Analysis of Emulsifiers in Foods by High Pressure Liquid Chromatography and Corona Charged Aerosol Detection

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Overview

Purpose: To develop HPLC methods for quantitation of emulsifiers in foods, using an HPLC system with a charged aerosol detector.

Methods: Two methods, using the Thermo Scientific™ Accucore™ C18 column and the Thermo Scientific™ Hypersil™ Gold Silica column, were used to quantify hydroxypropylmethylcellulose (HPMC, modified cellulose) and Lecithin (via phosphatidylcholine amount), respectively.

Results: Samples of foods or ingredients were analyzed for content of lecithin or HPMC using the developed methods.

Introduction

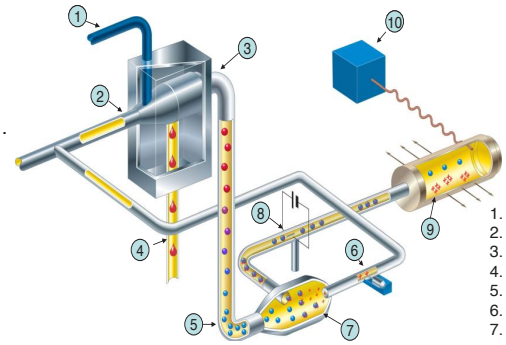
Emulsifiers are used to maintain a uniform suspension of immiscible materials. These compounds are typically surfactants, and can be designed for use in specific applications and products. Acylglycerols are used in food products containing oil and water (e.g. margarine, mayonnaise); lecithin is commonly found in chocolate and spray oils[1]; acid esters of monoglycerides are used for dough conditioners; and hydroxypropylmethyl cellulose (HPMC) is use to thicken dairy products and help improve flavor characteristics.[2] HPMC is also an important emulsifier used in the pharmaceutical industry.[3]

The analysis of emulsifiers is becoming increasingly important, for product quality, consistency and stability properties. High performance liquid chromatography (HPLC) is one of the more prevalent methods for analyzing these compounds. However, the majority of these analytes do not contain suitable chromophore characteristics for UV detection, which then requires the use of a universal detector, such as evaporative light scattering, refractive index, or charged aerosol detection. The Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector was used in the analyses of different emulsifiers that were extracted from food products.

The Corona Veo, a sensitive mass-based detector, is ideally suited for the direct measurement of emulsifiers, as they are non-volatile and non-chromophoric compounds. It offers excellent sensitivity (down to low nanogram amounts on column), a dynamic range of over 4 orders of magnitude, and similar inter-analyte response independent of chemical structure. As shown in Figure 1, the detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. The charge is then measured by a highly sensitive electrometer, providing reproducible, nanogram-level sensitivity. This technology has greater sensitivity, dynamic range and precision than ELSD and refractive index (RI), is gradient compatible and is simpler to operate than a mass spectrometer (MS). Compounds do not have to possess a chromophore (unlike UV detection) or be ionized (as with MS).

This sensitivity, combined with the linearity that is possible with use of the Corona Power Function, provides a unique and complete analytical solution for sensitive, reproducible, and routine analysis of non-chromophoric analytes. Several examples of emulsifier HPLC separations are detailed.

FIGURE 1. Schematic and functioning of charged aerosol detection.



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

Methods

Liquid Chromatography – Lecithin / DPPC

HPLC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 System with LPG3400-SD pump (normal phase), WPS3000 RS autosampler, and TCC3000 RS column oven
HPLC Column: Hypersil Silica 5 µm, 3.0 × 150 mm
Column Temperature: 50 °C
Mobile Phase A: Water, 18.2 MΩ·cm
Mobile Phase B: 2-Propanol
Mobile Phase C: iso-Octane
Flow Rate: 0.2–1.5 mL/min
Injection Volume: 2–10 µL
Detector: Corona Veo SD

Evaporator Temperature: 50°C Filter: 5 s
Data rate: 10 Hz PowerFunction: 1.40

Flow Gradient:

Time (minutes)	Flow (mL/min)	%A	%B	%C
-2.0	1.5	2	63	35
0.5	1.5	2	63	35
0.2	0.2	2	63	35
0.0	0.2	2	63	35
0.2	1.5	7	58	35
7.0	1.5	11	54	35
11	1.5	11	54	35
12	1.5	2	63	35

Standard and Sample Preparations
Standards and samples were dissolved in methanol / chloroform (1:9). If the solutions were clear, samples were used as is. If the sample was not clear, samples were centrifuged at 10,000 g for 5 minutes and the supernatant was used. If the sample was still cloudy, the samples were then centrifuge-filtered under the same conditions using a 0.2 micron membrane.

Liquid Chromatography – HPMC

HPLC System: UltiMate 3000 System with LPG3600-DGP pump, WPS3000 RS autosampler, and TCC3000 RS column oven
HPLC Columns: Imtakt Presto FF-C18, 2.0 µm, 150 x 4.6 mm
Column Temperature: 40 °C
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 2-Propanol
Flow Rate: 0.4 mL/min
Injection Volume: 2–10 µL
Detector: Corona Veo SD

Evaporation Temperature: 50 °C Filter: 3.6 s
Data rate: 5 Hz PowerFunction: 1.90

Flow Gradients:

non-Dairy: 5% B for 5 minutes before injection, hold 1 minute after injection, gradient to 100% B to 3 minutes, hold to 8 minutes, return to 5% to 9 minutes.

Dairy (extended gradient):
5% B for 5 minutes before injection, hold 1 minute after injection, gradient to 100% B to 3 minutes, hold to 8 minutes, gradient to 100% C to 9 minutes, hold 100% to 12.5 minutes, gradient to 100% B to 13 minutes, return to 5% B to 14 minutes.

Standard and Sample Preparations
Standards and samples were dissolved in water. If the solutions were clear, samples were used as is. If the sample was not clear, samples were centrifuged at 10,000 g for 5 minutes and the supernatant was used. Samples requiring more thorough cleaning were centrifuged-filtered using a 0.2 micron membrane.

Data Analysis
All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 6.8 SR 13.

Results

Sample Analysis- Lecithin

Lecithin was determined by the analysis of phosphatidyl choline in samples, as measured against a dipalmitoylphosphatidylcholine (DPPC) standard calibration curve. Because the material is comprised of various acyl groups, the peak shape between the samples and the DPPC standard were different, thus requiring a linear calibration curve.

A solution of DPPC, 1 mg/ mL was prepared in methanol/chloroform (1:9), sequentially diluted and analyzed for calibration. A chromatogram of the DPPC standard is shown in Figure 2. To provide a linear calibration fit for DPPC for amounts 39 – 10,000 ng on column (ng o.c.) a power function value (PFV) of 1.4 was identified. The linear calibration curve shown in Figure 3 had a linear regression coefficient, R^2 = 0.9996 and %RSD of 2.84.

Figure 2. HPLC-CAD chromatogram of DPPC, 2500 ng on column.

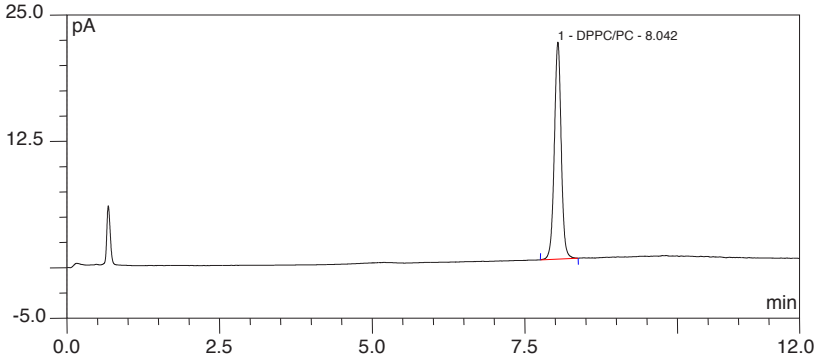
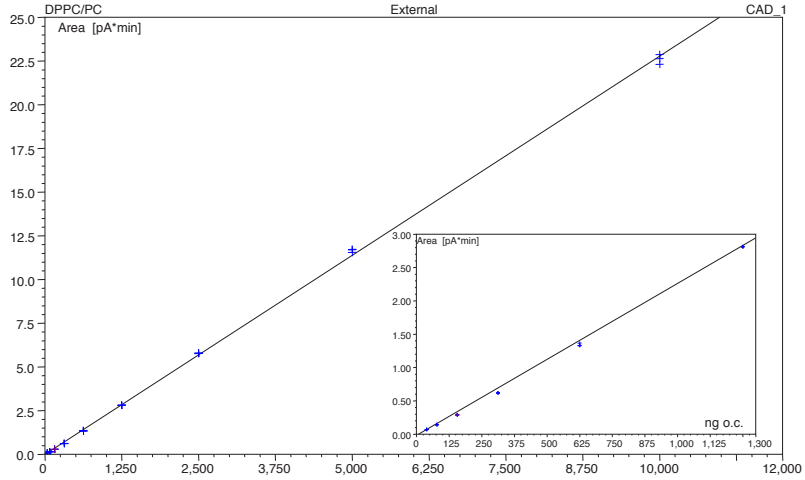


Figure 3. Linear regression fit of HPLC-CAD calibration data, 39 to 10,000 ng o.c., each amount in triplicate, inset 39-1250 ng o.c.



The linear fit spanned four order of magnitude. For sensitivity, the limit of quantitation, based on signal-to-noise ratio of 10, was determined to be 20 ng o.c.

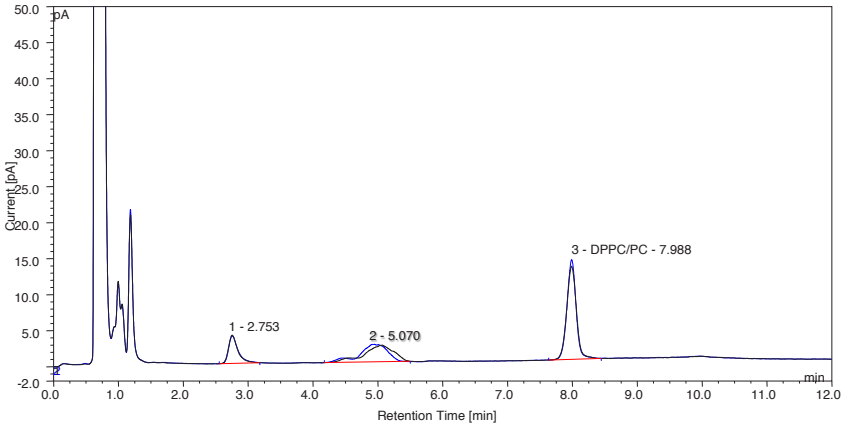
Three samples were dissolved, clarified, and analyzed, including lecithin, a granola bar, shown in Figure 4, and krill oil. The results are shown in Table 1, below.

Table 1. Phosphatidylcholine found in food samples.

Sample	Phosphatidylcholine found (mass-%)	Claim amount	Percent target
Lecithin, Laboratory Grade	47.4	N/A	N/A
Granola Bar	0.05	< 2%	N/A
Krill Oil	34.1	34.9*	97.7

*AOCS Official Method Ja 7c-07[4]

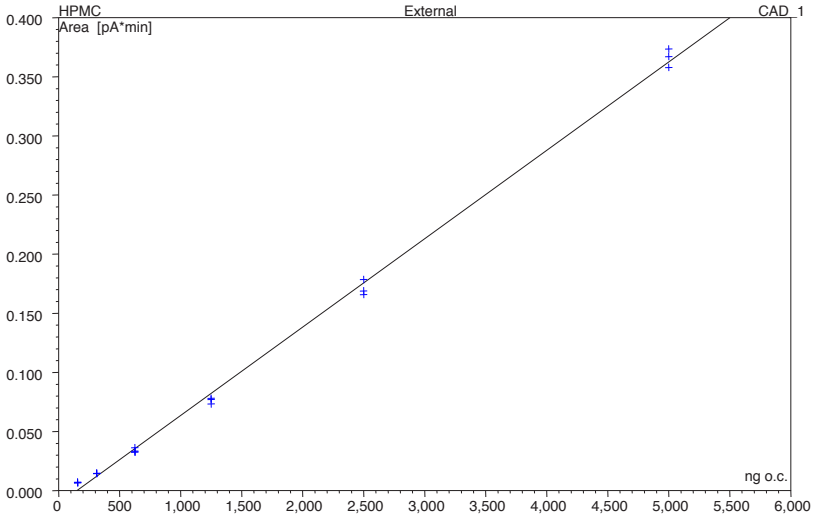
Figure 4. HPLC chromatogram of lecithin (DPPC/PC) in granola bar, extracted in methanol / chloroform (1:9), centrifuge-filtered.



Sample Analysis- HPMC

Hydroxypropylmethyl cellulose (HPMC) was dissolved in water and analyzed for calibration, from 156 to 5000 ng o.c., and the peak areas were fit to a linear regression line, as shown in Figure 5. The resulting correlation coefficient was 0.998 with a %RSD of 5.53. A PFV of 1.9 was used, and the estimated limit of quantitation is approximately 10 ng o.c. The peak area %RSD values ranged from 0.53 (625 ng o.c.) to 5.21 (156 ng o.c.).

Figure 5. Linear HPMC calibration curve, from 156 to 5000 ng o.c., each amount analyzed in triplicate.



Two samples were prepared and analyzed: a popsicle and a more complex frozen milk product, each contained less than 1% of HPMC ("modified cellulose"). The popsicle was melted and diluted, 99.5 mg (100 µL) into 900 µL water, and analyzed directly. The frozen milk product, however was diluted and vortex-mixed (44.7 mg in 900 µL of water) and centrifuge-filtered. Due to the added lipids of the milk product, an extended gradient was also used to clean the column of the lipids.

A spike-recovery sample of the dairy product was also prepared, using 47.9 mg of product and 10 µL of 10,000 ng o.c. standard solution (additional 1000 ng o.c.). The results of the two products and the spike recovery sample are provided in Table 2.

Table 2. HPMC found in food samples

Sample	HPMC found (mass-%)	Claim amount	Recovery (%)
Popsicle	0.05	< 1%	N/A
Dairy Product	0.21	< 1%	N/A
Spiked Dairy Product	835 ng o.c. (spiked)	1000 ng o.c. spiked	83.5

Figure 6. Overlaid HPLC-CAD chromatograms showing HPMC in a popsicle, melted and diluted, in duplicate.

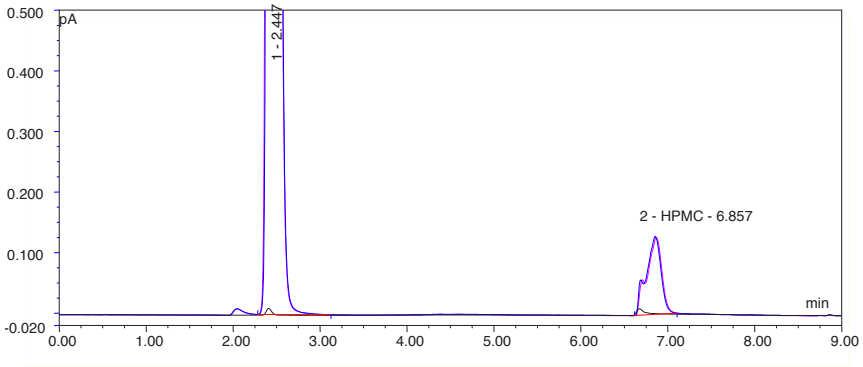
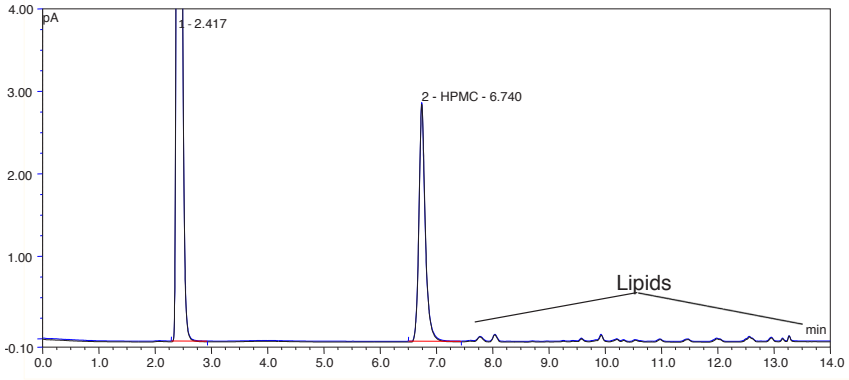


Figure 7. Overlaid HPLC-CAD chromatograms of HPMC in a frozen dairy product, 44.7 mg dissolved in 900 µL water, centrifuged, analyzed in duplicate.



Conclusions

Two HPLC methods were developed to analyze two, different emulsifiers commonly found in food products, lecithin and HPMC (modified cellulose). The lecithin method was able to determine the amount of phosphatidylcholine found in a food sample, in the ingredient itself, as well as in a natural nutraceutical product, krill oil, with results matching the official AOCS method for phospholipids. Sensitivity was 20 ng o.c. LOQ.

HPMC was calibrated over a wide range of concentrations, and the method was able to determine HPMC in two food products, including a spike-recovery of 83.5% and sensitivity to 10 ng o.c. LOQ.

Both of these analytes varied in their composition, mainly in their acyl groups, so linear calibration was required for accurate determinations. The Corona Veo charged aerosol detector was able to provide linear calibration curves for these analytes. The sensitivity and linear dynamic range provided useful, analytical data for these analytes that also lack a chromophore.

References

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