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Spectrohotometric Analysis
of Anacin Content

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Theory and Practice of Quantitative Chemistry
Chemistry 134

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Spectrophotometric Analysis of Anacin Content

John Mongan
March 16, 1997

Abstract
Spectrophotometric analyses of Unknown #40, a putative 500mg Anacin tablet, were performed to determine its organic content. At a 95% confidence level, UV-Vis molecular absorption spectroscopy found the tablet to contain 13(±4)mg of caffeine and fluorescence spectroscopy found the tablet to contain 115(±4)mg of acetylsalicylic acid and 1.6(±0.2)mg of salicylic acid.

Theory
Two techniques, UV-Vis spectroscopy and molecular fluorescence spectroscopy, were used to measure concentrations in this lab. UV-Vis spectroscopy is based on the linear relationship between concentration of analyte and absorbance of light at a given frequency. In fluorescence spectroscopy, analyte molecules are excited at a specifically chosen wavelength and emit light at different, typically longer wavelengths. The intensity of the light emitted at a given wavelength is related to the concentration of the analyte by the equation $I = \Phi I_0(1-e^{-\varepsilon cl})$, where $c$ is the concentration and all other values are constants, allowing concentration to be calculated from intensity. A more complete examination of the theory behind these types of spectroscopy is found in the lab manual.

The technique of absorbance spectroscopy can be applied to a wide range of molecules. Most organic molecules, including caffeine, salicylic acid and acetylsalicylic acid, have significant absorbance in the UV-Vis range. The technique is also quite sensitive: for a standard 1cm cuvette, the molecules studied in this lab are detected at concentrations of ca. $10^{-5}$M. The most significant drawback of absorbance spectroscopy is that absorbance is additive at any given frequency, so analyte concentrations cannot be measured in the presence of other molecules that absorb in the same range. Since substances of interest are often found in the presence of other organic, absorbing molecules, as in this lab, it is necessary to perform extraction procedures to separate the chosen analyte. These extractions inevitably increase the time required to take a measurement and the error associated with it. A further difficulty is that absorbances can be accurately measured only between about 0.4 and 1.2, so if no estimate of sample concentration is available, a number of trials may be required to achieve an appropriate dilution.

Fluorescence is a less common property of molecules. In general, only rigid aromatic molecules exhibit significant fluorescence. Since only excited molecules fluoresce, further specificity can be achieved by carefully selecting a wavelength for the incident light that will excite only the molecules of interest. If such a wavelength exists, analyte concentrations can be measured in the presence of other molecules, unlike with absorbance spectroscopy. In this case, the sample solution need only be free of particulates, so filtration is the only preparation procedure necessary, rather than complicated extractions. Although in this lab the concentrations measured by fluorescence spectroscopy are of the same order as those measured by absorbance.
spectroscopy, fluorescence spectroscopy has the potential to be a much more sensitive technique than absorbance for molecules that fluoresce efficiently. The major difficulty with fluorescence spectroscopy is that many molecules of interest do not fluoresce significantly, although this problem can often be alleviated by complexing the molecule with another substance that does fluoresce. Another minor issue with fluorescence spectroscopy is that the relation between emission intensity and concentration is well defined only at low concentration and is more complicated than that between absorbance and concentration. Acquiring a fluorescence emission spectrum also takes marginally longer than acquiring an absorption spectrum.

The molecules of interest in this lab are caffeine, salicylic acid and acetylsalicylic acid. Since caffeine does not have significant fluorescence, it is measured by UV-Vis absorbance spectroscopy. Salicylic acid and acetylsalicylic acid absorb in the same region as caffeine, so an extraction is necessary before this measurement can be made. Salicylic acid and acetylsalicylic acid each have a benzene ring and fluoresce (albeit inefficiently), so their concentrations are measured by fluorescence spectroscopy. The absorption peaks of these two substances differ sufficiently that they can be excited individually, so their concentrations are measured from a solution containing all three molecules of interest.

**Procedure**

Procedure generally followed that described in Experiment #4 of "Analytical Laboratory Experiments for Chemistry 134" by T. D. P. Stack, with a few exceptions.

Caffeine was extracted from the sample prior to analysis. 250.5mg of sample were dissolved in 8ml of chloroform. The solution was washed twice with cold 5% sodium bicarbonate and once with deionized water. The combined aqueous layers were twice back-extracted with chloroform. The combined chloroform solutions were passed through a pipet filter loaded with MgSO₄ and then diluted to a total volume of 25mL. Three 1:100 dilutions of this solution in absolute methanol were prepared for spectroscopic analysis. A fourth unknown solution was prepared by dissolving 20.5mg of unextracted sample in 10ml of absolute methanol and diluting this 1:10 with absolute methanol. Five standard solutions were prepared with known caffeine concentrations ranging from 4x10⁻⁵M to 1.2x10⁻⁴M according to the dilution scheme in Appendix 5. Absorbance spectra were recorded for all solutions as well as for pure methanol using a Spectral 420 UV/Vis spectrophotometer. Absorbance spectrum of pure methanol was subtracted from all other spectra. See Appendix 1 for spectra.

Five acetylsalicylic acid (ASA) and five salicylic acid (SA) standard solutions with known concentrations ranging from 2x10⁻⁵M to 2x10⁻⁴M were prepared according to the dilution scheme in Appendix 5. Three SA unknown solutions were prepared by dissolving ca. 50mg (not ca. 20mg as suggested by the lab manual) of sample in 15mL of 1% v/v acetic acid-chloroform solution. The solutions were then passed through glass wool pipet filters and diluted to 25mL. Three ASA unknown solutions were prepared by diluting an aliquot of each of the SA solutions 1:50 with 1% v/v acetic acid-chloroform solution. Spectra for the five SA standards, three SA unknowns and a 1% v/v acetic acid-chloroform blank were recorded from 350-500nm with excitation at 335nm using a Perkin Elmer LS 50B Fluorometer. Spectra for the eight ASA solutions and the same blank were recorded with the same instrument from 300-500nm with excitation at 270nm. Blank spectra were subtracted from each of the standard and unknown spectra. See Appendices 2 and 3 for spectra.
Results

Spectrophotometric Caffeine Content Results

<table>
<thead>
<tr>
<th>Trial</th>
<th>Absorbance at $\lambda_{\text{max}}$</th>
<th>Caffeine Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.148</td>
<td>2.86%</td>
</tr>
<tr>
<td>2</td>
<td>0.141</td>
<td>2.73%</td>
</tr>
<tr>
<td>3</td>
<td>0.112</td>
<td>2.17%</td>
</tr>
</tbody>
</table>

Statistical Analysis of Caffeine Content

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.59%</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.370</td>
</tr>
<tr>
<td>95% CL</td>
<td>2.6(±0.9)%</td>
</tr>
</tbody>
</table>

Calculation of caffeine content based on absorbance of standard solutions at $\lambda_{\text{max}}$ of 272.84nm. See Appendices 1 and 4 for absorbance spectra, regression of standard absorbances and calculation and statistical analysis of caffeine content.

Fluorometric Acetylsalicylic Acid Content Results

<table>
<thead>
<tr>
<th>Trial</th>
<th>Intensity at $\lambda_{\text{max}}$</th>
<th>Sample mass</th>
<th>Acetylsalicylic Acid Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>173.50</td>
<td>47.3mg</td>
<td>22.7%</td>
</tr>
<tr>
<td>2</td>
<td>183.49</td>
<td>48.7mg</td>
<td>23.4%</td>
</tr>
<tr>
<td>3</td>
<td>166.52</td>
<td>44.8mg</td>
<td>23.0%</td>
</tr>
</tbody>
</table>

Statistical Analysis of Acetylsalicylic Acid Content

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>23.1%</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.334</td>
</tr>
<tr>
<td>95% CL</td>
<td>23.1(±0.8)%</td>
</tr>
</tbody>
</table>

Calculation of acetylsalicylic acid content based on emission of standard solutions at $\lambda_{\text{max}}$ of 343.0nm. See Appendices 2 and 4 for emission spectra, regression of standard emissions and calculation and statistical analysis of acetylsalicylic acid content.

Fluorometric Salicylic Acid Content Results

<table>
<thead>
<tr>
<th>Trial</th>
<th>Intensity at $\lambda_{\text{max}}$</th>
<th>Sample mass</th>
<th>Salicylic Acid Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>152.21</td>
<td>47.3mg</td>
<td>0.30%</td>
</tr>
<tr>
<td>2</td>
<td>168.44</td>
<td>48.7mg</td>
<td>0.33%</td>
</tr>
<tr>
<td>3</td>
<td>145.30</td>
<td>44.8mg</td>
<td>0.30%</td>
</tr>
</tbody>
</table>
Statistical Analysis of Salicylic Acid Content

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.31%</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.013</td>
</tr>
<tr>
<td>95% CL</td>
<td>0.31(±0.03)%</td>
</tr>
</tbody>
</table>

Calculation of salicylic acid content based on emission of standard solutions at $\lambda_{\text{max}}$ of 442.5nm. See Appendices 3 and 4 for emission spectra, regression of standard emissions and calculation and statistical analysis of salicylic acid content.

Content of Putative 500mg Anacin Tablet

<table>
<thead>
<tr>
<th></th>
<th>Caffeine</th>
<th>Acetylsalicylic Acid</th>
<th>Salicylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>13(±4)mg</td>
<td>115(±4)mg</td>
<td>1.6(±0.2)mg</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The results of this lab were reasonably good. The 95% confidence levels were 2.6(±0.9)% caffeine, 23.1(±0.8)% ASA and 0.31(±0.03)% SA. These intervals correspond to relative errors of 34%, 3.5% and 9.7%, respectively. The precision of the ASA measurement is quite good. Although the relative error of the SA measurement is considerably higher, this is to be expected given that the extremely low SA content and the small samples used cause random errors to have a greater impact on the measurement. The caffeine measurement had quite low precision. There were two major contributing factors to this problem. First, the sample was diluted based on the assumption that it was 6-10% caffeine. As this assumption was high by a factor of two or three, the over-diluted sample solutions had absorbances of about 0.14 (see Appendix 1). Repeated measurements of the same sample showed that the spectrophotometer was capable of a precision of about ±0.01, which represents a significant relative error for solutions with low absorbance. A second difficulty was that the sample cuvettes were washed with acetone, which was discovered to absorb in roughly the same region as caffeine. Since the sample solutions had such low absorbances, contamination would have a large relative impact.

The accuracy of all sample measurements is fundamentally limited by the accuracy of the constants calculated from the standard solutions that are used to relate intensity or absorbance to concentration. The standard data points closely match the idealized absorbance and emission functions (see Appendices 1, 2 and 3), indicating minimal random error in the standard solutions. Any error in preparing the stock solutions from which the standard solutions were created would be equal for all standards of a given type and thus act as a systematic bias in the standardization process. The SA and ASA standards are particularly prone to such a bias since two dilutions were used to prepare their stock solutions. The magnitude of this systematic error could be determined by comparing multiple sets of standard solutions derived from distinct stock solutions, but in the absence of this comparison, it is difficult to estimate. As the stock solutions were prepared with as much care as possible, it seems likely that the error in the constants calculated from them is small, but probably still significant.
For the SA and ASA measurements, the relative error associated with the constants calculated from the standard solutions was of the order of 10-15% (see Appendices 2 and 3). Since these standards were also prone to systematic error, as discussed above, this was probably the largest source of inaccuracy. Another source of error was mechanical loss of the sample solution while attempting to pipet filter it. The high vapor pressure of chloroform, small diameter of the pipet filter and inexpert pipetting technique combined for a loss of ca. 1-2% of each of the sample solutions, which biased the SA and ASA results slightly low.

The inaccuracy due to error in standardization for the caffeine measurement was probably insignificant compared with the other errors involved. Besides the previously discussed difficulties with acetone contamination and over-dilution, the caffeine measurement was subject to loss in the extraction process performed to separate it from the SA and ASA. Caffeine was lost in the extraction due to residue left on the glassware, spillage during pipet filtering and, to a lesser extent, absorption by the MgSO₄ drying agent. This loss negatively biased the caffeine measurement and was probably the most significant source of inaccuracy.

A theoretical absorption spectrum for the sample was reconstructed from the calculated sample composition and quantitatively determined extinction coefficients (see Appendices 6 and 7). This theoretical spectrum can be compared to a measured absorption spectrum of the unextracted sample (see Appendix 1) as a cross-check of the experiment's accuracy. Since the content of SA is so low relative to ASA and caffeine, it does not significantly contribute to the summed spectra, so this comparison reveals nothing about the accuracy of the SA measurement. Caffeine has an absorption peak at ca. 270nm; the theoretical spectrum matches the experimentally measured spectrum closely in this region, indicating that the caffeine measurements were relatively accurate. At the ASA absorption peak of ca. 230nm, the absorbance of the experimental spectrum was approximately twice that of the theoretical spectrum (1.6 vs. 0.8). Assuming there are no significant errors in the extinction coefficients used to calculate the theoretical spectrum, this indicates that there may have been some relatively large systematic error in the ASA measurement. One possible source of such an error in the ASA measurement is the presence of caffeine in the sample solutions used for ASA measurement. An excitation wavelength of 270nm was used in the ASA measurements; however, this wavelength corresponds to an absorption peak of caffeine. If a significant portion of the incident light was absorbed by caffeine rather than ASA, it could negatively bias the measurement of ASA content. This hypothesis could be tested by measuring the emission intensity of a standard solution of ASA under the same conditions used in this experiment and then adding some caffeine to the solution to see if the emission was significantly decreased.

**Conclusion**

This experiment succeeded in measuring the content of an Anacin tablet with reasonable accuracy, although with lower precision that might be hoped for. Fluorescence and absorption spectroscopy were found to be useful techniques for measuring analyte concentrations. These techniques are non-destructive to the sample and highly sensitive, which is important when limited quantities of analyte are available. Furthermore, especially in the case of fluorescence spectroscopy, these techniques are often able to measure the concentration of a chosen analyte in the presence of other substances.
Spectroscopic measurements also take relatively little time, once the instrument is calibrated. These qualities distinguish these techniques from titrametric procedures, which are generally slower, more destructive and less forgiving of the presence of similar molecules in the analyte solution. Fluorescence and absorption spectroscopy do have some disadvantages. Because they are so sensitive, even very small amounts of contamination can cause large experimental errors. This problem was particularly evident with the absorption spectroscopy, where multiple trials were necessary before reasonable results were obtained. Another problem with spectroscopy is that the constants in the equations relating concentration to absorbance or emissions intensity are so dependent on solution conditions that it is almost always necessary to measure a set of standards before performing measurements on an unknown analyte.

The experiment was generally well designed. Unfortunately it was also extremely time consuming. Some time savings could be achieved by not having each group make every set of standards, but this will be a lengthy lab even then. As mentioned in the discussion section above, there was some mechanical loss of analyte involved in using the pipet filters. Use of larger diameter pipets to construct the pipet filters might minimize this problem somewhat. Finally, in future it would be best to wash cuvettes with methanol rather than acetone, as acetone contamination can cause errors in the UV-Vis absorption measurements.

References

Julia Frey. Lab Partner.


Stack, T.D.P. "Analytical Laboratory Experiments for Chemistry 134."
Appendices

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Appendix 1: Caffeine Spectra and Standard Regression

Caffeine Calibration Spectra

- 4.0E-5M
- 6.4E-5M
- 8.0E-5M
- 9.6E-5M
- 1.2E-4M

Wavelength (nm)

Absorbance

Caffeine Extinction Coefficients

- Extinction coefficient calculated from absorbance of 8.0E-5M standard solution

Wavelength (nm)

Extinction Coefficient

Caffeine Unknown Spectra

- Unknown 1
- Unknown 2
- Unknown 3
- Unextracted Unknown

Absorbance (Extracted Unknowns)

Absorbance (Unextracted Unknown)

Wavelength (nm)

Caffeine Absorbance Regression

Absorbance Data

Regression Results

\[
\hat{y} = m1 \cdot x + b
\]

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00000</td>
<td>0.0000</td>
<td>10010</td>
<td>164.73</td>
</tr>
<tr>
<td>4.0000e-05</td>
<td>0.37629</td>
<td>0.0048456</td>
<td>NA</td>
</tr>
<tr>
<td>6.4000e-05</td>
<td>0.59883</td>
<td>0.0048456</td>
<td>NA</td>
</tr>
<tr>
<td>8.0000e-05</td>
<td>0.83534</td>
<td>0.0048456</td>
<td>NA</td>
</tr>
<tr>
<td>9.6000e-05</td>
<td>0.99257</td>
<td>0.0048456</td>
<td>NA</td>
</tr>
<tr>
<td>0.00012000</td>
<td>1.1832</td>
<td>0.0048456</td>
<td>NA</td>
</tr>
</tbody>
</table>

\[
y = m1 \cdot x + b
\]

<table>
<thead>
<tr>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>10010</td>
</tr>
<tr>
<td>R</td>
<td>0.9974</td>
</tr>
<tr>
<td>Chi2</td>
<td>0.0048456</td>
</tr>
</tbody>
</table>

Absorbance

Concentration (10^-4)
Appendix 2: Acetylsalicylic Acid Spectra and Standard Regression
Appendix 3: Salicylic Acid Spectra and Standard Regression

Salicylic Acid Calibration Spectra

Salicylic Acid Unknown Spectra

Salicylic Acid Emission Curve Fit

Regression Results

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Intensity</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000000</td>
<td>0.0000</td>
<td>1875.5</td>
<td>125.76</td>
</tr>
<tr>
<td>2.0000e-05</td>
<td>75.28</td>
<td>2034.5</td>
<td>161.08</td>
</tr>
<tr>
<td>6.0000e-05</td>
<td>214.03</td>
<td>343.14</td>
<td>NA</td>
</tr>
<tr>
<td>1.0000e-04</td>
<td>343.14</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Chi sq 35.223 NA
R 0.99994 NA
Appendix 4: Calculation and Analysis of Sample Content

Initialization and functions:

\[
\text{ORIGIN} := 1 \\
\text{i} := 1 \ldots 3 \\
\text{s(data)} := \text{stdev(data)} \\
\text{length(data)} := \frac{\text{length(data)}}{\sqrt{\text{length(data)}} - 1}
\]

Caffeine composition analysis:

\[
\text{A}_i := c_i := \frac{A_i}{10010} \\
\text{caf}_i := \frac{c_i \cdot 100 \cdot 194.19 - 25}{250.5} \\
\text{caf} := \left( \begin{array}{c}
0.02865 \\
0.0273 \\
0.02168
\end{array} \right)
\]

\[
\text{mean(caf)} = 0.02588 \\
\text{s(caf)} = 0.0037
\]

\[
4.30 \cdot \text{s(caf)} = 0.0092 \\
\sqrt{\text{length(caf)}}
\]

Acetylsalicylic Acid composition analysis:

\[
\text{I}_{\text{ASA}_i} := M_i := \\
\text{c}_{\text{ASA}_i} := -\ln \left( \frac{I_{\text{ASA}_i}}{2711.3} \right) \\
\text{c}_{\text{ASA}_i} := \frac{1}{1384.5} \\
\text{c}_{\text{ASA}_i} := 1384.5 \cdot 50 \cdot 180.17 \cdot 0.025
\]

\[
\text{ASA}_i := \left( \begin{array}{c}
0.227 \\
0.234 \\
0.23
\end{array} \right) \\
\text{mean(ASA)} = 0.231 \\
\text{s(ASA)} = 0.00334
\]

\[
4.30 \cdot \text{s(ASA)} = 0.0083 \\
\sqrt{\text{length(ASA)}}
\]

Salicylic Acid composition analysis:

\[
\text{I}_{\text{SA}_i} := M_i := \\
\text{c}_{\text{SA}_i} := -\ln \left( \frac{I_{\text{SA}_i}}{1875.5} \right) \\
\text{c}_{\text{SA}_i} := \frac{2034.5}{1381.12 \cdot 0.025}
\]

\[
\text{SA}_i := \left( \begin{array}{c}
0.00304 \\
0.00328 \\
0.00305
\end{array} \right) \\
\text{mean(SA)} = 0.00312 \\
\text{s(SA)} = 1.351 \cdot 10^{-4}
\]

\[
4.30 \cdot \text{s(SA)} = 3.3547 \cdot 10^{-4} \\
\sqrt{\text{length(SA)}}
\]

Tablet analysis:

\[
\text{500} \cdot \text{mean(caf)} = 12.94 \\
\text{500} \cdot \text{mean(SA)} = 1.562 \\
\text{500} \cdot \text{mean(ASA)} = 115.272
\]

\[
\frac{4.30 \cdot \text{s(caf)}}{\sqrt{\text{length(caf)}}} = 4.5872 \\
\frac{4.30 \cdot \text{s(SA)}}{\sqrt{\text{length(SA)}}} = 0.1677 \\
\frac{4.30 \cdot \text{s(ASA)}}{\sqrt{\text{length(ASA)}}} = 4.1414
\]
Appendix 5: Standard Solution Dilution Schemes

### Caffeine Standard Solution Dilutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number of 0.100mL aliquots of stock sol.</th>
<th>Volumetric Flask Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0x10⁻⁵M</td>
<td>1</td>
<td>10mL</td>
</tr>
<tr>
<td>6.4x10⁻⁵M</td>
<td>2</td>
<td>25mL</td>
</tr>
<tr>
<td>8.0x10⁻⁵M</td>
<td>2</td>
<td>10mL</td>
</tr>
<tr>
<td>9.6x10⁻⁵M</td>
<td>3</td>
<td>25mL</td>
</tr>
<tr>
<td>1.2x10⁻⁴M</td>
<td>3</td>
<td>10mL</td>
</tr>
</tbody>
</table>

Stock solution was prepared by dissolving 77.7mg of caffeine in absolute methanol and diluting to 100mL. Aliquots were delivered with a 0.100mL positive displacement pipet.

### ASA Standard Solution Dilutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number of 0.100mL aliquots of stock sol.</th>
<th>Volumetric Flask Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0x10⁻⁵M</td>
<td>1</td>
<td>25mL</td>
</tr>
<tr>
<td>6.0x10⁻⁵M</td>
<td>3</td>
<td>25mL</td>
</tr>
<tr>
<td>1.0x10⁻⁴M</td>
<td>2</td>
<td>10mL</td>
</tr>
<tr>
<td>1.5x10⁻⁴M</td>
<td>3</td>
<td>10mL</td>
</tr>
<tr>
<td>2.0x10⁻⁴M</td>
<td>4</td>
<td>10mL</td>
</tr>
</tbody>
</table>

Stock solution was prepared by dissolving 90.1mg of acetylsalicylic acid in 1% v/v acetic acid-chloroform solution and diluting to 10mL. This solution was then diluted 1:10 in a 10mL volumetric flask. Aliquots were delivered with a 0.100mL positive displacement pipet.

### SA Standard Solution Dilutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number of 0.100mL aliquots of stock sol.</th>
<th>Volumetric Flask Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0x10⁻⁵M</td>
<td>1</td>
<td>25mL</td>
</tr>
<tr>
<td>6.0x10⁻⁵M</td>
<td>3</td>
<td>25mL</td>
</tr>
<tr>
<td>1.0x10⁻⁴M</td>
<td>2</td>
<td>10mL</td>
</tr>
<tr>
<td>1.5x10⁻⁴M</td>
<td>3</td>
<td>10mL</td>
</tr>
<tr>
<td>2.0x10⁻⁴M</td>
<td>4</td>
<td>10mL</td>
</tr>
</tbody>
</table>

Stock solution was prepared by dissolving 69.1mg of salicylic acid in 1% v/v acetic acid-chloroform solution and diluting to 10mL. This solution was then diluted 1:10 in a 10mL volumetric flask. Aliquots were delivered with a 0.100mL positive displacement pipet.
Appendix 6: Reconstructed Spectrum Concentration Calculations

Caffeine concentration:

\[
\frac{0.0205 \cdot \text{mean( caf } \% \text{) \cdot 0.01}}{10} = 2.732 \cdot 10^{-5}
\]

SA concentration:

\[
\frac{0.0205 \cdot \text{mean( SA } \% \text{) \cdot 0.01}}{10} = 4.636 \cdot 10^{-6}
\]

ASA concentration:

\[
\frac{0.0205 \cdot \text{mean( ASA } \% \text{) \cdot 0.01}}{10} = 2.62317 \cdot 10^{-4}
\]
Appendix 7: Reconstructed Absorption Spectrum

Reconstructed Absorbance Spectrum for Unextracted Unknown

Absorbance

Wavelength (nm)

220 240 260 280 300 320 340