Spectrophotometric determination of lycopene from tomatoes by extraction with hexane/ethanol/acetone and absorbance measurement at 503 nm.

Solvents. Acetone and hexane are HPLC grade from Fisher. The ethanol used is 200 proof absolute ethanol, which can be obtained from Spectrum chemicals (Gardena, CA). Mix in a ratio of two parts hexane to one part acetone and one part ethanol. Only mix as much solvent as you plan to use in the next few days and keep in a well stoppered bottle.

Procedure.
1. Starting with well homogenized tomato juice (prepared under vacuum to minimize the introduction of air bubbles), use a 100 µL Drummond micropipettor to take a sample. After drawing the sample into the pipetter, wipe any tomato juice from the outside of the glass bore with a kimwipe then inspect the pipetter to be sure no large air bubbles have been included. Dispense the sample into a 20 ml screw cap tube. Also prepare several blank samples with 100 µL water instead of tomato pulp.
2. Add 8.0 ml of hexane:ethanol:acetone (2:1:1) using a repipetter. Cap and vortex the tube immediately, then incubate out of bright light.
3. After at least 10 minutes, or as long as several hours later, add 1.0 ml water to each sample and vortex again.
4. Let samples stand 10 minutes to allow phases to separate and all air bubbles to disappear.
5. Rinse the cuvette with the upper layer from one of the blank samples. Discard, then use a fresh blank to zero the spectrophotometer at 503 nm (see comment #5 below). Determine the A503 of the upper layers of the lycopene samples.

Calculation of lycopene levels. Lycopene levels in the hexane extracts were calculated according to:

\[
\text{Lycopene (mg/kg fresh wt.)} = \left( A_{503} \times 537 \times 8 \times 0.55 \right)/\left(0.10 \times 172\right)
\]

\[= A_{503} \times 137.4\]

where 537 g/mole is the molecular weight of lycopene, 8 mL is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of tomato added, and 172 mM\(^{-1}\) is the extinction coefficient for lycopene in hexane.

If 100 µL of tomato juice is analyzed but the volume of mixed solvent used is something other than 8 mL (see comment #1 below), then the lycopene concentration can be calculated by:

\[
\text{Lycopene (mg/kg fresh wt.)} = A_{503} \times 17.17 \times V
\]

where V is the volume of mixed solvent added, in mL.

Comments on the procedure.
1. If only a few samples are to be analyzed, rather than setting up a repipetter, the solvent can be added using a 10 mL volumetric pipette. One mL of water can still be used to separate the
phases. The multiplication factor for calculating the lycopene concentration increases from 137.4 to 171.7.

If the sample material cannot be easily be pipetted, then it can be added by weight. In theory one could weigh out about 100 mg of tomato material and follow the procedure described above, but from a practical point of view it is easier and more accurate to weigh out a larger sample and then follow the original Sadler procedure for extraction. For this about 1.0 g of tomato material is accurately weighed into a 125 mL Erlenmeyer flask and 100 mL of mixed solvent is added with a graduated cylinder. The flask is sealed with a rubber stopper then, after at least 10 minutes of extraction, 15 mL of water is added to separate the phases and A503 of the upper phase determined. The lycopene concentration is given by:

\[
\text{Lycopene (mg/kg fresh wt.)} = A_{503} \times 171.7 / W
\]

where W is the exact weight of tomato added, in grams.

2. This extraction procedure is optimized for the amounts of lycopene typically found in undiluted juice from red tomatoes. To analyze products like paste or other products where the tomato material has been concentrated, the material must be diluted with water back to the consistency of juice. If not diluted the amount of lycopene in the assay may be too high, leading to either incomplete extraction or too high of an absorbance value. Formulated products containing more than just tomatoes can also be analyzed using this procedure but only with care. The dual wavelength procedure should be used and the density of the material checked if the sample is to be added by volume. The dual wavelength procedure should also be used for analyzing juice from tomatoes that differ from the typical red, fully ripe material used for processing.

3. The solvents used in this procedure are very prone to evaporation and must be kept in a well stoppered bottle. Keep this bottle closed as much of the time as possible. Optimally, fresh solvent should be mixed daily. The solvents are also flammable and prolonged exposure to the vapors is considered harmful. A fume hood should be used.

4. The mixed solvents are 50% hexane by volume. When separated into two phases, the upper phase is often referred to as the “hexane layer”, so one might expect it to have a volume equal to 50% of the original mixed solvent volume. Actually the upper layer is not pure hexane and has a volume equal to 55% that of the original mixed solvent volume.

5. Sometimes when the first sample is put in the cuvette tiny droplets or haziness will form. To avoid this, rinse the cuvette with a sample of upper layer from a blank sample. This removes any residual material in the cuvette that causes the haze to form. Sometimes the cuvette needs to be rinsed several times. Be sure the that the blank used to zero the spectrophotometer is clear.