Extraction of EPA Method 625.1 Semi-Volatile Analytes from Wastewater Using the Biotage® Horizon 5000, DryDisk® Solvent Drying System and TurboVap® II

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Introduction

The EPA has been monitoring organic pollutants within wastewater matrices since 1984 by developing their own methods and acceptance criteria. The basic, neutral and acidic extractions in EPA Method 625.1 are a part of the revised version of EPA Method 625.

EPA Method 625.1 organizes the target analytes into three different tables. Table 1 lists non-pesticide/PCB basic/neutral analytes and table 2 lists the acidic analytes which may be extracted from a sample matrix to determine analytes quantitatively and qualitatively. Table 3 contains additional analytes that can be extracted via 625.1. Since there are basic and acidic analytes that need to be recovered during the extraction, the method requires two pH adjustment steps when extracting the semi-volatile analytes from a sample. Initially, the water sample is adjusted to a pH less than 2 to extract the acidic and neutral analytes. Following the acidic extraction, the pH is adjusted to greater than 11, extracting the basic analytes from the sample. The large number of analytes in Tables 1–3 of this method makes testing difficult if all analytes are determined simultaneously. Therefore, it is necessary to determine and perform quality control (QC) tests for the "analytes of interest" only. Analytes of interest are those required to be determined by a regulatory/control authority or in a permit, or by a client. If a list of analytes is not specified, the analytes in Tables 1 and 2 must be determined, at a minimum, and QC testing must be performed for these analytes. The analytes in Tables 1 and 2, and some of the analytes in Table 3 have been identified as Toxic Pollutants (40 CFR 401.15), expanded to a list of Priority Pollutants (40 CFR 423, Appendix A).[1]

Within the revision of EPA Method 625.1, laboratories are allowed to extract samples via solid phase extraction. After extraction, the extracts must be dried and concentrated.

This application note will show recoveries of all the analytes within tables 1–2 of 625.1 and a few analytes from table 3 obtained using the Biotage® Horizon 5000 with Atlantic® 8270

One Pass solid phase extraction disks (P/N 47-2346-11), 8270 Carbon Cartridges Max-Detect cartridges (P/N 49-2620-01), and 1.0 Micron Atlantic® Fast Flow Pre-Filters (P/N FFAP-100-HS1) for analyte extraction. The DryDisk® Solvent Drying System and the TurboVap® II are used for solvent drying and concentration and analysis is by GC-MS.

Experimental

Each 1 L deionized water sample was prepared by adding 1 mL of hydrochloric acid, bringing the sample to a pH less than 2. A total of two method blanks were analyzed. The first method blank contained 50 µg/L of surrogates and the second method blank contained 100 µg/L of surrogates. A total of twelve samples were spiked with a 625.1 spike mix in addition to surrogates. Six of these samples were spiked at a concentration level of 50 µg/L with the remaining six spiked at a concentration level of 100 µg/L. Using the Biotage® Horizon 5000 (P/N SPE-DEX 5000), all the samples were extracted using the method outlined in table 1. Atlantic® 8270 One-Pass Disks in conjunction with the 8270 Carbon Cartridge Max-Detect were used as the solid phase extraction consumables. The Atlantic® 8270 One-Pass Disk consists of a mixed-mode chemistry, eliminating the need for the second adjustment to the pH of the sample. Instead, a 1% ammonium hydroxide rinse adjusts the pH of the disk. After the analytes that were retained on the disk have been collected, the 8270 Carbon Cartridge Max -Detect (P/N 49-2620-01) is eluted to recover the more volatile analytes within a sample.

Upon completion of the extraction, the samples were dried using the DryDisk® Solvent Drying System (P/N SDS-101-19/22) in conjunction with DryDisks® (P/N 40-705-HT) utilizing the parameters outlined in table 2. The dried extracts were transferred to 200 mL evaporation tubes with an endpoint of 0.9 mL (P/N C128506) for use in the TurboVap® II (P/N 415001). Because the final volume of the dried extracts exceeded 200 mL, the samples were added to the evaporation tubes in two portions. The first portion was concentrated to approximately 15 mL before adding the final extract volume as well as glassware rinses. The samples were evaporated using the parameters outlined in table 3.

After evaporation on the TurboVap® II, the samples were brought to 1 mL with methylene chloride and transferred to GC-MS vials. Internal standard was added to an aliquot of each of the completed 1 mL extracts and analyzed using the GC/MS using instrument parameters outlined in table 4.

Table 1. Biotage® Horizon 5000 extraction method.
Note: The method below was written in such a way as to be able to run those samples that may require a 5-µm Pre-filter, glass wool, and a fine mesh screen.

Step	Operation	Message	Attachment
1	Pause with Message	Part 1 of 3: Neutrals and Acids Elution. Have the Fast Flow Sediment Disk Holder (FFSDH) with One-Pass disk, 1 um filter, 5 um filter, top screen over the filters, 250 mL collection flask, and carbon cartridge installed. The down spout of the water in valve must push down on the top screen in the FFSDH. Click "Continue" to start Part 1.	None

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	Saturation Time	Soak Time (s)	Drain Time (s)
2	Condition SPE	Acetone	40	60	4	2	60	60
3	Condition SPE	*Reagent Water 2	20	60	4	2	60	60

Step	Operation	Sample Flow Rate (#)	Done Loading Sample Delay (s)
4	Load Sample	5	45

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
5	Wash Sample Container	Reagent Water	20	30	4	Off	2	5	30
6	Air Dry Disk Timer			360	6	Off			
7	Elute Sample Container	Acetone	20	20	4	Off	2	180	180
8	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	180	180

*"Reagent Water 2" is added to the list of configured solvents with the Waste Destination of "Solvent Waste" rather than using the factory programmed "Reagent Water", which is sent to "Water Waste".

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
9	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	120	120
10	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	120	120
11	Elute Sample Container	Methylene Chloride	17	15	6	Off	2	120	180

Step	Operation	Message	Attachment
12	Pause with Message	Part 2 of 3: Ion Exchange Elution. Remove the 250 mL collection flask containing the neutrals and acids elution. Stopper the flask and set aside for part 3. Then install a clean 125 mL flask to collect the ion exchange elution. Click "Continue" to start part 2.	None

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
13	Elute Sample Container	Acetone	20	20	4	Off	2	0	180
14	Elute Sample Container	1% Ammonium Hydoxide	20	30	4	Off	2	120	120
15	Elute Sample Container	Acetone	20	20	4	Off	2	180	120
16	Elute Sample Container	Methylene Chloride	17	15	4	Off	2	180	180
17	Elute Sample Container	Methylene Chloride	16	15	4	Off	2	120	180
18	Elute Sample Container	Methylene Chloride	16	15	4	Off	2	120	180
19	Elute Sample Container	Methylene Chloride	16	15	6	Off	2	120	180

Step	Operation	Message	Attachment
20	Pause with Message	Part 3 of 3: Carbon Cartridge Elution. Remove the carbon cartridge from the tubing lines. Connect the tubing ends together. Using a 20 cc syringe, plunge the carbon cartridge with air through the cap adapter to reseat the carbon bed on the frit. Replace the cap adapter with the funnel on the cartridge. Replace the disk holder with the cartridge. Replace the 125 mL flask with the 250 mL flask containing the neutrals and acids elution from part 1. Stopper the 125 mL flask. Click "Continue" to start part 3.	None

Step	Operation	Dry Time (s)	Pump Rate (#)	N_2 Blanket
21	Air Dry Disk Timer	60	6	Off

Step	Operation	Solvent	Approximate Solvent Volume (mL)	Purge Time (s)	Pump Rate (#)	N_2 Blanket	Saturation Time	Soak Time (s)	Drain Time (s)
22	Elute Sample Container	Acetone	25	20	4	Off	3	60	60
23	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
24	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
25	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
26	Elute Sample Container	Methylene Chloride	17	15	4	Off	3	60	20
27	Elute Sample Container	Methylene Chloride	17	15	6	Off	3	60	60

Table 2. Drying Parameters via the DryDisk® Solvent Drying System.

Parameter	Setting
Vacuum:	-8 ”Hg

Table 3. Evaporation Parameters for Drying via the Biotage TurboVap® II.

Parameter	Setting
Inlet Nitrogen Pressure:	87 psi
Gas Flow:	2.8 mL/min
Water Bath Temperature:	40 °C

Table 4. GC/MS Method.

Parameter	Setting
Injection Volume	1 µL
Inlet Temperature	280 °C
Injection Mode	Split
Split Ratio	10:1
Split Flow	12.5 mL/min
Gas Type	Helium
GC Column	Zebron™ ZB-Semi Volatiles (Phenomenex), 30 m, 0.25 mm, 0.25 µm
GC Mode	Constant Flow: 1.3 mL/min
Oven Program	45 °C hold for 1.0 minutes Ramp 15 °C/min to 270 °C Ramp 6 °C/min to 318 °C
MS Ions Monitored	35–550 AMU

TurboVap® II solvent evaporator.

Biotage® Horizon 5000.

Results and Discussions

Table 5 shows the total concentration time for each sample using the TurboVap® II.

Table 5. Concentration Times on the TurboVap® II.

Sample	Concentration (µg/L)	Time (Hour:Min.:Sec.)
1	50	1:51:56
2	50	1:42:00
3	50	1:56:36
4	50	1:37:25
5	50	1:47:41
6	50	1:55:53
7	100	1:48:43
8	100	1:39:12
9	100	1:48:30
10	100	1:53:53
11	100	1:46:59
12	100	1:55:49

When concentrating the samples, aluminum foil was used to cover each sample vial in order to limit interactions between the evaporating acetone and the water vapor molecules present from the warm water bath. Two holes were pierced on either side of the foil caps to allow the nozzle (N_2 flow) to enter, and evaporated solvent to escape, the vials. The concentration times only varied slightly between the samples. On average, the samples that initially contained approximately 303 mL of solvent were concentrated on the TurboVap® II to approximately 0.9 mL in 1 hour and 49 minutes.

Data for the method blanks and spiked samples, including average percent recovery and %RSD for each level (50 µg/L and 100 µg/L) are outlined in table 6. When reading table 6, the analytes are color coded based upon the legend outlined in the header. The list of analytes in table 6 below contains all the analytes from tables 1 and 2 and some from table 3 of EPA Method 625.1.

Table 6. Average Percent Recovery for 625.1 Analytes Spiked at 50 µg/L and 100 µg/L Using the Biotage® Horizon 5000 and the TurboVap® II.

Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
NDMA	N.D	65.43	6.34	N.D	51.38	25.51
Pyridine	N.D	38.26	11.03	N.D	36.87	8.63
2-Picoline	N.D	60.47	4.99	N.D	58.43	2.82
N-Nitrosomethyl ethylamine	N.D	80.59	5.77	N.D	75.02	7.06
Methyl methanesulfonate	N.D	66.34	7.60	N.D	57.53	11.85
2-Fluorophenol	39.63	73.03	9.34	76.36	57.43	7.23
N-Nitroso-diethylamine	N.D	81.11	5.04	N.D	78.78	0.95
Ethyl methanesulfonate	N.D	83.04	6.55	N.D	78.15	3.58
Phenol-d6	38.85	65.90	6.91	67.25	57.63	4.58
Phenol	N.D	68.00	4.23	N.D	60.05	6.35
Aniline	N.D	69.16	12.69	N.D	61.89	9.35
Bis(2-chloroethyl)ether	N.D	83.57	2.38	N.D	81.99	1.78
Pentachloroethane	N.D	71.28	7.23	N.D	68.72	2.91
2-Chlorophenol	N.D	83.36	5.62	N.D	77.78	3.18
Benzyl alcohol	N.D	85.15	3.52	N.D	88.84	10.41
o-Cresol	N.D	85.33	3.83	N.D	77.89	6.06
Bis(2-chloroisopropyl)ether	N.D	79.32	2.86	N.D	80.04	1.68
N-Nitroso-pyrrolidine	N.D	85.00	4.09	N.D	84.56	3.23
N-Nitroso-morpholine	N.D	79.90	6.72	N.D	83.69	7.41
Acetophenone	N.D	80.92	2.58	N.D	79.83	2.19
m+p-Cresol	N.D	83.14	3.98	N.D	74.41	6.77
N-nitrosodi-n-propylamine	N.D	83.02	2.56	N.D	82.18	2.16
Hexachloroethane	N.D	67.98	6.65	N.D	67.34	2.22
o-Toluidine	N.D	59.84	15.34	N.D	53.31	15.61
Nitrobenzene-d5	39.59	81.37	6.49	82.35	79.50	1.99
Nitrobenzene	N.D	79.48	6.30	N.D	77.96	2.45
N-Nitroso-piperidine	N.D	85.00	6.74	N.D	84.06	2.17
Isophorone	N.D	87.08	3.44	N.D	85.94	1.37
2-Nitrophenol	N.D	87.16	2.66	N.D	81.65	5.72
2,4-Dimethylphenol	N.D	87.12	3.56	N.D	86.57	1.82
Bis(2-chlorethoxy)methane	N.D	87.30	3.09	N.D	85.76	1.22
Benzoic acid	N.D	84.91	11.54	N.D	68.76	14.25

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
2,4-Dichlorophenol	N.D	88.33	3.60	N.D	87.00	3.53
1,2,4-Trichlorobenzene	N.D	75.61	3.84	N.D	73.59	2.80
Naphthalene	N.D	80.34	3.46	N.D	78.49	2.54
2,6-Dichlorophenol	N.D	86.86	5.29	N.D	84.33	3.07
4-Chloroaniline	N.D	68.70	13.31	N.D	60.09	11.36
Hexachloropropene	N.D	60.52	3.75	N.D	56.92	2.90
Hexachlorobutadiene	N.D	60.93	8.40	N.D	58.24	3.29
N-nitroso-di-n-butylamine	N.D	88.70	2.68	N.D	88.80	2.51
4-Chloro-3-methylphenol	N.D	91.36	3.31	N.D	91.81	4.09
cis-Isosafrole	N.D	83.02	4.66	N.D	81.29	3.15
2-Methylnaphthalene	N.D	79.98	3.21	N.D	77.43	2.99
Hexachlorocyclopentadiene	N.D	47.35	9.12	N.D	49.42	5.13
1,2,4,5 Tetrachlorobenzene	N.D	65.76	4.72	N.D	70.32	3.09
trans-Isosafrole	N.D	88.65	3.42	N.D	91.60	2.16
2,4,6-Trichlorophenol	N.D	90.99	2.61	N.D	93.65	3.42
2,4,5-Trichlorophenol	N.D	88.52	2.99	N.D	93.63	4.31
2-Fluorobiphenyl	37.44	78.78	2.78	77.06	81.22	2.93
Safrole	N.D	85.08	2.64	N.D	88.70	3.27
2-Chloronaphthalene	N.D	77.84	2.91	N.D	81.13	3.11
2-Nitroaniline	N.D	93.55	3.17	N.D	94.87	4.09
1,4-Naphthoquinone	N.D	73.24	4.47	N.D	81.86	7.60
Dimethyl phthalate	N.D	89.18	1.93	N.D	92.34	3.72
1,3-Dinitrobenzene	N.D	92.33	3.12	N.D	94.65	4.46
2,6-Dinitrotoluene	N.D	89.83	1.98	N.D	94.02	3.68
Acenaphthylene	N.D	82.88	2.54	N.D	85.56	2.67
3-Nitroaniline	N.D	85.43	2.68	N.D	87.64	4.49
Acenaphthene	N.D	81.55	2.39	N.D	83.84	2.75
2,4-Dinitrophenol	N.D	102.41	3.04	N.D	106.78	3.00
Pentachlorobenzene	N.D	73.09	1.92	N.D	79.27	2.95
4-Nitrophenol	N.D	94.00	1.57	N.D	97.93	6.46
Dibenzofuran	N.D	82.93	2.21	N.D	84.57	2.81
2,4-Dinitrotoluene	N.D	92.17	1.43	N.D	94.44	3.04
2,3,4,6-Tetrachlorophenol	N.D	93.30	2.31	N.D	95.22	2.77

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
1-Naphthylamine	N.D	56.04	16.37	N.D	59.31	14.02
2-Naphthylamine	N.D	57.26	18.08	N.D	57.06	11.75
Diethyl phthalate	N.D	91.04	1.87	N.D	92.85	2.72
Fluorene	N.D	84.46	2.31	N.D	86.09	2.78
4-Chlorophenyl phenyl ether	N.D	80.24	2.18	N.D	83.33	2.81
4-Nitroaniline	N.D	79.77	3.43	N.D	79.50	3.92
5-nitro-o-toluidine	N.D	72.07	11.38	N.D	76.02	6.89
2-Methyl-4,6-dinitrophenol	N.D	96.90	1.92	N.D	99.31	2.38
Diphenylamine	N.D	89.72	1.42	N.D	90.24	2.59
Azobenzene	N.D	86.88	2.58	N.D	88.78	3.11
2,4,6-Tribromophenol	43.13	99.77	6.09	92.32	96.62	3.66
1,3,5,-Trinitrobenzene	N.D	98.89	7.29	N.D	89.97	7.67
Phenacetin	N.D	106.16	5.18	N.D	100.52	3.70
4-Bromophenyl phenyl ether	N.D	90.17	4.99	N.D	89.19	3.01
Hexachlorobenzene	N.D	90.94	5.32	N.D	90.14	3.35
Pentachlorophenol	N.D	108.91	5.60	N.D	104.51	2.28
Pentachloronitrobenzene	N.D	95.32	5.02	N.D	93.37	2.24
4 Aminobiphenyl	N.D	54.51	22.72	N.D	54.37	10.55
Dinoseb	N.D	109.65	4.24	N.D	108.54	2.11
Phenanthrene	N.D	95.09	4.80	N.D	92.82	2.33
Anthracene	N.D	95.34	4.09	N.D	92.98	2.14
Carbazole	N.D	98.91	3.56	N.D	95.59	2.36
Di-n-butyl phthalate	N.D	104.60	3.49	N.D	100.73	1.90
Methapyrilene	N.D	93.73	4.78	N.D	92.66	2.63
Fluoranthene	N.D	97.78	4.10	N.D	93.39	2.67
Benzidine	N.D	29.04	38.08	N.D	48.31	10.12
Pyrene	N.D	95.73	3.18	N.D	92.24	2.33
p-Terphenyl-d14	44.76	101.64	3.42	92.25	98.14	2.36
Dimethylaminoazobenzene	N.D	109.40	4.36	N.D	105.87	2.09
3,3'-Dimethylbenzidine	N.D	29.31	30.84	N.D	31.59	11.28
Butyl benzyl phthalate	N.D	103.33	2.36	N.D	102.38	2.57
Acetylaminofluorene	N.D	118.53	4.03	N.D	117.59	1.72
3,3'-Dichlorobenzidine	N.D	66.92	8.44	N.D	63.95	7.86

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Legend	Table 1 Analytes	Table 2 Analytes	Table 3 Analytes	Table 4/6	Table 8 Analytes	Not in Table 8

Analyte	Blank 1: Surrogate level 50 µg/L	50 µg/L Spike Average Percent Recovery (n=6)	50 µg/L Spike %RSD	Blank 2: Surrogate level 100 µg/L	100 µg/L Spike Average Percent Recovery (n=6)	100 µg/L Spike %RSD
Benzo(a)anthracene	N.D	99.70	3.89	N.D	97.36	1.60
Chrysene	N.D	98.53	3.36	N.D	95.68	1.01
Bis(2-ethylhexyl)phthalate	N.D	110.09	2.98	N.D	106.74	2.43
Di-n-octyl phthalate	N.D	114.93	4.40	N.D	114.18	2.12
7,12-Dimethylbenz(a)anthracene	N.D	93.58	6.28	N.D	90.10	3.32
Benzo(b)fluoranthene	N.D	98.45	5.47	N.D	95.56	2.60
Benzo(k)fluoranthene	N.D	98.19	5.33	N.D	94.00	2.93
Benzo(a)pyrene	N.D	99.67	5.46	N.D	97.25	2.62
3-Methylcholanthrene	N.D	100.25	6.88	N.D	98.79	2.50
Indeno(1,2,3-cd)pyrene	N.D	99.55	8.01	N.D	97.60	4.30
Dibenz(a,h)anthracene	N.D	98.56	8.25	N.D	96.62	3.99
Benzo(ghi)perylene	N.D	99.28	7.65	N.D	96.11	4.17

The EPA acceptance criteria for table 1 and table 2 analytes are found in tables 4 and 5 respectively in EPA Method 625.1. For those compounds in tables 3 and 8, the EPA has elected not to set specific acceptance criteria themselves. Instead, the appropriate acceptance criteria are left to the laboratories to develop in one of several ways: based on laboratory control charts, using the range 60-140%, or using the guidelines outlined in section 8.4.5 of the 625.1 method itself. All the analytes in table 1 and table 2 of the EPA method passed the EPA method acceptance criteria for both concentration levels. The percent relative standard deviations indicated minimal variation in percent recovery for each sample set.

According to section 6.8.1 of EPA Method 625.1, a minimum of three surrogates are required for analysis as long as they do not interfere with target analytes. The six surrogates used in this application note passed the acceptance criteria set by the EPA method for all of the samples at 50 µg/L and three of the samples at 100 µg/L. The recovery of two surrogates, 2-fluorophenol and phenol-d6, from the other three samples at 100 µg/L fell below the lower passing limit of 60%, lowering the average. However, the EPA only requires a minimum of three surrogates for each sample. Even though two surrogates fell below the lower limit, four surrogates fell within the limits. The calculated percent relative standard deviation for each surrogate in the order found in table 7 was 7.23%, 5.48%, 1.99%, 2.93%, 3.66%, and 2.36%, indicating minimal variation between the samples.

Two method blanks were extracted with surrogates at different levels. The concentration of the surrogates for the first blank was 50 µg/L and the concentration of surrogates for the second blank was 100 µg/L. Neither showed any false positives for target analytes above the detection limit of 10 µg/L which is denoted by N.D, meaning “not detected.”

References

1. United States Environmental Protection Agency, Method 625.1: Base/Neutrals and Acids by GC/MS, available at www.epa.gov, December 2016.

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