

## THE GAS-LIQUID CHROMATOGRAPH AND THE ELECTRON CAPTURE DETECTION IN EQUINE DRUG TESTING

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### ABSTRACT

Three gas-liquid chromatographic (G.L.C.) procedures discussed have been designed around the four "esses" of detection tests — speed, sensitivity, simplicity, and specificity. These techniques are admirably applicable to the very low plasma drug levels encountered in blood testing under pre-race conditions. The methods are equally applicable to post-race testing procedures, where both blood and urine samples are tested.

Drugs can only rarely be detected by the electron capture detector (E.C.D.) without a prior derivatization step, which conveys to the drug(s) high electron affinity. Because of broad applicability, two derivatizing agents, heptafluorobutyric (HFBA) and pentafluoropropionic (PFPA) anhydrides are employed.

The three techniques, allowing broad coverage of various drug classes are:

- 1) direct derivatization of drugs to form strongly electron capturing amides and esters.
- 2) reductive fragmentation of drugs with lithium aluminum hydride to form alcohols, with conversion to ester derivatives.
- 3) oxidative fragmentation of drugs with potassium dichromate to form derivatizable groups, followed by direct derivatization.

### INTRODUCTION

Although the methods to be discussed in this paper will be referred to the broader concepts of post-race testing, they were developed principally for rapid blood analyses under pre-race testing conditions. Presently, in the Commonwealth of Kentucky, there is no pre-race drug testing. However, both blood and urine are secured from each horse to be tested.

The analytical laboratory is called upon to detect drugs whose therapeutic blood levels may range from a few picograms per millilitre to about a milligram per millilitre — a variation of one-billionfold\* in concentration levels. Obviously, the method adequate for reserpine detection might not be the method of choice for dipyrone or salicylic acid. Also, it would be highly desirable and forensically sound to develop "screening" methods for the parent drug as administered, as opposed to biotransformation or metabolic products.

The great majority of drugs sought by the equine

drug analyst lie in a concentration range of one nanogram per millilitre to one hundred micrograms per millilitre in blood or urine, with some notable exceptions. It is to find drugs in this concentration that most modern detection techniques are admirably suited.

Our laboratory employs a "modular" detection scheme for analyses of prohibited drugs. Samples, both blood and urine, are processed in screw-cap culture tubes, 125 mm or 150 mm in length. Biological sample volumes vary from 3 ml to 17 ml. In these tubes, depending upon the procedure(s), samples are hydrolyzed, liquid-liquid extractions are performed, derivatizations are accomplished and final wash steps, if necessary, are completed. Detection procedures employ derivatizations for both thin layer and gas-liquid chromatography; thin layer chromatography, gas-liquid chromatography, and ultra-violet spectrophotometry. Using this "modular" testing concept, the laboratory at the University of Kentucky has, in the first seven months of operation, confirmed one prohibited drug per 145 samples tested. Of the prohibited drugs found, 50% were detected by one or more of the gas-chromatographic procedures to be discussed in this paper.

\*In USA 1 billion = 1 thousand million, i.e.  $10^9$

A technique which we use in our laboratory, and which has been widely adopted for pre-race blood analyses employs gas-liquid chromatography (G.L.C.) with the electron capture detector (E.C.D.). As a gas chromatographic detector, the electron capture detector is very sensitive, perhaps 1,000 times more so than the commonly used flame ionization detector. The electron capture detector also detects only one-fifth as many substances as the flame ionization detector, and hence is a more specific detector.

The loss of standing current in the electron capture detector is a measure of the amount and electron affinity of the components in the carrier gas. This detector is extremely sensitive to certain molecules, such as organic and inorganic halogen-containing compounds.

Drugs can only rarely be detected by the electron capture detector (E.C.D.) without a prior derivatization step, which conveys to the drugs high electron affinities. Following derivatization, many drugs can be detected in biological fluids at very low concentrations. For simplicity, two derivatizing agents will be discussed, heptafluorobutyric and pentafluoropropionic anhydrides (HFBA and PFPA).

Three derivatization techniques will be discussed:

- (1) *direct derivatization* of drugs containing primary or secondary amine or phenolic or alcoholic groups form strongly electron capturing amide and ester derivatives.
- (2) *reductive fragmentation* followed by derivatization can be accomplished for some drugs which have groups which may be reduced with lithium aluminium hydride to form alcohols.
- (3) *oxidative fragmentation* followed by derivatization is valuable for drugs which can be oxidized to form derivatizable groups.

In Tables I and II are noted a partial list of the many drugs amenable to these procedures. Numerous other drugs which have caused detection problems could be included. The derivatization methods are applicable particularly to those drugs found in low nanogram per millilitre concentrations in biological fluids. The limits of this method are the lability of a compound, the availability of derivatizable sites on a molecule, to some extent molecular weight and extraction and/or derivatization conditions.

The acylation procedures described are used primarily, but not always, with basic drugs. These drugs constitute the bulk of prohibited drugs.

Figure 1 depicts direct acylation and reductive fragmentation followed by acylation using methylphenidate as the drug model. Figure 2 represents oxidative frag-

mentation followed by acylation, with promazine as the drug model.

TABLE I

A representative listing of drugs which may be directly derivatized with fluorinated acylating agents

Amphetamine	Codeine
Methamphetamine	Phenothiazine
Ephedrine	Procaine
Mephentermine	Benzocaine
Pentazocine	Desipramine
Morphine	Apomorphine
Ketamine	Mephesisin

TABLE II

A representative listing of drugs which may be acylated following (a) reductive fragmentation and (b) oxidative fragmentation

*Reductive Fragmentation*

Cocaine	Indomethacin
Scopolamine	Anileridine
Meperidine	Atropine

*Oxidative Fragmentation*

Propiopromazine	Triflupromazine
Promazine	Imipramine
Acetylpromazine	Doxepin

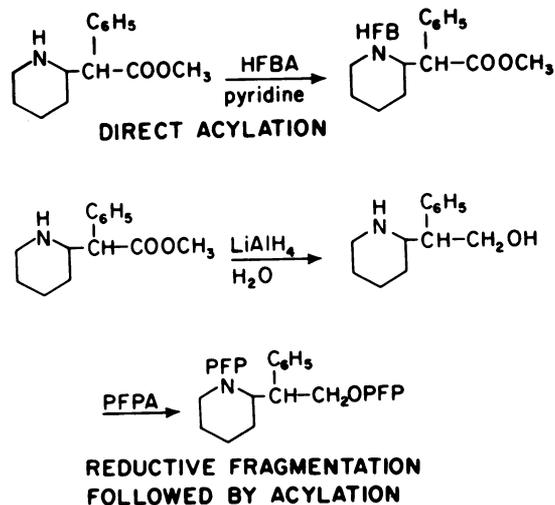
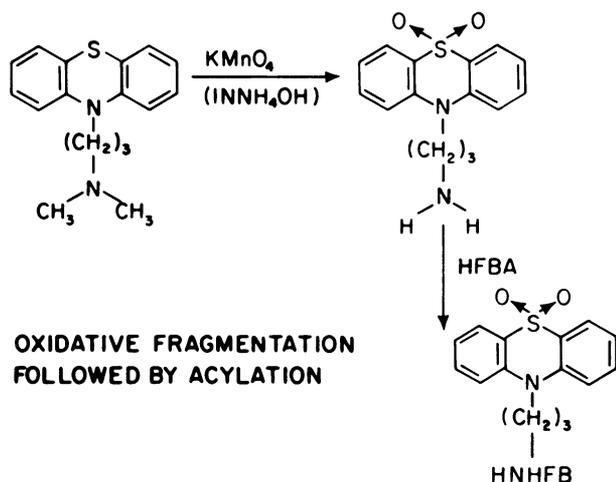


Figure 1

Acylation of methylphenidate by (a) direct derivatization with HFBA and (b) reductive fragmentation followed by direct derivatization with PFPA (Huffman and Blake, 1974).



*Figure 2*

*Acylation of promazine by oxidative fragmentation with  $KMnO_4$  followed by direct derivatization with HFBA (Blake and Tobin, 1976).*

Numerous variations of each procedure can be employed; — solvents such as cyclohexane, benzene, or dichloromethane, catalysts such as pyridine, ethylacetate or heat and washes using aqueous solutions of various pH may be used. The complexity of the acylation procedures may be adjusted to meet the conditions of testing, notably the time frame. Under pre-race conditions, if 80-100 blood samples are to be analyzed in four hours, certain procedures can be used, if five or six hours are available, more complex and more drug encompassing procedures may be used.

To explain the three procedures the "rule(s)" of "three and four" will be used.

*Direct derivatization* or acylation of a drug involves the extraction of 3 ml of plasma mixed with 3 ml of saturated sodium tetraborate into 3 ml of cyclohexane. To the cyclohexane phase is added 50 microlitres of heptafluorobutyric or pentafluoropropionic anhydrides (HFBA or PFP) and 50 microlitres of a pyridine/benzene catalyst. Derivatization reaction time is 4

minutes, after which the cyclohexane phase is washed with 4 ml of 0.5N sodium hydroxide or 8 ml of saturated tetraborate solution. The derivatized drug(s) in the cyclohexane phase are now ready for G.L.C. analysis.

*Reductive fragmentation* followed by direct derivatization employs the extraction of 3 ml of plasma mixed with 3 ml of saturated sodium tetraborate into 3 ml of cyclohexane. To the cyclohexane phase is added 100 microlitres of a saturated lithium aluminum hydride etherate to be followed by a 3-minute reduction reaction time. Twenty microlitres of distilled water is then added to the cyclohexane to stop reduction and destroy the excess  $LiAlH_4$ . From this point on, the procedure is identical to the direct derivatization cited. Pentafluoropropionic anhydride with a tetraborate wash is used where alcoholic sites are to be derivatized.

*Oxidative fragmentation* followed by direct derivatization involves the extraction of 8 ml of whole blood into 3 ml of dichloromethane. Prior to extraction, 3 ml of a 1N ammonium hydroxide solution saturated with potassium permanganate is added to the whole blood, well mixed, allowing for an oxidation period of 4 minutes. Following extraction the procedure follows that of direct derivatization, except that after a sodium hydroxide wash, the dichloromethane must be carefully evaporated to dryness, and the residue picked up in benzene prior to chromatography.

Gas-liquid chromatographic conditions employ short, three or four feet, glass columns packed with a non-polar phase such as 3% OV-1, 3% OV-101, or 3% SE-30. In the laboratory, the Varian machines with scandium-tritide foils have proved to be excellent units for routine analyses, although other chromatographs would suffice.

In summary, the detection of drugs in the biological fluids of racehorses has taken a new trend because of the development and availability in the last 10 years of excellent sensitive, analytical instrumentation. General derivatizing procedures for drugs have provided specific and sensitive gas chromatographic tests. Procedures have been and will further evolve toward smaller sample volumes using simpler, more specific, more instrumented and probably more automated techniques.

## REFERENCES

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