Determination of a Quaternary Ammonium Compound, Emepronium Bromide, in Human Urine by An Ion-Pair Extraction Method

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Abstract □ Emepronium bromide, an anticholinergic quaternary ammonium compound, was determined quantitatively in human urine by an ion-pair extraction method. This was constructed by the use of a theoretical approach, and favorable conditions were calculated from extraction constants and constants for side reactions. The principle of choosing selective extraction conditions is discussed on a theoretical basis; it has a general application in drug isolation from samples of such a complicated nature as biological objects. The present method includes extraction of the drug as perchlorate ion pair, purification of the extract, and determination with bromthymol blue. Using a concentration step in the method, a minimum concentration of 0.2 mcg./ml. urine could be determined with an absolute recovery of 98 ± 3%. Utilizing this method, the excretion of emepronium bromide in urine was followed after oral and rectal administration.

Keyphrases □ Emepronium bromide, determination in human urine—by ion-pair extraction method □ Ion-pair extraction method—determination of emepronium bromide in human urine □ Spectrophotometry—analysis

The isolation of quaternary ammonium ions from biological samples often can be made by an ion-pair extraction technique. Under suitable conditions, a very selective extraction method is obtained, which is of great importance when the object has such a complicated nature as the biological sample. Ion-pair extraction can also be used for transformation of the drug molecule to a form that makes the determination of low concentrations possible.

Investigation of the human urinary excretion of two quaternary antispasmodics by an ion-pair extraction method was made by Pfeffer *et al.* (1, 2). They used picric acid and tropaeolin 00 for the extraction and photometric determination. The recovery was low and varied greatly between subjects. This was assumed to be due to the presence of anions in urine with higher affinity than the anionic dyes for the quaternaries.

By the use of a theoretical approach in the method construction, more rapid and reliable results are obtained than by a purely empirical investigation technique. The calculation of favorable conditions can be based on extraction constants and constants for side reactions (3–7).

A demonstration of this principle is made in the present study, where an anticholinergic ammonium compound, emepronium bromide[1], is determined in human urine. The method is applied in a study of the excretion of the drug after oral and rectal administration.

Table I—Constants[a]

Anion	log E_{QX}	log E_{QHB}	log E_{Q_2B}	log $k_{diss.}$	pK'_{HB^-}
Perchlorate	3.58			−4.74	
BTB[b] (H_2B)		8.66	8.14		7.12

[a] Organic phase dichloromethane and aqueous phase phosphate or carbonate buffer solutions $\mu = 0.1$. [b] From *Reference 3*.

EXPERIMENTAL

Apparatus—The photometric measurements were performed with a Zeiss PMQ II Spektral-photometer in 10-mm. cells. The pH measurements were made with a Radiometer PHM 4 using glass and calomel electrodes.

Chemicals and Reagents—Dichloromethane of Fisher Certified ACS quality was equilibrated with water. Bromthymol blue (BTB) of analytical grade was purified according to Borg *et al.* (7). Tetrabutylammonium hydroxide, 0.1 *M*, in toluene–methanol was of BDH quality. All other chemicals were also of analytical grade. Phosphate and carbonate buffer solutions contained sodium as the only cation and had an ionic strength of 0.1. All aqueous reagents were equilibrated with dichloromethane.

Determination of Constants—The constants necessary for the construction of the method are given in Table I. The extraction constant of the emepronium ion pair with BTB, as well as the second acid dissociation constant of BTB, was determined by Schill (3).

The determination of the extraction constant of emepronium perchlorate was performed with samples containing equivalent amounts of emepronium and perchlorate ions, obtained by extracting an aqueous phase containing emepronium bromide and an excess of perchloric acid with dichloromethane. The organic phase was then equilibrated with an equal volume of 0.1 *M* NaH_2PO_4 for 1 hr.; after centrifugation the phases were separated. The emepronium content of the organic phase was determined by the BTB method (8) before and after equilibration. The concentrations of emepronium and perchlorate in the aqueous phase were then calculated from the difference between these measured concentrations. The found concentrations were used in the evaluation of the constants for the extraction and the dissociation of the ion pair as demonstrated earlier (9).

Analytical Method—A urine sample, 75.00 ml., is mixed with 10.00 ml. 2 *M* $HClO_4$ and 10.00 ml. dichloromethane in a 100-ml. glass-stoppered centrifuge tube. After shaking for 5 min. and centrifuging for 15 min., the organic phase is siphoned into a new centrifuge tube containing 10.00 ml. 0.1 *M* ClO_4^- in phosphate buffer pH 12. After shaking for 2 min. and centrifuging, the organic phase is siphoned into a centrifuge tube containing 5.00 ml. BTB (3×10^{-4} *M* in carbonate buffer pH 9.0). This mixture is shaken carefully for 5 min. to avoid emulsion, and is centrifuged for 20 min. The organic phase is siphoned and measured photometrically at 635 nm. after addition of 1 micro drop of 0.1 *M* tetrabutylammonium hydroxide into the cell. A blank from the same subject is prepared in the same way when no emepronium bromide is consumed. A theoretical recovery of 103% is obtained, depending on the solubility of dichloromethane in the urine sample (0.5 v/v%), for which compensation has to be done. Samples with a concentration down to 5×10^{-7} *M* (0.2 mcg./ml.) can be determined with the method.

Excretion Study—By the use of the described analytical method, urine blanks were determined for 12 volunteers employed in the excretion study. After administration of emepronium bromide, urine samples were collected every 2nd hr. and frozen for preservation.

[1] Cetiprin.

Table II—Total Urine Excretion of Emepronium Bromide 12 hr. after Administration

Formulation	Dose, mg.	Number of Subjects	Total Excretion mg.	δ	% of Dose
Tablets	200	12	1.11	±0.46	0.55
Suppository	100	11	2.96	±0.87	3.0

In a comparative study, the excretion was followed after an oral administration of 100 mg. at 7 a.m. and 100 mg. at 1 p.m. (therapeutic doses) and after a rectal single dose of 100 mg. at 7 a.m. In both these experiments, the subjects took the same doses the day before.

In another rectal experiment without premedication and with a lower dose, four of the test subjects were given 50 mg. of emepronium bromide in a suppository taken in the morning of the trial day. The excretion by urine was followed 12 hr. after administration.

RESULTS AND DISCUSSION

Calculation of Extraction Conditions—The drug concentration in the biological sample is often low, so it is essential to use a determination technique that gives the highest sensitivity possible. In the present method the BTB complex, with a molar absorptivity of 4.9×10^4, is used; it gives a minimum concentration of the complex of about 4×10^{-6} M (1.5 mcg./ml.) if reasonable precision in the photometric measurement is obtained. It is, however, possible to determine a lower concentration in the urine sample by the use of a large ratio (volume of aqueous phase)/(volume of organic phase) in the initial extraction step, which increases the concentration of the drug. In the present method the phase volume ratio is about 8, and the minimum urine concentration of the drug is then 5×10^{-7} M (0.2 mcg./ml.).

Suitable extraction conditions can be estimated from published data about the relation between the structure of the ion pair, the nature of the organic phase, and the extraction constant (4, 6, 10). In the actual case, a favorable system for the extraction of the quaternary ammonium compound from the urine sample was assumed to be found with perchlorate as the counterion in the ion pair and dichloromethane as the organic phase.

The calculation of extraction conditions was based on the extraction constant of emepronium perchlorate by the use of the following expression:

$$P_{QX} = 100/(1 + V_{aq.} \times V_{org.}^{-1} \times D_{QX}^{-1}) \qquad \text{(Eq. 1)}$$

where P_{QX} is the percentage extraction of the quaternary ammonium ion as ion pair with perchlorate; $V_{aq.}$ and $V_{org.}$ are the volumes of the aqueous and organic phases, respectively; and D_{QX} is the partition ratio defined by

$$D_{QX} = E^*_{QX} \times C_X \qquad \text{(Eq. 2)}$$

E^*_{QX} is the conditional extraction constant which can be calculated from the extraction constant, E_{QX}, and constants for side reactions. In this case, dissociation of the ion pair in the organic phase is the only side reaction, and E^*_{QX} can then be calculated from Eq. 3:

$$E^*_{QX} = E_{QX} + (E_{QX} \times k_{diss.})^{1/2} \times (C_Q \times C_X)^{-1/2} \qquad \text{(Eq. 3)}$$

As seen from this equation, E^*_{QX} will increase with decreasing values of $(C_Q \times C_X)$, which will favor the extraction in the low concentration range.

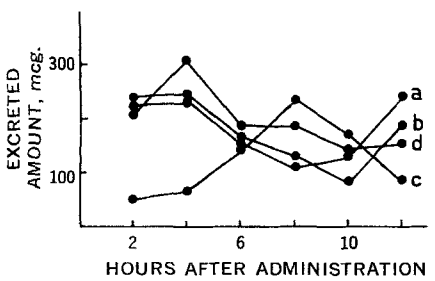

Figure 1—*Excretion of emepronium bromide in urine after oral administration of 2 × 100 mg. to four subjects (a, b, c, and d).*

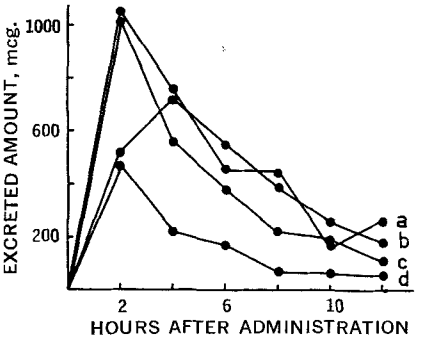

Figure 2—*Excretion of emepronium bromide in urine after rectal administration of 50 mg. to four subjects (a, b, c, and d).*

If a good selectivity is to be obtained by an extraction method, a system with unnecessarily high extraction capacity has to be avoided, since this will proportionally promote extraction of impurities. In general, an extraction degree of 99% is quite sufficient. An approximate calculation of extraction conditions by Eqs. 1 and 2 can, for the actual system, be based on the following values: $P_{QX} = 99$, $V_{aq.}/V_{org.} = 8$, and $E^*_{QX} = E_{QX} = 3.8 \times 10^3$ (Table I). These give a suitable perchlorate concentration of the aqueous phase, $C_X = 0.2$ M.

If a more exact calculation is wanted, E^*_{QX} has to be known. From Eq. 3, it follows that the value depends on the concentrations of the perchlorate and ammonium ion in the sample. The minimum sample concentration of Q^+ is according to the method 5×10^{-7} M, which gives a $C_Q = 5 \times 10^{-9}$ M after extraction. Calculations by Eq. 3 with this value of C_Q, $C_X = 0.2$ M, and $k_{diss.} = 10^{-4.74}$ (Table I) give $E^*_{QX} = 5.8 \times 10^3$, corresponding to a percentage extraction of 99.3.

The extraction conditions found by calculations were tested on aqueous solutions of emepronium bromide. The urine components urea, creatinine, and histidine, in normally occurring concentrations (11), were added to some samples. An emepronium recovery of 99 ± 2% was obtained in all cases after determination by the BTB method (8).

Isolation from Urine—When the perchlorate extraction was tested with urine samples free from emepronium bromide, in all cases the organic phase was found to contain components that gave response with BTB. This will give rise to a rather high blank and limit the sensitivity of the method.

It seemed, however, reasonable to assume that most of these coextracted substances were more polar than emepronium perchlorate with considerably smaller distribution ratios. If this was the case, the first extract from the urine could be purified by extraction with an aqueous phase of such perchlorate content that the emepronium would remain in the organic phase as an ion pair.

Calculations by Eqs. 1 and 2 proved that equal phase volumes and $C_{ClO_4} = 0.1$ M would prevent losses of emepronium. The aqueous phase was, in this case, alkalized (pH 12) to remove coextracted organic acids which might disturb the determination by the BTB method. At this pH, amines were extracted as bases. Tests showed that a single reextraction of this kind decreased the blank to one-fourth of its first value, while further extraction only gave small reductions.

The blank values varied considerably between tested subjects, with 0.15 as the highest and 0.04 as the lowest absorbance. Repeated tests showed small blank variations for each subject.

Photometric Determination—In the determination step the perchlorate ion in the organic phase was exchanged for a BTB anion. The main part of the perchlorate is present in the organic phase as an ion pair with emepronium but also to some extent with other urine components causing the blanks. The disturbance from components giving more polar BTB ion pairs than emepronium can be decreased by using a system without excessive extraction capacity. With a BTB reagent solution of a high pH, this is avoided and the influence of ion-pair forming amines will also diminish because of competitive base partition.

Emepronium can be extracted with both monovalent and divalent anions of BTB (3), but the extraction of the ion pair with the divalent anion is negligible at pH = 9.0. This can be shown by a logarithmic diagram using consecutive extraction constants according to Modin and Schill (12).

The BTB concentration necessary for a quantitative exchange of the perchlorate for BTB (HB^-) can be calculated by the use of α-

coefficients (3). The following equations will be valid:

$$D_{QHB} = E_{QHB} \times C_B \times \alpha_{HB}^{-1} \times \alpha_Q^{-1} \quad \text{(Eq. 4)}$$

α_Q is given by Eq. 5, where X^- means ClO_4^-:

$$\alpha_Q = ([Q^+] + [QX]_{org.} + [X^-]_{org.})/[Q^+] = 1 + E_{QX} \times C_X + (k_{diss.} \times E_{QX} \times C_X/C_Q)^{1/2} \quad \text{(Eq. 5)}$$

Eq. 5 is valid under the assumption that $[X^-]_{org.} = [Q^+]_{org.}$. If the constants in Table I, $C_X < 10^{-4.5}$, and $C_X/C_Q = 100$ are used, the calculation will give $\alpha_Q = 10^{0.55}$.

The calculation of α_{HB} at pH = 9.0 is easily made from Eq. 6:

$$\alpha_{HB} = C_B/[HB^-] = 10^{pH-pK'_{HB}} = 10^{1.88} \quad \text{(Eq. 6)}$$

When equal phase volumes are used, a percentage extraction of 99.0 is obtained if $D_{QHB} = 100$. If this value, E_{QHB} from Table I, and the found α_Q and α_{HB} are inserted in Eq. 4, a $C_B = 10^{-4.2}$ is obtained. This is the minimum concentration of BTB necessary for exchanging perchlorate for BTB in the emepronium ion pair. In the method, a slightly higher concentration is used to avoid losses due to small changes in the pH of the buffer.

The method was tested repeatedly on urine samples with added known amounts of emepronium bromide giving an initial concentration of the urine of 0.2 mcg./ml. and higher. Including the compensation for the solubility of dichloromethane in the urine, the absolute recovery of the method was 98 ± 3%, which is in good agreement with the theoretical recovery. For some single objects, somewhat lower precision in the determination was obtained due to incomplete phase separation.

Urinary Excretion—The described determination method was applied in some excretion studies; examples of excretion curves after oral administration of therapeutic doses are presented in Fig. 1. The comparative study of the total urinary excretion 12 hr. after oral and rectal administration is presented in Table II, where the mean values and the standard deviation between the subjects are given. A rather small part of the drug was excreted in the urine, the value for the rectal administration being higher than for the oral. In the rectal experiment, about half of the volunteers complained of side effects typical for anticholinergic drugs, *i.e.*, drynes in the mouth and accommodation disturbances.

In another rectal experiment, when the dose was lowered and no premedication was given, no side effects were noted. The four persons participating were identical with those in Fig. 1, and their excretion curves are given in Fig. 2. Maximal excretion in this case occurred within the first 2 hr. after administration.

Further studies of the absorption, metabolism, and excretion of emepronium bromide are in progress.

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Cactus Alkaloids X: Isolation of Hordenine and *N*-Methyltyramine from *Ariocarpus kotschoubeyanus*

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Abstract □ Hordenine HCl and *N*-methyltyramine HCl were crystallized from extracts of the cactus *Ariocarpus kotschoubeyanus* (Lemaire) Schumann. Other alkaloids, which have been found in closely related *Ariocarpus* species, were not detected in this species. Extraction of the isolated alkaloids by percolation gave higher yields than continuous extraction.

Keyphrases □ *Ariocarpus kotschoubeyanus*—isolation of hordenine, *N*-methyltyramine □ Hordenine—isolation from *Ariocarpus kotschoubeyanus* □ *N*-Methyltyramine—isolation from *Ariocarpus kotschoubeyanus* □ TLC—separation

Ariocarpus kotschoubeyanus (Lemaire) Schumann, like some other species of *Ariocarpus*, is commonly called pezuña de venado, pata de venado, chaute, or peyote in areas of northern and central Mexico where the cactus is indigenous (1–5). The common name of peyote and the purported poisonous nature of the plant (2, 4) aroused interest in this laboratory concerning its alkaloid chemistry. No previous phytochemical studies have been reported regarding *A. kotschoubeyanus;* however, *N*-methyl-3,4-dimethoxy-β-phenethylamine, hordenine, and *N*-methyltyramine have been identified and/or isolated from *A. fissuratus* (Engelmann) Schumann (6–8), *A. trigonus* (Weber) Schumann (9), and *A. retusus* Scheidweiler (10, 11), and *N*-methyl-4-methoxy-β-phenethylamine has been isolated from *A. retusus* (11).

In this investigation, alkaloids were initially extracted from defatted and basified plant material, using chloroform in large continuous extraction apparatuses; an anion-exchange resin was used to separate the purified alkaloids into phenolic and nonphenolic fractions (12). As evidenced by TLC, the phenolic fraction contained hordenine and *N*-methyltyramine, which were resolved by preparative TLC and crystallized as the hydro-