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Determination of piribenzil in urine containing a metabolite and dextropropoxyphene by an ion pair extraction method

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Piribenzil, an antispasmodic quaternary ammonium compound, has been determined quantitatively in human urine containing a metabolite of piribenzil and dextropropoxyphene. Ion pair extraction with bromothymol blue as counter ion was used and the extraction conditions were calculated from extraction constants and partition coefficients determined using methylene chloride as organic solvent. The determination was made by photometry in the range 5–50 μg of piribenzil methylsulphate/ml of urine. The recovery was 97% $\pm$ 3%. The co-extraction of urine components such as nicotine, tyramine, tryptamine and choline was investigated by determination of constants for the partition of these compounds.

In this paper we have used the principles of Schill (1965) for quantitative determination in human urine samples of the antispasmodic quaternary ammonium compound piribenzil. The samples also contained dextropropoxyphene and a metabolite of piribenzil, 1,1-dimethyl-2-hydroxymethylpiperidinium. Piribenzil was isolated and determined photometrically in the concentration range 5–50 μg per ml urine by ion pair extraction with bromothymol blue.

The disturbance by other urine components like nicotine and the endogenous substances tyramine, tryptamine and choline has also been studied.

EXPERIMENTAL

Apparatus. The photometric determinations were made with a Zeiss Spektralphotometer PMQ II and the pH measurements with a Radiometer pH Meter 4.

Reagents and chemicals. All substances were of analytical grade. The methylene chloride was shaken with water before use. Bromothymol blue was purified according to Borg, Modin & Schill (1968). Sodium phosphate and sodium borate buffer solutions were used as aqueous phases.

Determination of the partition ratio. The partition studies were performed according to Modin & Schill (1967), using a shaking time of 20 min in centrifuge tubes at 20°. The phases were separated with a capillary siphon.

RESULTS AND DISCUSSION

Determination of extraction conditions

Piribenzil was determined in urine samples containing >5 μg/ml of the drug. The sample also contained an amine, dextropropoxyphene, at less than 10% of the piribenzil concentration. A metabolite of piribenzil, 1,1-di-methyl-2-hydroxymethylpiperidinium, was also assumed to be present to about the same concentration as piribenzil.

In the actual concentration range ($>5 \times 10^{-6}$M) extraction and photometric determination of piribenzil as ion pair with bromothymol blue could be assumed to give both good sensitivity and sufficient degree of extraction (Modin & Schill, 1967).

The method was based on constants for the extraction since these give the necessary information about selectivity which is of significance for a biological sample.

The extraction equilibrium can be defined by the extraction constant, E_{HAHY}

$$E_{HAHY} = \frac{[HAHY]_{org}}{[HA^+] \times [HY^-]} \quad .. \quad .. \quad .. \quad (1)$$

where A represents ammonium compounds and H_2Y is bromothymol blue. The extraction is influenced by side-reactions which are protolysis and partition of the protolytic ion pair components in uncharged form. Compensation for these side-reactions can be made if the partition coefficients ($k_{d(A)}$ and $k_{d(H_2Y)}$) and the acid dissociation constants (K'_{HA^+}, K'_{H_2Y} and K'_{HY^-}) or functions of these constants are known.

The calculation of this compensation has been demonstrated by Modin & Schill (1967). For the partition of the ammonium compound, A, as ion pair the following expression is valid

$$D_{HAHY} = \frac{[HAHY]_{org}}{C'_A} = E_{HAHY} \times C'_Y \times (\alpha_{HA} \times \alpha_{HY})^{-1} \quad .. \quad (2)$$

C'_A and C'_Y represent the concentrations of A and H_2Y not extracted as ion pairs.

$$\alpha_{HA} = 1 + K'_{HA^+} (1 + k_{d(A)}) \times (a_{H^+})^{-1} \text{ (monovalent amine)} \quad .. \quad (3)$$

$$\alpha_{HY} = 1 + a_{H^+} (1 + k_{d(H_2Y)}) \times (K'_{H_2Y})^{-1} + K'_{HY^-} \times (a_{H^+})^{-1} \quad .. \quad (4)$$

The constants necessary for the calculation of the α-coefficients and D_{HAHY} are given in Tables 1 and 2. The partition ratio will be directed by the concentration of

Table 1. *Partition coefficients for amines and bromothymol blue.* Organic phase: Methylene chloride. Aqueous phase: sodium phosphate buffer solutions with an ionic strength of 0·1. A represents amines and H_2Y is bromothymol blue

	$-\log k_{d(A)} \cdot K'_{HA^+}$	$\log k_{d(H_2Y)}/K'_{H_2Y}$	pK'_{HY^-}
Dextropropoxyphene ..	3·05	—	—
Nicotine	6·50	—	—
Tryptamine	9·19	—	—
Tyramine	10·90	—	—
Bromothymol blue ..	—	5·40*	7·12*

* Taken from Schill (1964) and Schill & Marsh (1963).

bromothymol blue (C'_Y) and pH of the aqueous phase. The relation between the partition ratios of the ammonium compounds studied and pH of the aqueous phase are given in Figs 1 and 2 ($C'_Y = 10^{-3}$). For the quaternary ammonium ions the variation in D_{HAHY} is entirely due to changes in α_{HY} since $\alpha_{HA} = 1$ for these aprotic compounds. When $D_{HAHY} > 100$ and equal phase volumes are used more than 99% of the ammonium compound is extracted as ion pair with bromothymol blue. From Fig. 1, it follows that piribenzil can be extracted quantitatively at pH <9.

Table 2. *Extraction constants for ion pairs with bromothymol blue.* Organic phase: methylene chloride. Aqueous phase: sodium phosphate buffer solution with an ionic strength of 0·1. HA^+ represents all kinds of ammonium ions

Cation	$[HAHY]_{org} \cdot 10^5$	log E_{HAHY}
Dextropropoxyphene	2·5 – 5·7	10·29
Piribenzil	1·3 –18	7·20
Dimethylhydroxymethylpiperidinium ..	0·39– 1·3	4·10
Choline		3·10*
Nicotine	1·3 – 2·3	5·82
Tryptamine	4·0 –10·1	4·39
Tyramine	0·70– 1·90	2·51

* Taken from Schill (1965).

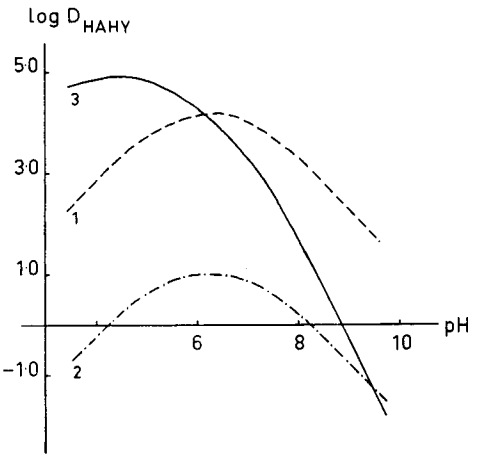

FIG. 1. Graphical illustration of the relation between the partition ratios of ion pairs with bromothymol blue and pH of the aqueous phase. 1. Piribenzil. 2. 1,1-Dimethyl-2-hydroxymethylpiperidinium. 3. Dextropropoxyphene.

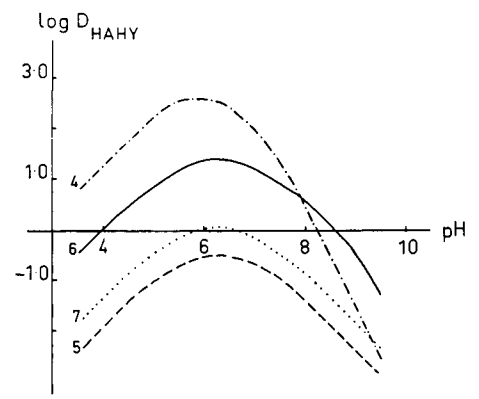

FIG. 2. Graphical illustration of the relation between the partition ratios of ion pairs with bromothymol blue and pH of the aqueous phase. 4. Nicotine. 5. Tyramine. 6. Tryptamine. 7. Choline.

The lower limit of the useful range is pH 7·5 since a lower pH will give a too high extraction of bromothymol blue in uncharged form.

The co-extraction of dextropropoxyphene and 1,1-dimethyl-2-hydroxymethylpiperidinium have within the useful pH-range a minimum at pH 9 where both substances have $D_{HAHY} = 0{\cdot}1$ corresponding to a percentage extraction of 10%. A suitable way to reduce this co-extraction is to treat the organic phase once more with an aqueous phase of 10^{-3}M bromothymol blue at pH 9·0. The recovery of piribenzil will then be almost quantitative while the disturbance by the two other components in the piribenzil determination is $\leqslant 1\%$.

Quantitative determinations on urine samples

When urine samples were assayed under conditions giving quantitative extraction of piribenzil very high blanks were obtained. It was assumed that these blanks were due to the presence of other ammonium compounds with lower extraction constants than piribenzil, but present in the sample in much higher concentrations. In principle, compensation for these blanks was possible but they would decrease the precision of the quantitative determination of piribenzil considerably.

According to this assumption a re-extraction of the organic phase with an aqueous phase of 10^{-3}M bromothymol blue with pH 9·0 should decrease the blank value without significant losses of piribenzil (as discussed above). Blank values decreased in absorbance from 1·07 at the first extraction to 0·12 at the third extraction. The recovery of piribenzil after two repeated re-extractions was 97% while the blank value decreased about 10 times.

A blank value of a sample containing piribenzil can be obtained after hydrolysing piribenzil by alkali (Beckmann, 1966). The hydrolysing procedure is given in the *General procedure*. The hydrolysis gives 1,1-dimethyl-2-hydroxymethylpiperidinium which is extracted in a negligible amount as discussed above.

A method for analysis of piribenzil in urine samples based on the discussions above is given under *General procedure*. A test on samples containing 5–50 μg piribenzil-methylsulphate per ml gave a recovery of 97 $\pm$ 3% which is in good agreement with the theoretically calculated. Dextropropoxyphene and 1,1-dimethyl-2-hydroxy-methylpiperidinium were also present in the samples.

Co-extraction of other ammonium compounds

In the determination of blank values it was observed that smokers gave significantly higher values and also a larger daily variation. Since this effect was supposed to be due to co-extraction of nicotine, the extraction constant for the ion pair between bromothymol blue and nicotine was determined as well as the partition coefficient of nicotine. The constants are given in Table 1 and 2 and variation of the partition ratio with pH of the aqueous phase is demonstrated in Fig. 2. From these data it can be calculated that 2·5% of the total concentration of nicotine in urine will be extracted to the organic phase as ion pair with bromothymol blue.

Tables 1 and 2 and Fig. 2 also give data necessary for the calculation of disturbances due to co-extraction of tyramine, tryptamine and choline. The distribution ratios have sizes such that a disturbing co-extraction can be expected especially when these substances are close to the upper limit of the normal range of variation.

General procedure

Determination of piribenzil + blank. 5·00 ml of the urine sample is added to 5·00 ml of a sodium borate buffer solution ($C_{H_3BO_3}$ = 0·4M) with pH = 9·0 containing bromothymol blue in a concentration of $10^{-2\cdot7}$. The extraction is with 10·00 ml methylene chloride in centrifuge tubes for 20 min. After centrifugation the organic phase is siphoned to another centrifuge tube containing 5·00 ml of the sodium borate buffer solution with bromothymol blue and 5·00 ml of water. Extraction is made as before and repeated once again. The organic phase from the third extraction is measured photometrically at 635 nm after the addition of a micro-drop of tetrabutylammonium hydroxide.

Determination of blank. To 5·00 ml of the urine sample 0·10 ml 10M NaOH is added. This solution is left for 20 min at room temperature. 0·10 ml 10M HCl is added and extraction and determination is performed according to the procedure above.

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