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Fully Automated Sensitive Quantitation of PFAS in Seafood for Regulatory Screening Using Triple Quadrupole LC/MS

Aimei Zou[1], Gwen Lim[2], and M. Lorna De Leoz[3]

[1]Agilent Technologies, Singapore

[2]CTC Analytics AG, Switzerland

[3]Agilent Technologies, United States

Introduction

Per- and polyfluoroalkyl substances (PFAS) are extensively found in marine life, posing health risks through seafood consumption.

Seafood is one of the regulated matrices by the U.S. FDA, EU, EURL POPs, and AOAC. Regulatory and standard-making bodies like the U.S. FDA, EFSA, EURL POPs, and AOAC have set stringent guidelines for PFAS levels in seafood.

Detecting trace PFAS level is challenging due to complex sample preparation like QuEChERS extraction followed by SPE cleanup, and evaporation/reconstitution. The manual steps can be labor-intensive and error-prone, affecting accuracy and reliability. This study developed a fully automated workflow for PFAS quantitation in seafood – shrimp.

Experimental

Instrumentation

An integrated 160 cm PAL3 Series 2 RTC autosampler coupled with a 6495D LC/TQ (Figure 1) was employed. The PAL3 platform performed automated sample preparation, while TQ data acquisition and analysis were conducted in parallel.

LC system comprised of two modules; an Agilent 1290 Infinity II high-speed pump and an Agilent 1290 Infinity II multicolumn thermostat.

The study utilized the following tools and modules: two PAL park stations with three liquid syringe tools, a dilutor tool, a micro-SPE tool, an LC/MS tool, a vortex mixer, a centrifuge, a dilutor multi, a tray cooler (for 2/10/20 mL vials), tray holders with rack R60 (for 10/20 mL vials), a micro-SPE tray (for 2 mL vials and micro-SPE cartridges), a solvent module, a fast wash module, and an LC injection valve.

The details of various consumables used for this study are provided elsewhere.[1]

Figure 1. CTC PAL3 Series 2 RTC autosampler with Agilent 6495D triple quadrupole LC/MS.

Experimental

End-to-end automation procedure

To evaluate the workflow performance, raw shrimp samples (4 g) were used. Sample preparation and LC/TQ injections were conducted based on a customized script. The PAL3 platform facilitated QuEChERS salting out-assisted solvent extraction, µSPE cleanup, dilution, and injection. Twelve calibration standards (1 to 50,000 ng/L) with 73 target analytes were prepared, each containing 34 surrogates and three ISTDs. Procedural blank (PB), matrix blank (MB), and spiked QCs (LSQ: 0.1, MSQ: 0.3, HSQ: 1.0 µg/kg) were analyzed to assess the automated workflow performance. The entire process was managed using MassHunter software.

Table 1: CTC PAL3 Series 2 RTC Autosampler and 1290 Infinity II LC Conditions

Analytical Column	ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm, (p/n: 959758-902)		
UHPLC Guard	ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, (p/n: 821725-901)		
Column Temp.	55 °C		
Injection Volume	10 µL		
PAL Tray Temp.	5 °C		
Mobile Phase A	5 mM Ammonium acetate in water		
Mobile Phase B	100% Methanol		
Flow Rate	0.4 mL/min		
Timetable	Time/min	%A	%B
	0.0	85	15
	1.0	85	15
	1.5	45	55
	5.5	30	70
	7.0	20	80
	12.0	0	100
	14.4	0	100
	14.5	85	15
Post Time	2.5 minutes		
PAL Injection Needle Wash	Multiwash; (S1:15:85 Methanol:water, S2: 1:1 Acetonitrile:2-propanol)		

Table 2: 6495D LC/TQ parameters

Ion Source	AJS ESI, Negative Mode
iFunnel Mode	Standard
Q1/Q3 Resolution	Unit
Cycle Time	720 ms
Nebulizer Gas	Temperature: 250 °C, Flow: 11 L/min
Nebulizer	25 psi
Sheath Gas	Temperature: 375 °C, Flow: 11 L/min
Capillary	3000 V (Negative)
Nozzle Voltage	0 V

Results and Discussion

Enhanced lab productivity

The entire automated workflow was managed using MassHunter software, which facilitated the creation of an online analysis worklist batch, and enabled parallel sample preparation and analysis, increasing overall lab productivity through automation and eliminating waiting time between runs.

Accuracy and precision of calibrations prepared by the PAL3 platform

The performance of automated calibrations was evaluated based on linearity, accuracy, and precision.

The linearity for all 73 analytes met the stringent criterion of $R^2 \geq 0.99$ with a minimum of five calibration points (four points for 10:2 FTCA and 8:2 FTCA). Figure 2 illustrates the calibration of PFNA and Figure 3 shows MRM overlay data for its surrogate and ISTD. The surrogate recoveries across the linearity range were well within 70 to 130%, and the ISTD response RSD was ≤20%.

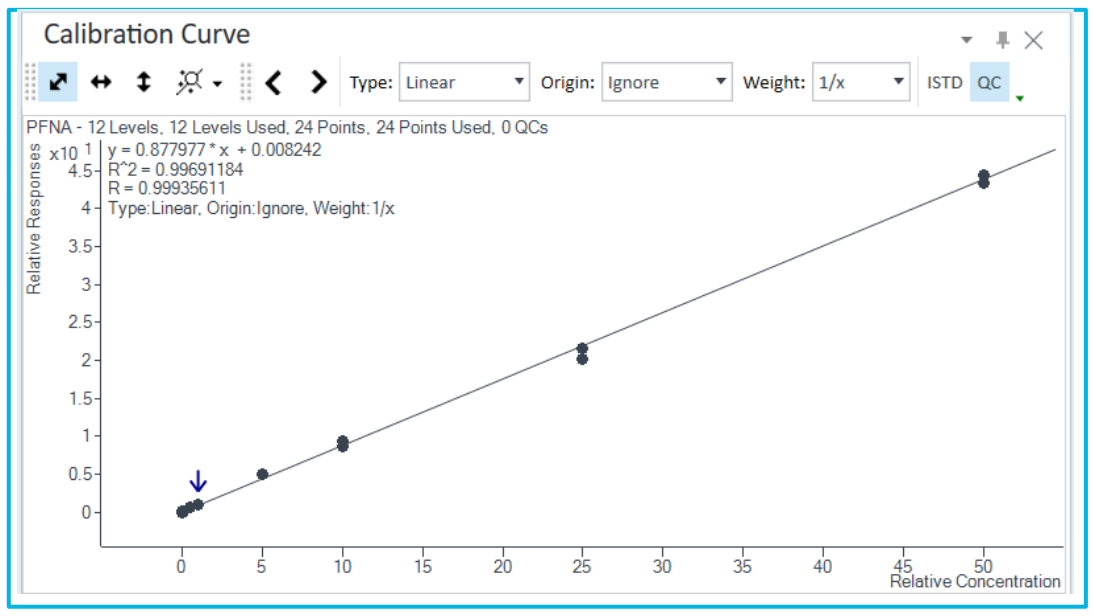

Figure 2: Linearity of PFNA across the full calibration range (Levels 1 to 12)

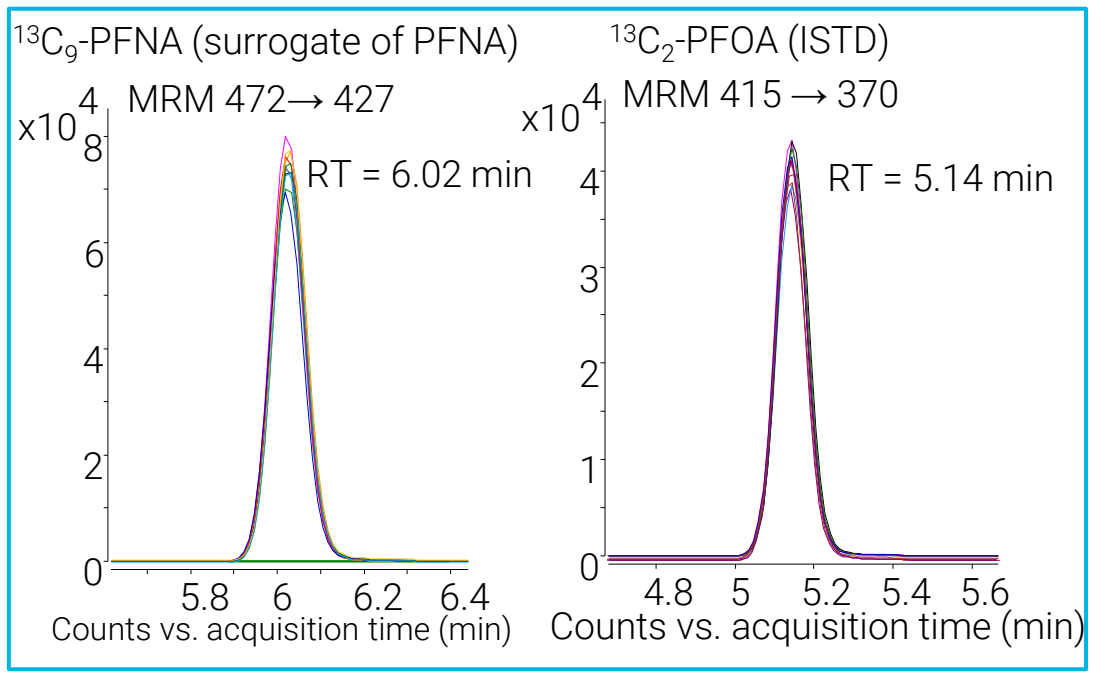

Figure 3: MRM Overlay of $^{13}C_9$-PFNA-surrogate (Left) and $^{13}C_2$-PFOA-ISTD (right) across 12 calibration levels

Sensitivity

Calculated MDL (MDL_{cal}) was derived from the U.S. FDA method. For the 28 regulated PFAS analytes from U.S. FDA, $MDL_{cal} \leq 10$ ng/kg was achieved, confirming the automated workflow's capability for U.S. FDA-validated analytical performance. The method LOQ_{vali} was obtained from spiked QCs, utilizing µSPE cleanup and a dilute-and-shoot approach.

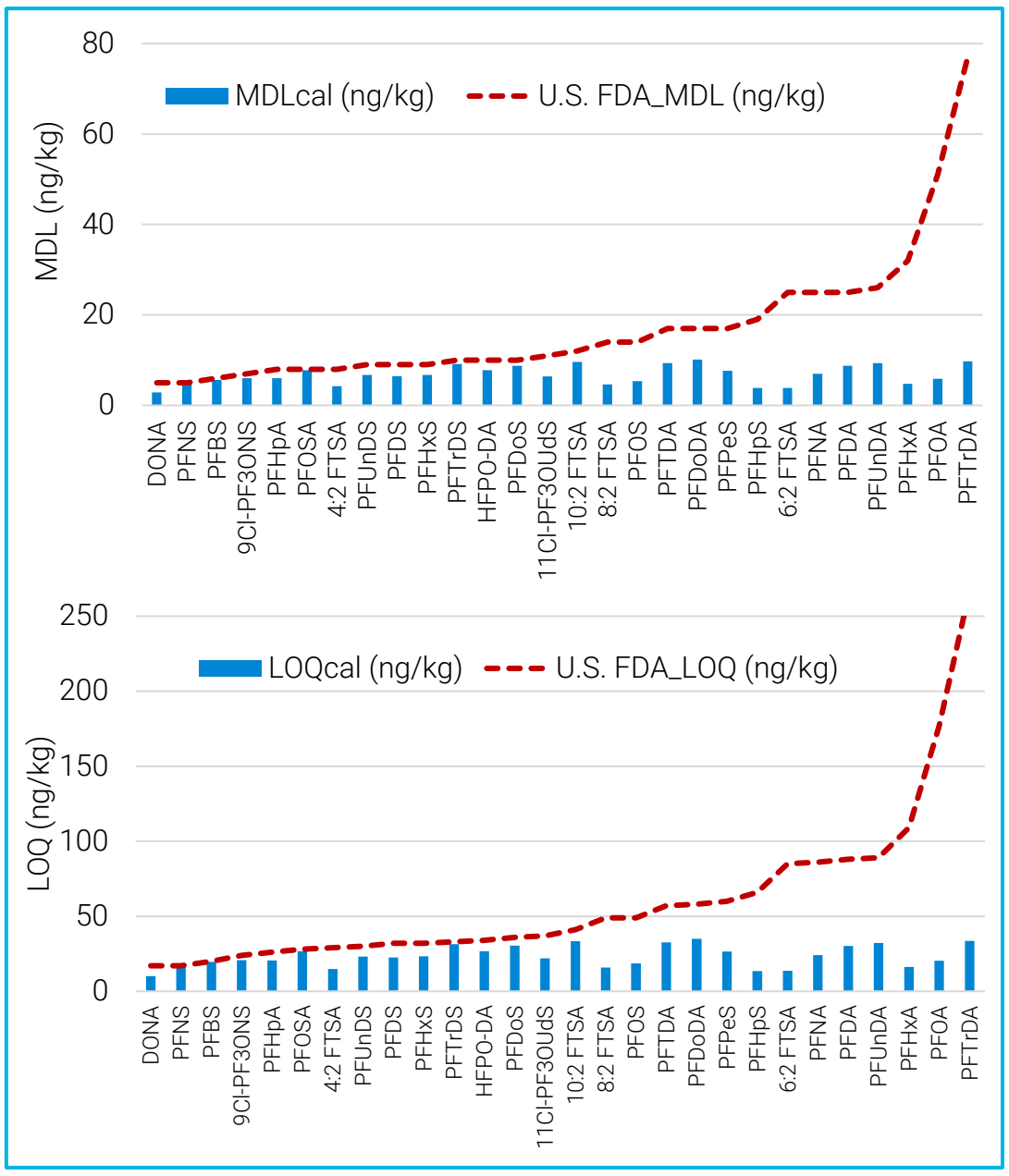

Figure 4: Sensitivity comparison for 28 mandatory PFAS targets from the U.S. FDA in terms of MDL (top) and LOQ (bottom).

Method applicability for Regulatory screening

Method LOQ_{vali} met the stringent requirements of the EU, EURL POPs, and AOAC for regulated compounds, with LOQ_{vali} values of 0.1 µg/kg for PFOS, PFNA, and PFOA, and 0.3 µg/kg for PFHxS. Due to the high positive residue of PFHxS in the shrimp matrix blank, the LOQ_{vali} for PFHxS was 0.3 µg/kg, precisely meeting the required specifications (Figure 5). For the remaining 26 mandatory targets from AOAC, the LOQ_{vali} was lower than the required LOQs (Figure 6).

Results and Discussion

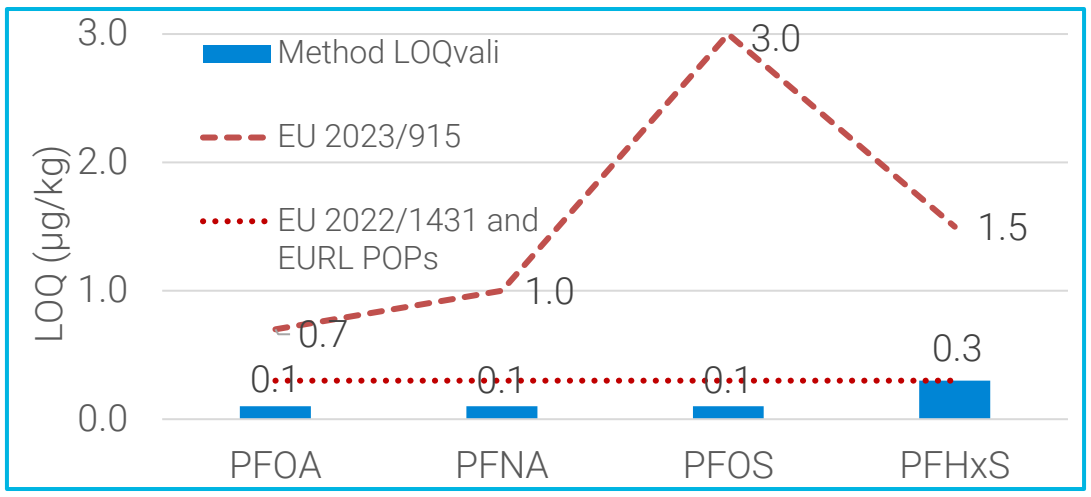

Figure 5: Comparison of method LOQ_{vali} with LOQ requirements/recommendations for PFOA, PFNA, PFOS, and PFHxS from EU and EURL POPs

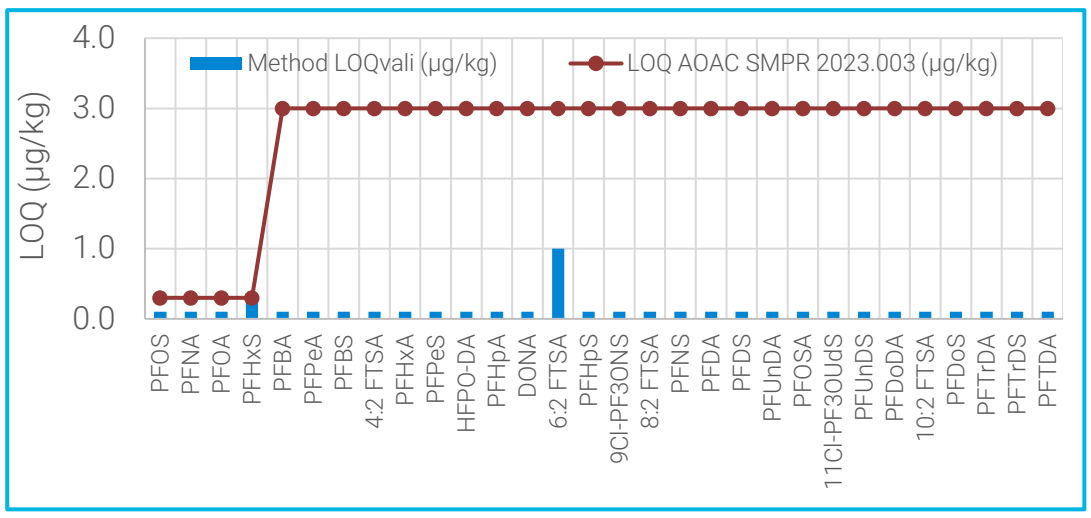

Figure 6: Comparison of method LOQ_{vali} with LOQ requirements for 30 mandatory PFAS analytes from AOAC.

Recovery and repeatability

The matrix-spiked QC recovery ranged from 65% to 135% for 58 analytes at LSQ, 65 analytes at MSQ, and 67 analytes at HSQ. Critical compounds PFOS, PFNA, PFOA, and PFHxS had MSQ recoveries within 80% to 120% (Figure 7). Over 93% of targets achieved recovery repeatability (RSD_r) of ≤19% (n=3), meeting the guidelines set by EURL POPs, U.S. FDA, and AOAC.

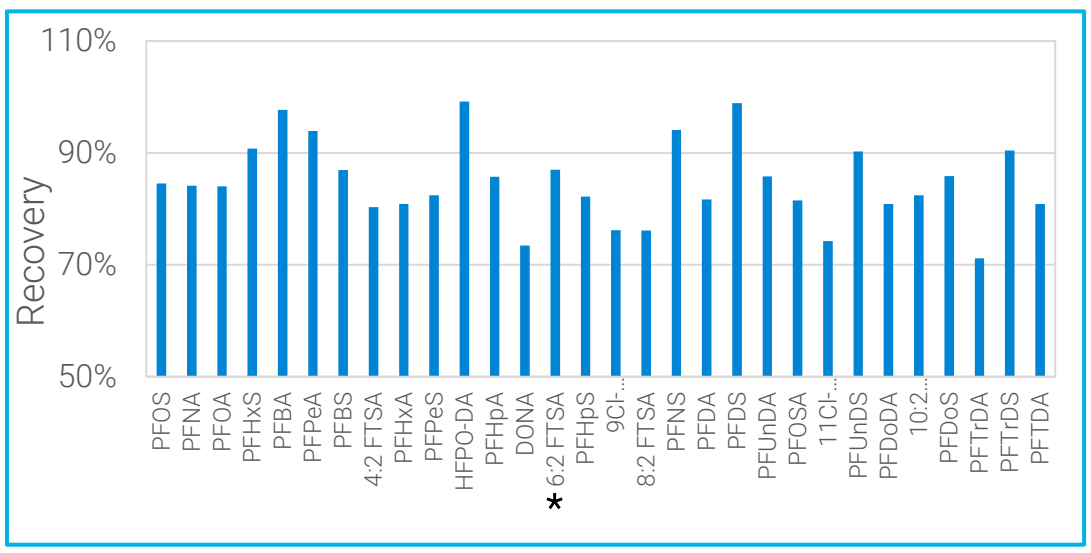

Figure 7: Distribution of MSQ recovery for mandatory PFAS analytes from EU, EURL POPs, and AOAC(*HSQ for 6:2 FTSA)

Interbatch reproducibility

The method reliability was confirmed by achieving a recovery reproducibility (RSD_R) of ≤ 20% at the MSQ level across three separate batch preparations for 68 out of 73 targets (93%), demonstrating highly reliable analytical results for most PFAS analytes using this fully automated system. Meanwhile, the 30 regulated PFAS compounds achieved an RSD_R of ≤ 12% (Figure 8).

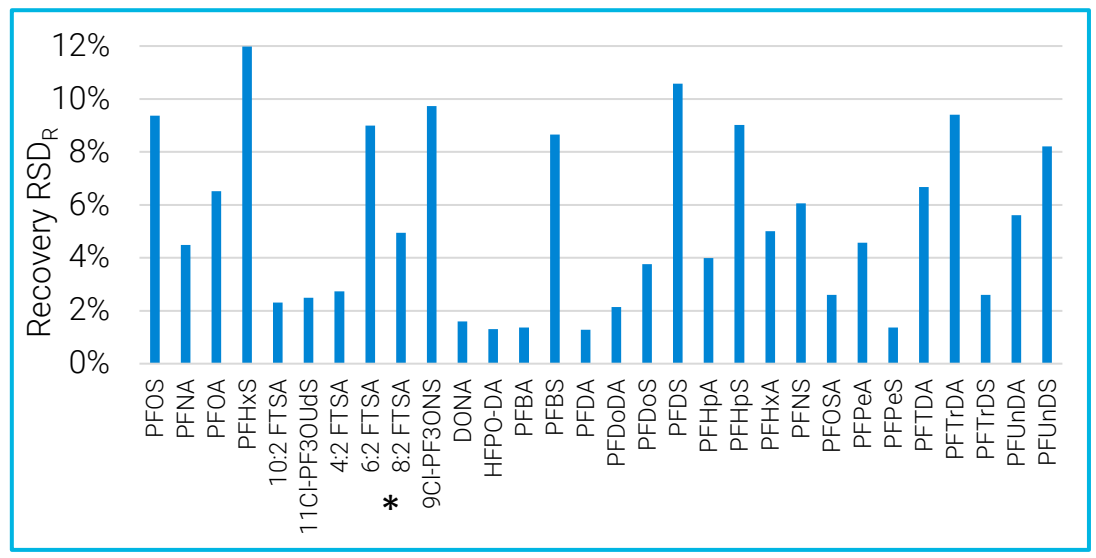

Figure 8: Interbatch recovery reproducibility at MSQ (0.3 µg/kg) for the 30 regulated PFAS (*HSQ was used to calculate RSD_R for 6:2 FTSA).

Conclusions

- The integrated system allows sample preparation and data analysis to run in parallel, offering a streamlined workflow and improving productivity for routine laboratory operations.
- The automated workflow significantly reduces manual intervention, minimizing human error and enhancing the precision of the analysis.
- The integration of automated sample preparation techniques with the highly sensitive 6495D LC/TQ ensures consistent and reproducible results, which are critical for regulatory compliance.
- The workflow demonstrated excellent analytical performance meeting the stringent regulatory requirements and recommendations for PFAS in seafood matrices set by the U.S. FDA, EU, EURL POPs, and AOAC.

References

1 For more details, refer to the Agilent publication titled "A Fully Automated Workflow for PFAS Analysis in Seafood for Regulatory Screening" (Publication Number: 5994-8011EN).

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