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Exp # 4

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Ultraviolet-visible Spectroscopy of a Mixture

Date experiment started: 2/8/2000
Date experiment completed: 2/8/2000
Date experiment write-up completed: 2/17/2000
Unknown Identification number: 6

Purpose:

The purpose of this experiment is to determine the ~~concentration~~ amount of aspirin, phenacetin and caffeine in an APC tablets by using UV/vis spectroscopy and the Beer's Law.

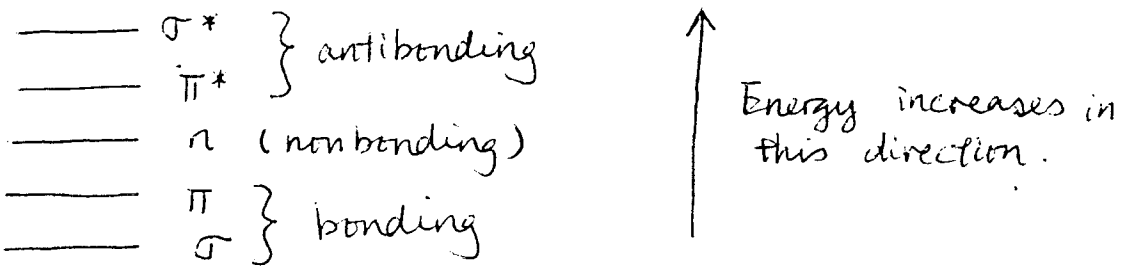
~~Theory~~

Introduction / Theory: (-3)

Ultraviolet-visible Spectroscopy is a very useful and powerful tool in identifying and quantifying the pure organic compounds, especially aromatics. Not only the UV/vis ~~Spectroscopy~~ is ~~of~~ has wavelength ranges from 100nm to 700nm in the light spectrum. The UV/vis lights have the energy that are approximately equivalent to the separation between 2 electronic states in energy scale. As the molecules absorb the UV/vis light, they will undergo electronic transitions. In general, $\sigma \rightarrow \sigma^*$ transition $n \rightarrow \sigma^*$ transition, $\pi \rightarrow \pi^*$ transition and $n \rightarrow \pi^*$ transition are the four general types of electronic transitions in organic molecules.(1),(4)

In $\sigma \rightarrow \sigma^*$ transition, a σ electron is moved into a σ^* antibonding orbital. This is the ~~light~~ highest ~~energy~~ electronic transition and occurs in the far-UV portion of the electromagnetic ~~transition~~ spectrum. This type of transitions ~~are~~ is usually hard to be observed using common laboratory spectrometers.(1)

The below figure shows the distribution of σ, σ^*, n, π and π^* orbitals in a typical molecule.

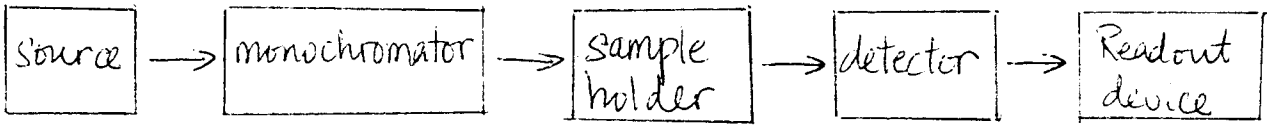


In $n \rightarrow \sigma^*$ transitions, a nonbonding electron is moved into ~~an~~ a σ^* antibonding orbital. These are still high-energy transitions that have λmax at about 200nm, which is at the near and far UV regions.

In $n \rightarrow \pi^*$ transitions, a nonbonding electron from lone pairs is excited to a π^* antibonding orbital. These transitions are considered to be lower in energy compare to the ~~previous~~ previous two types of transitions and occurs in the ultraviolet range.

The last transitions are the $\pi \rightarrow \pi^*$ transitions, in which ~~an~~ a π electron is promoted to a π^* antibonding ~~electron~~ orbital. These transitions needed the least energy and are well located in the visible spectrum of light.

Instrument: [1]



The light source that we will use should produce a useful continuum spectrum in the region of 200nm to 300nm. The lamp that produces this light spectrum is a deuterium lamp. This deuterium lamp is a low-voltage type in which an arc is formed between heated, oxide-coated filament and a metal electrode. The shape of the aperture ~~of~~ between the two electrodes constricts the discharge of a electron to a narrow path when 40V is applied. As a result, a ball of radiation is produced. [1],[3]

watch the style!

The monochromator acts as a wavelength selector that discompose the polychromatic light into ~~to~~ various monochromatic lights. Usually a reflection grating is used, in which it has ~~closed~~ closely ruled grooves on a reflective metal surface. Different wavelength of light striking this surface are reflected at different angle. because of?

Sample holder of this instrument is called cuvettes which have path length equals to 1cm and is transparent to the radiation from 200nm to 300nm. Quartz is usually the material that ~~made make~~ the sample holder is made of. To minimize the reflection loss, the window of the cell ~~is~~ are ~~normal~~ normal to the direction of the beam.

The detector of this instrument is used to measure the light that passes through the sample. The absorbance is obtained by measuring the power of a monochromatic light entering (P_0) and leaving (P) the sample and is given by

$$A = -\log_{10} T = -\log_{10} P/P_0$$

not clear

The power of light is measured by a photomultiplier tube which consist of an array of dynodes. These dynodes are arranged in such a way that the previous? dynode is maintain at a potential less than the later one, so the number of electrons kicked out by the transmitted photons will ~~multiply~~ be multiplied and the electrons are accelerated. This is then in a form current that can be measured by a electronic circuit.

The ~~de~~ readout device records the output from the detector and is usually a computer.

Beer's Law

In almost all the optical measurements of organic compounds, Beer's ~~is~~ Law is one of the most useful tools to analyze the result or spectrum. It is a quantitative expression that correlates the absorbance of light to the concentration of absorbing species, in which

$$A = b\varepsilon c \qquad \text{(eq 1)}$$

Units -2

where A is absorbance, ε is molar absorptivity, c is concentration and b is the path. Notice that A and ε are wavelength dependent. Thus at different wavelength A and ε will vary.

~~Usually~~ Sometimes, it is useful to know that ~~&~~ the absorbance can be defined as

$$A = -\log_{10} T \qquad \text{(eq 2)}$$

where T is the transmittance and is given by

$$T = P/P_0 \qquad \text{(eq 3)}$$

where P_0 and P ~~are~~ the power of light ~~entering~~ entering and leaving the sample.

However, Beer's law starts to fail when the concentration of the analyte is greater than 0.01M. This is due to the fact that, as we increase the concentration of the analyte solution, the distance between the molecules becomes shorter and shorter. This distance will shrink to a point that it can alter the ability of that molecule to absorbance a given radiation. As a result, a deviation in linear relationship between absorbance and concentration will ~~result~~ be induced,

The concentration of two species in a mixture can be determined by measuring the absorbance of the solution at two different wavelengths. The proper method used depends on the spectra that we obtain. In general, there are two separate methods to solve ~~these~~ the scenarios, ~~where The~~

These 2 scenarios are the spectra with minimal overlap and the spectra with significant overlap.

i) The spectra with minimal overlap

not clear

First, we consider the absorbance of compounds x and y. Compounds x and y have different values of λ_{max}, a wavelength where maximum absorbance occurs, and different values of molar absorptivity ε at various wavelengths. Let λ_1 and λ_2 be the λ_{max} of x and y respectively.

At λ_1, from eq 1, the Beer's Law, we know that

$A_{x_1} = \varepsilon_{x_1} [X] b$ (eq 4)

$A_{y_1} = \varepsilon_{y_1} [y] b$ (eq 5)

and at λ_2

$A_{x_2} = \varepsilon_{x_2} [X] b$ (eq 6)

$A_{y_2} = \varepsilon_{y_2} [y] b$ (eq 7)

For a mixture that contains x and y, at λ_1 the absorbance of the mixture, A_1, is given by

$$A_1 = \varepsilon_{x_1} [X] b + \varepsilon_{y_1} [y] b \quad \text{(eq 8)}$$

Also at λ_2, the absorbance of the mixture, A_2, is given by

$$A_2 = \varepsilon_{x_2} [X] b + \varepsilon_{y_2} [y] b \quad \text{(eq 9)}$$

To find the concentration of x and y, [X] and [y], we have to solve (eq 8) and (eq 9) simultaneously. In order to do this, we have to know the molar absorptivity of x and y at λ_1 and λ_2. These quantities can be determined by using standards of x and y and eq 4 to 7.

ii) Spectra with significant overlap.[1]

For a mixture, the absorbance (A_{mix}) is given by Beer's Law, at a particular wavelength, in which

$$A_{mix} = \varepsilon_x [x] b + \varepsilon_y [y] b \qquad \text{(eq 10)}$$

In order to obtain the concentration of x and y, [x] and [y], of a mixture, we have to prepare a standard x solution with concentration $[x]_s$ and standard y solution with concentration $[y]_s$. Again using (eq 1);

$$A_{x_s} = \varepsilon_x [x]_s b \qquad \text{(eq 11)}$$

$$A_{y_s} = \varepsilon_y [y]_s b \qquad \text{(eq 12)}$$

Then, we can solve for ε_x and ε_y and substitute them back into (eq 10). After rearranging the (eq 10), we get

$$\frac{A_{mix}}{A_{x_s}} = \frac{[y]}{[y]_s}\left(\frac{A_{y_s}}{A_{x_s}}\right) + \frac{[x]}{[x]_s} \qquad \text{(eq 13)}$$

Then, we make a calibration curve of ~~A_m/A_{x_s}~~ A_{mix}/A_{x_s} versus A_{y_s}/A_{x_s} at various wavelengths. Using the least square method to fit the data, we will find that the calibration curve has the slop equals to $[y]/[y]_s$ and intercept equals to $[x]/[x]_s$. From here, we can solve for [x] and [y] in the mixture.

Experimental section:

Instrument Name: Perkin-Elmer Lambda 3B
Instrument type: UV-Vis spectrometer

don't write this part as a cookbook recipe. Through the steps, commenting on difficulties that you encountered or just comments that you have about the procedure.

1) Sample preparation (A mixture of aspirin and phenacetin):
Weigh accurately one APC tablet and crush to a fine powder in a 150mL beaker. To this powder in the beaker, 80mL of chloroform will be added to dissolve the powder evenly. Next, the solution is transfered quantitatively into a 250mL separatory funnel. To exact the organic solution, two 40mL portions of cold 4% (w/v) sodium bicarbonate solution followed by 20mL of water are added. Next, the aqeous extracts are combined and washed with three 25mL portions of chloroform. Then, the organic liquids are combined and filtered through a filter paper wetted with chloroform into a 250mL volumetric flask. The solution is diluted to mark. Next, 10.00 mL of the solution is pipetted into a 100mL volumetric flask and is diluted to mark.

Aspirin solution:
First, we acidify the aqeous solution in the separatory funnel with 1M sulfuric acid. The acid should be added slowly in small portions. Do not mix vigorously until CO_2 evolution has almost ceased. Then, the aqueous solution is extracted with eight portions of 50mL chloroform. The chloroform extracts are filtered through chloroform wetted filter paper into a 500mL volumetric flask and diluted to mark. Next, 20mL of this solution is transferred to 100mL volumetric flask and diluted to mark with chloroform.

2) Standards Preparation

Aspirin Standards

A 1000 ppm aspirin stock solution is first prepared by dissolving 0.100 g of aspirin with 100mL of chloroform in 100mL volumetric flask. To prepare 100 ppm, 150 ppm and 200ppm of aspirin standards, 10mL, 15mL and 20mL of the stock solution are pipetted into 3 clean 100mL volumetric flasks respectively. Then, chloroform is used to dilute the solutions to marks.

Phenacetin and Caffeine Standards

0.100g of phenacetin is dissolved in 100mL of chloroform in a 100mL volumetric flask to prepare a 1000 ppm stock solution. 2ppm, 10ppm and 20 ppm of the phenacetin standards are prepared by pipetting 2mL, 10mL and 20mL of the stock solution into 3 separate 100mL volumetric flasks and diluted to mark with chloroform.

Caffeine Standards

To prepare 2 ppm, 10ppm and 20ppm of the caffeine standards, we follow the same steps in phenacetin standards preparation.

3) Data Analysis: [2]

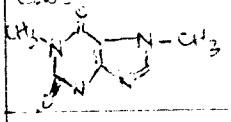
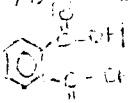
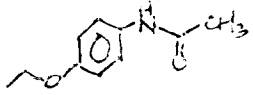
Least-square method is used to construct the calibration curves and to determine the concentration of aspirin, phenacetin and caffeine in a APC tablet. The following equation is used to find the error of the parameter $X_{unknown}$ of $y = mx + b$.

$$S_c = \frac{S_{ey}}{m}\sqrt{\frac{1}{M} + \frac{1}{N} + \frac{(\bar{y}_c - \bar{y})^2}{m^2 S_{xx}}} \quad (1)$$

where M is the number replication for each measurement, $\bar{y}$ is the average value of y, S_{ey} is the error of y and $\bar{y}_c$ is the value of y of the unknown.

$$\%wt = \frac{\text{Concentration} \times \text{Dilution Factor} \times \text{Volume}}{\text{wt. of the Tablet}} \times 100\%$$

4) MSDS:

	Chemical	Chemical Alerts & properties
1)	Caffeine 	harmful if inhaled or swallowed, may cause irritation; handle with proper protections
2)	Aspirin 	irritation will be induced if inhaled, contacted, & should be kept in a cool, ventilated area; BP(°C) = 140°C, MP = 135°C, classified as mutagen
3)	Phenacetin 	cancer causing; goggles, lab coat and gloves should be worn, put on; MP = 135°C; waste should be sent to RCRA approved for $\frac{1}{2}$
4)	Chloroform $CHCl_3$	strict protocols; must be absorbed through skin, be stored away from strong oxidants and bases and kept in dark, BP = 61°C; MP = -64°C
5)	Sodium bicarbonate $NaHCO_3$	use safety glasses & be investigated as mutagen, irritant to inhalation & skin and eye, MP = 0°C
6)	Sulfuric acid H_2SO_4	prevent generation of mists, wear use contact; corrosive; reacts violently with combustible and reducing materials; BP = 340°C; MP = 10°C

Data and Results:

Data:

Weight of the APC tablet: 0.5031g

Aspirin Standards:

λmax = 275nm

Concentration (ppm)	Absorbance
0 ppm	0.0000
100 ppm	0.7348
150 ppm	1.1638
200 ppm	1.5098
APC tablet	1.1870

Table 1

Caffeine Standards:

λmax = 273nm

Concentration (ppm)	Absorbance
0	0.0000
2	0.1068
10	0.4750
20	0.9398
APC tablet	0.6015

Table 2

Phenacetin Standards:

λmax = 274nm

Concentration (ppm)	Absorbance
0 ppm	0.0000
2 ppm	0.1315
10 ppm	0.6529
20 ppm	1.3301

Table 3

Note: 0 ppm solutions are the blanks used to zero the instrument.

ctra of Aspirin standards

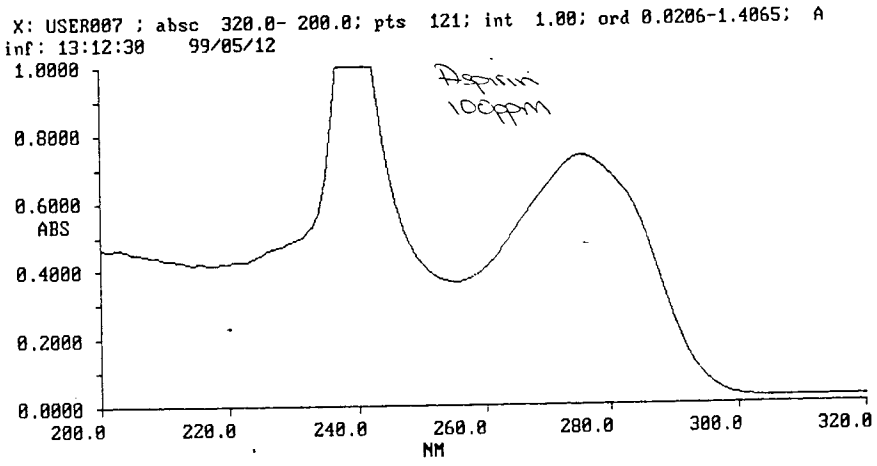


Figure 1.

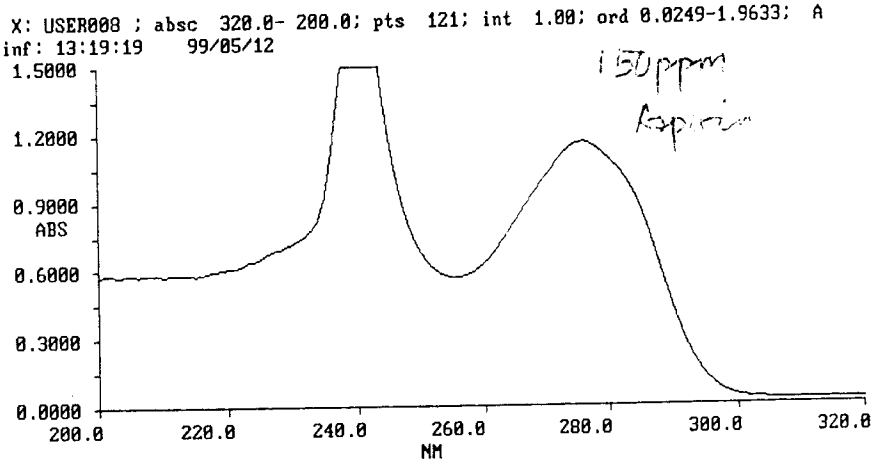


Figure 2.

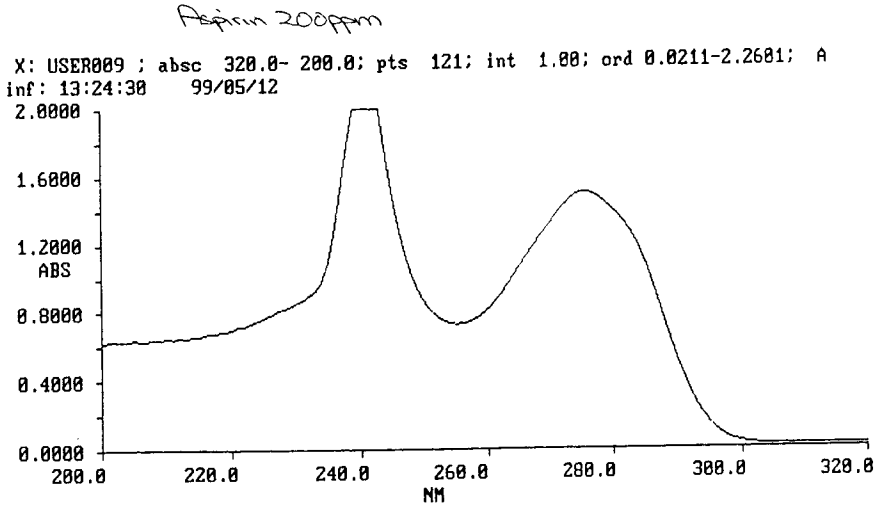


Figure 3

Spectra of Caffeine Standards

Caffeine
2ppm

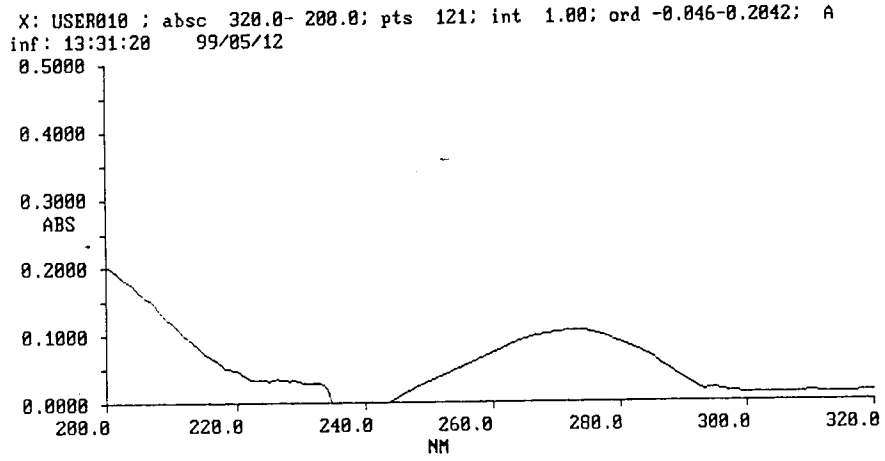


Figure 4

Caffeine
20ppm

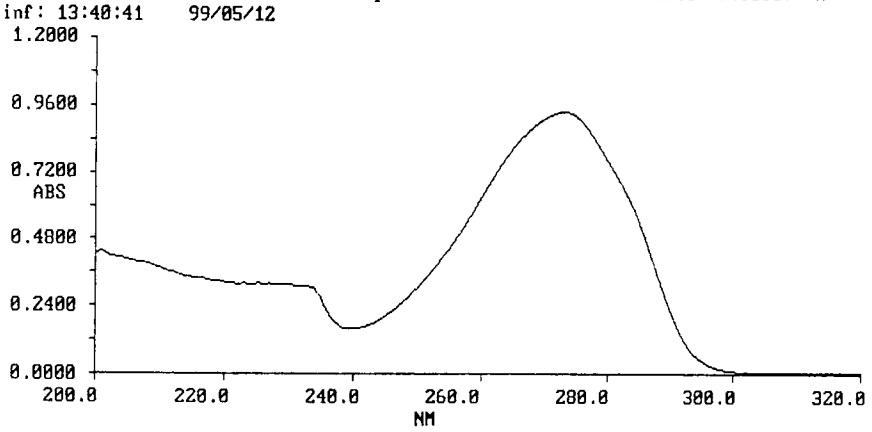
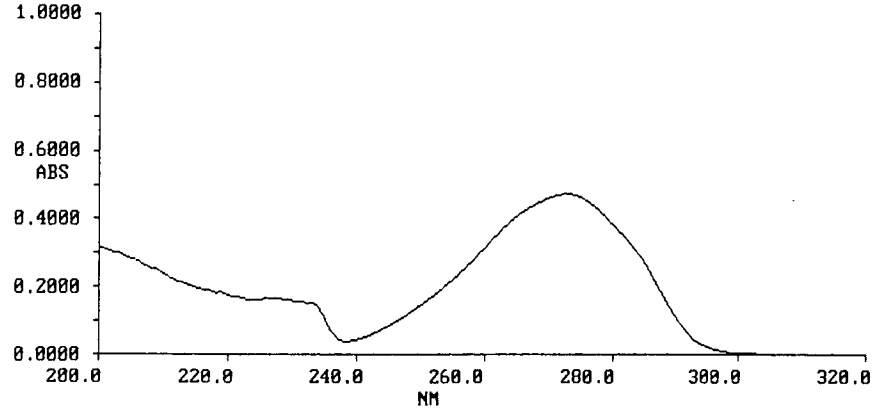


Figure 5

Caffeine
10ppm



Spectra of Phenacetin Standards:

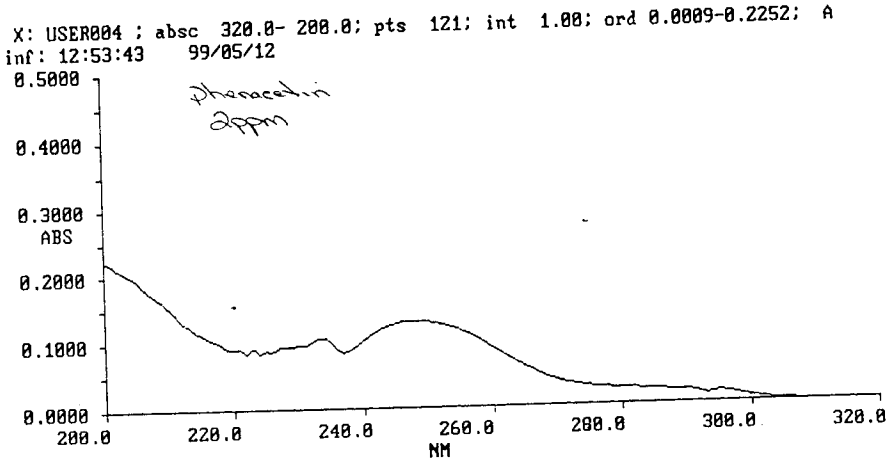


Figure 7

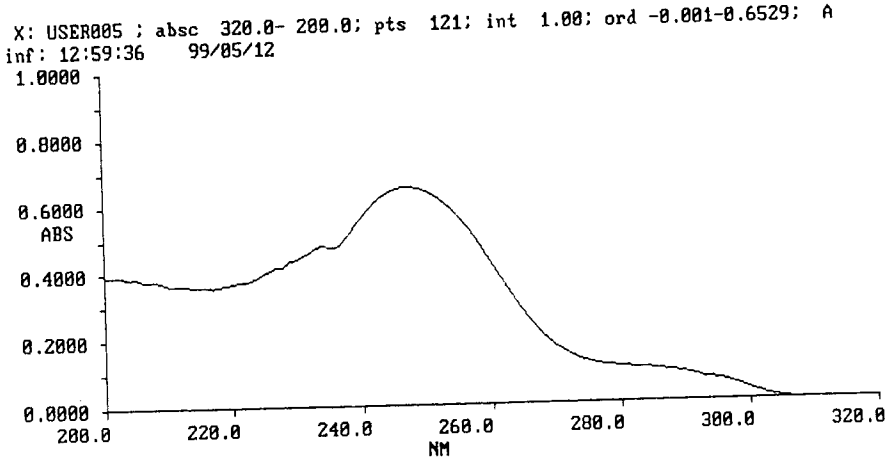


Figure 8

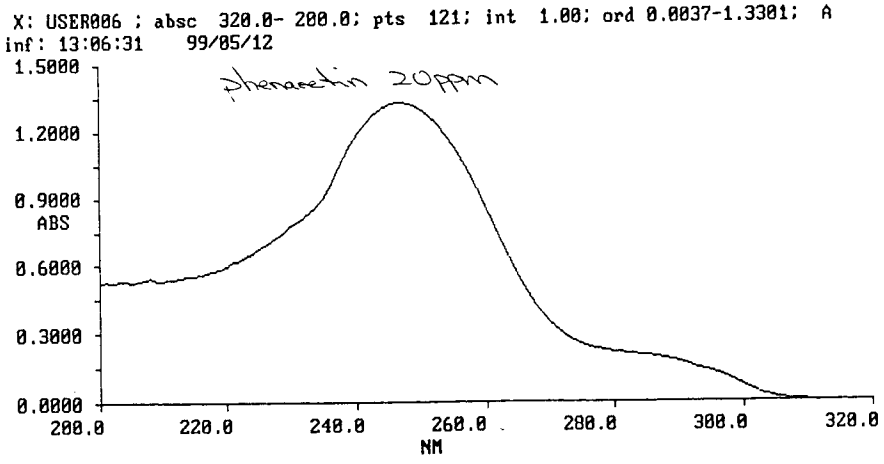


Figure 9

Spectra of unknown Organic Extract :

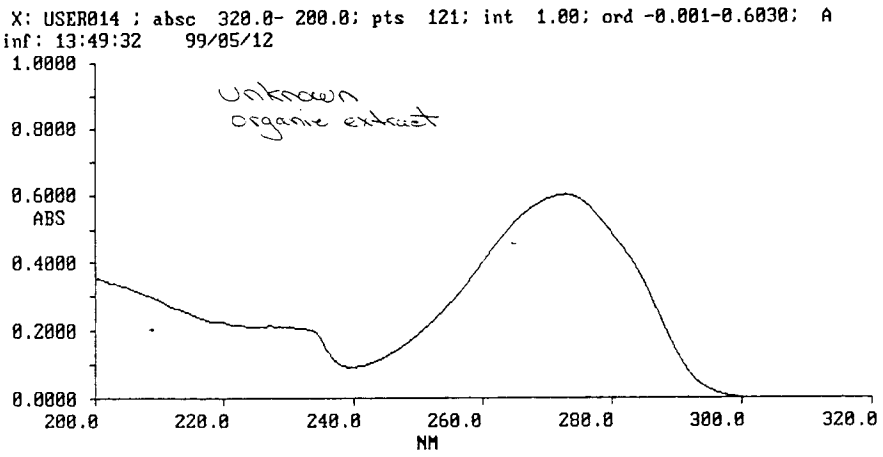


Figure 10

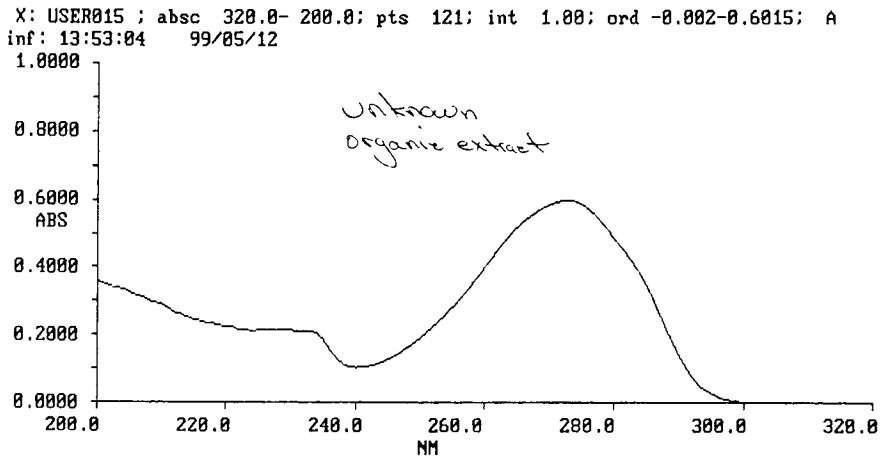


Figure 11

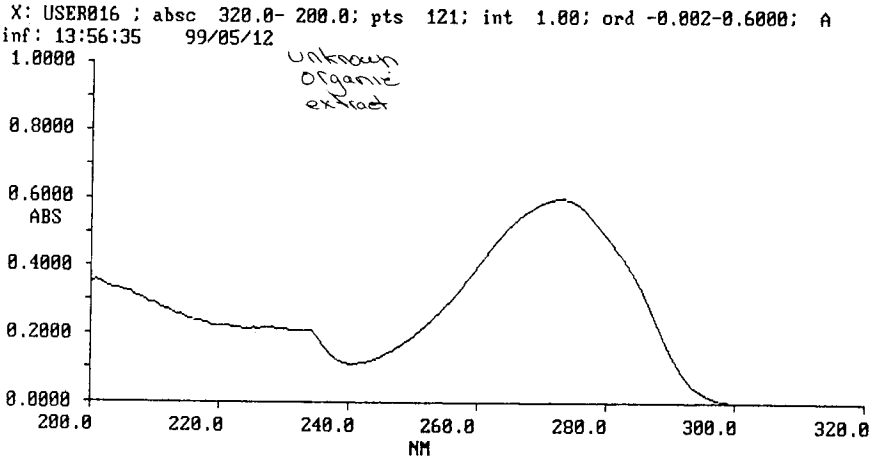


Figure 12

Spectra of unknown ~~aqueous~~ aqueous Extract =

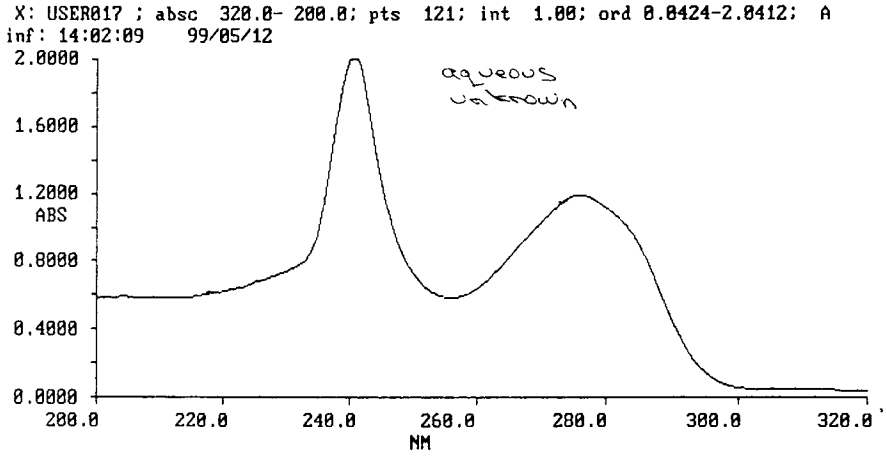


Figure 13

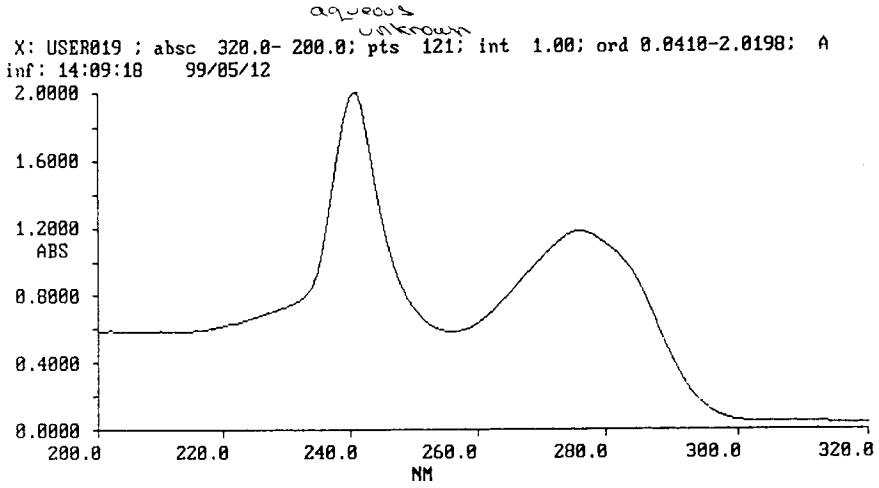


Figure 14

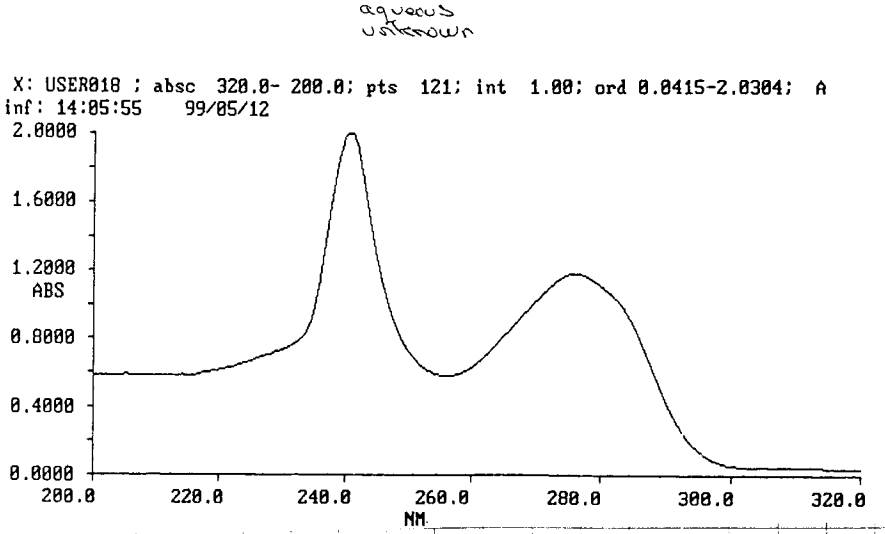


Figure 15

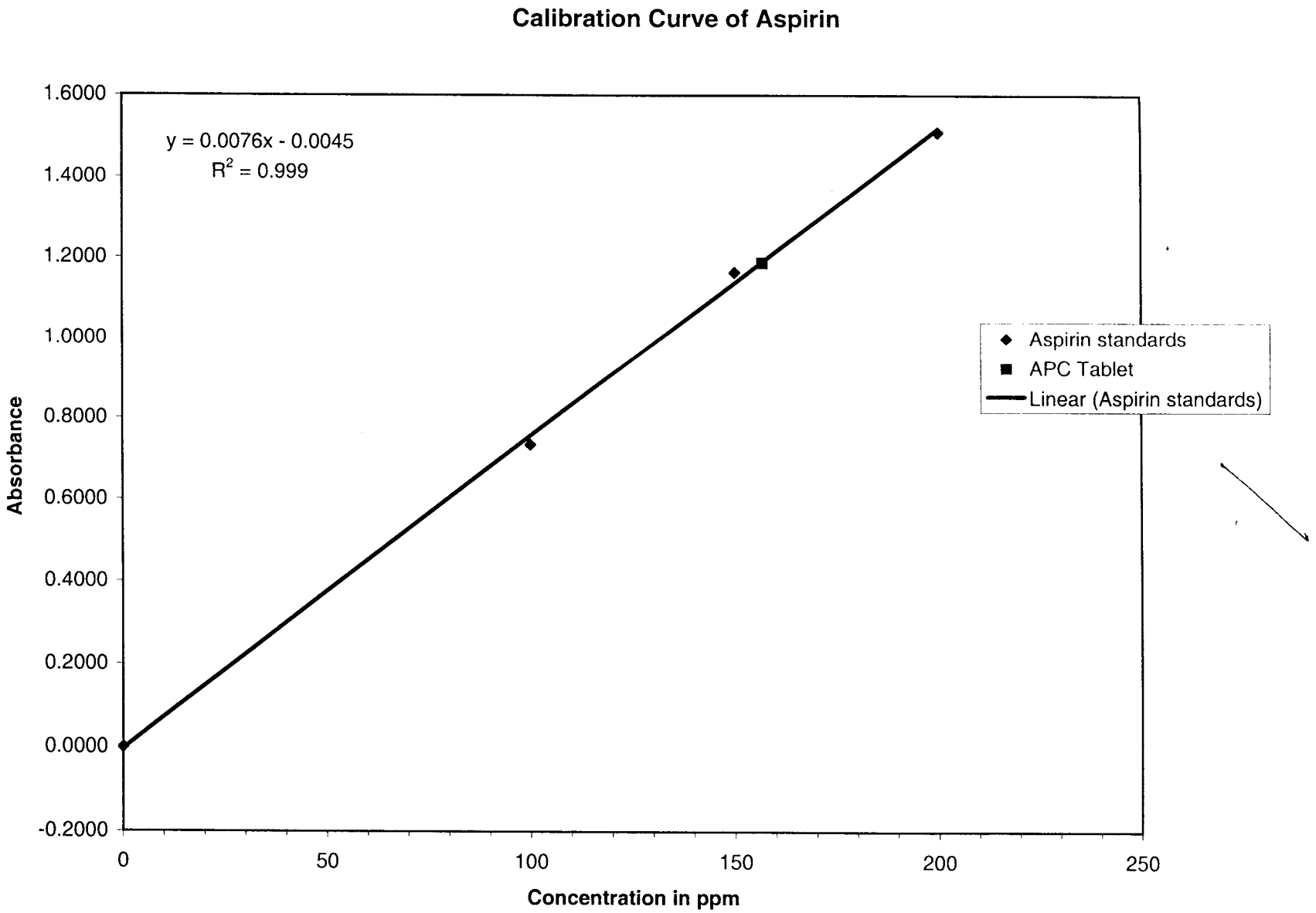

Calibration Curve of Aspirin
y = 0.0076x - 0.0045
R2 = 0.999
Absorbance
1.6000
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
-0.2000
0
50
100
150
200
250
Concentration in ppm
Aspirin standards
APC Tablet
Linear (Aspirin standards)

Figure 16

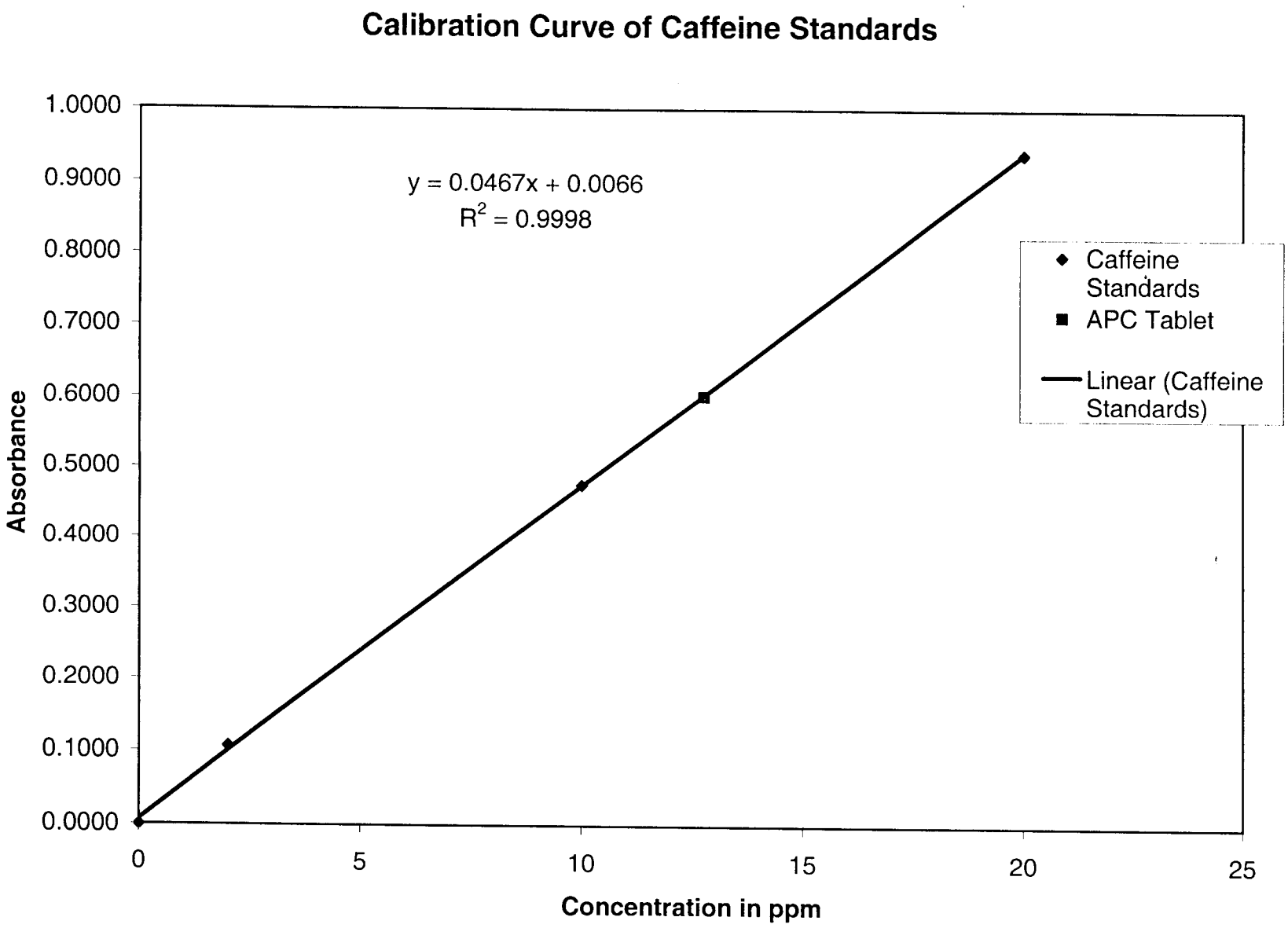

Calibration Curve of Caffeine Standards
y = 0.0467x + 0.0066
R2 = 0.9998
Absorbance
1.0000
0.9000
0.8000
0.7000
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0
5
10
15
20
25
Concentration in ppm
Caffeine Standards
APC Tablet
Linear (Caffeine Standards)

Figure 17

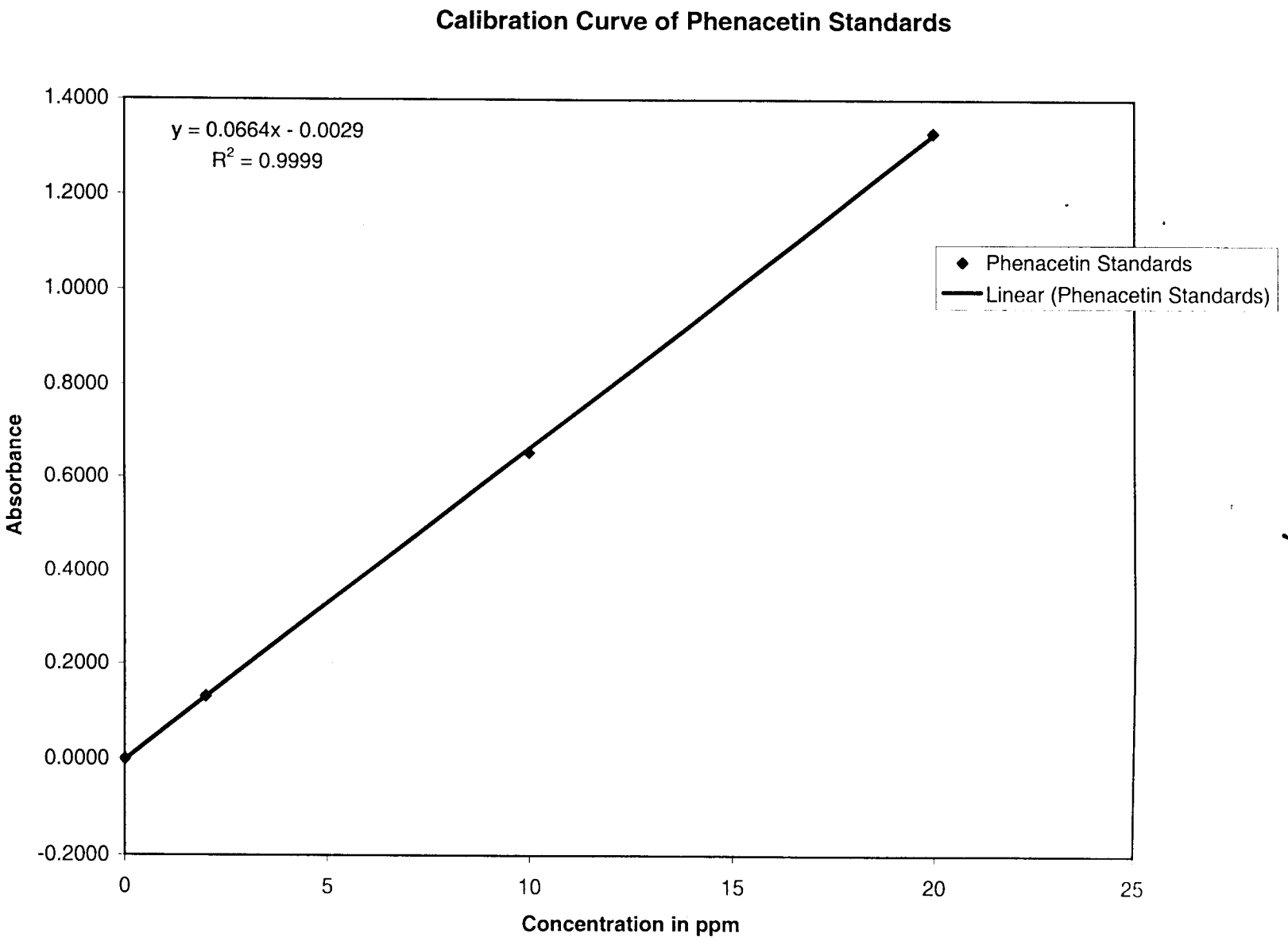

Calibration Curve of Phenacetin Standards
y = 0.0664x - 0.0029
R2 = 0.9999
Absorbance
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
-0.2000
0
5
10
15
20
25
Concentration in ppm
Phenacetin Standards
Linear (Phenacetin Standards)

Figure 18

Results:

Statistical Analysis:

	Absorbance					
Sample (extract)	**Trial #1**	**Trial #2**	**Trial #3**	**Average**	**Std. Dev**	**$S_e y$**
Aspirin (aqueuos)	1.1819	1.1856	1.1936	1.187	0.006	0.025
Caffeine (Organic)	0.6000	0.6015	0.6030	0.602	0.001	0.0068
Phenacetin (Organic)	Uncertain	Uncertain	Uncertain	Uncertain	Uncertain	Uncertain

Table 4

Summary of the Results of the Unknown:

Sample (extract)	Absorbance	Slope of the Best fit line	y-intercept	Conc. in ppm	Amount (mg)	%wt
Aspirin (aqueous)	1.187±0.006	0.0076±0.0002	-0.00±0.02	156±4	390±10	76%±2%
Caffeine (Organic)	0.602±0.001	0.0467±0.0004	0.007±0.004	12.7±0.2	31.8±0.4	6.31±0.08%
Phenacetin (Organic)	Uncertain	Uncertain	Uncertain	Uncertain	Uncertain	Uncertain

Table 5

Sample Calculation : (Aspirin)

Absorbance of Aspirin = 1.187 ± 0.006

$y = 0.0076x + 0.02$

$1.187 = 0.0076x + 0.02$

$x = 153.55$ ppm

error of $X = \frac{S_{e_y}}{m}\sqrt{\frac{1}{M} + \frac{1}{N} + \frac{(\bar{y}_c - \bar{y})^2}{m^2 S_{xx}}}$

$= \frac{0.025}{0.0076}\sqrt{\frac{1}{1} + \frac{1}{4} + \frac{(1.187 - 0.852)}{(0.0076)^2(2187.5)}}$

$= 3.82$

So, Concentration of aspirin = 156 ± 4 ppm

Amount of Aspirin = Concentration × DF × Volume (1 ppm = 1 mg/L)

$= (156 \pm 4)$ ppm $\times 5 \times 0.5$ L

$= 390 \pm 10$ mg

% wt = $\frac{\text{amount of aspirin}}{\text{wt of the tablet}} \times 100\% = \frac{390 \pm 10 \text{ mg}}{503.1 \text{ mg}} \times 100\% = 76\% \pm 2\%$

Discussion and Analysis of Results:

From Table 5, we found that there are 76% ± 2% and 6.31 ± 0.08% Aspirin and caffeine in the APC tablet. The reason that we are uncertain about the weight percent of phenacetin is because ~~we~~ there is no distinct peak at $\lambda = 247\,nm$ in the spectra of organic extract (Figure 10 to Figure 12). Hence, it is possible that there is no phenacetin in the APC tablet or the amount of phenacetin in the APC tablet are very small, in which it might overlap with the gausian shape peak of the caffeine. Since the sum of the percent weight of the ~~76%~~ aspirin and caffeine is not ~~greater than~~ equal to 100%, there are some molecular or organic binder that present in the Tablet.

Figure 1 to 3 and Figure 13 to 15 show the spectra of the aspirin standards and aspirin unknown. There are 2 distinct peaks present in the spectra at $\lambda = 275\,nm$ and $\lambda = 241\,nm$. We believed that these two peaks are due to the 2 distinct functional groups, carboxylic acid (-C(=O)-OH) and ketone (R-C(=O)-R'), in the aspirin, in which have $\lambda_{max} = 275\,nm$ and $241\,nm$ respectively.[2]

From Figure [illegible] to 20, we see that ~~there is a peak~~ [illegible] peak at [illegible] 240 nm. The [illegible] is due to the [illegible] of the cell [illegible] which is made of quartz. ?

Figure 16 to Figure 18 are the calibration curves of the aspirin, caffeine and phenacetin. All of them have R^2 value above [illegible]. This means that Beer's Law holds very well within the calibration range of these compounds.

To further confirm the content of our unknown (APC tablet #2), we can ~~separate the~~ phenacetin extract the caffeine from the sample by [illegible] adding (dilute?) to the ~~APC~~ [illegible] phenacetin and [illegible] mixture. This will extract the caffeine to an aqueous layer. Then we analyze the organic extract with [illegible] and [illegible] phenacetin in the tablet.

Question:

) Comment on the possibility that all three components of APC can be determined simultaneously. What do you base this conclusion on?

First, we have to know that the linear & relationship between concentration and absorbance in Beer Law hold in the range of concentration that we are interested in. Then, the spectrum must have 3 peaks that are not completely overlapped ~~each other~~. If these holds, we can use Beer's Law to write down ~~3 equations~~ an equation for 3 different wavelength where each of them absorb the most.

at λ_1 $\quad A_1 = \varepsilon_{x_1} b C_x + \varepsilon_{y_1} b C_y + \varepsilon_{z_1} b C_z$

at λ_2 $\quad A_2 = \varepsilon_{x_2} b C_x + \varepsilon_{y_2} b C_y + \varepsilon_{z_2} b C_z$

at λ_3 $\quad A_3 = \varepsilon_{x_3} b C_x + \varepsilon_{y_3} b C_y + \varepsilon_{z_3} b C_z$

We can find all the molar absorptivities ~~from the~~ measuring the standards at λ_1, λ_2 and λ_3. Then, we can solve ~~this~~ these equations. ii) and that would be complicated and resulting in errors

) What other solvents could be used for the solutions? What solvents would be much less useful? Why

Methanol, ether, ethanol will be useless since they [illegible] the [illegible] absorptions of carbonyl, carboxyl and amide, which are some of the functional groups of aspirin, caffeine and phenacetin. Dioxane, [illegible] would be [illegible] since they are [illegible] and [illegible] [illegible] ii)

rule # 1: the solvent must not absorb in the region of interest. did you check for this?

) Could you have determined the concentration of the aspirin in the aqueous solution? What would ~~be~~ your concerns be?

Since aspirin has a carboxylic acid functional group, it ~~can~~ acts as an acid. As we know, when acid is added into water, acid will dissociate, in which

$$HA \longrightarrow H^+ + A^-$$

$$K = \frac{[H^+][A^-]}{[HA]}$$

Now instead of one species, we now have 3 species and we have complicated the problem. ii)

~ OK

graphy

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