Journal of Chromatography, 150 (1978) 263–265

CHROM. 10,511

Note

Separation of perphenazine, its sulphoxide and its probable phenolic metabolites by electron-capture gas–liquid chromatography

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(Received July 28th, 1977)

The metabolism of piperazine-substituted phenothiazines appears to resemble that previously reported for chlorpromazine (CPZ)[1–3]. These compounds are converted into the sulphoxide and into their ring-hydroxylated derivatives. Hydroxylation of various phenothiazine derivatives with widely differing ring and side-chain substituents has been shown to occur in rat-liver preparations[4]. Dreyfuss and Cohen[5] reported that the nature of the side-chain does not markedly influence the occurrence and degree of ring-hydroxylation in CPZ and piperazine-substituted phenothiazines. Several studies have demonstrated that 7-hydroxychlorpromazine, 8-hydroxychlorpromazine and 7,8-dihydroxychlorpromazine are *in vivo* metabolic products of CPZ in mammals[1,2,6–12] and are pharmacologically active[13,14]. It has also been established that hydroxylated CPZ metabolites penetrate the blood–brain barrier[15]. Thus, separation and specific assay techniques for these compounds in pharmacological, pharmacokinetic and related studies is important. Fluphenazine sulphoxide and 7-hydroxyfluphenazine have been identified as the major metabolites of fluphenazine in faeces from dogs and monkeys[5,16]. Perphenazine (PPZ) is a close structural homologue of fluphenazine and is therefore expected to be metabolized according to a similar pattern, *viz.*, formation of a sulphoxide and ring-hydroxylated metabolites.

Recently, Larsen and Naestoft[17] described a GLC method for the determination of PPZ and its sulphoxide (PPZSO) metabolite in human plasma. This communication is concerned with the description of modifications to the method of Larsen and Naestoft[17], resulting in the separation of three probable phenolic metabolites, PPZ and PPZSO.

EXPERIMENTAL

Reagents

The following reagents were used: Spectroanalyzed grade methanol and toluene (J. T. Baker Chemicals, Phillipsburg, N.J., U.S.A.), analytical grade *n*-butyl

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acetate (Fisher Scientific, Fair Lawn, N.J., U.S.A.) and a specially purified grade of N,O-bis-(trimethylsilyl)acetamide (BSA) (Pierce Chemicals, Rockford, Ill., U.S.A.).

Standards

Authentic standards of PPZ and PPZSO were supplied by Schering Corporation (Bloomfield, N.J., U.S.A.) and authentic 7-hydroxyperphenazine (7-OH-PPZ), 8-hydroxyperphenazine (8-OH-PPZ) and 7,8-dihydroxyperphenazine (7,8-diOH-PPZ) were provided by the National Institute of Mental Health (Rockville, Md., U.S.A.). Standard solutions of the reference substances (50 μg/ml) were prepared in methanol; these solutions were refrigerated and protected from light.

Gas chromatography

A Hewlett-Packard Model 5830A gas chromatograph equipped with a ^{63}Ni (15 mCi) electron-capture detector was used in this study; the instrument was linked to a digital integrator (H.P. 18850 A). The stationary phase was 1% of OV-17 on high-performance Chromosorb W (100–120 mesh) packed into a 1.8-m coiled glass column (I.D. 4 mm; O.D. 6.3 mm). The column was conditioned at 350° for 65 h with an argon–methane (19:1) carrier-gas flow-rate of 50 ml/min. The column was operated at 280°, the detector at 300° and the injection port at 295°. The flow-rate of carrier gas was 50 ml/min.

Derivatization

A 20-μl portion of each standard solution was mixed in a 3-ml conical glass-stoppered tube and evaporated to dryness at 45° with a slow stream of nitrogen. The residue was dissolved in 1.5 ml of toluene, and to this solution were added 50 μl of a mixture containing 10 μl of BSA in 1 ml of toluene. The mixture was agitated in a vortex-type mixer for 1 min, then heated for 15 min at 60° and evaporated to dryness at 70° with a slow stream of nitrogen. The wall of the tube was rinsed with 0.5 ml of toluene by agitation in the mixer for 1 min, and the solvent was evaporated to dryness as described above. The residue was dissolved in 50 μl of *n*-butyl acetate, and 1 μl of this solution was injected into the gas chromatograph.

RESULTS

A typical chromatogram showing the separation of PPZ, PPZSO and the three probable ring-hydroxylated metabolites (8-OH-PPZ, 7-OH-PPZ and 7,8-di-OH-PPZ) is illustrated in Fig. 1. The sensitivity of the detector towards each compound may be inferred from the fact that each peak corresponds to 20 ng. Under the conditions used, the retention times of 8-OH-PPZ, 7-OH-PPZ, 7,8-di-OH-PPZ and PPZSO, relative to PPZ, were 1.38, 1.50, 1.64 and 2.94, respectively. The method can be extended to the identification of the phenolic metabolites of PPZ in biological materials, and the good sensitivity of the method suggests its application to the determination of these compounds in such samples.

ACKNOWLEDGEMENTS

We thank Schering Corporation Ltd. for providing us with authentic perphenazine and its sulphoxide. We are very grateful to Dr. A. A. Manian, of the National

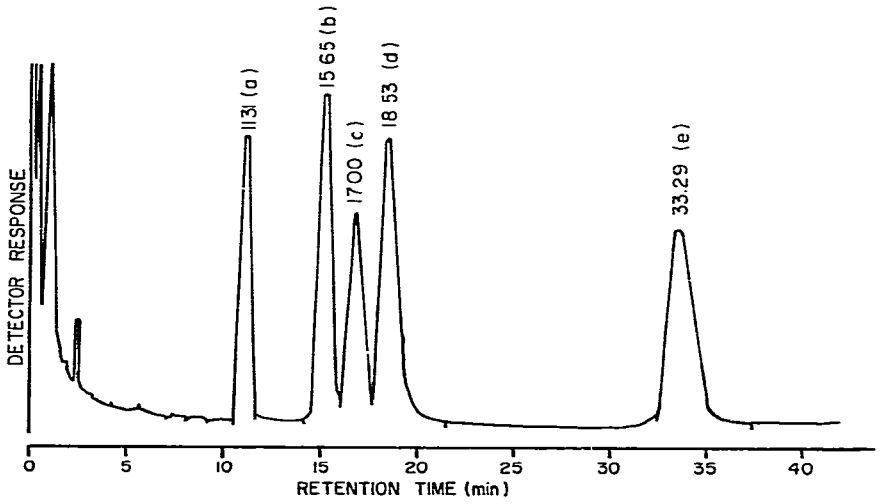

Fig. 1. Separation of a mixture of (a) PPZ, (b) 8-OH-PPZ, (c) 7-OH-PPZ, (d) 7,8-di-OH-PPZ, and (e) PPZSO.

Institute of Mental Health, for the kind gift of authentic samples of hydroxylated perphenazine compounds. Finally, we wish to acknowledge the expert technical assistance of Miss Angelique Bordeleau.

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