SARA Law **R**esearch **C**enter
International **J**ournal of **L**egal and **S**ocial **O**rder, https://www.ccdsara.ro/ijlso
ISSN 2810-4188, ISSN-L 2810-4188
Nº. 1 (2021), pp. 622-625

THE PROBATIVE VALUE IN THE MEDICO-LEGAL TOXICOLOGY OF PSYCHOTROPICS

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Abstract

In forensic toxicology, there are two types of tests: screening tests and confirmatory tests. Usually, the samples are first examined for the presence of psychotropics, then a more specific confirmatory test is performed to determine the exact substance and often its concentration. A confirmatory test should have a different methodology than the screening test and should be performed on another sample, if possible.

Keywords: *forensic toxicology, screening, confirmation, psychotropic*

INTRODUCTION

In forensic toxicology, there are two types of tests: screening tests and confirmatory tests. Samples are usually first screened for the presence of medications and/or intoxicating substances, then a more specific, confirmatory test is performed to determine the exact substance and, often, concentration. A confirmatory test should be a different methodology from the screening test and should be run on a different sample/specimen, if possible.

Prior to testing of any sort, the first step is preparation. For many methodologies, the drugs must be separated from the organic matrix in which they are suspended. This can be accomplished by heat, protein precipitation, liquid-liquid extraction, or solid phase extraction. In the postmortem setting, protein precipitation and liquid-liquid extractions are the most commonly used techniques.

I. SCREENING TESTS IMMUNOASSAY

Use: Can be used to screen for a large number of drugs; can be qualitative or semiquantitative.

Basic Principle: An antibody is designed to react against a particular drug or drug class. The specimen to be tested is combined with the antibody; the antibody

binds to the drug in question, yielding a positive screen if the drug is present. If the sample is combined with a known amount of labeled antigen, competitive binding can occur between the antibody, the known amount of labeled antigen, and the unknown amount of drug. The antibody-antigen reaction is then measured, allowing for a semiquantitative determination of the amount of drug present.

Types:

Radioimmunoassay (RIA).
Enzyme multiplied immunoassay (EMIT).
Fluorescent polarization immunoassays (FPIA).
Kinetic interaction of microparticles in solution (KIMS).
Enzyme linked immunosorbent assay (ELISA).

Advantages: Relatively easy to use and to perform; requires minimal, if any, sample preparation; good sensitivity even at low concentrations; can be performed using very small sample amounts.

Disadvantages: Limited specificity as can have cross-reactivity between drug(s) and structurally similar compounds; interfering substances may be present within the biological matrix, yielding either false positive or false negative results; requires that an assay has been developed and is available for the desired drug. Urine is the preferred matrix as it has less interfering substances than blood.

II. SPECTROPHOTOMETRY

Use: Not commonly used except for the determination of carboxyhemoglobin; historically also used for barbiturates and salicylates.

Basic Principle: Molecules will absorb/distort light of different wavelengths in particular ways. A spectrophotometer can measure the changes in the wavelength of light passing through a substance to determine the presence or absence of certain molecules within the matrix.

Types:

Ultraviolet (UV).
Visible spectra.
Infrared (IR).
Advantages: Ease of use.
Disadvantages: Lack of sensitivity and specificity.

III. CHROMATOGRAPHY

Use: When combined with a detector, can be used as a screening test for a large number of drugs.

Basic Principle: Drugs are dissolved into a mobile phase (gas or liquid), which is then passed through a stationary phase (i.e., a column) allowing for separation and isolation of the constituents of the sample. The time taken to traverse the stationary phase is recorded by a paired detector and compared to an internal standard, allowing for detection of each component within the sample.

Types:

Gas (GC): Uses time to traverse a packed column in a gas matrix; usually paired with a flame ionization detector (FID) or nitrogen phosphorous detector (NPD) for identification.

Liquid (LC): Uses migration distance in a liquid matrix; can be used on a solid media (Thin Layer) or liquid (HPLC); most commonly paired with ultraviolet detector but can also be paired with fluorescence or electrochemical refractive detectors for identification.

Advantages: Can vary packing material, temperature, mobile phase components, and flow rate to adjust sensitivity and specificity; can be paired with a detector to increase specificity; HPLC is run at normal temperatures (unlike GC which is run at elevated temperatures), and may preserve heat-labile components.

Disadvantages: Time consuming; requires significant sample preparation; equipment expensive.

IV. CONFIRMATORY TESTS/EXTERNAL MODELS

Confirmatory tests are performed when a drug has been identified by one of the screening tests. Confirmatory tests should be performed by a different methodology than the screening test and on a different sample, if possible, or, at least, a different extract of the same sample. The confirmatory test should also be more specific than the screening test. The gold standard for confirmatory testing in forensic toxicology is GC or LC paired with mass spectrometry. This paired method allows for mass spectral analysis of analytes after they have been separated and isolated by chromatography. Mass spectrometry is accomplished by fragmenting a molecule by a barrage of electrons and then analyzing the relative abundance of the fragments (electron ionization) or by ionizing molecules and analyzing the charge transference (chemical ionization).

If mass spectrometry is not available, the American Board of Forensic Toxicology (ABFT) allows for confirmation by the same system as identification as long as a different chemical derivatization and column and, thus, retention time is used. However, this is not recommended by the ABFT and may face scrutiny in a court of law. Confirmation of an immunoassay with another immunoassay is never acceptable.

In Romania, neither the amount of psychotropic substance(s) that influence the central nervous system (CNS) nor the screening and confirmation (dosing) methods that can be used are regulated. It is not acceptable for a person to be investigated or convicted without a quantitative material evidence that differentiates between habitual and accepted and desired consumption and/or an influence, or not, of mental functions.

V. TESTING PANELS

Most forensic toxicology laboratories offer five basic screening tests to determine the presence or absence of the majority of forensically significant drugs. These panels include (listed with examples of the drugs/drug types found):

Lower alcohols: methanol, isopropanol, acetone, ethanol Acid/neutral: barbiturates; meprobamate/carisoprodol; NSAIDs (ibuprofen, naproxen), salicylic acid, acetaminophen; valproic acid and phenytoin;

Basic (alkaline): psychoactive medications (antidepressants, anti-psychotics); methamphetamine/amphetamine/MDMA; benzodiazepines; antihistamines;

Cocaine: cocaine, benzoylecgonine, ecgonine methyl ester;

Narcotics: morphine, monoacetylmorphine, hydrocodone, codeine;

VI. ADDITIONAL TESTING

Additional testing may be available for THC or other specific drugs. Some substances require special testing procedures for identification. For example, digoxin is detected by RIA and carbon monoxide by UV spectrophotometry or Conway diffusion (semiquantitative). Screens for heavy metals are not uncommonly performed in postmortem cases and can be accomplished by multiple methods, including special atomic absorption, inductively coupled plasma mass spectroscopy (ICP-MS), and neutron activation. The Reinsch test can also be used to determine the presence of arsenic, antimony, bismuth, and mercury. It is recommended that the particular forensic laboratory be contacted regarding any specific requirements.

CONCLUSIONS

There are, as we have shown above, many screening methods, but they generally have a low specificity, with a reasonable possibility of false positive results. For this reason it is necessary to regulate, legislate and methods of confirmation. The institutions provided in art.188 C.P.P. should have the methods of confirmation (dosing), accredited R.E.N.A.R. on I.S.O. 17025. At this moment, there is no standardization at national level, regarding the dosage of psychotropics nor regarding their concentration, at which to influence the mental state.

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