Application Note

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Quantitative PFAS Analysis in Cosmetics Using the CTC PAL3 Series 2 RTC Autosampler with the 6495D Triple Quadrupole LC/MS System

Automation for PFAS screening in liquid foundations, lipsticks, and mascaras

Suitable for Agilent
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Abstract

This application note presents a fully automated, end-to-end workflow for the quantitative determination of per- and polyfluoroalkyl substances (PFAS) in cosmetic products, including liquid foundations, lipsticks, and mascaras. The workflow integrates a CTC PAL3 Series 2 RTC autosampler with an Agilent 6495D triple quadrupole LC/MS system to streamline calibration preparation, solvent extraction, micro-solid-phase extraction (micro-SPE) cleanup, and LC/MS analysis within a single automated platform. Method performance was assessed for 74 PFAS compounds, including substances regulated under EU Cosmetic Regulation, EU Persistent Organic Pollutants (POPs) Regulation, and Korean Ministry of Food and Drug Safety (MFDS) Guidelines for cosmetic products. The combined high-sensitivity ion source and triple quadrupole design enabled low method detection limits and validated limits of quantitation (LOQ_{vali}) of 2.5 µg/kg for 64 analytes, even in challenging cosmetic matrices. Matrix-spiked recoveries demonstrated consistent accuracy, with recoveries of 89 to 115% and relative standard deviations below 5% for key PFAS compounds. These results demonstrate that the integrated PAL3 Series 2 RTC autosampler and Agilent 6495D LC/TQ system deliver a robust, reproducible, and high-throughput solution for routine PFAS analysis in cosmetics, supporting reliable regulatory testing while reducing manual intervention and laboratory workload.

Introduction

PFAS are a diverse group of synthetic compounds characterized by highly stable carbon–fluorine bonds, which confer exceptional chemical resistance and persistence.[1] In cosmetics, PFAS may be added intentionally to provide a desired functionality or effect, such as emulsifying, film-forming, and water-resistant capabilities.[2,3] Unintentional PFAS contamination can also occur through raw material impurities, manufacturing equipment, processing aids, and environmental cross-contamination. As a result, consumers may be exposed to PFAS in cosmetics via dermal, ocular, and incidental oral routes, and these substances ultimately enter the environment and food chain over their lifecycle.

Regulatory agencies worldwide have begun tightening oversight of PFAS in cosmetics. Within the European Union, Regulation (EC) No 1223/2009 has restricted a wide range of PFAS in cosmetic products, aligning cosmetic safety requirements with broader chemical risk management strategies.[4] In parallel, various PFAS are regulated under the EU POPs Regulation, which establishes strict limits for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) for unintentional trace contaminants in substances, mixtures, and articles.[5] South Korea added five PFAS and their salts to restricted substances list for cosmetics in 2024[6]; France has implemented a full ban[7]; Canada has proposed a broad prohibition[8]; and the U.S. FDA, under the Modernization of Cosmetics Regulation Act (MoCRA), evaluated PFAS safety in cosmetics, with report released in December 2025.[9] These combined regulatory and industry initiatives are driving an increased need for robust analytical tools capable of detecting PFAS at trace levels in complex cosmetic matrices.

This application note demonstrates a fully automated workflow for the quantitative analysis of 74 PFAS compounds in cosmetic samples using a PAL3 Series 2 RTC autosampler coupled with an Agilent 6495D triple quadrupole LC/MS system. Seven commercially available cosmetic products across multiple brands—including liquid foundations, lipsticks, and mascaras—were evaluated. The workflow integrates automated calibration, solvent extraction, and micro-SPE cleanup, delivering high-throughput performance with excellent sensitivity, precision, and recovery to enable reliable PFAS screening in complex cosmetic matrices.

Experimental

Chemicals and reagents

LC/MS-grade ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure LC/MS-grade acetonitrile (ACN), methanol (MeOH), and water (H_2O) were obtained from Agilent Technologies.

Native and isotopically labeled PFAS standards were sourced from Wellington Laboratories Inc. (Guelph, ON, Canada) and Toronto Research Chemicals (Toronto, ON, Canada). Standards were supplied as individual stock solutions, mixed solutions, or powder form, depending on the compound.

Consumables

Consumables are a critical consideration for trace-level PFAS analysis, as material composition can contribute to background contamination and result in false-positive findings. To ensure data integrity, all consumables used in this study were evaluated and verified for PFAS suitability, following the consumable selection and testing approach described in previously published application notes.[10,11]

Instrumentation

An integrated PAL3 Series 2 RTC autosampler coupled with a 6495D LC/TQ (Figure 1) was employed for the fully automated workflow of PFAS quantitation from cosmetic matrix in this study.

The PAL3 platform was equipped with various tools and modules, providing the necessary capabilities to achieve its designated functions. The following tools and modules were used in this study:

- Two PAL park stations with three liquid syringe tools, dilutor tool, micro-SPE tool, and LC/MS tool
- Vortex Mixer
- Centrifuge
- Dilutor Multi
- Tray Cooler (for 2/10/20 mL vials)
- Tray Holders with rack R60 (for 10/20 mL vials)
- Micro-SPE Tray (for 2 mL vials and micro-SPE cartridges)
- Solvent Module and Fast Wash Module
- LC Injection Valve

The LC Injection Valve was configured on the PAL3 platform, and all liquid syringes were cleaned using a Fast Wash Module. All solvent tubing used in the PAL3 platform were PFAS free. Extra modules and tools can be added to meet specific sample preparation needs.

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

An Agilent 1290 Infinity II UHPLC system equipped with high-speed pump (part number G7120A) and multicolumn thermostat (part number G7116B) was used for chromatographic separation. A 6495D LC/TQ equipped with an Agilent Jet Stream technology ion source (AJS) was used for compound acquisition in negative/positive ionization mode.

The integrated PAL3 Series 2 RTC autosampler and 6495D LC/TQ, controlled by Agilent MassHunter acquisition software for LC/MS systems (version 12.2), was operated following the condition and parameters displayed in Table 1. Data analysis was conducted using Quantitative Analysis software, version 12.1

Table 1. Instrument operating conditions and MS source parameters.

Agilent 6495D MS parameters	
Ion Source	AJS ESI
iFunnel Mode	Standard
Polarity	Negative and positive
Q1 and Q3 Resolution	Unit
Cycle Time	720 ms
Gas Temperature	250 °C
Gas Flow	11 L/min
Nebulizer	25 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	11 L/min
Capillary (Negative/Positive)	3,000 V
Nozzle Voltage	0 V

CTC PAL3 Series 2 RTC Autosampler and Agilent 1290 Infinity II LC Conditions	
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 × 100 mm, 1.8 µm, 1200 bar pressure limit (p/n 959758-902)
UHPLC Guard	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200 bar pressure limit, UHPLC guard (p/n 821725-901)
Column Temperature	55 °C
Injection Volume	2 µL
PAL Tray Cooler Temperature	5 °C
Mobile Phase A	5 mM Ammonium acetate in water
Mobile Phase B	100% Methanol
Mobile Phase Flow Rate	0.4 mL/min
Timetable	Time/min %A %B 0.00 85 15 1.00 85 15 1.50 45 55 5.50 30 70 7.00 20 80 12.00 0 100 14.40 0 100 14.50 85 15
Stop Time	14.5 minutes
Post-Time	2.5 minutes
PAL Injection Needle Wash	Multiwash
Wash Solvent 1 (S1)	15:85 Methanol:water
Wash Solvent 2 (S2)	1:1 Acetonitrile:2-propanol

Workflow procedure

Automated calibration preparation: A total of 12 calibration levels were automatically prepared using the PAL3 Series 2 RTC platform, covering a concentration range of 1 to 50,000 ng/L for 74 PFAS analytes. Each calibration level included a constant concentration of 34 surrogate standards and three internal standards (ISTDs). A calibration blank was also prepared by spiking only the surrogates and ISTDs into the solvent mixture (MeOH/H_2O, 80:20 v/v), without any target analytes.

Upon completion of the calibration setup, the autosampler automatically initiated the worklist to begin analysis of the calibration standards.

Automated sample preparation methodology: Waterproof cosmetic products were reported to contain PFAS intentionally in some research.[2,12] In this study, seven cosmetic products from various brands—including liquid foundations, lipsticks, and mascaras—were locally sourced and selected for analysis. A liquid foundation exhibiting trace-level background PFAS (based on preliminary screening) was designated as the quality control (QC) sample to evaluate workflow performance. The remaining six products were analyzed as unknown samples to assess method applicability for PFAS screening in cosmetic matrices.

Approximately 0.20 ± 0.01 g of each cosmetic sample was manually weighed into a 10 mL sample vial and placed into the PAL3 autosampler tray. All subsequent preparation steps—including solvent extraction and extract cleanup—were fully automated by the PAL3 Series 2 RTC platform as illustrated in Figure 2.

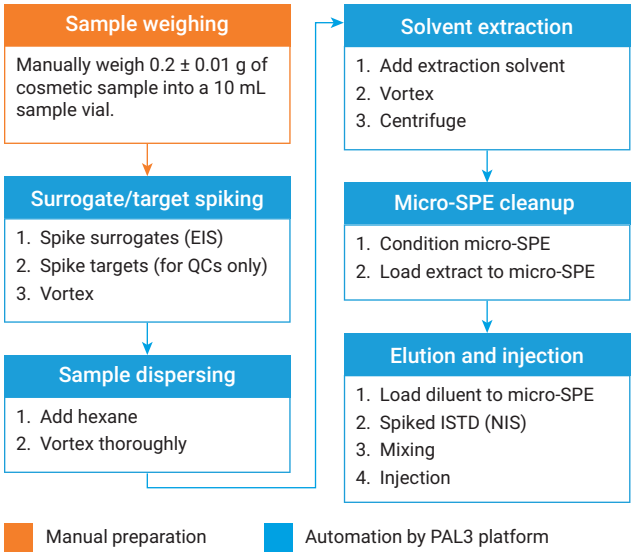

Figure 2. Automated sample preparation for cosmetic samples by the CTC PAL3 Series 2 RTC autosampler. (**Note:** 50 times dilution was involved due to the entire sample extraction process).

Initially, each sample vial was spiked with a surrogate standard mixture, used as extracted internal standards (EIS). For QC samples, a target compound mixture was also added. Hexane (0.2 mL) was then introduced as a nonpolar dispersing solvent, followed by vigorous vortex mixing to ensure thorough sample dispersion. QC samples were prepared at three concentration levels: low spike quantity (LSQ, 2.5 µg/kg), medium spike quantity (MSQ, 25 µg/kg), and high spike quantity (HSQ, 250 µg/kg). Each QC level was processed in triplicate using the PAL3 platform. A procedural blank (PB), prepared without sample matrix, was included to monitor potential PFAS contamination during the entire workflow. In addition, a matrix blank (MB) was prepared to evaluate background PFAS levels inherent in the cosmetic matrix.

Subsequently, 4.8 mL of solvent mixture (MeOH/ACN, 50:50) was added to the sample vial using the PAL Dilutor Module, followed by vigorous vortexing at 2,000 rpm in pulse mode. The sample was then centrifuged at 4,500 rpm for 5 minutes. A 100 µL aliquot of the supernatant was used to condition the micro-SPE cartridge and drained by air blowing. A second 100 µL of the supernatant was loaded onto the cartridge and passed through, followed by an additional 95 µL of diluent to further elute the cartridge. All eluents were collected into a 250 µL polypropylene injection vial equipped with a pre-slit cap.

Non-extracted internal standards (NIS, 5 µL) were then added to the injection vial and mixed prior to analysis. The LC/MS tool on the PAL3 platform directly injected 2 µL of the prepared extract into the Agilent 6495D LC/TQ system for data acquisition. Upon completion of injection, the system automatically performed post-wash steps and prepared the next sample. This online, fully automated workflow enables continuous operation, minimizes manual intervention, and increases overall laboratory productivity by eliminating idle time between runs.

Results and discussion

The analytical performance of the automated workflow—including MDLs, LOQ_{vali}, matrix-spiked recoveries, and relative standard deviations (% RSD)—is summarized in Table 2. Compounds highlighted in blue are regulated or restricted under the EU Cosmetic Regulation, the EU POPs Regulation, and the Korean MFDS guidelines on PFAS in cosmetics.

Workflow sensitivity

Achieving ultra-low background levels is critical for PFAS analysis, as contamination introduced by the analytical system, solvents, or sample preparation steps can directly impact method sensitivity. To evaluate potential background contributions from the entire automated workflow, a procedural blank (PB) was analyzed and compared with a solvent blank and a low-spike quantity (LSQ) sample. Figure 3 presents a total ion chromatogram (TIC) overlay of the solvent blank, procedural blank, and LSQ.

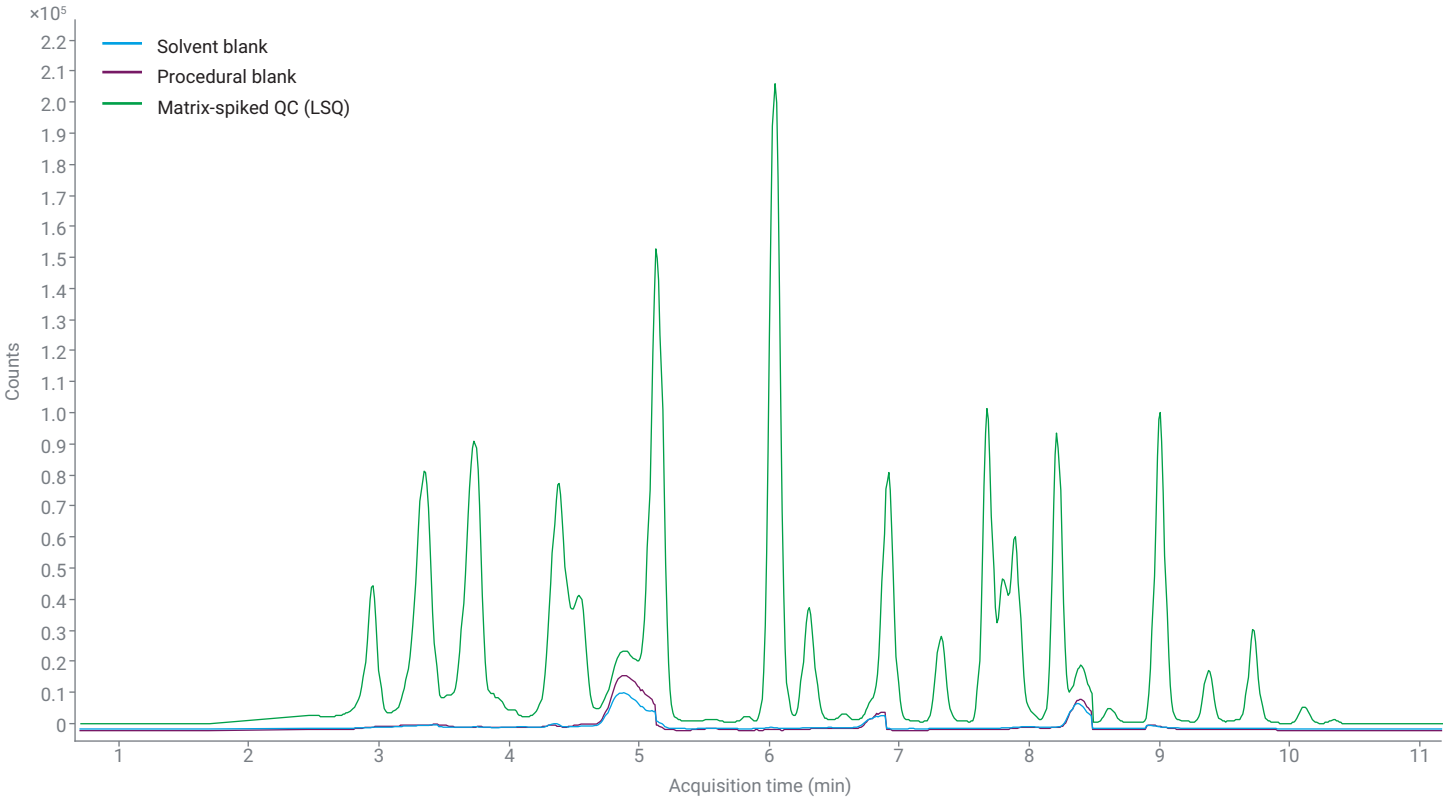

Figure 3. TIC overlay of solvent blank, procedural blank, and LSQ.

Compared to the LSQ sample, both solvent blank and procedural blank exhibited negligible PFAS background signals, demonstrating that the automated sample preparation workflow—together with the dedicated chemicals and PFAS-tested consumables—effectively minimizes contamination. These results confirm that the platform is well suited for trace-level PFAS analysis in cosmetic matrices and support the reliability of the calculated MDLs and LOQ_{vali} values.

MDLs were calculated by multiplying the standard deviation of nine replicates of matrix spiked QCs by a factor of 3.14. LOQ_{vali} were established from cosmetic sample spiked QC levels (LSQ, MSQ, HSQ) while simultaneously meeting the general criteria for compound identification and analytical performance.[13] As summarized in Table 2, 72 out 74 compounds showed MDLs < 1 µg/kg; MDLs for 6:2 FTCA and 8:2 FTCA were not determined (N.D.) due to matrix interference affecting compound identification.

Table 2. Analytical performance summary.

No.	Compound Name	CAS No.	MDL (µg/kg)	LOQ_{vali} (µg/kg)	Recovery at LOQ (%)	RSD of Recovery at LOQ (%)
1	PFBPA	52299-24-8	0.391	25	98	10
2	PFBA	375-22-4	0.186	2.5	101	7
3	PFMPA	377-73-1	0.104	2.5	99	5
4	PFPeA	2706-90-3	0.326	2.5	101	6
5	3:3 FTCA	356-02-5	0.477	2.5	122	6
6	PFBS	375-73-5	0.162	2.5	92	4
7	PFHxPA	40143-76-8	0.383	0.010*	85	5
8	PFMBA	863090-89-5	0.180	2.5	85	2
9	Cl-PFHxPA	N/A	0.361	0.05*	110	5
10	PFEESA	113507-82-7	0.183	2.5	91	2
11	NFDHA	151772-58-6	0.277	2.5	48	4
12	4:2 FTSA	757124-72-4	0.252	2.5	95	2
13	PFHxA	307-24-4	0.300	2.5	100	4
14	PFPeS	2706-91-4	0.270	2.5	75	3
15	HFPO-DA	13252-13-6	0.292	2.5	91	6
16	FBSA	30334-69-1	0.147	2.5	98	2
17	P5MeODIOXOAc	1190931-41-9	0.613	2.5	104	10
18	PFHpA	375-85-9	0.280	2.5	92	3
19	PFHxS	355-46-4	0.171	2.5	86	2
20	DONA	919005-14-4	0.158	2.5	91	2
21	PFOPA	40143-78-0	0.219	0.01*	120	4
22	5:3 FTCA	914637-49-3	0.308	2.5	95	5
23	6:2 FTUCA	70887-88-6	0.276	2.5	92	4
24	6:2 FTCA	53826-12-3	N.D.	0.5*	120	8
25	4-PFecHS	646-83-3	0.209	2.5	80	3
26	6:2 FTSA	27619-97-2	0.230	2.5	91	3
27	PFOA	335-67-1	0.224	2.5	97	3
28	PFHpS	375-92-8	0.179	2.5	82	4
29	MeFBSA	68298-12-4	0.536	2.5	95	7
30	FHxSA	41997-13-1	0.342	25	70	6
31	PFNA	375-95-1	0.238	2.5	89	4
32	PFOS	1763-23-1	0.294	2.5	89	4
33	8:2 FTUCA	70887-84-2	0.293	2.5	92	4
34	PFDPA	52299-26-0	0.402	250	129	9
35	7:3 FTCA	812-70-4	0.360	2.5	81	8
36	HFPO-TA	13252-14-7	0.301	2.5	85	5
37	8:2 FTCA	27854-31-5	N.D.	0.5*	117	7
38	9Cl-PF3ONS	756426-58-1	0.195	2.5	86	3
39	FOSAA	2806-24-8	0.124	2.5	110	6
40	8:2 FTSA	39108-34-4	0.233	2.5	90	3
41	PFNS	68259-12-1	0.281	2.5	85	4
42	PFDA	335-76-2	0.341	2.5	91	5
43	8:3 FTCA	34598-33-9	0.496	2.5	106	8
44	N-MeFOSAA	2355-31-9	0.421	2.5	91	6
45	MeFHxSA	68259-15-4	0.395	2.5	96	5
46	PFDS	335-77-3	0.336	2.5	81	6
47	PFUnDA	2058-94-8	0.354	2.5	86	8
48	N-EtFOSAA	2991-50-6	0.346	2.5	91	6
49	PFOSA	754-91-6	0.261	2.5	83	3
50	10:2 FTUCA	70887-94-4	0.295	2.5	92	4
51	11Cl-PF3OUdS	763051-92-9	0.326	2.5	89	4
52	PFUnDS	749786-16-1	0.195	2.5	71	5
53	PFDoDA	307-55-1	0.144	2.5	91	6
54	10:2 FTSA	120226-60-0	0.477	2.5	74	4
55	10:2 FTCA	53826-13-4	0.458	0.5*	116	5
56	6:6 PFPi	40143-77-9	0.260	2.5	78	10
57	PFDoS	79780-39-5	0.586	2.5	84	7
58	PFTrDA	72629-94-8	0.389	2.5	95	7
59	N-MeFOSA	31506-32-8	0.560	2.5	71	8
60	FDSA	N/A	0.409	25	67	5
61	MeFOSE	24448-09-7	0.506	2.5	91	9
62	PFTrDS	791563-89-8	0.606	2.5	72	9
63	6:2 diPAP	57677-95-9	0.262	2.5	94	4
64	PFTDA	376-06-7	0.377	2.5	85	8
65	6:8 PFPi	610800-34-5	0.619	2.5	30	8
66	N-EtFOSA	4151-50-2	0.372	2.5	90	5
67	EtFOSE	1691-99-2	0.314	2.5	85	6
68	6:2/8:2 diPAP	943913-15-3	0.446	2.5	46	9
69	8:8 PFPi	40143-79-1	0.479	2.5	31	3
70	PFHxDA	67905-19-5	0.311	2.5	92	6
71	8:2 diPAP	678-41-1	0.251	2.5	90	2
72	PFODA	16517-11-6	0.242	2.5	73	5
73	diSAmPAP	2965-52-8	0.265	2.5	101	4
74	Tetraconazole	112281-77-3	0.666	2.5	115	14

* LLOQ was defined.

In this context, GC/TQ can serve as a complementary technique to LC/TQ for the analysis of FTCAs in cosmetic matrix.[13] A total of 64 analytes met LOQ at 2.5 µg/kg, including key regulated compound PFOA and PFOS, which are below the thresholds specified under EU POPs Regulation for unintentional trace contaminant limits ≤ 25 µg/kg in substances, mixtures, and articles. These results demonstrate that the integrated PAL3 Series 2 RTC autosampler combined with an Agilent 6495D LC/TQ system provides the sensitivity required for trace-level PFAS determination in cosmetic matrices. Higher LOQs were obtained for PFBPA, FHxSA, PFDPA, and FDSA due to positive residue determined in MB. For six compounds exhibiting poor performance (recovery < 30%) in cosmetic matrices, the lowest level of quantitation (LLOQ) was defined in neat solvent instead.

Recovery and reproducibility

Matrix-spiked QC recoveries were used to evaluate the extraction efficiency and quantitative accuracy of the automated sample preparation workflow for PFAS analysis in cosmetic matrices. Recoveries for each QC level were calculated as mean percent recoveries (n = 6; three technical preparations with two injections per preparation). Across all QC levels (LSQ, MSQ, and HSQ), more than 82% of the 74 target analytes achieved recoveries within the range of 65–135%, demonstrating the high accuracy and extraction efficiency of the automated workflow for PFAS in cosmetics.

Notably, key regulated compounds highlighted in blue in Table 2—including PFHxS, PFOS, PFNA, PFOA, PFDA, and tetraconazole—exhibited LSQ recoveries ranging from 86 to 115%, which fall well within the commonly accepted recovery range of 80–120% for trace-level analysis. These results confirm that automated solvent extraction followed by micro-SPE cleanup provides effective and reliable sample preparation for PFAS determination in complex cosmetic matrices.

The entire workflow reproducibility was evaluated based on the relative standard deviation (RSD) of spiked QC recoveries (n = 6, three technical preparation and two injections per preparation) within a batch for LSQ, MSQ, and HSQ. As shown in Table 2, more than 90% of the targets achieved RSD < 15% across three QC levels, meeting the general analytical performance criteria for trace analysis of PFAS.[14] These results highlighted the excellent reproducibility of the automated workflow for PFAS analysis using the integrated PAL3 autosampler coupled with Agilent 6495D LC/TQ.

Cosmetic sample analysis

According to the OECD report (published in 2024) and other recent studies, a variety of cosmetic products—including shampoos, eye makeup, facial cleansers, lipsticks, and lip glosses—may contain PFAS that provide functional or performance-enhancing properties.[2,12] In this study, the native concentrations of PFAS in liquid foundations, lipsticks, and mascaras were evaluated to validate the applicability and reliability of the fully automated workflow for real-world cosmetic samples.

The results showed that multiple PFAS compounds belonging to the PFCA, PFECA, PFPA, PFSA, FASA, and FASAA classes were detected above MDLs in both mascara (Figure 4A) and lipstick samples (Figure 4B). These findings demonstrate that the automated workflow enables effective extraction and quantitation of PFAS in complex cosmetic matrices. The generated data provides practical and actionable information for cosmetic manufacturers and testing laboratories to monitor PFAS residues in processed and finished cosmetic products, supporting quality assurance and regulatory compliance.

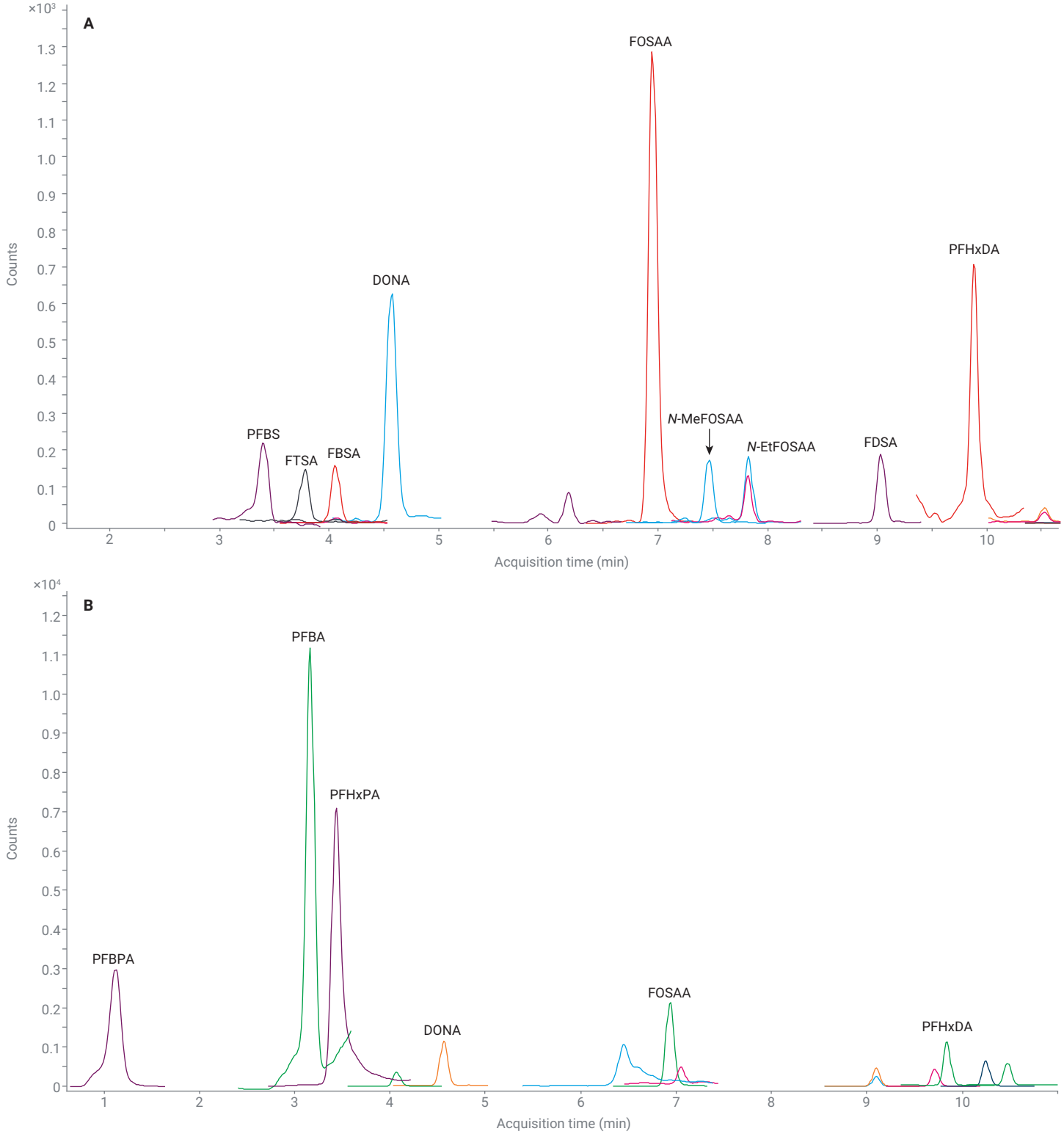

Figure 4. (A) MRM chromatogram of unspiked mascara sample extract. (B) MRM chromatogram of unspiked lipstick sample extract.

Conclusions

A fully automated, end-to-end workflow for PFAS analysis in cosmetic products was developed using the integrated CTC PAL3 Series 2 RTC autosampler coupled with the Agilent 6495D triple quadrupole LC/MS system. The workflow transfers traditionally labor-intensive steps—including calibration preparation, sample extraction, micro-SPE cleanup, and LC/MS analysis—to an integrated robotic platform, significantly reducing manual handling during sample preparation. The successful implementation of automated micro-SPE cleanup further streamlines the workflow and eliminates time-consuming manual operations.

Excellent analytical performance was achieved across key metrics, including method detection limits, validated limits of quantitation, matrix-spiked recoveries, and method reproducibility, demonstrating the system's capability to generate high-quality and reliable analytical data. By minimizing manual intervention, the automated workflow reduces the potential for human error while improving analytical precision and consistency.

The integrated platform enables parallel sample preparation and data acquisition, resulting in improved throughput and operational efficiency for routine laboratory testing. Combined with the high sensitivity and robustness of the Agilent 6495D LC/TQ system, the automated workflow provides a practical and reproducible solution for trace-level PFAS determination in complex cosmetic matrices, supporting routine monitoring, regulatory compliance, and consumer product safety.

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