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Analytical Method Validation in Forensic Assay

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Abstract: Reliable analytical data is an essential requisite for accurate interpretation of toxicological findings in the forensic science. If the analytical data is not reliable, it may lead to wrong legal processes for the defendant or the wrong treatment of the patient. Therefore, analytical methods to be used in forensic science require careful method development and validation. Method validation is the most important and key element in establishing reference methods and evaluating the ability of a laboratory to produce reliable analytical data. Newly developed or routine used analytical methods must validate according to the standard guidelines. Analytical method validation should include several performance factors such as: Selectivity, specificity, accuracy, precision, linearity, range, limit of detection (LOD), limit of quantification (LOQ), stability, ruggedness, and robustness etc.

Key words: Analytical methods, forensic science, criminal analysis, validation, determination, performance characteristics.

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Introduction

The Role of Analytical Methods in Forensic Science

Forensic science focuses on providing useful and result-oriented information and answering critical "who/what" questions for criminal justice system through the process that starts from crime scene and ends at court (Roux, 2012; Morgan, 2017, ss. 455-459).

Forensic analysis refers to a thorough investigation for detecting and determining the reasons and consequences of a security incident or violation of rules of the organization or country. The term of forensic science describes a multidisciplinary field that includes physics, chemistry, biology, computer science etc and helps to resolve questions of law. It has become an important part of the judicial system because it uses a wide range of sciences (United Nations Office on Drugs and Crime, 2009). It is responsibility of forensic scientists to work in cooperation with law enforcement officials and crime scene investigators in order to analyze physical evidences chemically and physically.

They utilize complex instruments, scientific and mathematical principles, and reference literature to analyze evidence for identifying both class and individual characteristics.

The essential principles and techniques of forensic studies are based on the natural sciences particularly in the areas of DNA and trace evidence. The forensic studies involve a multi-disciplinary approach that covers everything from biological methods to analytical chemistry techniques.

Analytical methods can be used to identify and quantify of drugs, poisons and/or their metabolites in biological fluids or tissues. For example; various chromatographic methods are employed for doping analysis in order to determine prohibited substances such as anabolic agents and stimulants from biological samples belonging to athletes (Rivier, 2003, ss. 69–82). When taking into consideration criminal investigations it can be seen that not only biological samples but also synthetic samples like cosmetic products or gunpowder can be the subject of analysis for forensic science (Burleson vd., 2009, ss. 4679–4683; Chophi vd., 2019). In scientific work, unreliable results could lead to false interpretations, and to unwarranted conclusions. If such errors are not obvious, they may stay undetected during a scientific study or case investigation. The basis for high quality data is reliable analytical methods. So, new analytical methods require careful method development and validation (Peters ve Maurer, 2002, ss. 1-9).

When the literature is examined it can be seen that there are numerous studies on the development and validation of analytical methods for forensic analysis. They focus on the determination of different compounds ranging from explosives (Koeberg vd., 2014, ss. 3–21; Barron ve Gilchrist, 2014, ss. 27–54), pesticides, (Gonçalves, 2017, ss. 8–13). drugs (Verplaetse ve Tytgat, 2012, ss. 136–145; Te-

rada vd., 2013, ss. 95–99), to gunshot residue (Gallidabino vd., 2019, ss. 1–14). In order to achieve a more sensitive and selective analysis there are studies based on the combination of chromatographic and spectrometric methods such as; Direct Immersion Solid-Phase Micro Extraction (DI-SPME)/ Liquid Chromatography-Time of Flight Mass Spectrometry (LC-TOFMS) (Majda vd., 2019), LC- quadrupole-time-of-flight mass spectrometry (QTOF-MS) (Grapp, 2018, ss. 63–73), gas chromatography (GC)/MS (Terada vd., 2013, ss. 95–99) and ion chromatography-high resolution mass spectrometry (IC-HRMS) (Gallidabino vd., 2019, ss. 1–14).

Aside from the analyses with research purposes; in order to assist criminal investigations and analyze evidential samples there are crime laboratories also known as forensic laboratories which are led by governments (Encylcopedia Britannica, 2019; Emniyet Genel Müdürlüğü Kriminal Daire Başkanlığı, 2019). They investigate a wide variety of compounds such as narcotics, explosives, inflammable matters, fire residues and gunshot residues by using thin layer chromatography, gas chromatography, LC, high performance liquid chromatography and MS [17]. (Emniyet Genel Müdürlüğü Kriminal Daire Başkanlığı, 2019).

The Importance of Method Validation in Forensic Science

In 2009, after the report on the state of forensic science of National Academy of Sciences was published, the validation term has become an important topic in both the forensic science community and the legal community (Academy vd., 2009).

Validation of an analytical method provide that the results of an analysis are reliable, accurate, consistent and perhaps more significantly that there is a degree of confidence in the results (Krull ve Swartz, 1999, ss. 1067–1080).

If an analytical method will be applied in any laboratory; revalidation or validation processes should be carried out in order to ensure the appropriateness of the method with the analyst or the laboratory media.

In revalidation process, verification involves fewer experimental processes than full validation. Any method newly introduced into a laboratory should well validated and documented. All analysts who will use the validated sensitive analytical methods, they must receive adequate training. Validation of analytical procedures requires that qualified and calibrated instruments, documented methods, reliable reference standards, qualified analysts and sample integrity (Ermer vd., 2005; Riley ve Rosanske, 1996).

In all scientific analysis methods, it is inevitable that human, device or equipment related errors will occur but on the other hand the rate of the errors determines the validity and reliability of the method **(**Murrie vd., 2019. Since the data obtained from forensic analyses is directly related to legal results, the error of the analysis becomes critical and cannot be tolerated (Chophi vd., 2019). For this rea-

son; in forensic science in the analysis of the evidence samples such as biological fluids, narcotics and other drugs, DNA and fingerprints etc. the validity and reliability of the analysis method gain a particular importance (Rawtani, vd., 2019).

When the results of a forensic analysis are presented to court the suitability, validity and reliability of the analytical method must be demonstrated even so; the judges may ask for additional information and expert opinion (Gruber vd., 2018, ss. 292–301). For example in the United States of America there are specific standards for error rates of the method by the United States Supreme Court in environmental forensics (Gruber vd., 2018, ss. 292–301).

The Parameters of Methods Validation

Before explaining the analy tical parameters of method validation it is important to understand that the validation process depends on the characteristics of the analytical methods; hence a validation process can be carried out considering the intended use of the method without checking all parameters which will be explained in details (Ozkan vd., 2017). In addition to these, it is possible to classify validation in three groups:

The first one is full validation, which is necessary if subject of analysis is a new compound or it is the first application of the method. The other one is partial validation that is carried out if there is a modification in the method, for example change of an instrument, process or matrix. The last one is cross validation and it is required in case of use of two or more analytical methods (Ozkan vd., 2017). At least the following validation parameters; selectivity, linearity, stability, accuracy, precision (repeatability, intermediate precision) and the lower limit of quantification (LLOQ) should be evaluated for quantitative bioanalytical processes. Additional parameters that can be relevant include limit of detection (LOD), recovery, reproducibility, and ruggedness. They should give in an accordance and discuss in an effort to prevent their misguided utilization and ensure scientific correctness and consistency among publications (Ermer vd., 2005; Riley ve Rosanske, 1996; Q2B Validation of Analytical Procedures, Taylor, 1983).

For the validation process of analytical methods should follow the characteristics included in the International Conference on Harmonization (ICH) guideline or European Pharmacopeia (EP), Japan Pharmacopeia (JP), and the United States Pharmacopeia (USP) or Food and Drug Administration (FDA) guidelines (Ermer vd., 2005; Riley ve Rosanske, 1996; Q2B Validation of Analytical Procedures, General Chapters). Moreover; there are available guidelines that provide guidance for all methods by International Union of Pure and Applied Chemistry (IUPAC), Eurachem, Association of Official Analytical Chemists (AOAC), Food and Agriculture Organization (FAO) International Atomic Energy Agency (IAEA), European Medicines Agency (EMA) and World Health Organization (WHO) (Barnett, 2013).

Selectivity

Validation process usually starts with the evaluation of selectivity and this parameter is related with the reliability of the method. It is the ability of an analytical method to clearly quantify the analytes in the presence of other component in the sample. Potential interfering substances in a biological matrix include endogenous matrix components, metabolites, and decomposition products. For selectivity, analyses of blank samples of the biological matrix such as plasma, urine should be obtained from at least six sources. For interference, each blank sample should be tested. The selectivity should be ensured at the lower limit of quantification (LLOQ) (Dogan-Topal, 2019, ss. 116–123).

Accuracy (Trueness)

The accuracy, according to ICH Q2, is a measure of the closeness of agreement between the value of the results of analysis and the accepted reference value. Accuracy determined by replicate analysis of samples containing known amounts of the analyte. Minimum three concentrations in the range of expected concentrations is recommended (Quality Control (QC) samples; lower limit of quantitation (LLOQ), low, medium, high). It should be measured using a minimum of five analysis per concentration. Accuracy is the combination of random and systematic error. According to ISO (International Organization for Standardization), accuracy depends on error and it is quantitatively expressed as BIAS. It usually express as a percentage.

During the process of an analytical method various errors may occur and affect the measurement results as well as the validation parameters. Gross errors are the most important type of errors and they can result with the cancellation of the analysis (Riley ve Rosanske, 1996). The reasons of gross errors can originate from instrumental problems or sample contamination/loss. Systematic errors can be related to reagents, instruments or analysts and they cause wrong results with faulty accuracy calculations. Random errors are types of errors that cannot be eliminated. Reasons of random errors are related with the uncontrollable variables of analysis conditions (Riley ve Rosanske, 1996).

Recovery studies which are used to determine the accuracy, are related with the extraction of the analyte from a biological matrix and the value which is obtained as the percentage of the analyte detected in the sample must be as much as close to 100% (González ve Alonso, 2020, ss. 115–134). In order to acquire an efficient validation study; the same concentration value should be used for precision, accuracy and recovery. Additionally calculating matrix effect and recovery simultaneously can increase efficiency (González ve Alonso, 2020, ss. 115–134).

Accuracy expresses the closeness between the average value of a series of measurements ($\bar{x}_i$) and a reference value μ_T. It is determined by a bias, relative bias or recovery together with their confidence intervals:

$$\text{Bias} = \bar{x}_i - \mu_T$$

$$\text{RelativeBias}(\%) = 100 \times \left(\frac{\bar{x}_i - \mu_T}{\mu_T} \right)$$

$$\text{Recovery}(\%) = 100 \times \frac{\bar{x}_i}{\mu_T} = 100 - \text{RelativeBias}(\%)$$

The mean value should be within 15% of the actual value for three levels QC samples except at LLOQ. Accuracy can be determined in different ways;

i- Using Appropriate Certified Reference Material:

The most appropriate method is to use a certified reference material to calculate the accuracy. But, in commercial industry, generally no certified reference materials are available for newly synthesized compounds. In biological fluids, the National Institute of Standards and Technology contains certified reference materials for drugs of abuse, but these cover a wide range of substances. The purity of reference material should be high as possible. The purification and characterization of the selected reference material should clarify by mass spectrometry, IR and spectrophotometry, etc.

ii- Spiking A Blank Matrix with Known Concentrations of Compound:

When the method is used to determine an analyte in a blank matrix media such as forensic samples or pharmaceutical dosage forms, the method of spiking with known concentrations of the pure compound can be used. For this method, characterized and pure known standard compound is required. When certified reference materials are not available or the matrix media is not known, a blank matrix media can be spiked with a known concentration of pure compound. At least three different levels (QC samples, low, medium, high levels) of concentrations of pure compounds should be added to a blank matrix media. At each level, five repeated experiments should be realized.

iii- Standard Addition of the Compound to the Matrix Media:

In forensic analysis, standard addition method could be utilized when matrix components or blank are not available. In this technique, a known amount of the pure compound should be added to the matrix media at various amounts. In this method, the sample matrix media contains the pure compound that analysed initially. Then, the same method is used to analyze the spiked amount of the pure compound. The difference between the spiked amount value and the measured amount owing to the spiking is a measure of the bias of the analytical procedure.

iv- Comparison with Results Using Another Validated And Already Published or Reference Method:

In order to assess the accuracy of the used method; it is useful to compare the acquired results with another validated or published or pharmacopoeia method as a reference. The accurate and precise reference method should be selected. If the pharmacopoeia method is available, it should be used primarily as a reference method for comparison. If not, the reference method may be chosen in the already published literature method or obtained from the pharmaceutical industry. The chosen reference method should be applied to the determination of the pure compound in forensic sample and pharmaceutical dosage forms.

Matrix Effect

Matrix effect is a validation parameter which is mostly related to MS analyses and it is described as "the changes and effects in the response of the measured analyte in the presence of compounds other than the analyte in the matrix" [32]. It is really important to evaluate matrix effect because even if there are not any visible interferences with other compounds there can be increased or decreased responses due to matrix effect. This parameter is quantified as the ratio of the peak response of analyte in the presence to the absence of the matrix ions (matrix factor (MF)). The acceptable MF value is between 0.8 and 1.2 while the ideal is 1.0 (González ve Alonso, 2020, ss. 115–134).

Precision

The precision is directly related with the random errors of an analytical method. It represents the closeness of every measures of an analyte when the procedures are applied repeatedly. Precision usually expressed as a variance, standard deviation, or Relative Standard Deviation (RSD) of the replicate analysis. It can be determined at each concentration level should not exceed 15.0%.

Precision can be considered at three levels and it can be called as; repeatability, intermediate precision, reproducibility.

i-Repeatability:

It is the lowest level degree of precision. It is also known as intra-assay precision which is obtained under the same method, the same operating conditions over a short time period. Repeatability should be determined in three separate runs of 6 replicates each for QC samples. The RSD value of repeatability should not exceed 1%. For determination of an impurity or trace amount assay, this value should not exceed 5.0%.

ii-Intermediate precision:

Intermediate precision can also be called as between-day, between-run or inter assay precision. The intermediate precision is obtained by within laboratory variations such as different stock solutions, different days, and different buffer solution etc. It indicates the total random error of the under different conditions that can be occurred during the routine application of the analytical method. The RSD value of intermediate precision should not exceed 2.0%. For determination of an impurity or trace amount assay, this value should not exceed 5.0%.

iii-Reproducibility:

The reproducibility can be called the precision between laboratories which is obtained by the same method under various conditions such as analysts, laboratories, equipment. The conditions can be utilized to obtain analytical results independent of each other consisting of the use of a set of analytical methods on a same sample, reagents and materials etc by different analysts with different apparatus, and materials in different laboratories. The RSD value of repeatability should not exceed 2.0%. For determination of an impurity or trace amount assay, this value should not exceed 10.0%.

Linearity and Range

ICH defines linearity of an analytical procedure as its ability to obtain test results that are directly proportional to the concentration of analyte in the sample. The calibration curves or response functions describes the relationship between concentration of analytes and the detector signal. This relationship can be any straight line or even not at all a straight line (quadratic, sigmoidal, exponential,…). The linearity criterion concerns the relationship between concentration values of the validation standards supposed to be known and concentration values obtained from the application of the analytical procedure to these validation standards. The range is defined by ICH as the interval from the upper to the lower concentration of analyte in the sample for which it has been showed that the analytical technique has an acceptable level of precision, accuracy and linearity. The concentration range should cover the target amount in the forensic sample or pharmaceutical dosage forms to be measured. For the linearity and range studies, standard solutions should be prepared at least five various concentrations (25, 50, 75, 100 and 150% of target concentration). At each level, three individually prepared replicate measurements should be analyzed. After experimental part, the mean, standard deviation, and RSD were calculated for each concentration. Plot concentration (x-axis) versus response from instrument (y-axis) for each concentration is drawn and calculate the regression equation with correlation coefficient (r) (or determination coefficient (r^2)). The acceptance value of r (or r^2) should be ≥ 0.999 for five concentration levels.

The valid analytical range of the method is that range of concentrations, which pass the linearity, precision and trueness and hence accuracy criteria.

Lower Limit of Quantification (LLOQ)

The lowest standard on the calibration curve should be accepted as the limit of quantification. The analyte response at the lower limit of quantification should be at five times the instrument response compared to blank response. The response should be discrete and reproducible with a precision of 20% and accuracy of 80-120% (Dogan-Topal vd., 2019, ss. 116–123).

Limit of Detection (LOD)

The LOD would be the lowest analyte concentration analyte concentration that can be detected and identified with a given degree of uncertainly. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected but not necessarily quantified as an exact value. It is a validation parameter for limit tests. The LOD is also defined as the lowest concentration that can be distinguished from the background noise with a certain degree of confidence. The LOD can be confused with the sensitivity of the method. The sensitivity is the slope of the calibration curve. The LOD is not a robust or rugged parameter and can be affected by minor changes in the analytical method such as temperature, supporting electrolyte, pH, matrix effects, instrumental conditions etc.

Limit of Quantification (LOQ)

LOQ is described as the determined lowest quantitative analyte amount in a sample with accuracy and precision in an individual analytical process by ICH. LOQ value may be calculated as the lowest concentration of the calibration as well as lower than the lowest concentration of calibration range.

Similar to LOD, LOQ can be obtained in three different ways;

i- Based on visual LOQ inspection: In visual inspection, LOQ is determined by the analytical method of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision.

ii- Based on Signal-to-Noise Ratio: Signal-to-Noise Ratio (S/N) method, there should be a baseline noise from the instrument. In this method, the LOQ is the minimum measurement concentration at which the analyte can be reliably quantified for a S/N of 10:1.

iii- Based on the Standard deviation of the response: In this method, LOD can be calculated by 3.3xSS/m where SS is the standard deviation m is the slope of the related calibration curve. LOQ can be calculated by 10xSS/m.

The estimate of standard deviation can be obtained by several approaches which are given as follows:

1- The magnitude of blank response is measured by analyzing an appropriate number of blank and the standard deviation is calculated from the obtained responses. Usually between 3 and 6 measurements are required for this calculation.

2- The standard deviation of the regression line (slope) can be used as "ss (σ)".

3- The standard deviation of the intercept of the regression line can be used as "ss (σ)".

4- The lowest calibration standard solution which produces a current response corresponding to the working analyte should be measured and appropriate number of times (usually between three and six). The obtained standard deviation is used in the above equation as "ss (σ)" for the calculation of LOD.

Another alternative way for practically assessing the LOD and LOQ is:

$LOD = x + 3.\sigma$

$LOQ = x + 10.\sigma$

where x and σ are the arithmetic mean and the standard deviation, respectively. σ is obtained from a set of blank measurements containing no analyte. In this way, at least ten independent blank measurements should be evaluated.

Robustness

As a short description, the robustness is related to intra-laboratory influences. According to ICH, the robustness expresses the ability of the method to stay unaffected by small and intentional changes, in the method, such as pH, ionic strength, temperature, percentage of organic solvent, injection volume etc. Any critical factors can be anticipated and controlled to ensure that the conditions of analytical method will fall within an undisturbed range. The acquired results of robustness study evaluate the reliability and quality of the validated method.

Ruggedness

The ruggedness include the ability of the method to stay unaffected by change of operational conditions between laboratories and from analyst to analyst. The ruggedness is not mentioned in the guideline of ICH, but the degree of reproducibility emphasizes in the description of ruggedness (González ve Alonso, 2020, ss. 115–134). Ruggedness is similar with the reproducibility which associated with inter-laboratory changes (Reichenbacher ve Einax, 2011). The results are given by RSD% values.

Stability

Drug stability in a biological medium is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of analytical process should evaluate as the stability of the analytes during sample collection and handling, after short-term (room temperature) and long-term (frozen at storage temperature) storage, and after going through freeze and

thaw cycles. Under these conditions, the stability of the analytes, standards and stock solutions should evaluate for analytical method.

Stability of a drug and its product has great importance because degradation process under various circumstances may lead to changes in characteristics (potency and purity etc.) of a drug and result with risky situations (Mustafiz vd., 2018, ss. 164–178). Determining the time and conditions for a drug to lose its efficacy and safety is very significant for both research analyses and drug development studies. Therefore in order to ensure the degradation profile (degradation products, pathways, mechanisms etc.) of a drug or drug product, drug degradation and stability studies which are also known as stress studies, forced degradation studies are carried out under numerous conditions such as high temperature, high humidity, high or low pH values, acidic or alkali conditions etc. (Zhou vd., 2017). In 1993 these stability studies were stated as obligatory in the guideline by ICH and explained to be performed under severe and accelerated conditions (Singh, 2013, ss. 71–88). ICH also describes the stability studies in terms of time period such as: 6 months for intermediate and accelerated studies, 12 months for long term studies (European Medicines Agency, 2003, ss. 1–20). Conditions for degradation and stability studies can be grouped as hydrolytic conditions in which hydrochloric acid, sodium hydroxide, sulphuric acids, potassium hydroxide were used in order to perform hydrolysis; oxidation conditions which is created with oxidizing agents such as hydrogen peroxide, oxygen, metal ions etc.; photolytic conditions that is used for evaluating photostability of a drug using ultraviolet or fluorescent exposure and thermal conditions that involve exposure to dry and/or wet heat at 40 – 80 °C (Blessy vd., 2014, ss. 159–165).

Bioanalytical Method Validation and Its Implications For Forensic Analysis

In the literature there are various researches on the development and validation of analytical methods for forensic analyses and most of them are chromatographic methods. They can be used alone or combine with other chromatographic or spectrometric methods. In their recent work Majda et al. developed a method based on DI-SPME/LC-TOFMS for the determination of large antidepressant drug groups (benzodiazepines (BZDs), selective serotonin reuptake inhibitors (SSRI's), serotonin and norepinephrine reuptake inhibitors (SNRI's) and tricyclic antidepressants (TCA's)) in the post mortem human blood and bone marrow (Majda vd., 2019). For validation studies the standard guidelines by FDA and Scientific Working Group for Forensic Toxicology were used and they calculated the linearity of the examined range firstly. After that the LOD and LOQ values were evaluated based on the constructed calibration curves. In order to calculate precision three different concentrations (50, 150, 300 ng/mL) were used and the measurements were repeated four times for each concentration for intraday study and twelve times for interday study in three days. Lastly, absolute matrix effect was examined

using biological materials and mobile phase. These parameters were calculated for each drug separately (Majda vd., 2019).

Gallidabino et al. used ion chromatography-high resolution mass spectrometry (IC-HRMS) for forensic analysis of ionic energetic material residues such as gunshot residue and explosives since explosion related attacks are the most significant threats of our century (Gallidabino vd., 2019, ss. 1–14). What makes their study novel and unique is the ability of the developed method to allow use of solvents of forensic extraction techniques for ionic energetic materials. As the sample application studies; the new method was applied to pre-blast residues of a black-powder substitute in palm sweat and fingermark and gunshot residue. For the validation of the method repeatability, LOD, LOQ and precision values of each compound were calculated ((Gallidabino vd., 2019, ss. 1–14).

For the forensic toxicological analysis of psychoactive substances such as haloperidol, phenobarbital, midazolam, propofol, morphine etc. in serum samples a LC-QTOF-MS based method was developed, validated and compared to GC-MS by Grapp et al. [15]hyphenated high-resolution mass spectrometry has gained interest as extensive and expandable screening approach. Here we present a comprehensive method for systematic toxicological analysis of serum by liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS. The newly developed method was found more sensitive and selective than GC-MS for the analysis of psychoactive drugs. LC-QTOF-MS procedure was applied to drug positive serum and post mortem femoral blood samples as forensic case specimens. In the method validation studies specificity, recovery, matrix effect and LOD parameters were evaluated. The recovery results of 26 substances were higher than 75% and various LOD values were obtained ranging from 0.002 mg/L to 0.025 mg/L (Grapp vd., 2018, ss. 63–73).

In a study by Gonçalves et al. an agricultural pesticide carbofuran and its metabolite 3-hydroxycarbofuran were determined using high pressure liquid chromatography with diode array detector (HPLC-DAD) (Gonçalves dv., 2017, ss. 8–13). Carbofuran and 3-hydroxycarbofuran are agricultural pesticides which have toxic effects with acetylcholinesterase inhibiton in mammals and forbidden in the United States and European Union. Those compounds can be used to intently intoxicate both humans and animals therefore their determination and identification are related to forensic analysis. The proposed method was applied to actual samples of stomach contents and liver of different animals which were investigated due to suspicion of poisoning by The Toxicology Diagnostic Laboratory of the School of Veterinary Medicine and Animal Science − University of Sao Paulo. The parameters of linearity, precision, accuracy, selectivity, recovery and matrix effect were studied to validate the method. In the linearity studies repeatability, LOD and LOQ were calculated (Gonçalves dv., 2017, ss. 8–13).

In their study; Xu et al. developed and validated high performance liquid chromatography coupled with (photodiode array and) LTQ ion trap/Orbitrap mass

spectrometry (HPLC-(PDA)-LTQOrbitrap) method for the forensic analysis of organic explosive compounds such as picric acid, 2,4,6-trinitrotoluene, ethylene glycol dinitrate etc. [8]n=1-n. Organic explosive material residues can be found in surfaces like post blast debris and skin which constitute complex samples with lots of other substances in addition to explosives therefore their forensic analysis is harder and developing a sensitive and selective method is more important. The validation process carried out according to EU Commission decision. LOD, selectivity, repeatability and reproducibility parameters were validated (Koeberg vd., 2014, ss. 3–21).

Conclusions

Forensic science involves for applying scientific methods and processes to solving crimes. The field of forensic and criminal sciences consists of different scientific branches such as chemistry, physics, biology, biotechnology etc. with its focus being on the identification, recognition, and evaluation of physical and chemical evidence. Nowadays, in the forensic science extraordinary scientific innovations and advancements have allowed it to become a highly developed science that involves a number of disciplines and thousands of forensic scientists specializing in everything. It has been used for clarifying uncover mysteries, solving crimes, and convict or exonerate suspects of crime for hundreds of years.

Validation is conducted to establish that a technique is reliable over a specified range of conditions, and is a necessary part of any quality assurance program in any forensic science laboratory. Laboratories perform validation processes to assure the reliability of results and to optimize forensic science techniques in the analysis of physical evidence.

Validation studies are key to understand the extent of the method therefore when those analytical methods are used in forensic analysis the analyst will have comprehensive knowledge of the method.

Traditional method-validation experiments described by ICH and other guidelines provide a reasonable assurance that the method performs as needed.

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