A note on the assay of chlorpromazine *N*-oxide and its sulphoxide in plasma and urine

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The amine oxide of chlorpromazine (chlorpromazine *N*-oxide: CPZNO) is an important metabolite (Beckett & Hewick, 1967; Beckett, Gorrod & Lazarus, 1971; Beckett, Essien & Franklin Smyth, 1974). Furthermore, the oxides of the primary and secondary amine analogues of chlorpromazine have been studied, although no reports have concerned the presence of any of these compounds in plasma. All phenothiazines can form sulphoxides, and this note concerns CPZNO, and its sulphoxide (CPZNOSO), in biological fluids.

A number of techniques have been applied to the assay of metabolites of chlorpromazine, chlorpromazine *N*-oxide (CPZNO) and chlorpromazine *N*-oxide sulphoxide (CPNZOSO), polarography being the most sensitive (Beckett & others, 1974), while the standard method for assay of chlorpromazine (CPZ) in plasma is heptane extraction and gas-chromatography (Curry, 1968, 1974). A problem in applying g.l.c. is decomposition of CPZNO to CPZ, demonomethylchlorpromazine, and the deaminated derivative *N*-alkyl-2-chlorophenothiazine, which occurs at 120°, well below the optimum g.l.c. temperature (Craig, Mary & Roy, 1964). In an analogous way, CPZNOSO forms sulphoxides of these compounds.

CPZNO and CPZNOSO are extractable from aqueous solutions into ether but not into heptane, and are reduced to CPZ and chlorpromazine sulphoxide (CPZSO) respectively when heated, or when treated with $Na_2S_2O_5$ or Fe (II) ions (e.g. as ferrous sulphate). Sulphoxides and amine oxides are both reduced by Ti (III) ions (e.g. as titanous chloride). No other known chlorpromazine metabolites regenerate CPZ when treated in this way. Thus it seemed that a simple g.l.c. assay of CPZNO and CPZNOSO might involve separation of plasma or urine samples into two equal fractions, reduction of any CPZNO and CPZNOSO present in one sample from each pair with $Na_2S_2O_5$, assay for CPZ and CPZSO by the standard g.l.c. method, and calculation of the original CPZNO and CPZNOSO by difference.

Accordingly, plasma and urine samples from three sources were studied. The samples were heparinized plasma (by venepuncture), and urine, from: (i) 10 acutely treated, responding schizophrenics previously untreated receiving 100 mg chlorpromazine 3 times daily for 36 days, samples being collected throughout the 36 days; (ii) 8 patients undergoing long-term treatment (more than 6 months) at low doses (20–25 mg three times daily); and (iii) 20 patients during and after long-term treatment (more than 2 years) at higher doses (50–200 mg, 3 times daily). (For clinical studies see Curry, Evans & others 1974; Loga, Curry & Lader, 1975.) All plasma samples were collected just before, or 2–4 h after routine oral doses of chlorpromazine. Urine samples were from 24 h samples. Blood-bank plasma, and urine contributed by a laboratory worker, were used for standards and blanks.

Samples were studied singly or as pooled samples (10 ml) of each type. The samples were studied in two portions. One portion of each sample was assayed for CPZ and CPZSO as described previously. To the other was added 1 ml of $Na_2S_2O_5$ solution (M) and 0·5 ml 2 N HCl. After standing the sample for 15 min, this tube was also assayed for CPZ and CPZSO by the established method. Calibration of the chromatograph was by means of the CPZ and CPZSO signals obtained from extracts of blank plasma and urine to which had been added CPZNO and CPZNOSO in concentrations ranging from 0–250 ng ml^{-1}. The blanks, recovery, accuracy and precision of this approach were as described in the original CPZ/CPZSO reports.

Fig. 1 shows g.l.c. traces obtained from 5 μl extracts of reduced and non-reduced urine from samples of type

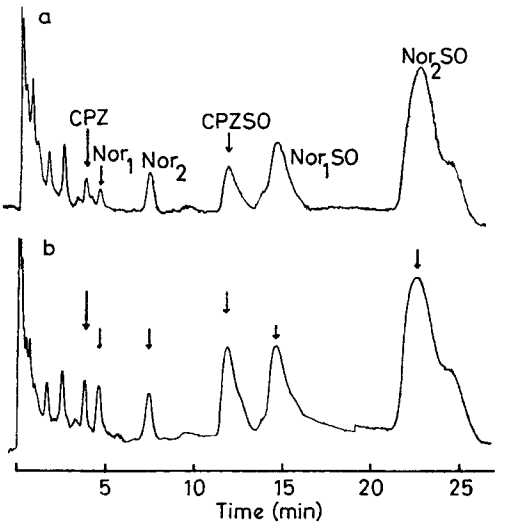

FIG. 1. G.l.c. traces of two 5 μl extracts of the same urine sample, one untreated (a), and one following reduction (b) with sodium metabisulphite. Abbreviations: CPZ, chlorpromazine; Nor_1 and Nor_2 indicate demonomethyl- and dedimethyl- respectively; CPZSO, chlorpromazine sulphoxide; SO, sulphoxide. Conditions as described previously.

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(iii) (above). CPZ and CPZSO and their demethylated analogues were clearly identified (by reference to standards) in the extract of the non-reduced sample. The extract from the reduced sample showed increased signals for CPZ and CPZSO, indicating that CPZNO and CPZNOSO were originally present. Additionally, there was an increase in the demonomethylchlorpromazine signal, and this was found to result from partial conversion of CPZNO to this compound when treated with $Na_2S_2O_5$, by g.l.c. and by thin-layer chromatography on silica gel using the standard method. This conversion, which yields formaldehyde, appears not to have been previously reported. It has obvious implications for CPZNO assays. In this case, standards and tests were assayed in parallel and a similar degree of conversion occurred in each case.

As implicit in Fig. 1, CPZNO and CPZNOSO were both found in urine. Concentrations of these compounds in all three patient groups ranged from 0·07 to 0·11 μg ml^{-1}, which was about the range of unmetabolized CPZ and CPZSO in the same urine samples. Concentrations of CPZNO in plasma were lower while no CPZNOSO was detected. Patients in group (iii) showed no CPZNO, no increase in the CPZ signals being detected even when a 10 ml pooled plasma sample was studied. In the other groups, CPZNO concentrations were 5·6–7·3 ng ml^{-1}, one third to one half of the CPZ concentrations 11·3–23·2 in the same patients. Thus CPZNO is sometimes present in plasma during treatment, although it appears to be cleared quite rapidly.

CPZNO is also extractable from plasma into ether, from which it is back extractable into 0·1N HCl. When present, it will therefore appear as CPZ and as desmonomethylchlorpromazine in g.l.c. traces of ether extracts of plasma designed specifically to detect CPZ.

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The compression properties of magnesium and calcium carbonates

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Tablets are traditionally made by either wet or dry granulation, but both methods are expensive in time, labour, space, equipment and power. Direct compression, which consists only of mixing and compaction stages, offers economies in all these areas, but depends for success upon certain properties of the final mixture. The technique, extensively reviewed by Livingstone (1970), Kanig (1970) and Khan & Rhodes (1973a), has, however, the disadvantage of requiring commercially available materials which are expensive compared with the more traditional tablet fillers. For this reason the compressional properties of several substances, traditionally found in development laboratories, were studied. This paper discusses the tableting properties of magnesium and calcium carbonates.

Materials: heavy magnesium carbonate, B.P. (Newalls Insulation Ltd, Washington, Co. Durham), magnesite, naturally occurring anhydrous magnesium carbonate, (Hopkins and Williams, Chadwell Heath, Essex) and heavy calcium carbonate (J. and E. Sturge Ltd, Birmingham). The heavy magnesium carbonate had a mean particle size of 55 μm. The magnesite was finer, 75% being less than 32 μm. The calcium carbonate —the coarsest grade available, had a mean particle size of 35 μm. These materials were blended with 5% sodium starch glycollate and 0·5% magnesium stearate and compressed on an instrumented single punch tableting machine (F3, Manesty Machines Ltd, Speke) fitted with 3/8 inch flat-faced punches. The instrumentation consisted of a four arm, semi-conductor strain gauge bridge (Kulite Semiconductor Ltd, Basingstoke) bonded onto the upper eccentric arm of the tableting machine. A constant direct current was applied from a bridge supply and balance unit (Fylde Electronic Laboratories Ltd, Preston), and the signal from the bridge was recorded using a storage oscilloscope (5103N series, Tektronix U.K. Ltd, Harpenden).

Tablet crushing strength was measured using an Erweka tester (Erweka-Apparatebau G.m.b.H., Offenbach, West Germany). Friability was measured by rotating the tablets 1000 times in a stainless steel

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