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# A TLC fluorescence derivatization assay for chlorpromazine and its non-conjugated hepatic microsomal metabolites

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**Abstract**: Procedures for the assay of chlorpromazine (CPZ) and its metabolites were compared. An improved assay, involving organic extraction, thin-layer chromatography and fluorescence derivatization, was developed. The compounds were extracted from microsomal protein suspensions into 15% *n*-propanol in dichloromethane by successive extractions at pH 2 and pH 12. CPZ and its mono- and di-desmethyl, 7-hydroxy, *N*-oxide, sulphoxide and *N*-oxide-sulphoxide metabolites were separated using TLC on silica gel developed with methanol–acetone–ammonia (50:50:1 v/v/v). The compounds were extracted from the TLC plate with chloroform–methanol (2:1 v/v) and subjected to oxidation with hydrogen peroxide. The highly fluorescent oxidized derivatives were identified as sulphoxides by their fluorescence characteristics. Amounts of derivatized CPZ and metabolites were measured using derivatized promazine as the internal standard.

**Keywords**: *Chlorpromazine; drug metabolism; thin layer chromatography; fluorescence derivatization; sulphoxides.*

## Introduction

Several assay procedures have been published for chlorpromazine (CPZ) and its metabolites. They include colorimetry [1], gas–liquid chromatography [2, 3], thin-layer chromatography (TLC) with UV [4] or radioisotope [5] detection, polarography [6], radioimmunoassay [7] and high-performance liquid chromatography with UV detection [8, 9]. There are also several assays based on fluorimetry. CPZ shows a weak intrinsic fluorescence that is increased slightly in alkaline solutions or at low temperatures [10], but various methods have been reported for converting CPZ and its metabolites into more intensely fluorescent derivatives [10–14].

In the course of a study of the metabolism of chlorpromazine by hepatic microsomal monooxygenases, it became apparent that there are serious limitations in all the available assays for the Phase I (oxidative) metabolites of chlorpromazine. The GC-ECD

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assay suffers from unpredictable detector sensitivity when assaying CPZ in biological material, and from an inability to detect the thermally unstable N-oxide metabolite [15]. The different fluorescence derivatization procedures are each unable to measure certain metabolites of interest (see Results). An improved and reliable procedure is described for the extraction, TLC separation and fluorescence derivatization of CPZ and its major oxidative metabolites.

## Experimental

### Materials

All chemicals and solvents were of analytical reagent grade, purchased from BDH Chemicals Ltd., Poole, UK, except for the following: hydrogen peroxide (laboratory grade, May & Baker, Dagenham, UK), dansyl chloride, NADP, DL-isocitric acid and isocitrate dehydrogenase (Sigma Chemical Co., Poole, UK) and methanol (HPLC grade, Rathburn Chemicals, Peeblesshire, Scotland, UK). Aluminium-backed silica gel TLC plates with fluorescent indicator (20 cm × 20 cm × 0.2 mm; Ref. No. 5554) were from E. Merck, Darmstadt, W. Germany.

CPZ and its metabolites were generous gifts from Dr A. A. Manian, Psychopharmacology Section, National Institute of Mental Health, Rockville, Maryland, USA. Promazine was kindly provided by Dr J. B. Houston, Department of Pharmacy, University of Manchester.

### Assay procedure for CPZ and metabolites

A 2 ml portion of a hepatic microsomal incubate of CPZ (see Fig. 1) was adjusted to pH 2 by addition of 100 μl 5N $H_2SO_4$; then 5 ml of 15% n-propanol in dichloromethane (DCM) and 50 μl of the internal standard, promazine (4 mM aqueous solution), were added. The solvent and aqueous phase were mixed by rotation at 60 rpm for 15 min and subsequently separated by centrifugation at 5000 rpm for 5 min in a bench centrifuge (MSE Super Minor). The upper aqueous layer and proteinaceous interface were transferred to a fresh tube and brought to pH 12 by the addition of 500 μl 2.5 N NaOH.

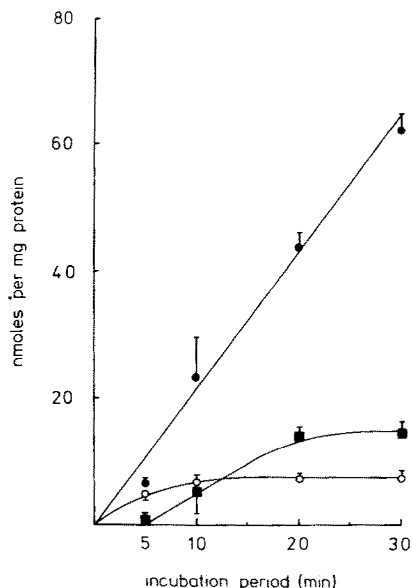


**Figure 1**
Time course for the metabolism of chlorpromazine by rat hepatic microsomes. Rats (male Sprague–Dawley, 250–280 g) were killed and their intact livers transferred to ice-cold buffer (0.15 M KCl in 0.01 M phosphate buffer, pH 7.4). Liver microsomes were prepared [26], and protein concentrations were measured [27]. Reaction mixtures, volume 2 ml in 0.1 M phosphate buffer pH 7.4, were prepared at 0°C in 12 ml polypropylene test-tubes, and contained 0.5 μmol NADP, 5 μmol DL-isocitric acid, 0.6 U isocitrate dehydrogenase, 10 μmol magnesium sulphate, 0.2 μmol chlorpromazine HCl and 1.5 mg microsomal protein. Reactions were initiated at 37°C with shaking and terminated by adding 100 μl 5N $H_2SO_4$. Promazine (50 μl of a 4 mM aqueous solution) was then added to each test-tube and extraction, TLC separation and fluorimetric quantitation performed. Each point (CPZNO (●), $Nor_1$CPZ (■), 7OHCPZ (○)) is the mean ± SD of three observations.

This alkaline aqueous phase was re-extracted with a further 5 ml of the same organic solvent as before. The final aqueous phase was discarded and the two organic phases were pooled and evaporated to dryness using a Büchler vortex evaporator (55°C, vortex setting 3).

The residue was dissolved in 25 μl of a mixture of chloroform–methanol (2:1 v/v) and applied to the TLC plates, which were developed in acetone–methanol–ammonia, sp. gr. 0.88 (50:50:1 v/v/v) under vapour-saturation conditions. CPZ, its metabolites and the internal standard (promazine) were located as background quenching spots under short wavelength UV light, and identified by their $R_f$ values. Each spot was cut out and sliced into small pieces into a separate polypropylene test-tube and 4 ml of chloroform–methanol (2:1 v/v) added. CPZ, metabolite or internal standard were extracted from the silica gel by rotation for 15 min at 60 rpm, followed by centrifugation for 5 min at 5000 rpm. A 3 ml aliquot of the solvent was transferred to a fresh tube and evaporated to dryness using the vortex evaporator (45°C, vortex setting 3). CPZ and its 7-hydroxy metabolite (7OHCPZ) were incompletely separated by the TLC system and were cut out and extracted from the TLC plate together. Similarly, CPZ sulphoxide (CPZSO) and promazine co-chromatographed and were removed and extracted together. Individual measurement of these co-extracted compounds was achieved by fluorimetry, as described below.

The residues were worked-up by one of three procedures: (i) The three residues containing individually separated CPZ-*N*-oxide (CPZNO), monodesmethyl CPZ ($Nor_1CPZ$) and didesmethyl CPZ ($Nor_2CPZ$) respectively were dissolved in 3 ml water and 100 μl 30% $H_2O_2$ added. Each tube was covered with a stopper pierced by a small hole and oxidatively derivatized for 10 min at 90°C in a water bath. After cooling to room temperature (important) the resulting fluorescence was measured using a Perkin–Elmer 3000 fluorescence spectrometer at an excitation wavelength ($\lambda_{ex}$) of 340 nm and an emission wavelength ($\lambda_{em}$) of 380 nm. (ii) The residue containing both CPZSO and the internal standard, promazine, was similarly dissolved in 3 ml water, but the fluorescence of the solution ($F_1$) ($\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm) was initially measured before either addition of $H_2O_2$ or heating. $H_2O_2$ (100 μl of 30%) was then added, derivatization performed as above, and a 300 μl portion of the solution transferred to a cuvette and diluted with 2.7 ml water. The fluorescence ($F_2$) was again measured at $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm. (iii) The residue containing both CPZ and 7OHCPZ was dissolved in 3 ml 20% glacial acetic acid in ethanol and 100 μl 30% $H_2O_2$ added. The solution was derivatized as above, cooled to room temperature and the fluorescence read twice, using $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm ($F_3$) and $\lambda_{ex}$ 360 nm, $\lambda_{em}$ 420 nm ($F_4$) respectively. Post-derivatization dilution was used for samples containing high concentrations of CPZ or metabolites (see Results). Fluorimeter excitation and emission bandwidths were set at 2.5 nm.

From the fluorescence of each compound was subtracted the fluorescence of a microsomal reagent blank. This was a suspension of boiled microsomes without CPZ, authentic metabolites or internal standard, assayed as described above. The extract of the microsomal reagent blank was taken through the TLC step of the assay and areas of the TLC plate corresponding in $R_f$ to CPZ and each of its metabolites were removed for derivatization etc. Each of these areas furnished the blank for the corresponding CPZ derivative and promazine; the blank fluorescence was different for each compound.

*Calculation of CPZ and metabolite concentrations*

The fluorescence due to the oxidized promazine after correction for the fluorescence

of CPZSO, and the fluorescence due to oxidized 7OHCPZ after correction for the fluorescence of oxidized CPZ, were calculated as follows:

Corrected promazine fluorescence ($F_p$) = $10F_2 - F_1$

Corrected 7OHCPZ fluorescence = $F_4 - 0.02F_3$,

where $F_1$ = CPZSO fluorescence (pre-derivatization: $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm),
$F_2$ = combined fluorescence of oxidized promazine + CPZSO (post-derivatization: $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm),
$F_3$ = CPZ fluorescence (post-derivatization: $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm),
$F_4$ = combined fluorescence of oxidized 7OHCPZ + CPZ (oxidized) (post-derivatization: $\lambda_{ex}$ 360 nm, $\lambda_{em}$ 420 nm).

The concentrations of CPZ and its metabolites were calculated from the ratios of the respective fluorescence values for CPZ, $Nor_1CPZ$, $Nor_2CPZ$, CPZNO and CPZSO and the corrected value for 7OHCPZ, relative to the corrected fluorescence of promazine, using standard curves (prepared daily) as described below. The internal standard technique conserved the scarce samples of authentic CPZ metabolites.

## Results and Discussion

*Extraction of CPZ and its metabolites from microsomal protein suspensions*

CPZ, $Nor_1CPZ$ and 7OHCPZ, chosen as representing the parent drug and its basic and acidic metabolites respectively, were extracted from separate microsomal protein suspensions once by 15% *n*-propanol in DCM. The extraction residues were each redissolved in solvent and assayed fluorimetrically for CPZ, $Nor_1CPZ$ and 7OHCPZ without using the TLC stage of the complete assay.

The extraction of CPZ, $Nor_1CPZ$ and 7OHCPZ from microsomal protein suspensions was highly dependent on the pH of the aqueous phase (Table 1). The highest recovery of CPZ occurred at pH 2 over the range of CPZ concentrations $10^{-5}$–$10^{-3}$M. However, no single pH value allowed optimal extraction of all three compounds: $Nor_1CPZ$ was also extracted best at an acidic pH, whereas 7OHCPZ required a neutral or alkaline pH. Furthermore, the expectation that an alkaline medium should favour the extraction from microsomal incubates of CPZ [5, 16, 17], which is a basic drug with $pK_a = 9.3$ [18], was not borne out. The procedure described (extraction at both acid and alkaline pH) was therefore adopted for the routine extraction of CPZ

**Table 1**
The effect of pH on the organic extraction of chlorpromazine, monodesmethylchlorpromazine and 7-hydroxychlorpromazine from suspensions of microsomal protein

Percentage extraction (mean ± SD) by 15% *n*-propanol in dichloromethane was calculated by comparison with the $H_2O_2$-derivatized fluorescence of equal unextracted quantities of CPZ, $Nor_1CPZ$ and 7OHCPZ. The concentrations of the compounds were CPZ, 150 μM; $Nor_1CPZ$, 37.5 μM; 7?HCPZ, 37.5 μM.

| pH | CPZ | $Nor_1CPZ$ | 7OHCPZ |
|---|---|---|---|
| 9.3 | 33.1 ± 2.4 (*n* = 6) | 69.0 ± 2.8 (*n* = 3) | 94.5 ± 6.5 (*n* = 3) |
| 7.4 | 47.0 ± 13.5 | 38.2 ± 1.7 | 104.0 ± 1.9 |
| 2.0 | 100.2 ± 3.0 | 97.4 ± 0.9 | 39.9 ± 5.1 |

and its metabolites from microsomal protein suspensions. Ballard and Tobin [19] have similarly found that organic extraction of CPZ from equine plasma is enhanced at low pH. This is in contrast to CPZ extraction from whole rat blood, which was found to be greatest at a basic pH (Blanchard *et al.*, unpublished work).

*TLC separation of CPZ and its metabolites*

Several TLC solvent systems have been reported to separate CPZ and its major oxidative metabolites on silica gel plates. Eight systems were evaluated [16, 17, 20–25] and methanol–acetone–ammonia (50:50:1 v/v/v) [25] was found to give the best separation between CPZ ($R_f$ = 0.59), $Nor_1CPZ$ ($R_f$ = 0.31), $Nor_2CPZ$ ($R_f$ = 0.76), 7OHCPZ ($R_f$ = 0.55), CPZSO ($R_f$ = 0.43), CPZNO ($R_f$ = 0.22) and CPZNO-sulphoxide (CPZNOSO) ($R_f$ = 0.13): it was therefore chosen for the assay procedure described above.

*Elution from silica gel TLC plates*

CPZ (30 nmol) and authentic $Nor_1CPZ$, $Nor_2CPZ$, 7OHCPZ and CPZSO (15 nmol each) were spotted separately on to TLC plates and extracted without chromatography, in order to test the efficiencies of four extraction solvents, *viz.* (i) chloroform–methanol (2:1 v/v), (ii) ethanol, (iii) methanol, and (iv) ether. The mixture of chloroform and methanol gave the highest recoveries, *viz.* CPZ: 90.7 ± 5.6%, $Nor_1CPZ$: 95.2 ± 6.2%, $Nor_2CPZ$: 85.4 ± 8.5%, 7OHCPZ: 75.4 ± 3.0% and CPZSO: 96.8 ± 8.7% (mean ± SD, $n$ = 3). CPZNO was not tested owing to an insufficiency of this compound.

*Fluorescence derivatization procedures for CPZ and its metabolites*

Although fluorescence derivatization of *N*-demethylated and hydroxylated CPZ metabolites using dansyl chloride has been reported [11], the method was found to be unsuitable for 7OHCPZ. Furthermore, this method cannot be used to quantitate CPZSO, CPZNO or CPZ, which do not react with the dansyl group. Fluorescence derivatization of CPZ and CPZSO has been achieved using 9-bromomethylacridine [12], but *N*-oxide and desmethyl metabolites of CPZ do not possess the necessary tertiary amine group. The use of fluorescein isothiocyanate is restricted to the desmethyl metabolites of CPZ [13]. The original report [14] has been confirmed that $KMnO_4$ is useful as an oxidative derivatizing agent for CPZ, but it is ineffective with 7OHCPZ (Blanchard *et al.*, unpublished work).

*Hydrogen peroxide oxidation*

Hydrogen peroxide ($H_2O_2$) has been used to oxidize CPZ and other phenothiazines to highly fluorescent products [10]. This method of CPZ oxidation has been improved, applied for the first time to some of the major CPZ metabolites and also used for promazine, chosen as the internal standard for the assay.

A major problem with the ethanol–acetic acid–$H_2O_2$ oxidation method [10] was a high reagent blank fluorescence at the wavelength optima for oxidized CPZ and $Nor_1CPZ$ ($\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm), arising from the ethanol. Substitution of water for ethanol not only reduced the background fluorescence, but also made the inclusion of acetic acid superfluous. An ethanol-free procedure was consequently adopted for oxidation of the non-hydroxylated metabolites of CPZ and for promazine, as described above. 7OHCPZ was not oxidizable in the ethanol-free medium. However, since the blank fluorescence of the ethanol–acetic acid-reagent was low at the wavelength optima for oxidized 7OHCPZ

($\lambda_{ex}$ 360 nm, $\lambda_{em}$ 420 nm), modified ethanolic–acetic acid reaction conditions were used for 7OHCPZ. These conditions were also used for CPZ, which was perforce derivatized with 7OHCPZ in the assay. Addition of 100 μl 30% $H_2O_2$ per 3 ml reaction was optimal for the oxidation of CPZ at all concentrations up to 200 μM.

CPZSO possessed a high intrinsic fluorescence that was not altered by $H_2O_2$ and was therefore assayed without oxidative derivatization. The excitation and emission fluorescence spectra of $H_2O_2$-oxidized CPZ, $Nor_1$CPZ, $Nor_2$CPZ, CPZNO and promazine and of non-oxidized, authentic CPZSO, $Nor_1$CPZ sulphoxide and $Nor_2$CPZ sulphoxide were all very similar, suggesting that oxidation of CPZ, $Nor_1$CPZ, $Nor_2$CPZ, CPZNO and promazine by $H_2O_2$ resulted in formation of the highly fluorescent sulphoxide derivatives (Table 2). It is likely that $H_2O_2$-oxidation of 7OHCPZ also

**Table 2**
Fluorescence wavelengths and intensities of chlorpromazine, its metabolites and promazine before and after oxidation with $H_2O_2$

| Compound | Before oxidation | | | After oxidation | | |
|---|---|---|---|---|---|---|
| | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) | Relative fluorescence | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) | Relative fluorescence |
| CPZ | 338 | 452 | 4 | 340 | 380 | 64 |
| $Nor_1$CPZ | 330 | 460 | 4 | 340 | 380 | 66 |
| $Nor_2$CPZ | 340 | 452 | 4 | 340 | 380 | 62 |
| CPZNO | 342 | 458 | 4 | 340 | 380 | 58 |
| 7OHCPZ | 346 | 460 | 2 | 360 | 420 | 68 |
| CPZSO | 340 | 380 | 63 | 340 | 380 | 61 |
| $Nor_1$CPZSO | 340 | 380 | 61 | 340 | 380 | 61 |
| $Nor_2$CPZSO | 340 | 380 | 69 | 340 | 380 | 66 |
| 7OHCPZSO | 360 | 420 | 77 | 360 | 420 | 63 |
| Promazine | 334 | 452 | 22 | 340 | 380 | 134 |

Solutions (5 μM) were in ethanol containing 20% glacial acetic acid (7OHCPZ and 7OHCPZSO) or in water.

occurred at the sulphur atom, for the fluorescence properties of its $H_2O_2$-oxidation product were identical to those of authentic 7OHCPZ sulphoxide, although different from the spectra of the sulphoxides of CPZ and its other metabolites (Table 2). The fluorescence developed showed a linear correlation with the concentration of CPZ, 7OHCPZ and $Nor_1$CPZ over the range 0–25 μM (abscissa and ordinate scales 0–25 μM and 0–60 fluorescence units respectively; correlation coefficients ranged from 0.99 to 1.00; intercepts ranged from 0.39 to 0.86; slopes varied from 1.79 to 2.18). Accurate measurement of higher concentrations (> 25 μM) of CPZ, its metabolites or promazine was achieved by diluting the samples after oxidation, with either the ethanol–acetic acid reagent (for CPZ and 7OHCPZ) or water (for all other compounds). The fluorescence of CPZ, 7OHCPZ and $Nor_1$CPZ developed after 10 min $H_2O_2$-oxidation was stable thereafter in the oxidizing medium, at 80°C under continuous excitation light, for at least 1 h. The fluorescence of $H_2O_2$-oxidized CPZ was stable for 18 h in the oxidizing medium under continuous excitation light at room temperature.

*Fluorimetric measurement of CPZSO in the presence of promazine and of CPZ in the presence of 7OHCPZ*

Because a normal TLC system did not adequately separate either CPZSO from promazine, or CPZ from 7OHCPZ, fluorimetry was used to differentiate between each

of these pairs of compounds. Fluorimetry was found to be much less time-consuming and more economical than the use of two-dimensional TLC to resolve these compounds. The TLC spot containing both CPZSO and promazine was eluted and the combined fluorescence measured both before and after derivatization. The fluorescence of promazine before derivatization was negligible compared with that of CPZSO at the wavelengths used ($\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm). The fluorescence of CPZSO ($F_1$) alone was directly proportional to concentration over the range 0–50 μM (correlation coefficient = 1.00) and was unaffected by the presence of up to 150 μM promazine. The fluorescence ($F_2$) of the post-derivatization solution was due to both CPZSO and oxidized promazine. Consequently, fluorescence due to oxidized promazine alone ($F_p$) was calculated by subtracting $F_1$ from $F_2$, taking into account the 10-fold post-derivatization dilution. $F_p$ was directly proportional to promazine concentration in the range 0–150 μM (correlation coefficient = 1.00).

The TLC-extraction residue containing both CPZ and 7OHCPZ was derivatized as described above. Since the oxidation products of CPZ and 7OHCPZ had different fluorescence characteristics, it was possible to differentiate between them. The fluorescence of the post-derivatization solution was measured consecutively at $\lambda_{ex}$ 340 nm, $\lambda_{em}$ 380 nm (fluorescence reading = $F_3$); and at $\lambda_{ex}$ 360 nm, $\lambda_{em}$ 420 nm (fluorescence reading = $F_4$). $F_3$ was directly proportional to the concentration of CPZ up to 150 μM and was unaffected by the presence of 7OHCPZ at concentrations up to 10 μM. However, the fluorescence of oxidized CPZ at the wavelengths used for 7OHCPZ (CPZ fluorescence reading = $F_5$) interfered with the measurement of oxidized 7OHCPZ. $F_5$ was directly proportional to the magnitude of $F_3$ at CPZ concentrations up to 150 μM ($F_5$ = 0.02 $F_3$; correlation coefficient = 1.00). Consequently, $F_5$ was calculated from $F_3$ and subtracted from $F_4$, to give a corrected measurement of 7OHCPZ.

*Internal standard fluorescence ratio curves*

The fluorescence ratios for CPZ and each metabolite are defined as the fluorescence of $H_2O_2$-oxidized CPZ or metabolite divided by the fluorescence of the $H_2O_2$-oxidized internal standard, promazine. In the exceptional case of CPZSO the pre-derivatization fluorescence, which was not increased by further oxidation, was divided by the fluorescence of $H_2O_2$-oxidized promazine. Standard curves were drawn of the fluorescence ratio versus the concentration (in a boiled microsomal suspension) of either CPZ or an authentic metabolite (analogous to the peak height ratio curves commonly used in gas and liquid chromatography). These standard fluorescence ratio curves showed a linear correlation with the concentrations of CPZ or authentic metabolites (7OHCPZ, CPZSO, CPZNO, $Nor_1CPZ$ and $Nor_2CPZ$) over the range 0–400 μM (ordinate scale 0–0.7; correlation coefficients from 0.98 to 1.00; intercepts from 0.00 to 0.13; slopes from 3.48 to 8.34). The amount of promazine added to each boiled microsomal suspension was 0.2 μmol.

*Recoveries, precision and detection limits of the assay*

Recoveries of CPZ (300 μM) and metabolites (37.5 μM each) taken through the complete assay procedure were: CPZ 73.0 ± 3.1%, $Nor_1CPZ$ 97.4 ± 6.4%, $Nor_2CPZ$ 39.1 ± 5.9%, 7OHCPZ 64.0 ± 10.2%, CPZSO 93.1 ± 7.8% (mean ± SD, $n$ = 3). The recovery of CPZNO was not measured owing to an insufficiency of the compound. The recovery of promazine (1.5 μM) was 89.6 ± 1.9%. Recovery calculations were based on

actual fluorescence values for each compound and did not use the fluorescence ratio standard curves. The detection limits of the assay for CPZ and metabolites in microsomal protein suspensions were: CPZ, 10 nmol; CPZNO, 9 nmol; $Nor_1CPZ$, 13 nmol; CPZSO, 7 nmol; 7OHCPZ, 6 nmol; and $Nor_2CPZ$, 13 nmol. These values are the amounts of the compounds, present in 2 ml microsomal suspensions, that gave a fluorescence reading twice that of a microsomal reagent blank after being taken through the complete assay procedure. The sensitivity of the assay can be considerably increased if necessary, by decreasing the volume of the derivatization reaction and using microcuvettes for fluorimetry.

*Microsomal metabolism of CPZ*

The new assay procedure was developed for a study of the hepatic microsomal metabolism of CPZ (to be reported in detail elsewhere). In order to demonstrate the applicability of the assay, the time course for the metabolism of CPZ by rat liver microsomes is shown in Fig. 1. The major enzymatic product was the *N*-oxide of CPZ. Its formation was linear with respect to time up to at least 30 min. Both $Nor_1CPZ$ and 7OHCPZ were also produced, but in neither case was the formation linear with respect to time. Moreover, there was an apparent initial lag phase of 5 min before $Nor_1CPZ$ was detected. No enzymatic formation of either $Nor_2CPZ$ or CPZSO was detected. However, a small amount of CPZSO arose due to spontaneous, non-enzymatic oxidation in blank incubations containing boiled microsomes and CPZ: this was quantified and the results of the enzymatic reactions corrected for this. These results for the metabolic formation of CPZNO and $Nor_1CPZ$ are similar to those previously reported by Beckett and Hewick [5], using TLC with $[^{35}S]$CPZ, and by Berman and Spirtes [3], using GC-FID.

Whilst this assay procedure was developed for use in microsomal drug metabolism experiments, it should also be applicable to other biological samples.

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