Journal of Chromatography, 160 (1978) 291–296

CHROM. 11,154

Note

Determination of antipsychotic drugs by gas–liquid chromatography with a nitrogen detector using a simple acetylation technique

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(Received April 27th, 1978)

Antipsychotic drugs have been widely used in treating schizophrenic patients for many years. It is well known that the concentrations of these drugs in blood can vary widely among patients receiving the same drug dosage[1,2]. Thus, simple, expedient, analytical methods sensitive enough to determine blood levels of these drugs in clinical trials would be valuable in patient therapy. Also, it has been suggested that for most of these drugs there is a "therapeutic window" or a range of drug concentration in plasma in which the drug is most efficacious[3].

In the last several years, with the advent of nitrogen detectors (thermionic nitrogen detector, for design see Kolb *et al.*[4]), it has become possible to measure subnanogram quantities of the drugs in plasma, *e.g.*, haldol[5], prolixin[6], tricyclic antidepressants[7], and cocaine[8]. Since most of the antipsychotic drugs contain either a secondary or a tertiary amine, they are well suited for determination by gas–liquid chromatography (GLC) using a nitrogen detector. Some of the hydroxylated antipsychotics have been difficult to elute from GLC columns in symmetrical peaks because of the polar nature of these compounds.

Acylation is by far the most common reaction used to derivatize hydroxyl-containing compounds ranging from sugars to drugs (for a review see ref. 9). Generally, acylation reactions have been used with a flame ionization detector (FID) or in the case of halogenated acylating agents with an electron capture detector (ECD). Here we describe a simple acetylation method for the assay of drugs by GLC equipped with N–P detectors.

EXPERIMENTAL

Materials

The following drugs were assayed and obtained from: acetophenazine maleate (Tindal) (Schering, Kenilworth, N.J., U.S.A.), fluphenazine (Prolixin), (National Formulary Board, Washington, D.C., U.A.A.), haloperidol (Haldol) (McNeil Labs., Fort Washington, Pa., U.S.A.), perphenazine (Trilafon) (National Formulary Board, Washington, D.C., U.S.A.), piperacetazine (Quide) (Dow Chemical, Indianapolis, Ind., U.S.A.).

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Methanol (spectrograde) and acetic anhydride (certified grade) were purchased from Fisher Chemical Co., (Chicago, Ill., U.S.A.), while the ethyl acetate (spectrograde) was obtained from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.).

Methods

A 1-mg sample (as the free base) of each of the above drugs was dissolved in 5 ml of methanol. To 5 μl of these solutions (which equaled 1 μg), 1.0 ml ethyl acetate was added and injected into the chromatograph as the unreacted drug assay (panel A in Fig. 2). Then 0.1 ml of acetic anhydride was added to each sample and heated for 15 min at 65°. Aliquots of 1 μl of the above reacted samples were injected in the chromatograph (Hewlett-Packard) equipped with a nitrogen detector using a 3 ft. × 2 mm I.D. column packed with either 3% OV-17 or 1% OV-101 on Chromosorb W HP (80–100 mesh). The temperature settings were: injection port and detector 300°. The column temperature and attenuation settings for each compound are given in Table I in the Results and discussion section.

RESULTS AND DISCUSSION

The structural formulas of the five hydroxylated antipsychotic drugs studied are given in Fig. 1. Four of the five drugs are hydroxylated at the aliphatic side chain, while haloperidol is hydroxylated at position 4 in the ring. Fig. 2 shows chromatograms of these drugs before and after acetylation. In each case 1 ng of the drug

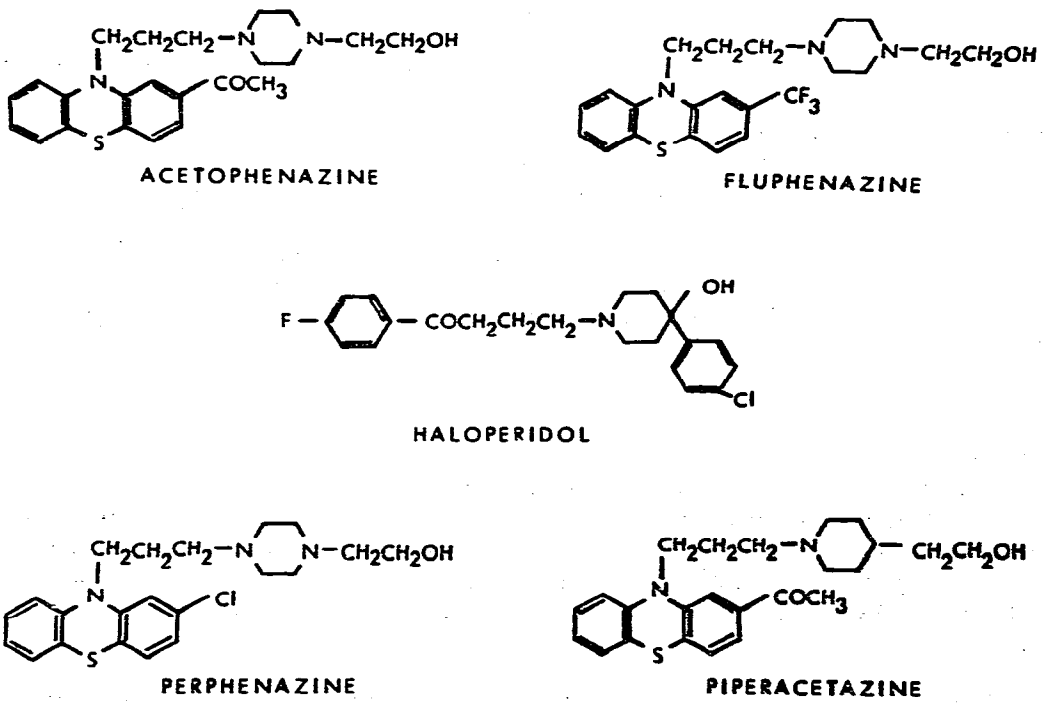

Fig. 1. Structural formulas of the five drugs assayed.

solution without reaction (panel A) is injected. At the temperature used, acetophenazine, fluphenazine and piperacetazine did not elute, although they may have eluted at higher temperatures. Unreacted haloperidol and perphenazine did elute at the temperatures used, but without the acetylation reaction the peaks were wide and unsymmetrical. After acetylation, however, the resolution improved considerably (Fig. 2, panel B). In these two drugs, it is worthwhile to note that acetylation did not change the retention time. Chromatograms of Fig. 2, panel B, were obtained by adding 0.1 ml acetic anhydride to 1 ml of the above solutions and heated at 65° for 15 min. In every instance, acetylation with acetic anhydride improved the shape of the resultant peak and increased the sensitivity by at least a few hundred times (Table I). In subsequent studies for optimum acetylation conditions, it was found that heating with acetic anhydride at 65° for varying periods of time did not substantially improve the acetylation. Thus it is reasonable to assume that the acetylation reaction is an on-column reaction. This step should be checked for each individual drug.

Here it should be mentioned that since most of the procedures employed for the extraction of these drugs from blood use a mixture of organic solvents rather than solely ethyl acetate, the extracted solvents should be dried after which a small volume of ethyl acetate–acetic anhydride (9:1) is added. Since most of these drugs are not soluble in the above mixture, heating the mixture at this stage to acetylate may be essential for some drugs to quantitatively solubelize the drug before injection.

We have had extensive experience using the above acetylation method for the determination of fluphenazine. Fig. 3 shows a typical standard curve for fluphenazine determination after acetylation. In these experiments, 10–40 ng fluphenazine were added to each reaction vial, dried under nitrogen, reacted as above in 0.1 ml ethyl acetate–acetic anhydride (9:1) at 65° for 15 min. Peak height was directly proportional to the amount of fluphenazine added. Thus from Fig. 3 it is apparent that acetylation reactions for nitrogen detector–GLC could easily be adopted for subnanogram levels of the above drugs. In fact, we have extended the acetylation procedure described here for the determination of fluphenazine for therapeutic levels in patient plasmas. The details of these studies will be communicated separately.

With the increased interest in the field of psychopharmacologic research such as drug pharmacokinetic studies or relating blood levels to clinical response, *i.e.*, per cent improvement of patients, there is a concommitant increase in the number of methods that are being adopted for determination of these drugs in blood. The most common methods were: radioimmunoassay[10], GLC[11], and high-performance liquid chromatography[7]. Recently, Snyder[12] extended his dopaminergic binding studies to the assay of these drugs. Radioimmunoassay and binding assay, which are expedient, have not been effectively studied for specificity. Conceivably, these would also determine some of the metabolites of the above drugs. HPLC methods have recently been introduced, which, although not without some problems, may very well be the common methods of the future. For the present time the method of choice is GLC by nitrogen detectors. Thus, a simple acetylation method as described above, increases the versatility of the nitrogen detectors for GLC, promising greater use of this method for the determination of subnanogram levels of most of these psychoactive drugs.

The chromatographic response produced by the acetylated drugs is greater than the non-acetylated ones, thus making it possible for adaptation for assay in the blood of patients for possible treatment.

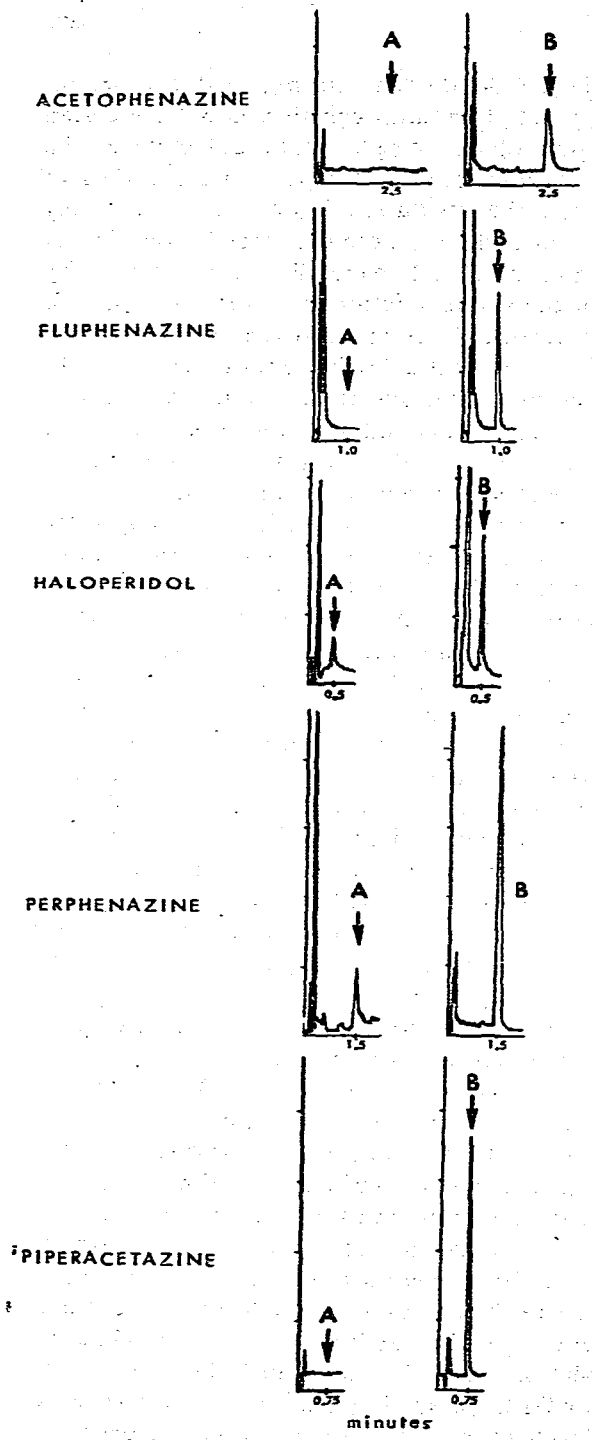

Fig. 2. Chromatograms of the five drugs assayed by GLC using a N–P detector. Panel A depicts chromatograms of the unreacted drugs, while Panel B contains chromatograms of drug samples acetylated with ethyl acetate–acetic anhydride (9:1) for 15 min at 65°. Acetophenazine, perphenazine and piperacetazine were run on a 3% OV-17 column at 250°, while fluphenazine and haloperidol were run on a 1% OV-101 column at 240°. The specific attenuation settings for each drug are given in Table I.

TABLE I

EFFECT OF ACETYLATION ON SENSITIVITY OF THE GLC ANALYSES OF PSYCHOACTIVE DRUGS

A: 1 ng (as a free base) of each drug was dissolved in 5.0 ml methanol and 5 μl of these solutions (which equaled 1 μg) were diluted with 1.0 ml ethyl acetate. One μl was then injected at the given attenuation. Numbers in parentheses are the units for peak height before acetylation of the drugs. B: To this solutions, 0.1 ml acetic anhydride was added and heated for 15 min at 65°. One μl was then injected into the GLC. Numbers in parentheses are the units for peak height after acetylation of the drugs.

Drugs	*Column*	*Temperature (°C)*	*Attenuation*	
			A	*B*
Acetophenazine (Tindal)	3% OV-17	250	8 (0)	64 (8.5)
Fluphenazine (Prolixin)	1% OV-101	240	64 (0)	64 (19.5)
Haloperidol (Haldol)	1% OV-101	240	16 (4)	8 (19.0)
Perphenazine (Trilafon)	3% OV-17	250	8 (8)	128 (48.0)
Piperacetazine (Quide)	3% OV-17	250	64 (0)	128 (33.5)

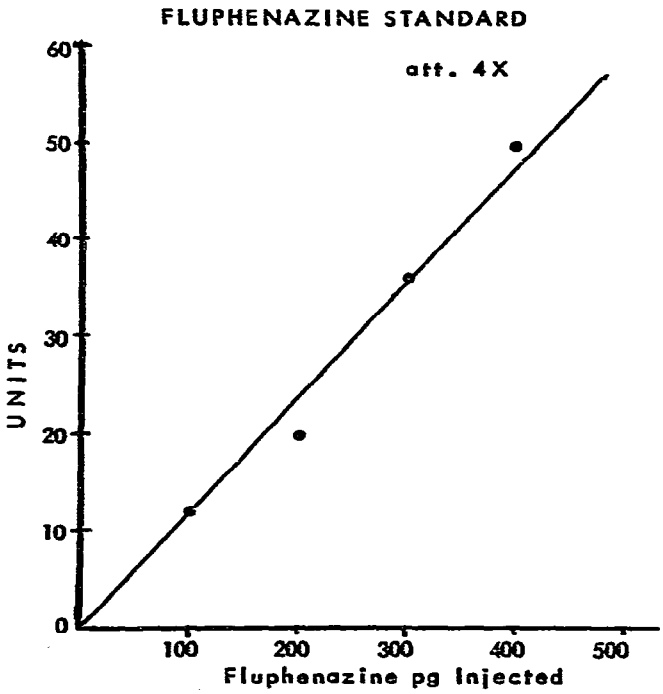

Fig. 3. Standard curve for fluphenazine.

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