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Rapid gas-liquid chromatographic estimation of antipyrine in plasma

L. F. PRESCOTT, K. K. ADJEPON-YAMOAH AND ELIZABETH ROBERTS

University Department of Therapeutics, The Royal Infirmary, Edinburgh, EH3 9YW, U.K.

A simple gas-liquid chromatographic method for the estimation of antipyrine in plasma is described. The drug is extracted from alkaline plasma into chloroform and after concentration is chromatographed directly using a mixed liquid phase of 0·5% SE 30 and 0·5% Carbowax 20M. The standard deviation of the method for plasma containing 10–50 μg ml^{-1} of antipyrine was 2·8%. Compared with the standard spectrophotometric assay, antipyrine plasma concentrations were lower and the mean plasma half life in 12 patients was slightly but not significantly shorter.

Hepatic drug metabolizing enzyme activity in man is commonly assessed by measurement of the plasma antipyrine (phenazone) half life. Antipyrine is particularly well suited for this purpose since it is well absorbed after oral administration, rapidly and evenly distributed and extensively metabolized by liver microsomal enzymes. In therapeutic doses it is safe and produces no subjective pharmacological effects.

Antipyrine in plasma is invariably estimated by measuring the change in optical density following conversion to 4-nitrosoantipyrine with nitrous acid (Brodie, Axelrod & others, 1949). This method suffers from the disadvantage that readings must be accurately timed to avoid error since the absorbance increases to a maximum about 20 min after the addition of nitrite and subsequently decreases. A simple gas-liquid chromatographic method has now been developed. Antipyrine is measured directly and the assay can be completed in a shorter time than the spectrophotometric method.

METHODS

To 1·0 ml of plasma containing 5–50 μg ml^{-1} of antipyrine in round-bottomed stoppered glass tubes is added 0·2 ml of 5N NaOH and 1·0 ml of chloroform containing 12·5 μg of phenacetin as the internal standard. The tubes are shaken mechanically for 10 min, centrifuged, and the upper aqueous phase and interface removed by aspiration. The chloroform extract is carefully decanted into a tapered centrifuge tube and the organic phase removed by placing the tubes in a water bath at 90° for 10 min. The residue is dissolved in 20 μl of chloroform with the aid of a vortex mixer and 1–3 μl aliquots injected into the gas chromatograph.

A Hewlett-Packard Model 402 gas chromatograph was used with a 6 ft × $\frac{1}{4}$ in o.d. glass "U" tube column packed with 80/100 mesh Gas-Chrom Q coated with 0·5% SE 30 plus 0·5% Carbowax 20M.* The column, injection port and flame ionization detector temperatures were 220, 240 and 250° respectively. The nitrogen carrier gas flow rate was 50 ml min^{-1} with hydrogen and air flow rates of 25 and 400 ml min^{-1}.

RESULTS

Gas-liquid chromatographic method

Under the above conditions, no interfering peaks were encountered with plasma extracts and the retention times of phenacetin and antipyrine were 1·2 and 2·2 min

* Applied Science Laboratories Inc., State College, Pa., U.S.A.

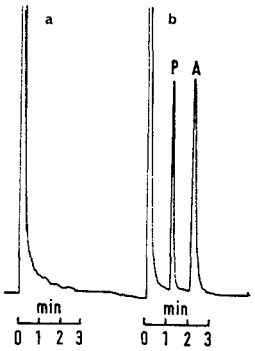

FIG. 1. Chromatograms of extracts of blank plasma (a) and plasma from a patient treated with antipyrine (b). The plasma antipyrine concentration was 18 μg ml^{-1}. Peaks P and A are phenacetin and antipyrine respectively. For conditions see text.

respectively (Fig. 1). A straight line plot passing through the origin was obtained when the peak height ratios of antipyrine to phenacetin were plotted against antipyrine concentration in the range 5–50 μg ml^{-1}. The recovery of antipyrine from aqueous solutions and plasma was the same and the mean standard deviation of the method for human plasma samples containing 10–50 μg ml^{-1} was 2·8% (Table 1). The recovery from plasma was consistently lower than that from water if extraction was carried out without the addition of NaOH.

An aqueous standard solution containing 25 μg ml^{-1} of antipyrine is run with the plasma samples and the drug concentration in an unknown sample (U) is given by the following formula

$$U = \frac{25Y}{X} \mu g \text{ ml}^{-1}$$

where Y is the peak height ratio of antipyrine to phenacetin of the unknown sample and X is the corresponding ratio for the aqueous standard.

Comparison with the spectrophotometric method

Sixty-seven plasma samples obtained from 12 patients at varying times after oral administration of 18 or 25 mg kg^{-1} of antipyrine were analysed by both the gas-liquid chromatographic assay and the spectrophotometric method of Brodie & others (1949)

Table 1. *Results of replicate gas-liquid chromatographic analyses of human plasma containing added antipyrine.*

Plasma antipyrine concentration (μg ml^{-1})	% recovery*	s.d. (%)
50	101·5	1·7
40	104·2	1·9
30	100·5	2·5
20	102·3	4·0
10	99·3	3·1
5	101·4	9·3
Mean	101·5	4·4

* Mean % recovery relative to an aqueous solution containing 25 μg ml^{-1} of antipyrine run with each set of plasma samples. Five plasma samples were analysed at each concentration.

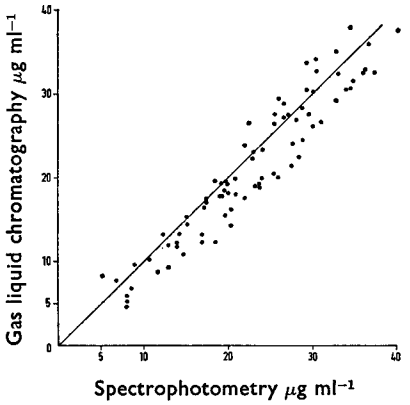

FIG. 2. Comparison of gas-liquid chromatographic and spectrophotometric estimation of antipyrine in plasma. 67 plasma samples obtained from 12 patients treated with antipyrine were assayed by both methods.

using the precipitation procedure. The results obtained by both methods were comparable, but the spectrophotometric method generally gave a higher value (Fig. 2). The mean difference was $13{\cdot}2 \pm 5{\cdot}8\%$ (s.d.). The plasma antipyrine half life was calculated in each of the 12 patients using both methods. A shorter mean half life was obtained by gas-liquid chromatography ($10{\cdot}6 \pm 3{\cdot}9$ h) than by spectrophotometry ($11{\cdot}4 \pm 3{\cdot}1$ h) but the difference was not statistically significant ($P > 0{\cdot}2$).

The mean standard deviation of the spectrophotometric method for 25 plasma samples containing 10–50 μg ml^{-1} of antipyrine was 7·7%.

DISCUSSION

The estimation of antipyrine in plasma by gas-liquid chromatography was mentioned recently by Shoeman, Kauffman & others (1972), but few details were given and an internal standard was not used. The simple assay described here is quicker than the standard spectrophotometric method and reproducibility is better. The drug is estimated directly without conversion to a derivative and the chromatographic step is completed within 3 min. There is no interference from slowly eluting peaks.

Further simplification was attempted by extracting the drug into a very small volume of chloroform so that injections could be made directly without the transfer and evaporation steps. Although this worked well with aqueous solutions, it was unsuccessful with plasma because of emulsion formation.

The spectrophotometric method gave higher antipyrine plasma concentrations and a slightly longer half life than the gas-liquid chromatographic assay. The explanation is not clear. However, there is a small but variable increase in absorbance when the sodium nitrite is added to blank plasma, and metabolites such as 4-hydroxyantipyrine and 3-hydroxymethylantipyrine (Yoshimura, Shimeno & Tsukamoto, 1968) might cause additional interference.

Acknowledgment

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