APPLICATION NOTE

Gas Chromatography

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GC-FID: Limit of Cetyl Alcohol and Aliphatic Alcohol Impurities According to USP

Introduction

Cetyl alcohol, also known as palmityl alcohol or 1-hexadecanol, is a long-chain aliphatic alcohol that is a common additive in foods, cosmetics, pharmaceuticals, personal care products (e.g., lotions and creams), and industrial fuels. Cetyl alcohol has many uses as a thickening agent, emulsifier, moisturizer, lubricant, and flavoring agent in product formulations, and is safe for human consumption, both orally and via contact with the skin[1]. Studies have found that minor impurities in cetyl alcohol might be the cause of dermatitis[2].

To ensure cetyl alcohol quality, the United States Pharmacopoeia (USP) has outlined a chemical purity assay within the USP-National Formulary (NF), Cetyl Alcohol monograph[3]. The monograph outlines procedures to assess instrument performance, measure cetyl alcohol content, and identify common aliphatic alcohol impurities (stearyl alcohol, lauryl alcohol, myristyl alcohol, and oleic alcohol) and other unidentified impurities using gas chromatography/flame ionization detection (FID). This application note shows the performance of the PerkinElmer GC 2400™ System with FID for the analysis of residual impurities in cetyl alcohol according to the USP monograph, while offering enhanced compound resolution and highly repeatable injections.

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Instrumentation

The GC 2400 System was configured with an FID and with PerkinElmer Elite 225 analytical column for the analysis of cetyl alcohol content, and identification of impurities. PerkinElmer SimplicityChrom™ Chromatography Data System (CDS) Software offers the user the convenience of automatically-generated suitability data.

PerkinElmer GC 2400 System

Experimental

Table 1: Chromatography conditions.

System		Part Numbers
Gas Chromatograph	PerkinElmer GC 2400 System	--
Injector	Capillary Split/Splitless (CAP) with PerkinElmer AS 2400™ Liquid Sampler	--
	Advanced Green Inlet Septum	N9306218
	Green Focus liner	N6502041
	5 uL Autosampler Syringe	N6402556
Detector	Flame Ionization Detector (FID)	--
	Grade 5 Hydrogen, 35 ml/min	--
	Grade 5 Air, 400 ml/min	--
	Grade 5 Nitrogen, 25 ml/min	--
Gas Filters	Triple Filter (Hydrogen & Nitrogen)	N9306110
	Moisture/Hydrocarbon Trap (Air)	N9306117
Analytical Column	Elite 225; 30 m x 0.25 mm x 0.25 µm	N9316177
Software	SimplicityChrom CDS Software	

Conditions	
Carrier	Grade 5 Hydrogen, 2 ml/min
Septum Purge	3 ml/min
Split	5:1 ratio*
Injection Volume	0.5 µL
Injector Temp.	270 °C
Detector Temp	280 °C
Oven	60 °C for 0 min, 20 °C/min to 180 °C, 10 °C/min to 220 °C, hold for 5 min

**Note: all tests were performed with a 5:1 split ratio. This differs slightly from the monograph, which recommends a split of 5:1 for the impurity test and resolution check, and a split of 100:1 for the assay.*

High purity (> 99 wt%) cetyl alcohol, stearyl alcohol, lauryl alcohol, oleyl alcohol, and myristyl alcohol were purchased from Millipore Sigma (Burlington, MA). 1-pentadecanol internal standard and 200 proof ethanol diluent were also purchased from Millipore Sigma. A natural commercial cetyl alcohol sample was purchased online from a cosmetics vendor.

The analytical column was conditioned following the procedures outlined in the PerkinElmer Capillary Column Quick Care Guide. To prepare 1 mg/ml of internal standard solution, approximately 100 mg of 1-pentadecanol was diluted into 100 ml of 200 proof ethanol in a volumetric flask. The solution was ultrasonicated at 50 °C for 5 minutes, whereupon all solids had dissolved. To prepare the standard solution, approximately 10 mg of lauryl, oleyl, stearyl, myristyl, and cetyl alcohol were weighed out into the same 10 ml volumetric flask. 10 ml of internal standard solution were added to dissolve the solutes, producing a mixture containing approximately 1 mg/ml of each component. To make the resolution check solution, the standard solution was further diluted with ethanol, producing a final test mix concentration of approximately 0.05 mg/ml. The commercial sample was prepared by dissolving approximately 10 mg of the sample into 10 mL of internal standard solution.

Analysis was performed on a GC 2400 System using SimplicityChrom CDS Software. Each solution was analyzed 5 times. The straightforward software interface allows for streamlined data acquisition and processing. The software's system suitability function automatically generates the required information for fulfillment of the monograph.

Results & Discussion

USP Retention Time

Retention time identification is critical when using flame ionization detection, as hydrocarbons' signal is otherwise indistinguishable.

Table 2: Retention time (RT) and relative retention time (RRT) repeatability study of the long-chain aliphatic alcohols in the resolution check solution. The RRT internal standard is 1-pentadecanol. Relative percent difference (RPD) is provided comparing internal RRT values against those in the USP monograph, equation shown below.

	Lauryl Alcohol	Myristyl Alcohol	1-Pentadecanol	Cetyl Alcohol	Stearyl Alcohol	Oleyl Alcohol
Run 1 RT (min)	5.56	6.57	7.11	7.69	8.94	9.08
Run 2 RT (min)	5.56	6.57	7.11	7.70	8.94	9.08
Run 3 RT (min)	5.56	6.57	7.11	7.69	8.94	9.07
Run 4 RT (min)	5.55	6.56	7.11	7.69	8.94	9.07
Run 5 RT (min)	5.55	6.56	7.11	7.69	8.94	9.07
Avg. RT (min) ± RSD	5.55 ± 0.02%	6.56 ± 0.02%	7.11 ± 0.02%	7.69 ± 0.02%	8.94 ± 0.01%	9.07 ± 0.01%
Run 1 RRT	0.78	0.92	1.00	1.08	1.26	1.28
Run 2 RRT	0.78	0.92	1.00	1.08	1.26	1.28
Run 3 RRT	0.78	0.92	1.00	1.08	1.26	1.28
Run 4 RRT	0.78	0.92	1.00	1.08	1.26	1.28
Run 5 RRT	0.78	0.92	1.00	1.08	1.26	1.28
Avg. RRT ± RSD	0.78 ± 0.01%	0.92 ± 0.01%	n/a	1.08 ± 0.01%	1.26 ± 0.01%	1.28 ± 0.01%
USP Literature RRT2 RPD*	0.79 1.13%	0.93 0.75%	1.00 n/a	1.09 0.75%	1.25 -0.57%	1.28 0.32%

$$*RPD = \frac{RRT_{PerkinElmer} - RRT_{USP}}{RRT_{USP}}$$

The GC 2400 System's advanced Pneumatic Pressure Controller (PPC) is capable of highly repeatable separations, allowing for reliable identification of compounds by retention time (RT), as shown in Table 2. A relative standard deviation of 0.01% - 0.02% was calculated for the average RT of each compound shown in Figure 1A.

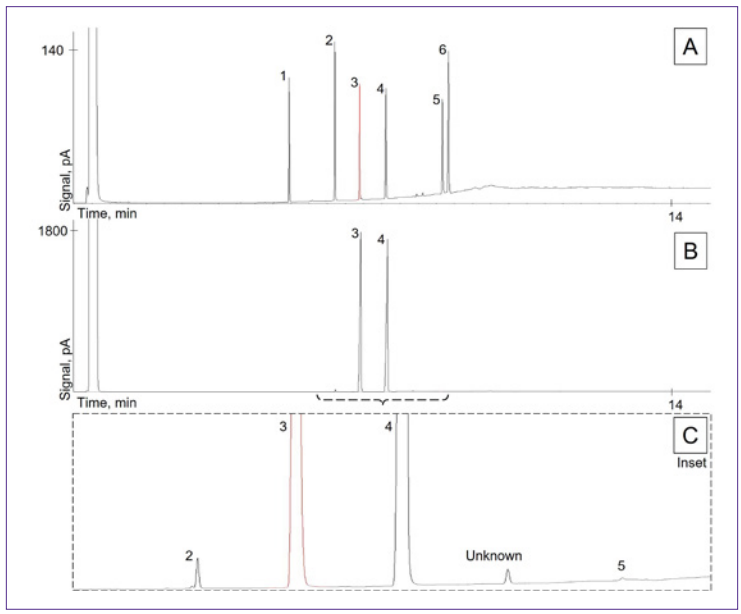

Figure 1: Chromatograms of (A) the resolution check solution, (B) the commercial cetyl alcohol sample, and (C) the inset of commercial sample highlighting low-level impurities. Compounds—1: Lauryl Alcohol; 2: Myristyl Alcohol; 3: 1-Pentadecanol internal standard; 4: Cetyl Alcohol; 5: Stearyl Alcohol; 6: Oleyl Alcohol.

Note: Sample B is at approx. 20x concentration versus resolution check solution, Sample A.

The USP monograph provides relative retention times (RRT) for each component against 1-pentadecanol internal standard; literature values are provided in Table 2. Excellent accuracy was obtained between internal and external (USP) values: approximately 1% or less. Excellent precision was also obtained with an average RRT standard deviation of 0.01% for each compound.

USP Resolution Check

The performance of the GC 2400 System, with an Elite 225 capillary column, greatly surpasses the USP method criteria for compound resolution. The method requires a myristyl alcohol and 1-pentadecanol resolution of at least 15, a cetyl and stearyl alcohol resolution of at least 30, and a stearyl and oleyl alcohol resolution of at least 2 in the resolution check solution. The results in Table 3 show that these values were exceeded, with improvements to each compound resolution by 45% (21.71), 33% (39.99), and 100% (3.99), respectively.

Table 3: Results of the resolution study based on USP resolution acceptance criterion. Results prove that the PerkinElmer system outperforms each acceptance criterion.

	Myristyl:1-Pentadecanol	Cetyl:Stearyl	Stearyl:Oleyl
Run 1 Resolution	21.69	39.08	3.93
Run 2 Resolution	21.90	40.10	4.02
Run 3 Resolution	21.57	41.37	4.09
Run 4 Resolution	21.83	39.59	3.91
Run 5 Resolution	21.56	39.81	3.99
Avg. Res. ± RSD	21.71 ± 0.70%	39.99 ± 2.15%	3.99 ± 1.82%
USP Res. Criterion	≥ 15	≥ 30	≥ 2
% Improvement	45%	33%	100%

USP Asymmetry

According to the USP asymmetry criterion, both 1-pentadecanol internal standard and cetyl alcohol must express a tailing factor between 0.80 (peak fronting) and 1.80 (peak tailing) in the standard solution. The ideal asymmetry has a value of 1.00 exactly. Table 4 portrays the near-perfect asymmetry obtained by the PerkinElmer GC 2400 System with Elite 225 column, with an average tailing factor of 1.04 for 1-pentadecanol and 1.02 for cetyl alcohol.

Table 4: Tailing factors (TF) for internal standard (1-pentadecanol) and target compound (cetyl alcohol) in standard solution.

	1-Pentadecanol	Cetyl Alcohol
Run 1 TF	1.03	1.01
Run 2 TF	1.01	1.03
Run 3 TF	1.05	1.03
Run 4 TF	1.06	1.02
Run 5 TF	1.04	1.04
Avg. TF ± RSD	1.04 ± 1.70%	1.02 ± 1.25%
USP TF Criterion	0.8 to 1.8	0.8 to 1.8

USP Area Ratio

The USP monograph requires assessment of the analytical repeatability using the area ratio of cetyl alcohol to 1-pentadecanol in the standard solution. A system suitability threshold of no more than 1% RSD in this area ratio is required by the method. The results in Table 5 show that this acceptance criterion has been met.

Table 5: Area ratio (AR) repeatability shows that the PerkinElmer™ system meets the USP monograph requirement.

	Cetyl:1-Pentadecanol
Run 1 AR	1.00
Run 2 AR	1.00
Run 3 AR	1.02
Run 4 AR	1.00
Run 5 AR	1.01
Avg. AR + RSD	1.01 ± 0.94%
USP AR Criterion	≤ 1.0% RSD

USP Purity Assay

The sample cetyl alcohol purity was determined using the following equation:

$$Purity = 100\% * \frac{R_{sample}}{R_{standard}} * \frac{C_{standard}}{C_{sample}} = 100\% * \frac{1.06}{1.01} * \frac{1.00\frac{mg}{ml}}{1.06\frac{mg}{ml}}$$

where R is the cetyl alcohol to 1-pentadecanol area ratio and C is the cetyl alcohol concentration in mg/ml. The monograph permits a purity of 90% to 102% in the sample. In our study, a cetyl alcohol purity of 99% was determined for the commercial sample.

USP Organic Impurity Test

Our sample of natural cetyl alcohol was analyzed according to Organic Purity Test 1 procedures. The sample chromatogram is presented in Figure 1B. The USP method permits aliphatic alcohol impurities to constitute up to 10% of the total peak area, whereas unidentified peaks may constitute up to 1%. Based on the results in Table 6, our commercial product meets these criteria, with a total aliphatic alcohol impurity of 0.76% and an unidentified impurity of 0.41%. All other minor peaks were beneath the threshold of 0.05% composition as delineated in the method and therefore were excluded from this calculation. Figure 1C shows a zoomed-in perspective of impurity peaks near the chromatogram's baseline.

Table 6: Peak area % composition of the commercial sample, excluding solvent and internal standard contributions.

	Myristyl Alcohol	Cetyl Alcohol	Stearyl Alcohol	Unknown Peak
Sample 1 Area	0.72%	98.78%	0.08%	0.41%
Sample 2 Area	0.69%	98.84%	0.05%	0.41%
Sample 3 Area	0.71%	98.82%	0.06%	0.42%
Sample 4 Area	0.75%	98.77%	0.07%	0.41%
Sample 5 Area	0.70%	98.83%	0.06%	0.41%
Avg. ± RSD	0.70% ± 3.24%	98.83% ± 0.03%	0.06% ± 16.51%	0.41% ± 0.78%

Conclusion

The USP-NF Cetyl Alcohol monograph is essential for determining impurities in cetyl alcohol additive. For the USP compliant analysis of residual impurities in cetyl alcohol, the GC 2400 System with FID Detector and Elite 225 column was able to meet or exceed system suitability criteria as outlined in the method, displaying consistent retention times for all compounds and repeatable, precise sample injections.

Resolution of target components has a particularly high-performance, with compound resolution of 133% to 200% of the target value. The performance shows high repeatability giving ultimate confidence for executing this analysis, even in high-sample throughput laboratories.

Data acquisition and analysis were performed with the SimplicityChrom CDS Software, which provides an intuitive, customizable user experience with multi-functionality and accessibility options.

The detachable touchscreen provides versatility and portability which optimizes time and ultimately lab productivity.

References

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