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Advantages of proficiency test items utilising real food matrices

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Abstract

Proficiency testing by interlaboratory comparison requires standardised test items to be distributed to all the participants. Since the participant laboratories are routinely receiving a variety of food types for analysis, a simple matrix test item would be more straightforward to produce.

However, this does not then reflect the types of samples received by the laboratory, which is a highly desirable element of the test. Real food matrix test items reflect not only the types of samples being routinely received and analysed by the laboratory but also the effect of critical method or preparation parameters. Several examples from a food analysis proficiency testing provider detail the advantages of issuing real food matrix test items. The effect of instructions to participants, their applied methods and critical parameters, and the additional benefits of incurred or contaminated materials are demonstrated.

Keywords

- Chemicals
- Food
- Laboratory proficiency testing
- Matrix
- Microbiology

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Introduction

Proficiency testing (PT) aims to objectively assess a laboratory's performance against a standard test item. In its simplest form, a standard solution (for analytical chemistry measurements) would serve this purpose. However, laboratories in the real world are not actually measuring standard solutions; they are measuring real samples made of complex matrices. A good proficiency test sample has to replicate, as far as possible, these real world samples. At the same time, the test sample has to be the same for each participating laboratory (which may be hundreds of laboratories).

A good complex matrix for a proficiency test item is one which inherently incorporates the analyte within its chemical structure, for example fat in processed food or veterinary drug residues in tissues. An alternative type of test item is one that contains the food matrix plus a separate standard of the analyte, which then has to be mixed by the participant prior to analysis. This is less desirable (since it does not replicate a real world sample) but has its advantages for the PT provider in terms of preparation and, possibly, performance assessment.

When considering a proficiency test item, the PT provider needs to know something about the methods that will be applied in its analysis. This is essential information because the results might be method-dependent and most food PT schemes set the assigned values on the basis of the participants' results. Fortunately, many methods can be applied to give the same answer (within defined acceptance criteria) but, even so, there might be a common parameter which is essential to be followed. A hydrolysis step for total fat determination is one example (discussed later).

A desirable feature of a PT scheme [ISO/IEC 17043:2010] is to capture information on participants' methods. This serves three purposes. First, it enables the PT provider to accept results from any method (so not to dictate to the participant which method to use). Secondly, it permits the PT provider to assess the results against specific method parameters, if there is evidence in the distribution of method-dependency. Thirdly, it allows participants to compare their method with those of the other participants to verify that a particular method works (or does not work).

Method dependency has a number of sources but they can broadly be categorised into:

- initial sample preparation,
- sample extraction,
- sample clean-up,
- and determination.

The first of these is often neglected in a PT because the samples are all homogenised (standardised) before dispatch to participants. Nevertheless, instructions to participants should still define how the sample is to be prepared initially, which often includes appropriate storage conditions and to mix the sample thoroughly before taking the analytical test portion. The determination step is less likely to be a major source of method dependency, since by this time the matrix effects will have been mitigated by appropriate sample extraction and clean-up procedures. In fact, it is the proper application of extraction and clean-up that is often critical to the success of a PT using complex matrix test items.

There are few exceptions and, for the sake of completeness, it is worth mentioning one. A PT for food contact materials necessarily standardises across a defined method [EN 1186-1:2002] and food simulant. The reason for this is that there is no extraction or subsampling, so the primary source of variation is the conditions under which the test is undertaken (time and temperature). This needs to be standardised for all participants for the test to be comparable.

This paper relates the range of method responses to results recorded in some FAPAS PTs

and discusses the advantages of utilising real food matrices from the perspective of the participants and the provider.

Previous examples of matrix or method dependency

It is not the purpose of this paper to provide a wide-scoping review of PT schemes that have found matrix or method dependency. However, it is worth highlighting two dependencies previously published by FAPAS.

■ Pesticides spiking

Many laboratories rely on formulation to provide an estimate of extraction efficiency or recovery. Typically, this involves spiking a blank matrix subsample with a known quantity of analyte immediately prior to analysis. While this approach is acceptable for immediate quality control purposes, it fails for pesticide residues PT materials. A number of weeks or months may elapse between the preparation of the test material and the actual running of the PT. During this time, pesticides may bind to the matrix such that there is a significant difference between theoretical spike values and actual consensus assigned values (and homogeneity mean values). This difference was determined [Sykes *et al.*, 2013] to be systematically a factor of 1.22 of spike value to assigned value.

■ Vitamins

Vitamin analysis has notable method dependencies that might not be apparent from an internal validation exercise. It is only when an interlaboratory comparison is applied that such method dependencies become visible across a large population of laboratories. One such example is that of vitamin B2 specifically in liquid supplement [Sykes *et al.*, 2013]. Due to the differing solubility, in a liquid supplement vitamin B2 is mostly in the form of riboflavin 5'-phosphate, not riboflavin. This requires a two-stage extraction process, firstly applying the common acid hydrolysis and, secondly, an enzymatic hydrolysis to release the phosphate. There is a clear and quantifiable distinction between those laboratories applying only the acid hydrolysis step and those applying both acid and enzymatic hydrolysis steps.

Instructions to participants

■ Total fat hydrolysis, example

One advantage of a PT is that participants generally are free to use their own routine method and this is not specified by the PT provider. However, as noted above, there remain some specific method parameters that might influence the results and the outcome of the PT. If they are critical, these need to be taken into account at the outset of the PT. In nutritional components analysis, the total fat determination is dependent on the use of a hydrolysis step. Although participants are still free to use their own method, an advisory instruction might be included relating to the use of valid data in the calculation of the consensus assigned value. One such example comes from FAPAS PT 25131 [FAPAS Reports], total fat in fish paste. The instruction to participants states, 'Please state if you have used acid hydrolysis or not. Results for total fat will only be included in the assigned value calculation if the use of acid hydrolysis is reported.' Out of 55 results reported for total fat, 32 (58%) claimed that acid hydrolysis was used in the extraction procedure and the assigned value (robust mean, 16.89 g/100 g) was calculated using only those data. If we take the data which haven't applied acid hydrolysis,

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the median value is 16.12 g/100 g. The median value of the acid hydrolysis data is also 16.89 g/100 g, and this is significantly different (P-value 0.0021 at 95% confidence). The observed distributions for the two separate sets of data are both symmetrical but when combined show a distinct skew (Figures 1a and 1b).

FIGURE 1a / Histogram of PT z-scores for total fat in FAPAS PT 25131, assigned value set using only data reporting the use of acid hydrolysis.

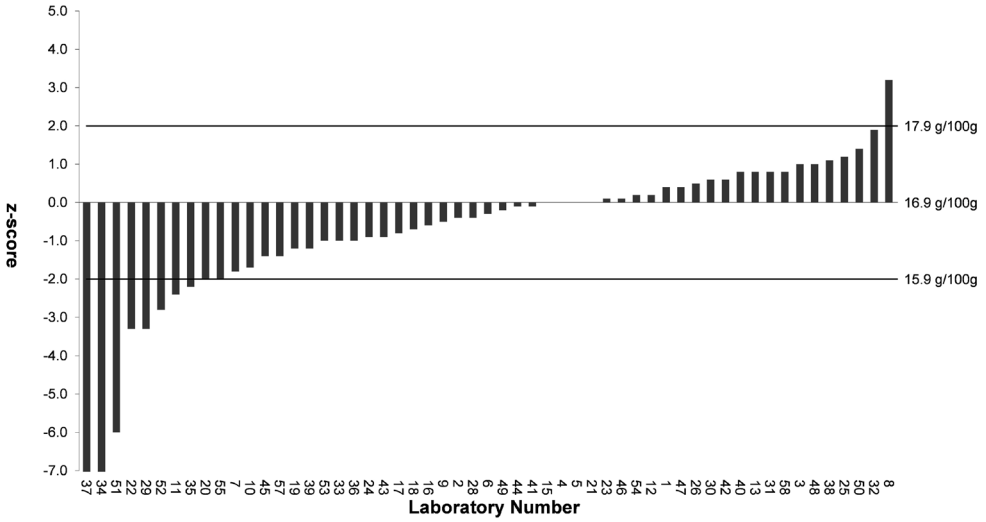
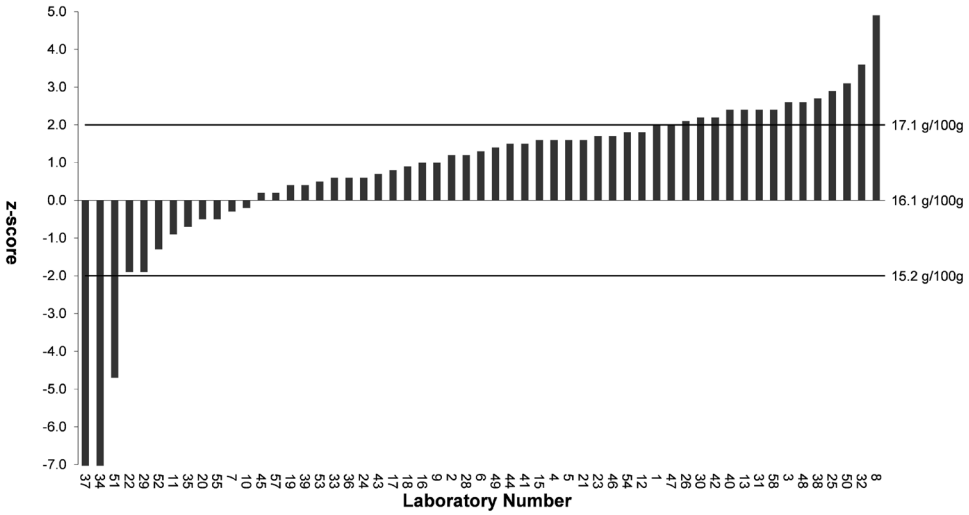


FIGURE 1b. Histogram of PT z-scores for total fat in FAPAS PT 25131, assigned value set using data not reporting the use of acid hydrolysis.



■ Food Microbiology, example

The FAPAS food microbiology PT samples are real food matrices incorporating the organisms and freeze-dried. Thus, the organisms are inherently part of the matrix which requires reconstituting before analysis. Instructions [FAPAS Reports] are provided to participants with regard to sample reconstitution and will typically include details as follows:

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Meat, egg, rice and green vegetable samples require rehydration in buffered peptone water (10 ml or 25 ml, depending on the test). The whole sample requires rehydration in the container provided and not be further subsampled. After mixing the buffered peptone water, the sample is left to stand for 30 min and is then ready for analysis, as a 10 or 25 g equivalent real sample. The use of buffered peptone water matches the initial diluent already used by the majority of laboratories.

Milk powder and animal feed samples require a larger volume of the laboratory's own diluent or pre-enrichment broth to give a reconstituted sample equivalent to 1/10 dilution of a real sample. The whole sample must be reconstituted in an appropriately large container or homogeniser bag, ensuring that the entire sample supplied has been transferred. A 30 min equilibration period is required.

Soft cheese and dry powder samples (black pepper, cocoa powder, flour, infant formula) are analysed without reconstitution. However, the whole sample must again be taken, not a subsample.

TABLE 1 / Target and background flora from PT FEPAS 202.

Proficiency test	Matrix	Target organism	Background flora
Enumeration of coliforms	Beef	*Escherichia coli*	*Micrococcus luteus* *Staphylococcus aureus* *Citrobacter freundii*
Enumeration of *Escherichia coli*	Beef	*Escherichia coli*	*Proteus vulgaris* *Micrococcus luteus* *Enterobacter aerogenes*
Enumeration of *Clostridium perfringens*	Milk powder	*clostridium perfringens*	*Bacillus coagulans* *Staphylococcus aureus* *Bacillus cereus*
Aerobic plate count and Enumeration of *Bacillus cereus*	Milk powder	*Bacillus cereus* *Lactobacillus plantarum* *Pseudomonas aeruginosa*	not applicable
Detection of *Salmonella* spp. test material A	Chicken	not applicable	*Citrobacter freundii* *Staphylococcus aureus* *Proteus vulgaris*
Detection of *Salmonella* spp. test material B	Chicken	*Salmonella Cerro*	*Citrobacter freundii* *Staphylococcus aureus* *Bacillus cereus*
Detection of *Campylobacter* spp. test material A	Chicken	*Campylobacter jejuni*	*Pseudomonas aeruginosa* *Bacillus cereus* *Proteus mirabilis*
Detection of *Campylobacter* spp. test material B	Chicken	not applicable	*Pseudomonas aeruginosa* *Bacillus cereus* *Proteus mirabilis*

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Real world samples arriving at the laboratory will not, generally, require this additional preparation procedure; they would be analysed as received. However, for the purposes of providing a stable and homogeneous test material, this slight difference to a real world sample is necessary. The matrix is real food, not a simulant, and when reconstituted is effectively indistinguishable from a homogenised wet food. Dry powder samples are, of course, identical to the matrix of a real world sample.

A further complication in the test material might be to deliberately introduce background organisms. A real world sample will inevitably contain other organisms in addition to the ones being targeted by the analysis, so why not replicate this in a PT sample? This is highly desirable because the correct target organisms need to be identified before enumeration. Even in a simpler detection test, a decision needs to be made by the participant on which of two test items is positive with regard to the organism being detected. A simple PT which is only positive with regard to the target organism and includes one completely blank sample (no organism at all) would not make for a very effective PT. An example of target and background organisms from a typical FAPAS food microbiology PT is provided in Table 1

Participants' reported methods

Proficiency tests differ from collaborative trials in that PT participants generally are free to use their own method of choice. This will be the method in routine use in their laboratory and, usually, the one that they hold accreditation for. Collaborative trials, by their very nature, are method-specific with carefully controlled and defined parameters. This might even dictate the exact chromatographic column to be used. The PT method of choice often will have been based on a previously published method, perhaps even an international standard. However, these are often modified by the laboratory to suit their particular circumstances, so rarely is a well-defined method followed to the letter. This in turn means that simply capturing a method reference is inadequate and that capturing specific method parameters is of more use to anyone with an interest in the PT report.

How much detail is required or useful to the reader is open to debate. Taking a simple moisture determination as an example reveals the potential complexity. The FAPAS PT 25131 (nutritional components in a fish paste test material) [FAPAS Reports] for moisture revealed that some 32 participants returned method details, out of 57 results. The method details are summarised in Table 2 (at end of article). (NB. returning method details is not a mandatory part of a PT, so will inevitably be incomplete.) The sample weight data demonstrates the scope for variation, with two laboratories taking between 1 and 2 g subsamples, 13 laboratories 2-5 g, 15 laboratories 5-10 g, and 2 laboratories 10-25 g. Of the 8 laboratories reporting that they follow an international standard method, 5 are in the 5-10 g category, 2 laboratories are in the 2-5 g category and one laboratory in the 10-25 g category. An order of magnitude range in subsample size for a PT sample is less likely to have an impact than a real world sample which is not proven to be homogenous. Nevertheless, if a PT sample is to replicate real world samples, methods applied in a PT ought to consider real world variables.

A further example highlights a difference within pesticide residues testing, which has seen a lot of development in recent years towards generic extraction methods. Methods of analysis for pesticide residues can broadly be categorised into those applying gas chromatographic separation (GC) or liquid chromatographic separation (LC). The extraction solvent of choice tends to match the chromatographic method, with acetonitrile being the common choice for LC and acetonitrile, acetone or ethyl acetate the solvent for GC (as a generalisation). This generalisation tends to apply regardless of the matrix and reflects the affinity of the pesticides being sought for GC or LC approaches.

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However, where cereals or their products are being analysed, either method benefits from pre-treatment of the sample with water. An amount of water added to the analytical sample will wet it sufficiently to maximise the extraction efficiency [SANCO, 2013]. As a consequence of this matrix effect, the PT should take this into account when participants report their results. The FAPAS method questions incorporate this additional information for cereals which isn't asked for in other matrix types.

There is insufficient data for a thorough examination of the effect of wetting in the PT data (the large majority of participants do report that they wet the sample first). A brief examination of the data for FAPAS PT 0995 (wheat flour containing deltamethrin, dimoxystrobin, fenamiphos sulfoxide, fenvalerate, isofenfos-methyl, tebuconazole) [FAPAS Reports] shows that there is a greater extraction efficiency from a wet sample by approximately 8% (data not shown).

Involuntary contamination

Many food analyses are concerned with detecting contamination of food items with components that should not be present. The associated PTs therefore attempt to replicate this by supplying a deliberately contaminated test sample. This works well for PTs in which there is one sample to quantitatively analyse the target contaminants. A complication can arise in detection PTs (qualitatively determining presence/absence) in which one sample is deliberately contaminated and a second one is not. The complication takes the form of unintended contamination of the base matrix, which is otherwise deemed to be 'blank'. This incurred contamination is to be avoided if possible but what better challenge is there to have not only a real food matrix but one that is genuinely contaminated? The challenge for the PT provider is to characterise that material, made more difficult where low level contamination is only detected by the most sensitive methods.

Two examples of unintentional contamination are in the fields of allergen and genetically modified (GM) materials testing. The problem of allergens contamination is well documented, certainly in the EU as far as labelling legislation [EU, 2012] is concerned. Many food producers demonstrate awareness of the problem with labelling that refers to the product being handled in a factory that also handles allergenic ingredients. It should come as no surprise, therefore, that sourcing uncontaminated ('blank') base materials for PT purposes is difficult.

■ GM materials

This problem of contaminated matrices is most starkly demonstrated in GM PT. Controls can be put in place in factories that handle food products but controls are less effective in the outside environment. This becomes evident when participants in a PT report the detection of a GM event that wasn't intended to be in the test sample.

A typical mixed flour GM PT will comprise a base matrix of organically-sourced soya, wheat and maize flours, into which have been spiked a number of GM flours (soya and/or maize). The GM flours are sourced directly from the original producer, so their provenance is known. Participants in the PT will be asked to detect and/or quantify genetic elements and specific GM events from a target list and their results reported together with their limit of detection (LOD) or limit of quantification.

Table 3a lists the summary of results from PT MU39 (April 2015) [FAPAS Reports]. The test material was spiked with GA21, MIR604 and MON89034 maize. No GM soya was used in the preparation of the test material. The qualitative results are typical of this type of PT, in that all participants have correctly detected GA21, MIR604 and MON89034 maize in the test material. The quantitative data from this PT are also sufficiently robust (not always the case for GM) that z-scores could also be issued to those participants that submitted quantitative data.

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The genetic elements p35S and tNOS were also detected by participants but this is not unexpected, since the spiked GM events would contain these elements.

TABLE 3a / Summary of qualitative results from PT GeMMA MU39, mixed flours.

Genetic element or event	Result	Agreement (%)
p35S	Positive	100
tNOS	Positive	100
Roundup Ready® soya	Positive	72
MON89788 soya	Negative	100
Bt176 maize	Negative	100
Bt11 maize	Negative	100
MON810 maize	Negative	100
GA21 maize	Positive	100
NK603 maize	Negative	100
TC1507 maize	Negative	100
MON863 maize	Negative	95
MIR604 maize	Positive	100
MON88017 maize	Negative	94
MON89034 maize	Positive	100

The result that stands out is that of Roundup Ready® soya (40-3-2) (RRS®), which 72% of participants have detected despite it not being deliberately used in the preparation of the test material. This is where the reported LOD by participants have in the past indicated a dependency of low LOD with positive detection. With more participants now reporting lower LODs (in the region of 0.01%), this dependency with positive detection is less clear. To illustrate this, Table 3b lists the first six laboratory results for RRS®. Two laboratories have reported an LOD of 0.01% and two have reported an LOD of about 0.05%. For both LOD levels, one laboratory has detected and one has not detected RRS®. These results highlight the low level endemic contamination of the environment with some GM materials but also the advantage of receiving a PT sample that truly reflects sampling circumstances.

TABLE 3b / First six laboratory results for RRS® (40-3-2) from PT GeMMA MU39, mixed flours.

Laboratory number	Result	LOD (%)
001	Detected	*
002	*	*
003	Detected	0.01
004	Detected	<0.045
005	Not detected	<0.05
006	Not detected	0.01

* no data entered

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■ Incurred chemical residues

The issue of GM contamination above tends to be limited to very low levels (probably <0.1%) for analytes which normally are being determined at about 1%. Higher level contamination (at levels normally being monitored) can be used to advantage in a PT. Several examples could be used to illustrate this, including veterinary drugs, mycotoxins and pesticides residues. The distinction between veterinary drug residues and the other chemical contaminants is that the former are largely banned substances in the human food chain whereas the others have tolerated limits. This in turn dictates how an incurred PT material can be produced to accurately mimic real matrices. Bulk materials for mycotoxins or pesticide residues can be screened for the possible presence of incurred residues whereas an animal has to be deliberately dosed to obtain an incurred veterinary drug.

In the case of veterinary drug residue test materials, a residue incurred through a dosing study serves two additional purposes for the PT. First, it will have metabolised to a certain degree and, second, it will be more tissue-bound than a drug spiked onto the matrix. Where the metabolites form part of the overall residue definition, this has obvious consequences for the overall drug residue determination. Most drug residue monitoring schemes will be determining tissue samples from animals that may have been dosed in uncontrolled circumstances. Hence, tissue-bound residues need to be determined using methods that apply appropriate extraction steps. An incurred drug residue PT sample will most closely resemble the type of samples actually received routinely by the participating laboratory.

An incurred drug residue PT adds complexity to the PT, not just in terms of the sample preparation but also with respect to the performance assessments. The PT provider can distinguish between results for the bound drug and results for the total (bound plus free) and highlight where improvements need to be made, especially for determining just the bound drug. Table 4 illustrates this with the summary results for PT 02240 [FAPAS Reports], the nitrofuran metabolite AMOZ in chicken muscle prepared from a dosing study. Forty participants reported results for the total AMOZ but just under half (19 participants) reported for the bound AMOZ. A further consequence of the bound AMOZ results was that the uncertainty of the consensus assigned value was high, reflecting the added difficulty of quantifying the bound drug.

TABLE 4 / FAPAS PT 02240 summary of results, nitrofuran metabolite AMOZ in chicken muscle.

Analyte	Assigned value µg/kg	Number of scores $\|z\| \leq 2$	Total number of scores	% $\|z\| \leq 2$
AMOZ (bound)	*0.99**	*10**	19	*53**
AMOZ (total)	2.16	35	40	88

* data issued for information only

Summary

A proficiency test sample ideally should resemble a real-life sample that would be received by the participating laboratory. This will not be an exact replica, since the PT test items must be homogeneous across all participants. Hence, a puree of lettuce, for example, will be the test sample, rather than a whole head of lettuce. However, there are further implications for the test samples in that method dependency will dictate critical parameters. The critical method parameters might be applied before the analysis begins (instructions to participants) or after

the PT closes (evaluation by the PT provider of data received). In reality, there is likely to be a combination of instructions to consider which allude to how the data will be assessed once all the participants' results are in. This is good practice for a PT provider in any case, as well as a requirement of the standard [ISO/IEC 17043:2010]. The provision of such instructions before the test begins means that they need not be followed exactly by participants but will indicate the effect on assessments if they are not followed.

In the case of the total fat hydrolysis and microbiology preparation instructions (detailed above), this serves dual purposes. It demonstrates how assessments will be affected and it provides equivalence to real food samples.

Capturing participants' method details is clearly desirable from the point of view of assessing results against critical method parameters. However, the example provided above additionally demonstrates the complexity of methods that exist in food testing laboratories. These complexities might have derived from necessity (availability of equipment or materials) or from the variety of food matrices that laboratories actually receive routinely. The PT samples which emulate the food matrices address the reality of the situation.

The production of a PT material and its characterisation is time consuming but essential to provide a realistic test item. A contaminated or incurred material (either intentional or not) can be utilised by the PT provider to advantageous effect. The examples above of GM and veterinary drug residues discuss this. This serves to highlight not just the performance of the participating laboratories but also to provide a true interlaboratory capability comparison.

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References

EN 1186-1:2002. Materials and articles in contact with foodstuffs – Plastics: Part 1: Guide to the selection of conditions and test materials for overall migration, 49 pp. https://standards.cen.eu

EU. 2012. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004, 2011R1169-EN-12.12.2011-000.001, 1-64. http://eur-lex.europa.eu/eli/reg/2011/1169/2011-12-12

FAPAS Reports, Fera Science Ltd. www.fapas.com

ISO/IEC 17043:2010. Conformity assessment – General requirements for proficiency testing, 39 pp. www.iso.org

SANCO. 2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. Document No. SANCO/12571/2013.

http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/qualcontrol_en.pdf

Sykes M, Croucher J, Smith RA. 2011. Proficiency testing has improved the quality of data of total vitamin B2 analysis in liquid dietary supplement, *Analytical and Bioanalytical Chemistry* 400:305-310.

Sykes M, Thompson M, Reynolds S. 2013. Pesticide residues in food-based proficiency test materials, spiking values vs. consensus assigned values, *Journal of Agricultural and Food Chemistry* 61:4205-4209.

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TABLES 2 / Participants' method information from FAPAS PT 25131, nutritional components in canned fish paste.

n = number of laboratories responding to this question

Moisture	n		n
Is the method used accredited?		**Apparatus used for constant weight**	
Yes	28	conventional oven	25
No	4	microwave oven	1
		vacuum oven	1
		oven forced air circulation	1
What is your method based on?		**Oven temperature (°C) for moisture determination**	
International Standard	8	<100	23
National Standard	13	≥100 - <105	21
In-house method	5	≥105 - <110	6
		≥110 - <150	2
Sample weight (g)		**Time heated for moisture determination (hours)**	
≥1 - <2	2	<1	1
≥2 - <5	13	≥1 - <5	13
≥5 - <10	15	≥5 - <10	11
≥10 - <25	2	≥10 - <24	5
		≥24 - <36	2
Moisture determination procedure		**Desiccator used to cool sample?**	
to constant weight	27	yes	31
4 h at 103°C	1	no	1

Ash	n		n
Is the method used accredited?		**Ash furnace temperature (°C)**	
Yes	26	≥500 - <550	11
No	3	≥550 - <600	17
		≥600 - <650	1
What is your method based on?		**Time in ash furnace (hours)**	
International Standard	7	<1	1
National Standard	12	≥1 - <5	6
Manufacturer/Kit instructions/Technical note	1	≥5 - <10	8
In-house method	5	≥10 - <15	5
		≥15 - <24	6
		≥24 - <48	2
Sample weight (g)			
≥1 - <2	4		
≥2 - <5	14		
≥5 - <10	10		
≥10 - <25	1		
Steps taken to avoid spattering			

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Ash	n		n
charred on hotplate before ashing	8		
crucible with lid	2		
pre-dried in oven	11		
pre-dried on steam bath	2		
slow temperature ramp	7		
charred on Bunsen burner	1		
none	1		

Total fat	n		n
Is the method used accredited?		**Pre-extraction stage?**	
Yes	27	Yes	7
No	4	No	24
What is your method based on?		**Pre-extraction time (hours)**	
International Standard	7	<1	6
National Standard	12	≥1 - <2	3
Paper Published in an international journal	1	≥2 - <3	3
Manufacturer/Kit instructions/Technical note	1	≥3 - <4	1
In-house method	8		
Sample weight (g)		**Extraction solvent components**	
≥1 - <2	7	diethyl ether	7
≥2 - <5	15	hexane	2
≥5 - <10	8	petroleum ether/spirit	20
≥10 - <25	1	chloroform: methanol (2:1)	1
		diethyl ether - petroleum ether	1
		none	1
Acid/alkaline hydrolysis used?		**Total extraction time (hours)**	
acid	20	<1	8
alkaline	1	≥1 - <2	8
none	10	≥2 - <5	9
		≥5 - <10	2
		>10	2
Total fat extraction method			
CEM	3		
Mojonnier	5		
SBR	1		
Soxhlet	9		
Soxtherm	1		
Tecator/Soxtec	4		
Weibull-Stoldt	3		
Acid hydrolysis	1		
acid hydrolysis +n-hexane extraction	1		
Folch et. al	1		
NMR	1		

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Nitrogen	n		n
Is the method used accredited?		**Digestion acid**	
Yes	18	hydrogen peroxide	2
No	1	sulphuric acid	26
What is your method based on?		**Catalyst**	
International Standard	6	copper (Cu)	17
National Standard	10	potassium (K)	8
Manufacturer/Kit instructions/Technical note	1	mercury (Hg)	2
In-house method	7	selenium (Se)	4
		titanium (Ti)	2
		3.5 g K2SO4 + 3.5 mg Se	1
		copper sulphate	1
		Kjeltab	1
Sample weight (g)			
<1	11		
≥1 - <2	15		
≥2 - <5	4		
Determination method			
Dumas	3		
Kjeldahl	18		
Kjeltec-Tecator System	7		
LECO	1		
Super Kjel	1		

Sodium	n		n
Is the method used accredited?		**Digestion**	
Yes	20	dissolve in acid	15
No	5	dissolve in water	2
		microwave digestion	6
What is your method based on?		**Pre-treatment**	
International Standard	7	caesium addition	3
National Standard	5	dissolution	2
Manufacturer/Kit instructions/Technical note	2	filtration	1
In-house method	4	heat	2
		heating with HCl	3
		internal standard addition	1
Sample weight (g)		**Determination**	
<1	5	cold vapour / hydride generation AAS	1
≥1 - <2	7	flame AAS	6
≥2 - <5	7	Flame Photometry	5
≥5 - <10	4	ICP	1
≥10 - <25	2	ICP-MS	4
		ICP-OES	5
		By calculation from sodium chloride	1

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Sodium	n		n
Sample preparation			
dry ashing	12		
wet ash	3		
acid digestion	1		
digestion	1		
digestion with nitric acid	1		
dissolved in water, filtration	1		
wet digest	1		

Chloride	n		n
Is the method used accredited?		**Time in ash furnace (hours)**	
Yes	17	≥1 - <5	1
No	9	≥5 - <10	2
		≥10 - <15	2
		≥15 - <24	2
What is your method based on?		**Steps taken to avoid spattering**	
International Standard	9	charred on hotplate before ashing	2
National Standard	7	crucible with lid	1
Manufacturer/Kit instructions/Technical note	1	pre-dried in oven	5
In-house method	3	slow temperature ramp	2
Sample weight (g)		**Sample preparation**	
≥1 - <2	4	cold water extraction	7
≥2 - <5	11	hot water extraction	4
≥5 - <10	7	nitric acid added	5
≥10 - <25	2	water added	6
		ammonia extraction	1
Was sample ashed?		**Determination**	
Yes	7	argentometric titration	7
No	17	chloride analyser	1
		Mohr	3
		potentiometric method	3
		thiocyanate titration	5
		Volhard	5
		ICP-MS	1
Ash furnace temperature (°C)			
<500	1		
≥500 - <550	4		
≥550 - <600	2		