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Drug analyses in poisoned patients—the need to be specific

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SUMMARY Methods for the qualitative determination of drugs in blood and urine which have been used over the past 20 years have on several occasions given rise to incorrect interpretation due to their lack of specificity, which has resulted in falsely high values being reported for commonly requested drugs. Modern methods provide an opportunity to ensure specificity in the future but analysts must continue to be aware of the potential pitfalls. Examples of non-specificity in colorimetric, UV spectrophotometric, and immunological techniques are described and the clinical implications are demonstrated.

Clinical chemistry is a twin-headed subject, advancing on the parallel fronts of improved analytical techniques and the application of these techniques in diagnosis, prognosis, and treatment. Recently, there has been a shift in emphasis in Europe away from the analytical and towards the clinical side. There are dangers in this approach, since the results upon which clinical advances are made must be produced by methods which measure what they purport to measure.

In the field of drug analysis, this has not always been the case, nor is methodology everywhere of an acceptable analytical standard. The reasons for this are not hard to find; drug estimations were derived from methods that were designed for screening purposes. Well-known examples are the FPN test for phenothiazines and the ferric chloride test for salicylate. These tests were of necessity rapid and were usually carried out on 'batches' of one specimen, often at night. There was little chance in such circumstances for the analyst to obtain guidance as to the acceptability of the methods in use. Broad differentiations into 'absent', 'trace', or 'significant amounts' of drug present were acceptable to the clinicians involved since treatment itself was based upon such classifications.

As quantitative methods became more widely available, more usable information was presented to clinicians, and clinical decision-making came to depend heavily on the results, notably those for salicylate and barbiturates, which made up the greater part of the drug-based poisonings in the late 1950s and early 1960s.

Since that time there has been a continual improvement in analytical techniques, led in part by the requirements placed upon the manufacturers of new drugs to have available a means of assaying the active principle. We are now in a position to measure more drugs more accurately than ever before but unfortunately we are still making mistakes, and clinicians are still being misled unwittingly by our analytical results.

In order to illustrate our continuing problems, let me consider a historical example, the reaction of salicylate with ferric chloride to give a purple colour known now as 'Trinder's' method. This method is accurate and linear when used for aqueous standard and is reasonably so in plasma if care is taken. Its advantage is that it is rapid and requires only a simple colorimeter. It is thus eminently suitable for emergency work.

The problem with ferric chloride is that it also gives a brownish colour with ketones, and a patient who is hyperventilating and whose plasma reacted with ferric chloride could be suffering from salicylate poisoning or be in ketoacidosis. With our currently available emergency methods we laugh at the possibility of confusion of these two conditions, but it has happened (in my experience as recently as 1969) and was confounded by the fact that gentisic acid (a metabolite of salicylate) is a reducing agent which will give a positive reaction when urine is checked with Clinistest tablets.

At about the same period there was great enthusiasm for the measurement of drugs in gastric aspirate rather than in plasma or urine, the argument being that only the unchanged drug is present and qualitative analysis is thus more simple. Unfortunately, aspirin (acetyl salicylate) does not react with ferric chloride until hydrolysed, and one was faced...
with the confusing picture of a sample containing particles of aspirin tablets which reacted only slightly to ferric chloride. This fact was unfortunately omitted from many standard texts at the time.

In 1957 Goldbaum developed the first reliable method for the determination of barbiturate in blood. The method, which depends upon the differential UV spectra of barbiturates at pH 10 and pH 14 and the disappearance of significant UV absorption at pH 1, was developed only when scanning UV spectrophotometers became available. Since the incidence of barbiturate self-poisoning at that time was high, the method was widely adopted and is still used in some centres.

Once clinicians became convinced of the relative reliability of the analyses in blood, attempts were made to study the effects of different forms of treatment for increasing the removal of barbiturate. Linton and his colleagues showed that forced alkaline diuresis could result in a great increase in the excretion of most barbiturates, the excretion of phenobarbitone and barbitone being increased most dramatically. These observations were challenged by Bloomer and Stork since they did not tie in with clinical observation. Controlled trials were carried out and were criticised since an 'effective' treatment was being withheld from those who could benefit from it.

In fact the observations of Linton et al., carried out scientifically and in good faith, were completely erroneous since they depended upon the estimation of barbiturate in the urine, and the method used was incapable of distinguishing between the unchanged barbiturate and the major (hydroxylated) metabolite. The results obtained for phenobarbitone and barbitone were correct, since these more polar drugs were excreted predominantly unchanged in the urine.

The simple mistake of assessing a new method only in water, blood, and spiked blood, and not in the urine of overdosed patients, gave rise to a major fault in clinical treatment which was not altered for over ten years. Similar problems occurred with methaqualone and glutethimide.

In the late 1960s paracetamol (acetaminophen) became widely available in the UK as a 'safe' analgesic, and the appearance of cases of severe and fatal hepatic necrosis following overdose caused widespread concern. Before treatment became available the aim was to obtain an assessment of prognosis soon after admission, since clinical signs and biochemical indicators of hepatocellular damage did not appear until 24–48 hours after ingestion of the drug.

Following the development of a UV method by Routh et al., it became clear that measurement of the plasma paracetamol concentration and relating this to the time of ingestion could give a good guide to prognosis. Unfortunately, the method required considerable skill and was not regarded as feasible in many centres. There were, however, methods for screening urine for paracetamol which involved the hydrolysis of the drug to p-aminophenol followed by a colour reaction with o-cresol or other similar phenols in alkali. These methods were simple and again required only a colorimeter. They were adapted to plasma, calibrated, checked on spiked plasma for accuracy and precision, and introduced in a large number of laboratories.

When methods for the treatment of paracetamol overdose, using thiol donors, became available several groups assessed the effectiveness of different substances and, as in the case of barbiturates, it became clear that all was not well. Miraculous 'cures' in patients with extremely high plasma paracetamol concentrations were observed in some centres and not in others. Correspondence in the journals became acrimonious and again the answer turned out to be reliance upon non-specific analytical methods.

Paracetamol is converted in vivo into several metabolites, all of which may be hydrolysed to p-aminophenol. For prognostic purposes only the unchanged paracetamol should be measured; the UV method of Routh et al. eliminated the metabolites which, being more polar, were not extracted into ether in the first part of the method. The colorimetric methods employed no extraction step and therefore all metabolites were measured.

The matter was finally cleared up after the development of an HPLC method for paracetamol by Adriaenssens et al. When we compared this method with the UV method and two commonly used colorimetric methods the error became clear, and its magnitude was appreciated, being as much as 300% overestimated in some cases.

In the last two years we have become interested in the drug clormethiazole, marketed in the UK, as an oral short-acting hypnotic, as an aid in the treatment of alcoholism, and as an intravenously administered sedative. Following studies in a number of cases of overdose, some of them fatal, it became obvious to us that the concentrations of drug measured by the forensic laboratory post mortem were considerably higher than those found by us just before the death of the patient. In addition, data from other centres on patients who had survived overdose attempts showed them to have plasma concentrations which, in our experience, had always resulted in severe coma or death due to cerebral anoxia.

Once again investigation of the method showed that the values obtained using GLC on a polyamide
column were much lower than those obtained using the standard UV extraction method, as published in a variety of analytical texts. Again, both methods were comparable when aqueous standards or spiked plasma samples were used.

Upon investigating further, using GLC on an OV17 column, it became clear that there were two components in the final extract in the UV method, one being a metabolite which had an almost identical UV absorbance. This fact had been noted some years previously by a group in Scandinavia but it had failed to be given its proper place in analytical texts.

Current problems exist more in the area of therapeutic drug monitoring than in toxicology but illustrate an important point concerning the use of immunological methods of drug analysis. We have been concerned to ensure that the results obtained for immunological methods would give a specific result which does not include inactive metabolites, endogenous compounds, or concurrently administered drugs. Immunoassays are popular with clinical staff and analysts owing to their capacity to cope with large numbers of specimens in a relatively short time.

There are three difficulties which we have observed when using immunoassays. The first is due to lack of specificity, and here the method for benzodiazepines in plasma gives the best example. Diazepam is metabolised to both a desmethylated and a hydroxylated form and also to a final metabolite which is both desmethylated and hydroxylated. All of these metabolites may be present in plasma and also all are marketed in their own right under various trade names. Chambers (unpublished observations) has shown that the cross-reactivity of one immunoassay system is such that the method is of no use for quantitative determination of any benzodiazepine in plasma and should be used only for forensic or toxicological studies of 'total benzodiazepines'.

Of more direct interest to clinicians is the use of immunoassays for the rapid determination of the chemotherapeutic drug methotrexate in the plasma of patients who are being treated with high-dose infusions. Methotrexate is metabolised to several forms, one of which, 7OH-methotrexate, is thought to be responsible for a number of side effects, notably that of renal damage.

We now have examples of patients presenting with side effects some days after the infusion when the methotrexate concentrations are almost below the limits of sensitivity of the immunoassay but which would seem to be innocuous. HPLC examination of these plasma specimens (Farid, unpublished observations) has revealed that they contain high concentrations of the 7-hydroxy-metabolite, the cross-reactivity of which is low compared with methotrexate itself.

The converse situation can occur when the antibody is specific to the parent drug and does not cross-react with an active metabolite whose concentration is of interest. The best example is that of the anti-convulsant drug carbamazepine, which has a 10-11-epoxide as the active metabolite. It has been suggested by Johannessen et al. that the clinical effectiveness of carbamazepine correlates better with the sum of the plasma concentrations of carbamazepine and its epoxide metabolite than with the parent drug alone. The immunoassay is rapid and convenient, but are we providing the correct answer to the clinician's questions if we analyse only carbamazepine?

Drug analyses in plasma and urine are now an established part of the repertoire of the clinical chemist. The data produced are being used to study the pharmacokinetics of therapeutic agents in individuals and in populations of patients. The results affect the manufacture, dosage schedules, and clinical care of patients.

We analysts must know the strengths and weaknesses of the techniques that we employ. We are now well versed in the need for accuracy and precision, but in drug analyses the new watchword must be 'specificity'.

References

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