REMEDi in Forensic and Legal Medicine Toxicology

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The automated HPLC analyzer of drugs and metabolites REMEDi HS Drug Profiling System (means Rapid EMErgency Drug Identification), started its way in Bio-Rad Laboratories, Hercules, California, USA in 1989. The multi-column HPLC system for on-line sample cleanup and liquid chromatographic separation of drugs offered an improvement over TLC and CGC methods requiring off-line cleanup. The REMEDi enabled the combination of sample preparation and analysis with computer-aided evaluation of retention data and UV-spectral data. Mass spectrometry detector (MSD) would be the ideal detector for REMEDi but interfaces available had had restricted applicability for broad-spectrum screening of multiple drug classes. The aim of REMEDi is to qualitatively detect and identify the probable presence of compounds of toxicological significance in urine. The REMEDi can now identify 937 drugs and metabolites (REMEDi HS System – Sofware Update Installation Guide, 4.3X.31/5.3X.31/5.1X.31, 2002). The toxicology screening by REMEDi frequently identifies also unsuspected drugs and metabolites in urine. However, broad-spectrum screening is a strategy employed by many toxicologists to determine unknown compounds in urine. Several techniques of toxicological analysis are available. They are TLC, TLC Toxi Lab, Abbott TDx, Triage TM8, ONTRAK, TOX/See, HPLC/DAD, GC/NPD, GC/FID, GC/MS, HPLC/MS, GC-GC/MS. TLC is a classical well-established chemical technique for broad-spectrum drug analysis, however, it is time-consumable method requiring subjective interpretation. Immunoassays provide positive results without any identity of a specific compound. Gas and liquid chromatography techniques offer speed and specificity. GC/MS is recommended as the reference method for the confirmation and verification of compounds and for the unequivocal characterization of molecules. The REMEDi is a broad-spectrum drug identification system using liquid chromatography with on-line sample preparation and analysis. A multi-column approach is used to extract, purify, separate and analyze drugs and metabolites in urine. Sample processing consists of dilution with an internal standard mixture and centrifugation. The two internal standards, Nordiazepam, N-ethyl (IS1) and Chlorpheniramine (IS2), are used to monitor the behaviour of chromatographic system. Upon injection of sample the prepared sample is combined with a buffer and passed through four chromatographic cartridges. What is REMEDi ? Where is REMEDi used ? The REMEDi expands the range of drugs and metabolites in biological fluids covered by TLC and immunoassays and provides a rapid preliminary report in hospital emergency and police investigation. REMEDi broad-spectrum screening of drugs and metabolites and the semiquantitative analysis are available upon request. A point of view on REMEDi is there. It appears that the ideal drug identification system uses a combination of immunoassay and REMEDi. Significant savings result from having to send fewer positive samples to the reference laboratory for confirmation. REMEDi eliminates errors due to the cross-reactivity at a far lower cost per unit (Fuentes, Block 1997). The Purification Cartridge extracts and concentrates the drugs while allowing proteins and salts to go to waste. The Mobile phase is introduced and sends the drugs through the Extraction Cartridge. Here, endogenous organic acids, hydrophobic neutral and anionic compounds are retained while the weakly acidic, neutral and basic compounds pass through. The Separation Cartridge I, is a reverse phase cartridge that separates weakly basic compounds. These include benzodiazepines, several tricyclic antidepressants,

phenothiazines and their methabolites. The Separation Cartridge II, differentiates basic compounds by cation exchange and separates more strongly basic compounds. These include amphetamines and similar stimulants, opiates and opioid analgetics and narcotics. Limitations of REMEDi. Drugs not seen on REMEDi are volatite toxic organic compounds (ethylalcohol, toluene, …), acidic organic compounds (paracetamol, chloroquine, delta 9-THC, ...), carbamates (meprobamate, …), most anabolic steroides, toxines of mushrooms, peptides, P,S heterocyclic organic compounds and all organic substances without UV absobtion. Drug identification is performed by a multiwavelength UV-scanning detector coupled with a sophisticated computer algorithm. A UV-scan from 200 to 300 nm is made. Sample spectra are then automatically compared with the library of known drug spectra stored in computer memory. The four optical and two chromatographic retention time data of analytes result in the identification of drugs and metabolites. The procedure is completed in less than 20 minutes. The detection limit is near 50 –300 ng.mL-1 for most drugs. A negative result does not imply the absence of a compound or compounds from the sample. A result of concern to the serious clinical or forensic situation should be confirmed by alternate method. The REMEDi provides increased sensitivity compared to TLC method. In a study involving 9 hospitals, REMEDi gave positive results in 94 % GC/MS confirmed occurences. The TLC gave positive results only in 56.4 % GC/MS confirmed positives (Fuentes, Block 1997). Anyway, these false negative results mean uncorrect results. REMEDi provides immediate access to preliminary results, eliminating the need for tedious classical methods. REMEDi software provides an expert system, reducing personal interpretation workload. The reference materials and calibrators have to be certified. The REMEDi fullfils all such requirements of GLP. The urine results established only the fact that sometime prior to death the toxicant had been present in the body. It can not determine the physiological effects of a toxicant. Correlation of urine values with physiological effects is poor. The physiologic effects correlate with the concentration of toxicants in blood. Those people having confirmed drug positive test results are dismissed from their jobs or police and court punished. The interpretation of postmortem blood concentrations of toxicants requires careful consideration. To ensure the integrity of urine testing forensic and legal medicine laboratories need certification and accreditation. As to the all forensic activities, every aspect of the laboratory activity, must be thoroughly documented. These are sample collection, quality control, qualification of personnel and reporting results. The toxicology laboratory must be thoroughly familiar with the facts and documents to build strategy to resolve uncertanities in authorizing positive or negative drug test results. The objective of the Toxicology laboratory in Nitra is to provide toxicology services to hospitals and police district offices throughout the Nitra region, where laboratory services are not available. The Toxicology laboratory in Nitra can analyze and interprete samples for blood alcohol concentration in hospitalized patients, drivers or other people violating the law. The samples of urine are screened for the presence of specific classes of drugs and their metabolites by immunoassays and by REMEDi. It is the aim of the Toxicology laboratory in Nitra to reach the frame of hospital and police requirements. The analysis of blood ethylalcohol, volatile toxic compounds and specific drugs and their metabolites will be given the priority when needed to answer particular legal and investigative issues. The confirmatory analysis could be performed on a later date with a lower priority within 14 days.Blood is the preferred sample for blood alcohol testing. Urine is the preferred sample in screening drugs and their metabolites. The standard accepted analytical methods of preliminary drug detection, drug screening, confirmation and quantitation in the Toxicology laboratory in Nitra are immunoassay, REMEDi HS System and capillary gas chromatography with FID and NPD detection. Results of toxicological analysis will be interpreted as how an average individual would or could be theoretically influenced by a drug or drugs. In special cases, psychologist or pharmacologist are invited as experts to the court trial. Quantitation of

drugs in blood samples is of limited value because of limited technical equipement of Toxicology laboratory in Nitra. Except of ethylalcohol in blood, there is limited source of information as for the quantity of drugs in blood in relation to the stage of influence. The therapeutical, toxic and lethal concentrations of drugs are different in every individual. The qualitative identification of a drug in urine sample is of some use in conjunction with the information on physiological observations at the time of police investigations or physician´s observation. The concentration of a drug in semiquantitative and quantitative value indicates the history of its use only. However, due to the variability of absorbtion, distribution, metabolism, excretion and elimination of drugs in individuals, generally, a weak correlation can be made between the presence of a drug in urine and concentrations of a drug in blood.

Case reports

Fig 1, Urine, INJ#1032, Autopsy No.108/2003
Case report 1
A 51-year-old man collapsed upon accute myocardian infarction. He was transported to Hospital Levice by help of his family. He had a history of hypertension and he was taking medication to control it. The emergency provided him cardiopulmonar resuscitation. Patient died 30 minutes after beginning of intensive resuscitation. At autopsy he had heavy coronary arterosclerosis and there was found thrombus in the left coronary artery. The blood ethanol 0,05 g.kg-1, the concentration of bisoprolol in urine 3 000 ng.mL-1 and bisoprolol in blood 200 ng.mL-1.

Fig 2, Urine, INJ#1073, Autopsy No.144/2003
Case report 2
A 23-year-old man was found dead in his bed at home by his grand father. His head was hidden in a black plastic bag tigtened around his neck. Inside the bag was located opened small propan-butan pressure bottle. His hands were tied up together by a rope. There was evidence of recent abuse of ethylalcohol. The external and internal autopsy findings confirmed the suffocation in unbreathable propan-butan atmosphere. Internally, heavy brain edema was revealed. The blood ethanol 2,05 g.kg-1, concentration of amphetamine in urine 3500 ng.mL-1, in blood 150 ng.mL-1 and the concentration of n-butane in blood 0,20 g.kg-1.

Fig 3, Urine, INJ#1085, Autopsy No.166/2003
Case report 3
A 52-year-old man was found dead in his garden. The body of the decedent was found in the sitting position at the bench with his head belted back. The surface of his naked body was thermal devastated by flame of an unidentified combustible fluid. There was found surface cutting scar on the flexor of the wright wrist. Near the body a kitchen knife and several drug blisters of Tramadol were found. The severe burn injuries (up to 80% of the body surface) were found. The cause of death was burn injury shock. The blood ethanol 0,15 g.kg-1, concentration of tramadol in urine 1000 ng.mL-1, concentration of tramadol in blood 100 ng.mL-1.

Fig 4, Urine, INJ#1055, Autopsy No.139/2003
Case report 4
A 37-year-old woman was found dead in her kitchen at home. External examination showed no significant external injuries or other remarcable findings. She suffered from pollinosis. The forensic pathologist determined the basic cause of death the mixed drug intoxication by codeine and maprotiline. The reports in the literature indicate that the most common adverse

effect of codeine is a respiratory failure, and of maprotiline, it is a myocardial insufficiency. The direct cause of death was accute cardiopulmonar failure. Since the deceased had expressed in her letter a desire to die, the mode of death was determined to be suicide. The blood ethanol 0,01 g.kg-1, concentration of maprotiline in urine 150 ng.mL-1 and codeine in urine 3 600 ng.mL-1. The concentration of maprotiline in blood 200 ng.mL-1 and codeine in blood 1000 ng.mL-1.

Fig 5, Gastric contents, INJ#1053, Autopsy No.139/2003
Case report 4 (cont.)
Autopsy of a dead body revealed drug-like particles in the stomach contents. These were identified like drug tablets of 25 mg Ludiomil (fa Ciba-Geigy, Switzerland). The concentration of maprotiline in gastric contents 3 500 ng.mL-1 and concentration of codeine 135 000 ng.mL-1.

Fig 6, Check mix, INJ#1010
Concentration of mixed urine calibrators. Nominal concentrations.

Diazepam	150 ng.mL-1
Nordiazepam N-Ethyl (IS1)	200 ng.mL-1
Amphetamine	220 ng.mL-1
Imipramine	200 ng.mL-1
Morphine	200 ng.mL-1
Chlorfeniramine (IS2)	200 ng.mL-1
Hydrocodone	200 ng.mL-1

Literature

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