

Analysis of Uric Acid Metabolites in Urine (or in synthetic urine) by HPLC-MS

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A. Overview

This experiment uses HPLC-MS to determine the presence and quantity of three compounds (uric acid, 6-aminouracil and allantoin; tyrosine is used as the internal standard) in urine.

Suggested or required equipment used:

- 6520 Accurate-Mass Q-TOF HPLC-MS with an Agilent 1200 Series Binary Pump
- Or Perkin Elmer single quad LC-MS
- Cogent Diol™ column, 150mm x 2.1mm ID, 4µm particle size (MicroSolv Tech. Corp. Leland, North Carolina, US), Catalog number: 40060-15P (required)

Note: Other LCMS systems can be substituted for the ones described above, but the HPLC column is essential.

B. Background

Urine analysis is a non-invasive way to evaluate a person's health. Allantoin, 6-aminouracil (6-AU) and uric acid (UA) are all compounds that have been detected in urine. UA is the end product of purine metabolism in humans and is discarded from the body by means of urine excretion. Although UA should naturally be present in everyone's urine, a relatively high concentration of UA in urine may indicate UA build-up in the body and be cause for concern; higher levels of UA in the body can cause several diseases, like gout and kidney stones. Furthermore, detecting allantoin and 6-AU in urine may also have physiological implications since these compounds are not natural end products of human metabolic processes and have been detected at higher levels in patients suffering from various health problems [1,2].

The Cogent Diol™ column is a unique column on the market which allows for retention and separation of the analytes of interest (allantoin, 6-AU and UA) using simple method conditions (without exotic mobile phase additives, or derivatization of the analytes) [3].

The experiment is designed for forensic, biochemistry and chemistry students. The LC-MS theory is part of the lecture and it is not covered in the description of the experiment. The experiment should be treated as a base to develop an LC-MS experiment suited to individual teaching settings.

Analysis of metabolites in synthetic urine samples was successfully done by San Jose State University students in the Chem 55L lab (Quantitative Analysis).

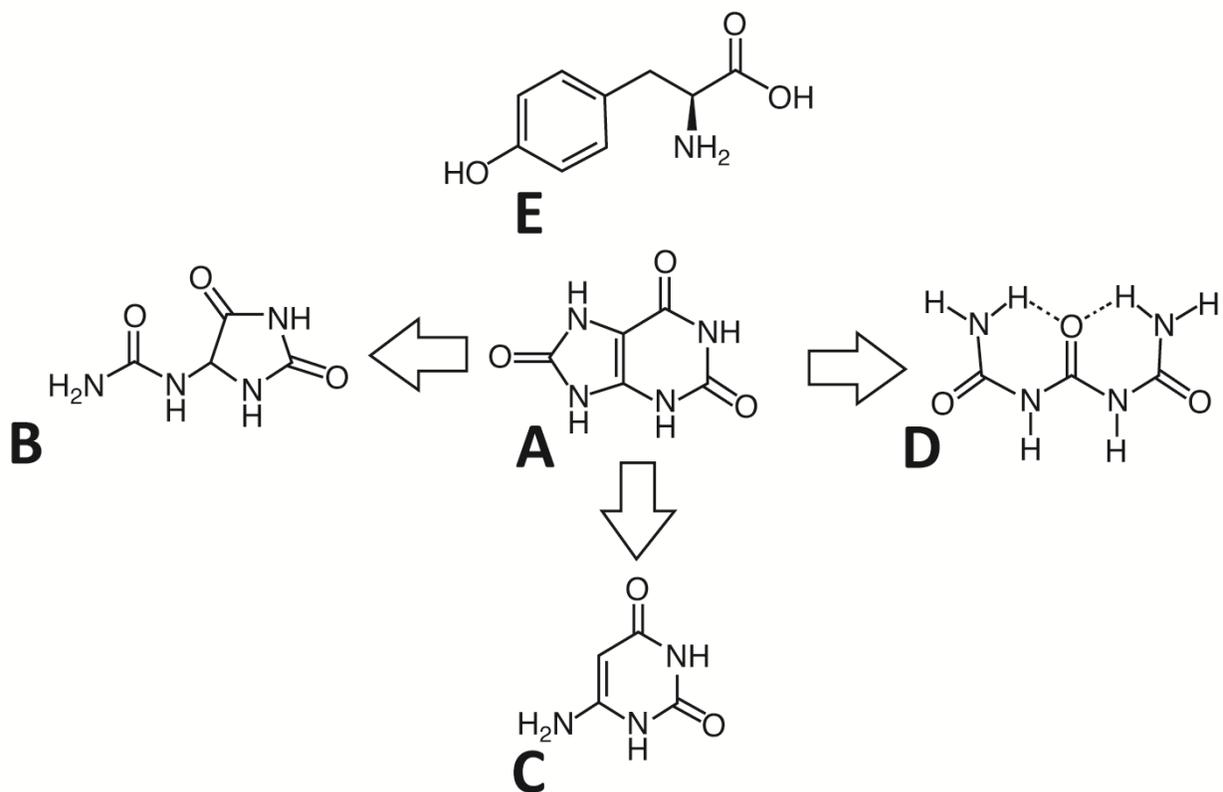


Figure 1. Structures of the analytes from the study: A) Uric acid, B) allantoin, C) 6-aminouracil, D) triuret, and E) tyrosine (internal standard). Please note that it was not possible to buy a triuret standard.

C. Equipment and chemicals

- 6520 Accurate-Mass Q-TOF LC/MS with an Agilent 1200 Series Binary Pump SL or other LC-MS instrument
- Cogent Diol column, 150mm x 2.1mm ID, 4um particle size, MicroSolv Tech. Corp. Leland, North Carolina, US, Catalog number: 40060-15P
- Pure standards:
 - allantoin (All), catalog number: 93791
 - 6-aminouracil (6-AU), catalog number: A50606
 - uric acid (UA): catalog number: U2625
 - D,L-Tyrosine (Tyr): catalog number: 145726
 were purchased from SigmaAldrich, US (SigmaAldrich.com)
- Synthetic urine – 500 mL bottle,

http://www.carolina.com/catalog/detail.jsp?prodId=695955&s_cid=ppc_products&utm_source=google&utm_medium=cpc&s_cid=ppc_gl_products&scid=scplp695955&sc_intid=695955&gclid=CPf_0fHriNECFUqSfgodFeoNDA

- HPLC vials - MicroSolv Tech. Corp. Leland, North Carolina, US, https://mtc-usa.com/Vials_Wisp.aspx
- Volumetric flasks
- Weighing bottles
- Spatula
- Centrifuge
- 1.5mL Microcentrifuge tubes
- 1000uL and 200uL micropipettes and tips
- Analytical balance
- HPLC-grade DI water/0.1% formic acid, formic acid, catalog number F0507 was purchased from SigmaAldrich, US (SigmaAldrich.com)
- acetonitrile/0.1% formic acid
- Syringes and syringe filters (Nylon 45 μm) - (MicroSolv Tech. Corp. Leland, North Carolina, US), syringes follow link: <https://mtc-usa.com/Luer-Lock-Syringes.aspx>, filters follow link: mtc-usa.com/wcode_filters_tech.aspx (Nylon, 4 mm filters were used)

D. Notes for the instructors

D.1 General notes

Experiment takes one lab period (3 and one-half hours). The pure standards were purchased from Sigma and the quantities are estimated to last for 1400 students.

For practical purposes assign determination of one analyte per student.

Have each student prepare their own calibration curve.

As a pre-lab assign:

1. Determination of m/z values for $[M+H]^+$ ions for each analyte – have students create a table.
2. Assign a calculation problem; for example: From mix of standards (1mg/mL) using micropipettes and HPLC vials prepare the following solutions for a calibration curve: 0.010 mg/mL, 0.020 mg/mL, 0.030 mg/mL, 0.040 mg/mL and 0.050 mg/mL, each solution containing 0.1 mg/mL of tyrosine (internal standard [4]). Have students WRITE A PROCEDURE (IN A TABLE) OF HOW TO PREPARE THE ABOVE 5 SOLUTIONS FOR A CALIBRATION CURVE AND HAVE THE TABLE APPROVED BY THE INSTRUCTOR at the beginning of the lab. Make sure that students will remember to include the unknown sample preparation in the table

D.2 Accuracy of the student's results

In Quantitative Analysis lab at San Jose State University students were given synthetic urine samples containing 0.015 mg/mL to 0.040 mg/mL of one analyte in the sample. Students created two calibration curves:

1. Peak area of the analyte vs concentration
2. Ratio of peak area to Internal Standard peak area (tyrosine) vs concentration
3. Students evaluated which calibration curve was better (higher R^2 value in regression equation) and used this calibration curve to report results. On average results were $\pm 2.5 - 10\%$ different from the

true value for 0.040 mg/mL samples and $\pm 6.7 - 20\%$ for 0.015 mg/mL samples. About 60% of the students received scores between 70 – 99 points.

D.3 Grading

The following grading scale was used

Key HPLC Points out of 100	Difference mg/mL
99	0.001
97	0.002
95	0.003
93	0.004
91	0.005
88	0.006
86	0.007
84	0.008

Additional points were assigned for selected calibration curve (for example $R^2 = 0.995 = -1$ point, etc) and report preparation

D.4 Waste disposal

During LC-MS runs most of the mobile phase is evaporated. Make sure that there are traps installed to collect the organic solvent portion of the mobile phase.

Collect all the extra solvents and analyzed sample solutions in a separate, labeled (according to your institutions requirements) waste container. Remember to write the date of the start of the accumulation of the waste. Keep waste containers in a secondary container (110% capacity of the original waste container).

E. Procedures

E.1 Preparation of Standard Solutions (LAB INSTRUCTOR)

NOTE: steps below refer to preparation of the solution of allantoin, but the steps apply to any compound for which you wish to make a standard solution.

1. Determine what standard concentration you'll make for allantoin. It is recommended to prepare a 0.1mg/mL solution. If you are using a less sensitive detector, please prepare 1 mg/mL solutions.
2. Place a clean, dry weighing bottle on an analytical balance and tare the balance. Weigh as much allantoin as you need and record the weight.
3. Transfer allantoin from the weighing bottle to a clean 100 mL volumetric flask. If necessary, use a Pasteur pipette and some DI water/0.1% formic acid to move any remnants of allantoin on the weighing funnel into the flask.

4. Using a beaker, add about 50 mL of DI water/0.1% formic acid to the flask (please do not fill up the flask). Swirl the water around to dissolve all of the allantoin*.
5. Fill the flask with DI water to the line. Cap the flask then invert and shake vigorously for a minute.
6. This solution is your standard solution. Store it in a refrigerator (at -20 °C).
7. Repeat steps 1-5 for tyrosine, 6-aminouracil, and uric acid. Transfer all prepared solutions into clean glass containers with a lid. Label containers with: Standard name, concentration, solvent, date of preparation, name of person who prepared solutions. Dispose of the solutions after one year. Place samples in the freezer until samples are ready to be used in the class.

* If any of the compounds don't dissolve completely in 100 mL room temperature water, prepare a water bath using a heating plate and 600mL beaker (you can use tap water for the bath). Hold the volumetric flask in a very warm bath to heat the solution. Remove the flask intermittently to shake up the solution. Continue heating and shaking until the compound has dissolved.

If the compound will not dissolve when heated, you'll need to start over and weigh out a smaller amount of the compound. If you start over, don't forget to calculate what the concentration will be for your new standard solution.

E.2 Internal Standard Calibration Curves [5] (STUDENTS)

1. Use standard solutions prepared by Instructor to prepare **at least 5** different calibration solutions for each target compound that each contain the same concentration of ISTD** tyrosine in our case (see Example 1 below for how to prepare different solutions of a compound that contain the same concentration of ISTD). You can approach this step in one of two ways:

** ISTD – Internal Standard

➤ *Research Oxidative-Stress-Induced Target Compound Concentrations in Urine*

Look into the approximate amount of each target analyte found in the urine of people undergoing oxidative stress (e.g. 70-100 µg/mL of uric acid). Prepare at least 5 calibration solutions for each compound that encompass their compound's concentration range in urine, e.g. a 30 µg/mL, 50 µg/mL, 70 µg/mL, 90µg/mL, and 110 µg/mL solution of analyte (uric acid, allantoin or 6 –aminouracil) would encompass the 70-100 µg/mL concentration range). Remember to add internal standard solution (the same concentration) to each calibration curve solution prepared.

AND/OR

➤ *Research Similar Experiments and/or Determine Concentrations through Trial and Error*

Make a wide range of different concentrations for each calibration curve. Be sure to make at least one of the concentrations very dilute, and another highly concentrated, i.e. one of the concentrations should be close to the detection limit, and the other should be nearly as concentrated as your standard solution. Don't worry if you haven't a clue what the detection limit may be; research and/or guess what concentration may be near the detection limit and prepare that. If the compound from the dilute solution you make isn't detected, make slightly more concentrated solutions until the compound is detected and can be analyzed.

2. Run every calibration solution through HPLC-MS in triplicate.
3. Using a data analysis program (e.g. MassHunter Qualitative Analysis), analyze each run to record peak areas for each calibration solution.
4. Plot **compound concentration** by ratios of **compound area to ISTD area** to make calibration curves for each compound (like the one depicted below). You may notice from your data that area increases linearly with increasing concentration until a certain point... at which point, the areas start to plateau. Only use responses in your calibration curves that have a linear relationship with concentration.

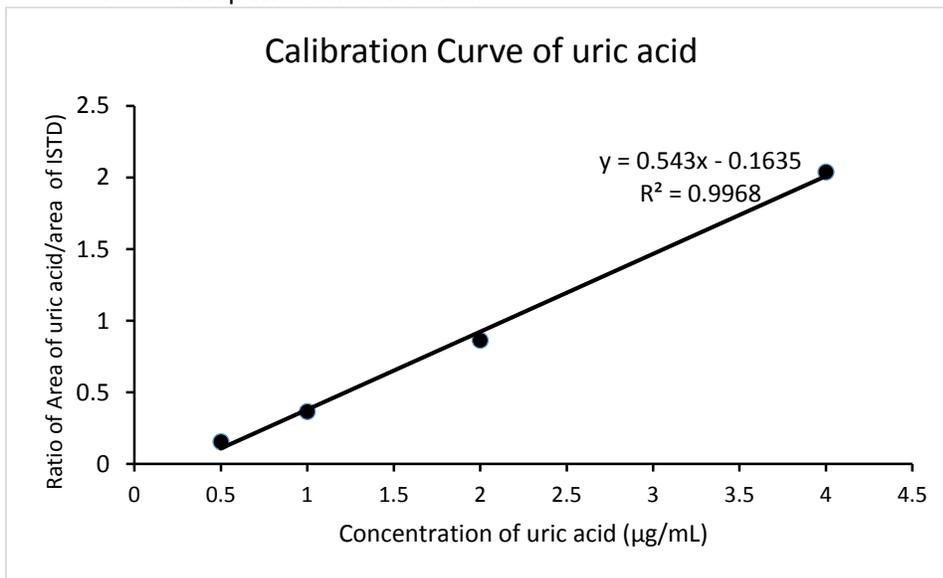


Figure 2. Example of calibration curve for uric acid

Calculations Example 1.

Problem: Using a 50 µg/mL concentrated standard solution of URIC ACID and a 50 µg/mL concentrated standard solution of ISTD, prepare (1) a 1 mL solution that is 2 µg/mL uric acid and 7 µg/mL ISTD, and (2) a 1 mL solution that is 4 µg/mL of analyte and 7 µg/mL ISTD. SHOW CALCULATIONS FOR THE SOLUTION FROM POINT 1 and POINT 2.

Use HPLC-grade DI water/0.1% formic acid for these solutions.

SOLUTION for point 2:

1 mL that's 4 µg/mL uric acid and 7 µg/mL ISTD

Calculations for uric acid

$$M_1V_1 = M_2V_2$$

$$(x)(50 \mu\text{g/mL analyte}) = (1 \text{ mL})(4 \mu\text{g/mL})$$

$$x = (1 \text{ mL})(4 \mu\text{g/mL}) / (50 \mu\text{g/mL})$$

$$x = 0.08 \text{ mL or } 80 \mu\text{L}$$

I.e. To make a 4 µg/mL concentration of uric acid from a 50 µg/mL concentration of uric acid, add 80 µl of the 50 µg/mL concentration to an HPLC vial and fill the vial to a final volume of 1 mL

Calculations for ISTD

$$M_1V_1 = M_2V_2$$

$$(x)(50 \mu\text{g/mL}) = (1 \text{ mL})(7 \mu\text{g/mL})$$

$$x = (1 \text{ mL})(7 \mu\text{g/mL}) / (50 \mu\text{g/mL})$$

$$x = 0.14 \text{ mL or } 140 \mu\text{l}$$

I.e. To make a 7 µg/mL concentration of ISTD from a 50 µg/mL concentration of ISTD, add 140 µl of the 50 µg/mL concentration to an HPLC vial and fill the vial to a final volume of 1 mL

THUS, to make a 1 mL solution that is both 4 µg/mL uric acid and 7 µg/mL ISTD... (follow quantities as shown in



$$(80 \mu\text{l} + 140 \mu\text{L} + 780 \mu\text{L}) = 1000 \mu\text{L or } 1 \text{ mL}$$

Figure 3. Sample preparation – following calculations in example 1.

E.3 Estimating Limit of Detection and Limit of Determination using a Calibration Curve [6,7] (Instructions refer to Agilent MassHunter software)

1. In excel, list the peak area data next to their corresponding calibration solution.

E.g.

Concentration (µg/mL)	Peak area
0	2.1
2	5.0
4	9.2
6	12.3
8	16.9
10	21.0
12	24.5

2. Go to the Data tab and click on the “Data Analysis” option near the top right corner of the page (if this option isn’t there, go to File > Options > Add-ins, then activate “Analysis ToolPak” so that it shows up).

See note below on loading Analysis ToolPak in Excel 2010.

3. A small box will pop up after you’ve clicked Data Analysis. Scroll down the list in the box to find “Regression”. Click on Regression and press OK.

4. Do the following in the Regression box that pops up: input the y-range (responses), input the x-range (concentrations), declare a confidence level (e.g. 95%), pick one box in the worksheet (using “output range”) for the results of this regression analysis to be displayed around, then click OK. The results should look like this:

Concentration (ug/ml)	Response	SUMMARY OUTPUT					
0	2.1	Regression Statistics					
2	5	Multiple R	0.998690404				
4	9.2	R Square	0.997382523				
6	12.3	Adjusted R Sq	0.996859028				
8	16.9	Standard Error	0.462832892				
10	21	Observations	7				
12	24.5	ANOVA					
			df	SS	MS	F	
		Regression	1	408.1289286	408.1289286	1905.237	
		Residual	5	1.071071429	0.214214286	1.19E-07	
		Total	6	409.2			
						Std Error of Y-Intercept	
		Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
		Intercept	1.546428571	0.315367457	4.903576889	0.004461	0.735751
		X Variable 1	1.908928571	0.043733598	43.64901769	1.19E-07	1.796508
						2.357106	2.021349
						0.735751	2.021349
						2.357106	

5. Calculate the Limit of Detection using this formula:

$$\text{LOD} = \frac{3 * \text{Standard Error of Y-Intercept}}{\text{Slope of the Line}}$$

(E.g. LOD = (3*0.3154)/1.9089 = 0.496 µg/mL)

6. Calculate the Limit of Quantitation using this formula:

$$\text{LOQ} = \frac{10 * \text{Standard Error of Y-Intercept}}{\text{Slope of the Line}}$$

$$\text{(E.g. LOQ} = (10 * 0.3154)/1.9089 = 1.652 \mu\text{g/mL)}$$

Note: Loading the Analysis ToolPak for Excel 2010.

The Analysis ToolPak is a Microsoft Office Excel add-in program that is available when you install Microsoft Office or Excel.

To use the Analysis ToolPak in Excel, however, you need to load it first.

1. Click the **Microsoft Office Button**  , and then click **Excel Options**.
2. Click **Add-Ins**, and then in the **Manage** box, select **Excel Add-ins**.
3. Click **Go**.
4. In the **Add-Ins available** box, select the **Analysis ToolPak** check box, and then click **OK**.
 - a. **Tip** If **Analysis ToolPak** is not listed in the **Add-Ins available** box, click **Browse** to locate it.
 - b. If you get prompted that the Analysis ToolPak is not currently installed on your computer, click **Yes** to install it.
5. After you load the Analysis ToolPak, the **Data Analysis** command is available in the **Analysis** group on the **Data** tab.

At this points there are no instructions for Mac users. Use Excel and formulas to do calculations.

E.4 Preparation of Unknown Samples (INSTUCTOR)

NOTE: The water used in these steps is HPLC DI water/0.1% formic acid

1. Use synthetic urine, diluted 1:10 with water.
2. Determine how much of unknown sample you need to prepare. Base your determination on the number of students in the class. You will need about 5 mL of the unknown sample per student. Using solution of standards prepared above prepare 3 samples at different concentration of allantoin, 6-AU, UA in the synthetic urine. For sample preparation see example below.

3. Transfer prepared samples into glass vials with lid. Label containers with: Sample name, concentration of all standards, solvent, date of preparation, name of person who prepared solutions. Outdate the solutions after one year. Place samples in the freezer until samples are ready to be used in the class.
4. Give out about 5 mL of unknown sample per student

Example of unknown samples preparation

Note: for all the pipetting use volumetric pipettes.

1. Standard solutions are 0.1 mg/mL of each allantoin, 6-AU, UA (see point D1 for preparation).
2. Using 50.00 mL volumetric pipette, add 50.00 mL of each standard into one container. Your standards are now diluted 1:3. Label solution as Mix of standards, concentration of all standards (0.033 mg/mL allantoin, 0.033 mg/mL 6-AU, 0.033 mg/mL UA), solvent, date of preparation, name of person who prepared solutions. Outdate the solutions after one year. When you are done refrigerate the glass containers at -20 °C until samples are ready to be used in the class.
3. For unknown #1: Pipette 50.00 mL of the diluted synthetic urine and 50.00 mL of the Mix of standards. Now your standards will be diluted 1:2 (0.017 mg/mL allantoin, 0.0017 mg/mL 6-AU, 0.017 mg/mL UA).
4. Unknown samples with different concentration of each analyte can be prepared as well. In point 2 above prepare mix of standards containing different concentrations of each analyte for example:
5. Using a 50.00 mL volumetric pipette, add 50.00 mL of allantoin standard, next to the same container add 25.00 mL of 6-AU and 15.00 mL of UA (total volume 80 mL). Your standards are now diluted in different ratios. Label solution as mix of standards, concentration of all standards, solvent, date of preparation, name of person who prepared solutions. Outdate the solutions after one year. When you are done refrigerate the glass containers at -20 °C until samples are ready to be used in the class.
6. Prepare unknown samples as in point 3 above, calculate concentrations for each analyte
7. Give out about 5 mL of one of the unknown samples per student

E.5 Preparation of Synthetic Urine Samples (STUDENTS)

1. Obtain about 5 mL of the synthetic urine sample
2. Filter the sample using syringe and syringe filters (Nylon 45 µm) into clean and dry weighing bottle

- Using synthetic urine sample and internal standard solution (tyrosine) prepare sample for LC-MS analysis in HPLC vial. Make sure that this sample contains the same concentration of tyrosine that your calibration solutions have.
- Run the sample at least 3 times through HPLC-MS.
- Using calibration curve that you constructed calculate concentration of assigned analyte(All, 6-AU or UA) in synthetic urine sample.

E.6 Example of analysis of standard solutions and urine samples

Analysis of Standard Solutions

Use the examples provided below to set up LC-MS runs and to separate all 4 standards.

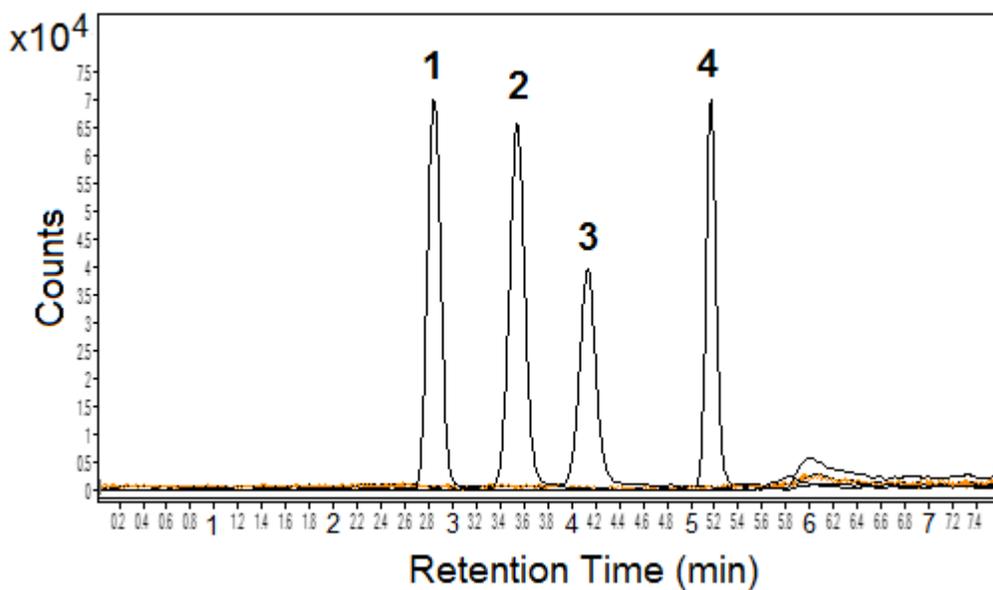


Figure 4. EICs of allantoin, 6-aminouracil, uric acid, and tyrosine

Peaks:

- Allantoin: $[M+Na]^+$ EIC(181.0332 m/z)
- 6-aminouracil: $[M+H]^+$ EIC(128.0455 m/z)
- Uric acid: $[M+H]^+$ EIC(169.0356 m/z)
- Tyrosine: $[M+H]^+$ EIC(182.0812 m/z)

Analytical Method:

Flow rate: 0.4 mL/min

Cogent Diol™ column: 4um, 100A, 150 x 2.1mm ID, SN: C1262, Part #: 40060-15P-2, Vendor: Microsolv Tech Corp.

Solvents:

A: DI water (0.1% FA)

B: Acetonitrile (0.1% FA)

Gradient:

Time (min)	%B
0.00	95
6.00	30
7.00	30
8.00	95

Post time: 4 min

Example of analysis of the urine samples

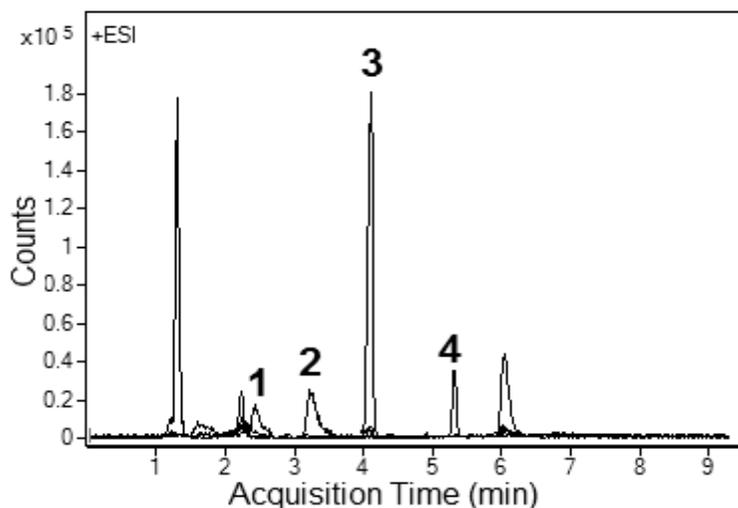


Figure 5. EICs of allantoin, 6-aminouracil, uric acid, and tyrosine

Peaks:

- 1 Allantoin: $[M+Na]^+$ EIC(181.0332 m/z)
- 2 6-aminouracil: $[M+H]^+$ EIC(128.0455 m/z)
- 3 Uric acid: $[M+H]^+$ EIC(169.0356 m/z)
- 4 Tyrosine: $[M+H]^+$ EIC(182.0812 m/z)

Method of analysis and gradient are the same as in analysis of the standards above. As can be seen in the chromatogram above there are several additional peaks at the same m/z values as standards. Peaks for allantoin, 6-AU, UA and Tyr were detected by comparison to of retention times with the standards.

E.7 Reporting the results of the experiment

Separate Excel files with a report page for the above experiment were prepared. Students are required to assign peaks to analytes in the mixture (by using m/z values for $[M+H]^+$ ions). Students record peak areas for analytes and construct calibration curves in the provided excel file. Based on the calibration curve students determine the concentration of one analyte in the synthetic urine sample.

RESULTS AND CALCULATIONS
 LC-MS Determination of 6-aminouracil (6-AU) in synthetic urine sample
 ANP chromatography

Name		Section	
Date		Locker No	
Lab partner' Names			

Instrument (Mft., Model No, I.D.)	
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Compound	Calculations for Unknown Sample			Calculations for Stock Solution (Mix 1)		
	6-AU	Allantoin	Tyrosine	6-AU	Allantoin	Tyrosine
Retention Time (sec.)						
Peak Width (sec.), $w_{0.1}$						
Theor. Plates, $N_{0.1}$						
Capacity factor, k $t_0 = 0.953 \text{ min}^*$						
Actual conc. (mg/mL)						

* verify the value with the instructor

Experimental conditions

Column Information	Solvents	Flow rate	Temp. (Deg. C)	ANP Gradient	Injection μL

Calibration Curve data (Peak area- PA, actual concentration -mg/mL)

Cal. Curve levels	1	2	3	4	5
6-AU actual conc.					
6-AU peak area					
Allantoin actual conc.					
Allantoin peak area					
Tyrosine actual conc.					
Tyrosine peak area					
6-AU PA/Tyr PA				Intercept	Slope
Equation for trend line					
6-AU				Intercept	Slope
Equation for trend line					
Allantoin PA/ Tyr PA				Intercept	Slope
Equation for trend line					
Allantoin				Intercept	Slope
Equation for trend line					

Urine Sample (Unknown)

Compound	Peak Area
6-AU	
Allantoin	
Tyrosine	

Cal. Curve used	6-AU/Tyr	6-AU	All PA/Tyr PA*	Allantoin
Calculated conc. 6-AU (mg/mL)				

E.8 Reading Micropipettes [8]

A micropipette can be set to expel a specific volume, and that volume will be displayed on a small rectangular window. See Figure 1 below.

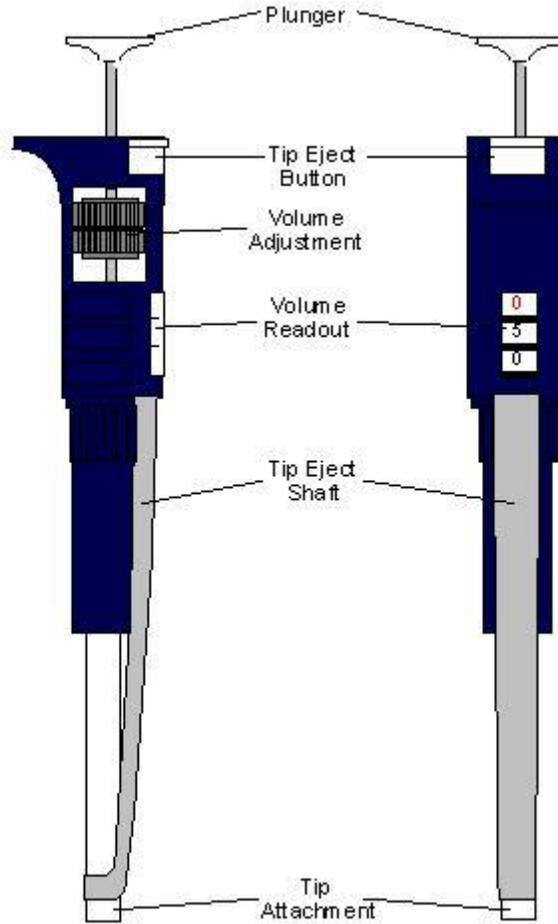


Figure 1. Labelled diagram of a typical micropipette

Each of the three boxes that comprise the window represent a different place value, and the place values depend on the type of micropipette you're using (as shown in Figure 2).

P10 and P20 the last digit is decimal					
P10	P10	P20	P200	P1000	P1000
	1	1	1	1	0
2	0	8	1	0	7
5	0	2	0	0	1
2.5 μL	10.0 μL	18.2 μL	110 μL	1000 μL	710 μL

Figure 2. Volume readouts for different types of micropipettes

For instance, if you were using a 200 μL micropipette – called a P200 – the top box would represent the hundreds place, the middle box would represent the tens place, and the final box would represent the ones place. Thus, a readout of “082” (read from top to bottom) would mean that the pipette is set to draw 82 μL of liquid. A 10 μL pipette on the other hand – or P10 – with the same readout would be set to draw 8.2 μL of liquid.

Micropipettes can only expel a volume accurately within a certain range. P1000s can expel 200-1000 μL accurately, while P20s can expel 20-200 μL accurately, and so on. The range of volumes that a pipette should be used for is usually stated on the volumetric pipette itself. Do not use a pipette to expel a volume out of its accuracy range – doing so will damage the instrument.

G. Glossary

Calibrate: to calibrate is to check, adjust, or determine a measurement's or measuring device's accuracy by comparing it to a standard.

Calibration Solution: a solution used to create a calibration curve that's made by diluting a standard concentration to a desired concentration.

Chromatography: a technique used to separate the compounds in a solution. Chromatography entails using a gas or liquid – aka the “mobile phase” – to carry a solution through a solid material – or “solid phase”. The solid phase possesses a different affinity for each compound, and this attraction affects the time by which the compounds travel to a detector. The compounds with the lowest affinity to the stationary phase travel through it the fastest and are detected first, while the compounds with the greatest affinity to the solid phase are detected last.

Qualitative Analysis: determining the quality of the components in a sample (i.e. qualitative analysis is figuring out WHAT compounds are in a solution).

Quantitative Analysis: determining the quantity of each component in a sample (i.e. quantitative analysis is figuring out HOW MUCH of each compound is present in a solution).

Limit of Detection: the lowest amount of analyte in a sample that can be observed and that is statistically different from the noise.

Limit of Quantitation: the lowest amount of analyte in a sample that can be detected and quantified (how much of the compound is present). The limit of quantitation is usually greater than the limit of detection.

Mass Spectrometry: a technique used to analyze the mass and quantity of compounds in a solution via their masses and charges. In mass spectrometry, a solution's compounds are charged and guided to a detector by means of magnetic and electric fields. The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields. The ion signal is processed into mass spectra.

Noise: the random fluctuations occurring in the signal that affect the accuracy with which a compound is detected and quantified.

Standard Solution: a solution with a known concentration of a solute. A standard solution is made by weighing out a specific amount of a compound and dissolving it to a certain volume.

Target analyte: the compound that you have targeted for analysis. In this experiment, allantoin, 6-aminouracil, uric acid, and triuret are the target analytes.

References

- [1] Kim KM, Henderson GN, Frye RF, Galloway CD, Brown NJ, Segal MS, Imaram W, Angerhofer A, Johnson RJ. 2008. Simultaneous determination of uric acid metabolites allantoin, 6-aminouracil, and triuret in human urine using liquid chromatography “mass spectrometry. *Journal of Chromatography.B, Analytical Technologies in the Biomedical and Life Sciences* 877(1-2):65-70.
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