

Evaluation of ascorbic acid as a standard for the Oxygen Radical Absorbance Capacity (ORAC) Assay

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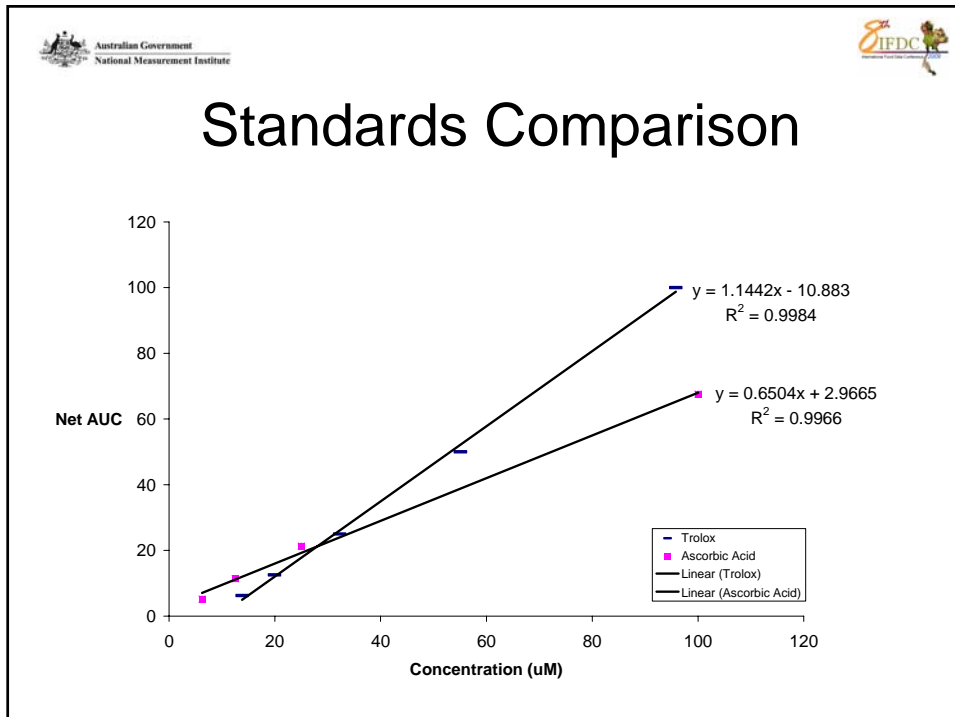
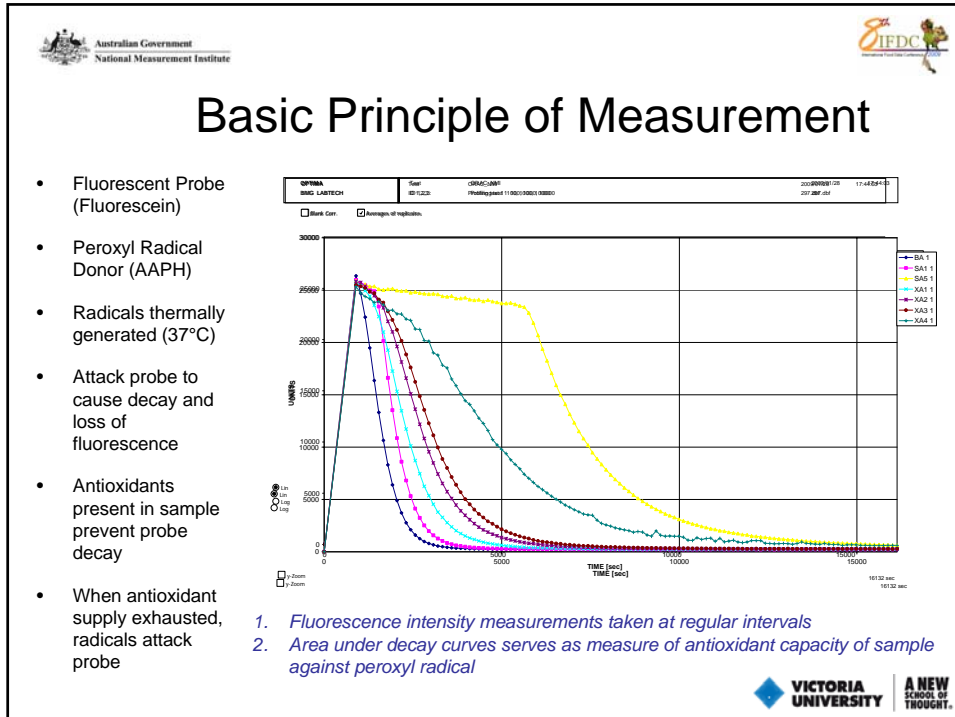
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Introduction



- Oxygen Radical Absorbance Capacity assay
- Measure of antioxidant capacity of a sample against a specific radical species (peroxyl radical)
- Increasing in popularity
 - Health claims on product packaging
- Increasingly popular choice for antioxidant capacity measurements of food



Validation Summary

Validation parameters for both Trolox and ascorbic acid analytes over a calibration range of 6.25-100µM for both standards

Validation Parameters	Measurand	Replicates	Statistical Indicators
Linearity & Repeatability of Standards	Trolox	7	$R^2 = 0.9996$; $\%RSD_{(CL \leq 3\%)} = 2.22$
	ascorbic acid	7	$R^2 = 0.9986$; $\%RSD_{(CL \leq 3\%)} = 2.32$
Limit of Detection	Trolox	10	$(\bar{x}_{(Net AUC)})13 \pm 0.66$; $LOD = 2.0\mu M$
	ascorbic acid	10	$(\bar{x}_{(Net AUC)})12 \pm 0.69$; $LOD = 2.0\mu M$
Instrument repeatability	Phosphate buffer	30	$(\bar{x}_{(AUC)})21 \pm 0.86$; $\%RSD_{(CL \leq 3\%)} = 0.04$
Repeatability of Samples	Trolox	7	$MDL_{(\mu M)} = 1.4$; $\%REC_{(CL 80-120\%)} = 110$; $\%RSD_{(CL \leq 3\%)} = 1.1$
	ascorbic acid	7	$MDL_{(\mu M)} = 2.1$; $\%REC_{(CL 80-120\%)} = 110$; $\%RSD_{(CL \leq 3\%)} = 1.6$

(\bar{x}) = mean, MDL = method detection limit, %REC = % recovery of 10µM spike of standard in natural mineral water matrix, %RSD = % relative standard deviation/coefficient of variation, AUC = area under the curve, CL = control limit, LOD = limit of detection, R^2 = correlation coefficient for linear regression plot

Measurement Uncertainty

Measurement uncertainty estimation for ORAC values in the Trolox scale

Net AUC used to get result	Typical ORAC values over calibration range (µM T.E)	± Combined uncertainty	Error as %
35	27.8	4.3	15%
45	38.3	4.2	11%
75	69.7	4.5	7%
95	90.6	5.0	6%
100	95.8	5.1	5%

(µM T.E = micro mol per liter of Trolox equivalents)

Measurement uncertainty estimation for ORAC values in the ascorbic acid scale.

Net AUC used to get result	Typical ORAC values over the calibration range (µM A.E)	± Combined uncertainty	Error as %
25	37.9	5.8	15%
30	46.4	5.9	12%
40	63.5	6.0	9%
50	80.7	6.4	8%
60	97.8	7.1	7%

(µM A.E = micro mol per liter of ascorbic acid equivalents)



Data Interpretation

ORAC Calculation

$$I \quad AUC = \left(\left[\frac{f_1}{f_0} + \frac{f_2}{f_0} + \frac{f_3}{f_0} \dots \frac{f_i}{f_0} \right] + 0.5 \right) \times CT$$

(f_0 = initial fluorescence reading, f_i = fluorescence reading at cycle i , and CT = cycle time in minutes)

$$II \quad NetAUC(sample) = \left(\frac{AUC_{sample} - AUC_{blank}}{AUC_{Trolox} - AUC_{blank}} \right)$$

$$III \quad ORAC / kg = \left[\frac{netAUC_{sample} - c}{m} \right] \times DF$$

(where the equation, $y = mx + c$, is for a plot of $netAUC$ vs concentration of standards (Trolox or Ascorbic acid) and DF = dilution factor of sample)

Dense liquids

$$\frac{x\mu M \text{ equivalents}}{kg} \times \frac{1kg}{1000g} = \frac{x\mu M \text{ equivalents}}{g}$$

To convert to $x\mu M$ equivalents/g to $x\mu M$ equivalents/mL density factor is considered

$$IIIa \quad \frac{x\mu M \text{ equivalents}}{\left(\frac{g}{density(g/mL)} \right)} = x\mu M \text{ equivalents} \times \frac{density}{1mL}$$



Reporting in Mass Units

Conversion to mass units

Determine moles of ascorbic acid in the sample (based on the concentration in IV)

$$IV \quad C = x\mu M = \left(\frac{x\mu M}{1 \times 10^6 \mu M} \right) \times 1M = x \times 10^{-6} M$$

$$V \quad n = C \times V$$

(where n = moles, C = concentration of ascorbic equivalents in molar units, V = volume in litres)

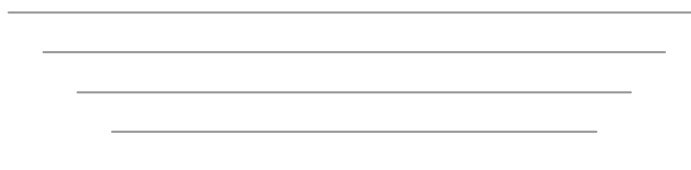
$$VI \quad m = n \times MM$$

(where m = equivalent mass of Ascorbic acid, n = moles of Ascorbic acid (V), MM = molar mass of Ascorbic acid)

Results

Sample ID	ORAC _(TAC) μmol T.E/g	ORAC g/kg T.E	ORAC _(TAC) μmol A.E/g	ORAC g/kg A.E	
Cranberry Juice	12.0	3.0	21.0	5.3	
Orange Juice	15.0	3.8	25.0	6.3	
Fresh Orange Juice	33.0	8.3	55.0	13.8	
Ribena (ready to drink)	8.0	2.0	14.0	3.5	
Fresh Lime Juice	9.0	2.3	17.0	4.3	
Rooibos Tea	8.3	2.1	14.0	3.5	
Black Tea	19.0	4.8	29.0	7.3	
Apple Juice	6.4	1.6	9.6	2.4	
Prune Juice	52.0	13.0	85.0	21.3	
	ORAC _(TAC) μmol T.E/g	ORAC g/kg T.E	ORAC _(TAC) μmol A.E/g	ORAC g/kg A.E	
Dark Chocolate (70%)	ORAC _(lipophilic)	3.8	1.0	15.0	3.8
	ORAC _(hydrophilic)	21.0	5.3	37.0	9.3
	ORAC _(TAC)	25.0	6.3	52.0	13.1

(TAC = total antioxidant capacity; T.E = Trolox equivalents; A.E = ascorbic acid equivalents) Results are taken as mean of n=7 replicates. Results are reported to 2 significant figures, and rounded up where necessary.



Significance

- Results understandable to layperson
 - Added benefit of using Ascorbic acid units
 - Naturally occurring
 - Common in plant based foods
- Easier to communicate ORAC values in mass units
 - Molar units not widely used in NIP labels



Conclusion

- **Ascorbic acid viable standard**
 - Validated
 - Hydrophilic
 - Lipophilic
 - Comparable MU budget
- **More effective communication of antioxidant capacity measurement**
 - mass units vs. molar units
 - Solids
 - g/kg vs. $\mu\text{M T.E/kg}$ (alternatively $\mu\text{M A.E/kg}$)
 - Liquids
 - g/L vs. $\mu\text{M T.E/L}$ (alternatively $\mu\text{M A.E/L}$)



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