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A Forensic view: Quality Control Norms for Analytical Instruments

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ABSTRACT:
A wide variety of Analytical Instruments are available for identification and quantification of various drugs and poisons in Forensic Toxicology Laboratories. The LC-MS/MS (Liquid Chromatography- Mass Spectrometer with tandem Mass) is an emerging technique in the field of Forensic Toxicology, which provides the ability to analyze a high range molecular weight; high polarity range of compounds with high sensitivity and specificity, in less time. But in case of LC-MS or LC-MS/MS, many factors influence the mass spectra of organic molecule during analysis. The collision induced dissociation of organic molecules by Atmospheric Pressure Chemical Ionization (APCI) or Electro-Spray Ionization (ESI) show the need of standardization of experimental conditions for building up a reference library. The different mass filters like Time of Flight (TOF), Ion Trap, Single or Triple Quadruple, Magnetic Sector Mass Analyzers in single or multiple mass spectrometry coupled with High Performance Liquid Chromatography(HPLC) give their unique pattern of mass spectra which are not easy to compare with each other. So, when confirmation of identity of a compound is required, it is very difficult to rely on the definite set of library data. In this article, the attention on the above problem is directed explicitly to Forensic Toxicology. The standard protocol that should be followed and the number of Identification Points or Information Points (IPs) that should be taken into account for confirmation of identity of a chemical compound by LC-MS/MS has been discussed.

Keywords: Analytical Instrumentation, LC-MS/MS, Quality Criteria, Identification Point, Forensic Toxicology.

INTRODUCTION:

The two important tasks of Forensic Toxicologists are providing indication of abuse of illicit drugs in murder cases or in poisoning cases. The analysis of biological fluids or tissues is the essential step for determining whether a person has been administered any forbidden substance or not. The outcomes of the laboratory analyses have a vital economic, social, and/or the personal consequences as it may affect the penalty of an accused. To cope-up with the Criminal-Justice System, the forensic scientists should adopt the analytical techniques which can withstand the judicial scrutiny in the court of law. The toxicologists are expected to bring an unambiguous evidence for the presence of structurally well-defined substance in the particular biological matrixes [1].

Standard rules or operating procedures concerning the execution of the analytical techniques and proper interpretation of results is to be followed for getting unequivocal evidence from Forensic Toxicology laboratories. The analyst should fully understand the instrument performance and should be familiar with the integral components of method validation of the analytical instruments, i.e. signal-to-noise ratio (S/N), lower limit of quantitation (LOQ), upper limit of quantitation (ULOQ), limit of detection (LOD), accuracy, precision, interference and robustness [2].

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MAINTAINANCE OF GOOD LABORATORY PRACTICES IN FORENSIC TOXICOLOGY LABORATORY

Undoubtedly, an expert opinion from Forensic Toxicology Laboratories has great impact on the Justice Delivery system; therefore maintenance of standards of a Forensic Toxicology Labs for analysis of samples must meet the requirement of Criminal Justice System. The toxicological Laboratories should be equipped with the latest analytical Instruments. The man power required for the routine toxicological analysis must be decided keeping in view the equipment in use, technical ability and the experience of the analyst. Forensic Toxicologists or analysts should be trained and experienced in working with latest analytical techniques. The Analytical Instruments used in Forensic Toxicology Laboratories should be calibrated at regular intervals. Standard operating procedures should be strictly followed for the laboratory tests. The results of the sample should be compared with the standard. Comparison with the theoretical value and library data should be avoided. The expert opinion about the case should be peer reviewed by senior and experienced toxicologists before it is released. Complete laboratory analysis must be validated and well documented. The Forensic Toxicology Laboratories should have regular inspections by approved accreditation panel and it should be certified for proficiency testing to meet the Global standards.

SYSTEMATIC TOXICOLOGY ANALYSIS

The usual practice of toxicological analysis begins with the extraction of drugs and poisons from biological matrices. There are various extraction methods, such as Stass-Otto method or their different modifications or modern methods especially Solid Phase Micro-Extraction(SPME)or Enzyme Digestion [3]. Extraction is followed by purification of the analytical extract to avoid any error. In the process of extraction degraded protein, fat etc. must be avoided, which may interfere in the identification and quantitation process.

Screening or color test is done to identify the group or class of drug or poison, however individualization of a particular drug is done by Thin Layer Chromatography comparing R_f factor with the standard and other confirmatory tests[3]. The major problem is encountered in the determination of small quantity of drugs and poison present in biological matrices. Multiple steps of extraction and purification may cause loss of these drugs and poisons resulting into the false negative results which may be because the drugs extracted are below the lower detection limit of instruments like UV-Visible Spectrophotometer, Gas Chromatograph, High Performance Thin Layer Chromatograph, High Performance Liquid Chromatograph, Raman Spectrophotometer [4-7].
LC-MS/MS which is able to detect the sample at ppb (parts per billion) level is therefore one of the techniques of choice.

COMPARISON OF DIFFERENT CHROMATOGRAPHIC AND MASS SPECTROMETRIC TECHNIQUES

One can choose choose between GC-MS and LC-MS for identifying a substance of forensic interest depending upon the nature of the compound. Certain factors influence the detection ability of drugs in various chromatographic systems, they are: thermal stability, polarity of the extracted substances, the detector sensitivity etc. The molecular ions detected in one method may not be reproducible when the physico-chemical detection approach is changed. For example, the ions detected by LC-ESI-MS may not be reproducible with LC-APCI-MS. It is the

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responsibility of the laboratory or the toxicologist to make the final choice of several procedures and their combinations.

The coupling of High Performance Liquid Chromatography (HPLC) with Atmospheric Chemical Ionization (APCI) or Electro-Spray Ionization (ESI) has widened up the new possibilities of determining the toxicologically relevant compounds. However, the mass spectra obtained from LC-MS are not satisfactorily reproducible to set up a good library of reference spectrum [8]. In case of GC-MS, the ionization energy applied are constant, i.e. 70eV. Hence the 70 eV electron impact mass spectra are unique for a molecule [9]. But in case of LC-MS, the fragmentation depends on various factors, such as the chromatographic condition applied, the nature of eluent, the MS condition applied, the geometric configuration of LC-MS interface etc. Hence it's not possible to apply a single optimized ionization voltage for a wide variety of toxic compounds of forensic interest as the fragmentation pattern for APCI and ESI are quite different [9]. Even if same ionization energy is applied, practically, the LC-MS/MS spectra are Instrument specific.

Latest analytical approach has been coupling of HPLC with high resolution Time of Flight (TOF) mass analyzer as compared to LC-MS with triple quadruple mass analyzer, magnetic sector mass analyzer, Ion trap mass analyzer. This method includes the acquisition of high resolution mass spectra and the comparison of the unknown with a reference library containing more than 400 toxic substances of forensic interest.

QUALITY CRITERIA FOR COMPOUND IDENTIFICATION

For Systematic Toxicological Analysis (STA), the general criteria for using any analytical technique should be followed [10]. i.e., the analytical method that has been chosen should be capable of distinguishing between the compound of interest and all known interfering substances that may possibly occur in the compound of interest. The physico-chemical behavior of the sample should be same as that of the reference standard material. Every analytical technique has its own identification power and a numerical value of Identification Points (IPs), can be imputed in each measurement . For example, the value of retention factor in TLC (Thin Layer Chromatography) offers less information as compared to the retention time in GC combined with Mass Spectrometer for identification of a substance. On the basis of IPs, a most appropriate method should be selected [11] for getting the confirmation of identity of a compound; multiple numbers of analytical techniques are used to gather more and more information varying from partition co-efficient in GC to fragmentation pattern in mass spectroscopy. For confirmation of Identity the minimum number of information point or identity point (IP) should be three [11]. The IP earned from GC-MS, LC-MS, LC-MS/MS and LC-MS^3 is summarized in the Table 1.

Table 1: Information Point earned from different Mass Spectra's

Analytical technique	Number of ions	IP
GC-MS (EI or EC)	i (No. of ions detected)	i
LC-MS	i	i
LC-MS/MS	1 precursor ion, 2 daughter ion	4
LC-MS^3	1 precursor ion, 1 daughter ion, 2 grand daughter ion	5.5

The compound of interest is identified by comparing the information obtained by each individual steps of a particular analytical

technique with a reference standard by running in the same instrument, under identical experimental conditions. While using LC, the retention time of the sample and standard should match with a maximum tolerance of ±5 second. However the relative retention time (RRT) of the sample and standard should match with a tolerance of ±5/A, where A is absolute retention time of the internal standard in second [9, 12]. In case, no reference standard is available, some forensic scientists consider that correct identification is not possible as the published mass spectra may not be always correct and there are certain drawbacks in using the library reference spectra. The reference spectra those are available in the library are limited in number. The impurity present in the compound or co-eluting with the compound may cause a mismatch. Certain instrumental differences may produce ions of different intensities, which give a poor matching. Reference libraries are not always comprised of whole mass spectra. Hence only a few characteristic ions can be retained. The retrieval system shows a random result if the unknown substance is not available in the reference library. Hence the library databases can be used only with extreme caution even for the very preliminary identification of the compound.

CONCLUSION

The outcome of the study suggests Liquid Chromatography with multiple Mass Spectrometry (LC-MS or LC-MS/MS) system play a more crucial role than GC-MS as a preferable technique for identification of organic molecule in biological specimens, particularly in the field of Forensic Toxicology. Further, some specific additional considerations are necessary for using LC-MS or LC-MS/MS to achieve the reproducibility besides analyst's personal expertise on extraction, isolation and identification of organic substances.

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