

Automated Sample Preparation Using the PAL3 RTC System for EPA 8270E Semivolatile Organic Analysis by GC/TQ

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Abstract

Growing demand for SVOC analysis at laboratories reveals the analysis bottlenecks, e.g., a lack of practical sample preparation experience, low sample throughput, and high consumption of chemical solvents. Online automated sample preparation is gaining attention as a solution to address these laboratory challenges. An automated workflow solution for quantitation of SVOCs in water samples, combining calibration, sample preparation, and detection was developed on Agilent gas chromatography/triple quadrupole mass spectrometer (GC/TQ) using the PAL3 robotic tool change (RTC) system in this study.

Introduction

Semivolatile organic compounds (SVOCs) analysis is widely implemented at analytical laboratories. The SW-846 Compendium EPA 8270 method provides guidelines on conditions and quality control (QC) checks to ensure successful analysis of SVOCs using gas chromatography/ mass spectrometer (GC/MS). Furthermore, the EPA 8270E method, which was released in 2018, includes GC/MS with an MS/MS detector. The MS/MS selectivity ensures better lower limit of quantitation (LLOQ) thus delivers reliable analysis results¹.

Growing demand for SVOC analysis at laboratories reveals the analysis bottlenecks, e.g., a lack of practical sample preparation experience, low sample throughput, and high consumption of chemical solvents. Online automated sample preparation is gaining attention as a solution to address these laboratory challenges. This application note presents a proofof-concept on a novel automated sample preparation workflow modified based on EPA 3510C method² (that is a procedure for isolating organic compounds from aqueous samples), followed by Agilent gas chromatography/triple quadrupole mass spectrometry (GC/TQ) analysis according to EPA 8270E. The novel sample preparation workflow involved liquid-liquid extraction (LLE) of water samples using dichloromethane (DCM) at two specific pH conditions. The combination of these two extracts was then analyzed by the GC/TQ. Surface water samples were prepared automatically and online by the PAL3 robotic tool change (RTC) system prior to GC/TQ analysis. Likewise, working calibration standards, method blank samples, and matrix-spiked QC samples were also prepared automatically by PAL3. Then, 100 analytes of SOVCs were tested and evaluated in this study.

Experimental

Instrumentation

An Agilent 7000 series triple quadrupole mass spectrometer was coupled to an Agilent 8890 GC with a back split/splitless inlet (SSL) and splitless inlet liner (part number 5190-2293, 900 μ L, single taper, wool, Ultra). The ion source was equipped with a 9 mm diameter drawout lens (part number G3870-20449). The system was autotuned using the etune algorithm embedded in the Agilent MassHunter software version 10.1. Table 1 contains the conditions and operating parameters for both GC and MS.

A PAL3 Series II RTC system (Figure 1) was used as a liquid handling platform for the calibration/sample preparation and injection onto the GC/TQ system in the study. The PAL3 system was equipped with a vortex mixer and various tray holders and racks (for 2 mL, 10/20-mL vials). Various liquid syringe tools were used fitting different volumes of PTFE coated smart syringe. Solvent module and fast wash module were also configured with the PAL3 system. The integrated PAL3-GC/TQ system was controlled by Agilent MassHunter Workstation GC/MS Data Acquisition 10.1, offering an easy user experience with a single software system. The operating window is captured in Figure 2.

Table 1. Agilent 8890 GC and 7000 series instrument parameters.

GC Conditions							
Injection Volume	2.0 μL						
Column	Agilent J&W DB-UI8270D, 30 m x 250 μm x 0.25 μm (part number 122-9732)						
Inlet Temperature	250 °C						
Injection Mode	Pulsed splitless						
Carrier Gas	Helium, constant flow, 1.2 mL/min						
Transfer Line Temperature	320 °C						
Oven Program	40 °C hold for 0.5 minutes						
	25 °C/minute to 260 °C, hold for 9.3 minutes						
	5 °C/minute to 280 °C, hold for 13.3 minutes						
	25 °C/minute to 320 °C, hold for 18.9 minutes						
MS Parameters							
Acquisition Mode	dMRM						
Ion Source Temperature	320 °C						
Quadrupole Temperature	150 °C						
Ionization	El mode						
EMV Mode	Gain factor (10)						
Solvent Delay	1.5 minutes						
Cycles Per Second	15						



Figure 1. PAL3 RTC system on an Agilent 7000 series GC/TQ.



Figure 2. The integrated PAL3-GC/TQ system operating window by MassHunter software 10.1.

Calibration preparation by the PAL3 system

A total of 10 calibration levels were automatically prepared by the PAL3 system for the experimental work in this study. The automated procedure is illustrated in Figure 3. A stock standard solution containing 100 analytes based on the EPA 8270E target list was manually prepared at the concentration of 300 µg/mL (ppm) in DCM. Agilent semi-volatiles internal standard (part number ISM-563-1, 2000 ppm) was manually diluted by DCM at 40 ppm as ISTD. Both stock standard solution and ISTD were loaded onto the predefined vial positions according to the method parameters. As indicated in Figure 3, the 3 intermediate standard solutions were prepared from the stock standard solution by the PAL3 system, and then were used to prepare the 10 levels of working calibration standards from 0.01 to 20 ppm. Lastly, 5 µL of ISTD were then spiked into each vial of calibration standards at a final concentration of 2 ppm.

Sample preparation by the PAL3 system

Surface water was used as a sample to test out the performance of automated sample preparation by the PAL3 system. According to EPA 3510C, manual liquid-liquid extraction (LLE) using a separatory funnel is defined for aqueous samples, which involves large sample size and high chemical/reagents consumption. Based on the LLE described in EPA 3510C, an automated workflow was modified and developed on the PAL3 system in this study. The automated sample preparation workflow is shown in Figure 4.

1 g of NaCl was manually weighed into a 20 mL vial followed by adding 15 mL of the water sample. The vial was capped securely and placed on the sample rack (PAL R60 rack for 10/20-mL vial). The rest of the sample preparation workflow steps were then done by the PAL3 system. The analytes from the water sample were enriched 10-fold during the workflow.



PAL Parameters

Figure 3. Automated preparation for calibration standards by the PAL3 system.



Figure 4. Automated sample preparation by the PAL3 system.

Online analysis sequence

As illustrated in Figure 5, a batch of online analysis sequence includes working calibration standards, method blank (MB), which is unspiked matrix blank, and matrix-spiked QC samples. First, 10 points of calibration standards were prepared by the PAL3 and subsequently analyzed via GC/TQ. Next, MB was prepared by the PAL3 and immediately injected into GC/TQ for quantitative analysis. In the meantime, PAL3 moved forward to the next sample preparation while GC/TQ was continually working on the analysis of MB. As a result, the integrated PAL3-GC/TQ system allowed sample preparation and sample analysis to proceed in a parallel mode. Thus, the overall lab productivity was increased through automation and eliminating waiting time between runs.

Results and discussion

Compound identification

The acquisition method including multiple reaction monitoring (MRM) transitions, collision energy, and retention time (RT) used for this study was based on the existing well-developed method from a previous application note³. Figure 6 shows a representative MRM chromatogram of the 100 analytes at 5 μ g/mL (Cal 8) prepared by the PAL3 system. The symmetric sharp peaks demonstrate the efficient chromatographic separation of targets within the retention time window.



Figure 5. Online analysis sequence on the integrated PAL3-GC/TQ system.



Figure 6. Representative MRM chromatogram for 100 analytes at 5 µg/mL (Cal 8) and ISTDs at 2 µg/mL in DCM prepared by the PAL3 system.

Initial calibration performance

Initial calibration (ICAL) performance was evaluated in terms of linearity, response factor (RF), and accuracy of calibration standards. The results are summarized in Table 2. The overall working range of the method for all analytes was determined to be 0.01 to 20 μ g/mL, while some data points for certain compounds may be deleted at the low and high ends of the calibration range to meet the method performance criteria based on EPA 8270E. In this study, 96% of compounds achieved R>0.995 (LR mode) with minimum 5 points and 97% of compounds met the accuracy requirement for each calibration level. The %RSD of RF is within 20% for all analytes, demonstrating the excellent performance done by the integrated PAL3-GC/TQ system for automated calibration preparation and acquisition analysis.

The ISTD was also assessed to determine if the method sensitivity and stability was maintained throughout the whole process. 5 μ L of ISTD mixture was added to each calibration level and matrix-spiked QC to reach the final concentration of 2 μ g/mL. The absolute RT change for ISTDs was within the regulatory recommendation of \leq 30 secs. The response of all ISTDs in the individual standard was obtained within 70 to 150% of average response throughout the final calibration range, meeting the EPA performance criteria¹.

Method sensitivity based on LLOQ

The method sensitivity was evaluated based on the LLOQ in this work. The lowest point in the ICAL is defined as LLOQ that met the performance criteria including linearity, RF, and accuracy¹. The summary of the LLOQ for all analytes is listed in Table 2. The LLOQ of 100 analytes was distributed across 0.01 to 0.5 µg/mL as shown in Figure 7. Overall, 39 out of 100 compounds obtained LLOQ \leq 0.02 µg/mL, demonstrating the excellent sensitivity of the method developed on the PAL3-GC/TQ.

Method blanks

Method blanks (MBs) must be carried out through all stages of sample preparation and analyzed for the compounds of interest as a safeguard against lab contamination caused from the sample, the reagents used, and the preparation workflow. In this study, duplicate MBs were prepared by the PAL3 system following the same method script except for the addition of analytes/surrogates. Target concentration for all compounds in MBs was obtained less than 50% of the LLOQ, although positive presence was observed for certain compounds, demonstrating that lab contamination was controlled to the desired level.

Compound Name	Quantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (µg/mL)	Recovery (%)	RSD of Recovery (n=3)
1,2,4-Trichlorobenzene	179.9 -> 109.0	0.9998	5.64	1.04	0.7%	0.01	115	4%
1,2-Dichlorobenzene	146.0 -> 111.0	0.9999	4.69	1.12	0.5%	0.01	109	5%
1,3-Dichlorobenzene	146.0 -> 111.0	0.9997	4.50	1.12	0.2%	0.01	105	5%
1,3-Dinitrobenzene	168.0 -> 75.0	0.9993	7.16	0.07	6.6%	0.2	102	12%
1,4-Dichlorobenzene	146.0 -> 111.0	0.9998	4.56	1.09	0.5%	0.01	105	8%
1,4-Dinitrobenzene	168.0 -> 75.0	0.9987	7.09	0.04	3.0%	0.2	114	4%
1-Bromo-2-nitrobenzene	156.9 -> 75.9	0.9995	6.56	0.20	0.8%	0.01	122	9%
1-Chloronaphthalene	162.0 -> 127.1	0.9988	6.88	1.45	2.7%	0.02	129	6%
1-Methylnaphthalene	142.0 -> 114.9	0.9998	6.48	1.59	0.7%	0.01	117	3%
1-Naphthylamine	143.1 -> 115.1	0.9951	7.70	0.29	4.3%	0.2	51	30%
2,2'-oxybis[1-chloropropane]	121.0 -> 77.0	0.9998	4.77	0.05	1.7%	0.02	108	16%
2,3,4,6-Tetrachlorophenol	230.0 -> 165.9	0.9980	7.72	0.08	7.5%	0.5	64	3%
2,3,5,6-Tetrachlorophenol	230.0 -> 165.9	0.9988	7.68	0.07	0.6%	0.5	60	4%
2,4,5-Trichlorophenol	195.8 -> 97.0	0.9995	6.70	0.35	10.2%	0.5	63	7%
2,4,6-Trichlorophenol	195.8 -> 97.0	0.9985	6.66	0.46	1.8%	0.05	64	6%
2,4-Dichlorophenol	162.0 -> 63.0	0.9996	5.57	0.98	2.0%	0.01	59	11%
2,4-Dimethylphenol	107.1 -> 77.1	1.0000	5.36	0.99	1.6%	0.01	67	14%
2,4-Dinitrophenol	184.0 -> 79.0	0.9931	7.50	0.01	3.6%	0.5	53	2%
2,4-Dinitrotoluene	165.0 -> 63.0	0.9998	7.59	0.09	3.8%	0.5	100	9%
2,6-Dinitrotoluene	165.0 -> 63.0	0.9990	7.19	0.11	1.4%	0.2	121	9%

Table 2. Analytical performance summary for analytes.

Compound Name	Quantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (µg/mL)	Recovery (%)	RSD of Recovery (n=3)
2-Acetylaminofluorene	222.9 -> 181.1	0.9966	11.82	0.07	6.7%	0.5	119	5%
2-Chloronaphthalene	162.0 -> 126.9	0.9992	6.86	2.48	1.8%	0.05	118	5%
2-Chlorophenol	128.0 -> 64.0	0.9998	4.37	0.35	2.0%	0.01	54	3%
2-methyl-4,6-dinitrophenol	198.0 -> 121.0	0.9949	7.99	0.03	3.6%	0.5	53	7%
2-Methylnaphthalene	142.0 -> 141.0	0.9984	6.39	2.81	0.2%	0.01	117	4%
2-Nitroaniline	138.0 -> 92.0	0.9988	6.96	0.14	1.4%	0.5	104	9%
2-Nitrophenol	138.9 -> 81.0	0.9987	5.34	0.25	1.2%	0.01	61	9%
2-Picoline	93.1 -> 66.0	0.9997	3.20	0.28	2.3%	0.01	40	5%
3-Methylcholanthrene	268.1 -> 252.1	0.9997	15.61	0.81	1.2%	0.5	101	7%
4,4'-DDD	234.8 -> 164.9	0.9986	11.04	1.46	1.5%	0.1	123	6%
4,4'-DDE	245.8 -> 176.0	0.9984	10.56	1.15	1.3%	0.1	116	6%
4,4'-DDT	234.8 -> 164.9	0.9985	11.51	0.89	1.1%	0.05	99	8%
4-Aminobiphenyl	168.1 -> 167.1	0.9986	8.68	0.21	8.6%	0.5	59	11%
4-bromophenyl phenyl ether	248.0 -> 141.0	0.9994	8.44	0.52	1.9%	0.1	112	6%
4-chloro-3-methylphenol	107.0 -> 77.0	0.9998	6.23	0.61	1.1%	0.05	55	13%
4-Chloroaniline	127.0 -> 65.0	0.9991	5.77	0.48	2%	0.02	19	14%
4-Chlorophenyl phenyl ether	141.1 -> 115.1	0.9968	7.94	0.45	0.7%	0.02	123	2%
4-Nitroaniline	138.0 -> 108.1	0.9996	7.97	0.14	6.4%	0.5	76	8%
7,12-Dimethylbenz[a]anthracene	256.1 -> 241.1	0.9998	14.45	1.51	1.5%	0.1	121	5%
Acenaphthene	152.9 -> 77.0	0.9997	7.44	0.17	0.6%	0.01	113	6%
Acenaphthylene	151.9 -> 102.0	0.9998	7.27	0.17	0.3%	0.01	114	8%
Aldrin	262.7 -> 192.6	0.9997	9.69	0.15	1.4%	0.01	105	5%
Aniline	93.0 -> 66.0	0.9999	4.27	0.68	0.9%	0.01	23	15%
Anthracene	177.9 -> 152.0	0.9958	8.94	0.93	4.5%	0.2	118	5%
Azobenzene	77.0 -> 51.0	0.9975	8.10	1.42	2.3%	0.2	119	7%
Benz[a]anthracene	228.1 -> 226.1	1.0000	12.36	1.60	1.2%	0.5	122	7%
Benzo[a]pyrene	252.1 -> 250.1	0.9998	15.04	1.79	3.5%	0.1	113	5%
Benzo[b]fluoranthene	252.1 -> 250.1	0.9997	14.46	2.20	1.4%	0.5	120	4%
Benzo[g,h,i]perylene	276.1 -> 274.1	0.9998	17.39	1.67	3.8%	0.5	112	5%
Benzo[k]fluoranthene	252.1 -> 250.1	0.9979	14.47	1.80	0.1%	0.5	114	5%
Benzyl alcohol	108.0 -> 79.0	0.9999	4.65	0.50	0.8%	0.02	59	22%
BHC-alpha	180.8 -> 144.9	0.9995	8.44	0.50	2.3%	0.02	109	7%
BHC-beta	180.8 -> 144.9	0.9994	8.65	0.38	1.7%	0.1	117	6%
BHC-delta	218.8 -> 182.8	0.9936	8.97	0.39	1.5%	0.1	106	6%
BHC-gamma	218.8 -> 182.9	0.9991	8.74	0.35	3.0%	0.1	112	8%
bis(2-Chloroethoxy)methane	93.0 -> 63.0	0.9998	5.46	1.75	1.2%	0.01	112	10%
bis(2-Chloroethyl)ether	93.1 -> 63.0	0.9999	4.31	0.86	0.5%	0.01	101	19%
Bis(2-ethylhexyl) phthalate	149.0 -> 65.0	0.9997	12.44	1.40	1.7%	0.02	124	5%
Butyl benzyl phthalate	149.0 -> 65.0	1.0000	11.38	0.91	2.4%	0.05	128	6%
Chrysene	226.1 -> 224.1	0.9985	12.38	0.73	7.8%	0.1	111	8%
Dibenz[a,h]anthracene	278.1 -> 276.1	0.9995	16.88	0.86	4.6%	0.5	104	6%
Dibenzofuran	167.9 -> 139.1	0.9959	7.61	1.53	0.2%	0.5	116	7%
Dieldrin	262.9 -> 193.0	1.0000	10.70	0.14	1.9%	0.05	113	6%

Compound Name	Ouantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (ug/mL)	Recovery	RSD of Recovery (n=3)
Diethyl phthalate	149.0 -> 65.0	0.9997	7.83	1.03	0.5%	0.1	114	8%
Dimethyl phthalate	163.0 -> 77.0	0.9999	7.13	0.96	0.4%	0.1	105	11%
Di-n-butyl phthalate	149.0 -> 65.0	0.9923	9.46	3.15	1.5%	0.1	125	5%
Di-n-octyl phthalate	149.0 -> 65.0	0.9993	13.90	1.94	2.7%	0.1	133	5%
Diphenylamine	167.0 -> 166.2	0.9985	8.06	0.80	3.1%	0.2	115	8%
Endosulfan I	241.0 -> 206.0	0.9997	10.41	0.08	2.0%	0.1	121	8%
Endosulfan II	240.7 -> 205.9	0.9999	11.05	0.05	1.0%	0.05	115	6%
Endosulfan sulfate	271.6 -> 236.7	0.9963	11.52	0.21	1.6%	0.02	113	8%
Endrin	262.7 -> 190.5	0.9995	10.94	0.03	0.7%	0.05	94	12%
Ethyl methanesulfonate	109.0 -> 78.9	0.9999	3.91	0.26	1.7%	0.01	79	5%
Fluoranthene	200.9 -> 199.9	0.9996	10.17	0.62	2.3%	0.1	121	7%
Fluorene	166.0 -> 165.1	0.9954	7.95	1.80	0.8%	0.1	121	6%
Heptachlor	273.6 -> 238.7	0.9999	9.37	0.19	0.7%	0.02	104	5%
Heptachlor epoxide	352.7 -> 216.7	0.9996	10.03	0.03	1.8%	0.05	112	7%
Hexachlorobenzene	283.7 -> 213.8	0.9997	8.49	0.51	2.5%	0.1	110	5%
Hexachlorobutadiene	224.7 -> 189.9	0.9998	5.83	1.26	0.9%	0.01	112	1%
Hexachlorocyclopentadiene	236.7 -> 143.0	0.9987	6.53	0.12	0.5%	0.05	87	4%
Hexachloroethane	200.9 -> 165.9	1.0000	4.99	0.88	1.0%	0.01	105	3%
Isophorone	82.0 -> 54.0	1.0000	5.27	0.82	1.4%	0.01	112	11%
Methoxychlor	226.9 -> 211.9	0.9995	12.24	0.23	0.5%	0.05	103	8%
Methyl methanesulfonate	80.0 -> 64.9	0.9999	3.91	0.05	2.0%	0.01	80	5%
Naphthalene	128.1 -> 102.1	0.9998	5.72	1.37	0.6%	0.01	113	5%
N-Nitro-o-toluidine	152.0 -> 106.0	0.9993	7.96	0.10	6.9%	0.5	71	10%
N-Nitrosodiethylamine	102.0 -> 85.0	0.9999	3.70	0.07	2.8%	0.01	97	5%
N-Nitrosodi-n-butylamine	84.1 -> 56.0	0.9998	6.08	0.14	0.2%	0.02	113	12%
N-Nitrosodi-n-propylamine	113.1 -> 71.0	0.9998	4.88	0.05	2.1%	0.02	109	12%
N-Nitrosomethylethylamine	88.0 -> 42.0	0.9999	3.25	0.11	2.3%	0.01	66	5%
N-Nitrosomorpholine	116.0 -> 86.0	0.9999	4.90	0.10	2.1%	0.05	55	13%
N-Nitrosopiperidine	114.0 -> 84.1	0.9998	5.19	0.14	2.8%	0.02	106	7%
N-Nitrosopyrrolidine	100.1 -> 55.1	0.9996	4.87	0.07	0.7%	0.05	69	13%
p-Dimethylaminoazobenzene	225.1 -> 120.1	0.9995	10.82	0.26	2.2%	0.5	144	6%
Pentachloronitrobenzene	248.8 -> 213.8	0.9997	8.70	0.17	1.2%	0.1	105	6%
Phenanthrene	177.9 -> 152.0	0.9985	8.91	1.32	2.6%	0.2	119	3%
Phenol	94.0 -> 66.1	0.9996	4.21	0.50	0.5%	0.01	19	18%
Pronamide	173.0 -> 145.0	0.9976	8.73	1.07	1.2%	0.5	116	6%
Pyrene	201.1 -> 200.0	0.9997	10.45	0.84	1.2%	0.2	119	7%
Thionazin	143.0 -> 79.0	0.9997	7.91	0.13	2.8%	0.1	118	8%
1,4-Dichlorobenzene-d4 (ISTD)	149.9 -> 114.9	N.A.	4.55	N.A.	N.A.	N.A.	N.A.	N.A.
Acenaphthene-d10 (ISTD)	161.9 -> 159.9	N.A.	7.40	N.A.	N.A.	N.A.	N.A.	N.A.
Chrysene-d12 (ISTD)	240.0 -> 235.9	N.A.	12.36	N.A.	N.A.	N.A.	N.A.	N.A.
Naphthalene-d8 (ISTD)	135.9 -> 107.9	N.A.	5.70	N.A.	N.A.	N.A.	N.A.	N.A.
Perylene-d12 (ISTD)	263.9 -> 259.9	N.A.	15.04	N.A.	N.A.	N.A.	N.A.	N.A.
Phenanthrene-d10 (ISTD)	187.9 -> 160.0	N.A.	8.88	N.A.	N.A.	N.A.	N.A.	N.A.

Matrix-spiked QC recovery

Three technical replicates of matrix-spiked QC (n=3, 2 µg/mL in the final extract) were prepared by the PAL3 system in order to evaluate the reproducibility and robustness of the automated sample preparation. Each QC was analyzed by GC/TQ in duplicates account for the homogeneity of the QC solution and the repeatability of spiked recovery. The recovery values and %RSD are summarized in Table 2. Overall, 96% of compounds met recovery 50 to150%, and 98% of compounds obtained RSD of recovery <20% as shown in Figure 8A and 8B, respectively. The obtained results indicate that this automated protocol developed on the PAL3-GC/TQ is suitable, offering good reproducibility and robustness for SVOC analysis according to EPA 8270E.



Figure 7. LLOQ distribution of 100 compounds.



Figure 8. Matrix-spiked QC recovery (A) at 2 µg/mL in the final extract and %RSD of recovery (B).

Conclusion

An automated workflow solution for quantitation of SVOCs in water samples, combining calibration/sample preparation and detection was developed on Agilent gas chromatography/ triple quadrupole mass spectrometer (GC/TQ) using the PAL3 robotic tool change (RTC) system in this study. The analytical performance parameters were evaluated based on EPA 8270E, meeting acceptance criteria for more than 90 out of 100 compounds. The PAL3 system provides various tools and modules enabling the automated preparation of calibration standards and samples to meet diverse customer needs, resulting in less manual work for the user. Agilent 7000 series triple quadrupole mass spectrometer coupled to 8890 GC offers excellent selectivity and sensitivity to target analytes. This newly developed automated workflow on the integrated PAL3-GC/TQ system offers an easy to use and more environmentally friendly solution for users by reducing chemicals/standards consumption as well as waste. This automated solution will enhance lab productivity and reduce costs significantly.

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