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Note

Chromatographic properties of imines formed from dedimethylchlorpromazine and various aldehydes and ketones

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Metabolites of chlorpromazine (CPZ) are of considerable importance in the action of the parent drug. A gas–liquid chromatographic (GLC) method for the assay of CPZ and some of its metabolites, notably demonomethylchlorpromazine (Nor_1CPZ) and dedimethylchlorpromazine (Nor_2CPZ) was published in 1968[1]. The columns used in this assay are of OV-17, and although a clear separation of CPZ, Nor_1CPZ and Nor_2CPZ was originally described, a more recent report has shown that effective separation of the two demethylated compounds is not always achieved[2]. It occurred to us that these compounds might be chromatographing as derivatives, formed by reaction of the amines with impurities in the amyl alcohol used in the extraction of CPZ and its metabolites from plasma, and this paper describes a full investigation of this possibility.

EXPERIMENTAL

CPZ (as hydrochloride) was a gift from May and Baker (Dagenham, Great Britain). The demethylated analogues were obtained from Dr. A. A. Manian (National Institute of Mental Health, Rockville, Md., U.S.A.). Primary standards were prepared by dissolving weighed quantities in methanol to give solutions of 1 mg/ml. Aldehydes and ketones were obtained commercially and were used without further treatment.

A Pye Model 104 gas chromatograph equipped with a 7 mCi Ni^{63} electron capture detector was operated in the pulsed voltage mode (49 V, 150 μsec pulse space). The glass column (7 ft. × 4 mm I.D.) was treated with dimethyldichlorosilane by standard methods and packed with 3% OV-17, on Chromosorb W HP (80–100 mesh) (laboratory prepared). High-purity nitrogen was the carrier gas with an inlet pressure of 35 p.s.i.g. Column and detector ovens were at 270° and 310°, respectively. Samples were injected directly on to the column.

Nor_2CPZ was diluted to 10 μg/ml in methanol. The aldehydes and ketones (0.1 ml of each) were added to samples (0.5 ml) of the dilution, mixed, and stood at room temperature for variable times. Aliquots (1–10 μl) were injected into the chromatograph. The retention time of the major GLC peak arising from each solution was measured for comparison with that of Nor_2CPZ.

Samples (0.1 ml) of the original solutions of CPZ, Nor_1CPZ and Nor_2CPZ were placed in one tube and evaporated together under a stream of nitrogen, and the residues were taken up in blood-bank plasma (10 ml). This solution was further diluted in plasma to give solutions for analysis. Plasma samples (1 ml) were assayed as previously described[1,2], except that (1) amyl alcohol previously shown not to react with Nor_2CPZ was used, (2) the final extraction solution contained either 15% amyl alcohol and 0.1% isovaleraldehyde in toluene or 15% amyl alcohol and 10% methyl ethyl ketone (MEK) in toluene.

RESULTS

Fig. 1A shows a GLC trace of a mixture of 40 ng of CPZ (a), Nor_1CPZ (b) and Nor_2CPZ (c) in methanol. While CPZ is readily separated from the other two compounds, the demethylated analogues overlap in such a way as to make their independent assessment impossible. This contrasts with the separation originally reported, when the solvent included amyl alcohol.

Fig. 1B shows a GLC trace of a mixture of the same materials with MEK added. Peaks a and b are apparently unchanged, c has disappeared and d has appeared. Sampling of the solution at invertals showed that the change occurred almost completely within 90 min of addition of MEK.

Fig. 1C shows a GLC trace of a mixture treated with the solvent from a bottle of amyl alcohol (stated to be principally isoamyl alcohol) which had been open for several months. Peak c again disappeared and this time e appeared at a retention time longer than that of d. This reaction required 1–2 h for completion.

Fig. 1D shows the GLC trace obtained when isovaleraldehyde was the additive. Again, the reaction required 1–2 h for completion. The retention times of peaks e and f are identical.

A series of new peaks appeared when various aldehydes and ketones were

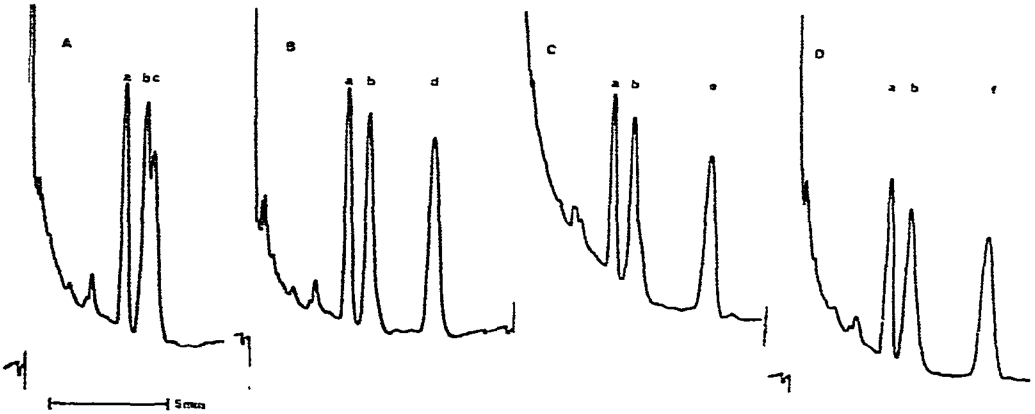

Fig. 1. GLC of (A) a solution containing (a) chlorpromazine, (b) Nor_1CPZ and (c) Nor_2CPZ; (B) the solution of (A) treated with MEK showing the presence of the imine (d); (C) the solution of (A) treated with amyl alcohol to produce peak (e); (D) the solution of (A) treated with 0.1% isovaleraldehyde to produce peak (f) which has a retention time identical to that of (e).

TABLE I

RELATIVE RETENTION TIMES OF THE PRINCIPAL PEAKS IN VARIOUS SOLUTIONS OF Nor_2CPZ AND A RANGE OF ALDEHYDES AND KETONES

Compound	*Relative retention time*
Aldehydes	
Acetaldehyde	0.98
n-Butyraldehyde	1.40
Isobutyraldehyde	1.15
n-Valeraldehyde	1.75
Isovaleraldehyde	1.52
Ketones	
Acetone	1.22
MEK	1.42
Diethyl ketone	1.67
Methyl propyl ketone	1.67
Methyl isopropyl ketone	1.49
Methyl butyl ketone	1.98
Methyl isobutyl ketone	1.76
Methyl amyl ketone	2.42
Methyl hexyl ketone	2.98

TABLE II

RECOVERIES OF CPZ, Nor_1CPZ AND Nor_2CPZ FROM PLASMA SOLUTIONS WHEN MEK OR ISOVALERALDEHYDE ARE ADDED

Recoveries are mean percentage values ± standard error (n = 4 or 5).

Strength of plasma solution (ng/ml)	*Additive*	*Recovery (%)*		
		CPZ	*Nor_1CPZ*	*Nor_2CPZ*
500	MEK	96.5 ± 5.3	98.8 ± 6.3	43.0 ± 1.7
500	Isovaleraldehyde	90.2 ± 2.1	82.2 ± 2.1	82.1 ± 1.3
200	Isovaleraldehyde	90.6 ± 4.8	67.2 ± 4.8	67.4 ± 5.2

used. In each case, Nor_2CPZ apparently disappeared and a new compound with a retention time longer than that of Nor_2CPZ appeared. The retention times of the principal peaks in each solution, relative to that of Nor_2CPZ are shown in Table I.

Plasma samples containing CPZ, Nor_1CPZ and Nor_2CPZ were extracted and assessed using dilutions of the primary standards, treated with MEK or isovaleraldehyde, the criterion of assessment being recovery, represented by the percentage of the amount originally present in the plasma solutions found in the final extracts after calculation corrections for solvent aliquots had been made. The recoveries are listed in Table II.

DISCUSSION

Nor_2CPZ reacted with a wide range of aldehyde and ketones. Nor_1CPZ showed retention time changes (not reported) only at very high concentrations of the aldehydes after long periods. Reaction of Nor_2CPZ was generally complete within 2 h.

Aldehydes and ketones condense with primary amines to form imines[3]:

$$RCHO + R'NH_2 \rightarrow RCH{=}NR' + H_2O \qquad (a)$$

$$RCOR' + R''NH \rightarrow RR'C{=}NR'' + H_2O \qquad (b)$$

Aldehydes with an α-hydrogen will also react with secondary amines to produce enamines:

$$RCH_2CHO + R_2NH \rightarrow RCH{=}CH\text{–}NR_2' + H_2O$$

The primary amine Nor_2CPZ apparently reacts with aldehydes and ketones to form imines which are stable to the conditions of GLC and produce good shaped peaks with little tailing. As may be seen in Table I, the retention time of the peak may be manipulated by choosing different aldehydes and ketones. In certain cases, where a biological extract of the primary amine gave rise to a trace with interfering peaks, the carbonyl compound could be chosen to give a retention time corresponding to a part of the trace relatively free of peaks. The production of imines allows the separation of Nor_2CPZ from Nor_1CPZ and the quantification of these compounds after they have been extracted from plasma. The aldehyde or ketone chosen for this separation may be varied depending on the problems encountered. For example, since the analysis of chlorpromazine and its metabolites involves the extraction of the compounds into a small volume of solvent instead of the more customary practice of solvent evaporation and re-solution of the residue, the aldehyde or ketone must be immiscible with water, *i.e.* acetone cannot be used (although the imine formed between Nor_2CPZ and acetone was discussed in the original paper[1]).

Of the ketones tested, MEK appears at first sight to be the most promising. It reacted readily with pure solutions of Nor_2CPZ to produce a compound which gave a good peak shape with a convenient retention time. It does not affect the recovery of CPZ or Nor_1CPZ but when added to the final extraction solvent only partially reacted with Nor_2CPZ. However, since amyl alcohol is used in the assay and is known to give peaks which can be used to quantify the Nor_2CPZ content, the obvious choice is isovaleraldehyde. This has the advantage that there will be no complications caused by Nor_2CPZ reacting with any isovaleraldehyde present in the amyl alcohol. Care must be taken to ensure that the concentration of isovaleraldehyde is such that it will not form an enamine with Nor_1CPZ. Adding 0.1% isovaleraldehyde to the 15% amyl alcohol–toluene solution appears to give a suitable concentration.

Imine formation has been exploited in the assay of primary amine catecholamines, when pentafluorobenzaldehyde was chosen to render the products suitable for electron capture detection[4].

This paper has been concerned with the assay of Nor_2CPZ. However, amyl alcohol (principally isoamyl alcohol) is widely used as an additive to solvents used in assays of amine drugs. Since our samples apparently contained isovaleraldehyde, it is reasonable to suppose that other samples will also contain this impurity. The implications for a wide range of drug assays are obvious.

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