

Supporting Information (S-1)

A Complete Structure Investigation of an Organic Molecule for Chemistry Students; An Analysis of Aspartame

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Student Handouts

Laboratory procedures

10 UV analysis

1. A stock solution of 1.3 mg/25 mL aspartame is prepared by the instructor.
2. Check that the cuvettes can be used in the UV area (cut-off at 230 nm).
3. Use automated pipettes to add 0.5 mL stock solution and 2.0 mL methanol directly to the cuvette.
4. Take a background spectrum of methanol from wavelengths 230 nm to 400 nm.
- 15 5. Follow the instrumental procedure for running the aspartame spectrum.
6. Acquire similar spectra for compounds with and without an extended chromophore, like ibuprofen and salicylic acid, and compare their molar absorption coefficient (ϵ) at the wavelength maximum to the spectrum of aspartame.

The UV analyses were performed at ambient temperature, and the spectra were processed using UVProbe 2.62 software.

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IR analysis

1. Before you start preparing the KBr-tablet containing aspartame: clean the equipment that will come in direct contact with your tablet using acetone (including the spatula, mortar and parts of the tablet press) and let air dry in a cupboard.
- 25 2. Add a small amount of aspartame (small spatula) and *dry* KBr (oven-dried at 95 °C overnight) in a 1:9 ratio, to a mortar and mix and grind the mixture into a fine powder using a pestle.
3. Add a small amount of powder to the tablet press and press the powder at a pressure of 12 tons for 1 minute, following the procedure shown by the instructor. Be aware that the tablet breaks easily.
4. Check that the IR-instrument has the transmission unit inserted.
- 30 5. Take a background spectrum of air.
6. Place the tablet in the tablet holder and place the holder in the transmission unit of the IR instrument.
7. Record and process the spectra following the instrument procedure.

The IR analyses were performed at ambient temperature, the instrument was used with a resolution of 4 cm⁻¹, and the
35 spectra were processed using Omnic software.

NMR analysis

1. Add 10-20 mg of aspartame to a reagent tube and dilute the sample with ca. 0.75 mL of deuterated water (D₂O) using a glass pipette.
- 40 2. Use the pipette to carefully mix the solution.
3. Observe the following: does aspartame dissolve in D₂O? If not, add a few drops of concentrated HCl and study what happens.
4. Transfer the solution to an NMR tube using a glass pipette and cap the tube. Ensure that a tag describing the tubes' content and your group name always remains attached to the tube whenever it is not placed inside the NMR
45 instrument.
5. Perform the ¹H-NMR analysis and spectral data processing following the instrument procedure. Use the solvent peak at 4.80 ppm as the reference peak¹. Include phase- and baseline correction, peak assignment and integration in the processing of the spectra.
- 50 6. The ¹³C-NMR and DEPT spectra are handed out because the concentration of the sample is low, and the spectral data uptake will be very time consuming.

The ¹H-NMR experiments were performed at ambient temperature, and the spectra were processed using MestReNova software.

GC-MS analysis

1. Add a spatula of 1-8 mg aspartame in a weighing boat and transfer to a 10 mL volumetric flask.
2. Dilute the sample with methanol to the 10 mL mark and cap the flask.
3. Make sure the solid is dissolved. Turn the flask and/or place the flask in a beaker with hot water to accelerate the process.
4. Further dilute the sample with methanol to a concentration suitable for your MS-instrument.
5. Transfer a diluted sample to a GC vial using a pipette and mark it properly.
6. Apply the temperature program specified in Table S1 and follow the instrumental procedure for analyzing the aspartame sample.

Table S1. Temperature program for the GC analysis of aspartame

Rate (°C/min)	Temperature (°C)	Hold time (min)
	50	1.00
20.0	200	5.00
20.0	250	4.00

GC parameters:

Splitless injection, or a split ratio suitable to your MS-instrument.

Column: Thermo Scientific TG-5MS, 30 m x 0.25 mm x 0.50 μ m.

The MS spectra were processed using Xcalibur software.

Report instructions

Keep the interpretation of the spectral analysis for each analytical method clear and concise. Use tables frequently to compare literature values to observed signals, as demonstrated for an IR spectrum in Table S2.

Table S2. Expected and observed IR absorption for various functional groups

Functional group	Expected absorption ² (cm ⁻¹)	Observed absorption (cm ⁻¹)
Aldehyde (-CHO)	1720-1740 (s, sh)	

You do not have to submit a full report. Only the “Results and Discussion” and the “Conclusion” sections in the report will be evaluated. Below are points of elements that should be included in the discussion of the molecule for each analytical method:

UV analysis

1. What can you generally say about the compound based on the UV spectrum? Consider aspects such as aromaticity, aliphatic chain and chromophore of the molecule.
2. Calculate the molar absorption coefficient (ϵ) for the molecule at λ_{max} .
3. Is this a good method for a complete structure determination of the molecule?

IR analysis

1. Which characteristic peaks do you observe in the spectrum? Include only the most important findings; do not identify all the peaks.
Use the following notation for the peaks (br = broad, sh= sharp) and for the intensity (s = strong, m = medium, w = weak) of the absorptions.
2. Is this a good method for a complete structure determination of the molecule?

NMR analysis

1. Interpret the ^1H -NMR spectrum. Comment on the position of the peaks, splitting patterns and their integrals. Do they meet your expectations?
2. Interpret the ^{13}C -NMR spectrum. Comment on the position of the peaks. Supply with the information from the DEPT spectra.
3. Comment on the choice of solvent for the analysis.
4. Is this a good method for a complete structure determination of the molecule?

GC-MS analysis

1. Describe the spectrum. Does it contain many fragment peaks? Can you see the molecular ion peak?
2. Interpret the spectrum. Which fragment losses can you identify?
3. Determine the double bond equivalence (DBE) of the compound.
4. Is this a good method for a complete structure determination of the molecule?

Additionally, include a risk assessment of the activities in the project and refer to relevant literature in the text.

Oral presentation

Select two molecules from the project (aspartame, salicylic acid, acetylsalicylic acid, ibuprofen, and an unknown paraben) and present your analysis in a PowerPoint presentation with a maximum duration of 10 minutes. Following your presentation, there will be a 10-minute discussion with the teacher and laboratory assistant, where questions based on the spectral data and your written report will be addressed. This oral presentation serves as preparation for the final oral examination at the end of the term.

References

1. Cambridge Isotope Laboratories, Inc. NMR Solvent Data Chart. [Online]. Available: <https://cil.showpad.com/share/54zWw3avN5TUYa6uMSY32> [Accessed June 27, 2025].
2. Fleming, I.; Williams, D. *Spectroscopic methods in organic chemistry*, 7 ed., Springer Nature Switzerland AG, 2019, pp109-121.