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ENHANCING THE LABORATORY QUALITY ASSURANCE USING THE MYKIMIA PROFICIENCY TESTING PROVIDER (PTP) FOOD SCHEME DATA

(Meningkatkan Jaminan Kualiti Makmal dengan Menggunakan Data Ujian Kemahiran Skim Makanan MyKIMIA PTP)

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ABSTRACT

Accuracy, reliability, defensibility, and timeliness are all aspects of laboratory quality. The outcomes must be error-free in order to produce high accuracy data. Valid methods and calibrated equipment are referred to as reliability. To dispute the laboratory results, all possible errors were identified and the measurement uncertainties were estimated. The certificate of analysis or report must be issued timely. Laboratories in Malaysia was referred to the ISO/IEC 17025 for accreditation purpose. There are three essential elements defined as the backbone of the laboratory quality system. Validate analytical methods, accreditation involving third part auditing and participation in proficiency testing (PT). For this study, PT data is used as a tool to assess laboratory quality management systems' competency. Even if their performance is satisfactory according to the PT provider's evaluation criteria, participants in PT can interpret their results to detect inaccuracies and possibilities for continuous improvement. One of the PT schemes from MyKIMIA Proficiency Testing Provider (PTP) selected for the case study, the overall percentage of satisfactory for the Food Analysis Scheme (FODAS) 1-20 Benzoic acid (BA) and Sorbic acid (SA) in cordial was in the range from 82% to 88%. Statistic performance evaluation was based on a calculation of Z score for Test 1, Test 2 and Test 4. The laboratories shall recalculate the Z score based on the PTP assigned value and fit for purpose standard deviation proficiency assessment (SDPA). Zeta score can be calculated to check the plausibility of the laboratory's measurement uncertainty estimate. The outlier data received that cause the poor performance include human errors, random errors, and systematic errors. Investigation on the errors is consistently based on four main factors: manpower, method, material, and machinery (4M).

Keywords: quality assurance; proficiency testing; ISO 17025; Z score

ABSTRAK

Ketepatan, kebolehpercayaan, kebolehtahanan dan ketepatan masa adalah semua aspek kualiti makmal. Hasilnya mestilah bebas ralat untuk menghasilkan data berketepatan tinggi. Kaedah yang sah dan peralatan yang ditentukur dirujuk sebagai kebolehpercayaan. Untuk mempertikaikan keputusan makmal, semua kemungkinan ralat telah dikenal pasti dan ketidakpastian pengukuran dianggarkan. Sijil analisis atau laporan mesti dikeluarkan tepat pada masanya. Makmal di Malaysia telah dirujuk kepada ISO/IEC 17025 untuk tujuan akreditasi. Terdapat tiga elemen penting yang ditakrifkan sebagai tunjang utama sistem kualiti makmal. Mengesahkan kaedah analisis, akreditasi yang melibatkan pengauditan pihak ketiga dan penglibatan dalam ujian kecekapan (PT). Untuk kajian ini, data PT digunakan sebagai alat untuk menilai kecekapan sistem pengurusan kualiti makmal. Walaupun prestasi mereka memuaskan mengikut kriteria penilaian penyedia PT, peserta dalam PT boleh mentafsir keputusan mereka untuk mengesan ketidaktepatan dan kemungkinan penambahbaikan berterusan. Salah satu skim PT daripada MyKIMIA Proficiency Testing Provider (PTP) yang dipilih untuk kajian kes, peratusan keseluruhan yang memuaskan untuk FODAS 1-20 asid Benzoik (BA) dan asid Sorbik (SA) dalam kordial adalah dalam julat 82% hingga 88%.

Penilaian prestasi statistik adalah berdasarkan pengiraan skor Z untuk Ujian 1, Ujian 2 dan Ujian 4. Makmal hendaklah mengira semula skor Z berdasarkan nilai yang ditetapkan PTP dan sesuai untuk tujuan penilaian sisihan piawai kecekapan (SDPA). Skor Zeta boleh dikira untuk menyemak kebolehpercayaan anggaran ketidakpastian pengukuran makmal. Data terpencil yang wujud yang menyebabkan prestasi buruk termasuk ralat manusia, ralat rawak, dan ralat sistematik. Penelitian terhadap kesilapan secara konsisten biasanya berdasarkan empat faktor utama: tenaga kerja, kaedah, bahan dan jentera (4M).

Kata kunci: jaminan kualiti; ujian kecekapan; ISO 17025; skor Z

1. Introduction

Quality management systems are required to assure laboratory quality. It provides the management tool within the organization. There are three essential elements as described by researchers (Earnshaw *et al.* 2009). First is using validated analytical methods based on appropriate laboratory-developed methods or standard methods like American Public Health Association (APHA), Association of Official Analytical Chemists (AOAC) and others. Second is the accreditation involving third party auditing by a Accreditation of Bodies (AB). In Malaysia, the Department of Standards Malaysia is the national AB. Lastly, it involves the participation of Proficiency Testing (PT) in the certified PT provider scheme.

PT is a method of evaluating a laboratory's protocols and the analyst who follows those protocols in order to demonstrate competency. PT compares participant performance evaluations to pre-established criteria chosen by the PT provider, as defined by ISO/IEC 17025 and ISO/IEC 17043. ISO (International Organization for Standardization) is a non-profit organization that develops standards to assure product quality, safety, and efficiency. ISO certification verifies that a company's management system, manufacturing process, service, or documentation steps meet all the standards' requirements. Over the last few years, significant progress has been achieved in implementing ISO/IEC 17025 quality management system in testing laboratories. According to the Department of Standards Malaysia's annual report for the year 2019, they are 4.3 % of new accredited testing laboratories which comprises of 24 out of 553 laboratories. For the year 2020, it has increased to 4.4 % where there are now 25 new laboratories out of 562 laboratories.

In Malaysia, laboratory accreditation programmes are known as "Skim Akreditasi Makmal Malaysia" (SAMM) where established. SAMM policy is based on a specific title. One of the titles related to PT is SAMM Policy 4 (SP4), elaborated participation in PT activities. Participating in PT is a necessary component of meeting the SP4 criteria in the laboratory for ensuring the validity of the results. The laboratories required to monitor the PT plan assessed the results and compared them to those from other available and appropriate laboratories. Therefore, laboratories are urged to select PT from a reputable and experienced provider.

Since 2004, the Department of Chemistry Malaysia (DOC) has provided food preservative samples for comparison between laboratories for branch use. The scheme was opened to other government and private laboratories after two years in 2006. ISO/IEC 17043 accreditation was obtained in 2017 and DOC introduced as the first certified PT provider in Malaysia. For continuously improvement, MyKIMIA PTP was established in 2020 to conduct proficiency testing more efficiently in Malaysia and ASEAN country. Various scopes including chemistry, biology, halal and forensic science have been accredited. Therefore, each PT scheme has its own set of designs and processes to deal with PT materials and participating laboratories.

The benefits of participating in PT can be defined based on four M factors: Man, Method, Material and Machine (4M).

(1) Man refers to the competent and trained personnel, conducted adequate training to avoid human errors such as clerical errors from PT result submission. Apply the principle of Good Laboratory Practice (GLP) is practiced voluntarily to improve the laboratory work (Peric *et al*. 2014).
(2) Method refers to the validity of the adopted test method; either verification or validation records shall be made readily available, the participating laboratories' methods were declared and comparable. One of the typical causes of poor performance is primary sampling (Brookman & Mann 2021). Thus, the laboratory needs to make sure the first step is right to obtain a good performance.
(3) Material refers to the metrological traceability, laboratory shall be completed with certified reference material or reference material and maintain the validity of the measuring system such as analytical balance, micropipette and others. Calibration curve was affected by the expired standard or degraded standard solution. Standard solution prepared freshly was recommended.
(4) Machine refers to the calibrated and well-maintained equipment including measuring instruments, software and auxiliary apparatus that are required for correct performance of the analysis which can influence the results. It can achieve the measurement accuracy and measurement uncertainty required to provide a valid result.

Testing laboratories will gain an advantage in terms of improving their laboratory quality system when participating in PT. Thus, PT as a useful screening tool in quality management system where laboratories can be objective and measure their performance independently (Teresita *et al*. 2011). Through their participation, any inconsistencies or outliers in the results obtained could help identify areas needing improvement.

When the laboratories receive the PT reports, the laboratory shall evaluate and interpret their own results to avoid misinterpretations or over-interpretations. Good laboratory practice must be applied in every PT cycle and result must be compared continuously from previous completed schemes to measure the performance over time (Brookman & Mann 2021). Laboratory takes advantage of its performance evaluation to increase the quality assurance. Whatever findings from the PT scheme report or data indicate useful information throughout the analysis conducted.

2. Methods

This case study chose benzoic acid and sorbic acid as food preservative analytes. This scheme was named FODAS 1-20 Benzoic acid and Sorbic acid in cordial for 2020. The establishment of this scheme has been an annual event for MyKIMIA PTP since 2006 until now. It is commonly applied in many food analysis laboratories. PT is an important tool for laboratories involved in meeting the requirements and for accredited or non-accredited laboratories to improve their competency in the quality management system.

2.1. *Subscription of a PT Scheme*

The participating laboratory subscripted the scheme through Proficiency Testing Online System (PTOS) and payment made for the selected scheme.

2.2. *The procedures of PT material distribution*

PT material preparation procedures follows the PT design to ensure that stable material was used. The material used for this PT scheme is the real sample matrix, which is cordial. The concentration of benzoic acid was unmodified. Determination of the concentration was done and a known concentration of sorbic acid was spiked into it. Refering to the Malaysia Food Regulation 1985, Sixth Schedule (Regulation 20) Table I, both preservatives' legal allowance is less than 800 mg/kg under soft drink requiring dilution (level before dilution). A random number of each type of sample is chosen and analyzed to confirm that they are sufficiently homogeneous to be distributed for analysis. The PT material is considered homogeneous if the between sample standard deviation is less than 0.3 × standard deviation for proficiency assessment (SDPA).

MyKIMIA PTP announced through the PTOS news tab a week before the PT material is sent. The supplier will make sure that the packaging of the item is perfect to ensure the stability of the material and the characteristics of the material remain. PT materials send via national courier service. It is also assured that specific transportation constraints complied with, such as dangerous goods regulations or customs requirements. Online acknowledgement via PTOS as soon as possible when the laboratory receives the PT material. This procedure is to ensure that the PT material has reached the intended recipient in good condition.

2.3. *The protocol of result submission*

Results from participating laboratories are to be submitted through PTOS by the participants before the closing date. The closing date is usually set four weeks from the date of the PT material dispatch. Participants shall submit results on time. Any late result submission will not be accepted and entertained. Participants are fully responsible for the results submitted where no alterations are allowed after the closing date. They are responsible for any collusion or falsification of results as well.

2.4. *The performance evaluation*

The performance evaluation is based on the assigned value (AV), SDPA, and Uncertainty. AV means the value attributed to a particular property of a proficiency test material. The approach that MyKIMIA PTP uses to determine the assigned value is to obtain the consensus results from all participants and a robust analysis is used to identify the outliers. The protocol guideline based on ISO 13528 statistical methods was used by the provider who is fully responsible for evaluating the quantitative PT scheme. It is necessary to consider the often-abnormal distribution of the provided findings when determining the best estimate of the correct value (James 2015). The robust Mean as AV was determined after six iterations (procedures in Annex C Algorithm A of ISO 13528) and robust standard deviation was determined too with the condition that each value does not change at their third significant figure. Thus, the standard uncertainty of the assigned value is estimated as Equation (1), where s^* is the robust standard deviation and p is the number of participants.

$$u(Xpt) = 1.25 \times \frac{s^*}{\sqrt{p}} \quad (1)$$

The SDPA is used for the calculation of the Z-score. The method used to determine it may vary depending upon the scheme and test material. One of the approaches used by the provider is based on the Horwitz equation to obtain the percentage coefficient of variation (%

CV) as follows, where C is the concentration of the analyte in Equation (2). Then, SDPA, with the symbol of σ, is calculated as Equation (3).

$$\%\ \mathrm{CV} = 2(1 - 0.5\log C) \tag{2}$$

$$\sigma = \mathrm{CV} \times Xpt \tag{3}$$

Z scores primarily evaluated the performance of the participating laboratories. The formula of the scoring system for Z score is as follows,

$$Z = \frac{(xi - Xpt)}{\sigma} \tag{4}$$

xi is the participant's result, Xpt is the assigned value, and σ is SDPA. If the Z score is equal and less than 2, it implies a satisfactory result. If it is equal and more than 3, it implies unsatisfactory performance and generates an action signal. If the score is between 2 and 3, it indicates questionable performance and generates a warning signal.

Figure 1 shows the workflow summary for the participating laboratory from the scheme subscription until apply the root cauce analysis (RCA) if obtain the questionable or outlier result.

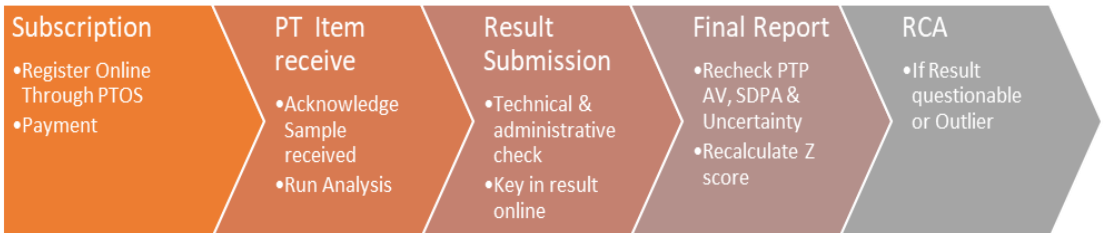


Figure 1: The proficiency testing summary workflow for the participating laboratory

3. Results and Discussion

There were 2 sets of PT items sent out to the participating laboratories: item A and item B. Each item contained 2 analytes: benzoic acid (BA) and sorbic acid (SA). There were 2 approaches for this scheme, which are quantitative and qualitative approach. For this study, only the quantitative approach will be discussed, there are Test 1, Test 2 and Test 4 as shown in Figure 2.

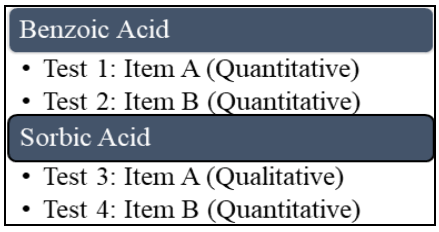


Figure 2: List of test

There were thirty-four participating laboratories involved in this case study. All of them submitted the Test 1 and Test 2 results and only thirty-three participating laboratories submitted the Test 4 results.

A total of thirty laboratories achieved a satisfactory Z-score for Test 1 as shown in Table 1. The percentage of the satisfaction obtained is 88%. A score of 80 percent of outcomes within permitted limits is considered satisfactory performance (Edson *et al.* 2007). Test 4 achieved 82% satisfactory results, 12% questionable and 6% unsatisfactory results, which means that the quality performance from the participating laboratories achieved a satisfactory level. For continuous improvement purposes, the laboratories can still improve their quality system based on PT data from the final report. Continue for the zeta score calculation for determine the validity value of the measurement uncertainty.

When the participating laboratories receive the PT report, the lab shall examine the following values: AV, the fitness of purpose of SDPA, standard Uncertainty of the AV and recalculate the Z score with the provider's AV and fit of purpose SPDA if the finding is questionable or is an outlier. Refer to Brookman and Mann (2021), the fit for the purpose of SDPA decided by the participating laboratory then compare and evaluate the relevance of the scheme.

Table 1: Distribution of Z-score from all participating laboratories

Z Score	No. of Participating Laboratories (% in parenthesis)		
	Test 1	Test 2	Test 4
$\|Z\|\leq 2$	30 (88%)	29 (85%)	27 (82%)
$2<\|Z\|<3$	2 (6%)	2 (6%)	4 (12%)
$\|Z\|\geq 3$	2 (6%)	3 (9 %)	2 (6%)
Total	34 (100%)	34 (100%)	33 (100%)

Table 2 shows an overview of the results based on arithmetic and robust analysis. The AV for Test 1 is 478 mg/kg and it was observed that it is 1.8 % higher than the arithmetic mean (469 mg/kg). A basic mean value (arithmetic mean) is insufficient because it is too readily impacted by extreme values (Earnshaw *et al.* 2009). For Test 2 and Test 4, show that the AV are higher than the arithmetic mean. As an example of Test 1 for the laboratory code 247 in Figure 3: Comparison among the AV from the PTP which use the Algorithm A of ISO 13528 robust mean = 478 mg/kg, arithmetic mean = 469 mg/kg and robust mean without alteration = 480 mg/kg, the Z score obtained are 2.27, 2.53, 2.20 respectively. Its prove that the AV not crucially if the performance for most of the participating laboratories achieve satisfactory result.

MyKIMIA PTP determines the standard deviation of the proficiency assessment by using the general model Horwitz equation to obtain the percentage of CV. As shown in Table 2, the SDPA for Test 4 is 13.0, which is 48.5% lower if compared with the robust standard deviation, 19.3. It means that the satisfactory acceptance percentage is higher compared with the SDPA=13. As an example, in Figure 4, laboratory code 247 has a provider Z-score of 2.71. The laboratory obtained satisfactory results if it used the robust standard deviation as their fit of purpose SDPA. The Z score was 1.8 after calculation using Equation (4), making it satisfactory.

Table 2: Overview of the results from arithmetic and robust analysis.

	Test 1	Test 2	Test 4
No. of results	34	34	33

Assigned Value, AV (mg/kg)	478	363	163
Expanded Uncertainty, 95% (k=2)	14	10	8
Arithmetic Mean, mg/kg	469	353	159
Robust Mean (mg/kg)	480	361	163
Arithmetic Std. Dev	91.0	68.6	33.9
Robust Std. Dev	32.0	25.0	19.3
SDPA	33.5	25.4	13.0
SDPA Set at CV (%)	7	7	8

No.	Laboratory Code Number	Benzoic Acid (mg/kg)					
		Item A	z-score	Expanded Uncertainty	Item B	z-score	Expanded Uncertainty
28	247	554	**2.27**	10	418	**2.16**	10
29	254	5.015	**-14.14**	0.784	3.515	**-14.15**	0.784
30	259	510	0.96	1.53	360	-0.12	1.53

Figure 3: Partial table 1a results and z-scores of FODAS 1-20 KIMIA PT final report No.: 2/2020

No.	Laboratory Code Number	Sorbic Acid (mg/kg)				
		Item A	Expanded Uncertainty	Item B	z-score	Expanded Uncertainty
26	236	0	-	137	-1.98	39.6
27	242	0	0.17X	170	0.55	0.17X
28	247	0	10	198	**2.71**	10
29	254	0	5.265	1.585	**-12.40**	5.265
30	259	0	1.87	150	-0.98	1.87

Figure 4: Partial table 1b results and z-scores of FODAS 1-20 KIMIA PT final report No.: 2/2020

The assessment of whether a laboratory's result is considered satisfactory, questionable, or unsatisfactory, therefore strongly depends on the value of the SDPA used in the denominator of the Z-score equation as show in Equestion (4). The bigger the SDPA the smaller the Z-score and, therefore, the better the apparent performance of the laboratory in the PT round. The laboratory has to answer whether the SDPA is chosen on a sound basis and whether it is fit for the laboratory's purpose.

Figure 5 shows the summary of the Z-score for Test 1, Test 2 and Test 4. It shows that laboratory code 254 contributed lower results and is far from the mean. During the analysis process, human error may cause these results and must be further investigated by the laboratory. In addition, findings must be documented for record and improvement purposes (Kuselman *et al.* 2014).

For laboratory code 254, the human error, in this case, did not follow Appendix 1 of FODAS 1-20 Kimia PT final report. The instruction in laboratory item no. 8 states that the expected range value for the BA is from 200 mg/kg to 800 mg/kg. The result submitted by the participating laboratory code 254 was 5.015 mg/kg and 3.515 mg/kg for Test 1 and Test 2, respectively refer to the Figure 3, and 1.585 mg/kg for Test 4. Based on a survey (Ellison & Hardcastle 2012), human error, specifically transcription errors, is one of the leading issues in testing. Another example of a human error is the calculation error. It covers improper formula entry, which is considered human error rather than a software error.

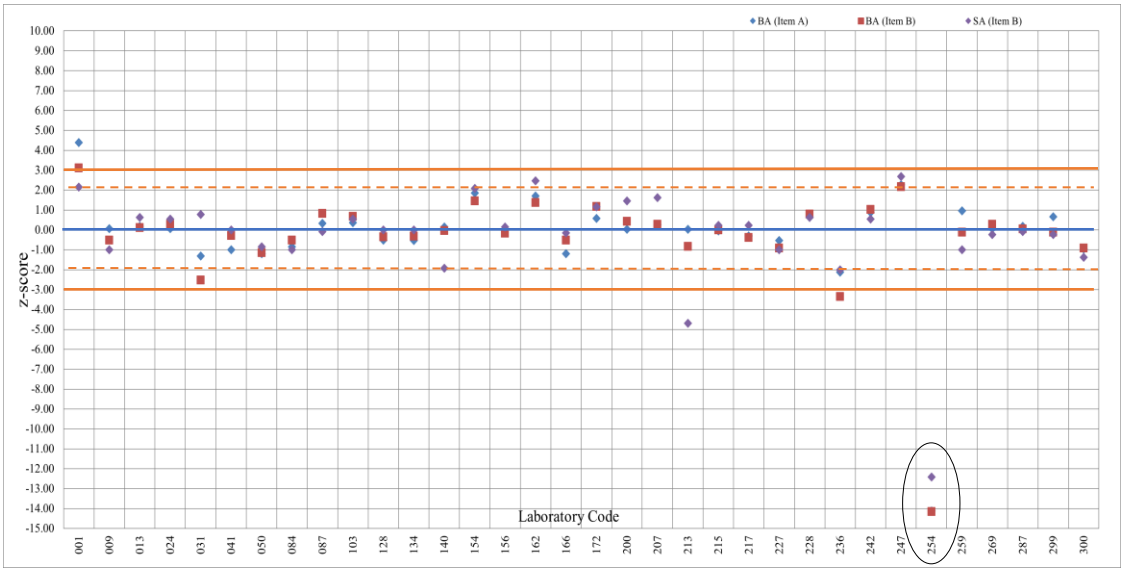
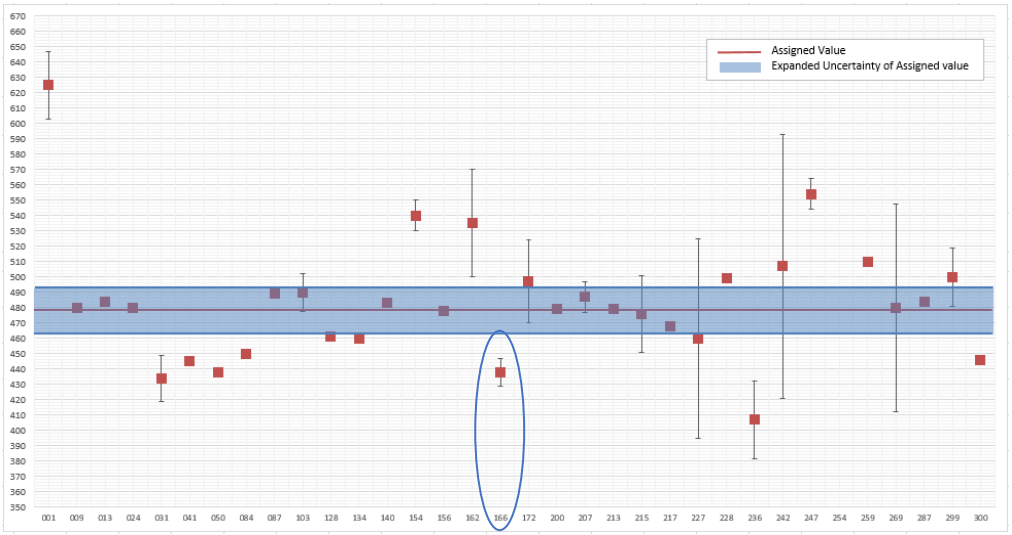


Figure 5: Summary z score Test 1, Test 2 and Test 4

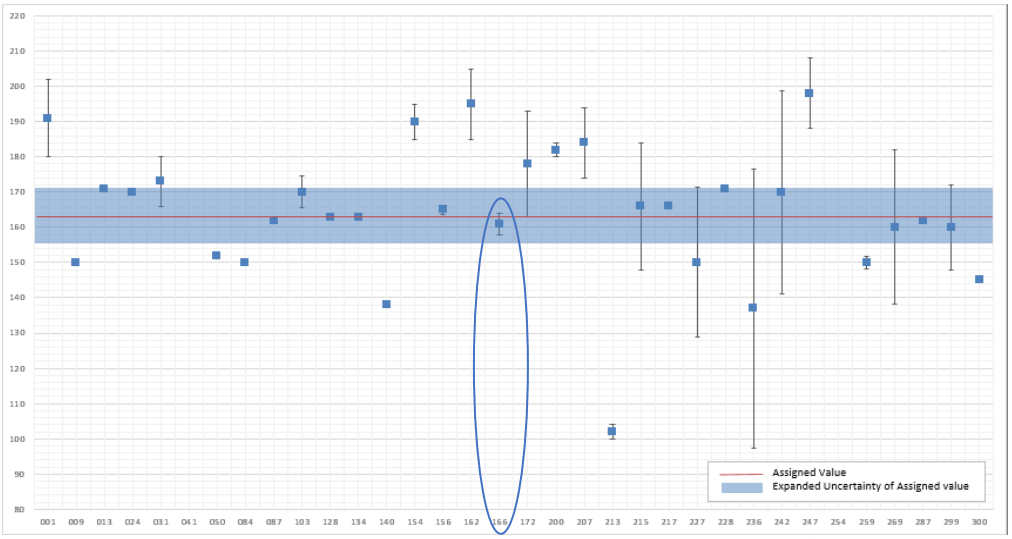
The basic concern for PT is accuracy because inaccuracy contains systematic and random errors. A laboratory can determine whether imprecision or bias is the reason for its inaccuracy. The random error could occur when the results fall on either side of the mean, which can be minimized by replicating measurements and sound techniques. Both humans and equipment can cause it. Schedule equipment maintenance is compulsory based on the ISO/IEC 17025, especially the analytical instruments. Referring to the method declaration of participating labs, some labs use the AOAC standard methods, which High Performance Liquid Chromatography (HPLC) is the analytical instrument for the BA and SA determination. Calibration needed and involved the standard solution, if the standard solution used is degraded, a calibration drift might occur. Therefore, validity storage and standard solution intermediate check is required to ensure accuracy. For the outliers in the results obtained, it is recommended that the laboratories investigate the possible bias in their method, e.g., increase the sample size, check on the extraction efficiency of the method, and increase the number of extraction cycles (Majors 2013). Systematic error can be avoided by applying good laboratory practice. For the random error, temperature variation and pipetting errors happen frequently. Laboratories need to conduct root cause analysis to investigate the questionable and unsatisfactory results. Based on a chemical analysis study in China, technical tasks involving calibration problems and the problem of managing reagents used is 37.4 % (Li *et al.* 2018). This indicates a relatively high percentage on this problem.

From the uncertainty view, the associated expanded uncertainties for both preservatives were not fully declared by the participating laboratories. None of the laboratories agreed well with the assigned value within its respective measurement uncertainty for the BA as shown in Figure 6 and only one laboratory (Laboratory code 166) for the sorbic acid, as shown in Figure 7 is within its respective measurement uncertainty. Estimating the measurement uncertainty is compulsory to fulfill the requirement of ISO/IEC17025 for all the accredited laboratories. The laboratory needs to identify the contribution of the uncertainty during the process, which starts from sampling and includes the significant error to estimate the measurement uncertainty (MU).



Note: One participant marked as an outlier (Lab code: 254) is not seen on the graph above due to a value outside of the y-axis range.

Figure 6: All participating laboratories' results, assigned value and associated expanded uncertainties (mg/kg) for Test 1 (BA item A)



Note: One participant marked as an outlier (Lab code: 254) is not seen on the graph above due to a value outside of the y-axis range.

Figure 7: All participating laboratories' results, assigned value and associated expanded uncertainties (mg/kg) for Test 4 (SA item B)

The zeta score, ζ helps to check the plausibility of the laboratory's measurement uncertainty, u estimate. It is calculated as follow:

$$\zeta = \frac{xi - Xpt}{\sqrt{u^2(xi) + u^2(Xpt)}} \tag{5}$$

The assessment criteria for satisfactory, questionable, and unsatisfactory results are the same as for the z-score. For example, Lab code 166 for Test 1 (BA item A) result = 438 mg/kg and the reported expanded uncertainty is 9 mg/kg. Thus, the result of ζ score is -4.80,

which shows unsatisfactory performance. The conclusion was that the lab's MU was underestimated and did not include all significant sources of uncertainty.

Most of the participating laboratories did not offer complete data of the methods used, making it difficult for the PT provider to evaluate outcomes with the methods used. There were, however, examples where many collaborating laboratories used the same technical parameters for an analyte and received a wide range of results. Outlier results can be caused by issues with analytical methodologies, poorly calibrated equipment, and under-optimized instrument operating parameters, as well as faulty sample extraction and interferences (Portugal *et al.* 2011). In addition, random errors might occur due to bubbles in reagents or reagent lines applied for the HPLC operator.

Based on Eurachem, when a laboratories' results in a PT scheme are not satisfactory, it allows management to investigate areas where future testing could be better. Additional operator training, adopting new or modified measuring techniques, data quality control improvements, equipment upgrades, calibration, or replacement are all viable options. In investigating the error consistently, the focus will be based on the four main categories: manpower, method, material, and machinery (4M) as mentioned earlier.

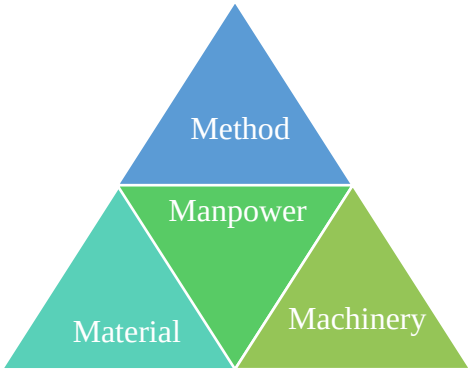


Figure 8: 4M factors

Stefanelli *et al*. (2013) provide the finding of calculating measurement uncertainty, MU using proficiency test data after applying two alternative methodologies, namely the top-down procedure and the bottom-up procedure. They used analytical data from five different editions of the PT on pesticide residues in olive oil (from 2007 to 2011). PT is not just a way to increase competency; it may also be used to improve accuracy in other ways.

4. Conclusion

BA and SA are the common analytes for food preservatives and are mainly analyzed using HPLC. Although the laboratories' performance in the program was higher than 80%, the non-performance laboratories need to look into total errors, including random and systematic errors. Identified them and estimated the uncertainties to include in the results. Application of zeta score is useful, such as the lab code 166 was underestimated uncertainty and the lab shall identified all the contribution of the uncertainty during the testing and recalculate the value of MU. Recalculate the AV based on the PTP protocol is important to make sure the value obtained by the laboratory is correct and it’s not affected the participating laboratories whereas the fit for purpose SDPA needed to decide by the laboratory to obtain the satisfactory results, such as lab code 247. No significant different for the AV but found different if SDPA chosen between robust standard deviation or others approaches.

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