Quantitative Determination of Drugs in Blood

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ABSTRACT

The quantitative determination of drugs in blood becomes more and more important. One reason is that threshold values for “driving under the influence of drugs” are in the discussion. Several points e.g. the differences between blood and serum or active and inactive stereoisomeres must be taken into consideration. The available methods are able to determine drugs with a SD of about 10% which is much higher than for the determination of alcohol in blood. It is additionally necessary to lower the limits of detection especially for THC into a range of about 0.5ng/ml with a signal/noise ratio of more than five at this concentration. It is not necessary to prescribed specific methods but internal and external quality control programs are essential elements in quantitative determination of drugs.

INTRODUCTION

In forensic and toxicological analyses, identification of the substances involved is of major importance. In the case of illegal drugs the suspected often denies having consumed any or the information given may be intentionally or unintentionally wrong as on this sector of the illegal market there is never a guarantee that particular ingredients are actually involved. However, in addition to actual substance identification quantitative determination, especially in blood samples, is becoming more and more important. The quantitative determination of drugs in blood samples is necessary if limiting values in respect of ability to drive a vehicle are discussed.

SITUATION IN GERMANY

Limiting values for assessing driving ability are currently in force for alcohol only. As alcohol and other intoxicating agents are regarded in Germany as being equivalent with respect to traffic laws, limiting values are being considered for drugs and medication. In the case of illegal drugs, the limiting values could be established at the minimum level of efficacy. In the moment it is illegal to drive a vehicle with an alcohol concentration of 0.8‰ or more (0.8‰ Law). But the punishment is harder with a concentration of 1.1‰ or more. The german government is now preparing an extending of the 0.8‰ Law on drugs. In the consequence one person will be guilty if in his blood a specially named analyte is present. From the presence it is concluded that the person involved had consumed the corresponding drug only a short time before he was driving a motor vehicle. It is not necessary that there are any signs of an intoxication observed by the police man or by the physician.
In the first step in the new law only the drugs heroin, cocaine and cannabis will be included and therefore morphine, benzoylecgonine and THC will be the analytes to be determined in blood.

One can be convinced that traffic safety could be increased by defining risk limiting values for drugs with the consequence, that the person involved is not allowed to drive a motor vehicle for one to three months if the analyte was determined. But from the toxicological point of view not all problems are solved till now.

PROBLEMS WITH QUANTIFICATION

Quantitative determinations should by nature always be as precise and accurate as possible. However, particularly high standards should be applied when limiting values are involved that, if exceeded, can result in persons being penalised even in the absence of further evidence. Below, a number of factors are treated that should be taken into account if a high standard of quantitative determination is to be achieved.

Up to now the simplest form of quantitative determination is the combination of a single stage liquid-liquid extraction followed by chromatography and detection using a system that guarantees a linear relationship between quantity of substance and signal produced at a high degree of stability. These criteria are fulfilled by e.g. a HPLC DAD system. Under the given conditions, a standard substance with a relative standard deviation of 2.5% can be determined. Subsequent to extraction of a particular ingredient, it is difficult to achieve a relative standard deviation of less than 10% without using an internal standard. With an extracted internal standard, the relative standard deviation can be reduced to 2.5 - 3 %.

This enables a degree of precision comparative to that achieved in chromatographic blood alcohol analysis to be obtained.

A relative standard deviation of about 10% can be achieved up to a signal to noise ratio of less than 10. A signal to noise ratio of less than 5, however, results in a relative standard deviation of 20 - 30% for individual determinations as reproducible integration is no longer possible. The relative standard deviation is difficult to improve upon, even when using double determination procedures. Therefore for many substances of interest the sensitivity of the HPLC DAD system is not sufficient.

In gas-chromatography, there is a high degree of stability of retention time or retention index. Mass spectrometric detection systems, however, normally show a limited linear relationship between substance quantity and signal produced; and, in addition, the stability of such systems is relatively low. Thus, the sensitivity can fluctuate rather strongly. In such cases exact quantification is only possible using an internal standard. In mass spectrometry, the deuterated compounds of the substances can be chosen as internal standard as they possess the same physical-chemical properties. Hence, quantitative determination of opiates, amphetamines, cocaine and cannabis should generally be carried out with their deuterated compounds added prior to sample processing. But for cannabis delta-8 THC and delta-8-THC-carboxylic acid also can be used successfully (Staak et al., 1993).

In an external quality control study with 5 laboratories good results were achieved. The standard deviation was at a concentration of 4.9 ng THC pro ml of serum only 0.42 (8.6%) (Daldrup, 1995). There were deuterated and not deuterated internal standards and different methods for sample processing used. These results indicate that the problems for
quantitation of THC in serum are solved or at least can be solved. On the other hand it should be mentioned, that the standard deviation is much higher if not serum but blood is analyzed (30.9%) at a concentration of 3 ng/ml.

PROBLEMS WITH BLOOD OR SERUM

Quantitative determinations in the area of pharmacokinetic analysis are normally carried out on serum samples, blood not being a homogeneous substance. Nevertheless in the penal code only limiting values for blood and not for serum are defined. In Germany blood alcohol analysis is thus also normally carried out on serum; this value is then used to calculate blood alcohol concentration using a factor determined from the water content of blood and serum. This procedure can only be applied to other substances if these are distributed in the aqueous phases in the same way as alcohol. However, this is the exception rather than the rule. Benzodiazepines are contained exclusively in serum and not at all in the erythrocytes. Thus, the benzodiazepine concentration in serum is 1.8 times higher than in blood (Osselton et al. 1980). According to a personal information of VON MEYER, a factor of about 2 should be assumed between serum and blood in the case of cannabis. Our investigations on opiates do not reveal such a differing degree of distribution.

In any case, an indication must be provided as to whether blood or serum was used in a particular analysis and whether the rate of recovery was taken into account. Longer term, serum/blood factors will have to be used at least for certain classes of substance. These, however, still have to be precisely defined.

PROBLEMS WITH THE METABOLISM

With most narcotics, the qualitative identification of active ingredients or characteristic metabolites ensures complete clarification. However, this does not apply to opiates. In the body, codeine is demethylated to produce morphine; hence, morphine is always detectable subsequent to the ingestion of codeine. Heroin available on the street market contains the alkaloid acetyl codeine. Thus, both codeine and morphine can normally be detected in blood subsequent to heroin consumption. In order to be able to decide whether heroin or codeine has been consumed, both substances must be quantitatively determined; qualitative analysis is insufficient in this case. After a consume of codeine - at least in the first step - persons will not be punished without signs of an intoxication.

PROBLEMS WITH STEREOISOMERES

In contrast to naturally occurring substances, synthetic substances are normally present in the racemate form, providing they are optically active. This is the case e.g. with amphetamine or methadone. In such cases, quantitative analysis should not just determine both active and non-active substances. On the other hand, e.g. in the case of methadone, it is not known whether in the case in question the racemate or exclusively the effective form had been taken. On the other hand, the d,l-quotient does not remain constant subsequent to the ingestion of the racemate (Käferstein and Sticht 1994). In urine samples with
methamphetamine and amphetamine Cooke (1994) determined a 1:d ratio over 1 for methamphetamine but a 1:d ratio less than 0.5 for amphetamine.

In my opinion it is therefore necessary to define limiting values in traffic law for the active stereo-isomers only. The chromatographic separation of relatively low concentrations of stereoisomers in blood is a somewhat complex process. Stereospecific immunochemical detection methods could be of advantage in such cases. According to experience gained with immunoassays up to now, it should not prove too difficult to prepare highly specific antibodies to e.g. active laevo-methadone or active d-amphetamine e.g. d-methamphetamine. A detection limit of 0.01 mg/l or lower for serum for an individual substance need not be utopian for a non-radioactive immunoassay. Even now, cannabis can be determined in urine with homogeneous assays like EMIT or FPIA with cut-offs or 0.02 mg/l. Even lower detection limits should become possible in the near future with in homogeneous assays like those based on magnetic particles for the detection of pesticides.

CONCLUSION

It can be established that the requirements placed on the quantitative determination of narcotics in blood will certainly increase. This is not only because of the establishment of limiting levels for assessing driving ability. Those methods currently available enable at best (HPLC) quantification with a relative standard deviation of less than 3 %. In future instrument system should be required that can detect even lower levels of substance whilst offering improved separation performance in respect of the analytical column. For this reason a lowering of the standard deviation for GC-MS is anything but utopic. In addition to chromatographic analysis, immunochemical methods should be used more and more provided the manufacturers can substantially increase the sensitivity of their non-radioactive immunoassays.

Both internal and external quality control procedures are essential for quality assurance purposes. The latter should be applied on as wide a basis as possible and also internationally. Up to now variation quotients in such studies for alcohol are about three times lower than for most of the relevant drugs. Therefore many remains to do to make not only really quantitative determinations of alcohol but also of drugs in blood. A precise quantitative determination of the pharmacologically active substances should be the condition for establishing limiting values for drugs in traffic laws.

REFERENCES