Quantitation of the Residual Solvent Naphtha in a Pharmaceutical Soft Gelatin Capsule Product by Equilibrium Headspace Gas Chromatography

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Abstract

An equilibrium headspace gas chromatographic (EHS–GC) method for the quantitation of the residual solvent naphtha in a pharmaceutical soft gelatin capsule product is described. The drug product, which is an encapsulated oil suspension, is exposed to the solvent naphtha during the manufacturing process. Headspace gas chromatography is the preferred method for the analysis of residual solvents in bulk pharmaceutical and drug products because the sample matrix is not introduced into the gas chromatographic system, resulting in a simpler and cleaner assay method. In EHS–GC sampling, the gas phase above the sample matrix is analyzed after it has reached thermal equilibrium. The EHS–GC method parameters are optimized by monitoring the *n*-octane gas–liquid partition coefficient of naphtha in the sample matrix via the EHS–GC phase ratio variation method of Ettre et al. (*Chromatographia* 35[1,2]: 73–84 [1993]). The method selectivity, linearity, detection and quantitation limits, standard and sample reproducibility, recovery from placebo, and effect of sample matrix are described.

Introduction

Headspace gas chromatography (GC) has been increasingly used for the analysis of organic volatile impurities (OVIs) and residual solvents in bulk pharmaceuticals and drug products since the technique was proposed as an alternate method (USP chapter 467, method IV) by the United States Pharmacopoeia (1–7). Headspace GC is the preferred method for the analysis of residual solvents in bulk pharmaceutical and drug products because the sample matrix is not introduced into the GC system, resulting in a simpler and cleaner assay method. Headspace GC has several advantages over other residual solvent analysis techniques, including the direct analysis of a wide range of samples (liquids, gases, solids, sludges, and pastes) with minimal sample handling or preparation, increased sensitivity over liquid injections (from 10- to 500-fold improvement), increased reproducibility and precision with automated systems, increased column lifetime, and reduced instrument maintenance because nonvolatiles are not

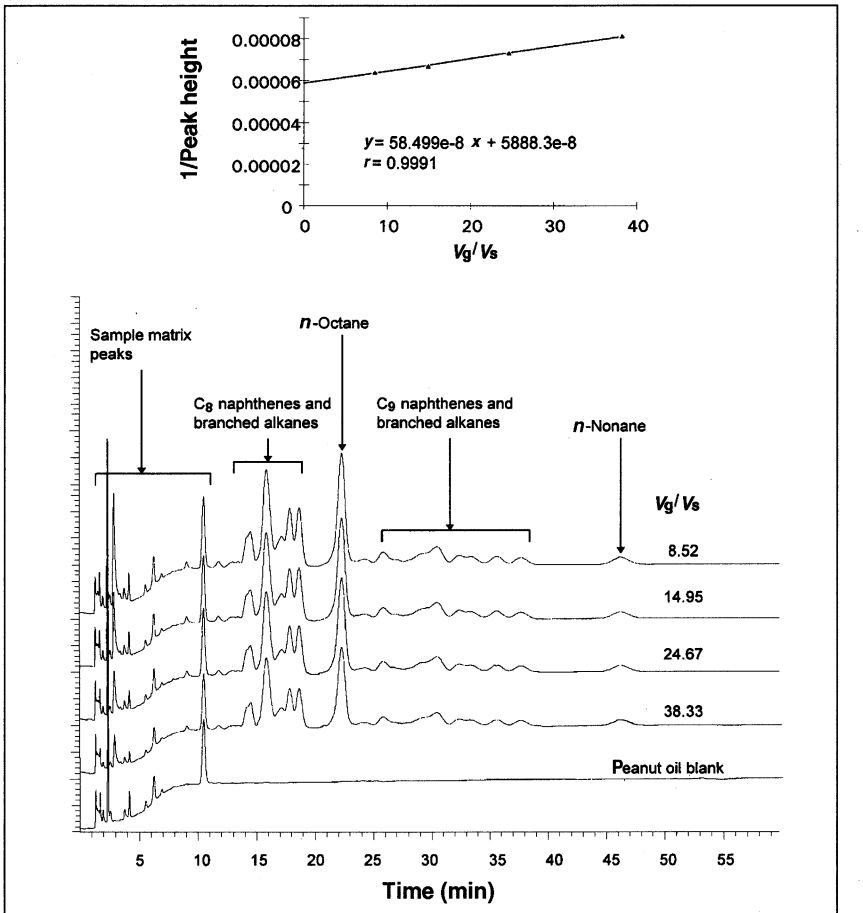

Figure 1. EHS–GC chromatograms of the naphtha standards at four different phase ratios (V_g/V_s) after equilibration for 60 min at a headspace oven temperature of 110°C and the corresponding plot of V_g/V_s versus 1/peak height. A peanut oil blank chromatogram is provided for comparison.

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Table I. Instrumentation and Experimental Parameters Used for the EHS–GC Quantitation Method For Residual Solvent Naphtha

Gas chromatograph:	HP 5890 Series II GC with EPC
Column:	80/120 Carbopack B/3% SP-1500 packed in a 10-ft × 1/8-in. stainless steel column (Supelco)
Carrier gas:	Helium at 20 mL/min (approx. 19 mL/min flow from the headspace transfer line, the remainder from the GC with the EPC on)
Detector:	Flame ionization
Temperature program:	150–200°C at 8°C/min, held for 53.7 min at 200°C
Injector temperature:	200°C
Detector temperature:	250°C
Headspace sampler:	HP 7694 (HP G1290A stand-alone)
Carrier gas pressure:	50.5 psi at 150°C oven temperature
Oven temperature:	100°C
Vial equilibration time:	60 min
GC cycle time:	65 min
Vial shake level:	2 (high)
Loop/transfer line	
Temperature:	110°C
Sample loop:	1 mL
Vial pressurization:	18 psi, 0.13 min
Loop fill time:	0.15 min
Loop equilibration time:	0.15 min
Injection time:	0.20 min
Headspace vials:	10 mL

introduced into the GC (3–12).

Equilibrium headspace GC is based on the thermostatic partitioning of a volatile compound (or compounds) in a closed vial between the sample matrix (liquid or solid) and the surrounding gas phase (i.e., the vial headspace), followed by the transfer of an aliquot of the vial headspace containing the gas phase volatile analyte(s) to the GC for analysis. The term *equilibrium* is added to the phrase *headspace GC* in order to stress the fact that the volatile compound has reached its equilibrium concentration between the gas phase and the liquid phase (i.e., sufficient time has passed at a given headspace oven temperature) (13–15). The equilibration of the volatile analyte between the sample matrix and the headspace is controlled by the analyte's gas–liquid partition coefficient (11–15). Therefore, equilibrium headspace GC (EHS–GC) method development and optimization require the measurement of the volatile analyte's gas–liquid partition coefficient (K) as a function of the EHS–GC conditions. The proper selection of the EHS–GC conditions, primarily temperature and sample volume, can enhance the concentration of the volatile compound(s) in the headspace and provide for the more accurate analysis of trace sample concentrations (8–15).

An EHS–GC method for the quantitation of the residual solvent naphtha in a pharmaceutical soft gelatin capsule product is described. The drug product, which is an encapsulated oil suspension, is exposed to the solvent naphtha during the manufacturing process. Naphtha is produced from crude oil by distillation, typically within the temperature range of 60–320°F. The naphtha used in this study consisted of primarily C_8–C_9 hydrocarbons with a boiling range of 245–290°F and an average molecular weight of 115. The method selectivity, linearity, detection and quantitation limits, standard and sample reproducibility, recovery from placebo, and effect of sample matrix are described.

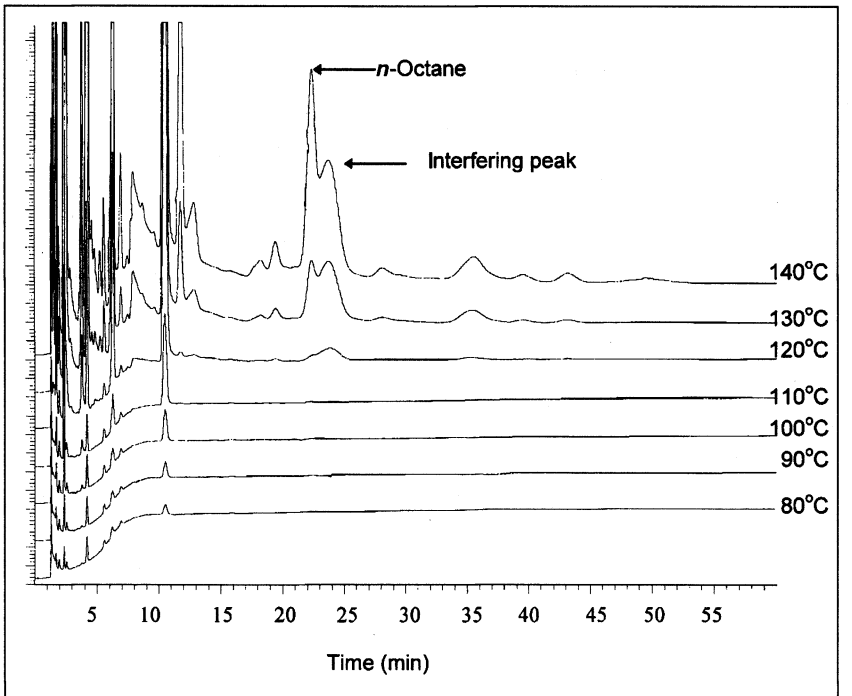

Figure 2. EHS–GC chromatograms of several peanut oil blank solutions after equilibration for 60 min at various headspace oven temperatures.

Experimental

Materials

The reference standards ASTM crude oil qualitative standard (a mixture of approximately 10% each of *n*-propane, *n*-butane, *n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, and *n*-nonane), the qualitative reference naphtha standard (Supelco cat. #4-8265, a mixture of paraffins, isoparaffins, aromatics, naphthenes, and olefins), and the branched alkanes C_6–C_9 kit #221D (PolyScience, Niles, IL) containing 3-methyl-heptane (97%) were all obtained from Supelco (Bellefonte, PA). The reference standard *n*-octane (99+% anhydrous) was obtained from Aldrich Chemical (Milwaukee, WI). Naphtha was obtained from Ashland Chemical (Columbus, OH). NF-grade peanut oil was used without further purification.

GC

The characterization and identification of the hydrocarbon composition of naphtha was performed by capillary GC on an HP 5890 series II GC

with electronic pressure control (EPC) using the nonpolar capillary GC column Petrocol DH50.2 (50 m × 0.20-mm i.d., 0.50-µm phase film) (16) obtained from Supelco. The GC parameters used to analyze both of naphtha and the qualitative reference naphtha standard (Supelco cat. #4-8265) were a column temperature program of 35°C for 30 min increased to 200°C at 2°C/min and held for 15 min, injector and detector temperatures of 200°C, helium carrier gas at 19–21 cm/s, flame-ionization detection (FID), and a 1-µL injection volume using a 200:1 split ratio.

Quantitation of residual naphtha in the pharmaceutical soft gelatin capsule product was performed on a headspace GC system that consisted of an HP 7694 headspace sampler (HP G1290A stand-alone) interfaced with an HP 5890 series II GC with EPC (4–7). An industrial solvent-resolving packed column, 80/120 Carbopack B/3% SP-1500 packed in a 10-ft × $^1/_8$- in. stainless steel column (17–19) obtained from Supelco was used for the residual naphtha assay. The GC parameters used for the residual naphtha assay are given in Table I. The GC temperature program was optimized for resolution of the *n*-octane peak in the naphtha solvent on the 80/120 Carbopack B/3% SP-1500 packed column.

Selection of headspace oven temperature and effect of sample matrix

GC parameters used during the selection of the headspace oven temperature were the same as listed in Table I except the headspace oven temperature ranged from 80 to 140°C, and the loop and transfer line temperatures were maintained at 10°C higher than the headspace oven temperature. For each temperature, four different samples were analyzed, ranging from 0.2 to 1.0 g of the 40-ppm naphtha standard solution. The naphtha standard stock solution (800 ppm) was prepared by transferring 100 µL (accurately weighed, *W*) of naphtha into a 100-mL volumetric flask containing peanut oil. The naphtha working standard solution (40 ppm) was prepared as a 20-time dilution of the naphtha standard stock solution. Because peanut oil is very viscous, it is difficult to accurately transfer and measure by volume. Therefore, the weight of the peanut oil (W_1) and the standard stock solution (W_2) were determined when dilutions were made, and the weights were used to calculate the concentration (C_S in ppm) of the diluted standard solutions (*n*-octane or naphtha) using the following equation:

$$C_S = \frac{1000\ (\mu g)}{1\ (mg)100\ (mL)}\ W_S \frac{W_2\ (mg)}{W_1\ (mg) + W_2\ (mg)} = 10 W_S \frac{W_2}{W_1 + W_2}\ (ppm) \quad \text{Eq 1}$$

For the effect of the capsule shell on the quantitation of residual naphtha, four samples were prepared with approximately 0.2, 0.4, 0.6, and 0.8 g of 40-ppm naphtha standard solution and one, two, three, and four empty soft gelatin capsule shells, respectively. For the effect of the drug on the quantitation of residual naphtha, the drug (in an amount equivalent to six sample capsules) was added to a 40-ppm naphtha standard solution.

Quantitation of residual naphtha in the drug product

The *n*-octane working standard solution (4 ppm) was prepared via a 20-time dilution of the *n*-octane standard stock solution (80 ppm). The *n*-octane standard stock solution (80 ppm) was prepared by transferring 10 µL (accurately weighed, *W*) of *n*-octane into a 100-mL volumetric flask containing peanut oil. The 4-ppm *n*-octane working standard corresponded to 40-ppm naphtha (based on 10% *n*-octane in naphtha). For standard solutions, 1.25 g (1.35 mL) of the standard solution was accurately weighed into the 10-mL headspace vial. For samples, six soft gelatin encapsulated drug product capsules (each containing approximately 0.225 mL of the drug–peanut oil suspension fill) were cut in half with serrated-edged scissors directly into the 10-mL headspace vial (refer to the *Selection of sample volume* section in *Results and Discussion* for the effect of this nonquantitative transfer technique). Samples and standards were analyzed by EHS–GC using the parameters in Table I.

Linearity and recovery standard preparation

From the above-mentioned *n*-octane stock solution (80 ppm), solutions representing an *n*-octane concentration range of 0.9–4.5 ppm (nine solutions) were prepared on two different days and analyzed by the EHS–GC parameters in Table I. This covers the concentration range of the residual naphtha in the drug product samples of approximately 9–45 ppm (based on 10% *n*-octane in naphtha). From the above-mentioned naphtha stock solution (800 ppm), solutions representing a naphtha concentration range of 3.4–34 ppm (10 solutions) were prepared and analyzed using the EHS–GC parameters in Table I.

The recovery standards were prepared by combining the drug product capsule fill suspension from many sample capsules and spiking portions of the resulting capsule fill suspension with the naphtha stock solution. A series of capsule fill suspensions containing naphtha ranging from 9 to 40 ppm (five solutions) were prepared on two different days and analyzed by EHS–GC using the parameters in Table I. The recovery results were corrected for the amount of residual naphtha found in the unspiked capsule fill suspension (5.4 ppm naphtha).

Results and Discussion

Characterization and identification of naphtha

Naphtha is a petroleum distillate that consists of mostly C_8–C_9 hydrocarbons including straight-chain alkanes, branched-chain alkanes, and cyclic alkanes or naphthenes. The characterization of the hydrocarbon composition of naphtha was performed by capillary column GC versus Supelco's qualitative reference naphtha standard composed of a known mixture of paraffins, isoparaffins, aromatics, naphthenes, and olefins that ranged in size from propane (C_3) to *n*-dodecane (C_{12}). From the known hydrocarbons of Supelco's qualitative reference naphtha standard, 73 hydrocarbons, accounting for nearly 77% by peak area, were identified in a typical lot of naphtha (Table II). The largest component in naphtha was *n*-octane (nearly 15% peak area); therefore, *n*-octane was selected to quantitate residual naphtha in the pharmaceutical soft gelatin capsule product.

Naphtha's *n*-octane concentration

The *n*-octane concentrations in four different lots of naphtha (40 ppm in peanut oil) from three different refineries were determined versus an *n*-octane external standard using the headspace

GC conditions listed in Table I, and the results are shown in Table III. The *n*-octane concentration in four lots of tested naphtha ranged from 11.9 to 16.0% (weight percent). Because the *n*-octane concentration is not the same for different lots of naphtha, it is recommended that *n*-octane be used as the external standard. Furthermore, to avoid underestimating the residual naphtha concentration in the drug product, the *n*-octane concentration of 10% in naphtha was selected for all calculations.

Naphtha's gas–liquid partition coefficient versus headspace oven temperature

The GC peak response of the analyte in the gas phase is directly related to the initial analyte concentration in the sample matrix by the following equation (11–15):

$$PR = \frac{fC_S}{K + V_g/V_S} \qquad \text{Eq 2}$$

where PR is the analyte peak response, f is the analyte-specific response factor, C_s is the initial analyte concentration in the sample matrix, K is the partition coefficient of the volatile analyte between the sample matrix and the gas phase, and V_g/V_s is the phase ratio of the volume of the headspace (V_g) and the sample volume (V_s).

The analyte peak response (PR) from the GC headspace injection is directly proportional to the initial sample concentration (C_s) and thus provides the basis of analyte quantitation using EHS–GC. In addition, the analyte peak response is inversely proportional to the sum of the analyte's partition coefficient (K) and the sample phase ratio (V_g/V_s). Thus, optimizing the analyte peak response (and sensitivity) for the EHS–GC assay requires GC headspace conditions that produce a low analyte partition coefficient value and a reproducible sample phase ratio (13–15).

Equilibrium headspace GC offers a convenient method for the determination of the analyte's gas–liquid partition coefficient (K) by using the EHS–GC phase ratio variation method (15). Taking the reciprocal of Equation 2 and rearranging yields (15):

$$\frac{1}{PR} = \frac{K}{fC_S} + \frac{1}{fC_S}\left(\frac{V_g}{V_S}\right) \qquad \text{Eq 3}$$

which is in the form of the linear equation $y = mx + b$. Therefore, the partition coefficient (K) can be experimentally obtained by measuring the peak response (PR) while varying the sample phase ratio (V_g/V_s) with a constant analyte concentration (C_s). A plot of $1/PR$ versus V_g/V_s is a straight line with a slope equal to $1/fC_s$ and a y-intercept equal to K/fC_s. Thus, the partition coefficient (K) can be obtained by the following equation (15):

$$K = \frac{\frac{K}{fC_S}}{\frac{1}{fC_S}} = \frac{y\text{-intercept}}{\text{slope}} \qquad \text{Eq 4}$$

Table II. Lot A Naphtha Capillary GC Analysis Summary

Component	Area (%)	Component	Area (%)
n-Pentane	0.03	1,1-Ethylmethylcyclopentane & 2,2,4-trimethylhexane	0.10
2- & 3-Methylhexane	0.01	*trans*-1,2-Dimethylcyclohexane	2.86
n-Heptane	0.17	*trans*-1,3-Dimethylcyclohexane	0.03
Methylcyclohexane & *cis*-1,2-dimethylcyclopentane	0.28	*cis*-1,4-Dimethylcyclohexane	3.56
1,1,3-Trimethylcyclopentane & 2,2-dimethylhexane	0.10	*n*-Octane	14.86
Ethylcyclopentane	0.04	Isopropylcyclopentane & 2,4,4-trimethylhexane	0.15
2,5-Dimethylhexane & 2,2,3-trimethylpentane	0.26	*cis*-1,2-Ethylmethylcyclopentane	0.22
2,4-Dimethylhexane	0.45	2,3,5-Trimethylhexane	0.15
1-*trans*-2-*cis*-4-Trimethylcyclopentane	0.17	2,2-Dimethylheptane	0.37
3,3-Dimethylhexane	0.24	cis-1,2-Dimethylcyclohexane	1.50
1-*trans*-2-*cis*-3-Trimethylcyclopentane	0.22	2,4-Dimethylheptane	1.19
2,3,4-Trimethylpentane	0.09	4,4-Dimethylheptane	0.11
Toluene & 2,3,3-trimethylpentane	0.11	Ethylcyclohexane & *n*-Propylcyclopentane	7.73
2,3-Dimethylhexane	1.05	2-Methyl-4-ethylhexane & 2,6-dimethylheptane	2.45
2-Methyl-3-ethylpentane	0.28	1,1,3-Trimethylcyclohexane	2.08
2-Methylheptane	5.57	2,5-; 3,3-; & 3,5-Dimethylheptane	2.86
4-Methylheptane	2.56	Ethylbenzene	0.21
3-Methyl-3-ethylpentane	0.20	*m*-Xylene	0.69
3,4-Dimethylhexane	0.10	*p*-Xylene	0.04
cis-1,3-Dimethylcyclohexane	10.73	2,3-Dimethylheptane	1.99
3-Methylheptane & 1-*cis*-2-*trans*-3-trimethylcyclopentane	1.15	3,4-Dimethylheptane (stereoisomers)	1.07
3-Ethylhexane & *trans*-1,4-dimethylcyclohexane	1.51	4-Methyloctane	2.62
1,1-Dimethylcyclohexane	0.25	*o*-Xylene	2.75
trans-1,3-Ethylmethylcyclopentane & 2,2,5-trimethylhexane	0.51	*n*-Nonane	4.36
cis-1,3-Ethylmethylcyclopentane	0.42	C_9 naphthenes (eight peaks)	5.60
trans-1,2-Ethylmethylcyclopentane	0.70	Unknowns (74 peaks)	23.25

Optimization of the headspace oven temperature was accomplished by measuring the *n*-octane gas–liquid partition coefficient (K) of naphtha (by the phase ratio method [15] and Equations 3 and 4) as a function of the oven temperature (from 80 to 140°C). Naphtha (40 ppm) standard was analyzed at four different phase ratios (V_g/V_s) and the peak height of the *n*-octane peak was used to calculate the *n*-octane gas–liquid partition coefficient (K) of naphtha in peanut oil. Figure 1 shows the EHS–GC chromatograms of the naphtha standards at four different phase ratios analyzed after a 60-min equilibration at a headspace oven temperature of 110°C along with the corresponding plot of V_g/V_s versus 1/peak height. A peanut oil blank reference chromatogram exposed to the same EHS–GC conditions is also included in Figure 1 for comparison. The *n*-octane gas–liquid partition coefficient versus headspace oven temperature results shown in Table IV indicate that the gas–liquid partition coefficient decreased with increasing temperature, which increased the peak response and method sensitivity. For example, the peak response increased 180% when the headspace oven temperature was increased from 80 to 100°C.

The peak response depends on the partition coefficient; a higher peak response will be

observed for a smaller partition coefficient value (Equation 2). The partition coefficient depends mainly on the headspace oven temperature (T), according to the following equation:

$$\log K = a + b\frac{1}{T} \qquad \text{Eq 5}$$

where a and b are system-dependent constants from which the slope (b) is a direct function of the heat of the analyte solution (11,15). If narrow temperature ranges are used, the linear dependence of $\log K$ versus $1/T$ is usually maintained (11,15). Equations 2 and 5 suggest that the sensitivity of EHS–GC can be optimized by increasing the headspace oven temperature. However, this temperature-induced sensitivity enhancement is limited by the effect of temperature on the sample matrix.

Sensitivity enhancement via increasing the headspace oven temperature is limited by the interfering peaks from the sample matrix that occur at elevated temperatures (see next section). Sample matrix interference prevented the experimental determination of the *n*-octane gas–liquid partition coefficient of naphtha above 110°C (Figure 2). However, by using Equation 5 and the plot of $\log K$ versus $1/T$ for the experimentally determined values of K in the temperature range from 80 to 110°C (Figure 3), the values of K at temperatures higher than 110°C were calculated (Table IV). The validity of the relationship represented in Equation 5 is thus demonstrated by the linear regression analysis (correlation coefficient of 0.99861) from the $\log K$ versus $1/T$ plot (Figure 3) and also represents a check of the correctness of the experimentally determined *n*-octane gas–liquid partition coefficient values for naphtha using the EHS–GC phase ratio variation method (15).

Table III. *n*-Octane Concentration in Different Lots of Naphtha from Three Different Refineries as Determined by Packed-Column GC* Versus an *n*-Octane External Standard, Both in Peanut Oil

Naphtha lot number (refinery)	*n*-Octane (%, wt)
Lot A (refinery 1)	16.0
Lot B (refinery 1)	11.9
Lot C (refinery 2)	14.0
Lot D (refinery 3)	11.9

* Parameters listed in Table I.

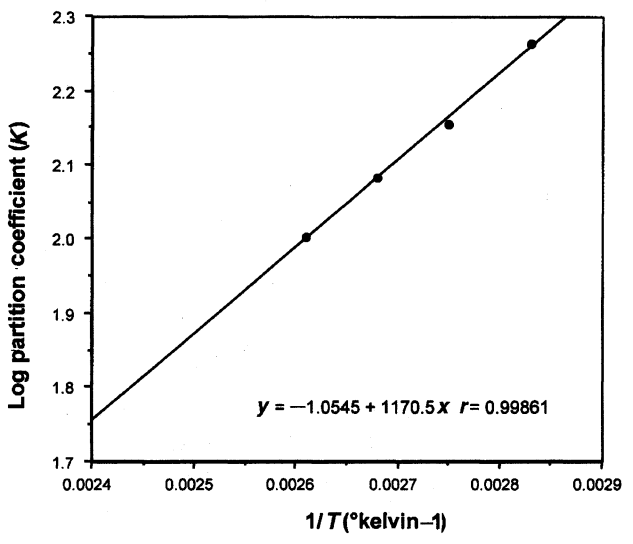

Figure 3. Logarithm of *n*-octane gas–liquid partition coefficient (K) versus the reciprocal of absolute temperature ($1/T$). Data obtained from Table IV.

Effect of sample matrix

Peanut oil is known to chemically degrade at elevated temperatures (50–200°C was examined) into at least 99 detectable volatile compounds including hydrocarbons, aldehydes, fatty acids, alcohols, ketones, furans, esters, and lactones (20). Several of these volatile hydrocarbon compounds are also found in naphtha including *n*-octane, which is used to quantitate residual naphtha in the soft gelatin encapsulated drug product. Although the contribution to the *n*-octane peak response from the peanut oil was relatively small below 110°C, it became significant above 120°C due to peanut oil degradation, as shown in Table IV and Figure 2. In addition, a broad peak adjacent to the *n*-octane peak was detectable at 120°C and grew with increasing oven temperature (Figure 2). This broad peak adjacent to the *n*-octane peak made naphtha quantitation above the 110°C headspace oven

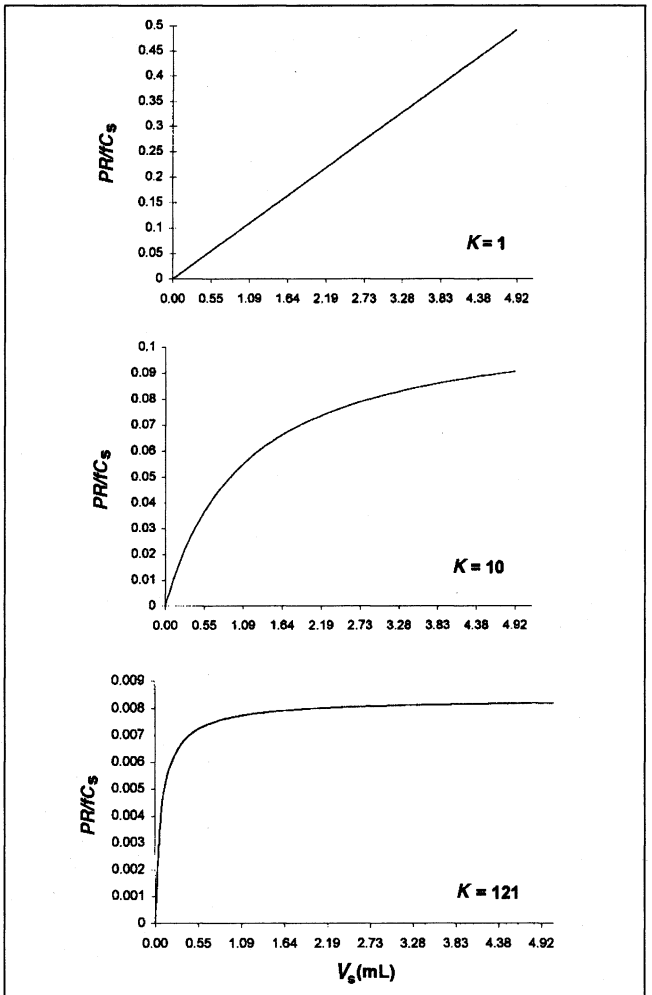

Figure 4. Dependence of the peak response (PR/fC_s) on the sample volume (V_s) at various K values.

temperature unreliable. Based on the data in Table IV, an oven temperature of 100°C was chosen, which provides an *n*-octane partition coefficient of 121 along with a relatively small contribution (0.8%) to the *n*-octane peak due to peanut oil degradation.

The residual naphtha method EHS–GC sample is a composite of six drug product capsules in one EHS–GC vial, thus the effect of the soft gelatin capsule shell on the naphtha partition coefficient was evaluated. The *n*-octane partition coefficient of the naphtha standard (40 ppm) solution with six empty soft gelatin capsule shells at 100°C was 127.2 ± 12.5 (Table IV), which is experimentally equivalent to the *n*-octane partition coefficient of naphtha standard (40 ppm) solution without capsule shells at 100°C (121.2 ± 8.2, Table IV). Therefore, the naphtha partition coefficient is not dependent on the presence of the soft gelatin capsule shells under the conditions employed. In addition, the effect of the drug's presence on the *n*-octane peak response was examined. The addition of the drug to peanut oil resulted in a 1.4% peak area contribution to the *n*-octane peak (0.8% was due to peanut oil contribution) under the EHS–GC conditions listed in Table I.

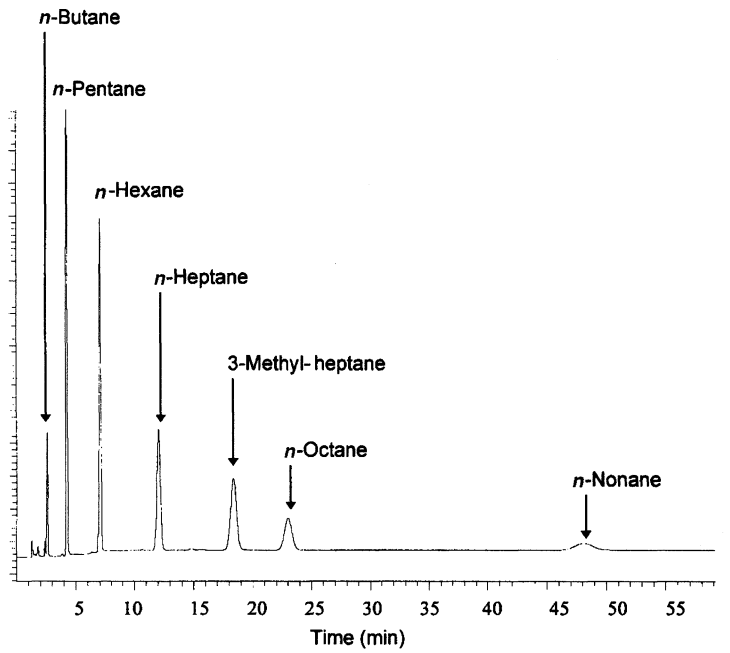

Figure 5. Typical EHS–GC chromatogram of selectivity standard solution containing *n*-butane, *n*-pentane, *n*-hexane, *n*-heptane, 3-methyl-heptane, *n*-octane, and *n*-nonane using the EHS–GC parameters listed in Table I.

Table IV. Effect of Headspace Oven Temperature (80–140°C) on the *n*-Octane Gas–Liquid Partition Coefficient of Naphtha (40 ppm) in Peanut Oil and the Contribution of Peanut Oil to the *n*-Octane Peak Response

Temperature (°C)	Slope $\left(\frac{1}{fC_S}\right) \times 10^8$	*y*-Intercept $\left(\frac{K}{fC_S}\right) \times 10^8$	Correlation coefficient (*r*)	Partition coefficient (*K*)	Contribution from peanut oil to *n*-octane peak response (%)
80	77.139	14128.8	0.9998	183.2 ± 2.5	0.8
90	71.709	10260.9	0.9971	143.1 ± 7.8	0.8
100	63.044	7632.9	0.9978	121.1 ± 8.2	0.8
110	58.499	5888.3	0.9991	100.7 ± 4.4	0.6
120				82.8*	undetermined
130				70.5*	19
140				60.0*	43
100 (capsule shells present)	30.161	3836.2	0.9975	127.2 ± 12.5	0.8

* The partition coefficient was calculated from Equation 5 and the plot of log K versus 1/*T* (°Kelvin – 1) using the experimental data from 80 to 110°C. The resulting linear equation was log $K = 1170.5\ (1/T) - 1.055$, and the correlation coefficient was 0.99861 (see Figure 3).

Sample volume selection

In addition to the gas–liquid partition coefficient, the peak response also depends on the sample volume (V_s) according to Equation 2 (13). Because sample preparation requires the cutting of six drug product sample capsules directly into a headspace vial, loss of some product capsule fill on the cutting implement is inevitable. In other words, the sample volume will change slightly from sample preparation to sample preparation. This raises the issue that poor sample-to-sample reproducibility may result from a sample-volume-dependent peak response. However, a closer examination of the relationship of the peak response (*PR*), partition coefficient (*K*), and sample volume (V_s) indicates that the sample volume can be selected to increase the sensitivity and to ensure a good sample-to-sample reproducibility (13). For example, for a 10-mL headspace vial, Equation 2 can be rewritten as (13):

$$\frac{PR}{fC_S} = \frac{1}{K - 1 + 10/V_S} \qquad \text{Eq 6}$$

Figure 4 shows theoretical plots of the peak response (PR/fC_s) for a given component (i.e., fixed *f* and fixed C_s) versus sample volume (V_s) for $K = 1$, $K = 10$, and $K = 121$ according to Equation 6. Clearly the peak response was more sensitive to the change of sample volume (V_s) when the partition coefficient (*K*) value was small. For a *K* value of 121 (the partition coefficient of naphtha at 100°C), the peak response increased rapidly with increasing V_s when V_s was smaller than 0.5 mL. However, the peak response was almost independent of V_s when V_s was greater than 1.0 mL. This indicates that a smaller sample volume (less than 0.5 mL) will result in poor sample-to-sample reproducibility, whereas a larger sample volume (greater than 1.0 mL) can tolerate small changes in the sample volume. For example, the relative standard deviation (RSD) of the peak response due to a deviation of 0.1 mL from a sample volume of 1.35 mL was less than 0.5%, according to Equation 6. Thus, a sample volume of 1.35 mL was chosen, which corresponds to 1.25 g of the

standard solution (the density of peanut oil is approximately 0.92 g/mL) and is equivalent to combining six drug product sample capsules (each capsule contained approximately 0.225 mL of capsule fill) in a 10-mL headspace vial.

Selectivity

The chromatographic system separated *n*-octane from the other alkanes, including *n*-butane, *n*-pentane, *n*-hexane, *n*-heptane, 3-methyl-heptane, and *n*-nonane (Figure 5). The alkanes found in naphtha are *n*-heptane, 3-methyl-heptane, *n*-octane, and *n*-nonane. In addition, the capsule shell, the drug substance, and the peanut oil did not generate interfering peaks around the *n*-octane peak under the conditions listed in Table I. Approximate retention times (t_r), relative retention times (RR_t) of the above-mentioned alkanes, the resolution factor between the *n*-octane peak and the 3-methyl-heptane peak, and the tailing factor of the *n*-octane peak on two different GC column lots are shown in Table V. The retention time differences of the hydrocarbons between column 1 and column 2 was due to slightly different conditioning of these two columns. However, the relative retention times, resolution factor, and tailing factor remained the same (Table V). Representative chromatograms of an *n*-octane standard (4 ppm) solution and a drug product sample (containing six drug product capsules) are shown in Figures 6 and 7, respectively.

Linearity

The linearity of the *n*-octane chromatographic response (peak area and peak height) versus the concentration of *n*-octane and naphtha in peanut oil was evaluated. Solutions representing an *n*-octane concentration range of 0.9–4.5 ppm (nine solutions, corresponding to 9–45 ppm naphtha based on 10% *n*-octane in naphtha) and a naphtha concentration range of 3.4–34 ppm (10 solutions) were analyzed by the EHS–GC conditions listed in Table I. The linearity of *n*-octane response (peak area and peak height) for both the *n*-octane and naphtha standard solutions were evaluated by linear regression analysis, and the results are summarized in Table VI.

Table V. Approximate and Relative Retention Times of C_4–C_9 Hydrocarbons, Resolution Factor Between *n*-Octane and 3-Methyl-heptane Peaks, and Tailing Factors of the *n*-Octane Peak on Two Different GC Packed Columns*

	Column 1		Column 2	
	Retention time (t_r) (min)	Relative retention time (RR_t) (min)	Retention time (t_r) (min)	Relative retention time (RR_t) (min)
n-Butane	2.40	0.10	1.90	0.13
n-Pentane	4.24	0.18	3.11	0.21
n-Hexane	8.00	0.31	5.30	0.35
n-Heptane	12.04	0.64	8.59	0.57
3-Methyl-heptane	18.37	0.80	12.41	0.83
n-Octane	23.01	1.00	15.02	1.00
n-Nonane	48.09	2.08	29.13	1.94

	Column 1	Column 2
Resolution factor between *n*-octane and 3-methyl-heptane peaks	4.26	3.95
Tailing factor of the *n*-octane peak	1.04	1.04

* GC parameters are listed in Table I.

Detection and quantitation limits

When *n*-octane was used as the external standard, *n*-octane was detectable to a level of at least 0.05 ppm (corresponding to 0.5 ppm naphtha). Based on the reproducibility of the peak responses of five consecutive injections from different vials, each containing the same *n*-octane standard, the quantitation limit of *n*-octane peak was 0.09 ppm (corresponding to 0.9 ppm naphtha) with peak response RSDs of 4.5 and 4.2% for area and height, respectively. When naphtha was used as the external standard, the *n*-octane peak was detectable to a level of at least 0.34 ppm naphtha. Based on the reproducibility of the peak responses of five consecutive injections from different vials, each containing the same naphtha standard, the quantitation limit for the *n*-octane peak was 1.44

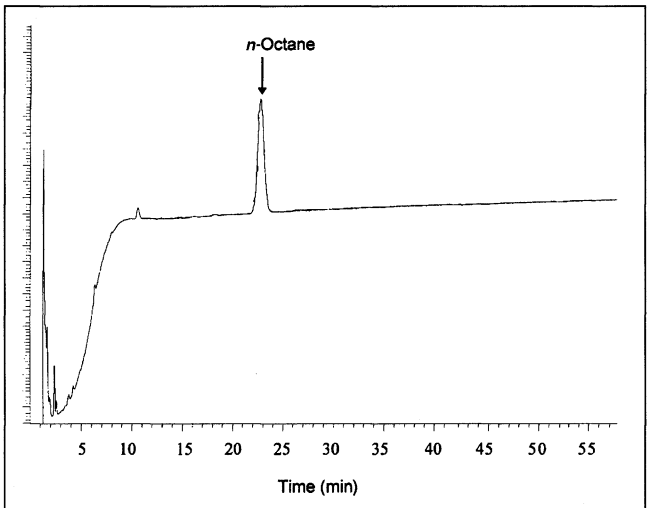

Figure 6. Typical EHS–GC chromatogram of an *n*-octane standard (4 ppm) solution using the EHS–GC parameters listed in Table I.

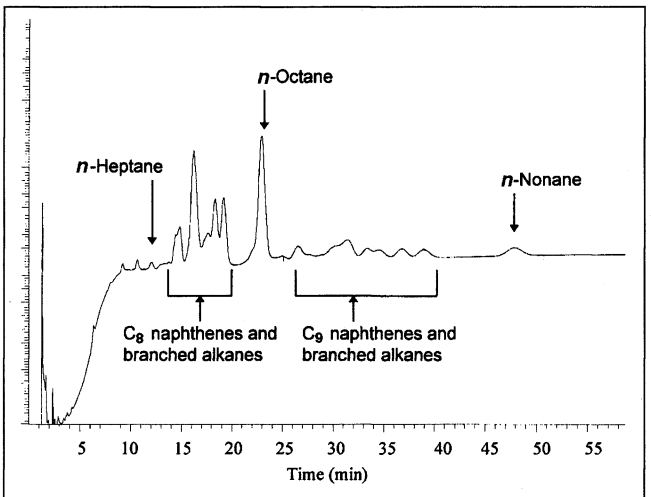

Figure 7. Typical EHS–GC chromatogram of a pharmaceutical soft gelatin capsule product sample (composite of six drug product capsules) using the EHS–GC parameters listed in Table I.

ppm naphtha with peak response RSDs of 5.3 and 3.1% for area and height, respectively.

Recovery

The accuracy of the assay was investigated by analyzing a series of drug product capsule fill suspensions with a naphtha concentration ranging from 9 to 40 ppm (five suspensions) using the EHS–GC conditions listed in Table I. The linear regression analysis of the observed versus theoretical naphtha concentration data is summarized in Table VII. Linearity was observed across the concentration range tested as indicated by linear correlation coefficients greater than 0.999 and insignificant *y*-intercepts. The naphtha recoveries, determined from the peak area data, were 106.4% (7.3% RSD) and 110.6% (2.7% RSD) for each of the two days, whereas the recoveries, determined from the peak height data, were 109.3% (5.6% RSD) and 112.5% (0.9% RSD) for each of the two days.

Table VI. Linearity Data for *n*-Octane and Naphtha Standards*

Component in peanut oil	Peak type	Slope	*y*-Intercept	Correlation coefficient	Average response factor (RSD [%])
n-Octane	area (day 1)	81632.1	1631.6	0.99991	82607 (1.3)
n-Octane	area (day 2)	73239.6	7568.7	0.99977	76798 (2.5)
n-Octane	height (day 1)	1814.5	38.1	0.99993	1834 (1.2)
n-Octane	height (day 2)	2680.6	172.7	0.99995	2764 (1.8)
Naphtha	area (day 1)	14509.0	–8037	0.99965	13897 (3.3)
Naphtha	height (day 1)	284.4	–30.4	0.99917	282 (2.2)

* GC parameters are listed in Table I.

Table VII. Linearity Data for the Analysis of Naphtha Recovery from the Drug Product Capsule Fill Placebo*

Component in capsule fill placebo	Peak type	Slope	*y*-Intercept	Correlation coefficient	Recovery (RSD [%])
Naphtha	area (day 1)	1.224	–2.665	0.99909	106.4 (7.3)
Naphtha	area (day 2)	1.178	–1.148	0.99954	110.6 (2.7)
Naphtha	height (day 1)	1.212	–1.985	0.99915	109.3 (5.6)
Naphtha	height (day 2)	1.133	–0.128	0.99992	112.5 (0.9)

* GC parameters are listed in Table I.

Table VIII. Residual Naphtha Assay Method Reproducibility Using the EHS-GC Parameters Listed in Table I

Drug product lot	Residual naphtha (ppm)*	RSD (%)	Number of six-sample composite sets
Lot A	31.1	10.1	4
Lot B	19.6	26.5	4
Lot C	34.8	8.7	3
Lot D	14.1	23.8	4
Lot E	11.6		2
Lot F	10.3		2
Lot G (day 1)	11.2	15.5	3
Lot G (day 2)	11.3	18.3	6

* Quantitated versus an external n-octane standard and based on a 10% *n*-octane content in naphtha.

Reproducibility

In EHS–GC, the sample (or standard) placed in a vial can only be analyzed once because the removal of an aliquot of the headspace changes the analyte concentration, and a second aliquot would give lower results. Therefore, replicate standard analysis means that aliquots from the same standard solution were placed in separate vials for analysis. Standard reproducibility was examined from the peak responses of six consecutive injections from different vials, each containing 1.25 g of standard (*n*-octane at 4 ppm and naphtha at 30 ppm). Replicate analysis (six replicates) of the *n*-octane standard resulted in peak response RSDs of 1.7 and 1.5% for area and height, respectively. Replicate analysis (six replicates) of the naphtha standard resulted in peak response RSDs of 2.2 and 1.1% for area and height, respectively.

Replicate sample analysis means that sets of six drug product capsules from the same sample lot were placed in separate vials for analysis. The reproducibility of the analysis of residual naphtha in the same lot of the pharmaceutical soft gelatin capsule product was examined by analyzing multiple sample sets of the drug product lot G on two separate days (Table VIII). The average residual naphtha for lot G on two different days differed by less than 1% (day 1, 11.2 ppm; day 2, 11.3 ppm). However, the sample precision from the different sample sets on the same day had a much larger variation. Analysis of replicate drug product sample sets (lot G) resulted in RSDs of 15.5 and 18.3% for days 1 and 2, respectively. Because good reproducibility for both the *n*-octane and naphtha standards was observed, and the peak responses were independent of slight variations in sample volume, the large sample RSD represents the actual variation of the residual naphtha content in the different drug product sample sets. Similar variations (RSDs ranged from 8.7 to 23.8%) were found in all drug product lots (lots A–G) tested (Table VIII).

Conclusion

An equilibrium headspace GC (EHS–GC) method has been developed and validated for the quantitation of the residual solvent naphtha in a pharmaceutical soft gelatin capsule product. The EHS–GC method parameters were optimized by monitoring the *n*-octane gas–liquid partition coefficient (K) of naphtha in the sample matrix via the EHS–GC phase ratio variation method (15). The EHS–GC residual naphtha method was demonstrated to be selective, sensitive, precise, and linear with a detection limit of 0.5 ppm naphtha and a quantitation limit of 0.9 ppm naphtha.

References

1. K.J. Dennis, P.A. Joseph, and J. Dokladalova. Proposed automated headspace method for organic volatile impurities (467) and other residual solvents. *Pharm. Forum* **18(1):** 2964–72 (1992).
2. *Organic Volatile Impurities/Chemical Tests, Method IV.* USP 23. USP 467: 1746–47 (1995).
3. Z. Penton. Determination of residual solvent in pharmaceutical preparations by static headspace GC. *J. High Res. Chromatogr.* **15:** 329–31 (1992).
4. R.L. Frior, P.L. Wylie, L. Cobelli, and M. Bergna. Performing USP method 467 using the HP 7694 headspace sampler. Hewlett-Packard, Application Note 228-237: Publication No. 5091-7757E, May, 1993.
5. S. Brillante, R. Firor, P. Wylie, I.L. Chang, and D. DiUbaldo. USP headspace analysis of organic volatile impurities in bulk pharmaceutical chemicals. Hewlett-Packard, Application Note 228-309: Publication No. 5963-7109E, July, 1995.
6. D. Buckmaster, R. Firor, and P. Wylie. Gas analysis by headspace-gas chromatography: Quantitative analysis of fluorocarbon propellant ratios in aerosol. Hewlett-Packard Company, Application Note 228-248: Publication 5091-8947E, September, 1993.
7. A.E. Gudat and H.P. Sievert. Analysis of volatile compounds in excipient material. Hewlett-Packard Company, Application Note 228-208: Publication No. 5091-5610E, October, 1992.
8. P.L. Wylie. Trace analysis of volatile compounds in water using the HP 19395A headspace sampler. Hewlett-Packard, Application Note 228-240: Publication No. 5953-1820, February, 1985.
9. Z. Penton. Optimization of conditions in static headspace GC. *J. High Res. Chromatogr.* **15:** 834–36 (1992).
10. V. Naughton and G. O'Neil. Enhancing the sensitivity of residual solvents in pharmaceuticals by static headspace analysis. Static Headspace Application Note, Tekmar Company.
11. B.V. Ioffe and A.G. Vitenberg. Head-Space Analysis and Related Methods in Gas Chromatography, John Wiley and Sons, 1984, pp. 1–66.
12. S. Vieths. Headspace gas chromatography of highly volatile compounds. *Capillary Gas Chromatography in Food Control and Research.* R. Wittkowski and R. Matissek, Eds. Technomic Publishing, 1993, pp. 277–324.
13. L.S. Ettre and B. Kolb. Headspace gas chromatography: The influence of sample volume on analytical results. *Chromatographia* **32(1–2):** 5–12 (1991).
14. B. Kolb, C. Welter, and C. Bichler. Determination of partition coefficients by automatic equilibrium headspace gas chromatography by vapor phase calibration. *Chromatographia* **34(5–8):** 235–40 (1992).
15. L.S. Ettre, C. Welter, and B. Kolb. Determination of partition coefficients by automatic equilibrium headspace gas chromatography utilizing the phase ratio variation method. *Chromatographia* **35(1–2):** 73–84 (1993).
16. High resolution detailed hydrocarbon analysis by capillary column gas chromatography. Supelco GC Bulletin 868A: Supelco, 1994.
17. Separating industrial solvents using packed GC columns. Supelco GC Bulletin 803, Supelco, 1983.
18. Analyzing trace amounts of solvents in water. Supelco GC Bulletin 816A, Supelco, 1984.
19. Resolving industrial solvent mixtures on packed and capillary GC columns. Supelco GC Bulletin 824A, Supelco, 1987.
20. T.Y. Chung, J.P. Eiserich, and T. Shibamoto. Volatile compounds identified in headspace samples of peanut oil heated under temperatures ranging from 50 to 200°C. *J. Agric. Food Chem.* **41:** 1467–70 (1993).

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