solvents.

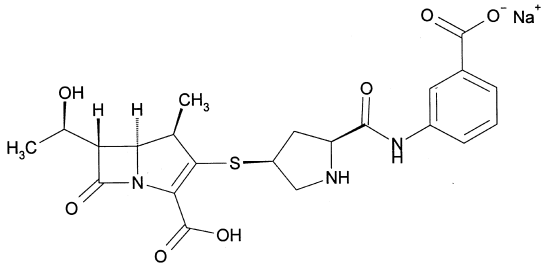

Fig. 1. Structure of the antibiotic L-749,345.

An alternate method of sample analysis, suitable

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for the determination of organic volatiles, is headspace gas chromatography [1]. The sample is in a condensed phase (liquid or solid) while direct analysis is carried out on the equilibrium gaseous (vapor) phase. Thus, headspace analysis is a suitable means to determine levels of volatiles in samples which contain non-volatile components which otherwise would remain on the column, degrade in the injection port or generate degradates which interfere with the analysis. This approach may also be used effectively in samples which contain major volatile analytes which interfere in the analysis and components having different volatilities.

There are three methods commonly used in headspace gas chromatography [2–4]: static headspace, multiple headspace (MHE) and dynamic headspace (purge and trap). Static headspace gas chromatography was chosen as the method of headspace sampling in this study for several reasons. One reason is for simplicity of method development. Multiple headspace methods require extensive, lengthy method development procedures and are more applicable for undissolved solid samples. Another reason is that static headspace methods are more easily automated for the analysis of a large number of samples in a timely fashion. Dynamic headspace methods often are not readily automated and require repeated cleaning of fragile glassware. Also, static headspace methods have wide applicability for use with a liquid matrix containing the dissolved solid sample which is the mode of sample preparation used in this study. Lastly, the sensitivity requirements for the volatile analytes studied were not stringent; therefore, it was not necessary to use dynamic headspace methods which are inherently more sensitive, but have other discussed drawbacks.

Static headspace sampling is a single stage gas extraction of a volatile component from a solution [2]. The solution, with volume V_L and concentration of the analyte C_L^0 is placed into a container of fixed volume *V*. The container is held at a constant temperature until the volatile analytes have equilibrated between the liquid and gaseous phases. At equilibrium, the analytes concentrations in the liquid and gas phases are C_L and C_G, and the volumes of the liquid and gas phases are V_L and V_G, respectively. The phase ratio, β, is equal to the ratio of the volumes of the gas and liquid phases, V_G/V_L. The quantity of the substance C^0 extracted from the solution, expressed as peak area *A*, in the gas phase depends on the relationship of β and the value of the distribution coefficient, *K*. The distribution coefficient is equivalent to the ratio C_L/C_G. For optimum sensitivity it is desirable to have *K* as small as possible, with the majority of the analyte present in the gaseous phase. Eq. (1) [5] which describes the principles of static headspace is given by the following expression:

$$A \cong C^0/K + \beta \quad (1)$$

Response of volatile components can be enhanced to determine trace concentrations in the sample with the proper selection of equilibrium conditions, primarily the parameters of equilibration temperature and thermostating time. Also, the sensitivity can be increased by adjusting the pH, ''salting out'' [2] or raising the thermostating temperature.

The use of static headspace gas chromatography to determine the level of residual solvents in drug forms has been demonstrated [6–10]. This paper will not only describe the steps undertaken to develop and validate a residual solvent static headspace gas chromatographic method for L-749,345, but also demonstrate how the equilibrium conditions used in the residual solvent method were tailored to provide a limit test for trace levels of methylene chloride in the drug substance in the presence of significant levels of alcohols which interfered in the analysis using conventional direct gas chromatographic methods.

2. Experimental

2.1. Reagents and materials

L-749,345 was obtained from Process Research, Merck Research Laboratories. Solvents used were >99% purity and purchased from the following sources: methanol, *n*-propanol, dimethylformamide (DMF), methylene chloride from Fisher Scientific (Fairlawn, NJ, USA) and punctilious ethanol from Quantum Chemical Corp., (Newark, NJ, USA). Water was Millipore HPLC grade. The headspace vials were 22-ml capacity (Perkin–Elmer, Norwalk,

CT, USA) and PTFE coated butyl rubber septa, crimp cap and star spring (Perkin–Elmer) were used to seal the vials. All pipetting was performed using Finn Autopipets.

2.2. Chromatographic systems and methods

2.2.1. Headspace GC instrumental conditions

The headspace experiments were performed on an Autosystem Perkin–Elmer gas chromatograph equipped with a flame ionization detector and an HS-40 headspace injector (Perkin–Elmer). The GC column used was a DB-1 fused-silica column (J&W Scientific, Folsom, CA, USA), dimensions 0.32 mm×30 m and 5.0-μm film thickness. Chromatographic data were collected by the PE–Nelson Access*Chrom Data System (Perkin–Elmer–Nelson Systems, Cupertino, CA, USA).

The GC and headspace parameters used for the determination of residual methanol, ethanol and *n*-propanol are given as follows: the GC temperature program was 35°C isothermal for 8 min, then 25°C min^{-1} to 125°C. The injector temperature was maintained at 180°C with a split ratio of 25:1 and FID detector temperature of 250°C. The headspace injector parameters were static mode, 85°C equilibration temperature, 15 min thermostating time, 2 min pressurization time, 0.06 min injection time, 125°C needle temperature, 135°C transfer line temperature, 1.0-ml sample volume and sample weight of 20 mg. Helium carrier gas was used with a column head pressure of 24 psi.

The GC and headspace injector method parameters chosen for determination of trace levels of methylene chloride in the presence of methanol, ethanol and *n*-propanol are given as follows: the GC temperature program was 35°C isothermal for 8 min, then 25°C min^{-1} to 200°C. The injector temperature was maintained at 135°C with a split ratio of 25:1 and FID detector temperature of 250°C. The headspace parameters were static mode, 50°C equilibration temperature, 6 min thermostating time, 0.5 min pressurization time, 0.06 min injection time, 125°C needle temperature, 95°C transfer line temperature, 1.0-ml sample volume and sample weight of 135 mg. Helium carrier gas was used with a column head pressure of 24 psi.

2.2.2. Direct GC instrumental conditions

Direct injection gas chromatography, performed for the accuracy determination in the validation section, was performed on a Model 5890 Hewlett–Packard (San Fernando, CA) Series II gas chromatograph equipped with packed column injector, a Model 7673 autosampler and flame ionization detector. The GC column used was a RTX-1701 (Restek Corp., Bellefonte, PA, USA) fused-silica column, dimensions 0.53 mm×60 m, 1.5-μm film thickness and a 5-m retention gap. The direct GC method temperature program was 35°C to 45°C at 2°C min^{-1} then 5°C min^{-1} to 70°C and then increased 20°C min^{-1} to 210°C with a final hold time of 10 min. The packed column injector was maintained at 200°C and FID detector temperature at 250°C. Helium carrier gas was used with column head pressure of 10 psi. The concentration of the samples was 10 mg ml^{-1} using water–DMF (20:80) diluent and the volume injected was 2.0 μl.

2.3. Standard and sample preparation

2.3.1. Sample preparation used for headspace determination of methanol, ethanol and n-propanol

A 15–20 mg amount of drug substance was accurately weighed into duplicate headspace vials, 1.0 ml of water was added and the vial was sealed, vortexed for 0.5 min to dissolve the sample and then analyzed. Standard preparation: the 1.0% (v/v) stock solution was prepared by pipetting 100 μl each of methanol, ethanol and *n*-propanol into a 10-ml volumetric flask and diluting to volume with water. The 0.0005, 0.001, 0.005, 0.01 and 0.02% (v/v) standard solutions were prepared by serial dilution. A 1.0-ml volume of each standard solution was pipetted into duplicate headspace vials, sealed, vortexed for 0.5 min and analyzed.

2.3.2. Sample preparation used for the headspace determination of methylene chloride

A 135-mg amount of drug substance was accurately weighed into duplicate headspace vials, 1.0 ml of water and 10 μl of DMF were added and the vial was sealed. The solution was vortexed for 0.5 min to dissolve the sample and then analyzed. Standard preparation: a 100-μl volume of methylene chloride was pipetted into a 10-ml volumetric flask and

diluted to volume with DMF to prepare the 1.0% (v/v) stock solution. The 0.01% (v/v) standard solution was prepared by serial dilution. A 1.0-ml volume of water and 10 μl of the 0.01% (v/v) methylene chloride standard were pipetted into a headspace vial to prepare the 0.0001% (v/v) standard solution. The solution was vortexed 0.5 min and analyzed. Preparation of the 10 ppm (w/w) methylene chloride spiked sample: a 135-mg sample was accurately weighed into a headspace vial, 1.0 ml water and 10 μl of the 0.01% (v/v) methylene chloride standard were added and the vial was sealed. The solution was vortexed for 0.5 min and analyzed.

2.3.3. Sample preparation for the direct determination of residual methanol, ethanol and n-propanol

A 100-mg amount of sample was accurately weighed into a 10-ml volumetric flask, 2 ml of water was added, the solution was vortexed to dissolve the sample and diluted to volume with DMF. Standard preparation: aliquots (100 μl each) of methanol, ethanol and *n*-propanol were pipetted into a 10-ml volumetric flask and diluted to volume with water–DMF (20:80) to prepare the 1.0% (v/v) stock solution. The 0.0005, 0.001, 0.005 and 0.01% (v/v) standard solutions were prepared by serial dilution. The samples and standards were placed into autosampler vials and analyzed.

The amount of each solvent found in the above experiments was expressed as a weight percent of L-749,345 is given by Eq. (2):

$$\text{Wt\% i} = \frac{\text{Area cts i}_{\text{sample}}}{\text{Area cts i}_{\text{standard}}} \times \text{Vol\% i}_{\text{standard}} \times \frac{\text{Sample volume, ml}}{\text{Sample weight, g}} \times \text{Density i, g ml}^{-1} \quad (2)$$

where i = analyte.

3. Results and discussion

Several parameters must be considered when developing a headspace residual solvent method: volatility of the analytes, solubility of the sample in a suitable diluent, choice of an appropriate GC column to adequately separate the analytes, sensitivity requirements and lastly, the headspace parameters of equilibration temperature, thermostating time, sample volume and pressurization time.

3.1. Residual solvent headspace method-determination of methanol, ethanol and n-propanol

Methanol, ethanol and *n*-propanol are suitably volatile with b.p.'s of 65°, 78° and 97°C, respectively. Water was chosen as the diluent since the analytes and sample are readily soluble and it does not show a response in the flame ionization detector. The DB-1 medium bore, 5 μm thick film column was selected because it provided good separation and peak shapes of the analytes. The desired sensitivity was <100 ppm for each component.

The headspace parameters that needed to be optimized were the equilibration temperature, pressurization time and thermostating time. A 0.01% (v/v) methanol, ethanol and *n*-propanol standard was used for all the experiments and a separate vial was used for each determination.

The optimal equilibration temperature was determined by varying the equilibration temperature from 50 to 85°C. The area counts vs. equilibration temperature are shown in Fig. 2. The area counts increased with the equilibration temperature. The temperature which provided maximum response of the analytes was 85°C; a temperature >85°C was not tested due to thermal degradation (evaporation) of the sample aqueous matrix [9]. It is necessary to keep the volume of the liquid matrix constant for quantitative studies.

The equilibration temperature was maintained at 85°C and the pressurization time varied from 0 to 16 min. The plot of area counts vs. pressurization time is shown in Fig. 3. The area counts increased with the pressurization time up to 8 min then leveled off. A pressurization time of 2 min was selected for the analysis because the increase in area counts with pressurization times >2 min was small and determined not to be significant enough to merit increasing the analysis time.

The thermostating time was varied from 0 to 30 min holding the equilibration temperature at 85°C and pressurization time at 2 min. The area counts

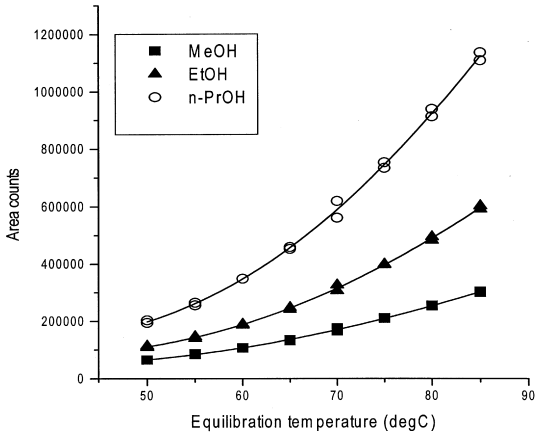

Fig. 2. Headspace GC method plot of area counts vs. equilibration temperature. Method conditions: J&W Scientific DB-1 fused-silica column (0.32 mm×30 m, 5.0-μm film thickness); temperature program of 35°C isothermal for 8 min then 25°C min^{-1} to 125°C; 25:1 split ratio; FID detector temperature of 250°C; injector temperature of 180°C; headspace parameters: static mode; equilibration temperature varied from 50 to 85°C; 15 min thermostating time; 2 min pressurization time; 0.06 min injection time and 1.0 ml sample volume. Solution analyzed: 1.0 ml of the 0.01% (v/v) methanol, ethanol, *n*-propanol standard solution in water.

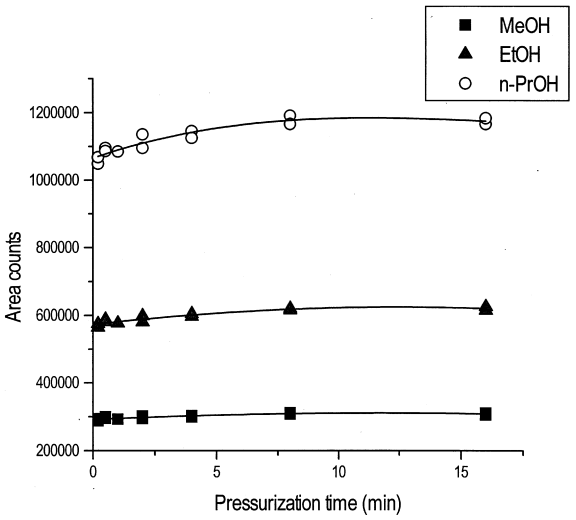

Fig. 3. Headspace GC method plot of area counts vs. pressurization time. Method conditions: J&W Scientific DB-1 fused-silica column (0.32 mm×30 m, 5.0-μm film thickness); temperature program of 35°C isothermal for 8 min then 25°C min^{-1} to 125°C; 25:1 split ratio; FID detector temperature of 250°C; injector temperature of 180°C; headspace parameters: static mode; equilibration temperature 85°C; 15 min thermostating time; pressurization time varied from 0 to 16 min; 0.06 min injection time and 1.0 ml sample volume. Solution analyzed: 1.0 ml of the 0.01% (v/v) methanol, ethanol, *n*-propanol standard solution in water.

increased up to 15 min then remained approximately the same (within experimental error) after 15 min; for that reason, a thermostating time of 15 min was selected.

In a different experiment, the water diluent was saturated with sodium sulfate to increase the response of the analytes by the ''salting out'' effect. The response of methanol, ethanol and *n*-propanol was increased by approximately two-fold, however, the sensitivity of the method was found to be adequate without the addition of sodium sulfate.

A typical comparison chromatogram of the water blank, 0.01% (v/v) standard solution and sample is shown in Fig. 4, using all method parameters given in Section 2.2.1. The analytes are well-resolved and the peak shapes of the components in the sample are good.

3.2. Method validation

Linearity was evaluated from duplicate injections of methanol, ethanol and *n*-propanol over the concentration range 0.05 to 0.0001% (v/v). The resulting plots for methanol, ethanol and *n*-propanol are linear over the entire range with $r^2 = 0.99996$ for methanol, $r^2 = 0.99995$ for ethanol and $r^2 = 0.99996$ for *n*-propanol.

Chromatographic precision was determined by repeated analysis of a standard mixture which contained 0.01% (v/v) each of methanol, ethanol and *n*-propanol. Six consecutive injections were made. The %R.S.D. of methanol, ethanol and *n*-propanol were 0.8%, 0.7% and 1.0%, respectively.

The limit of detection (LOD) is defined as the concentration at which the $S/N \geq 3$ and the limit of quantitation (LOQ) where $S/N \geq 10$ [11]. Analysis of the 0.0001% (v/v) solution revealed a S/N ratio >10 for methanol, ethanol and *n*-propanol. Therefore, the LOD for each of these solvents is $<0.0001\%$ (v/v), equivalent to <0.004 wt% and the LOQ for each is 0.0001% (v/v), equivalent to <0.012 wt%.

To determine method recovery, three separate samples of L-749,345-002C containing residual methanol at <0.1 wt%, ethanol at 0.7–1.0 wt% and *n*-propanol at <0.1–0.3 wt% were spiked with

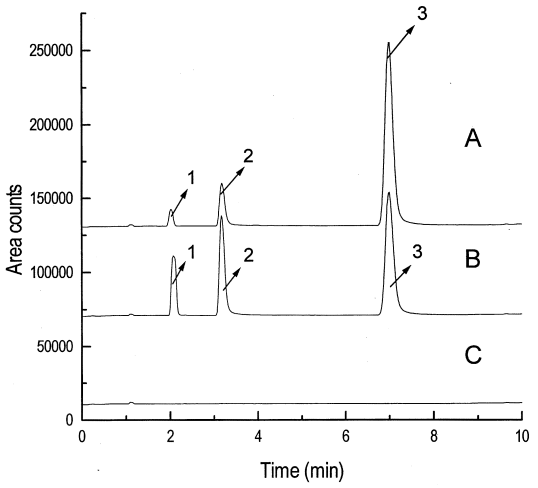

Fig. 4. Typical headspace GC chromatograms. (A) Chromatogram of a sample of L-749,345. Method conditions used were: J&W Scientific DB-1 fused-silica column (0.32 mm×30 m, 5.0-μm film thickness); temperature program of 35°C isothermal for 8 min then 25°C min^{-1} to 125°C; split ratio of 25:1; FID detector temperature of 250°C; 180°C injector temperature; headspace parameters: static mode; 85°C equilibration temperature; 15 min thermostating time; 2 min pressurization time; 0.06 min injection time and 1.0 ml sample volume. Sample preparation: 20 mg dissolved in 1.0 ml water. (B) The 0.01% (v/v) standard solution. Method conditions were the same as in (A) except 1.0 ml of the standard solution was analyzed. (C) The water blank. Method conditions were the same as in (A) except that 1.0 ml of water diluent was analyzed. Peaks: 1=methanol; 2=ethanol; 3=*n*-propanol.

Table 1
Accuracy of the headspace GC method

	Headspace GC	Direct injection GC method
Sample 1		
Wt% methanol	0.02	<0.01
Wt% ethanol	0.05	0.05
Wt% *n*-propanol	0.16	0.16
Sample 2		
Wt% methanol	0.02	<0.01
Wt% ethanol	0.87	0.92
Wt% *n*-propanol	0.02	0.02
Sample 3		
Wt% methanol	0.03	<0.01
Wt% ethanol	0.87	0.84
Wt% *n*-propanol	0.02	0.02

Headspace GC method conditions given in Section 2.2.1. Direct injection GC method conditions are given as follows: Restek RTX-1701 fused-silica column (0.53 mm×60 m, 1.5-μm film thickness and 5-m retention gap); temperature program: 35°C to 45°C at 2°C min^{-1} then 5°C min^{-1} to 70°C and then increased to 20°C min^{-1} to 210°C with a final hold time of 10 min; FID detector temperature of 250°C and injector temperature of 200°C; sample preparation: 10 mg sample dissolved in 1 ml of water–dimethylformamide (20:80) with 2.0-μl injection volume.

standards of methanol, ethanol and *n*-propanol at the 0.30 wt% level. The recoveries were 100–104% for methanol, 85–115% for ethanol and 100% for *n*-propanol.

The accuracy of the method was determined by analyzing three different samples of L-749,345-002C both using the headspace GC method and the direct GC method. The results of the testing are given in Table 1. There was excellent agreement between the headspace GC and direct injection GC methods supporting the accuracy of the headspace procedure.

System suitability criteria were established for the method based upon examination of the existing database: maximum 2% R.S.D. for the area counts of methanol, ethanol and *n*-propanol from three separate consecutive injections of the 0.01% (v/v) standard solution; maximum peak tailing factors of 2 for methanol, ethanol and *n*-propanol; and a minimum 2 for the resolution between methanol and ethanol.

3.3. Limit test for trace levels of methylene chloride in samples containing significant amounts of ethanol

The desired limit of detection of methylene chloride is <10 ppm (w/w). However, resolution of trace levels of methylene chloride in L-749,345 containing >1.0 wt% of ethanol was not adequate using commercially available chromatographic column stationary phases with direct injection gas chromatographic methods to obtain an LOD of ≤10 ppm. Consequently, the headspace gas chromatographic method was adapted to provide a 10 ppm limit test for methylene chloride by comparison to methylene chloride spiked samples.

Methylene chloride has a b.p. of 41°C, significantly lower than methanol, ethanol and *n*-propanol. Water containing 10 μl of DMF added to solubilize the methylene chloride was selected as the diluent. The DB-1 medium bore, 5 μm thick film column was selected because it provided good separation and

peak shape of methylene chloride, methanol, ethanol and *n*-propanol.

The method parameters chosen for the method are given in Section 2.2.1. A thermostating temperature of 50°C decreases the response of the methanol, ethanol and *n*-propanol peaks while the methylene chloride peak is not affected. The ethanol peak is well-separated from methylene chloride allowing for 10 ppm methylene chloride spiked samples to be detected, see Fig. 5. The analysis of three different lots of L-749,345 revealed that there was <10 ppm methylene chloride present in each of the samples.

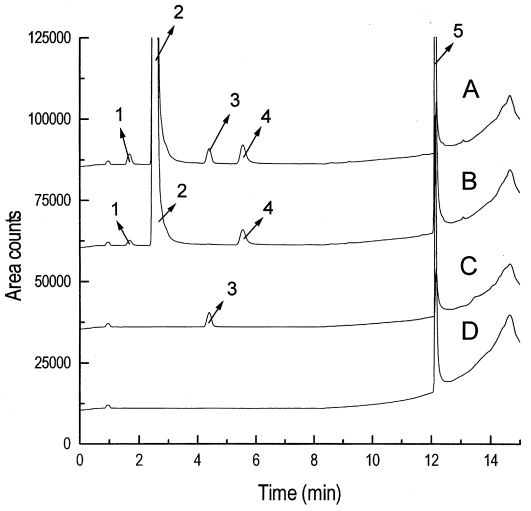

Fig. 5. Headspace GC chromatograms for the methylene chloride limit test. (A) Chromatogram of 10 ppm methylene chloride spiked L-749,345. Method conditions: J&W Scientific DB-1 fused-silica column (0.32 mm×30 m, 5.0-μm film thickness); temperature program of 35°C isothermal for 8 min then 25°C min^{-1} to 200°C; 25:1 split ratio; FID at 250°C; 135°C injector temperature; headspace parameters: static mode; 50°C equilibration temperature; 6 min thermostating time; 0.5 min pressurization time; 0.06 min injection time and 1.0-ml sample volume. Sample preparation: 135 mg sample dissolved in 1 ml water and 10 μl of the 0.01% (v/v) methylene chloride standard solution. (B) Chromatogram of L-749,345. Method conditions the same as (A) except that the sample was dissolved in 1.0 ml water and 10 μl dimethylformamide. (C) Chromatogram of the 0.0001% (v/v) methylene chloride standard solution. Method conditions were the same as (A) except that 1.0 ml of water and 10 μl of the 0.01% (v/v) methylene chloride standard solution was analyzed. (D) Chromatogram of the water:dimethylformamide blank. Method conditions were the same as (A) except that 1.0 ml water and 10 μl dimethylformamide was analyzed. Peaks: 1=methanol; 2=ethanol; 3=methylene chloride; 4=*n*-propanol; 5=dimethylformamide.

4. Conclusion

The headspace GC residual solvent method for L-749,345 provided several advantages compared to the direct GC residual solvent method. It effectively eliminated the column degradation problem encountered with the direct injection GC method and therefore is a more accurate and rugged method to determine the levels of residual solvents. Some economic advantages are that the column lifetimes are extended and the time necessary to perform maintenance on the GC system reduced with the headspace GC method. Also, the headspace GC method parameters (equilibration temperature and thermostating time) compared to the direct GC parameters offer greater flexibility in achieving the separation of closely eluting analytes.

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