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Note

A thin-layer chromatographic method for the quantitative determination of quinidine in human serum

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The usefulness of quinidine as an antiarrhythmic agent is limited by the difficulty of determining an adequate dosage and a suitable dosing interval[1]. Serum concentrations between 4 and 8 μg/ml are generally accepted to be therapeutic levels. It is therefore imperative to be able to determine quinidine levels in blood with precision and accuracy. Methods described in the literature usually make use of an extraction procedure[2], direct reading of the fluorescence of quinidine in deproteinated serum or plasma[3], or the separation of quinidine on thin-layer chromatographic (TLC) plates, extraction of the appropriate bands and subsequent reading of the fluorescence on a spectrofluorimeter[4].

This paper describes a simple but highly accurate and precise method for determining quinidine. It consists of partially deproteinating a small volume of serum, spotting the supernatant on a TLC plate together with known standard amounts of quinidine, developing the plates and measuring the fluorescence of the quinidine spots directly on the plate by using a suitable instrument. Quantitation is effected by preparing a standard graph of quinidine concentration *versus* peak height.

EXPERIMENTAL

Reagents

The reagents used were of guaranteed reagent grade (Merck, Darmstadt, G.F.R.).

Apparatus

A Perkin-Elmer MPF3 spectrofluorimeter equipped with a thin-layer chromatogram scanning attachment was used to measure the fluorescence of the spots on the thin-layer plates using the following operating conditions: light source, xenon lamp; excitation wavelength, 335 nm; emission wavelength, 445 nm; emission filter, 430 nm; excitation slit width, 10 nm; scanning speed, "high" (4 cm/min); paper speed, "low" (2.5 cm/min). The emission slit width, amplifier sensitivity, zero suppression and sample adjustment were set so as to obtain approximately 80% of full-scale deflection on the recorder when the strongest spot in the chromatogram was being scanned.

The other apparatus used consisted of silica gel 60 TLC plates, dimensions

10 × 20 cm (Merck) and 5-μl disposable glass micropipettes (Clay Adams, Division of Becton, Dickinson & Co., Parsippany, N.J., U.S.A.).

Stock solutions

Stock solutions were made up in absolute methanol and stored at −20°. Stock solutions containing 1, 2, 4 and 8 μg of quinidine per 100 μl of absolute methanol were prepared as follows. The quinidine was weighed on a Mettler ME22 electronic microbalance and the solution made up with methanol so as to contain 10 mg of quinidine in 10 ml of absolute methanol. By further appropriate dilutions, the above stock solutions were prepared. These stock solutions can be stored at −20° for about 2 weeks, but greater accuracy can be obtained by making up fresh solutions every time determinations are carried out.

Standard solutions

Four standard solutions were prepared by evaporating under nitrogen 100 μl of each stock solution in screw-capped bottles and then adding 1 ml of fresh, drug-free human serum. The containers were closed and left at room temperature for 30 min with occasional agitation in order to allow the quinidine to dissolve completely. These standard solutions were made up freshly for each series of determinations.

Preparation of serum

A 50-μl volume of serum (standard or unknown) was measured accurately into a small glass-stoppered conical centrifuge tube. To the serum was added 100 μl of methanol in order to precipitate the proteins. The contents of the tube were mixed thoroughly by means of a Whirlimixer and centrifuged for 1 min to produce a clear supernatant.

Spotting the plates

Of the clear supernatant fluid, 5 μl were removed with a clean micropipette

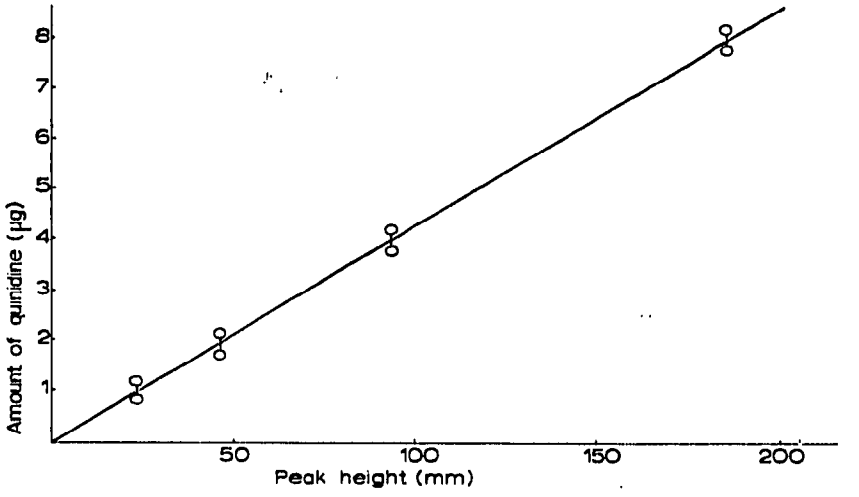

Fig. 1. Standard graph of peak height *versus* amount of quinidine.

TABLE I

RECOVERY OF QUINIDINE FROM HUMAN SERUM

Quinidine added (μg)	*Quinidine recovered (μg)* *	*Mean recovery ± S.D. (μg)*
2.2	2.15, 2.25, 2.20, 2.20	2.20 ± 0.04
2.4	2.30, 2.30, 2.25, 2.20	2.26 ± 0.05
4.0	3.90, 4.00, 4.20, 4.05	4.04 ± 0.13
5.6	5.82, 5.72, 5.68, 5.68	5.73 ± 0.07

* Four replicate determinations.

and applied to the TLC plate in one smooth application. The liquid was allowed to run on to the plate by gravity and the natural capillary action of the plate only. In this fashion, standard sera and unknown sera, alternating in duplicate, were applied to a single 10 × 20-cm plate, allowing four determinations on unknown sera to be carried out in duplicate on one plate.

RESULTS

The fluorescence peak heights measured for the standard solutions were used to plot a standard graph of peak height *versus* quinidine concentration (Fig. 1). By means of this graph, the recovery of quinidine from human serum samples was determined (Table I).

Specificity

A variety of drugs, including commonly prescribed antiarrhythmic agents, were tested for possible interference in this determination, but under the conditions described no interference was found.

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