DETERMINATION OF BENZODIAZEPINES: THE PRESENT-DAY SCENE

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Methods that have been used for determining benzodiazepines in biological fluids include GC, HPLC, direct DPP, RIA and RRA methods*, of which GC, HPLC and RRA have proved the most valuable in clinical research on benzodiazepines. For low concentrations GC is particularly suitable, with ECD and possibly a SCOT column and a solids injector. Some hydroxylated benzodiazepines have to be derivatized. For thermally unstable compounds such as these, HPLC-UV is advantageous, although less sensitive than GC-ECD. HPLC with fluorescence detection is feasible but requires derivatization. HPLC with EC detection has so far been unpromising. HPLC could be useful for drug enantiomers.

RRA's can detect both parent drug and pharmacologically active metabolites, preferably with assay on the sample direct. Special attention is needed to receptor quality (brain-membrane preparation).

Since their advent in 1960, benzodiazepines have proved valuable due to their anxiolytic, hypnotic, sedative, anticonvulsant, muscle-relaxant and amnestic properties. Whilst they have a common spectrum of pharmacological effects, pharmacokinetic behaviour shows remarkable differences that may be clinically important, and accordingly has been widely studied, with a range of assay procedures. This article discusses those that have been found most valuable in clinical research on benzodiazepines.

PHYSICOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF BENZODIAZEPINES

The 1,4-benzodiazepine nucleus is common to almost all the drugs concerned:

* DPP, differential pulse polarography; RRA, radioreceptor assay; EC, electrochemical.
The various derivatives differ in the substituents at the 1, 2, 3, 4, 7 and 2' positions. Generally alkyl substituents are found at 1, hydroxy substituents at 2, 3 and 4, and halogen or nitro substituents at 7 and 2'. Analytically it is convenient to classify the benzodiazepines into three groups. Class I contains what might be considered the 'classical' benzodiazepines such as diazepam and chloridazepoxide. Class II, e.g. oxazepam and temazepam, have a hydroxy substituent in the 3 position. Class III are the so-called triazolo and imidazolo(benzo)diazepines, distinctive in having a ring structure attached to the diazepine moiety at 1 and 2; examples are triazolam and midazolam.

Analytically this classification is important for several reasons. Firstly, the physicochemical properties of the three classes appear to be somewhat different. Thus, class II compounds appear to be somewhat more polar and are rather unstable thermally, which may impose constraints on the choice of analytical techniques. Pharmacologically there are important differences, notably in potency: generally this appears to be least for class II, the plasma concentrations needed to get hypnotic action being in the range ~100-1000 ng/ml for oxazepam. The class I benzodiazepines are of intermediate potency, the requisite concentrations being ~20-100 ng/ml for nitrazepam, and class III are the most potent, the concentrations being ~0.1-10 ng/ml for triazolam and brotizolam. Obviously these potency differences affect the detection limits needed in analysis and hence the selection of the analytical method.

Being lipophilic drugs, benzodiazepines are eliminated from the body through metabolic conversion in the liver. In this respect the three classes show notable differences that are analytically relevant. Compounds of classes I and III are eliminated by metabolic oxidation, which may result in pharmacologically active metabolites. For example, diazepam gives rise to the active metabolites N-desmethyl-diazepam (contributing notably to overall pharmacological activity), oxazepam and temazepam. This emphasizes the need for selective analytical procedures. On the other hand the class II benzodiazepines are generally metabolized by conjugation reactions (in particular glucuronidation), resulting in formation of inactive metabolites; these do not cause significant analytical interference since they are very different physicochemically from the parent compounds.

ASSAY TECHNIQUES FOR BENZODIAZEPINES IN BIOLOGICAL MATERIAL

Techniques that have been used include GC, HPLC, TLC, direct DPP, RIA, and RRA [1]. In clinical research, GC, HPLC and RRA have proved especially valuable. [#B-1 by S.H. Curry, this vol., is pertinent.-Ed.]

GC assay

GC-ECD procedures are the most widely used (e.g. [2-4], and J.A.F. de Silva in Vol. 7, this series). The electronegative halogen or