

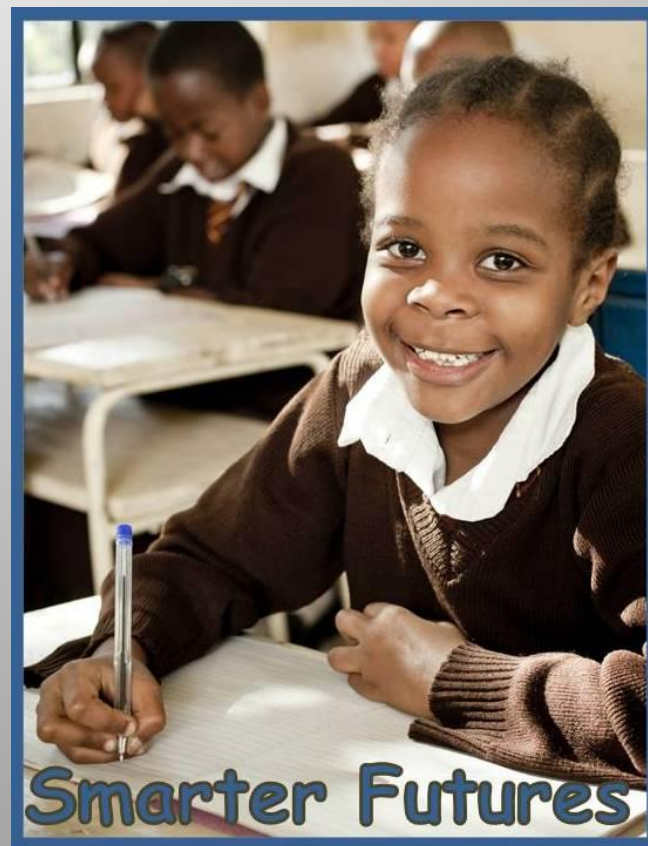
CHEMICAL ASSAYS

Philip Randall

pcubed@mweb.co.za

Tanzania

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$$\text{Total Error} = \sqrt{\text{Sampling Error}^2 + \text{Analytical Error}^2}$$

A Simple Example

- Sampling error is $\pm 0.4\%$
- Analytical error is $\pm 0.2\%$

Total Error

$$= \sqrt{\text{Sampling Error}^2 + \text{Analytical Error}^2}$$

$$\text{Total Error} = \sqrt{(0.4)^2 + (0.2)^2} = 0.45 \%$$

$$\text{Total Error} = \sqrt{(0.4)^2 + (0.1)^2} = 0.41 \%$$



Analysed 4
times

$$\text{Total Error} = \sqrt{(0.2)^2 + (0.2)^2} = 0.28 \%$$



Sampled 4
times

$$\text{Total Error} = \sqrt{(0.2)^2 + (0.1)^2} = 0.22 \%$$

Chemical Analysis

- Is only as good as the sample itself
- Requires skilled analysts
- For fortification - requires relatively expensive to very expensive equipment and consumables
- Is time consuming and
- Most importantly its expensive

Validity of Analysis

- Inspectors frequently take a grab sample - as they are overworked as well – so the sample is not representative but it is considered legal
- Mills are not pharmaceutical level processors and fortified foods are not like vitamin tablets (every single one the same) – we can get mills to that level of homogeneity but not economically

- The general public isn't like an astronaut taking pills and pastes they eat bulk quantities of a food vehicle i.e. Bread which has undergone a further mixing process
- The analyst takes 0.5g of sample and tries to find the micronutrients – the consumer eats 200g of sample and lets the body find the micronutrients

A Case in Point

- 2 internationally accredited (for vitamin and mineral analysis) laboratories plus 5 pre-mix supplier laboratories participate in a ring trail to assess how much reliance can the RSA Department of Health place on an external analysis for prosecution purposes.
- The 2 accredited laboratories had already been verified against the Canadian accredited reference laboratory for such analysis.

- For the purpose of the following study CV was taken at 1 standard deviation
- For compliance verification it would be expected for a laboratory to report to 1.96 (2) standard deviations i.e. at 95% confidence level

Method

- Laboratories are provided with freshly prepared pre-mixes which are then adulterated to be below the legal limit.
- Each lab receives 2 original, but different, pre-mix formulations, 2 adulterated by 10% and 2 adulterated by 20%
- Each of the above is provided to the laboratory on 2 or 3 different occasions i.e. Blind duplicate or triplicate samples

Results

- Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc
- Each laboratory correctly identifies the 100%, 90% and 80% samples.
- The coefficient of variation (CV) within anyone laboratory was <5%
- The CV between laboratories was typically 10-12% depending on micronutrient

Conclusion

- If you fool around with fortification pre-mix any reasonably competent laboratory will catch you out.

On Fortified Product??

- Same experimental design using pre-mixes designed to be used at 200g/MT i.e. 1:5000
- Samples prepared in laboratory using the same food vehicle (wheat flour) but the 2 different pre-mixes (avoids variability in intrinsic value issues) and made thoroughly homogenous.

Results

- Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc
- Individual laboratory CV's >10% so even within a laboratory compliance verification questionable.
- Between laboratory CV's >40%

Conclusions

- Group could definitely not distinguish even at 20% adulteration level so disputes are inevitable.
- Compliancy or not would depend on luck

Methodology

- HPLC – Numerous methods
- Spectroscopy
- Microbiology

- Method often depends on concentration

- Two accredited laboratories provide different results on the same sample – who is correct?
- Codex “Special Foods” and Margarine contain specified methods for Vitamin analysis
- Spot HPLC ?????

CODEX Standard 234 - 1999 contain method amendments adopted 2007

- Fluorometry
- Colorimetry
- Spectrophotometry
- Microbioassay
- Rat bioassay

Accessed March 2011

HPLC 95% Confidence ±	Product	Pre-Mix
Vitamin A	17.1	20.4
Thiamine	8.2	20.6
Riboflavin	9.0	8.8
Nicotinamide	9.4	10.0
Pyridoxine	12.2	7.8
Folic acid	17.4	10.0
Iron	10.7	11.1
Zinc	7.5	7.5

So we scrap chemical assays?

- No – vital role to play in fortification programme.
- Ensure pre-mix is “fit for purpose” – note this is different to “conforms to specification” (concrete life jacket)
- Recognise the limitations of wet chemistry and use it not abuse it.

In Context

- RSA study in 2005 found that four (4) registered suppliers of wheat flour and maize meal pre-mix into the country where compliant with “conformance to specification” on all the vitamins and minerals
- Same study subjected those pre-mixes to accelerated storage conditions of 40°C; 75% RH for 30 days using an environmental cabinet

- Pre-mix was placed in paper bags same as used for retail sale of wheat flour and maize meal
- Pre-mix was analysed by three (3) internationally accredited (for vitamin and mineral analysis) laboratories for Vitamin A at days) 0, 15 and 30

- Suppliers A and B = pre-mixes (wheat and maize) had a **RETENTION** of Vitamin A of $\approx 80\%$ after 30 days
- Supplier C = pre-mixes (wheat and maize) had a **LOSS** of Vitamin A of $\approx 90\%$ after 30 days
- Supplier D = had a retention of Vitamin A in one pre-mix of $\approx 80\%$ but had a loss in the other of $\approx 90\%$

- Both the wheat flour and maize meal pre-mixes had the same micronutrient compounds but in slightly different proportions.
- It was concluded that it was not due to micronutrient interaction
- Chance remark from one supplier indicated probable reason for this anomaly

- Supplier C sometimes bought Vitamin A from the same source as supplier D but on other occasions from a different source
- Very strong indications that original source of Vitamin A makes the difference between “conformance to specification” and “fitness for use”

IRONY

- South African millers had insisted upon “proving” fortification would not affect organoleptic properties and would survive the distribution chain.
- Conducted a 12 month trial using multiple grades of wheat flour and maize meal (with their respective pre-mixes) under a wide variety of distribution conditions and concluded that they had no problems BUT

- They had only used one source of pre-mix (Supplier A)
- Then suddenly the RSA market is open to multiple suppliers – all of whom are registered, and deemed credible, on the basis of “conformance to specification” investigations

- RSA has now made an amendment to the regulations requiring suppliers to, confidentially, inform the Department of Health who they are sourcing their micronutrients from and to advise them if they change sources.
- Food Control inspectors now check not only IF millers are fortifying but also WHOSE pre-mix they are using

Rapid Tests

- Iron
- Vitamin A

- Still working on EDTA methodology
- Vitamin A test requires “care”