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New technology in TDM and toxicology

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Abstract - Society makes ever increasing demands for more sensitive, specific, quantitative analyses of the many substances that affect the community's health and well-being. Scientists have responded to these demands by adapting advanced technology to achieve these goals. Automated equipment that simultaneously analyzes many specimens for several analytes with excellent accuracy and specificity is now in common use. The necessary high sensitivity required to detect nanogram and picogram quantities can be achieved using mass spectrometry coupled to GC, HPLC and SCF. Coupled to the technical advanced is an increasing awareness that many substances, produced and used as racemates, may be more effective if the more active anantiomer is used. Chiral separation of these entantiomers enables the investigation of their respective activities. This could lead to more effective use, if the necessary separation process could be performed economically. Succeeding speakers will address these technologic advances.

NEW TECHNOLOGY IN TDM AND TOXICOLOGY

Is my patient poisoned? Has an employee been using drugs? Laboratories are asked to help answer these questions with increasing frequency. One basis for an answer to the first question is the physicians clinical experience. Clinicians presume that their physical examination and their assessment of the patient's history is sufficient to establish a diagnosis and they institute the necessary supportive therapy. Seldom do they request a stat toxicological analysis of blood and/or urine to confirm their presumptive diagnosis. Most laboratories depreciate the laboratory's ability to provide helpful data in real time. This attitude is understandable because too often the laboratory is not prepared to analyze specimens for the many substances that may be involved. Very few laboratories have the experienced staff and elegant equipment essential to perform the required analyses 24 hours a day, every day of the year. To simplify the problem, some physicians request a stat analysis for the specific substances they think may be involved. For 1 year, our laboratory compared the physician's requests with the results obtained from a comprehensive testing program used in the laboratory as a routine procedure. One third of the time, the physician's presumption was correct, 30% of the analyses indicated another unsuspected drug was found in addition to the ordered drug, in 20% of the analyses an unsuspected drug was present, and in 16% of the requests no toxic substance was detected. These data suggest that the phusician's ability to correctly identify the agent(s) involved in a suspected poisoning is less than optimal and that a comprehensive toxicological analysis can be helpful. What is the present state of the art? Can realistic data be provided at reasonable cost, in real time, by technologists with limited training? A resounding YES is the answer. A comprehensive toxicological analysis is not feasible. A routine analysis is possible. It should include those drugs for which antidotes are available and those drugs whose clinical effects are a function of their concentration in a user's blood. Included in the first group are acetaminophen, methanol, ethylene gycol, cyanides, carbon monoxide, opioids, organic phosphates, and the various heavy metals. In the second group one would include the sedatives, the benzodiazepines, the tricyclic antidepressants and those substances included in therapeutic monitoring programs.

Time honored classical spot tests and microdiffusion techniques can identify many of these substances. Classical thin layer chromatography has seen many new advances. Today semi-automated procedures are available that will separate the analytes from their biological matrix and proceed with spotting and development. Another innovation that helps relatively naive analysts interpret the chromatogram is a computerized data analysis instrument.

In a companion paper, Steven Binder will present the details of the automated HPLC analysis for some 300 drugs that he helped develop. As he indicates, this novel instrument enables personnel with limited laboratory skills to provide a reliable analysis in about 30 minutes. From his brief presentation you will learn that a reasonably staffed and equipped laboratory can provide acceptable reports that can be helpful to a clinician concerned with the diagnosis and treatment of a patient suspected to be a victim of poisoning.

The second of the two questions originally proposed reflects growing societal concerns about drug abuse. More and more companies are subjecting their employees and prospective employees to tests designed to indicate if they are using specified substances that are considered to be undesirable and detrimental. Solving this problem requires a different approach to that just suggested for clinical toxicology which concerns infrequent analysis of blood and/or urine for many analytes. Drug abuse programs involve a large number urine specimens that are to be analyzed for a limited number (3 to 8) substances. The number of specimens processed a drug testing laboratory varies from 100 to 5000. Specificity and sensitivity demands are high, as is the necessity to meet forensic criteria. The mandate thorough documentation of each step in the analysis under rigid quality assurance standards. Not only must each batch of specimens contain negative and positive controls and a calibrator but internal and external blind specimens are also required. One false positive report is the basis for discrediting a laboratory.

Novel techniques are required to meet this challenge. One such is bar coding. Each specimen is labelled with a bar code wich not only identifies the specimen but also indicates which analytes are to be tested when the specimen is placed in a suitable automated chemical analyzer. These instruments are remarkably reliable and are capable of processing up to 120 to 300 specimens an hour for up to 10 analytes in each specimen. Admittedly these instruments are very expensive but their cost is easily amortized by their high volume throughput and minimal demands for specimen and reagent volumes. Some laboratories report their cost for reagents for 1 test approximates $0.05. These efficient units can be used for initial immunoassays which isolate presumptive positives and permit the rejection of the negatives.

The presumptive positives must be confirmed by GC/MS, if present standards are to be met. Modern advances permit robotic processing of the specimens so that the drug is isolated from the urine matrix, derivatized, and a suitable aliquot is analyzed by GC/MS, which can be accomplished with automatic sample handling equipment. A computer retrieves the resulting data and yields quantitative data based on rigid specifications for acceptability. The exacting steps in the preparation of material suitable for GC/MS analysis can be, and usually is, performed by skilled analysts rather that by a robot whose time has not yet come. Dr. Gelpi points out there are many compounds that are more amenable to HPLC than GC - the anabolic steriods for one example. Again with painstaking care, very small amounts of these compounds can be reliably quantitated using HPLC/MS. His presentation will amplify these introductory remarks.

Having demonstrated that specimens can be analyzed accurately, the interpretation of the result is a real challenge. Only one aspect of that problem will be discussed now. That problem relates to assessing the relative activity of the two forms present in the racemic mixtures of drugs in common use.

Recognition that enantomers of drugs have different pharmacological properties has led to the development of methods for their separation. Most drugs are presently sold as racemates, mixtures of two enantiomers. One, the eutomer, is more active than the other, the distoner. As evidence is developed to indicate that the therapeutic advantages of the eutomer exceed those for the racemate, efforts will be made to produce and market the more effective eutomer. Methods have been developed that permit these separations, and Dr. Porter presents that material in his treatise.

I have presented an overview of some new analytical techniques that will lead to improved laboratory performances. My colleagues will present more details.