Olympic Drug Scandal (UV/Vis): Teacher Resource

One of the pills from the Olympic competitors was found to be illegal in sport; however, it is not illegal in small doses. You have been asked by the Olympic committee to determine if the amount of drug in the competitor's urine sample is above the legal limit.

Method:

A concentration curve of the drug must be made. A stock solution of known concentration will be provided. Make up the following solutions of different concentrations to create a set of standards.

1. Prepare a blank, by filling a cuvette with deionised water.
2. Test a sample of the stock solution on the machine, using the wavescan function (3), to find the wavelength of maximum absorbance (λ_{max}).
    - Run a full spectrum, from 200-900 nm, on a sample of the stock solution.
    - Look for the highest peak and record the value for λ_{max} on the worksheet provided.
    - The students should find a value of 273 nm, for caffeine.
3. Prepare Standards for UV/Vis Analysis
    - Using volumetric pipettes, measure out 1, 2, 3, 4 and 5 mL concentrations of the stock solution into each of 5 test tubes, following the Table 1, provided in this script.
    - Using volumetric pipettes, add deionised water to the test tubes to make each sample 5mL, following Table 1.
4. Measure the absorbance of each solution of known concentration and the unknown "urine sample", using the single wavelength function (1). Record the values on the worksheet provided.

Materials required:

Chemicals:

- Deionised water
- Stock solution of caffeine
- Unknown solution of caffeine, approx 200 ppm

Apparatus:

- Scanning UV/Vis spectrometer
- Volumetric or graduated pipettes
- Volumetric flasks or test tubes
- Disposable cuvettes
- Disposable pipettes
- Wash bottle

- Gloves

Paperwork:

- Lab scripts
- Risk assessments and hazard sheets
- Evaluation forms

Stock solutions:

For smaller groups and for use with smaller volumes (often with graduated pipettes):

The stock solution should be made by dissolving 125 mg of caffeine into a 250 mL volumetric flask, making a 500 ppm solution. The solutions for the concentration curve should be made by adding 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of the 500 ppm stock solution to each of five test tubes. These should be topped up to 5 mL total. A table showing the proper amounts is shown in the full lab script, Table 1.

For larger groups or with larger glassware available (often with volumetric glassware):

The stock solution should be made by dissolving 2000 mg of caffeine into a 1.000 L volumetric flask. The solutions for the concentration curve should be made by adding 5 mL, 10 mL, 15 mL, 20 mL, and 25 mL of the 2000 ppm stock solution to each of five 100.0 mL volumetric flasks. These should then be topped up to the full 100.0 mL with deionised water. A table showing this is below:

Concentration	Amount of Stock (mL)	Amount of H_2O (mL)
100ppm	5.00	95.00
200ppm	10.00	90.00
300ppm	15.00	85.00
400ppm	20.00	80.00
500ppm	25.00	75.00

Analysis of the data:

1. Plot the concentration vs. absorbance on a sheet of graph paper or using an excel spreadsheet. Use this calibration graph to determine the concentration of the unknown sample.
    - The concentration determined from the absorbance plot should come out to the value around 200 ppm. The demonstrators should have the correct value.
2. Compare the concentration from the unknown that you determined by the graph with the data points in the worksheet table. Does this answer make sense? Can you estimate where the answer should be by using the table?
    - There have been instances where the hand drawn graphs have not obtained the correct answer, even though the data points are correct. This is due to the inexperience of drawing graphs. Excel graphs will fix this. But also just a discussion of estimation from the data points and where the error would come in to effect.
3. Using the concentration determined and the λ_{max} values for the drugs below, can you determine if the competitor has too much of the drug in his system?
    - Ephedrine is prohibited when its concentration in urine is greater than 10 micrograms per millilitre (10 ppm)[1]. λ_{max} for ephedrine is 206nm [2].
    - Pseudoephedrine is prohibited when its concentration in urine is greater than 150 micrograms per millilitre (150 ppm)[1]. However it is still on the monitoring list. λ_{max} for pseudoephedrine is 206nm [2].
    - Caffeine was prohibited when it's concentration in urine was above 12 micrograms per millilitre (12 ppm). This is approximately equivalent to 8 cups of coffee, drunk in one sitting. In 2004, the World Anti-Doping Agency (WADA) took caffeine off the list of performance enhancing drugs[3]. λ_{max} for caffeine is 273nm [2]. Students should determine that caffeine is the drug in the "urine sample".
4. What would you recommend to the British Olympic Association about this competitor?
    - The compound should be determined to be caffeine and therefore the amount of caffeine in the sample should be far over the limit of 12 ppm. This could lead to a debate about how much over eight cups of coffee would give the athlete an advantage and if the competitor should be banned from the competition. Since caffeine is only on the monitoring list, the athlete would probably not be banned. Perhaps start a discussion about what is performance enhancement, is it okay if everyone does it, and further ethical questions.

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[1] World Anti-Doping Agency. "2012 Prohibited List", http://www.wada-ama.org/Documents/World_Anti-doping_Program/WADP-Prohibited-list/2012/WADA_Prohibited_List_2012_EN.pdf, accessed 30 April 2012.
[2] Hellriegel, C., H. Händel, M. Wedig, S. Steinhauer, F. Sörgel, K. Albert, U. Hozgrabe. *Journal of Chromatography A*, **914**, (2001), 315-324.
[3] MacMichael, S. "WADA president to urge re-banning of caffeine". *Road.cc*, 11 Aug 2010. http://road.cc/content/news/21341-wada-president-urge-reconsideration-lifting-caffeine-ban, Accessed 30 April 2012.

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