HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPCL)

Paper III CA 2: Interpretation of HPCL Chromatogram

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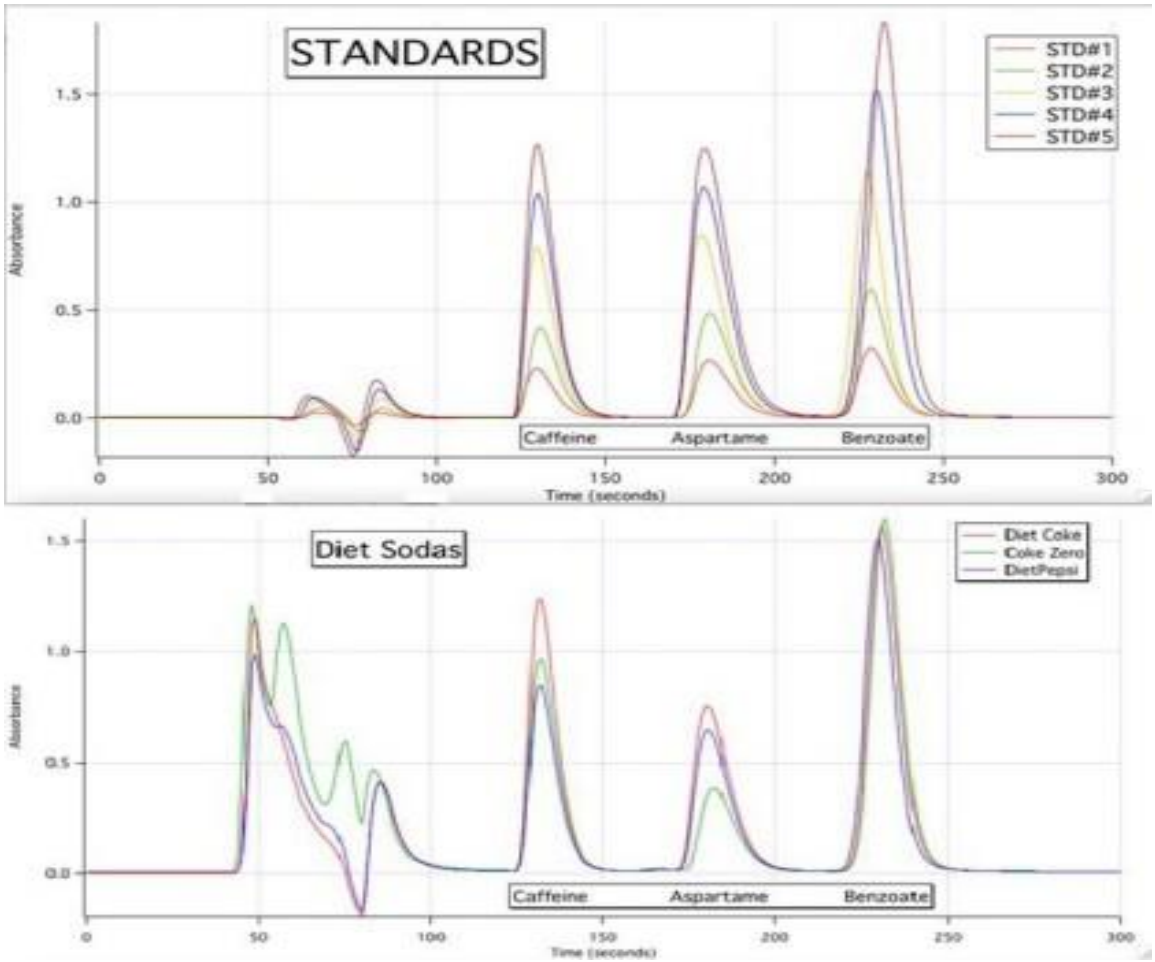
Interpretation of given HPCL Chromatograms

Standard: Aspartame

Unknown: Coke Zero in Aspartame



Aspartame Samples	Peak Height (Absorbance)	Peak width (seconds)	Concentration (mg/L)
Standard 1	0.267	12.11	122.2
Standard 2	0.487	11.76	244.4
Standard 3	0.847	11.25	366.6
Standard 4	1.070	11.85	488.8
Standard 5	1.250	12.98	611.0
Unknown (Coke Zero)	0.386	11.25	-

Peak analysis

1. The standard chromatogram displays clear peaks for each aspartame concentration.
2. The aspartame peak in Coke Zero is distinctly separated.
3. There is no co-elution detected in either chromatogram.
4. The standard chromatogram shows background noise between 50 to 100 seconds.
5. The chromatogram for the diet soda exhibits significant noise from approximately 40 to 100 seconds.
6. The aspartame peaks appear as skewed, asymmetric curves.
7. Both chromatograms maintain stable baseline readings.
8. The peaks in the standard chromatogram are well-defined for each concentration of aspartame.

Approximate Retention time

1. Aspartame Standard 1= 185 seconds
2. Aspartame Standard 2= 183 seconds
3. Aspartame Standard 3= 180 seconds
4. Aspartame Standard 4= 182 seconds
5. Aspartame Standard 5= 184 seconds
6. Aspartame in Coke Zero= 185 seconds

Calculation of Peak Area

Peak area for Standard 1

- Peak Height (PH): 0.267 (mm)
- Peak width at half height (PW): 12.11s
- Peak Area = $PH \times PW$
  = 0.267 × 12.11 = **3.23 mm^2**

Peak area for Standard 2

- Peak Height (PH): 0.487 (mm)
- Peak width at half height (PW): 11.76s
- Peak Area = $PH \times PW$
  = 0.487 × 11.76 = **5.73 mm^2**

Peak area for Standard 3

- Peak Height (PH): 0.847 (mm)
- Peak width at half height (PW): 11.25s
- Peak Area = $PH \times PW$
  = 0.847 × 11.25 = **9.53 mm^2**

Peak area for Standard 4

- Peak Height (PH): 1.070 (mm)
- Peak width at half height (PW): 11.85s
- Peak Area = $PH \times PW$
  = 1.070 × 11.85 = **12.69 mm^2**

Peak area for Standard 5

- Peak Height (PH): 1.250 (mm)
- Peak width at half height (PW): 12.98s
- Peak Area = $PH \times PW$
  = 1.250 × 12.98 = **16.23 mm^2**

Peak area for Coke Zero

- Peak Height (PH): 0.386 (mm)
- Peak width at half height (PW): 11.25s
- Peak Area = $PH \times PW$
  = 0.386 × 11.25 = **4.34 mm^2**

Calculation of Percentage

Sample	Peak Area of Standard 1	Peak Area of Standard 2	Peak Area of Standard 3	Peak Area of Standard 4	Peak Area of Standard 5	Peak Area of Coke Zero
Caffeine	2.2	4.0	7.27	9.56	12.08	9.04
Aspartame	3.23	5.73	9.53	12.69	16.23	4.34
Benzoate	3.44	5.49	9.88	14.5	18.06	15.18

Percent Aspartame in Coke Zero

= ($Area\ of$ Aspartame $curve\ for$ Coke Zero ÷ $Area\ of\ the\ total\ curve$) × 100

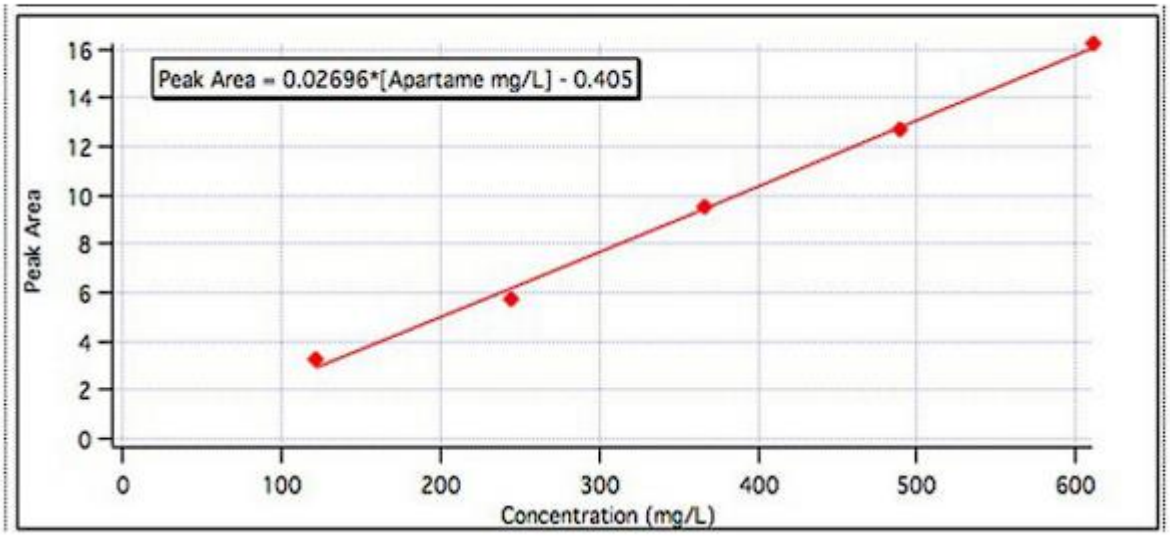
= (4.34 ÷ (3.23 + 5.73 + 9.53 + 12.69 + 16.23)) x 100

= (4.34 ÷ 47.41) x 100

= 9.154 %

Estimation of Aspartame Concentration in Coke Zero

The calibration curve for Aspartame



Peak Area of Aspartame for Coke Zero = 4.34mm^2

Peak Area of Aspartame Standard 1 = 3.23mm^2 (This is the closest to the unknown peak)

The Concentration of Aspartame in Standard 1 is 122.2 mg/L

∴ The concentration of aspartame present in as is equal to 122.2 mg/L

But the Diet Soda was diluted by a dilution factor of 2. Thus, the actual concentration of Aspartame in would be:

122.2 mg/L × 2 = 244.4 mg/L