

# FAMEs Analysis of Oils by GC-FID Coupled with Fully Automated Sample Preparation using a PAL3 Series 2 RTC

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## Abstract

This work describes workflows to improve process safety, optimizes throughput, and minimizes errors in the analysis of fatty acid methyl esters (FAMEs). These analyses are routinely performed in governmental, quality control (QC), and contract research laboratories worldwide. The resulting data often informs nutrition labeling, helping consumers make informed dietary choices. The physiological effects of fatty acids depend on their structural characteristics and detailed compositional analysis is essential. The Agilent automated sample preparation workflow using the PAL3 Series 2 RTC autosampler and the Agilent 8890 gas chromatography (GC) system with flame ionization detector (FID) delivered reproducibility and accuracy in the analysis of FAMEs.

## Introduction

The analysis of fatty acid methyl esters (FAMES) is a widely used technique for characterizing lipid profiles in various food products, including oils, meats, and seeds. Fats are composed of a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids, each with varying carbon chain lengths.<sup>2</sup> This type of analysis is routinely performed in governmental, quality control (QC), and contract research laboratories worldwide. The resulting data often informs product labeling. Among the analytical techniques available, gas chromatography (GC) with flame ionization detection (GC-FID) is one of the most common methods for determining fatty acid composition in foods.

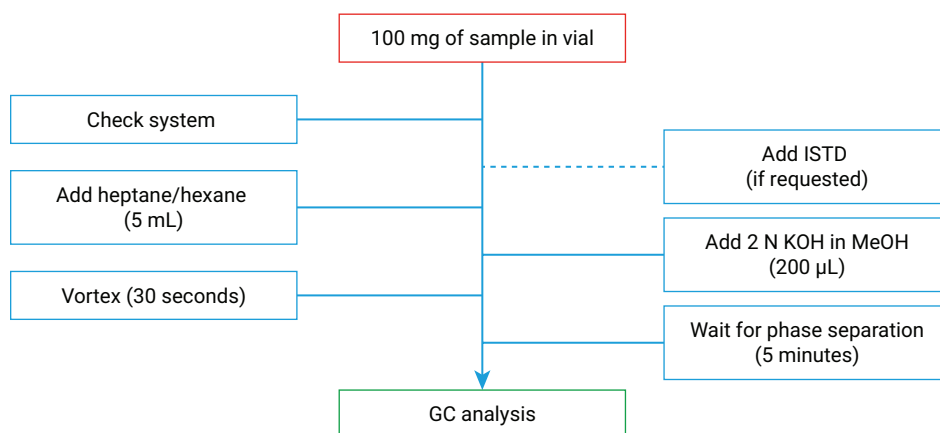
This application note describes the use of automated sample preparation and analysis of FAMES using the PAL3 Series 2 RTC Autosampler system coupled with Agilent 8890 GC system equipped with an FID. The results shown here focus on two different methods of derivatization for FAMES extraction: (1) base catalyzed reactions using potassium hydroxide (KOH) (part number M5795AA #001) and (2) base plus acid catalyzed reactions using boron trifluoride (BF<sub>3</sub>)<sup>1</sup> (part number M5795AA #002). For ease of use, both processes have been transcribed into dedicated sample preparation methods. These methods are part of the Agilent automated sample preparation workflow suite of solutions. These workflows improve process safety, optimizes throughput, and minimizes errors.

## Experimental

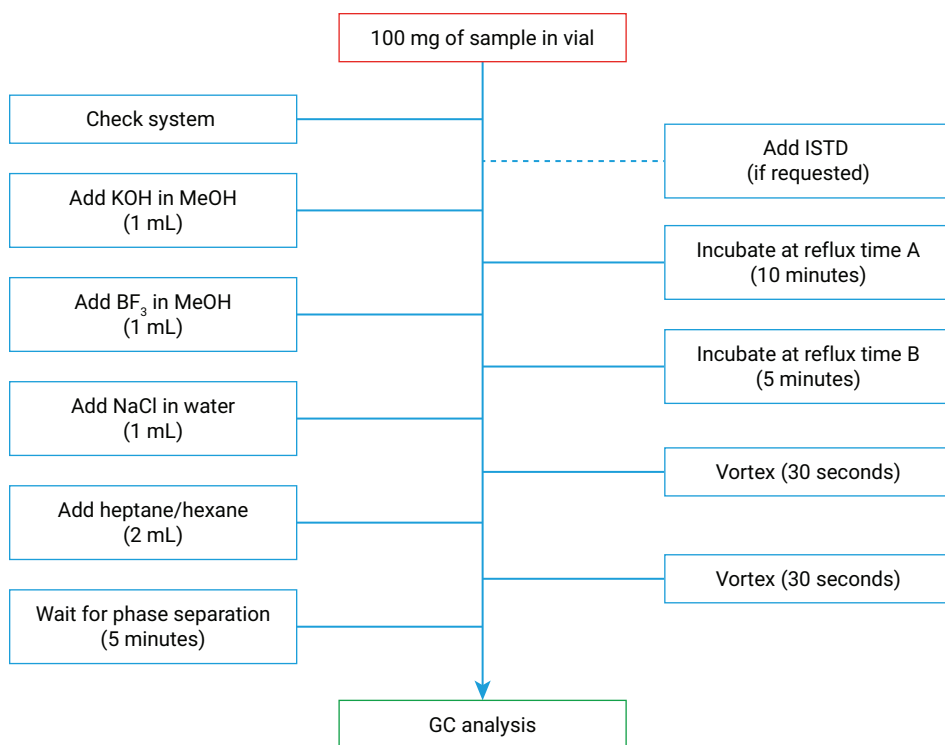
The 37 component FAME mix standard was purchased from Sigma-Aldrich (part number CRM47885), and three consumer oil products were purchased from a local grocery store. The samples were prepared and injected into the GC using the PAL3 Series 2-RTC system. The analysis was performed using an 8890 GC equipped with an Agilent DB-FastFAME GC column, 30 m × 250 μm, 0.25 μm, (part number G3903-63011) and configured with an FID using Agilent OpenLab CDS v2.7+.

Two different Agilent automated sample preparation workflows were tested to extract FAMES from oils. The M5795AA #001 workflow used a quick base catalyzed reaction (Figure 1). While M5795AA #002 workflow used an acid-base catalyzed reaction (Figure 2). Included in each of the workflows are FAMES analytical method, parameters show in Table 1, and a corresponding data process method. For ease of use a FAMES workflow consumables kit (part number 5150-2201) was utilized during the experiment (Table 2).

The oil samples were purchased from a local grocery store. For sample preparation, 100 mg of sample was weighed, put into a 10 mL vial, and capped. Sample vials were placed into the PAL3 VT15 sample rack.



**Figure 1.** Detailed description of the sample preparation done with the Agilent automated sample preparation method (M5795AA #001) using a methanolic KOH solution. The red block indicates the need for user input where 100 mg of sample was accurately weighed and put into a 10 mL vial.



**Figure 2.** Sample preparation steps taken using the Agilent automated sample preparation method (M5795AA #002) using a methanolic KOH solution in addition to methanolic BF<sub>3</sub> solution.

**Table 1.** GC-FID parameters used for FAMEs analysis.

Parameter	Value
Injection Volume	1 µL
Inlet (Split/Splitless)	275 °C (50:1 split)
Oven Program	70 °C (hold for 0.5 min), 60 °C/min to 165 °C (hold for 0.5 min), 10 °C/min to 200 °C (hold for 0.5 min), 5 °C/min to 230 °C (hold for 2 min)
Column Flow	3.0 mL/min (hydrogen)
FID Temperature Setpoint	300 °C

**Table 2.** The Agilent FAMEs workflow consumables kit (part number 5150-2201) includes the following items.

Part Number	Description
5183-4759	Advanced Green Inlet Septa, 11 mm 50/pk
5190-3163	Inlet liner, UI, single taper with glass wool, 5/pk
5181-3323	Ferrule, 0.4 mm id, 85:10 Vespel/graphite, 10/pk
5190-6145	DB-FastFAME GC column, 30 m × 0.25 mm, 0.25 µm (7 in. cage)
5188-5392	10 mL Screw top vials
5188-2759	Magnetic caps
8010-1353	2.5 mL Liquid Sampling syringe with removable needle (D18/57)
8010-1383	F1000 replacement needles (23/57/C)

**Table 3.** List of optional consumables that are recommended for ease of use.

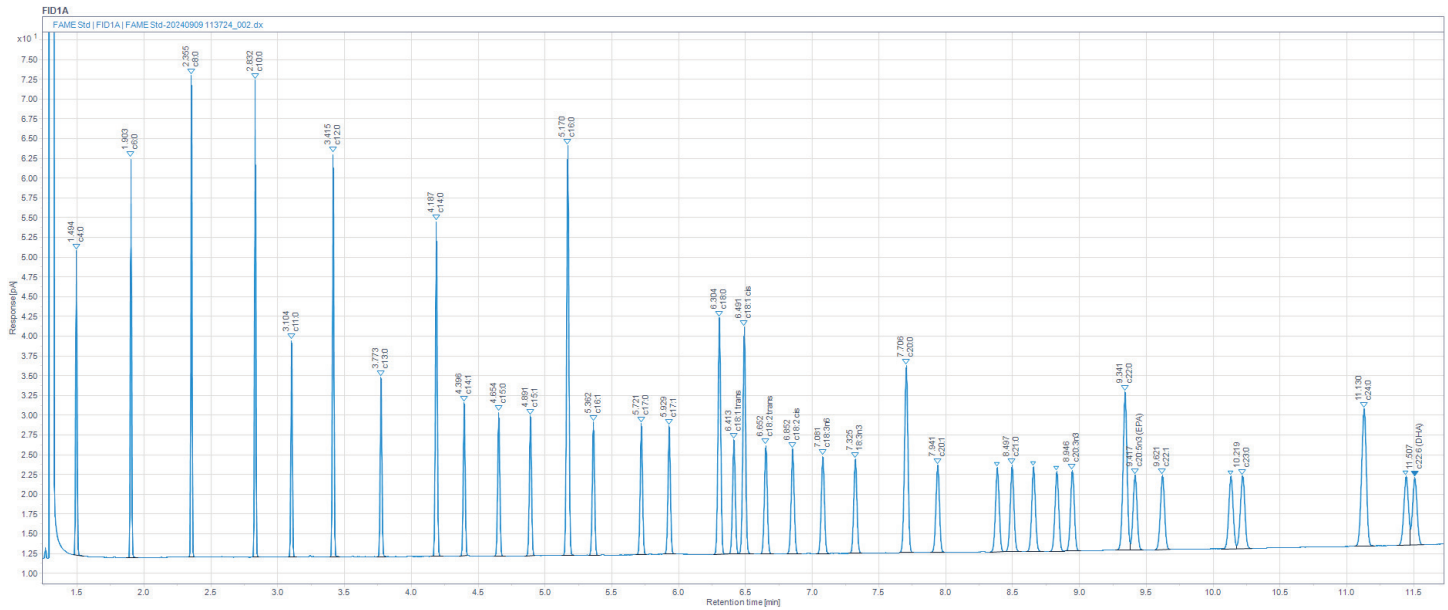
Part Number	Description
G3440-81011	Agilent self-tightening column nut, inlet/detector
G3440-88000	Agilent capillary column depth guide
5200-0176	FID Jet, universal fit, 0.011 in. id
8010-1352	1 mL Liquid sample syringe

## Results and discussion

Figure 3 shows the results from 37 compound FAMES standard using the analytical and processing methods included in the Agilent FAMES workflow. The results show well resolved peaks (resolution >1.5) with analysis time of less than 15 minutes. Table 4 lists the elution order of the 37 compound FAMES mix on the Agilent DB-FastFAME GC column.

**Table 4.** Elution order for the 37 component FAMES mixture on an Agilent DB-FastFAME GC column.

Elution Order	Analyte	Elution Order	Analyte	Elution Order	Analyte
1	c4:0	14	c17:0	27	c20:3n6
2	c6:0	15	c17:1	28	c20:4n6
3	c8:0	16	c18:0	29	c20:3n3
4	c10:0	17	c18:1 trans	30	c22:0
5	c11:0	18	c18:1 cis	31	c20:5n3 (EPA)
6	c12:0	19	c18:2 trans	32	c22:1
7	c13:0	20	c18:2 cis	33	c22:2n6
8	c14:0	21	c18:3n6	34	c23:0
9	c14:1	22	18:3n3	35	c24:0
10	c15:0	23	c20:0	36	c24:1
11	c15:1	24	c20:1	37	c22:6 (DHA)
12	c16:0	25	c20:2		
13	c16:1	26	c21:0		



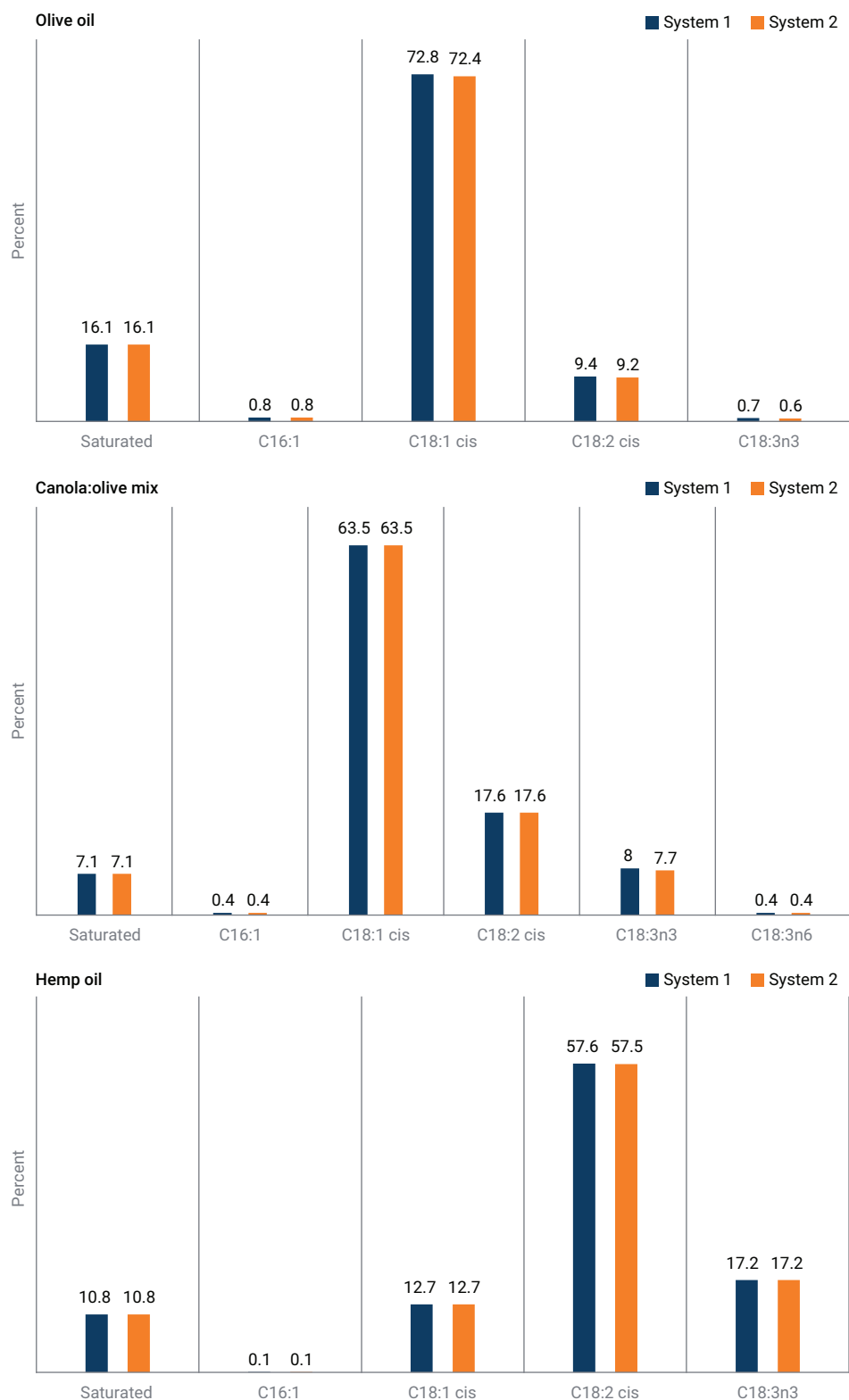
**Figure 3.** Chromatographic performance for a 37 component FAMES mixture on a 30 m x 250 µm, 0.25 µm Agilent DB-FastFAME column using GC-FID.

Figure 4 shows the results from three different store-bought oils using the KOH solution. The results show stable results from system to system for all three samples. The calculated values of the saturated and unsaturated fats in the samples are comparable to expected concentrations found in literature. Table 5 highlights the robustness of the automated sample preparation methodology. Repeatability tests were carried out using five samples of olive oil on two different PAL3 Series 2-RTC systems. The calculated RSD% shows highly reproducible results.

Similar studies on method reproducibility and accuracy were carried out using the KOH-BF<sub>3</sub> solutions. The results are comparable to what was achieved using the simplified KOH solution method.

**Table 5.** Repeatability studies on select compounds on olive oil sample (N = 5), put through the automated sample preparation, show highly reproducible results independent of system dependency.

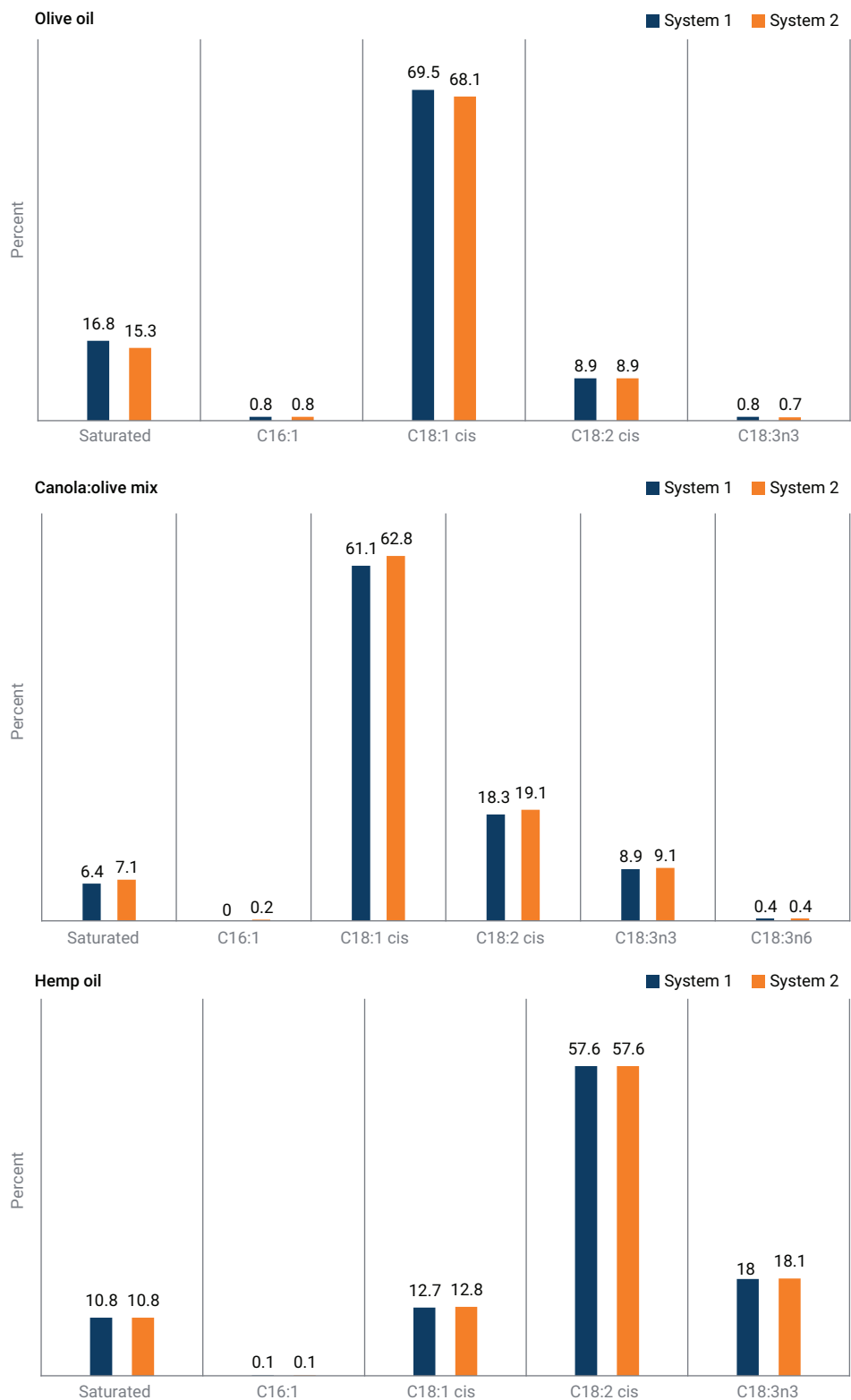
	System 1 Peak Area RSD%	System 2 Peak Area RSD%
C16:0	2.2	2.9
C16:1	2.2	2.9
C17:0	3.0	3.0
C17:1	3.7	3.3
C18:0	2.2	2.9
C18:1 cis	2.1	2.9
c18:2 cis	2.1	2.9
C18:3n3	2.8	3.6
C20:0	2.3	2.6



**Figure 4.** Precision study was carried out on three store-bought oils using M5795AA #001 workflow.

**Table 6.** Repeatability studies using olive oil (N = 5) show highly reproducible results across multiple systems.

	System 1 Peak Area RSD%	System 2 Peak Area RSD%
C16:0	0.91	3.54
C16:1	1.11	5.34
C17:0	3.01	3.62
C18:0	0.70	1.87
C18:1 cis	0.64	1.67
c18:2 cis	0.75	3.06
C18:3n3	2.37	4.31
C20:0	2.41	2.34



**Figure 5.** Precision study was carried out on three store-bought oils using M5795AA #002 workflow.

## Conclusion

In summary, the current study demonstrated excellent reproducibility and accuracy using automated sample preparation and analysis of FAMES. Two different sample preparation methods were demonstrated, both delivering comparable and reliable results. The base-catalyzed method enables rapid analysis but is not suitable for samples containing free fatty acids. In contrast, the acid-catalyzed method, while more extensive, is compatible with a broader range of lipid classes, including free fatty acids, phosphoglycerides, and triglycerides. The Agilent automated sample preparation workflow suite of solutions deliver a comprehensive package by combining preconfigured hardware, optimized analytical methods, and the necessary consumables to ensure efficient and reliable results.

## References

1. Godina, L. Analysis of Oil and Fat Containing Foods by Fully Automated Sample Preparation Using a PAL3 Coupled with 7890 GC and a 5977 MSD System According to AOAC 996.01. *Agilent Technologies application note*, 5991-9107EN, **2018**.
2. Zou, Y. H. Improving the Analysis of 37 Fatty Acid Methyl Esters. *Agilent Technologies application note*, 5991-8706EN, **2023**.

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