Residual Solvents in Spironolactone by Headspace GC/FID and Agilent J&W DB-Select 624UI

Application Note

Pharmaceuticals

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Abstract

This application note highlights the utility of the Agilent J&W DB-Select 624UI GC column and nitrogen as carrier gas for the analysis of residual solvents in spironolactone by static headspace GC/FID. Residual solvents in spironolactone API include methanol, ethanol, acetone, tetrahydrofuran, ethyl acetate, pyridine, and N,N-dimethylformamide. The internal standard used for this method was 1-propanol. The Ultra Inert column provided excellent peak shapes for these active compounds and the method showed good repeatability of both retention time and peak area.

Introduction

Spironolactone, which is marketed primarily under the brand name Aldactone in most countries, is a synthetic, steroidal antimineralocorticoid agent with additional anti-androgen and weak progestogen properties. This medicine has some indirect estrogen and glucocorticoid effects, and is primarily used as a diuretic and antihypertensive, but also for reducing elevated or unwanted androgen activity in the body. It acts predominantly as a competitive antagonist of the aldosterone (or mineralocorticoid) receptor, and belongs to a class of pharmaceutical drugs known as potassium-sparing diuretics [1].

According to the China Pharmacopoeia (2010), methanol, ethanol, acetone, tetrahydrofuran, ethyl acetate, pyridine, and N,N-dimethylformamide are frequently used in the pharmaceutical industry for manufacture of active pharmaceutical ingredients (APIs) of spironolactone [2]. These organic residual solvents should be monitored to ensure the safety of pharmaceutical products.

Helium is the most popular carrier gas in GC for a number of reasons, but mostly because it is highly inert, nonexplosive and delivers satisfactory chromatographic efficiency. The growing concern about dwindling supplies of helium and its associated increasing cost is leading many chromatographers to evaluate the use of alternative gases such as hydrogen and nitrogen. This application demonstrates the analysis of residual solvents in spironolactone using gas chromatography with headspace and an Agilent J&W DB-select 624UI column.

Experimental

Analyses were performed on an Agilent 7890 GC equipped with a flame ionization detector (FID) and an Agilent 7697A Headspace Sampler. Organic solvents, including methanol, ethanol, acetone, tetrahydrofuran (THF), 1-propanol, ethyl acetate, pyridine, and N,N-dimethylformamide (DMF), were obtained from J&K Chemical (Shanghai, China). Dimethyl sulfoxide with ≥99.9 purity was purchased from Sigma-Aldrich (Shanghai, China).

Standard solutions

The internal standard solution (ISS) was made by adding 1-propanol to dimethyl sulfoxide (DMSO) to produce a solution of 1 mg/mL, and mixed well. The standard stock solution was made by adding standards of residual solvents in DMSO to produce a solution containing 1 mg/mL methanol, ethanol, acetone, and ethyl acetate, 0.07 mg/mL THF, 0.02 mg/mL pyridine, and 0.09 mg/mL DMF.

To make the standard solution, 5 mL of the stock solution was transferred to a 20 mL headspace vial. One mL ISS was added, and diluted with DMSO to 10 mL. The vial was sealed.

Sample preparation

One gram of spironolactone was weighed accurately into a 20 mL headspace vial. One mL ISS was added, then diluted with DMSO to 10 mL and the vial sealed.

To prepare the spiked sample solution, 1 g spironolactone was weighed into six 20 mL headspace vials and 1 mL ISS of internal standard solution was added to each vial. A 5-mL stock solution was transferred into each vial and diluted with DMSO to 10 mL. The vials were then sealed.

Conditions

Column:	Agilent J&W DB-Select 624UI, 30 m × 0.53 mm, 3 µm (p/n 125-0334UI)
Carrier:	Nitrogen, constant flow mode, 4.5 mL/min
Oven:	40 °C for 8 min, 40 to 200 °C at 45 °C/min, 200 °C for 3 min
Sample loop:	1 mL
Temperature:	HS oven 80 °C, loop 110 °C, transfer line 130 °C
Vial equilibration time:	30 min
Inlet:	200 °C, split ratio 3:1
Detector:	FID at 250 °C
Sampler:	Agilent 7697A Headspace Sampler
Instrument:	Agilent 7890A GC

Supplies

Vials:	Flat bottom crimp cap headspace vials, 20 mL, 100/pk (5182-0837)
Vial caps:	Headspace crimp caps and septa, 20 mm, 100/pk (p/n 5183-4477)
Crimper:	Manual crimper, 20 mm (p/n 5040-4669)
Septa:	Non-stick Bleed and Temperature Optimized (BTO) (p/n 5183-4757)
Liner:	Straight, Ultra Inert liner (p/n 5190-4047)
Ferrules:	Graphite ferrules, 10/pk (p/n 500-2118)
Column nuts:	Universal 0.45 to 0.53 mm column, nuts, 2/pk (p/n 5181-8830)

Results and Discussion

The carrier gas can influence GC separation. Although nitrogen can provide the best efficiency, small changes in the average linear velocity result in large changes in efficiency, according to the nitrogen Van Deemter curve (Figure 1). The optimal velocity (Uopt) is a bit below average linear velocity, which means a longer analysis time. However, unlike hydrogen and helium, nitrogen is the least expensive carrier gas, and is readily available without worry of explosion. Nitrogen can be substituted for helium as carrier gas to gain satisfactory chromatographic efficiency and reasonable run times in many applications when a suitable flow rate is used.

Figure 2 shows the chromatograms of standard spiked sample and sample solution. The concentrations of target compounds ranged from 0.01 to 0.5mg/mL due to varying target limits for different residual solvents. The amount of pyridine in the sample solution (average 0.001 mg/mL) found was within limits. Figure 2 shows that the analysis time for this application was approximately 14 minutes when using nitrogen as carrier gas at a 4.5 mL/min flow rate. All peaks were well resolved on the DB-Select 624UI column. Due to the ultra-inert character of the column, peak shapes for the target compounds were sharp and symmetrical.

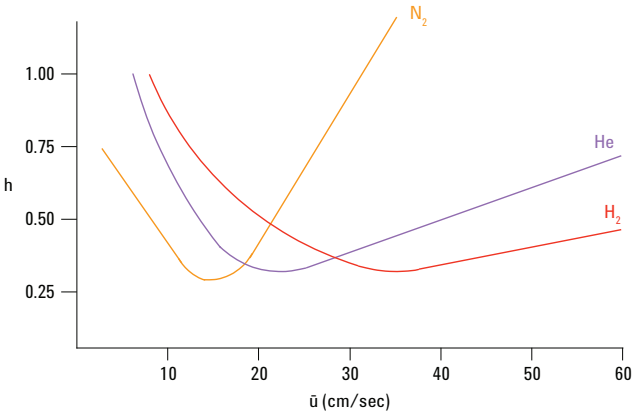

Figure 1. Van Deemter plot of helium, nitrogen, and hydrogen.

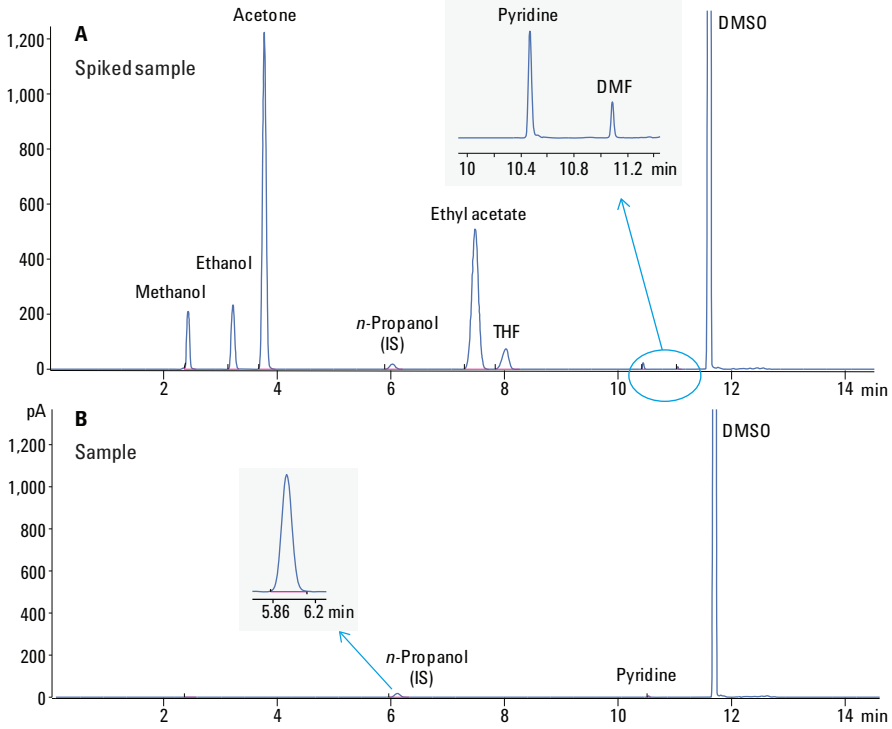

Figure 2. Chromatograms of standard spiked sample (A) and sample solution (B) using an Agilent HS/GC/FID system and an Agilent J&W DB-Select 624UI column.

Table 1 shows the performance and repeatability results for the peaks in the chromatogram of the spiked sample. Table 1 also shows efficiency and resolution results.

The USP-tailing factor for each compound was quite close to 1. Most notably, pyridine tails on most GC columns but the average USP tailing value of 1.19 on the DB-Select 624UI column is demonstrated. Good repeatability of retention time and area (RSD better than 1.64%) indicated high reliability of the method.

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Table 1. Performance and repeatability results.

		Retention time (min)		Area (mAU*s)				
No.	Compound	RT	RSD (%) (n = 6)	Area	RSD (%) (n = 6)	Theoretical plate (N)	Resolution (Rs)	USP Tf
1	Methanol	2.43	0.04	801.66	1.11	8,456	/	1.07
2	Ethanol	3.22	0.04	974.77	1.22	12,281	7.25	1.04
3	Acetone	3.77	0.03	5585.24	0.85	14,915	4.72	1.01
4	1-propanol (IS)	6.03	0.02	144.69	0.74	16,177	14.65	1.04
5	Ethyl acetate	7.49	0.02	4329.34	0.56	17,353	7.13	1.00
6	Tetrahydrofuran (THF)	8.05	0.02	583.61	0.90	20,956	2.52	0.86
7	Pyridine	10.47	0.01	37.10	0.98	756,377	18.47	1.19
8	N,N-Dimethylformamide (DMF)	11.09	0.02	10.55	1.64	1,126,295	14.58	1.01

Conclusions

Residual solvents in spironolactone were analyzed by gas chromatography with headspace and an Agilent J&W DB-Select 624UI column. Nitrogen was used as the carrier gas. The DB-Select 624UI column demonstrated excellent inertness as indicated by the sharp and symmetrical peaks for alcohols and pyridine. This method for the analysis of residual solvents in spironolactone API was economical, reliable, and exhibited good performance and repeatability.

References

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