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# GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF PERAZINE, THIORIDAZINE AND THIORIDAZINE METABOLITES IN HUMAN PLASMA

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## SUMMARY

A gas–liquid chromatographic method for the detection of perazine, thioridazine and its major metabolites in human plasma is presented. Repeated extraction, an internal standard and a temperature program with flame ionization detection make possible accurate and reproducible results with patients on therapeutic doses of these drugs. Examples of chromatograms after extraction of plasma are given.

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## INTRODUCTION

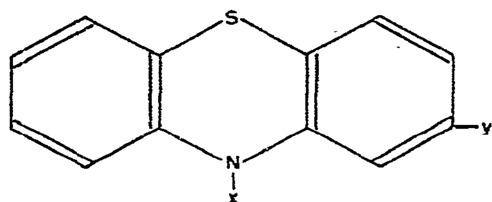
The importance of the study of drug levels in the plasma of patients is ever increasing. More and more psychiatrists are trying to rationalize the pharmacological treatment of their patients by adjusting dosage regimens to drug levels in plasma.

Thioridazine and perazine, both phenothiazines, are used in the treatment of acute and chronic schizophrenic patients. Thioridazine is metabolized by sulfoxidation and N-demethylation. Two of the metabolites formed in this way are pharmacologically active and are present in sufficient amounts in the plasma of the patients treated with this drug to be of clinical importance (see Fig. 1).

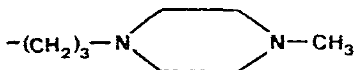
Several arguments can be put forward to prove the necessity of measuring the plasma levels of the active metabolites of thioridazine at the same time as the original compound[1–3].

We therefore tried to develop a single method for the rapid determination of these substances and the two pharmacologically active metabolites in human plasma.

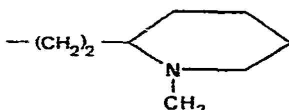
Fluorimetric determinations[4] do not differentiate between thioridazine and its metabolites. Thin-layer chromatography[4] and high-pressure liquid chromatography[5] give good results but demand more precautions and skill than gas–liquid chromatography (GLC). The gas chromatographic method described by Curry and Mould[6]




Phenothiazine nucleus
y substituents: $-S-CH_3$ (thioridazine)
$-SO-CH_3$ (mesoridazine)
$-SO_2-CH_3$ (sulforidazine)
$-H$ (perazine)




x substituent for thioridazine and its metabolites




x substituent for perazine

Fig. 1. Chemical structures of the four compounds which are determined using the present method.

makes no use of an internal standard for quantitation and cannot be used for the determination of the active metabolites of thioridazine. For perazine no specific gas chromatographic method has been described.

## EXPERIMENTAL

### *Reagents*

Benzene, trimethylchlorosilane and methanol were used for the silanization of glassware. Heptane and isoamyl alcohol were used for the extraction process. Pure perazine was obtained from Promonta (Hamburg, G.F.R.). Thioridazine and its metabolites were obtained from Sandoz (Basle, Switzerland). All other products were of reagent grade.

### *Glassware*

All glassware involved was silanized by the following method: after the normal cleaning procedure and ultrasonic treatment, the glassware was soaked for 1 h in 5% trimethylchlorosilane in benzene, then rinsed with benzene, methanol and water, distilled in glass, and dried.

### *Apparatus*

A gas chromatograph (Pye Unicam, Series GCV) with a flame ionization detector (FID) was used. A glass column, 1.83 m × 4 mm I.D., was packed with 3% XE-60 on 80–100 mesh Gas-Chrom Q (Pye Unicam). The injection port temperature was 260°, the column temperature was 240° and the detector block was at 290° for the perazine determination. For thioridazine and its metabolites a temperature

program was run as follows: initial temperature at 240° for 15 min, increasing at 10°/min to a final temperature of 270°, which was maintained for 30 min. To maintain a steady baseline, a second column, identical to the first, was put in the oven and its detection signal was inversed and fed into the same amplifier as used for the first column on which the injections were performed.

The nitrogen carrier-gas flow-rate was 30 ml/min; for the flame the hydrogen gas flow-rate was 30 ml/min and the air flow-rate 300 ml/min. The electrometer range was $32 \cdot 10^{-11}$.

*Extraction procedure*

To 5 ml of plasma, 0.1 ml of 10 *N* sodium hydroxide was added; the plasma was then heated for 15 min at 100° in a hot-water bath. A first extraction was performed by adding 5 ml of *n*-heptane containing 1.5% isoamyl alcohol, shaking for 30 min and separating the two phases by centrifugation for 10 min at 1086 g. This was repeated twice. The three organic phases were transferred to a second set of glass-stoppered test tubes and shaken for 30 min with 3 ml of 0.1 *N* hydrochloric acid. After centrifugation (10 min, 3000 rpm) the organic phase was discarded and 0.15 ml of 10 *N* sodium hydroxide was added to the aqueous solution, which was again extracted three times, as described above using *n*-heptane containing 1.5% isoamyl alcohol.

After centrifugation, the organic solutions were evaporated to dryness under nitrogen. The sample was dissolved in 10 µl of *n*-heptane containing 1.5% isoamyl alcohol and 2 µl was injected into the gas chromatograph. The stoppered samples were stored at −20°.

*Quantitation*

Since there were no patients taking both drugs at the same time, it was always possible to use one of them as an internal standard for the quantitation of the other. Quantitation of the amount of perazine present in the plasma was done by using thioridazine as an internal standard, and vice versa. A known amount of the internal standard was added to the plasma samples before the extraction procedure. The peak areas of both products on the chromatogram were calculated by multiplying the peak height by the width at half peak height; from the ratio of the peak areas of perazine and the internal standard, the amount of product present in the samples could be calculated. A calibration factor was obtained by analyzing samples to which known amounts of both internal standard and test product were added. The calibration samples underwent the same extraction procedure as used for the unknown samples.

For the quantitation of thioridazine and its active metabolites, perazine was used as an internal standard and three calibration curves had to be made.

## RESULTS

Relative retention times for perazine (with thioridazine as an internal standard) and for thioridazine and its metabolites (using perazine as an internal standard and running the temperature program described above) are given in Table I.

The efficacy of extraction is summarized in Table II. The lower limit of detec-

TABLE I

RELATIVE RETENTION TIMES

| *Substance examined* | *Internal standard* | *Relative retention time* |
|---|---|---|
| Perazine | Thioridazine | 0.41 |
| Thioridazine | Perazine | 2.47 |
| Mesoridazine | Perazine | 5.81 |
| Sulforidazine | Perazine | 6.38 |

TABLE II

EFFICACY OF EXTRACTION AND ACCURACY OF THE GLC DETERMINATION FOR SUBSTANCES ADDED TO BLANK PLASMA

All determinations were done five times.

| *Substance* | *Quantity added (ng/ml plasma)* | *Mean assay (ng/ml plasma)* | *Relative standard deviation (%)* | *Relative error (%)* |
|---|---|---|---|---|
| Perazine | 1000 | 980 | 5.5 | −2.0 |
| | 500 | 488 | 10.5 | −2.4 |
| Thioridazine | 1000 | 986 | 4.5 | −1.4 |
| | 500 | 480 | 9.9 | −4.0 |
| Mesoridazine | 1000 | 975 | 6.0 | −2.5 |
| | 500 | 520 | 11.0 | +4.0 |
| Sulforidazine | 1000 | 978 | 5.8 | −2.2 |
| | 500 | 483 | 10.7 | −3.4 |

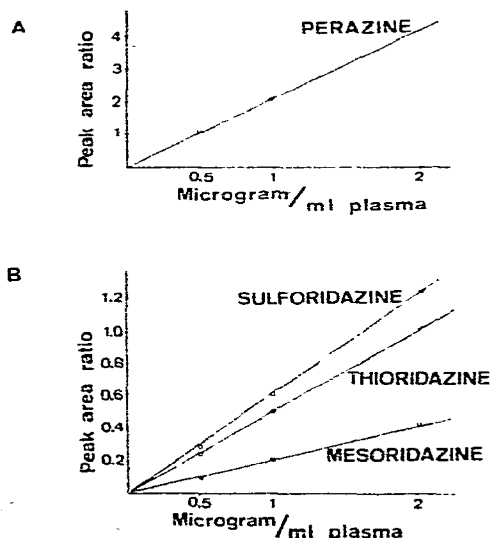



Fig. 2. Calibration curves for perazine determination using thioridazine as an internal standard (A) and for determination of thioridazine and its metabolites, using perazine as an internal standard (B).

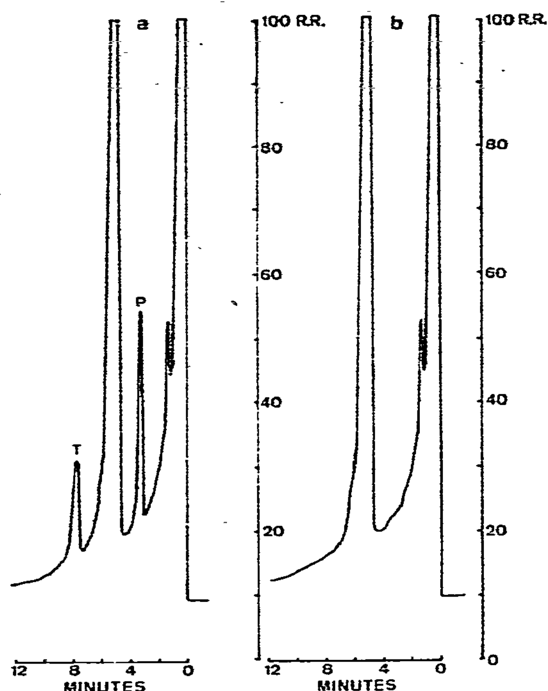



Fig. 3. Gas chromatograms of extracted human plasma. (a) Perazine determination in a patient taking four times 100 mg perazine daily. P = Perazine; T = thioridazine (internal standard, 1 $\mu$g/ml), perazine concentration, 0.77 $\mu$g/ml (12 h after the last dose). (b) Blank plasma. R.R. = Recorder response.

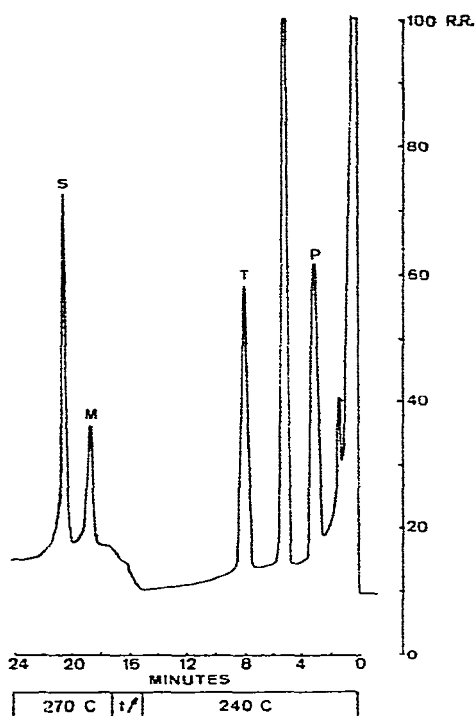
tion, using 5 ml plasma samples is approximately 100 ng/ml for perazine and thioridazine and 150 ng/ml for mesoridazine and sulforidazine.

For good quantitation, using an internal standard, a linear relationship between the ratio of the peak areas of internal standard and compound and the quantity of the compound is necessary. This is illustrated in Fig. 2.

The remaining figures give examples of chromatograms after extraction of blank plasma (Fig. 3), after adding known amounts of the compounds (Fig. 4) and after extraction of plasma samples of patients who had been on a normal oral dose for some time (Fig. 5).

## CONCLUSION

The main differences with the original method, described by Curry and Mould[6] are: (1) the extraction procedure has been modified so that higher yields of the compounds are present in the final injection mixture; (2) an internal standard, also going through the extraction procedure, makes quantitation easier, more reproducible and more accurate; (3) the use of a temperature program makes it possible to determine the active metabolites as well as the original compound; (4) this method can also be used for the determination of other important phenothiazines, as demonstrated in the present study.



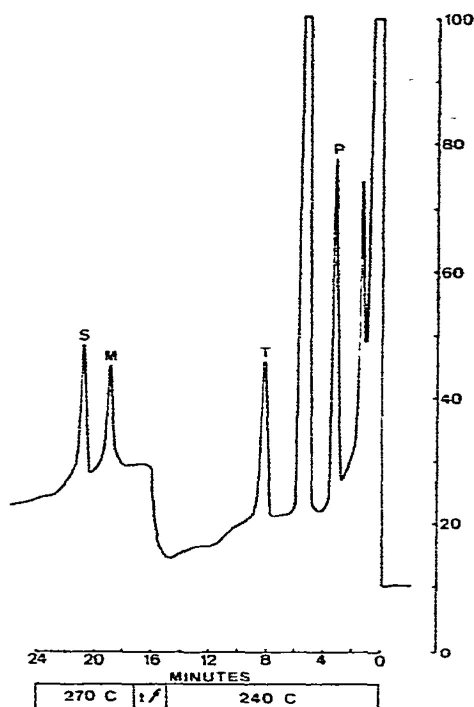




Fig. 4. Gas chromatogram of extracted human plasma after adding known amounts of the compounds. P = Perazine (2 μg/ml); T = thioridazine (1 μg/ml); M = mesoridazine (1 μg/ml); S = sulforidazine (1 μg/ml); R.R. = recorder response.

Fig. 5. Gas chromatogram of extracted human plasma of a patient taking four times 100 mg thioridazine daily. Plasma taken 12 h after the last dose. P = Perazine (internal standard, 1 μg/ml); T = thioridazine (concentration, 0.94 μg/ml); M = mesoridazine (concentration, 1.36 μg/ml); S = sulforidazine (concentration, 0.64 μg/ml); R.R. = recorder response.

Many laboratories have the equipment for GLC, which is already used for determinations of drug levels in plasma, so that the present method can easily be adopted.

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