Mainly because of sample complexity and the relatively complicated concentration and extraction procedures generally required to obtain workable extracts, the analysis of organic micropollutants in water is one of the more difficult fields of analytical chemistry in which to work. In this field, the development and application of advanced instrumental techniques are still in a relatively early phase and most of them are generally stretched to the limits of their capabilities in terms of, for example, resolution and sensitivity.

The work has been carried out under contract from the Department of the Environment and is published with their permission.

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Drug Metabolism and Analysis

The following is a summary of the paper presented at a meeting of the Midlands Region and the Loughborough University Student Chemical Society held on February 21st, 1978, at Loughborough University of Technology.

Analytical Chemistry and Drug Metabolism

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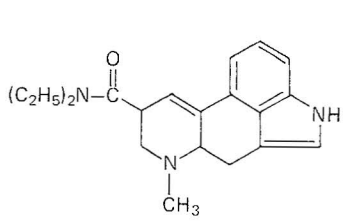
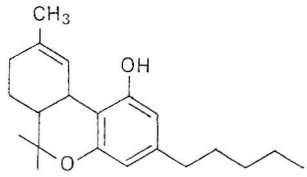
A knowledge of the metabolic routes and pharmacokinetics of a drug may be of considerable use in forensic toxicology. The identity of a drug in a biological fluid can be confirmed by the presence of its metabolites and the relative proportions of these compounds in blood and urine can give an indication of how much drug was taken and when. This paper describes some of our studies on the assay of lysergide (LSD), Δ^9-tetrahydrocannabinol (THC) and their metabolites in biological fluids.

Drug metabolites are normally more polar than the parent compound and can be conjugated to form glucuronides, sulphates, etc. In many instances the first step of an analysis is to hydrolyse the biological sample by enzymes, acid or alkali to obtain unconjugated



LSD



THC

metabolites. These metabolites can then be extracted into organic solvents at various pH values to give extracts containing the acidic, neutral and basic metabolite fractions. The individual metabolites can then be identified and quantified by chromatographic and spectroscopic procedures.

Radioactively labelled drugs are very useful in the early stages of drug metabolism studies because direct counting of the biological materials gives quantitative data for the metabolites without the need for extraction procedures. The use of tritium-labelled drugs with liquid scintillation counting gives very high sensitivities of detection. Unfortunately, the position of labelling may also be a position for metabolic attack or the tritium label may be labile. In these instances the use of ^{14}C-labelled materials may be preferred. Although sensitivity is lost in this way, the use of the radioactively labelled drug still means that metabolic and kinetic data can be obtained easily without the need to develop specific methods involving other techniques.

Thin-layer chromatography is often used for separating drugs and metabolites, but gas - liquid chromatography (GLC) may be preferred because it has the advantages of greater resolution and sensitivity. By using a mass spectrometer in the multiple-ion detection mode as the GLC detector, only a few picograms of material may be needed for an assay. This type of approach has been used for the determination of THC in plasma[1] and it can also be used for the assay of its metabolites.

Because LSD is thermolabile, high-performance liquid chromatography (HPLC) has been found to be superior to GLC for the separation of this drug and its metabolites. Reversed-phase HPLC columns are particularly useful as substances elute according to their polarity (*i.e.*, the order of elution is conjugates first, then non-conjugated metabolites and finally the parent drug). A fluorimeter can be used as the HPLC detector when determining LSD because of its excellent fluorescence characteristics. If a valve is incorporated between the column and the detector a chromatographic peak may be trapped in the fluorimeter flow cell and the excitation and emission spectra can then be obtained.[2] Hence the information gained from HPLC - fluorimetry is not only the elution volume of the compound, but also its spectral characteristics. Another property of LSD that can be used for characterisation is its conversion into the non-fluorescent lumi-LSD with ultraviolet (UV) irradiation. The identity of the compound can thus be confirmed by constantly irradiating the trapped eluate in the flow cell with UV light so that the fluorescence decreases as the LSD is decomposed.

In contrast to the situation with LSD, cannabinoids are not naturally fluorescent but some, *e.g.*, cannabinol (CBN), can be converted into highly fluorescent compounds when irradiated with UV light. This phenomenon has been used in an on-line photochemical derivatisation method for the detection of CBN in biological fluids.[3] When a sample is chromatographed the HPLC eluate passes into a photochemical reactor, where it is subjected to a high flux of UV light for a few seconds, which converts CBN into a fluorescent derivative. The eluate then passes to a fluorimeter, which detects the fluorescent photoproduct with a sensitivity of less than 1 ng. If the biological sample is re-chromatographed with the lamp in the photochemical reactor turned off, only the naturally fluorescent compounds are seen. The comparison of the two chromatograms gives a very high specificity to the method.

Radioimmunoassays (RIAs) have been reported for both THC[4] and LSD.[5] This technique has the advantages that it can be used directly on both plasma or urine without extraction, it has a high sensitivity down to the 1 ng ml^{-1} level, it requires only small sample volumes (10–100 μl) and both drug and metabolites are normally assayed together. The RIA for THC, for example, cross-reacts with a number of its metabolites, which is an essen-

tial characteristic when assaying urine because THC itself is not present in this body fluid (only the metabolites are present).

The combination of HPLC with RIA gives the advantages of both techniques. Several millilitres of urine or plasma can be injected on to a reversed-phase column provided that, with plasma, the protein has been removed by centrifugation after the addition of a few volumes of methanol. The HPLC eluate is collected in a fraction collector after the column has separated the parent drug and metabolites from each other. The RIA is then used as the HPLC detector. This approach has been developed successfully for the identification and assay of LSD[2] and cannabinoids[6] in biological fluids and is routinely in use for forensic samples. Fig. 1 shows a typical separation of THC and its metabolites in a plasma sample from a subject who had smoked THC. The large first peak has the retention volume corresponding to THC-11-oic acid and metabolite conjugates. The other areas of cross-reaction observed in the chromatogram have retention volumes corresponding to THC, CBN and monohydroxylated and dihydroxylated metabolites.

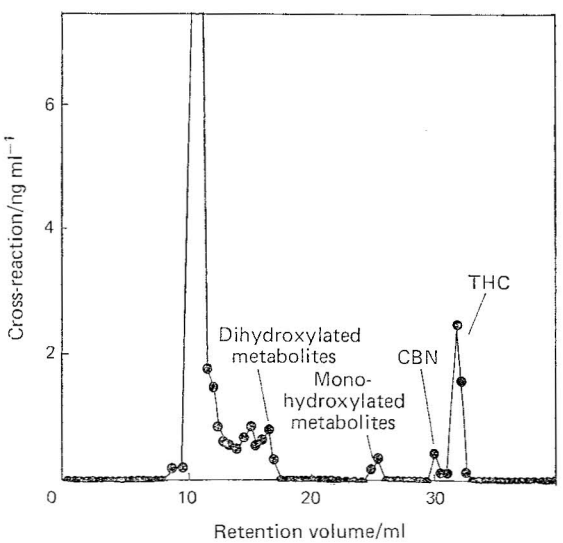


Fig. 1. An HPLC - RIA chromatogram of a plasma sample from a subject who had smoked THC.

There is probably no best single technique for studying the metabolism of a drug—the best combination of techniques should be chosen from a knowledge of the known or expected physicochemical properties of the drug and its metabolites.

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