Defining the Retention Times of 209 PCB Congeners Using GCxGC-TOFMS

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Key Words: PCB, Critical Pair Separation, Ion Ratio, GCxGC-TOFMS

1. Introduction

Polychlorinated biphenyls (PCBs) are one of the most widely recognized persistent organic environmental pollutants. They are suspected carcinogens and are known to cause liver damage[1]. Because the molecular structures of the 209 PCB congeners are very similar to one another, they have been a challenge to completely separate using one-dimensional gas chromatography. This structural similarity also leads to nearly identical mass spectra for congeners with equivalent numbers of chlorine substitution.

Comprehensive two-dimensional gas chromatography (GCxGC) is a powerful evolution of gas chromatography that allows the user to separate compounds on two different stationary phase chemistries without compromising the separation on either phase. The coupling of a GCxGC system with a LECO Pegasus® 4D time-of-flight mass spectrometer (TOFMS) produces an instrument with significant improvements in peak capacity, peak detectability, and peak identification capabilities. This article describes the use of a GCxGC-TOFMS instrument to determine the primary retention time (t_r') and secondary retention time (t_r'') for each of the 209 PCB congeners. In addition, a user library with the mass spectra of each congener was created in order to see how successful the ChromaTOF® software would be in appropriately labeling critical pair congeners, which are the most difficult congener pairs to separate.

2. Experimental Conditions

A PCB sample was prepared individually for each of the 209 congeners. In this study, the PCB congeners are labeled according to IUPAC conventions. The PCB congeners were diluted to 20 μg/mL in isooctane solvent and placed in individual 2 mL vials. Previous work using 1D GC allowed for an estimate of the total retention time for each congener accurate to within 60-90 seconds. For the critical pair analyses, small portions of the individual congener samples were combined to make two-congener solutions of roughly 10 μg/mL concentration.

The analysis was performed using an Agilent 6890 GC coupled with a Pegasus 4D TOFMS detector. The column set consisted of a 40 m, 0.18 mm i.d., 0.18 μm RTX-PCB film (Restek Corp.) for a primary column and a 1 m, 0.10 mm i.d., 0.10 μm RTX-17 film (Restek Corp.) for a secondary column. The overall temperature program was a 0.50 minute hold at 70°C followed by a ramp to 150°C at 10°C/minute, then ramped at 1°C/minute to 250°C, then ramped at 4°C/minute to 275°C and held for 15 minutes. However, since many PCB congeners eluted more quickly and did not require the full temperature program timeframe, the GC method for each congener analysis was designed to end approximately 5-10 minutes after the congener was expected to elute. The secondary oven was set to a 15°C temperature offset. A split/splitless inlet was used in the split mode with the split determined roughly by elution time (100:1 for early eluters, 50:1 for late eluters, and 20:1 for very late eluters) due to late eluters producing broadened peaks with reduced peak height. Helium carrier gas was used with a corrected constant flow of 1.2 mL/minute. Transfer line temperature and inlet temperature were held at 280°C. The modulator temperature offset was 25°C with a modulation period of 4.00 seconds (1.10 hot pulse time and 0.90 cold pulse time per stage). The LECO ChromaTOF software package was used for acquisition control and data processing and additional retention time processing was done using Microsoft Excel.

The mass range for the TOFMS was 45-550 m/z with an acquisition rate of 100 spectra/s. The ion source chamber temperature was 200°C with an electron energy of -70 eV. The solvent delay for the MS method was tuned to be about 5 minutes shorter than the expected elution time of the PCB congener. This was done to protect the source and to minimize the usage of modulator coolant and gases.

3. Results

Retention times in the primary and secondary dimensions were recorded for each of the 209 PCB congeners and are listed in Appendix A. Figure 1 displays the retention times for the PCB congeners organized by the number of chlorines in the represented PCB congener. In general, the more chlorines that are present, the longer the retention time is in both dimensions, although definable regions for the specific number of chlorines is not readily visible – only the 1 Cl congeners and the 10 Cl congener can exist in a t_r' region void of all other congeners. For example, the t_r' time span of 2400-3000 seconds contains both 2 and 3 Cl congeners, while the span of 4400-5000 seconds contains 4, 5, and 6 Cl congeners.

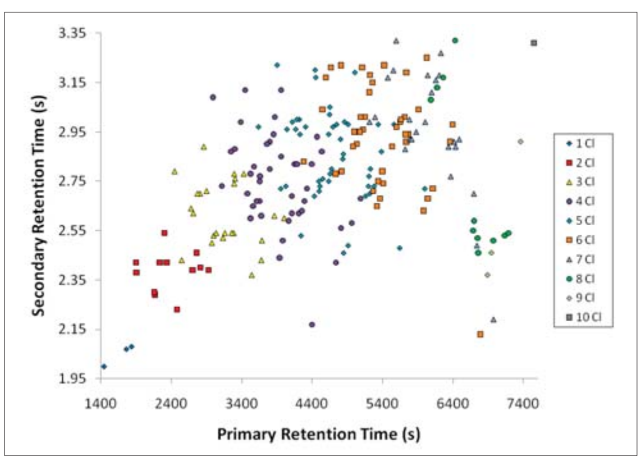

***Figure 1.** Plotted above are the peak apexes representing the two-dimensional retention times for the 209 PCB congeners. The legend indicates the number of chlorines contained in the represented PCB congener.*

This retention information was used to predict critical pairs of congeners that may be difficult to separate. There were 15 congener pairs that had the same primary retention time and 63 congener pairs within one or two modulation periods of each other. A new user library was created using the mass spectra generated during the initial analysis of the PCB congeners in order to see if the critical pairs could be accurately identified based on mass spectra alone.

The congener pairs of interest were tested and the data was processed. Figures 2 and 3 show contour plots of some of the critical pairs with surface plot insets and the congener structures. Note that the separation of these critical pairs is done almost entirely on the second column. While the user library typically failed to identify the correct congeners present, the top hit was always one with the correct chlorine number. As shown in Table 1, the 15 congener pairs with identical t_r' consisted of 8 pairs with the same Cl content and 7 pairs that are non-homogenous in Cl content. Except for the trichloro/trichloro (congeners 21 and 33) critical pair, the analysis of the 8 critical pairs with the same Cl content samples found two different congeners with the appropriate number of Cl present. Even the congener pair of 58 and 67 with only a 0.07 s Δt_r" produced two tetrachloro congeners during data processing.

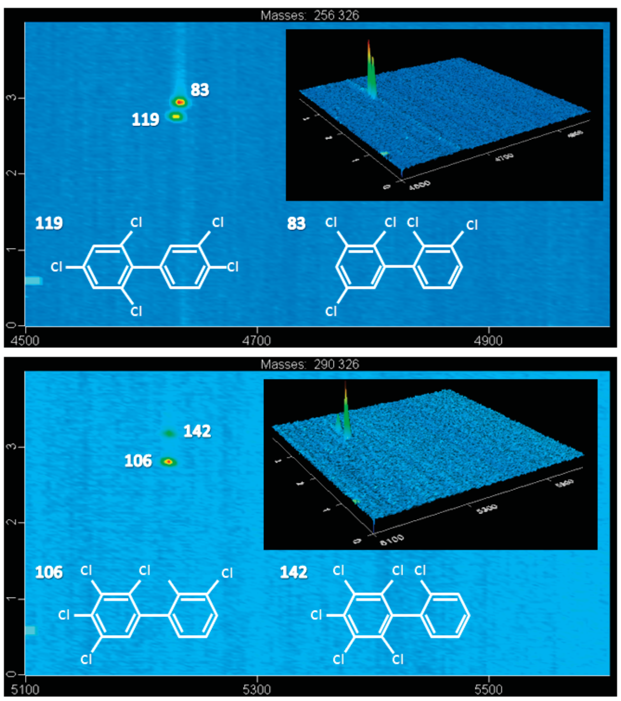

Figure 2. The contour plots of the PCB critical pairs 83/119 and 106/142 using the summed unique masses of the two congeners with the structures displayed and surface plot displayed in the inset.

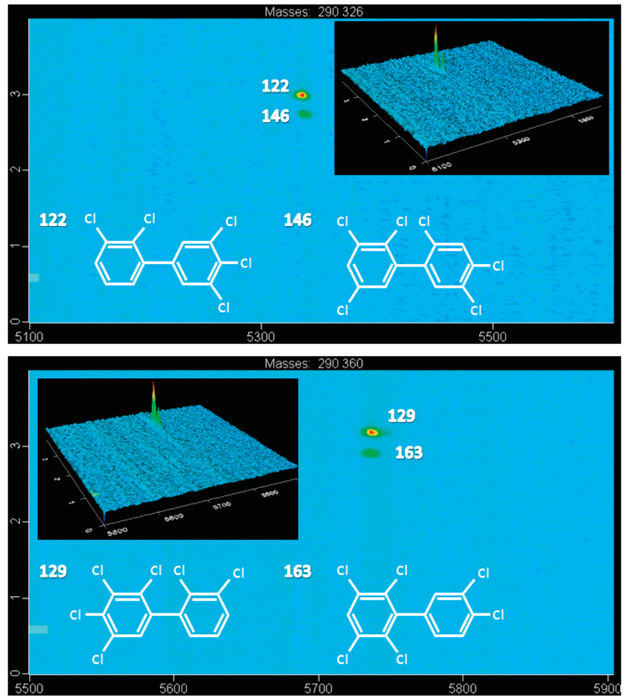

Figure 3. The contour plots of the PCB critical pairs 122/146 and 129/163 using the summed unique masses of the two congeners with the structures displayed and surface plot displayed in the inset.

Table 1. Results for identical t_r' critical pair analyses. The red printing in the Δt_r" indicate differences in the second dimension retention that are below 0.20 seconds. The Test Results column indicates the congeners of the top hit and Cl Result column indicates the number of Cl atoms in the top hit. Correct hits are typed in green.

t_r' (s)	Congeners	Cl Content	Δt_r'' (s)	Test Results	Cl Result
3292	21,33	tri/tri	0.04	24	tri
3524	69,43	tetra/tetra	0.18	64,51	tetra/tetra
3800	59,42	tetra/tetra	0.11	63,52	tetra/tetra
3960	64,40	tetra/tetra	0.27	69,53	tetra/tetra
4112	67,58	tetra/tetra	0.07	55,57	tetra/tetra
4632	119,83	penta/penta	0.19	119,98	penta/penta
4908	120,110	penta/penta	0.49	113,121	penta/penta
5736	163,129	hexa/hexa	0.28	169,134	hexa/hexa
3676	39,75	tri/tetra	0.18	39,57	tri/tetra
5088	77,149	tetra/hexa	0.33	58 (poor sample)	tetra
5212	188,134	hepta/hexa	0.12	190,139	hepta/hexa
5224	106,142	penta/hexa	0.38	112,143	penta/hexa
5336	146,122	hexa/penta	0.23	139,113	hexa/penta
6040	162,174	hexa/hepta	0.50	169 (poor sample)	hexa
6696	199,170	octa/hepta	0.11	199,180	octa/hepta

For the critical pairs where the number of Cl in the congeners were different, the correct congener types were found in the 5 cases where the samples produced usable signals. For the pair of 162 and 174, the heptachloro PCB 174 had too little concentration in the new sample to be found during data processing, though it could be differentiated by eye due to the large Δt_r". A similar situation occurred for the 77/149 pair.

The 63 critical pairs separated by 1 or 2 modulation periods in t_r' were broken down to 23 same-Cl number pairs and 40 different-Cl number pairs. Because the different-Cl critical pairs were fairly easily separated in the identical-t_r' study, they weren't explicitly investigated—there was only time for a portion of the 23 same-Cl number pairs to be tested with results shown in Table 2.

Table 2. Results for some critical pairs separated by 1 or 2 modulation periods on the primary dimension. The red printing in the Δt_r" indicate differences in the second dimension retention that are below 0.20 s. The Test Results column indicates the congeners of the top hit and Cl Result column indicates the number of Cl atoms in the top hit. Correct hits are typed in green.

t_{r1}' (s)	t_{r2}' (s)	Congeners	Cl Content	Δt_r'' (s)	Test Results	Cl Result
1900	1904	4,10	di/di	0.04	9	di
3284	3292	28,33	tri/tri	0.20	35,31	tri/tri
3652	3656	47,65	tetra/tetra	0.08	62,51	tetra/tetra
3656	3660	65,62	tetra/tetra	0.02	69,63	tetra/tetra
3676	3684	39,38	tri/tri	0.08	39,24	tri/tri
4216	4224	88,95	penta/penta	0.06	89,90	penta/penta
4392	4400	55,80	tetra/tetra	0.65	63,80	tetra/tetra
4440	4448	89,84	penta/penta	0.03	89	penta
4492	4496	90,101	penta/penta	0.04	95	penta
4644	4648	125,86	penta/penta	0.03	96,125,90	penta/penta/penta
4648	4652	86,112	penta/penta	0.27	99,90	penta/penta
4812	4820	136,154	hexa/hexa	0.43	145,148	hexa/hexa
4832	4840	117,115	penta/penta	0.02	121,106,110	penta/penta/penta
4840	4844	115,111	penta/penta	0.40	107,104	penta/penta
5080	5088	147,149	hexa/hexa	0.06	139	hexa
5196	5204	107,123	penta/penta	0.04	125,125	penta/penta
5216	5224	109,106	penta/penta	0.10	125,110	penta/penta
5256	5264	131,133	hexa/hexa	0.44	159, 133	hexa/hexa
5652	5660	130,164	hexa/hexa	0.01	148,143,163	hexa/hexa/hexa
5728	5736	160,163	hexa/hexa	0.03	156,166,152	hexa/hexa/hexa
5728	5736	160,129	hexa/hexa	0.25		
6172	6176	201,204	octa/octa	0.00		
6748	6756	196,203	octa/octa	0.06		

In four cases, only a single congener was found. Surprisingly, another four cases found three different congeners, though only two were present. Of the 20 critical pairs studied in the Table 2 group, only 5 had a correctly identified congener, and none of the pairs had both congeners correctly identified. The similarity values for both the correctly-identified and incorrectly-identified congeners were typically in the range of 650-850 with only a few cases where >900 similarity occurred.

The frequent occurrences of misidentified congeners led to the possibility that using ChromaTOF's Ion Ratio Calculation capability could be used to get better matches. The critical pair congeners 43/69 were used to demonstrate this feature. As shown in Table 1, congener 69 was identified as congener 64. In the generated User Library, the ion ratios between masses 150 and 292 were approximately 0.455 for congener 64 and 0.380 for congener 69, which is sufficiently different to warrant their use in identification. A single-point Calibration Method was created to make use of the Ion Ratio Calculation property column. Before the Calibration Method was included in data processing for the 43/69 critical pair set, the similarity for congener 64 was 865 and the similarity for congener 69 was 854. Once the data set was reprocessed with the Calibration Method included, the similarity for congener 69 increased to 886, which was enough to put it in the top hit position. Figure 3 depicts the chromatogram of the critical pair analysis, the Peak Table, and the Hit Table after processing using the Calibration Method. The Congener # field is blank for the top hit because the mainlib and replib libraries were used in the Calibration Method rather than the User Library that contains the Congener # user field information, though 2,3',4,6-tetrachloro-1,1'-biphenyl does correspond with congener 69.

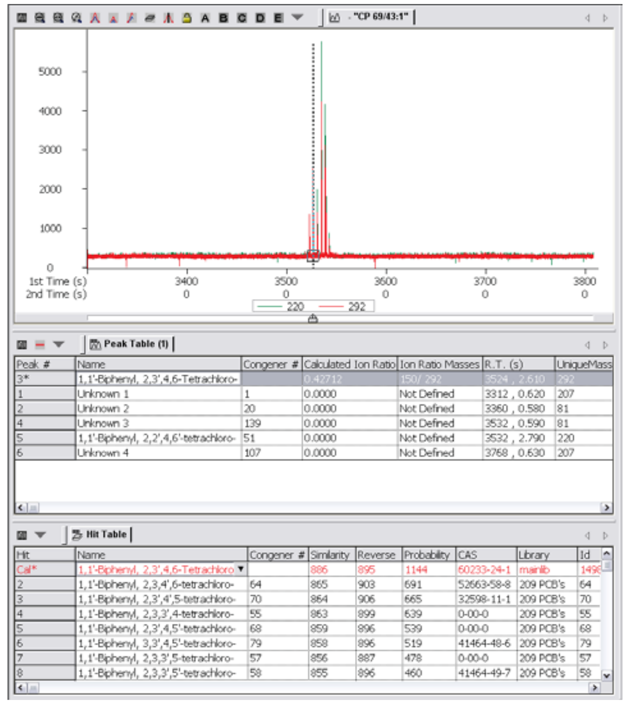

Figure 3. The chromatogram of the critical pair congeners 43 and 69 with associated Peak Table and Hit Table is displayed. The use of Calculated Ion Ratio increased the similarity of congener 69 to 886, allowing it to take the top hit slot.

4. Conclusion

This article described the analysis of 209 individual PCB congeners using the LECO Pegasus 4D GCxGC-TOFMS instrument with ChromaTOF software. The study resulted in valuable t_r' and t_r" information for the identification of PCBs present in a sample. Based on t_r information, only one, two, or rarely three possible congeners will be contenders for the correct congener number. The user library created to look specifically at the PCBs did not have great success in correctly identifying the congener number based on the top hit from mass spectra alone, which is not unexpected since the spectra are so similar amongst same-Cl numbered congeners. However, combining the t_r and Calculated Ion Ratio information from a Classification Method leads to confident congener identification.

5. References

[1]Safe, S. *Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action.* Crit. Revi. Toxicol., 1984, 13 (4), 319-395.

Appendix A. List of tr' and tr" for the 209 PCB congeners. The Cl Count column indicates the number of chlorine substitutions in the PCB congener.

PCB #	Cl Count	t_r'	t_r''	total t_r	PCB #	Cl Count	t_r'	t_r''	total t_r	PCB #	Cl Count	t_r'	t_r''	total t_r
1	1	1448	2.00	1450.00	38	3	3684	2.51	3686.51	75	4	3676	2.61	3678.61
2	1	1764	2.07	1766.07	39	3	3676	2.43	3678.43	76	4	4188	2.82	4190.82
3	1	1836	2.08	1838.08	40	4	3960	3.12	3963.12	77	4	5088	2.68	5090.68
4	2	1900	2.42	1902.42	41	4	3868	3.01	3871.01	78	4	4808	2.56	4810.56
5	2	2300	2.54	2302.54	42	4	3800	2.91	3802.91	79	4	4740	2.42	4742.42
6	2	2236	2.42	2238.42	43	4	3524	2.78	3526.78	80	4	4400	2.17	4402.17
7	2	2168	2.29	2170.29	44	4	3756	2.90	3758.90	81	4	4964	2.58	4966.58
8	2	2332	2.42	2334.42	45	4	3388	2.99	3390.99	82	5	5004	3.19	5007.19
9	2	2156	2.30	2158.30	46	4	3448	3.12	3451.12	83	5	4632	2.94	4634.94
10	2	1904	2.38	1906.38	47	4	3652	2.67	3654.67	84	5	4448	3.17	4451.17
11	2	2700	2.39	2702.39	48	4	3572	2.81	3574.81	85	5	4856	2.99	4858.99
12	2	2756	2.46	2758.46	49	4	3608	2.67	3610.67	86	5	4648	3.05	4651.05
13	2	2812	2.40	2814.40	50	4	3116	2.73	3118.73	87	5	4800	2.92	4802.92
14	2	2484	2.23	2486.23	51	4	3300	2.88	3302.88	88	5	4216	3.00	4219.00
15	2	2928	2.39	2930.39	52	4	3560	2.65	3562.65	89	5	4440	3.20	4443.20
16	3	2860	2.89	2862.89	53	4	3248	2.87	3250.87	90	5	4492	2.75	4494.75
17	3	2712	2.62	2714.62	54	4	2992	3.09	2995.09	91	5	4304	2.97	4306.97
18	3	2680	2.64	2682.64	55	4	4392	2.82	4394.82	92	5	4428	2.69	4430.69
19	3	2448	2.79	2450.79	56	4	4468	2.93	4470.93	93	5	4180	3.00	4183.00
20	3	3304	2.76	3306.76	57	4	4064	2.59	4066.59	94	5	4040	2.96	4042.96
21	3	3292	2.78	3294.78	58	4	4112	2.69	4114.69	95	5	4224	2.94	4226.94
22	3	3428	2.78	3430.78	59	4	3800	2.80	3802.80	96	5	3900	3.22	3903.22
23	3	3000	2.53	3002.53	60	4	4536	2.87	4538.87	97	5	4692	2.97	4694.97
24	3	2808	2.70	2810.70	61	4	4152	2.82	4154.82	98	5	4164	2.96	4166.96
25	3	3160	2.54	3162.54	62	4	3660	2.77	3662.77	99	5	4548	2.76	4550.76
26	3	3132	2.52	3134.52	63	4	4212	2.62	4214.62	100	5	4024	2.73	4026.73
27	3	2772	2.70	2774.70	64	4	3960	2.85	3962.85	101	5	4496	2.71	4498.71
28	3	3284	2.54	3286.54	65	4	3656	2.75	3658.75	102	5	4116	2.99	4118.99
29	3	3036	2.54	3038.54	66	4	4328	2.72	4330.72	103	5	3952	2.72	3954.72
30	3	2548	2.43	2550.43	67	4	4112	2.62	4114.62	104	5	3636	2.97	3638.97
31	3	3256	2.54	3258.54	68	4	3980	2.51	3982.51	105	5	5560	2.98	5562.98
32	3	2900	2.71	2902.71	69	4	3524	2.60	3526.60	106	5	5224	2.80	5226.80
33	3	3292	2.74	3294.74	70	4	4288	2.67	4290.67	107	5	5196	2.75	5198.75
34	3	2976	2.50	2978.50	71	4	3852	2.94	3854.94	108	5	4672	2.81	4674.81
35	3	3860	2.61	3862.61	72	4	3936	2.44	3938.44	109	5	5216	2.72	5218.72
36	3	3540	2.37	3542.37	73	4	3480	2.70	3482.70	110	5	4908	2.98	4910.98
37	3	4000	2.60	4002.60	74	4	4256	2.63	4258.63	111	5	4844	2.46	4846.46

PCB #	Cl Count	t_r'	t_r''	total t_r
112	5	4652	2.78	4654.78
113	5	4508	2.73	4510.73
114	5	5380	2.87	5382.87
115	5	4840	2.86	4842.86
116	5	4744	2.98	4746.98
117	5	4832	2.84	4834.84
118	5	5276	2.73	5278.73
119	5	4632	2.75	4634.75
120	5	4908	2.49	4910.49
121	5	4240	2.53	4242.53
122	5	5336	2.98	5338.98
123	5	5204	2.77	5206.77
124	5	5156	2.69	5158.69
125	5	4644	3.02	4647.02
126	5	5996	2.72	5998.72
127	5	5640	2.48	5642.48
128	6	6028	3.25	6031.25
129	6	5736	3.19	5739.19
130	6	5652	2.99	5654.99
131	6	5256	3.15	5259.15
132	6	5424	3.22	5427.22
133	6	5264	2.71	5266.71
134	6	5212	3.11	5215.11
135	6	5000	2.95	5002.95
136	6	4812	3.22	4815.22
137	6	5600	2.97	5602.97
138	6	5716	3.01	5719.01
139	6	5120	2.96	5122.96
140	6	5148	3.01	5151.01
141	6	5536	2.89	5538.89
142	6	5224	3.18	5227.18
143	6	5108	3.21	5111.21
144	6	5036	2.90	5038.90
145	6	4668	3.21	4671.21
146	6	5336	2.75	5338.75
147	6	5080	2.95	5082.95
148	6	4732	2.78	4734.78
149	6	5088	3.01	5091.01
150	6	4544	3.04	4547.04
151	6	4980	2.89	4982.89
152	6	4596	3.17	4599.17
153	6	5412	2.74	5414.74
154	6	4820	2.79	4822.79
155	6	4280	2.83	4282.83
156	6	6356	2.91	6358.91
157	6	6392	2.98	6394.98
158	6	5764	2.94	5766.94
159	6	5984	2.63	5986.63
160	6	5728	2.94	5730.94
161	6	5364	2.68	5366.68
162	6	6040	2.68	6042.68
163	6	5736	2.91	5738.91
164	6	5660	3.00	5663.00
165	6	5320	2.65	5322.65
166	6	5908	3.04	5911.04
167	6	6112	2.72	6114.72
168	6	5396	2.79	5398.79
169	6	6788	2.13	6790.13
170	7	6696	2.70	6698.70
171	7	6204	3.18	6207.18
172	7	6336	2.89	6338.89
173	7	6228	3.27	6231.27
174	7	6040	3.18	6043.18
175	7	5784	2.92	5786.92
176	7	5552	3.20	5555.20
177	7	6156	3.16	6159.16
178	7	5720	2.88	5722.88
179	7	5472	3.17	5475.17
180	7	6416	2.91	6418.91
181	7	6096	3.11	6099.11
182	7	5780	3.00	5783.00
183	7	5876	2.95	5878.95
184	7	5292	3.01	5295.01
185	7	6004	2.99	6006.99
186	7	5592	3.32	5595.32
187	7	5812	2.92	5814.92
188	7	5212	2.99	5214.99
189	7	6976	2.19	6978.19
190	7	6740	2.49	6742.49
191	7	6488	2.92	6490.92
192	7	6368	2.77	6370.77
193	7	6436	2.89	6438.89
194	8	7132	2.53	7134.53
195	8	6972	2.51	6974.51
196	8	6748	2.52	6750.52
197	8	6260	3.17	6263.17
198	8	6684	2.55	6686.55
199	8	6696	2.61	6698.61
200	8	6428	3.33	6431.33
201	8	6172	3.12	6175.12
202	8	6084	3.08	6087.08
203	8	6756	2.46	6758.46
204	8	6176	3.13	6179.13
205	8	7188	2.54	7190.54
206	9	7360	2.91	7362.91
207	9	6948	2.46	6950.46
208	9	6892	2.37	6894.37
209	10	7552	3.31	7555.31

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