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Quantitative analysis of 4′-chloro-2-ethylaminopropiophenone, 4′-chloro-2-aminopropiophenone and the corresponding aminoalcohols in a mixture of the four compounds

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A method specific for the quantitative analysis of 4′-chloro-2-ethylaminopropiophenone, and 4′-chloro-2-aminopropiophenone in the presence of their corresponding aminoalcohols in urine is described. The instability of the aminoketones makes extraction from alkaline solutions impractical. Hence they are quantitatively reduced to their corresponding alcohols and analysed using gas-liquid chromatography.

Some α-aminoaromatic ketones, like diethylpropion (2-diethylaminopropiophenone), are used as drugs. Their assay is difficult because they are unstable in alkaline solution and therefore they cannot be extracted from urine before gas-liquid chromatography without some decomposition occurring. The related aminoalcohols like methylephedrine, ephedrine and norephedrine are, however, stable in alkaline solution and can be so analysed (Beckett & Wilkinson, 1965).

We have devised a method of quantitative analysis for the aminoketone 4′-chloro-2-ethylaminopropiophenone (compound I, Table 1), the aminoketone (compound III) and aminoalcohols (compounds II and IV) in the presence of each other in urine in which they are found after oral doses of compound I are given to man. The assay is applicable to other α-aminoketones.

EXPERIMENTAL

Apparatus

Perkin-Elmer Model F11 Gas Chromatograph (F.I.D.). Hitachi Perkin-Elmer Model 159 recorder. Lab-tek Aliquot Mixer. Centrifuge tubes with ground glass stoppers. Evaporating tubes with finely tapered base (Beckett, 1966). Pye Dynacap pH meter.

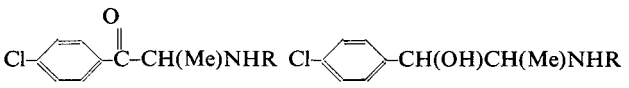
Materials and reagents

Compound I HCl, compound II HCl, compound III HCl and compound IV HCl were supplied by Smith, Kline and French Labs., Philadelphia. The buffer contained 52·4 g dipotassium hydrogen orthophosphate and 46·8 g of sodium dihydrogen phosphate in 200 ml of water. The internal marker solution contained pethedine HCl (9·69 μg/ml water). 20% NaOH. Sodium borohydride. Freshly distilled Analar diethyl ether.

Chromatography

A 1 metre stainless steel tube ($\frac{1}{8}$ in.o.d.) packed with 80–100 mesh Chromosorb G

Table 1. *Structures of compounds investigated*



Compound	Aminoketone Structure	Aminoalcohol Chemical names
I	Ketone R = Et	4′-Chloro-2-ethylaminopropiophenone
II	Alcohol R = Et	1-(4-Chlorophenyl)-1-hydroxy-2-ethylaminopropane
III	Ketone R = H	4′-Chloro-2-aminopropiophenone
IV	Alcohol R = H	1-(4-Chlorophenyl)-1-hydroxy-2-aminopropane

(acid-washed, dimethyldichlorosilane treated) which was coated with 10% KOH and 10% Apiezon L was used. The column was conditioned for 24 h at the following operating conditions, oven temp. 157°, injector block temp. *ca* 160°, hydrogen pressure 14 lb/in²; air pressure 24 lb/in²; nitrogen flow 70 ml/min; stream split ratio *ca* 1 : 8. The column was then silanized with 2 × 5 μl hexamethyldisilazane.

Procedure

(A) *Analysis in urine of the aminoketones (I and III) in the presence of the aminoalcohols (II and IV).* The stable aminoalcohols (II and IV) were measured by extracting them into ether, followed by gas-liquid chromatographic analysis (Analysis 1). Compounds I and III were determined by reducing them in the urine to produce the corresponding aminoalcohols II and IV (Analysis 2).

Analysis 1 (for aminoalcohols). A urine solution (4 ml) containing compounds I–IV was placed in a centrifuge tube containing the internal marker solution (1 ml) and NaOH (0·5 ml). The compounds were extracted with 4 × 2·5 ml of ether using a shaker (2 min), centrifuged (5 min) and the ether extracts were transferred to a 15 ml Quickfit test tube (tapered base), concentrated (*ca* 50 μl) in a water bath (40°) and the tube was placed in ice. 5 μl of the concentrate was analysed by gas-liquid chromatography. The drug to marker ratios for the two alcohols were determined by measuring the heights of the peaks and these ratios were multiplied by the appropriate calibration factor, determined as described below, to give the concentration (μg/ml) of drug in the urine.

Analysis 2 (for aminoketones). A second aliquot (4 ml) was placed in a centrifuge tube with the internal marker solution (1 ml). The tube was placed in an ice bath for 10 min, buffer solution (0·5 ml) was added and the tube returned to the ice bath for 5 min. Sodium borohydride (*ca* 10 mg) was added, the tube was slowly inverted once and returned to the ice bath for 20 min. The addition of sodium borohydride was repeated twice at 20 min intervals. The NaOH (0·5 ml) was added and the compounds (II and IV) were extracted, analysed and the concentrations calculated as above. The value for each compound obtained in Analysis 2 (ketones I and III, and alcohols, II and IV measured as alcohols II and IV) less the value obtained for the alcohols II and IV all in Analysis 1, give the amount of reduced ketones I and III calculated as the corresponding alcohols.

B) *Calibration factor of compounds II and IV*

Urine (4 ml) containing known quantities of compounds II or IV, or both, was placed in a centrifuge tube. The compounds were extracted, analysed and the drug to marker ratio for each compound calculated using procedure A1. The calibration

factor (μg base/ml urine ÷ the drug to marker ratio) was calculated. The coefficient of variance was calculated for the calibration factor obtained using both amino-alcohols.

(C) *Stability of compounds I-IV in alkaline urine*

Urine (4 ml) containing known quantities of compounds I–IV plus NaOH (0·5 ml of 20%) was allowed to stand (2 h) at room temperature. The internal marker (1 ml) was then added and the contents of the tubes were analysed using procedure A.

(D) *Stability of compounds I–IV stored in urine*

A volumetric flask containing known quantities of compounds I–IV had urine and solutions of either HCl or NaOH added to adjust the urine pH to 4·5, 6·5 or 8·5. The solution was placed in clear glass bottles and stored at either room temperature in the laboratory or in darkness at 4°. "Blank" urine samples were prepared and stored as above.

(E) *Error of injection*

An ethereal concentrate containing internal marker and either compound II or IV was repeatedly injected into the chromatograph. All drug to marker ratios for each alcohol were averaged and the coefficients of variance were calculated.

RESULTS AND DISCUSSION

Injection of an ethereal solution, containing compound II or IV and the internal marker, into the chromatograph (procedure E) led to a ±2% coefficient of variance (8 samples). Extraction of compound II from urine followed by chromatography resulted in a coefficient of variance of ±2·5% (8 samples), whereas with compound IV there was a ±6·2% coefficient of variance (8 samples). This latter error for compound IV could have been decreased with the use of an appropriate gas-liquid chromatographic column and solvent for extracting, but, for the purpose of analysis one column and solvent which could be used satisfactorily for compound II in the presence of ketones and urinary constituents was adopted.

The aminoketones (I and III), but not the aminoalcohols (II and IV), were unstable when left in 4 ml of alkaline urine (0·5 ml of 20% NaOH) for 2 h. Typical results before and after this storage were compound I, 1·56/0·21; compound II, 1·53/1·46; compound III, 1·58/0·24 and compound IV, 1·62/1·57 μg/ml. Thus there would have been negligible decomposition of the aminoalcohols during their extraction from alkaline urine into ether.

At acid pH values (4·5 and 6·5) the aminoketones and aminoalcohols were stable in the dark at 4° for at least 2 days (ketones) and 5 days (alcohols) without interfering

Table 2. *Determination of the compounds I–IV in urine*

Compound	Amount of compound added to urine (μg/ml)				Amount of compound determined in urine (μg/ml)			
	I	II	III	IV	I	II	III	IV
	1·56	1·53	1·58	1·62	1·76	1·46	1·71	1·53
	3·13	1·53	1·58	—	3·11	1·45	1·44	—
	1·56	3·05	—	1·62	1·38	3·13	—	1·60
	1·56	—	3·15	1·62	1·49	—	3·23	1·47
	—	1·53	1·58	3·24	0·01	1·49	1·65	3·31
	1·56	3·05	1·58	—	1·49	2·93	1·37	—

peaks developing in the urine. Thus, immediate analysis of urine samples is not necessary provided the sample is acidic.

Straight line calibration curves were obtained for the aminoalcohols within the experimental error previously mentioned. As the aminoketones were quantitatively reduced to the alcohols the quantitative determination of compounds I–IV in urine (Table 2) was within the experimental error discussed above and thus, this method of analysis is suitable for following the distribution and metabolism of compound I in man.

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