Abstract. Intoxications, as a rule, are related to the medical-legal area; some toxic agents occupy prominent place as the main ones responsible for the occurrence of deaths. In legal medicine, both human and animal, the major challenge faced is the elucidation of the cause of death and the time of death when corpses are found, indicating possible exposure to toxic agents, which are intentionally added most often, in order to cause irreversible damage to the victim. In this context the methods of toxicological analysis involving poisoning are widely studied and disseminated, there are numerous literature reviews on analytical validation processes in the most diverse areas, but reviews of forensic literature are scarce and outdated. Wrong or even unreliable analytical reports can lead to misleading conclusions, culminating in irreparable financial, academic or judicial damages. Since the validation processes are essential in laboratory routines and that forensic analytical methods applied in the legal area are important for the elucidation of xenobiotic intoxication tables, the purpose of this review is to discuss validation processes with a focus on analysis forensic, since the results from this type of analysis must be irrefutable and unequivocal and an error of result...
can lead to irreparable damage to the victim. In this review it is clear that there is no harmonized standardization of a concept of analytical validation, and both national and international regulations often fail to come to terms with merit figures that are paramount in an analytical validation process.

**Keywords:** Validation; Analytical methodology; Forensic analysis; Intoxications.

**Resumo.** As intoxicações, via de regra, estão relacionadas com a área médico-legal; alguns agentes tóxicos ocupam lugar de destaque como os principais responsáveis pela ocorrência de óbitos. Em medicina legal, tanto humana quanto animal, o grande desafio enfrentado é a elucidação da causa mortis e do tempo da ocorrência da morte quando os cadáveres são encontrados, indicando possível exposição aos agentes tóxicos, os quais são adicionados intencionalmente, na maior parte das vezes, com a finalidade de causar danos irreversíveis à vítima. Nesse contexto os métodos de análises toxicológicas envolvendo intoxicações são amplamente estudados e divulgados, inúmeras são as revisões de literatura sobre processos de validação analítica nas mais diversas áreas, porem revisões de literatura a respeito da área forense são escassas e desatualizadas. Os laudos analíticos errados ou mesmo não confiáveis podem levar a conclusões equivocadas, culminando, assim, em prejuízos financeiros, acadêmicos ou judiciais irreparáveis. Uma vez que os processos de validação são essenciais nas rotinas laboratoriais e que métodos analíticos com finalidade forense aplicados na área legal são importantes para a elucidação de quadros de intoxicação por xenobióticos, o objetivo dessa revisão é discutir os processos de validação com o enfoque em análises forenses, uma vez que os resultados provenientes desse tipo de análise devem ser irrefutáveis e inequívocos e um erro de resultado pode levar a danos irreparáveis a vítima. Nesta revisão fica claro que não existe uma padronização harmônica de um conceito de validação analítica, e as regulamentações tanto nacionais quanto internacionais muitas vezes não entram em um acordo das figuras de mérito que são primordiais em um processo de validação analítica.

**Palavras Chave:** Validação; Metodologia analítica; Análise forense; Intoxicações.

### 1. Introduction

The increasing demand for laboratory tests and the need to guarantee high quality and reliability indexes in the medical equipment obtained, as well as comparability and traceability, are being increasingly recognized and demanded in the most diverse spheres. The credibility of a chemical analysis is guaranteed by the care with which the analyst surrounds himself, in order to generate a result that expresses the closest possible measure of the real value. 

A. Fukushima et al.
Incorrect or even unreliable analytical results can lead to misleading conclusions, culminating in irreparable financial, academic or judicial damages. Therefore, for an analytical method to be reliable, and allow generating interpretable information about the sample, it must be submitted to an evaluation called validation or verification\textsuperscript{1-4}. Table 1 shows some concepts of what is validation.

| Reference | Concept |
|-----------|---------|
| USP, 2014\textsuperscript{5} | "Validation of methods ensures their credibility during routine use, and is sometimes referred to as the process that provides documented evidence that the method accomplishes what it is meant to do." |
| BRASIL, 2012\textsuperscript{6} | "The validation is the confirmation by test and provision of objective evidence that the specific requirements for a particular intended use are met; may be subdivided into partial validation which is the implementation of part of the validation tests as a result of modification in the validated bioanalytical method, aiming to demonstrate the maintenance of the performance and reliability of the method and the total validation that is the accomplishment of all the validation tests of a bioanalytical method." |
| Eurachem Working Group, 2012\textsuperscript{7} | "Validation is the process of defining an analytical experience and confirming that the method under investigation has a performance capability consistent with what the application requires" |
| MAPA, 2011\textsuperscript{8} | "The validation of a determined analytical procedure is aimed at demonstrating that it is adequate to the proposed objectives, that is, that the performance parameters evaluated meet the recommended acceptance criteria. This is an experimental and fully documented study. Validation aims to guarantee the metrological quality of analytical results, giving them traceability, comparability and reliability for decision-making." |
| WHO, 2016\textsuperscript{9} | The systematic validation of an analytical procedure is to demonstrate that it is under the conditions in which it should be applied |
| ISO/TEC 17025, 2005\textsuperscript{10} | "Confirmation by tests and presentation of objective evidence that certain requirements are fulfilled for a given intentional use" |
| BRASIL, 2003\textsuperscript{11} | The validation must guarantee, through experimental studies, that the method meets the requirements of the analytical applications, ensuring the reliability of the results." |

Brazilian regulators, as well as other countries, currently require laboratories to perform validation of their analytical methods according to their official documents that establish the merit figures required in the process\textsuperscript{2}. 

A. Fukushima et al.
Briefly, the validation of a method is one of the basic elements in quality systems, integrating the programs of good laboratory practice. The planning of a validation process begins with analytical development and can be transferred through a co-validation process\textsuperscript{1,2}.

The validation objectives to ensure that the method used during the analysis is adequate in the identification and quantification of the analytical goal, not being static, depending on the analytical challenge to be solved\textsuperscript{1,2}. It applies in several areas such as chemical and biological analysis, questionnaires, observations, or in any situation that can quantify a parameter.

In forensic toxicological analysis, the Society of Forensic Toxicologists has established the necessary merit figures to perform the validation of quantitative analytical methods. They are: specificity/selectivity, limit of detection (LD), precision (intra-laboratories-repeatability and/or inter-laboratories-reproducibility), linearity, application interval, accuracy, recovery, uncertainty of the measurement, stability and some parameters such as limit of quantification and robustness can also be used in qualitative and quantitative analysis. This recommendation is also used by other national and foreign regulators\textsuperscript{4,6,11,12}.

Chromatographic separation techniques, such as gas chromatography, high performance liquid chromatography and capillary electrophoresis, are widely used in laboratory chemical analysis, especially those related to toxicology, as they have the capacity to provide qualitative data (identifying the investigated agent), in several types of matrices, whether they come from the environment, food, pharmaceuticals, biological matrices, among others\textsuperscript{2}.

A well-established and documented validation process ensures objective and unambiguous evidence that methods and systems are appropriate for the intended use, in particular for regulatory agencies and the judiciary, providing a reliable enough result to make a decision.

Since the validation processes are essential in the laboratory routines and the forensic analytical methods applied in the legal area are important for the elucidation of xenobiotic intoxication, the purpose of this review is to discuss the validation processes with the analytical approach since the results from this type of analysis must be irrefutable and unequivocal and an error of result can lead to irreparable damage to the victim.
2. Method
For the accomplishment of this article a bibliographical survey was made in guidelines, original journals and revision articles obtained in the databases Scielo, PubMed, ScienceDirect and national and international regulations between the years of 1994 and 2017. The terms sought are “forensic validation toxicology methods”, these terms were searched in Portuguese and English. A total of 2256 papers were found related to the topic, of which 17 guidelines and / or resolutions, 15 original journals and 6 review articles were selected, which presented the terms selected as the main objective of the study. Duplicate articles were excluded.

3. Presentation of the national and international scenario
There are several international and national guidelines, as well as laws and regulations that suggest the safety parameters or merit figures that should be adopted for chemical analysis. Table 2 briefly presents the merit figures that should be evaluated in chemical analysis, according to the guidelines suggested by some international and national regulatory agencies.

The following are the different safety parameters and merit figures used in chemical analysis.

4. Linearity
Linearity is defined as the ability of a method to generate results directly proportional to the concentration of the analyte, which must be studied at an appropriate interval. This makes it possible to establish the relation between a "measure" dependent variable as a function of an independent variable "concentration".\textsuperscript{1,17,18}

In order to experimentally evaluate linearity, it is necessary to construct graphs using the least squares method (linear regression), which in most cases are called calibration curves. Thompson, et al. (2002)\textsuperscript{19} recommends the use of the term linear graph, linear range or dynamic range. The Agency for Health Surveillance (ANVISA), through the Board of Directors' Resolutions\textsuperscript{6} which deal with validation of analytical and bioanalytic methods in Brazil, calibration curve denomination, as well as most of the other international agencies\textsuperscript{1,17,18}.
Table 2. Merit figures that are evaluated in chemical analysis, according to the guidelines suggested by some international and national regulatory agencies

| Reference | Linearity | Precision | Accuracy | Selectivity | ME | Recovery | LLQ | HLQ | DL | QL | HO | Stability | Robustness | MU |
|-----------|-----------|-----------|----------|-------------|----|----------|-----|-----|----|----|----|-----------|------------|----|
| USP, 2014\(^b\) | X | X | X | X | - | - | - | X | X | - | - | - | - | - |
| BRASIL, 2012\(^a\) | X | X | X | X | X | X | X | X | - | - | X | X | - | - |
| Eurachem Working Group, 2012\(^7\) | X | X | X | X | X | X | - | - | - | X | X | X | X | - |
| MAPA, 2011\(^8\) | X | X | X | X | X | X | - | - | - | X | X | - | - | - |
| WHO, 2016\(^9\) | X | X | X | X | - | - | X | X | - | - | - | - | - | - |
| UNODC, 2011\(^13\) | X | X | X | - | X | X | X | X | - | - | X | X | - | - |
| GTFCh, 2009\(^14\) | X | X | X | X | X | X | - | - | X | X | - | X | - | X |
| SOFT, 2006\(^3\) | X | X | X | X | X | X | - | X | X | X | X | - | - | - |
| ISO/IEC 17025, 2005\(^10\) | X | X | X | X | X | X | - | - | X | X | X | - | - | - |
| LANÇÃS, 2004\(^15\) | X | X | X | - | X | X | X | X | X | - | X | X | - | - |
| BRASIL, 2003\(^11\) | X | X | X | X | - | - | - | X | X | - | - | X | - | - |
| DIERAUF, 1994\(^16\) | X | X | X | - | - | - | - | - | - | - | - | - | - | X |

Legend: ME – matrix effect, LLQ – lower limit of quantification, HLQ – high limit of quantification, DL – detection limit, QL – quantification limit, HO – homogeneity, MU – measurement uncertainty
The calibration curve is the method of quantification often used in the
determination of the concentration of a given analyte\textsuperscript{1,20}. The construction of the
 calibration curve, in most cases, is performed through the measurement of the signal
emitted by the equipment used, depending on the masses or concentrations of the
analyzed analyte in the matrix\textsuperscript{21}.

The calibration curve obtained by the linear regression method is expressed
by the equation of a line (Eq.1):

\[
y = \alpha x + b \\
r = 1 \\
r^2 = 1,
\]

(1)

where \( y \) is a dependent variable (method response), \( x \) is an independent variable
(measured), \( \alpha \) is the angular coefficient (slope of the line, indicates the sensitivity of
the method), \( b \) is the linear coefficient (expressed as the intersection of the line with
the \( x \) and \( y \) axes), \( r \) is the correlation coefficient (indicates linearity), \( r^2 \) is the
determination coefficient (indicates linearity).

The mathematical expression of this straight line is presented by the angular,
linear, correlation and determination coefficients normally obtained by linear
regression and described in the form of the straight equation\textsuperscript{21}.

A line can be constructed from only one point in the space\textsuperscript{2}, but in the
analytical validation processes, the number of points accepted in the graph to trace
this line varies between five\textsuperscript{19} and six points\textsuperscript{1,6}, which should not include the zero
point in the curve, due to the possible associated errors. The construction of this line,
in practice, occurs by the serial analysis of calibrators, that is, solutions of different
concentrations that contemplate the range of concentration of interest in the work\textsuperscript{2},
therefore varies according to the purpose of the analysis.

According to Chasin et al.\textsuperscript{1,18}, for analysis of biological material involving
poisonings, the recommended concentration range should range from the limit of
quantification (LQ) to 1,000 \( \mu \text{g/mL} \); on the other hand, for residue analysis the
concentration range of interest can only cover an order of magnitude.

If the concentration range establishes linearity with a coefficient of
determination (\( r^2 \)) of up to 0.980 for biological matrix and of 0.999 for pharmaceutical
products, this can be called the dynamic range\textsuperscript{2,6,15,17}. Thus, the verification of the
Another measure that is important in the construction of a calibration curve is the angular coefficient \( a \), a parameter that evaluates the sensitivity of the method; can be used, for example, for the comparison between two different analytical methods. The one with the highest value of \( a \), in modulo, has a greater inclination of the line and, consequently, has a greater sensitivity. Figure 1 shows the influence of the coefficient on the sensitivity of the analytical method. Thus, it is noticed that method B is more sensitive than A, because a same range of concentration variation, method B is able to present a greater response.

Another parameter related to the linearity and the application range, which is the interval between the upper and lower concentration values of the substance under examination, provided that it meets the accuracy and precision requirements\(^{25,26} \). There is no consensus on the use of a range of applications among the various regulations, as can be seen in Figure 1.

In chromatographic methods, three different techniques can be used to construct the calibration curve: external standardization, internal standardization and matrix overlay with standard addition.

**Figure 1.** Angular coefficient \((a)\) of the analytical methods A and B, equation of the lines and determination coefficients \((r^2)\).
External standardization relates the response to be quantified in the matrix (area of the substance) with the responses obtained from solutions prepared with the analytical standard of known concentrations. Thus, the calibration curve is obtained from multiple dilutions of a standard in a solvent. In order to obtain the concentration of the test substance present in the matrix, it is obtained by comparing it with the calibration curve\textsuperscript{25,27,28}. Historical curves are the calibrations performed only once during the determination or quantification process of a particular analyte. These curves are not commonly used for this type of standardization because they are sensitive to errors during sample preparation and dilution of the patterns\textsuperscript{25,27,28}.

The internal standardization uses a substance called the internal standard with physical and chemical characteristics similar to the analyzed analytes. These are added to the sample in known concentration or mass so as not to interfere in the analysis. Therefore, the internal standard must necessarily be exempt from the matrix studied, be available in high purity, the sample should be added in a concentration similar to the concentrations of the substances to be analyzed and have a good chromatographic resolution\textsuperscript{15,26}.

The construction of the curve using internal standardization is done by calculating the ratio between the responses obtained from the internal (constant) standard by the analyte (variable) depending on the reasons between the constraints established by the application range. The sample is analyzed in the same way, i.e., by adding the fixed amount of internal standard\textsuperscript{25-27}. This type of standardization is recommended for methods that have small variations of their parameters, and is widely used to correct injection in the gas chromatographic method\textsuperscript{28}.

The matrix superposition is the addition of the analyte standard to various concentrations in an identical or similar matrix free from the substance. The construction of the calibration graphic lists the areas obtained with the concentrations of the standards. Therefore, both internal and external standardization can be applied to matrix superposition, being used to evaluate the effect that possible interferences present in the matrix would generate on the analytical method\textsuperscript{27}.

The parameters evaluated in the matrix overlap are: extraction recovery and the selectivity or detection of the substance of interest, which provide a better correspondence in relation to the composition of the sample, since it mimics an actual situation of analysis of the same\textsuperscript{27}. Sometimes, there are drawbacks, such as
the high cost of analysis, as well as the underestimation of the effects of co-extracts\(^{29}\).

Although calibration evaluating the matrix effect is reliable, it evaluates only the effect of a single matrix, but there are analytical situations in which the composition difference between matrices should be considered\(^{27}\).

When it is not possible to obtain an analyte free of the analyte of interest, it is recommended to use the standard add-on technique\(^{27}\), in which known concentrations of the analyte of interest are added in known quantities in the matrix before preparation. These are used to obtain the response of the method by constructing a calibration curve relating the amounts of the substance added to the sample with the respective areas obtained. The point where the axis of the ordinates corresponds to the response of the analytical method of the substance being determined without any addition of the standard. The concentration of the analyte is defined by the extrapolation of the abscissa axis\(^{30}\). This technique is complex and involves many steps and can be used when it is difficult to find an adequate internal standard for the analysis\(^{31}\).

5. Accuracy

According to Resolution 27 of 2012 of ANVISA\(^{6}\), the determination of accuracy must be evaluated in two ways: in the same race (precision in-run) and in at least three different races (accuracy intercurrent). In order to evaluate the accuracy in each run, it is necessary to perform at least five replicates of the analyzed points, with at least five concentrations comprising lower limit of quantification, low quality control, medium quality control, high quality control and quality control of dilution. The test for accuracy assessment should cover races on different days.

The accuracy must be mathematically demonstrated by means of the coefficient of variation (CV\%), not exceeding 15\% (fifteen percent), except for the lower limit of quantification whose values are less than or equal to 20\% (twenty percent); the calculation of the CV and made by the following mathematical equation (Eq. 2):

\[
CV = \frac{\text{Standard Deviation}}{\text{Mean} \times 100}
\]
The calculation of the CV of both the intracorrelated and intercurrent accuracy must take into account all the values obtained. If the variations are above the regulation in the legislation, it is necessary to repeat the test.

### 6. Accuracy

In order to evaluate the accuracy, an experiment similar to the one of precision must be carried out, being evaluated in two ways: in a metamorphosis (intracurrent accuracy) and in at least three different races (intercurrent accuracy), it is necessary to perform at least five replicates of the analyzed points, such as at least five concentrations comprising the lower limit of qualification, low quality control, medium quality control, high quality control and dilution quality control. The performance of the accuracy test performed should cover races on different days.

The accuracy and mathematically presented by means of the calculation of the Relative Standard Error (RSE), being calculated by the following formula (Eq. 3):

\[
RSE = \frac{(\text{Experimental mean concentration} - \text{Nominal value}) \times 100}{\text{Nominal value}}
\] (3)

The accuracy must be mathematically demonstrated by means of the RSE, not exceeding 15% (fifteen percent), except for the lower limit of quantification whose values are less than or equal to 20% (twenty percent);

The calculation of the RSE of the intracurrent and intercurrent accuracy must take into account all the values obtained. If the variations are above the regulation in the legislation it is necessary to repeat the test.

### 7. Selectivity

According to Resolution 27 of 2012 of ANVISA\(^6\), to evaluate the selectivity must consider the existence of coelutions of substances that could be common in biological matrices. For the selectivity acceptance, the criterion of chromosome resolution of at least 2% in relation to the retention time between the analytes is adopted.

In the selectivity assays and the analysis of biological matrix samples obtained from at least six different sources, however, in the case of whole blood, five
normal and one lipemic samples should be used. If other biological matrices are used, their characteristics should be evaluated and tested.

8. Residual and matrix effect
The selectivity can be divided into residual effect tests and matrix effect tests. For evaluation of the residual effect it is recommended to perform at least three injections in the chromatograph of the same white matrix (matrix extracted without addition of standard), one before and two soon after the injection of one or more processed high limit of quantification matrices. These results should be compared with the results of the lower limit quantification.

For accepting the residual effect, the retention time of the analyte should be less than 20% of the analyte response when compared to the matrices processed in the lower limit of quantification. However, for the internal standard, interfering peaks are allowed at retention time of less than 5% of its response. If the effect of the matrix is unavoidable, analytical steps should be taken to correct this effect so that it does not interfere with the accuracy and accuracy of the method.

To perform the matrix effect test, it is recommended the analysis of samples of biological matrices processed and later added of analyte and internal standard, as well as solutions in the same concentrations of the samples of low dose control point and high dose control point.

When the biological matrix is whole blood, six samples from different sources must be analyzed, four of which are normal and two are lipemic. In relation to other biological matrices used, six samples from different sources should be analyzed.

To evaluate the acceptability for each sample, the matrix factor (MF) must be calculated according to the formula (Eq. 4):

\[
MF = \frac{\text{Matrix analyte response} / \text{IS response in matrix}}{\text{Solution analyte response} / \text{IS response in solution}}
\]

Legend: IS – Internal Standard

The coefficient of variation (CV) of the MFs for all samples should be less than 15% for the acceptance.
9. Recovery
The recovery is used to measure the efficiency of the extraction procedure within a limit of variation. The recovery tests should be done by comparing the results of the analytes of samples collected from three concentrations (low, medium and high) contemplating the range of linearity of the method. The results obtained should be compared with the results from analysis of standard solutions not extracted, which represent 100% recovery. The calculation of the recovery must be done according to the area ratio of the extracted and not extracted pattern, for both the analyte and the internal standard, separately.

10. Lower limit of quantification
The lower limit of quantification is the smallest amount of analyte in the matrix that can be determined quantitatively with acceptable accuracy. Usually is considered as the lowest concentration of the calibration curve.

11. High limit of quantification
The high limit of quantification is defined as the largest amount of analyte in the matrix that can be determined quantitatively with acceptable accuracy and accuracy. Usually is considered the highest concentration of the calibration curve.

12. Homogeneity (Fidelity)
Heterogeneous matrices are a challenge for the analyst, since they guarantee a reliable quantitative result and basic premise of the toxicological analysis. Therefore it is necessary to carry out tests to verify if the sample to be analyzed is considered homogeneous or heterogeneous in relation to the analyte that will be searched. Thus, a sample of six distinct points is made in triplicate, performing the quantitative analysis, in order to verify the concentration variations between the sampling points.

If the result is not accurate, this matrix is considered heterogeneous and should be analyzed as a whole if possible, or a homogenization process must be carried out, within its possibility, in order to guarantee a reliable result, reflecting the real values of quantification in the whole.

A. Fukushima et al.
13. Stability
Stability of the analyte in the biological matrix should be demonstrated by freeze-thaw cycles for short-term stability, long-term stability and post-processing stability, only by varying the time or processing stage of the matrix.

In order to carry out the stability studies proposed by RDC 27 of 2012 of ANVISA, the conditions of storage, preparation and analysis of the samples under study should be reproduced using a set of biological matrix samples added with analyte solutions and the internal standard. For this purpose, a minimum of three samples of Low Quality Control (LQC) and three samples of High Quality Control (HQC) must be used, which must be analyzed immediately after its preparation and after being submitted the applicable energy conditions.

The acceptance parameter and the use of only samples whose result of the analysis immediately after its preparation is within + 15% of the nominal value of the analyte and the internal standard.

14. Strength
It may be defined as strength of an analytical method the ability of the analytical method to resist variations in results when small modifications are performed in the experimental conditions initially described. Therefore, a method can be considered robust if its results are not affected by small modifications in its analytical passages.

15. Conclusion
A validated analytical method ensures that the procedure, from the equipment up to the analytical sequence, and the documentation is accepted as correct and reliable, regardless of the area.

The validation of analytical methods is of great importance in assuring the quality of the analysis, in all areas of knowledge, as it ensures the reliability of its results and the quality of the processes, leading to safety to consumers.

The validation an analytical method and transfer data of methods that play a fundamental role in the area of forensic toxicology since the analysis in this context must be irrefutable and the award unequivocal. The validation is important in the maintenance of the chain of custody being paramount not only to ensure the authenticity of the material evidence in the criminal prosecution, but also to protect

A. Fukushima et al.
the parties involved, guaranteeing the authenticity and solidity of their work. Another
important aspect in this context is the long-term stability data, must be produced in
acceptable scientific standards, but adequate to the reality of the forensic matrix. For
this reason and the need to satisfy the requirements of the regulatory authority, all
analytical methods must be duly validated and documented, even though there is no
consensus among these authorities. The objective of this article was to provide a
simple approach to the correct scientific knowledge to improve the quality of the
process of development and validation of the analytical method. This article provides
an idea about the criteria for preparation, procedure and sample acceptance for all
validation parameters analytical methods. The applications of the analytical method
and the transfer of the method are also taken into account in this article. These
several essential characteristics of development and validation for analytical
methodology have been discussed with a view to improving standards and
acceptance in this area of research.

References
1. Chasin AAM, Nascimento ES, Ribeiro–Neto LM, Siqueira MEPB, Andraus MH, Salvadori
   MC, Fernicola NAG, Gorni R, Salcedo S. Validação de métodos em análises
toxicológicas: uma abordagem geral. Rev Bras Toxicol. 1998; 11(1):1-6.
2. Ribani M, Bottoli CBG, Collins CH, Jardim ICSF, Melo LFC. Validação em métodos
cromatográficos e eletroforéticos. Quím Nova. 2004; 27(5):771-780.
   https://doi.org/10.1590/S0100-40422004000500017
3. SOFT/AAFS. Forensic toxicology laboratory guidelines Committee Report. Society of
   Forensic Toxicologists and Toxicology Section of the American Academy of Forensic
   Science. 2006. Disponível em: http://www.soft-tox.org/files/Guidelines_2006_Final.pdf.
   Acesso em: 13 mai 2018.
4. UNODC. World Drug Report. 2009. Disponível em: http://www.unodc.org/documents/wdr/WDR_2009/WDR2009_eng_web.pdf. Acesso em:
   17 mai 2018.
5. The United States Pharmacopoeia (USP36-NF31) Rockiville: The United States
   Pharmacopoeia Convention, 2014.
6. BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC nº 27, de
   22 de maio de 2012. Dispõe sobre os requisitos mínimos para a validação de métodos
   bioanalíticos empregados em estudos com fins de registro e pós-registro de
   medicamentos. Disponível em: <http://portal.anvisa.gov.br/wps/wcm/connect/
1. Fukushima et al. 564310004b60537e891f9baf8fdded4db/RDC+27+12+Valida%C3%A7%C3%A3o+de+M%C3%A9todos+Bioanal%C3%ADticos.pdf?MOD=AJPERES> Acesso em: 04 mar 2018.
7. Eurachem Working Group. Key challenges in internal quality control. 2012. Disponível em:https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf. Acesso em: 16 mai 2018.
8. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Instrução Normativa MAPA nº 51, de 04 de novembro de 2011. Diário Oficial [da] União, Brasília, DF, 17 maio 2015. Seção 1, 55 p.
9. World Health Organization (WHO). Guidelines on validation. 2016. Acesso em: 20 mai 2018.
10. International Organization for Starndardization (ISO). Acreditação de laboratórios. 2005. Disponível em: http://www.inmetro.gov.br/credenciamento/acre_lab.asp. Acesso em: 17 maio 2018.
11. BRASIL. Ministério da Saúde. Resolução nº 899, 29 de maio 2003. Determina a publicação do “Guia para validação de métodos analíticos e bioanalíticos”; fica revogada a resolução nº 475, de 19 de março de 2002. Diário Oficial [da] União, Brasília, DF, 29 maio 2003. Seção 1, 15 p.
12. BRASIL. INMETRO. Portaria n. 191, de 10 de dezembro de 2003. Diário Oficial [da] União, Brasília, DF, 10 dez 2003. Disponível em: <http://www.inmetro.gov.br/legislacao/rtac/pdf/RTAC000871.pdf>. Acesso em: 17 maio 2018.
13. UNODC. World Drug Report. 2009. Disponível em: http://www.unodc.org/documents/wdr/WDR_2009/WDR2009_eng_web.pdf. Acesso em: 17 maio 2018.
14. GTFCh Scientific committee quality control. Guideline for quality control in forensic-toxicological analyses. Toxichem Krimtech. 2009; 76(3):142-176.
15. Lanças FM, Carrilho E, Cantu MD, Costa ME, Hilebrand S. Validation of non aqueous capillary electrophoresis for simultaneous determination of four tricyclic antidepressants in pharmaceutical formulations and plasma samples. J Chromatogr B. 2004; 799:127-132. https://doi.org/10.1016/j.jchromb.2003.10.037
16. Dierauf AL. Pinniped forensic, necropsy and tissue collection guide. U.S. Department and Atmospheric Administration National Marine Fisheries Service, Washington, D.C., 1994.
17. Chasin AAM. Parâmetros de confiança analítica e irrefutabilidade do laudo pericial em toxicologia forense. Rev Bras Toxicol. 2001; 14(1):15-21.
18. Chasin AAM, Chasin M, Salvatori M. Validação de métodos cromatográficos em análises toxicológicas. Rev Bras Cienc Farm. 1994; 30(2):49-53.
19. Thompson M, Ellison SLR, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl Chem. 2002; 74(5):835-855. https://doi.org/10.1351/pac200274050835

20. Barros-Neto B, Pimentel MF, Araújo MCU. Recomendações para calibração em química analítica - Parte I. Fundamentos e calibração com um componente (calibração univariada). Quím Nova. 2002; 25(5):856-865. https://doi.org/10.1590/S0100-40422002000500024

21. CUSTODIO, R.; ANDRADE, J. C.; AUGUSTO, F. O. O ajuste de funções matemáticas a dados experimentais. Química Nova, Campinas, v. 20, p. 219-225, 1997. https://doi.org/10.1590/S0100-40421997000200016

22. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A. 2003; 987(1): 57-66. https://doi.org/10.1016/S0021-9673(02)01536-4

23. Jenke DR. Chromatographic method validation: a review of current practices and procedures. General concepts and guidelines. J Liq Chromatogr Relat Technol.1996; 19(5):719-736. https://doi.org/10.1080/10826079608005533

24. Green MJ. A Practical guide to analytical method validation. Anal Chemi. 1996; 68(9):305A-309A. https://doi.org/10.1021/ac961912f

25. Krull I, Swartz M. Validation viewpoint: quantitation in method validation. LC-GC. 1998; 16:1084-90

26. Geetha G, Raju BKNG, Kumar V, Raja MG. analytical method validation: an updated review. IJAPBC. 2012; 1(1):64-71.

27. Cuadros-Rodríguez L, Gámiz-Gracia L, Almansa-López EM, Bosque- Sendra JM. Calibration in chemical measurement processes. II. A methodological approach. Trends Analyt Chem. 2001; 20(11):620-636. https://doi.org/10.1016/S0165-9936(01)00111-X

28. Lanças FM, Rissato SR, Galhiane MS. Analysis carbaryl and carbofuran in tobacco samples by HRGC, HPLC and CZE. J High Resol Chromatogr. 1996; 19:200-206. https://doi.org/10.1002/jhrc.1240190405

29. Egea-González FJ, Torres MEH, López EA, Cuadros-Rodríguez L, Vidal JLM. Matrix-effects of vegetable commodities in electron-capture detection applied to pesticide multiresidue analysis. J Chromatogr A. 2002; 966(1):155-165. https://doi.org/10.1016/S0021-9673(02)00707-0

30. Berg K, Wood-Dauphinée S, Williams JJ, Gayton D. Measuring balance in the elderly: preliminary development of an instrument. Physiother Can. 1989; 41: 304-311. https://doi.org/10.3138/ptc.41.6.304

A. Fukushima et al.
31. Snyder RL, Lirkland JJ, Glajch JL. Practical HPLC method development. 2.ed. New York: John Wiley and Sons: 1997. 760p. https://doi.org/10.1002/9781118592014