Enzymatic Testing in the Winery

Eric Wilkes

FOSTER'S GROUP

Widely Used

- G/F
- Malic
- Acetics
- Citrics
- Ascorbics
- NOPA
- Ammonia
- And lots and lots more

Wide Range of Equipment

- Spectrometers
- Plate readers
- Flow systems
- Discreet auto-analysers

- All work in different ways but essentially the same rules apply.

The Range Problem

- “my plate reader methods gives me sugar results for 0 to 100 g/L without dilution”
- Yeah, right.
- Most of the enzymatic methods have ranges of around one order of magnitude.
- eg a typical G/F kit has a range of 4 to 80 µg per sample.
- For G/F 80 µg translates to a maximum 0.8 g/l assuming that you are using 0.1 mL sample size.

The Range Problem

- For Malic acid with a working maximum of 30 µg a similar sample size gives you a maximum 0.3 g/L working concentration.
- To get accurate results you must dilute into the linear range of the method.
- Otherwise you may not have enough reagent to get reliable and repeatable results.
- A 1 in 10 dilution for both gives ranges typical for wine.
- You may need to use different dilutions for different samples.

Spectroscopic Considerations

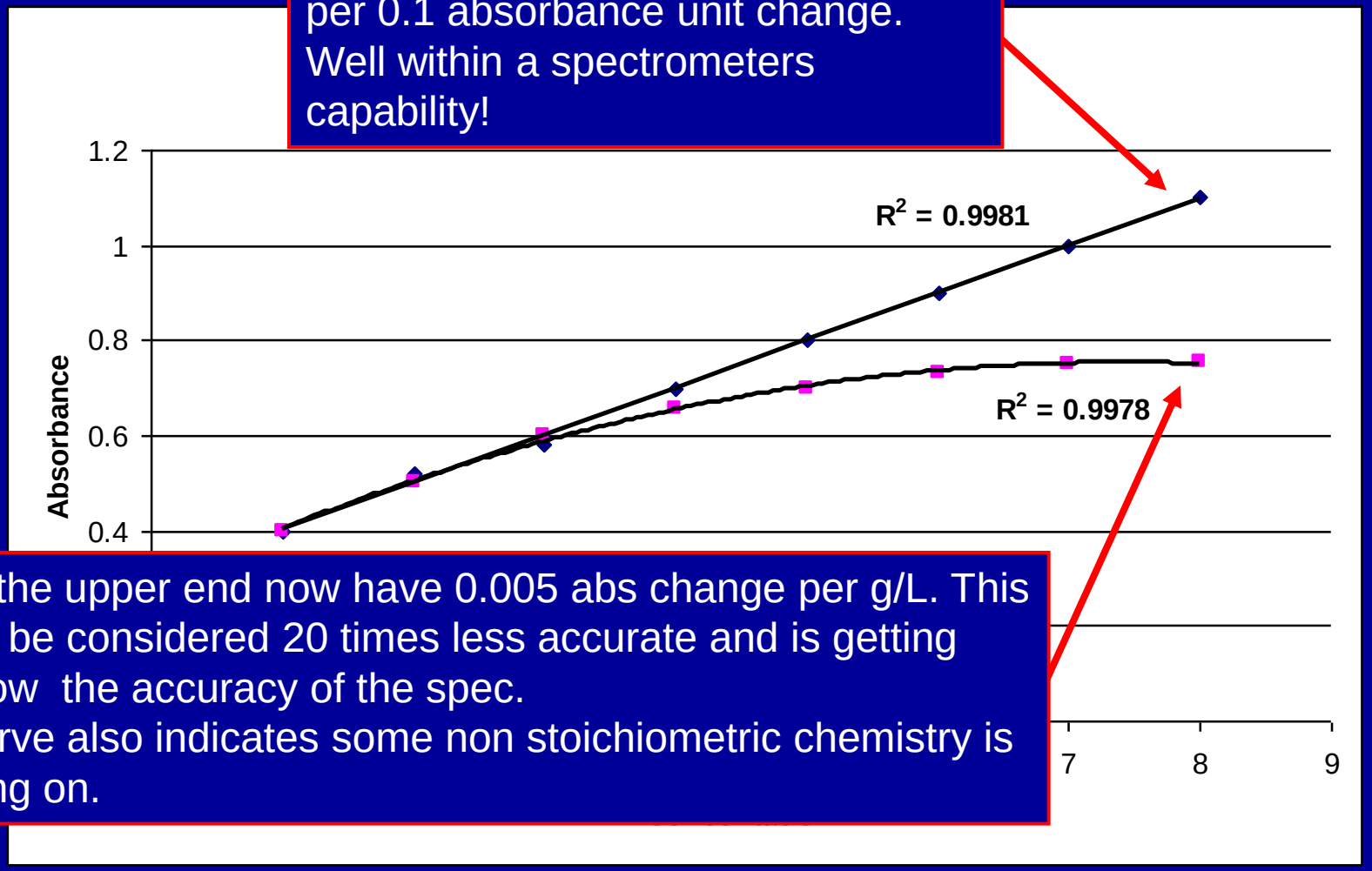
- The Beer-Lambert law is a linear relationship (not curved).
- Absorbance measurements should be done between 0.2 and 0.8 absorbance units (1 at the maximum).
- Reading outside this range can lead to increased errors including curved and inconsistent responses.
- E.g. using the supplied method 0.8 g/L GF gives an absorption of ~1.1 units, OK just.

Calibrations Curves

- They need to be done over the linear range for the reagents (I know, I keep saying this but it is important!).
- Using curved calibrations suggests that other chemical or spectroscopic effects are coming into play.
- Leads to big errors at higher concentrations.

Curved v's Linear

At the upper end still getting 1 g/L per 0.1 absorbance unit change. Well within a spectrometers capability!



- At the upper end now have 0.005 abs change per g/L. This can be considered 20 times less accurate and is getting below the accuracy of the spec.
- Curve also indicates some non stoichiometric chemistry is going on.

Enzymes

- Lots of different brands these days.
- Vary in stability, shelf life, sensitivity.
- They need to be handled with a lot of care.
- Refrigerate.
- Make up only when needed.
- Do not expose to undue physical shocks.
- Throw out after the use by date.

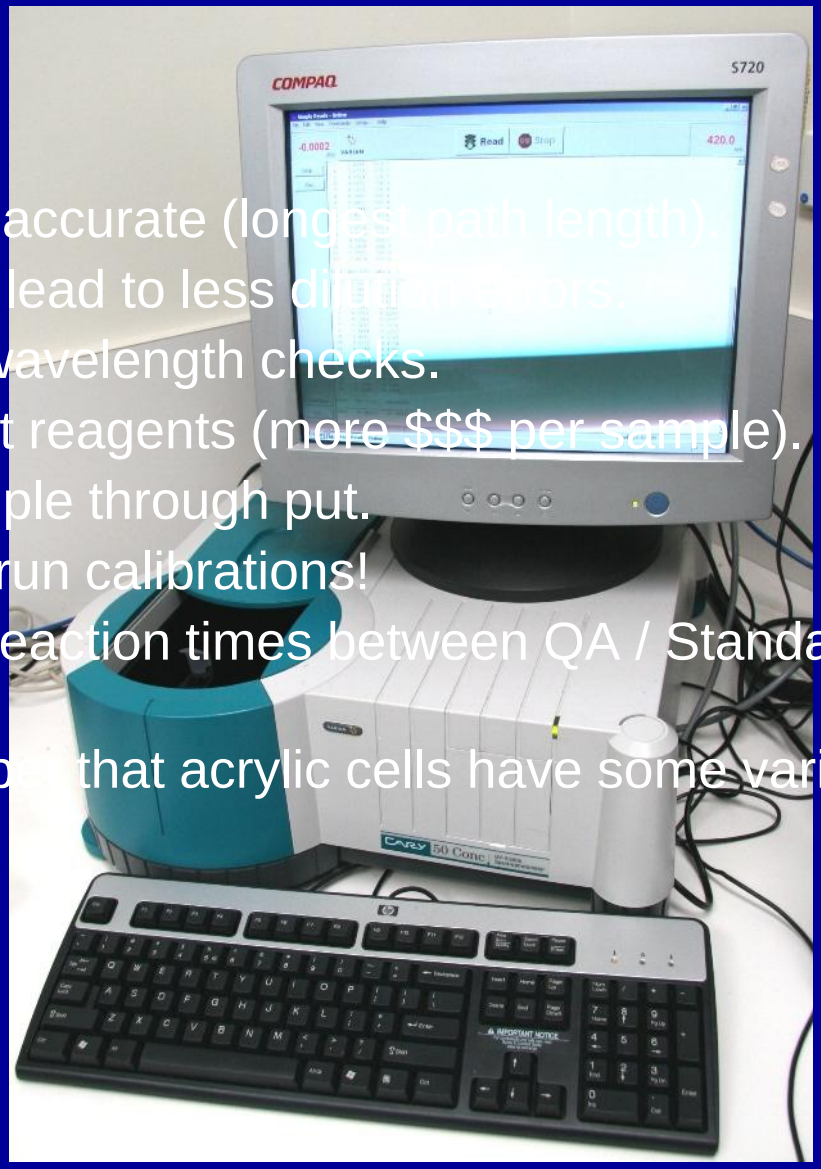
Validate

- Always fully validate a new enzyme or method.
- Include
  - **Repeatability (in one day and over a week)**
  - **Matrix effects (different wines and levels)**
  - **Range (linearity, and response)**
- Develop your SOP around the validation (ie appropriate dilution).

QA

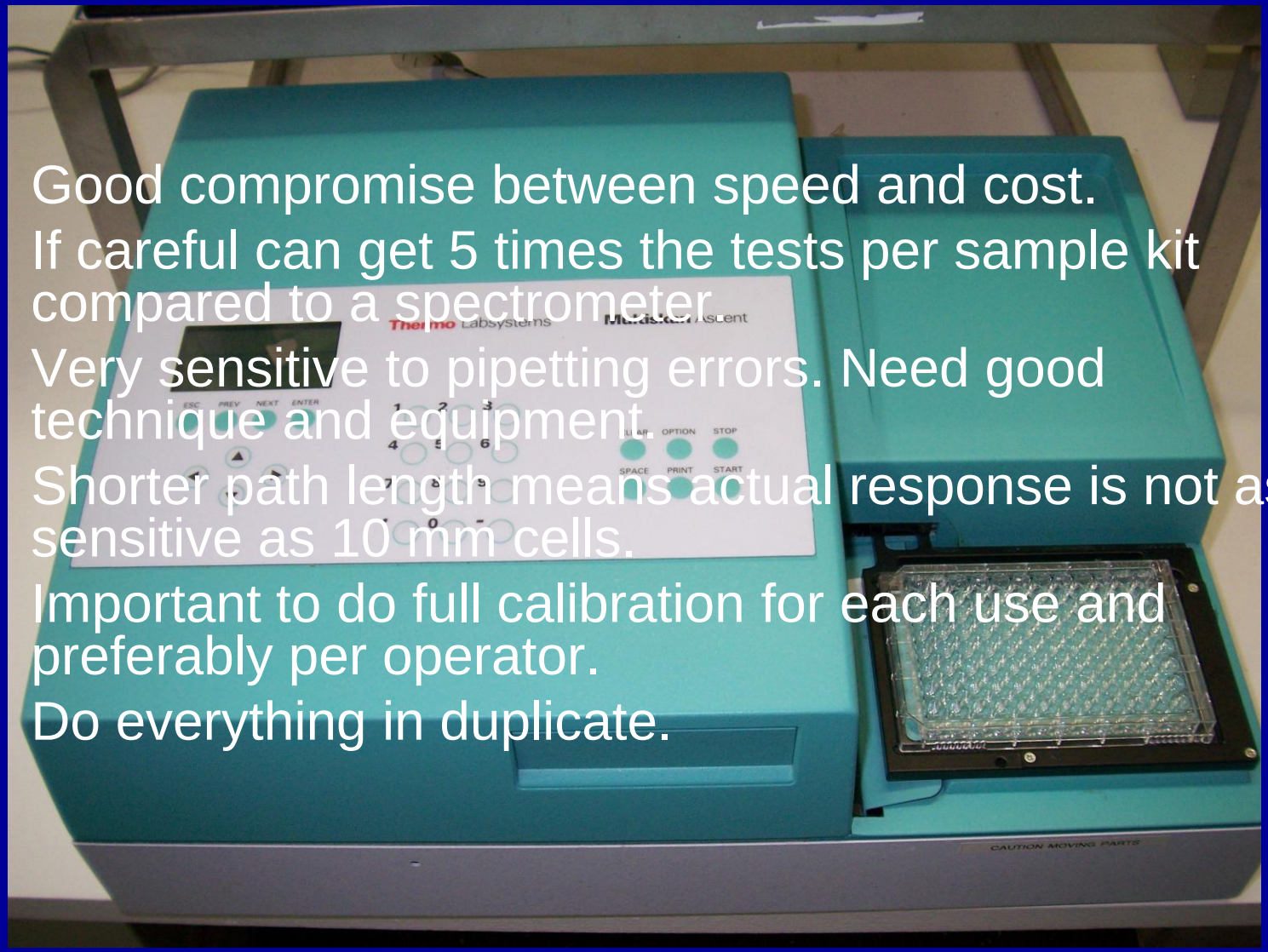
- DO IT!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
- Best to do a calibration for every run (two point if worse comes to worse). Enzymes can change slightly from batch to batch.
- Also use wine QA checks, ideally one high one low. Standard adds are good.
- Don’t use straight analyte samples as your QA, does not pick up unexpected interferences or range changes. Use known samples.

Spectrometers



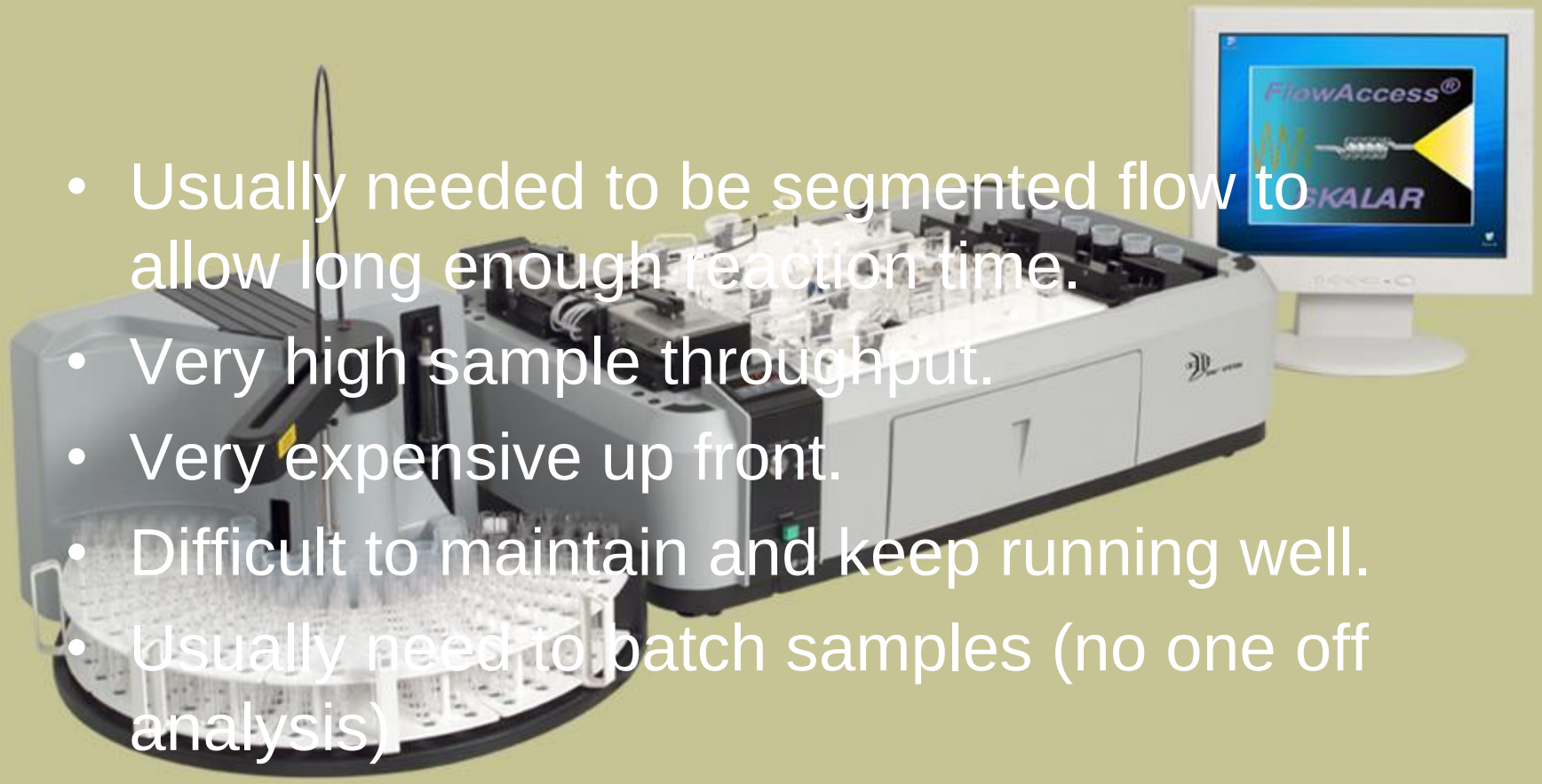
- Can be very accurate (longest path length).
- Big volumes lead to less dilution errors.
- Need to do wavelength checks.
- Use the most reagents (more $$$ per sample).
- Slowest sample through put.
- Still need to run calibrations!
- Try to keep reaction times between QA / Standards the same as samples.
- Also remember that acrylic cells have some variation in path length!!

Plate Readers



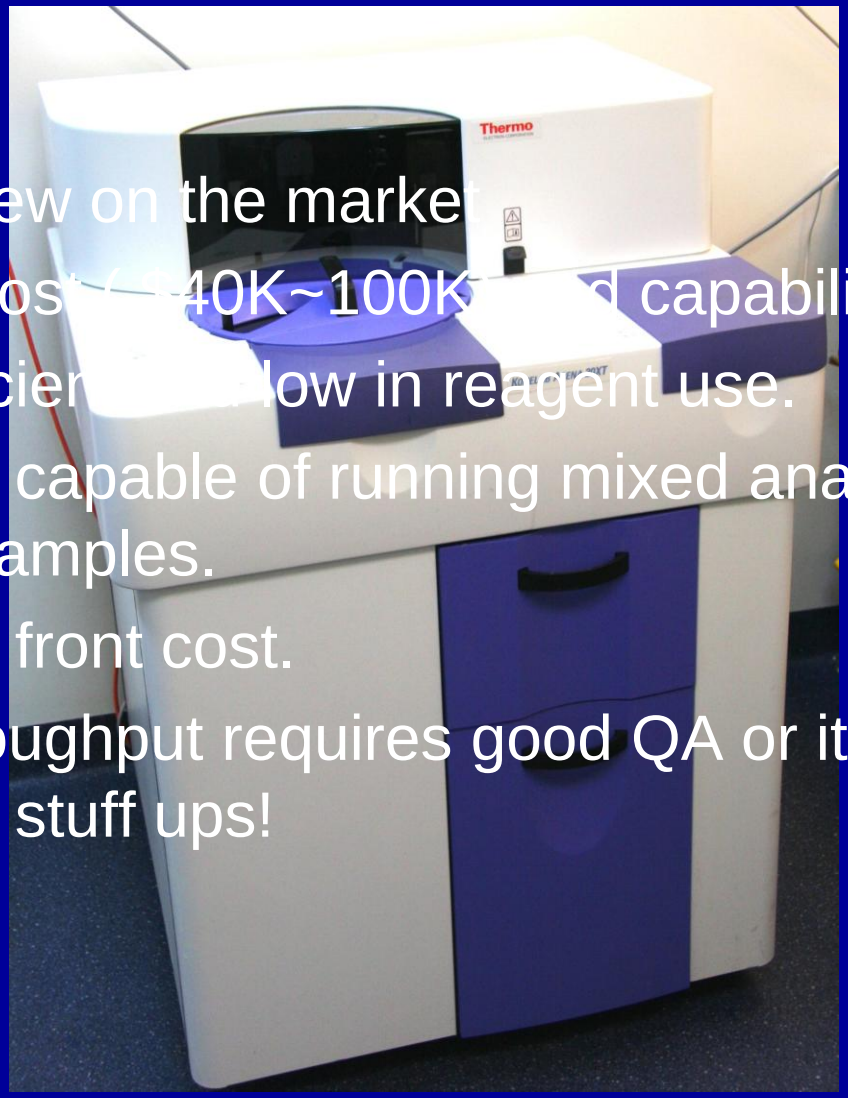
- Good compromise between speed and cost.
- If careful can get 5 times the tests per sample kit compared to a spectrometer.
- Very sensitive to pipetting errors. Need good technique and equipment.
- Shorter path length means actual response is not as sensitive as 10 mm cells.
- Important to do full calibration for each use and preferably per operator.
- Do everything in duplicate.

Flow Analysers



- Usually needed to be segmented flow to allow long enough reaction time.
- Very high sample throughput.
- Very expensive up front.
- Difficult to maintain and keep running well.
- Usually need to batch samples (no one off analysis)

Discreet Auto-analysers



- Quite a few on the market
- Vary in cost (~40K~100K+) and capability.
- Very efficient and low in reagent use.
- Most are capable of running mixed analyte sets and one off samples.
- Major up front cost.
- High throughput requires good QA or it can translate to bigger stuff ups!

Malics

"You said it was moving, it went from 3.5 to 3.3, now it is 3.6!"

- Most reagent kits have undiluted ranges of 0.01 to 0.3 g/L for 0.1mL samples.
- Errors of around ±0.005 g/L are generally quoted based on spectroscopic accuracy.
- Usually dilute samples 1 in 10 but loose accuracy at the sub 0.1 g/L level.
- Need to define the definition of dry and dilute or not appropriately.
- If you are testing at sub 0.1 g/L concentrations need to decolourise with pvpp.
- 1 in 10 dilutions can give reasonable results above 3 g/L, but lots more errors and inconsistent results.

Glucose / Fructose

- Most reagent kits have undiluted ranges of 0.05 to 0.8 g/L for 0.1mL samples.
- Errors of around ±0.02 generally quoted based on spectroscopic accuracy.
- 1 in 10 dilution gets range to 0.5 to 8 which is reasonably typical for wine.
- Tend to have a lot more problems with sweet wine. Must dilute to get into appropriate range.
- This leads to much greater errors (1 in 100 can give 2 g/L variations)
- Must invert for sparkling wine samples to convert sucrose.

Acetic Acid (VA)

- Generally accepted that VA is at least 95% acetic acid (ethyl acetate does not get measured by traditional methods).
- A number of enzyme schemes exist, however the acetate kinase version seems to give the most linear results.
- 1 in 10 dilution gives a range in the region of 0.1 to 1 g/L.
- Reagents are very very sensitive. Best made up at last moment and checked with each run.

Take home messages!

- Look after reagents.
- Get linear calibrations.
- Dilute to fit samples into appropriate ranges.
- Do QA!
- Never trust reagents, always check!

Thank you

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