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A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index

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Abstract

A new means for assessing analytical protocols relating to green analytical chemistry attributes has been developed. The new tool, called GAPI (Green Analytical Procedure Index), evaluates the green character of an entire analytical methodology, from sample collection to final determination, and was created using such tools as the National Environmental Methods Index (NEMI) or Analytical Eco-Scale to provide not only general but also qualitative information. In GAPI, a specific symbol with five pentagrams can be used to evaluate and quantify the environmental impact involved in each step of an analytical methodology, mainly from green through yellow to red depicting low, medium to high impact, respectively. The proposed tool was used to evaluate analytical procedures applied in the determination of biogenic amines in wine samples, and polycyclic aromatic hydrocarbon determination by EPA methods. GAPI tool not only provides an immediately perceptible perspective to the user/reader but also offers exhaustive information on evaluated procedures.

Keywords: green analytical chemistry, NEMI, Eco-Scale, GAPI

1. Introduction

There is no doubt analytical laboratories have an essential role to play in environmental protection through monitoring of pollutants in air, water or soil. On the other hand, analytical activities involve the use of many reagents and solvents, thus generating toxic residues. For these reasons, green analytical chemistry (GAC) was introduced in 2000 to reduce or to remove the side effects of analytical practices on operators and the environment [1]. This idea has attracted a great deal of interest among chemists, particularly those concerned with making laboratory practices in analytical chemistry environmentally friendly [1, 2]. As it is a great challenge to reach an acceptable compromise between increasing the quality of results and improving environmental friendliness of analytical methods, it is important to follow the guidelines and principles of green analytical chemistry (presented in Supplementary Materials, Figure 1SM) which have been introduced and provide a framework for GAC [2]. However, some problems with GAC exist one of the most pressing being the lack of well-established methods of “greenness” assessment [3]. The calculations that provide an answer as to whether an analytical procedure can be regarded as green should be performed utilizing tools that serve such assessment.

Analytical protocols are used to generate data in all fields of application. Due to the fact that these data are applied as a basis for decision making, their validity is extremely important [4]. Thus, consistent quality of obtained data by these analytical methods is obligatory. To ensure this quality, a tool called life-cycle assessment (LCA) can be used [5]. The transfer of the life cycle idea to analytical methods is illustrated in Figure 2 SM. Life cycle of an analytical method includes quality-by-design (QbD) approaches in method development, validation and operational use and may be considered as a link between method development and method validation [4,6]. In addition, the life-cycle approach can be broken down into three stages, namely method design, method qualification and continued method verification.

More recently, regulatory bodies have increased their awareness of life-cycle management for analytical methods and so the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), as well as the United States Pharmacopeial (USP) Forum discussed the registration of new guidelines that include life-cycle management of analytical methods. This alleviates the effort required in method performance verification and post-approval changes, as well as minimizing the risk of method-related out-of-specification results [4], and in turn helps reduce method costs during its life cycle.

One of the oldest tools used to assess the greenness of analytical procedures is the National Environmental Methods Index (NEMI) [7]. In this tool, analytical procedures are evaluated by using the greenness profile symbol divided into four fields (presented in Supplementary Materials, Figure 3SM). Although, NEMI as a greenness assessment tool has advantages (easy to read by potential users), it is also has some drawbacks. The NEMI symbol presents each threat as being below or above a certain value, therefore it cannot be regarded as being quantitative. Moreover, preparation of a symbol, especially if many, non-typical chemicals are used in the procedure, is time consuming because the presence of each compound has to be checked on one or more lists (EPA's TRI list, Resource Conservation and Recovery Acts lists, etc.)[8, 9].To improve the NEMI tool, Guardia et al. [10] proposed an additional pictogram to classify, using a color scale, three levels of evaluation of the greenness of a method. Another means for assessing chemical methods, including analytical procedures relative to green chemistry attributes, has been developed by Raynie et al. [11]. The assessment categorizes risk potential into five categories: health, safety, environmental, energy, and waste, based on toxicity, bioaccumulation, reactivity, waste generation, corrosivity, safety, energy consumption, and related factors (Figure 4SM). All of the criteria are presented on a pentagram and marked green, yellow or red depending on the impact to the environment.

A further approach is Analytical Eco-Scale [12]. This tool is based on penalty points subtracted from a base of 100. The higher the score, the greener and more economical the analytical procedure is. A summary of the procedural penalties is presented in Supplementary Materials, Table 1SM.

The Analytical Eco-Scale has several advantages but also many drawbacks including: no information about the structure of the hazards is obtained; lack of information on the causes of environmental impact of the analytical procedure, such as the use of solvents, other reagents, occupational hazard or generation of waste.

All of tools discussed assess the “greenness” of protocols and have their own advantages and disadvantages, and ideally the best solution is to apply them all to gain as much information as possible. However, in reality, such an approach is very time-consuming. Therefore, a new tool called GAPI (Green Analytical Procedure Index) is proposed which can evaluate the green character of an entire analytical methodology, from sample collection to final determination. Utilizing all the advantages of the aforementioned pictograms as well as Eco-Scale, GAPI affords not only general but also qualitative information. The discussion on how to use GAPI has been based on the assessment of analytical procedures used in the determination of biogenic amines in wine samples. Other examples presented here include the application of GAPI to analytical protocols used in the determination of organic compounds such polycyclic aromatic hydrocarbons (PAHs) in samples of specified matrix composition (e.g. water).

2. Application of the Green Analytical Procedure Index

The number of stages of each analytical procedure strongly depends on the sample properties, as well as the analytical method to be used for final determination. In general, the greater the number of steps involved, the less green the analytical methodology is which is obvious considering the increased energy use and larger volume of waste product. Obviously some stages are unavoidable, thus it is a vital to search for more environmentally benign analytical methodologies. In order to choose a methodology from the literature it is recommended to use multicriteria decision analysis (MCDA) [13] such as TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution) [14] or PROMETHEE (Preference Ranking Organization Method for Enrichment Evaluation) [15]. These tools allow comparison and ranking of up to a twelve analytical procedures according to their greenness. However, if only two or three procedures are to be compared for greenness of methodology, GAPI is an ideal tool as it presents and assesses whole analytical procedures, from sampling to final determination.

2.1. Stages of analytical procedure description

Sample collection is the first step of every analytical procedure [16]. The time delay between sampling and obtaining analytical results is crucial for the greenness of an analytical procedure. Therefore, the greenest approach to this step is *in-line* sampling, for example, using a portable XRF device. Other modes of sample collection are *on-line* and *at-line* sampling which represent a medium-green approach, and off-line sample collection which according to Tobiszewski et al. [16] should be avoided.

The second step of the analytical procedure that protects the samples from possible physical and chemical changes is preservation. This process is of high importance for the overall quality of the analysis, because it assures the integrity of the sample [12], however, it is not a green stage in any analytical procedure, as it requires energy and/or the use of chemicals. Three methods of preservation are known: chemical, physical and physico-chemical.

Transport of a sample is another stage that needs to be discussed when considering the GAC idea. For reasons of energy consumption (vehicle fuel), time used, emission pollutants from vehicles, and so on, it is not recommended and could be avoided by applying field analysis. Transport of the sample is directly related to sample storage, which also depends on laboratory capacity for performing analysis.

Sample preparation is at the heart of any analytical procedure when considering the green nature of a method because it usually comprises several operations in which non-green reagents, such as organic solvents or strong acids, are often required. The best solution is to use direct methods, where sample preparation is not required. Unfortunately, the most common methods of sample preparation are extraction, post-extraction or derivatization.

While the ideal situation would be to not have to extract a sample, isolation and enrichment processes are often required. The good news is that the field of extraction has been widely explored and great progress has been made to make this process more green. Considering GAC principles, the best option is to perform solvent-free extraction [17, 18], followed by reduction of solvents (applying shorter, smaller diameter chromatographic columns [19], using factors that enhances the extraction such as microwaves, pressure, vortex, etc. [20, 21]), or replacing organic solvents with ‘‘green solvents’’, e.g., supercritical fluids or subcritical water [19, 22, 23]. Without any doubt, it is also good to use microextraction techniques such as liquid-phase microextraction (LPME), hollow-fiber liquid phase microextraction (HF-LPME), and single-drop microextraction (SDME) or even better nano-extraction than perform it at macro scale [24].

The final step of the anaytical procedure is determination and quantification of analytes using analytical techniques. The ideal choice, as mentioned above, is to use direct techniques including NIR spectroscopy, electro-thermal atomic absorption spectrometry (ETAAS), laser ablation (with ICP-OES or ICP-MS), etc. Otherwise, indirect methods, needing sample preparation, have to be used. However, even here some improvements can be achieved, for instance, miniaturization of analytical methods [25] can bring many advantages with respect to GAC principles by reducing the use of toxic reagents and resulting in a decrease in waste generation [8, 26].

2.2. Green Analytical Procedure Index description

The GAPI tool applies a pictogram to classify the greenness of each stage of an analytical procedure, using a color scale, with three levels of evaluation for each stage. In GAPI, a specific symbol (Figure 1) with five pentagrams could be used to evaluate and quantify-from green through yellow to red-the low, medium and high environmental impact involved for each step of the methodology. Each field reflects a different aspect of the described analytical procedure and the field is filled green if certain requirements are met. The visual presentation of the assessment tool allows individual researchers to make their own value judgments about conflicting green criteria. Hence, this assessment tool is most valuable in comparing procedures. Green Analytical Procedure Index parameters description is presented in Table 1.

2.3. Assessment of selected analytical procedures applied to biogenic amine determination in wine samples

To present how the GAPI tool can be used, reported analytical procedures for biogenic amines (BAs) determination were evaluated. For comparison, to demonstrate how the GAPI could replace other tools used to assess the “greenness” of an analytical methodology, NEMI, tools reported by Raynie et al.[11], and Eco-Scale are also applied.

2.3.1. Evaluated analytical procedures

Four reported analytical procedures applied to biogenic amine determination in wine samples [27-29] are subjected to "greenness" assessment using the GAPI tool. Schematic representations of these procedures are presented in the Supplementary Material (Figure 5SM).

2.3.2. Assesssment of selected procedures using reported tools

Analytical procedures for the determination of biogenic amines were evaluated using known tools to assess "greenness" of the methodology used, namely: NEMI, tool reported by Raynie et al. [11], and Analytical Eco-Scale.

Figure 2 shows the NEMI pictograms for the evaluated procedures. Three pictograms are the same for Procedures 1, 2 and 3 which suggest that the green character of these methodologies is very similar. The worst analytical procedure appears to be Procedure 4 (methodology based on HPLC technique).

Green assessment profiles of evaluated procedures created by tool reported by Raynie et al. [11] are presented in Figure 3. This assessment profile presents more information, so differences between evaluated procedures are more visible. Procedures 1 and 2 have similar "green" character, and are placed in the middle, between Procedure 3 and Procedure 4. Procedure 4 appears to display the worst "green nature". Although, this tool presents more information than NEMI, the pictograms cannot be regarded as being semi-quantitative. To obtain more qualitative information, Analytical Eco-Scale penalty points (PPs) should be calculated for each procedure evaluated (Table 2).

Considering the PPs calculated for each procedure, it is obvious that Procedure 3 can be assigned as green (85 PPs). Procedure 2 also gives satisfactory results (69 PPs). Analytical Eco-Scale agrees with the conclusion that Procedure 4 is the worst in terms of green character. This shows that Analytical Eco-Scale is a good semi-quantitative tool for laboratory practice and educational purposes. However, even though this approach compares different parameters and steps in the analytical process, it still does not provide comprehensive information about the evaluated protocols. In order to supplement this information, GAPI has been introduced. GAPI provides information on the whole procedure, from sampling through transport, storage and sample preparation to final determination. Additionally, information on whether quantification is a part of the evaluated procedure is given. At a glance, a chemist familiar with the GAPI tool, can quickly choose the best analytical methodology for their purpose. The green assessment profile for the evaluated procedures, using the GAPI tool, is presented in Figure 4.

Considering the GAPI pictograms, a similar result to that obtained using Analytical Eco-Scale analysis can be deducted. The greenest nature represents analytical Procedure 3, followed by Procedure 2, Procedure 1 and finally, Procedure 4. However, GAPI pictograms present more information, it shows: whether an extraction process is necessary, and if so, it shows the type of extraction process and its scale; information on whether any additional process is necessary; and finally, whether the methodology can be used for qualification as well as for quantification. Considering the examples of analytical procedures in this paper, the first choice of methodology for biogenic amines determination in wine should be to use Procedure 3 (CE-DAD) which is the most green here. This methodology is a direct method, without an extraction process, and moreover uses small amounts non-toxic compounds. The amount of waste is also low. However, this procedure is only beneficial for qualification. In this case, when quantification is required, Procedure 2 should be applied.

2.4. Application of GAPI to analytical procedures for PAH determination

In this sub-section, examples of analytical methodologies, recommended by EPA and NEMI for their greenness, are evaluated using the GAPI tool. These examples present methods for determining PAHs in different kinds of water samples (environmental, drinking, waste water). The procedures covered are:

- Determination semi-volatile organic compounds by gas chromatography/mass spectrometry GC/MS): METHOD 8270C [30];
- determination of polycyclic aromatic hydrocarbons in drinking water by liquid-solid extraction and HPLC with coupled ultraviolet and fluorescence detection: METHOD 550.1 [31];
- Determination of PAHs in water by immunoassay: METHOD 70620 [32].

The evaluation of EPA and NEMI procedures for PAH determination in water (waste water, drinking water, environmental water) using GAPI is presented in Figure 6SM (and Table 2SM, Supplementary Material).

Taking into consideration all procedures for PAH determination in water samples recommended by the EPA, it is visible at first glance that Method 70620 can be considered greener that the other two methodologies. This is mainly because an extraction procedure is not necessary, and direct analysis is performed. It can also be seen that this method uses much less energy than other two, as well as using smaller aliquots of reagents, and so, generates smaller amounts of waste. The main critical point of Method 70620 is sample preservation and storage, which is worst that in Method 8270C and Method 550.1. It should be noted that Method 70620 is only a screening method and does not allow for quantitative analysis, therefore, Method 8270C should be chosen, but this method is far from being environmentally friendly. The poor score for GC-MS arises from the use of hazardous solvents and could be improved by replacing these chemicals with their green alternatives.

3. Summary

The growing interest in green analytical chemistry requires a fresh perspective on tools to assess analytical procedures. And although the GAC principles used to guide development are straightforward, they are not useful in assessing and comparing analytical processes for their health and environmental impact. Similar assessments may neglect one or more of the major concepts in the 12 Principles of GAC, for example, NEMI neglects energy considerations and is not a qualitative tool. GAPI, on the other hand, as a "green" assessment tool of analytical protocols, rates analytical methods against waste amount and type, chemical health and environmental hazard, and energy requirements. Reagents, procedures, and instrumentation are evaluated. Moreover, it presents information on the entire analytical procedure, from sampling, through sample preparation to final determination. The visual presentation of GAPI allows for an at-a-glance comparison of several methods and easy selection of the greenest method for a particular study. The proposed GAPI assessment can be a good semi-quantitative tool for laboratory practice and educational purposes. The GAPI tool not only provides an immediately perceptible perspective to the user/reader, but also gives exhaustive information on evaluated procedures. Due to the characteristics of this green assessment tool, GAPI is recommended to be applied in the search for new, greener methodologies, because it clearly and evidently indicates the weakest points in analytical procedures.

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Figures

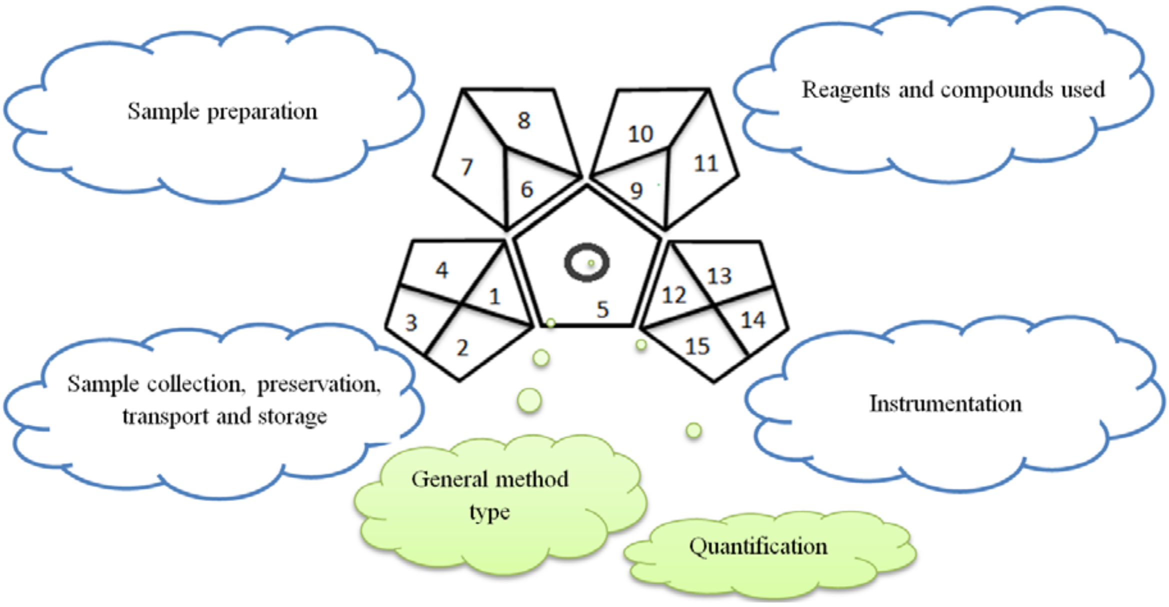

Figure 1. Green Analytical Procedure Index pictogram with description.

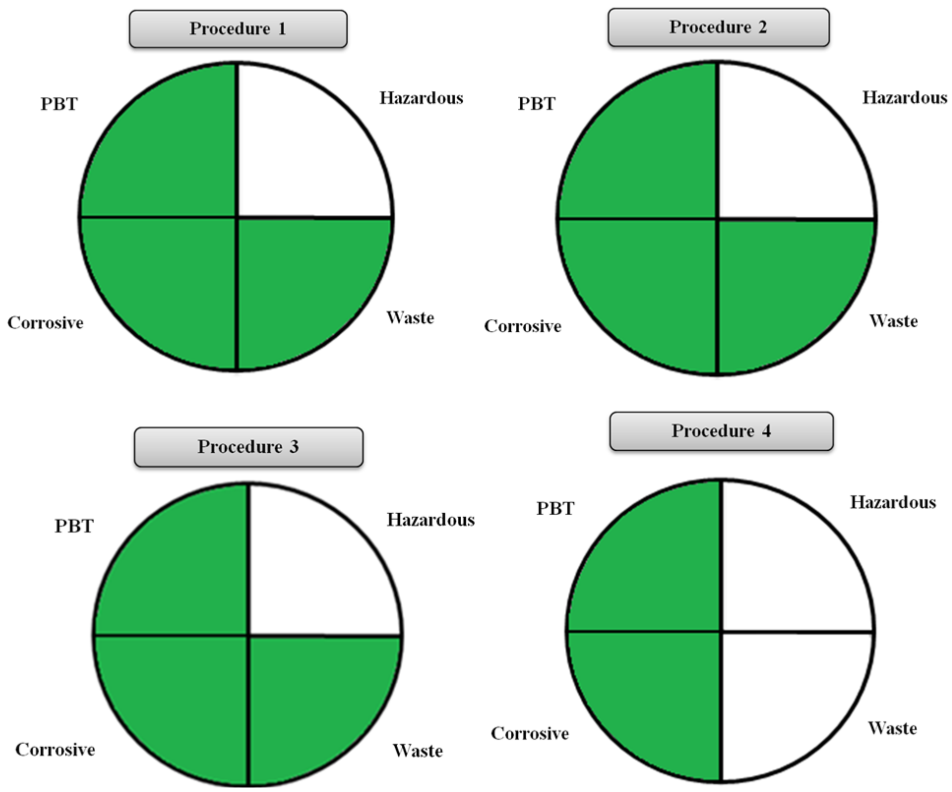

Figure 2. The NEMI pictograms for assessment of “greenness” of selected analytical procedures.

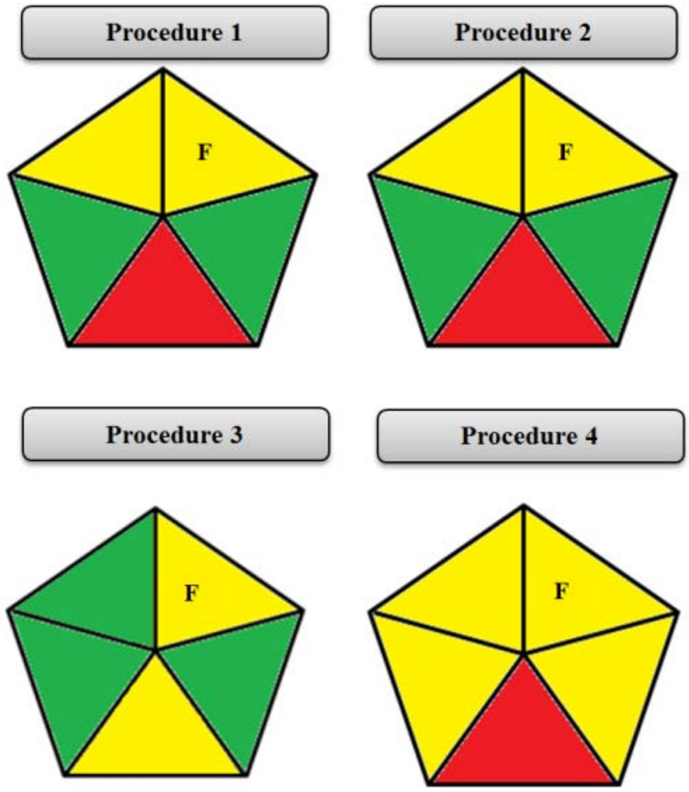

Figure 3. Assessment of the green profile of evaluated procedures for determining BAs in wine samples by using tool reported by Raynie et al. [11]

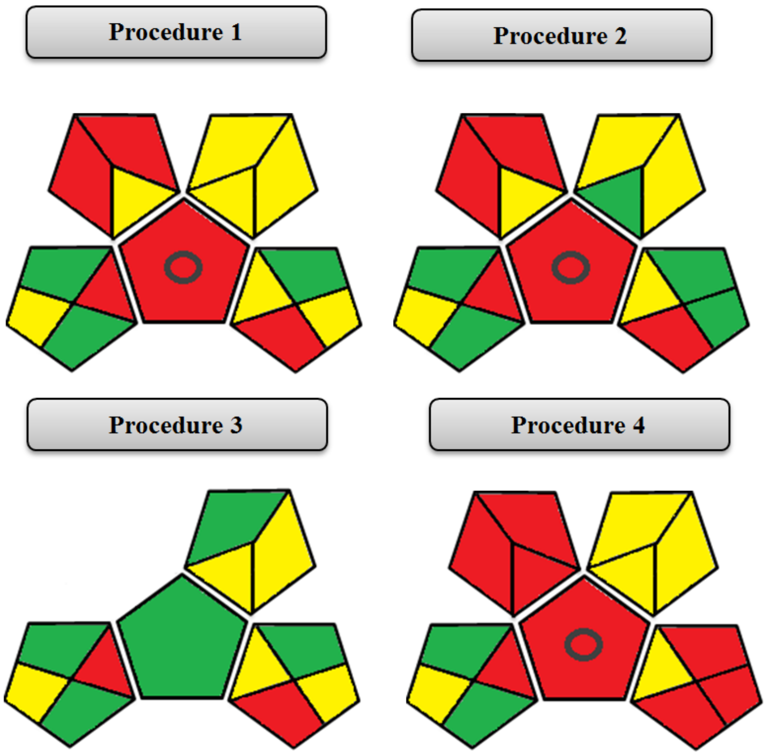

Figure 4. GAPI assessment of the green profile of the evaluated procedures for determining BAs in wine samples.

Tables

Table 1. Green Analytical Procedure Index parameters description.

Category	Green	Yellow	Red
Sample preparation			
Collection (1)	In-line	On-line or at-line	Off-line
Preservation (2)	None	Chemical or physical	Physico-chemical
Transport (3)	None	Required	-
Storage (4)	None	Under normal conditions	Under special conditions
Type of method: direct or indirect (5)	No sample preparation	Simple procedures, eg. filtration, decantation	Extraction required
Scale of extraction (6)	Nano-extraction	Micro-extraction	Macro-extraction
Solvents/reagents used (7)	Solvent-free methods	Green solvents/reagents used	Non-green solvents/reagents used
Additional treatments (8)	None	Simple treatments (clean up, solvent removal, etc.)	Advanced treatments (derivatization, mineralization, etc.)
Reagent and solvents			
Amount (9)	<10 mL (<10 g)	10-100 mL (10-100 g)	>100 mL (>100 g)
Health hazard (10)	Slightly toxic, slight irritant; NFPA health hazard score =0 or 1.	Moderately toxic; could cause temporary incapacitation; NFPA = 2 or 3.	Serious injury on short-term exposure; known or suspected small animal carcinogen; NFPA = 4.
Safety hazard (11)	Highest NFPA flammability or instability score of 0 or 1. No special hazards.	Highest NFPA flammability or instability score of2 or 3, or a special hazard is used.	Highest NFPA flammability or instability score of 4.
Instrumentation			
Energy (12)	≤0.1 kWh per sample	≤1.5 kWh per sample	>1.5 kWh per sample
Occupational hazard (13)	Hermetic sealing of analytical process	-	Emission of vapours to the atmosphere
Waste (14)	<1 mL (<1 g)	1-10 mL (1-10 g)	>10 mL (<10 g)
Waste treatment (15)	Recycling	Degradation, passivation	No treatment
ADITIONAL MARK: QUANTIFICATION			
Circle in the middle of GAPI: *Procedure for qualification and quantification*		No circle in the middle of GAPI: *Procedure only for qualification*	
NFPA: National Fire Protection Association			

Table 2. The penalty points (PPs) for evaluated procedures determining BAs in wine samples.

PROCEDURE 1		**PROCEDURE 2**	
Reagents	**PPs**	**Reagents**	**PPs**
NaOH (150 µL)	1	Pyridine	1
Phosphate buffer 0.5 M	0	Internal standard	4
Internal standard	4	HCl (55 µL)	3
HCl (2 mL)	4	Chloroform (400 µL)	2
Acetonitrile (1 mL)	4	Isobutyl chloroformate (110 µL)	8
Toluene (350 µL)	6	MeOH (215 µL)	6
Isobutyl chloroformate (110 µL)	8		
MeOH (75 µL)	6		
	Σ 37		Σ 26
Instruments	**PPs**	**Instruments**	**PPs**
Transport	1	Transport	1
GC-MS	2	GC-MS	2
Occupational hazard	0	Occupational hazard	0
Waste	3	Waste	1
	Σ 6		Σ 4
Total PPs: 43		**Total PPs:** 31	
Score: 57		**Score:** 69	
PROCEDURE 3		**PROCEDURE 4**	
Reagents	**PPs**	**Reagents**	**PPs**
Methanol	6	Polyvinylpyrrolidine 0.5 g	0
Internal standard	4	Internal standard	8
Borate buffer (20 mM)	0	HCl (10 mL)	6
Sodium dodecyl sulfate	0	Na_2CO_3 (0.5 mL)	0
		Dansyl chloride (1.6 mL)	8
		Acetone (> 10 mL)	8
		Acetonitrile (> 5 mL)	8
		Water	0
	Σ 10		Σ 38
Instruments	**PPs**	**Instruments**	**PPs**
Transport	1	Transport	1
CE-DAD	2	HPLC-fluorimetric detection	2
Waste	2	Occupational hazard	0
		SPE	2
		Hot-plate	2
		Drying instrument	2
		Waste	5
	Σ 5		Σ 14
Total PPs: 15		**Total PPs:** 52	
Score: 85		**Score:** 48	