Characterization of Castor Oil by HPLC and Charged Aerosol Detection

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ABSTRACT

Castor oil is a natural oil which, in its native state, has many uses ranging from personal care products (laxative, cosmetics, topicals), to chemical manufacturing (raw materials), and to industrial materials (lubricants, hydraulic fluids, dielectric fluids, textiles, paints, coatings). The composition of castor oil is unique in that it contains a triglyceride formed from the omega-9 unsaturated fatty acid, 12-hydroxy-9-*cis*-octadecenoic acid (ricinoleic acid, R). Ninety percent of the total triglycerides are triricinoleate (RRR).

The castor bean is the natural source of castor oil. Unfortunately, it also contains ricin, a highly toxic protein. As a result, the castor plant is being genetically manipulated to eliminate the production of ricin and increase the oil yield. To provide a measurement of oil quantity and quality for this research, a high-performance liquid chromatography (HPLC)-charged aerosol detection method was developed that can be used to accurately determine the amount of triglycerides that are present in a castor oil sample, and to fully characterize individual lipid components. Presented here is a simple, reliable, and direct method for the characterization of castor oil from different commercial sources. Results are obtained with only sample dilution and analysis.

Castor seeds.

INTRODUCTION

Commercially, castor oil is produced only from castor seed. Typically 50% of the seed weight is oil,[2] of which 90% of the fatty acid content is ricinoleate[3] and 70% of the oil is composed of RRR (Figure 1). This oil, with its unique properties, has a diversity of uses, including as a specialty lubricant in model aircraft engines. Currently, researchers are evaluating ways to improve castor oil's lubricity through chemical modifications.[4] Furthermore, extensive research is being performed on the castor seed to increase the yield or content of ricinoleate or to reduce the amount of the poisonous protein, ricin, that is present in the plant and seed. A recent research paper described identification of the tetraricinoleate, RRRR, which is the RRR ester of triricinoleate and is normally present as approximately 0.5% in the oil.[5] Because this molecule contains 33% more ricinoleate than RRR, having the seed contain more RRRR would significantly increase the amount of this fatty acid in the harvested oil. To assist in the biochemistry for the formation of this product, the regioisomer of the ester (found on the center hydroxyacid) was identified in 2008.[6]

With research and bioengineering focused on the castor plant to achieve these goals, a sensitive HPLC method would enable the determination of highly subtle changes that may likely occur in the resulting oil. Based on an HPLC-evaporative light scattering detection (ELSD) method from the USDA, the performance of charged aerosol detection for the analysis of castor oils is characterized here.
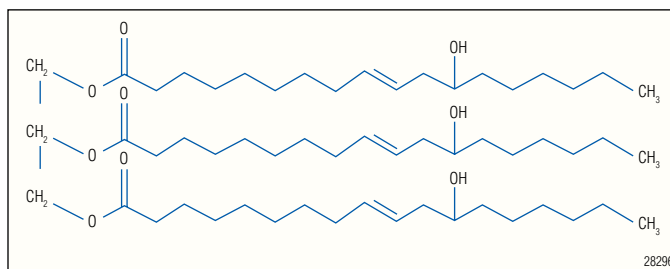

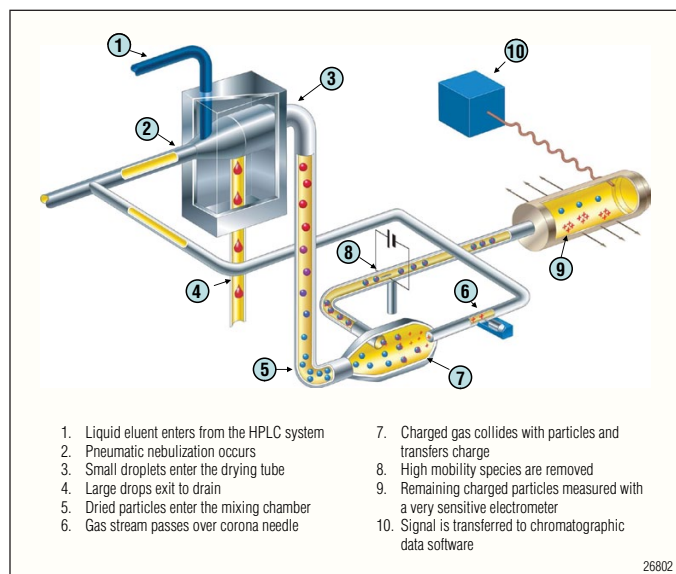Figure 1. Structure of triricinoleate.

Figure 2. Schematic of CAD flow paths.

1. Liquid eluent enters from the HPLC system
2. Pneumatic nebulization occurs
3. Small droplets enter the drying tube
4. Large drops exit to drain
5. Dried particles enter the mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and transfers charge
8. High mobility species are removed
9. Remaining charged particles measured with a very sensitive electrometer
10. Signal is transferred to chromatographic data software

The Charged Aerosol Detector (CAD®) is a sensitive mass-based detector especially well suited for the determination of this class of analytes: nonvolatile and without a chromophore. As shown in Figure 2, the detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles that become charged in the mixing chamber. The charge is then measured by a highly sensitive electrometer, providing reproducible ng-level sensitivity. This technology has greater sensitivity and precision than evaporative light scattering (ELS) and refractive index (RI), and it is simpler and less expensive to operate than a mass spectrometer (MS).

METHODS

HPLC Method Conditions

HPLC System:	Dionex 3000 RSLC
Column:	Dionex RSLC 120 C8, 150 × 2.1 mm, 2.2 µm
Column Temp.:	50 °C
Mobile Phase A:	Methanol/water (900:100)
Mobile Phase B:	Isopropanol
Flow Rate:	0.7 mL/min

Gradient:

Time (min)	%B
0	0
3	15
20	20
25	90
28	100
28	0
33	0
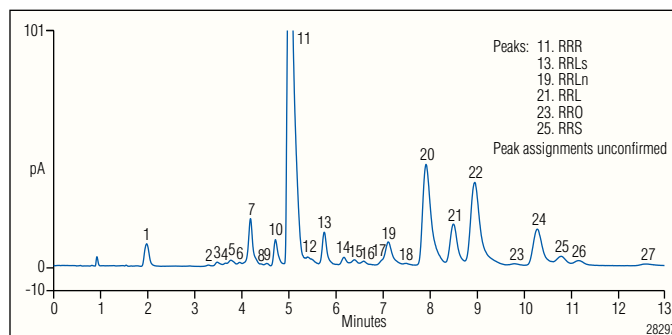
Figure 3. Chromatogram of castor oil A, 14,000 ng on column.

Peaks: 11. RRR
13. RRLs
19. RRLn
21. RRL
23. RRO
25. RRS
Peak assignments unconfirmed

Inj. Volume:	5 µL at 20 °C
Detector:	Charged aerosol for UHPLC
Nebulizer Heater:	20 °C
Filter:	High
Sample Solvent:	Alcohol, denatured

Sample Preparation

Stock solutions of castor oil were prepared at 1.0 mg/mL concentration in alcohol and mixed by vortex mixer.

RESULTS AND DISCUSSION

Castor oil samples were diluted in alcohol prior to analysis. No external standards were used for this analysis in an attempt to use the charged aerosol detector's unique homogeneity of relative response factors across analytes. This can sometimes be complicated with all nebulizer-based detectors, as analyte response is dependant on nebulizer efficiency, which changes with the amount of organic solvent present in the column eluent. However, because the gradient used in this method is largely organic with little change in water content, response factors should be similar to those seen with analyses where nebulizer efficiency is maintained (e.g., using the inverse gradient technique).[7]

One of three commercial castor oil products was used for calibration studies and to evaluate the relative RRR content against known literature values. In Figure 3, a chromatogram of 500 ng on column (o.c.) of castor oil is shown. Twenty-eight different analytes were separated.

To calibrate response, both peak areas and amounts on column are needed. Without a standard analyte, the only amount known was the amount of total castor oil and the peak areas of the many analytes. RRR, being the most abundant analyte in the sample, was used for the initial calibration. Thus, the first calibration curve used the total amount of castor oil analyzed, plotted against the RRR peak area. Calibration data were fit using an inverse second-order polynomial. The correlation coefficient, r^2, was 0.9999, and precision was found to be high, with RSD values of 0.05–0.79% for RRR across the entire range of amounts analyzed.
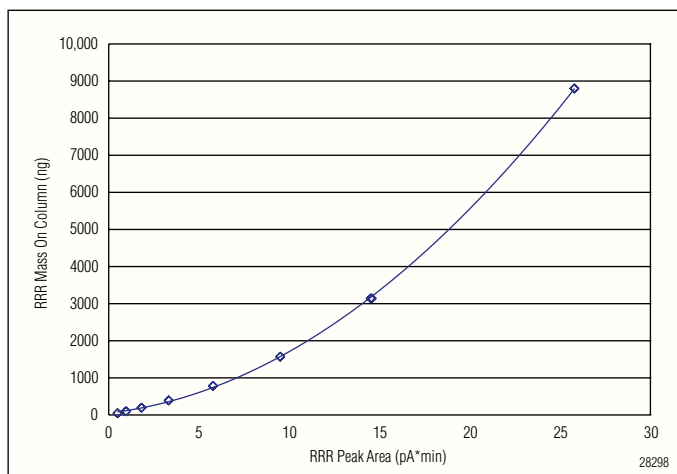
Figure 4. Calibration curve for estimated RRR mass o.c. vs RRR peak area, from 49 to 8800 ng o.c. (n = 3)

Using this calibration curve, mass values were then found for each analyte peak. The sum of all analyte masses was greater than was originally analyzed, due to the assignment of RRR as the entire mass of castor oil (14,000 ng o.c.). A scaling factor had to be applied to yield a total mass of all analytes of 14,000 ng o.c. Subsequently, the amount of RRR found in 14,000 ng of castor oil was 8800 ng, or 63%. This is similar to the literature value of 70%.[1] Using this calibration curve, the limits of quantitation and detection were found to be 6 and 2 ng o.c. of RRR, based on signal-to-noise ratios of 10 and 3, respectively.

A new calibration plot was then used, shown in Figure 4, based on the 8800 ng of RRR found in the 14,000 ng castor oil. From this plot, estimates of mass for the larger analyte peaks, shown in Figure 5, were made, and the results are shown in Table 1.
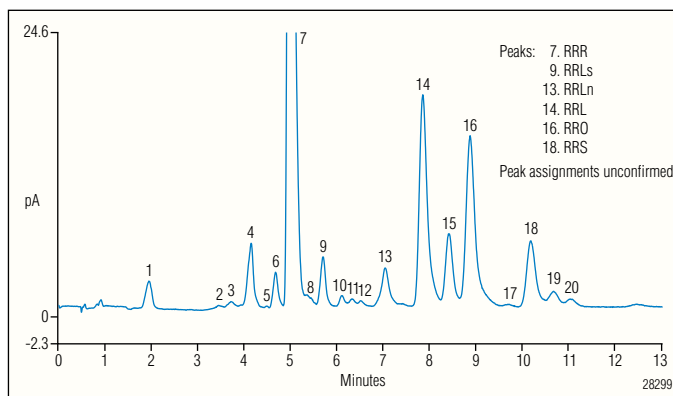

Figure 5. Chromatogram of castor oil A at 500 ng o.c. Numbered peaks were tracked for impurity calibration estimates.

Table 1. Composition of Castor Oil by HPLC-Charged Aerosol Detection Estimates			
Peak*	Analyte	Mass Percent	Literature Mass Percent[1]
1		0.9	
2		0.5	
3		0.6	
4		1.7	
5		0.4	
6		0.9	
7	RRR	63	71
8		0.6	
9	RRLs	1.1	0.7
10		0.5	
11		0.5	
12		0.5	
13	RRLn	1.4	0.15
14	RRL	10.4	6.6
15		2.5	
16	RRO	9.6	8.8
17		0.4	
18	RRS	2.8	1.1
19		0.8	
20		0.6	

R = ricinoleate, Ls = lesqueroleate, Ln = linolenic, L = linoleic, O = oleic, S = steric

*See Figure 5 for peak identification; peak assignments were made by analogy to assignments made in the literature.1
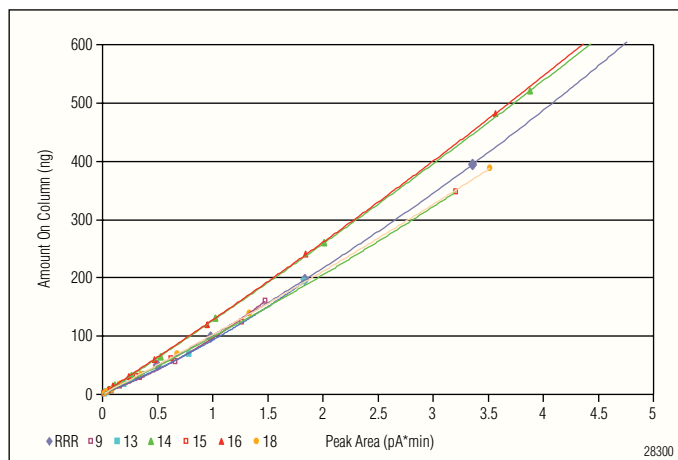
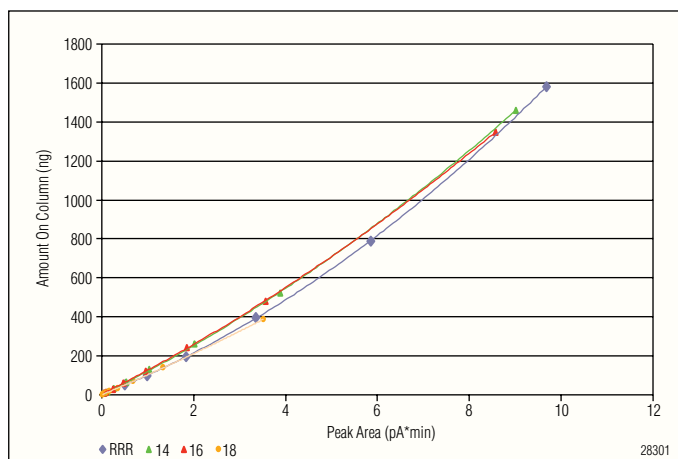Figure 6. Response curves based on estimated analyte amounts for low-amount analytes.

Figure 7. Response curves based on estimated analyte amounts for high-amount analytes.

A visual comparison of the different calibration curves, where analyte peak area was plotted against the estimated analyte mass for selected analytes, is shown in Figures 6 and 7. There are no drastic differences in the curves between RRR and other analytes as expected. This relatively uniform response is largely due to both the close similarity of the analytes contained in this sample, and the fairly consistent amount of organic present in the mobile phase. This second point has been shown to be an important factor in the relative uniformity of response of similar analytes across a gradient.[7]

The lower percentage of RRR that was found and shown in Table 1 may be attributable to the greater sensitivity of charged aerosol detection over ELSD: with greater sensitivity, a higher number of minor analytes can be detected and a more accurate measure of their mass may also be found. Both of these factors could result in a lower amount of RRR in the castor oil samples.

For the identification of these and other analytes, the mobile phases used are compatible with MS, which enables identification using this
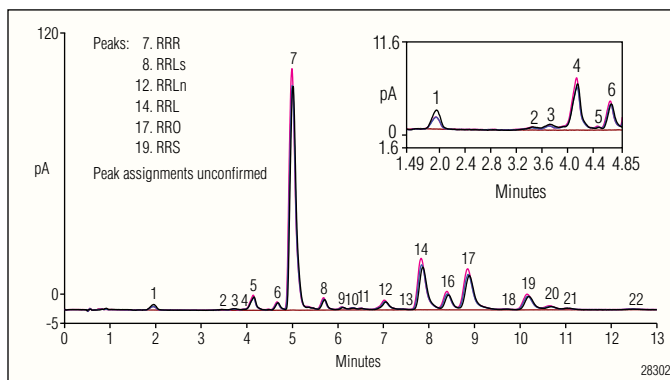

Figure 8. Comparison of three castor oil samples at 14,000 ng o.c. by HPLC-charged aerosol detection. Castor oil A is black, B is blue, C is pink, and blank is brown.

mode of detection. Once peaks are identified, the charged aerosol detector can be used as a routine detector to detect changes in relative peak heights and fingerprinting, or to detect new analytes that may not ionize well or ionize less consistently in an MS detector. For example, single acid triglycerides, from C7:0 to C22:0, exhibited a consistent relative response factor range of 1–2.4 by charged aerosol detection as compared to the wider range of 1–211 for atmospheric pressure chemical ionization MS (APCI-MS).[7]

An example of examining the possible differences of three castor oil samples, three castor oils, A, B, and C, were analyzed and compared as a means of examining lot-to-lot variability. In Figure 8 the castor oil chromatograms are overlaid, showing the similarity between all product samples, with the most significant difference found in Sample A: peak 1 was relatively greater than the same peak in the other two castor oil samples. These differences in composition may be important during studies involving hybrids or newly modified castor plants.

CONCLUSION

A reversed-phase HPLC-charged aerosol detection method was developed and successfully applied to the analysis of a wide range of triglycerides found in castor oil. The composition of the major analytes was determined without the use of external standards and these results compared favorably to those obtained by HPLC-ELSD.

By using charged aerosol detection, the relative analyte composition of castor oil was made using the similarity of response between different analytes. The sensitivity of the system enables the detection of low-level amounts of analytes (to 2 ng o.c.), that could not be achieved using other forms of detection (ELSD, UV, MS).

The method as outlined enables characterization of castor oil without the use of external standards. This analysis can provide fast, simple, comparative results for castor oils that originate from experimental castor plants. Characterization of total castor oil and the relative amounts of different components of the sample are obtained using a single calibration curve. The detector is simpler to operate, less costly than MS, and generally more sensitive than ELSD.

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