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Determination of low concentrations of the quaternary ammonium compound thiazinamium methylsulphate in plasma and urine

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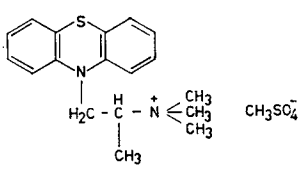
A sensitive and selective method for the quantitative determination of the quaternary ammonium antiacetylcholine-compound thiazinamium methylsulphate (Multergan) in plasma and urine is described. The procedure is based on ion pair extraction of the compound with iodide as the counter ion. This is followed by gas chromatography using an alkali flame ionization detector. The detection limit is 2 ng ml^{-1} with a recovery of 88·0 ± 6·2% from plasma, 91·4 ± 4·6% from urine. The described method can also be applied to other quaternary ammonium compounds.

A number of methods for the determination of thiazinamium methylsulphate (I) in biological fluids have been published (Berti & Cima, 1954; Kleinsorge, Thalman & Rösner, 1959; Kerckhoffs & Huizinga, 1967) but are of limited application because of the high water solubility of the drug. Isolation of a quaternary ammonium compound from plasma or urine can be accomplished however using an ion pair extraction technique (Schill, 1964, 1965a,b, 1974; Schill, Modin & Persson, 1965; Persson & Schill, 1966; Modin & Schill, 1967; Borg, 1971) and in most cases estimation is completed by spectrophotometry. Apart from a clean up procedure to remove the coextracted plasma or urine components, lack of sensitivity in the spectrophotometric detection can prove disadvantageous (Eksborg, Persson & others, 1971; Allgén, Ekman & others, 1960). A gas chromatographic procedure using an alkali flame ionization detector provides for a higher selectivity and sensitivity, and with the method, described in this paper, it is possible to measure plasma concentrations in the nanogram range.



I

MATERIALS AND METHODS

Apparatus. All gas chromatographic measurements were performed on a H.P. 5750 Research Gas Chromatograph, equipped with a H.P. High Sensitivity (rubidium

bromide) Nitrogen Detector model 15161-B, and connected with a H.P. recorder model 7123 B and a H.P. integrator model 3373 B with baseline correction (Hewlett Packard, Avondale, PA.).

An all glass system with graphite ferrule connections was used, column length 180 cm, i.d. 2 mm. Stationary phase 3% S.P. 2100 (Supelco, Inc.) on Chromosorb G-(A.W.-D.M.C.S.)-High Performance 80–100 mesh (Johns-Manville). Column temperature: 240°. Injection port temperature: 300°. Detector temperature: 400°. Carrier gas: helium 50 ml min^{-1}. Detector gases: air 180 ml min^{-1}, hydrogen 30 ml min^{-1}. As additional make up gas helium, 30 ml min^{-1}, was added in the detector.

pH measurements were made with a Philips digital pH-meter P.W. 9408; spectrophotometric measurements with a Zeiss Spectrophotometer P.M.Q. II. Where mixing or shaking was indicated, a Vortex mixer was used.

Reagents. Thiazinamium methylsulphate (3554 R.P.) and chlorpromazine hydrochloride were obtained from SPECIA (Rhône Poulenc) Paris, France. Potassium iodide Suprapur and ethanol Pro Analysi were used without purification; 1,2-dichloroethane Uvasol (spectrophotometric grade) was purified by distillation in glass before use (E. Merck, Darmstadt), heparin solution contained 5 mg (= 500 U) ml^{-1} in distilled water. Silyl-8 G.L.C. column conditioner was obtained from Pierce Chemical Company.

Glassware was cleaned by standing overnight in chromic acid and then rinsed with distilled water. Phosphate detergents were avoided because of the high sensitivity of the detector for phosphor compounds.

Extraction constants were determined by the method of Modin & Schill (1970). Cations and anions were dissolved in sodium phosphate buffer (pH = 6·5; ionic strength = 0·1). The organic phase was equilibrated with buffer solution before use. Aqueous solutions and the organic solvent were shaken in centrifuge tubes (30 min: 25°). After centrifugation and separation of the phases with a capillary siphon, the concentration of the ion pair in the organic phase and of the cation in the aqueous phase were determined from the absorbance at 300 nm. The absorption maximum at 254 nm could not be used because of interference of iodide ion.

Determination of thiazinamium methylsulphate in plasma. Blood samples of approximately 10 ml were obtained by venous puncture and collected in tubes containing 1 drop of heparin solution. After centrifugation 4·0 ml plasma was removed to a centrifuge tube of 50 ml capacity with a Quickfit stopper. Potassium iodide solution (0·5 ml of 1 M) was added and the pH adjusted to 10 by means of a 1 N sodium hydroxide solution; the mixture was then shaken (10 s), 1,2-dichloroethane (20·0 ml) was added and the plasma was extracted (30 s). The phases were separated by centrifugation (20 min) at 6000 *g* and the plasma layer was aspirated by means of a Pasteur pipette. Of the remaining dichloroethane layer 15·00 ml was transferred to a conical glass tube and the dichloroethane evaporated at 70° under a gentle stream of nitrogen. The residue was dissolved in ethanol (150 μl), containing chlorpromazine HCl as a gas chromatographic standard; 10 μl (or in low concentration ranges 20 μl) were injected into the gas chromatograph.

Determination of thiazinamium methylsulphate in urine. The volume and the pH of each urine fraction was measured and 0·50 ml pipetted into a 50 ml centrifuge tube as above. Distilled water (1·5 ml) was added and the pH adjusted to 7·0 with 1 N sodium hydroxide solution. Potassium iodide solution (0·5 ml, 1 M) was added and after mixing 1,2-dichloroethane (20·00 ml) was added. The urine was extracted

by vigorous shaking (30 s). The two phases were then separated by centrifugation (20 min) at 6000 *g* and the analysis completed as described in the procedure for plasma.

RESULTS AND DISCUSSION

(A) *For ion pair formation and extraction* of quaternary ammonium compounds from aqueous solutions organic or inorganic anions (e.g. Cl^-, Br^-, I^-, ClO_4^-) can be used (Schill, 1974). Of these only the inorganic anions had acceptable gas chromatographic properties. For practical reasons we chose iodide as counter ion.

When an aqueous solution of potassium iodide is added to an aqueous solution of thiazinamium cations a 1:1 ion pair, thiazinamium iodide, is formed. This was confirmed by mass spectrometry, infrared spectrometry and elemental analysis. Suitable extraction conditions can be estimated from the structure of the ion pair, the nature of the organic phase, the extraction constants and the constants for side reactions (Persson & Schill, 1966). The partition of thiazinamium between the organic and the aqueous phase is expressed by the distribution ratio $D_{ThI} = \frac{c'_{ThI_{org}}}{c'_{Th^+_{aq}}}$ or

$$D_{ThI} = E_{ThI} \times c'_{I^-_{aq}}$$

where $c'_{ThI_{org}}$ = the total concentration of thiazinamium cation, present in the organic phase as ion pairs with iodide. $c'_{Th^+_{aq}}$ = the concentration of the thiazinamium cation in the aqueous phase after equilibrium. $c'_{I^-_{aq}}$ = the concentration of the iodide anion in the aqueous phase after equilibrium. $E^*_{ThI} = \frac{c'_{ThI_{org}}}{c'_{Th^+_{aq}} \times c'_{I^-_{aq}}}$ (the conditional extraction constant).

When E_{ThI} is known the value of $c_{I^-_{aq}}$ to give $D_{ThI} = 100$ (~99% of the ion pair in the organic phase) can be determined.

Determination of the extraction constants of thiazinamium. The strength of the solvation of the ion pair depends on the properties of both the solute and the solvents, particularly the ability to form hydrogen bonds (Schill, 1974). From a number of organic solvents, chloroform, dichloromethane and dichloroethane were chosen for the extraction of thiazinamium iodide. For any solvent the conditional extraction constant E^*_{ThI} can be calculated for any concomitant side reaction—in this case the dissociation of the ion pair in the organic phase. Therefore,

$$E^*_{ThI} = E_{ThI} + \left[(E_{ThI} \times K_{diss})^{\frac{1}{2}} \times (c'_{Th^+_{aq}} \times c'_{I^-_{aq}})^{-\frac{1}{2}} \right]$$

showing that E^*_{ThI} will increase with decreasing values of $(c_{Th^+_{aq}} \times c'_{I^-_{aq}})$ (Borg, 1969). This is an advantage because the therapeutic plasma levels are very low (nanogram range). The various constants for thiazinamium iodide in chloroform, dichloromethane and in 1,2-dichloroethane are given in Table 1. Although the highest extraction constant is obtained with dichloromethane, the value of E^*_{ThI} in the range 50–500 ng ml^{-1} is much higher with dichloroethane, due to the dissociation of thiazinamium iodide in the organic phase. Thus when the concentration of thiazinamium

Table 1. *Extraction and dissociation constants of thiazinamium iodide in different solvents.*

	Chloroform	Dichloromethane	Dichloroethane
$[Th^+]$	$9{\cdot}9 \times 10^{-5}$	$9{\cdot}9 \times 10^{-5}$	$9{\cdot}9 \times 10^{-5}$
$c'_{I^-_{aq}}$	$9{\cdot}5 \times 10^{-4}$–$9{\cdot}5 \times 10^{-3}$	$9{\cdot}5 \times 10^{-4}$–$9{\cdot}5 \times 10^{-3}$	$1{\cdot}7 \times 10^{-3}$–$9{\cdot}5 \times 10^{-3}$
$c_{ThI_{org}}$	$9{\cdot}3 \times 10^{-6}$–$4{\cdot}7 \times 10^{-5}$	$2{\cdot}8 \times 10^{-5}$–$7{\cdot}4 \times 10^{-5}$	$1{\cdot}3 \times 10^{-5}$–$6{\cdot}8 \times 10^{-5}$
E	$0{\cdot}99 \times 10^2$	$2{\cdot}88 \times 10^2$	$1{\cdot}22 \times 10^2$
Log E	1·99	2·46	2·09
K_{diss}	*	*	$7{\cdot}3 \times 10^{-5}$
log K_{diss}	—	—	−4·1

* Values for K_{diss} in chloroform and dichloromethane are negligible.

is approximately 5×10^{-7} (the average therapeutic plasma level) and $c'_{I^-_{aq}} = 1{\cdot}8 \times 10^{-2}$, log E* = 2·5 in dichloromethane and 3·1 in dichloroethane.

The percentage recovery (P) of thiazinamium cations from an aqueous solution can be expressed as

$$P = \frac{100}{1 + \dfrac{V_{aq}}{V_{org} \times D_{ThI}}} \qquad \text{(Schill, 1974)}$$

where V_{aq} = volume of the aqueous phase, V_{org} = volume of the organic phase.

For a concentration of thiazinamium of approximately 5×10^{-7} a recovery of 99% (D = 27·2 for the described procedure) is achieved for a $c'_{I^-_{aq}} = 1{\cdot}86 \times 10^{-2}$. In practice with this concentration of iodide ions the recovery was 89·5%. Higher concentrations of iodide ion gave no further recovery, probably because of absorption on glass and/or accumulation at the interface between the aqueous and the organic layer.

Isolation from plasma. With human plasma, chloroform and dichloromethane were unsuitable because of coextraction of a number of plasma components, resulting in high blanks. Dichloroethane proved suitable at pH = 10*. The initial concentration of iodide necessary for quantitative extraction of thiazinamium cations (approximately 5×10^{-7} mol) from water was calculated to be $1{\cdot}86 \times 10^{-2}$ mol. However, a quantitative extraction from a complex sample like plasma was found to require a higher iodide concentration, probably due to additional side reactions, e.g. protein binding of the iodide, coextraction of other iodide ion pairs (Westerlund & Karset, 1973). The amount of iodide anions taken away by side reactions may vary from patient to patient. In practice a concentration of 0·1 M appeared to be sufficient to get an adequate recovery.

Isolation from urine. Because of higher concentrations of thiazinamium, 0·50 ml urine samples were examined. These were diluted with 1·5 ml distilled water and the pH adjusted to 7, higher values as with plasma, were unnecessary.

(B) *Gas chromatographic procedure.* Thiazinamium iodide undergoes immediate quantitative demethylation in the injection port at 300° into the volatile compounds promethazine and methyl iodide. This has been confirmed by combined gas chro-

* Extraction constants at pH 10 are almost the same as those at pH 6·5.

matography–mass spectrometry. The retention time of promethazine is 350 s and of chlorpromazine (standard) 700 s (Fig. 1).

In order to avoid degradation in the gas chromatograph, glass columns, glass-lined injection ports and glass-lined detector entrances were used. The column system was silanized before use with Silyl-8. A rubidium bromide flame ionization detector (A.F.I.D.) is selective for nitrogen compounds (Donike, Jaenicke & others, 1970; Goudie & Burnett, 1973; James & Waring, 1973; Natush & Thomas, 1973; Breimer & van Rossum, 1974; Riedmann, 1974a,b) and under the conditions of use gave a 50-fold higher response than a normal F.I.D.

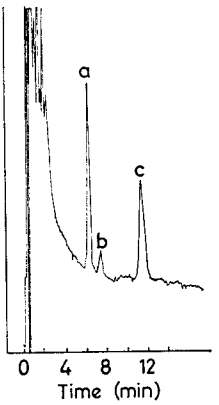


FIG. 1. Gas chromatogram of a plasma extract. a = promethazine formed by demethylation of thiazinamium iodide (30 ng). b = di-(2-ethylhexyl)phtalate, contaminant from the PVC cap liner (1·5 μg). c = gas chromatographic standard chlorpromazine HCl (25 ng).

The optimal use of an A.F.I.D. requires exact adjustment of several parameters. The distance between the rubidium bromide crystal and the top of the burner jet is of major importance and must be adjusted with precision. There is a pronounced decrease in sensitivity if the crystal surface is not clean. The surface must be cleaned regularly before use with a special brush. After prolonged use a white coating of SiO_2, the oxidation product of the silicon rubber stationary phase and of the Silyl-8, appears on the crystal surface. It can be removed with very fine sand paper. The flow rate of the detector gases are also critical. The selectivity can be optimized by adding extra carrier gas into the detector. The optimal operating conditions can be achieved by adjusting the parameters and monitoring the detector response of an 1 μl injection of a mixture of 500 mg octadecane and 1 mg azobenzene per 100 ml n-hexane. Suitable conditions have been achieved when the response of the azobenzene is several times (e.g. 5 $\times$) the response of the octadecane. Replacement of the dichloroethane is necessary since alkali flame ionization detectors are contaminated by halohydrocarbons.

For plasma extracts a calibration curve of 5–50 ng thiazinamium iodide versus 25 ng chlorpromazine HCl, and for urine extracts a calibration curve of 50–500 ng thiazinamium iodide versus 250 ng chlorpromazine HCl was used.

Selectivity, sensitivity, recovery and reproducibility. When normal human plasma or urine was examined as described in the procedure, no blank values were observed. The main metabolite of thiazinamium, which was found to be thiazinamium sulphoxide, is much more polar than the parent drug and is not extracted from plasma

or urine in this way (Jonkman, Wijsbeek, Hollenbeek Brouwer de Boer, Greving, van Gorp & de Zeeuw, unpublished). So far, except for promethazine and chlorpromazine, no other drugs have been found to interfere with the present procedure.

Plasma concentrations of 2 ng ml^{-1} can be detected and accurate quantitation can be done at concentrations of 20 ng ml^{-1}, and above. The recovery from plasma is 88·0 ± 6·2% (n = 40); from urine a recovery of 91·4 ± 4·6% (n = 40) has been obtained.

Experience with other quaternary ammonium compounds indicates that the method has general applicability. This is due to the good extractability of the iodide ion pair and to the fact that this ion pair allows g.l.c. analysis because it easily demethylates into the more volatile tertiary amine.

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