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Automated Liquid-Liquid Extraction and Determination of Fatty Acids Composition in Infant Formula according to AOAC® 2012.13 using a Robotic Autosampler

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Abstract

Liquid-liquid extractions are used to extract and concentrate analytes from aqueous matrices. This extraction technique is widely accepted, as shown by its inclusion in many official methods. Analytical laboratories are looking to automation to help reduce solvent usage and increase sample throughput while ensuring the high quality of the resulting data.

The GERSTEL MultiPurpose Robotic Smart Series Sampler (MPS), commonly used for sample introduction into GC or HPLC, can perform a wide variety of sample preparation techniques using a single instrument and controlling software. The sampler can be configured as part of a GC or LC system or as a bench-top workstation.

In this study, the automation of the liquid-liquid extraction of fatty acid methyl esters in infant formula using the MPS is discussed. Two key GERSTEL options make automated extraction possible: (A) The Motion 40 agitator option that allows samples to be rapidly and effectively mixed, and (B) the M50 pump module that enables the precise delivery of the neutralization solution. The MAESTRO software enables the precise controlled timing required by this method. Automation of the extraction of infant formula samples and subsequent GC-FID determination of the FAMES listed in the official AOAC® 2012.13 Official Method℠ [1] are examined, and resulting precision and accuracy data are provided.

Introduction

Infant formula is crucial in ensuring the healthy development of babies who cannot be breastfed for various reasons. It serves as a vital alternative, providing essential nutrients necessary for growth and development during the early stages of life. One of the key components in infant formula is its fatty acid composition, which mirrors that found in breast milk. Fatty acids are integral for developing the nervous system, cognitive function, and immune response in infants. Therefore, ensuring the proper fatty acid composition in infant formula is critical to mimic the benefits of breast milk accurately.

Determining the correct fatty acid composition involves meticulous research and testing to replicate the nutritional benefits infants would receive from breastfeeding. This process is crucial because variations in fatty acid levels can directly impact an infant's growth and long-term health outcomes. Properly balanced fatty acids, such as omega-3 and omega-6, contribute to brain development and overall cognitive function, making them essential for infant nutrition. As such, ensuring that infant formula meets these nutritional standards is not only essential for the immediate health of infants but also for their future well-being and development. Methods, such as the one found within the official AOAC 2012.13 Official Method℠ are critical for understanding and optimizing the fatty acid composition of infant formula.

GERSTEL AppNote 267

Experimental

Materials

Two (2) powdered infant formula samples containing 25.5% fat and 27.1% fat, respectively, were obtained for use during the evaluation of the automated extraction procedure. The following equation was used to determine the amount of infant formula to extract for each replicate:

$$\frac{\text{50 mg = Theoretical Fat (g)}}{\text{100 g Product * Sample Weight (mg)}}$$

For the powdered infant formula sample with 25.5% fat, 196 mg of sample was weighed into three (3) separate 40 mL vials (Supelco, clear, screw top, 28 x 98 mm with PTFE/Silicone septa, #27089-U), 2 mL of deionized water was added to each vial, the vials were sealed with GERSTEL magnetic screw caps (093640-122-00) and then mixed to dissolve. For the powdered infant formula sample with 27.1% fat, 184.5 mg of the sample was weighed into three (3) separate 40 mL vials, 2 mL of deionized water was added to each vial, and the vials were sealed and then mixed to dissolve. To prepare a blank sample, 2.0 mL of deionized water was pipetted into a 40 mL vial.

Methanolic sodium methoxide (5% w/v) was prepared by combining 20 mL of sodium methoxide (25% wt% in methanol, Sigma, 15256-25ML) with 80 mL of methanol (Millipore, LiChroSolv, 1.06035.2500) and mixing well. This solution was transferred into a glass reservoir vial and placed onto the MPS robotic/MPS robotic^{PRO} sampler.

The neutralization solution was prepared by dissolving 20.0 grams of sodium hydrogen citrate sesquihydrate (Sigma, 359084-250G) and 30.0 grams of sodium chloride (Sigma, S9888-500G) in a 250 mL glass bottle using 200 mL of deionized water and mixing well. This solution was then incorporated into the GERSTEL Valco M50 pump module.

The internal standard solution was prepared by dissolving 200 mg of glyceryl tritridecanoate (Sigma, T3882-500MG) and 200 mg of methyl undecanoate (Sigma, U0250-5G) using 100 mL of tert-butyl methyl ether (Sigma, 306975-1L) and mixing well. This solution was transferred into a glass reservoir vial and placed onto the MPS robotic/MPS robotic^{PRO} sampler.

A 37-component FAME Mix (Supelco #CRM47885) was used to establish chromatography and match retention times during the GC-FID analyses of the samples. All other solvents were reagent grade.

Instrumentation

The automated Prep Sequence was performed using a MPS robotic/MPS robotic^{PRO} sampler with the GERSTEL Multi-Position Dilutor, Valco M50 pump module, and Motion 40 options as shown in Figure 1. Before their GC-FID analyses, each vial was centrifuged for 5 minutes at 1500 rpm. Three (3) mL of the clear, top layer was transferred to a 10 mL screw top vial containing 200 mg of anhydrous calcium chloride. Seven (7) mL of hexane was added to the vial, the vial was mixed, and the dried extract was transferred to a 2 mL vial for GC-FID analysis. The GC-FID analyses of the transesterified extracts were performed using a GERSTEL MPS robotic^{PRO} autosampler with a 10 µL syringe, a GERSTEL CIS 4 cooled inlet system with LN_2 option, and an Agilent 8890 GC/FID.

Figure 1: MPS robotic^{PRO}/MPS robotic sampler with the GERSTEL Multi-position dilutor, Valco M50 pump, and Motion 40 agitator options.

Analysis Conditions

CIS 4	Baffled liner 40 °C (0 min), 12 °C/sec, 260 °C (3 min) Split: 20:1
Column	100 m CP-SIL 88 (Agilent), d_i = 0.25 mm, d_f = 0.20 µm 1.2 mL/min, constant flow
Oven	80 °C (2 min), 4 °C/min, 225 °C (25 min)
FID	280 °C

GERSTEL AppNote 267

Automated MPS Prep Sequence for Liquid-Liquid Extraction of Infant Formula Samples

1. The MPS adds 5 mL of the internal standard solution in MTBE into the sample vial.
2. The MPS adds 5 mL of the 5% methanolic sodium methoxide solution into the sample vial.
3. The MPS mixes the sample vial for 10 seconds at 500 rpm.
4. After approximately 180 ±10 seconds, the MPS adds 2 mL of hexanes into the sample vial.
5. After 210 seconds, the MPS adds 10 mL of the neutralization solution at 35 mL/min into the sample vial.
6. The MPS mixes the sample vial for 10 seconds at 500 rpm.

Results and Discussion

When using this method, the time at which the hexane and then the neutralization solution are added to the sample is critical for the reproducible determination of fatty acid composition in infant formula samples. As shown in Figure 2, the MAESTRO Prep Sequence Scheduler shows that the use of the GERSTEL Valco M50 Pump module, which allows solutions to be delivered at flow rates up to 35 mL/min, as well as the use of WAIT commands within MAESTRO software, ensured that all solutions can be added within required time periods.

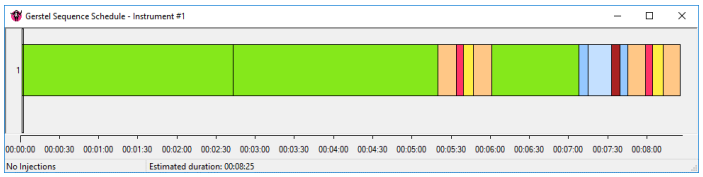

Figure 2: Example MAESTRO Prep Sequence Scheduler showing the timing of the additions of the various solutions during the automated liquid-liquid extraction.

During the automated method optimization, it was found that gentle mixing of the sample is required to avoid generating an emulsion. The user's ability to control the speed of the GERSTEL Motion40 agitator option allows the mixing speed to be optimized. During the automated method, a mixing speed of 500 rpm was used.

Figure 3 shows the comparison chromatograms of the 37-component FAMES standard and the extracted blank. The retention times of the same components in the chromatogram from the standard were found to match those of the methyl undecanoate (22.7 min) and glyceryl tritridecanoate (26.9 min) internal standards.

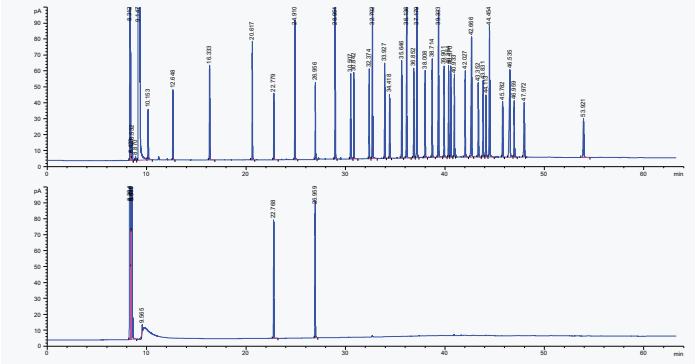

Figure 3: Comparison chromatograms of 37 component FAMES standard and extracted blank.

Figures 4 and 5 show the chromatograms from the extracted infant formula sample replicates having 25.5% fat and 27.1% fat, respectively. The major peaks observed in the chromatograms of the extracted sample replicates were chosen to establish the reproducibility data. The precision data for the powdered infant formula samples is shown in Table 1. Precision data for the samples generated from the automated extraction were found to average 2.86% and 10.0% for each infant formula sample, respectively.

GERSTEL AppNote 267

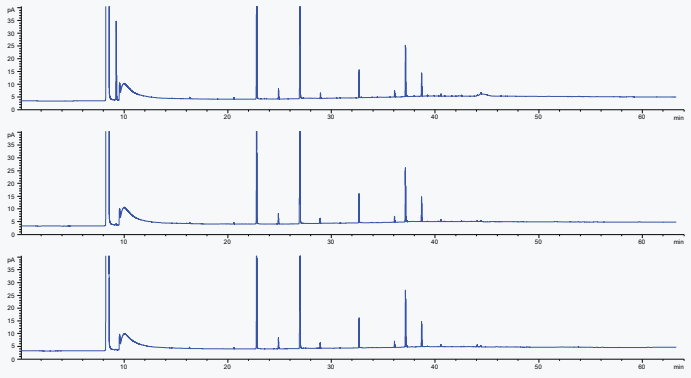

Figure 4: Replicate chromatograms from extracted infant formula (25.5% fat).

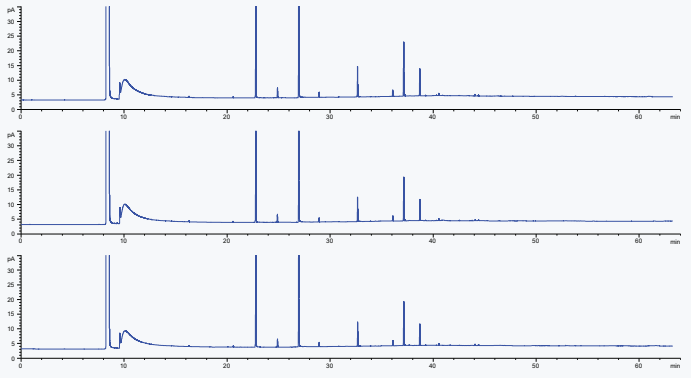

Figure 5: Replicate chromatograms from extracted infant formula (27.1% fat).

Table 1: Precision data obtained from the automated liquid-liquid extraction method for n=3.

Free Fatty Acid Name	FAME conc. in Standard [µg/mL]	Rentention Time [min]	Peak Area 25.5% - 1	Peak Area 25.5% - 2	Peak Area 25.5% - 3	Peak Area %CV	Stoichio-metric Factor	Re-sponse Factor	Average Individual FA(g) / 100g sample 25.5%
undecanoic acid	204	22.7	221	229	233	2.66	0.930	1.00	4.74
lauric acid	408	24.8	10.0	10.4	10.8	3.52	0.935	0.905	0.197
tridecanoic acid	203	26.9	263	273	277	2.67	0.939	0.855	4.86
myristic acid	407	28.9	5.37	5.33	5.47	1.34	0.942	0.783	0.0891
palmitic acid	610	32.6	26.6	27.8	28.7	3.78	0.948	0.680	0.399
stearic acid	407	36	6.00	6.03	6.14	1.20	0.953	0.623	0.0806
oleic acid	407	37.1	50.6	53.2	54.8	3.93	0.953	0.614	0.692
linoleic acid	202	38.6	23.7	24.9	25.5	3.77	0.957	0.629	0.333
					Average	2.86			

Free Fatty Acid Name	FAME conc. in Standard [µg/mL]	Rentention Time [min]	Peak Area 27.1% - 1	Peak Area 27.1% - 2	Peak Area 27.1% - 3	Peak Area %CV	Stoichio-metric Factor	Re-sponse Factor	Average Individual FA(g) / 100g sample 27.1%
undecanoic acid	204	22.7	229	223	226	1.34	0.930	1.00	5.04
lauric acid	408	24.8	8.48	6.93	6.88	12.3	0.935	0.905	0.150
tridecanoic acid	203	26.9	272	265	268	1.32	0.939	0.855	5.16
myristic acid	407	28.9	4.50	3.55	3.54	14.3	0.942	0.783	0.0683
palmitic acid	610	32.6	25.3	20.5	20.2	13.0	0.948	0.680	0.340
stearic acid	407	36.0	5.42	4.45	4.41	12.0	0.953	0.623	0.0677
oleic acid	407	37.1	46.6	37.2	37.3	13.3	0.953	0.614	0.565
linoleic acid	202	38.6	23.7	19.1	19.1	12.9	0.957	0.629	0.298
					Average	10.0			

Conclusions

As a result of this study, we were able to show:

- Automation of the liquid-liquid extraction of fatty acid methyl esters in infant formula was readily automated using the GERSTEL MPS robotic/MPS robotic^{PRO} sampler and determined using an Agilent 8890 GC-FID.
- The automated Prep Sequence was found to successfully prepare and mix the transesterified extracts.
- The automated extraction method proved to be precise. Precision data averaged 2.86% and 10.0% for each infant formula sample, respectively.

References

[1] Official Methods of Analysis of AOAC INTERNATIONAL (2023) 22nd Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 2012.13.