Difficulties and Pitfalls in Analysis of Benzodiazepines

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AIMS: Benzodiazepines are widely used medicaments toxicologically significant due to their frequent misuse. The aim of the presented report is to point out possible problems and difficulties we encounter during forensic-toxicological as well as clinical-toxicological analyses of benzodiazepines in biological material.

METHODS AND RESULTS: Besides well-known benefits, the use of immunochemical methods for screening tests of benzodiazepines in urine also poses certain problems, that may significantly influence further toxicological analyses of the biological material and eventually lead to misinterpretation of obtained results. When using these methods for screening tests on urine, relatively high number of false-positive and, more importantly, false-negative results are found compared to other groups of medicaments or drugs of abuse. As example, the false-negative results obtained using FPIA screening method (AxSym system, Abbott) even in the cases of intoxication by certain benzodiazepines, e.g. by flunitrazepam or bromazepam, are typical. (Especially flunitrazepam is one of the most significant benzodiazepines from the viewpoint of its toxicity.) Moreover, in a few cases when a screening test on urine and/or stomach contents showed negative results, some benzodiazepines were identified by the subsequent analysis in blood. In this respect, when only urine and, possibly, stomach contents are analysed, the laboratory may easily come up with erroneous results. The TLC method, often used for identification of benzodiazepines in urine (after its acid hydrolysis) in recent time, should nowadays be used as another screening method only. In order to identify benzodiazepines in urine, it is desirable to apply a different, more specific method, such as gas chromatography with mass detector (GC-MS) etc. First, however, the urine sample must undergo enzymatic hydrolysis, followed by solid phase extraction (SPE) and subsequent derivatization of isolated benzodiazepines. As far as the quantitative analysis of benzodiazepines in blood is concerned, gas chromatography with electron capture detector (GC-ECD), a method sensitive enough even for determination of therapeutic concentrations of commonly used benzodiazepines, has been successfully applied. Moreover, the extraction of blood samples for this analysis using the so-called “freeze out” method is simple and cost-effective.

CONCLUSIONS: In order to reveal possible benzodiazepine therapeutic application or poisoning, it is absolutely necessary to be aware of all the problems and difficulties that can be encountered, especially when using immunochemical screening methods. These problems are not fully respected mainly by those clinical-biochemical laboratories which perform only screening immunochemical tests. They quite often interpret their results incorrectly, not only from the aspect of passing false-negative results, but also from the aspect of incorrectly evaluated positive findings presented as the “concentration of benzodiazepines”.

KEYWORDS: *Benzodiazepines, Immunoassay, GC, ECD, MS*

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