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THE ESTIMATION OF QUINIDINE IN HUMAN PLASMA BY ION PAIR EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid, sensitive, accurate method for determination of quinidine in plasma has been developed using ion-pair extraction and high-performance liquid chromatography. The method, which is capable of distinguishing between quinidine and dihydroquinidine, involves acidification of plasma with perchloric acid, extraction with methyl isobutyl ketone and chromatography of the carbonate-washed extract on a silica gel column with a mobile phase of methylene chloride—hexane—methanol—perchloric acid (60:35:5.5:0.1) followed by fluorometric detection. The procedure is sensitive to below 50 ng/ml (coefficient of variation 6.6%) and compares favourably with a standard spectrofluorometric method when tested with plasma from volunteer subjects.

INTRODUCTION

The cardiac depressant quinidine is used widely in the treatment of certain cardiac arrhythmias. Due to the narrow range between its effective and toxic concentrations (3—5 μg/ml) [1] there is a need for a rapid, accurate and sensitive procedure for monitoring plasma levels of this drug, clinically.

Quinidine is presented as different salts in a variety of dose forms which generally contain 3—10% dihydroquinidine [2, 3]. In humans both of these substances undergo biotransformation [4] with the major urinary metabolites being either 2′-quinidinone, and 3-hydroxyquinidine or the dihydro analogues.

The fluorometric method of Cramér and Isaksson [5] used for over a decade for the assay of quinidine in plasma was found to be non-specific by several workers [6—8]. Armand and Badinand [8] modified the extraction procedure to remove most of the interfering metabolites. However, neither these procedures nor the more recent gas chromatographic procedures of Valentine

et al. [9] and Midha and Charette [10] differentiated quinidine from dihydroquinidine.

An additional problem with the use of direct spectrofluorometric procedures in the clinical situation occurs because cardiac patients may be exposed to several drugs which may interfere in assays. Recently such an interference has been described with triameterene [11, 12] which has extraction and fluorescence characteristics similar to those of quinidine.

The present paper describes the resolution of these problems by application of recent developments in ion-pair extraction and chromatography [13] together with the separatory power of high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

Quinidine sulphate was obtained from Burroughs Wellcome, (La Salle, Canada); its dihydroquinidine content was 9.3%. 2'-Quinidinone and 3-hydroxyquinidine were the generous gift of Dr. F.I. Carroll (Research Triangle Institute). Dated plasma, obtained from the Canadian Red Cross, was used for blanks and calibration curves.

The solvents used for chromatography were from Burdick and Jackson (Muskegon, Mich., U.S.A.). All other chemicals were of reagent grade, purchased locally.

Chromatography

Equipment. The chromatographic apparatus used consisted of a Rheodyne Model 7120 injector, Waters Model 6000A pump and Schoeffel Model FS 970 fluorescence detector.

Conditions. The column (250 × 3 mm I.D.) was packed by a balanced-density slurry method using silica gel (LiChrosorb Si-60, 5 μm, E. Merck, Darmstadt, G.F.R.). The mobile phase dichloromethane—hexane—methanol—70% perchloric acid (60:35:5.5:0.1) was pumped at a flow-rate of 3 ml/min, producing a back pressure of 3500 p.s.i.g. (Flow-rates of 2 ml/min, used in some experiments, gave equivalent results). The excitation wavelength of the detector was set at 325 nm, and the emission monitored using a sharp-cut filter at 420 nm. The gain of the detector was adjusted to give a peak-to-peak noise of 0.5% full scale (f.s.) at the sensitivity of 0.05 μA f.s. and the time constant setting at minimum. Monitoring at 0.02 or 0.01 μA f.s. was done with the time constant set at nominal 2 sec.

Solutions

Dilutions of perchloric acid (1 *M* and 3 *M*) were prepared from the 70% reagent (approx. 13 *M*) with fluorescence grade water. Methyl isobutyl ketone (MIBK) saturated with an equal volume of 1 *M* perchloric acid was prepared shortly before use.

Standards

Quinidine sulphate was dissolved in water at the concentration of 1 mg/ml.

Appropriate dilutions of this solution with blank plasma were made to give the desired concentrations.

Treatment of the human volunteers

Four healthy male volunteers, who abstained from food and water overnight, were administered 200 mg quinidine sulphate (commercial formulations). Venous blood was withdrawn into heparinized evacuated containers (Vacutainers; Becton-Dickinson, Toronto, Canada) just prior to the dose and at appropriate intervals during the next 30 h. The blood samples were immediately centrifuged and the separated plasma kept at —18° until analysis.

Procedures

To 0.5 ml plasma, in a 10 ml round-bottomed glass tube fitted with a PTFE-lined screw cap, 0.25 ml 3 *M* perchloric acid was added. After mixing briefly (Vortex), 1 ml of MIBK (perchloric acid saturated) was added, the mixture was shaken vigorously (Evapo-Mix, Buchler Instruments, U.S.A.) for 5 min and centrifuged for 5 min.

The organic layer was transferred to another tube and the aqueous phase (including the precipitate) re-extracted as above with 1 ml MIBK. The organic phase was combined and washed by shaking briefly (Vortex) with 0.3 ml saturated aqueous potassium carbonate solution and centrifuged. Exactly 100 μl of the washed extract was used for chromatography.

The fluorometric procedure of Armand and Badinand [8] was used with only minor modifications [14].

Quantitative analyses

The amount of quinidine in plasma samples was estimated by comparing the peak height obtained for the sample to a calibration curve, constructed daily, using spiked blank plasma. This curve took into account the actual quinidine sulfate content (90.7% of the weighed amount) of the standard material used.

Statistical evaluations

Correlation coefficient (r^2) and regression were determined by the linear least squares method ($y = b_0 + b_1 x$).

RESULTS AND DISCUSSION

Extraction

Quinidine, a basic substance, can be extracted into organic solvents either at alkaline pH [5] or in the presence of an ion-pair. The former method is somewhat delicate as alkaline solutions of plasma proteins have a tendency to emulsify when shaken vigorously with organic solvents. Furthermore, quinidine-free base is non-fluorescent and requires back-extraction into sulfuric acid for quantitation.

Ion-pair extraction, on the other hand can be accomplished with relative ease, provided an appropriate counter-ion and extraction solvent are used.

For the following reasons the counter-ion of choice in these experiments

was perchloric acid: being a strong acid it remains dissociated in water in the absence of buffers, giving rise to perchlorate ions; it prevents emulsification by virtue of its low pH; it forms a strongly fluorescent organic-soluble ion-pair with quinidine, with some solubility even in non-polar solvents such as hexane (personal observations).

Table I shows the distribution coefficients for quinidine perchlorate between plasma containing 1 *M* perchloric acid and various solvents. Of the solvents tested MIBK appeared to be the most efficient. More polar ketones were not investigated because of the mutual solubility of the organic and aqueous phases.

TABLE I

DISTRIBUTION COEFFICIENT OF QUINIDINE PERCHLORATE BETWEEN PLASMA AND VARIOUS SOLVENTS

Solvents used: Methyl isobutyl ketone (MIBK); ethyl *n*-butyl ketone (ENBK); di-*n*-propyl-ketone (DPK) and benzyl alcohol—toluene (4:1) (BzOH—Tol).

Quinidine (μg/ml plasma)	Solvent	V_{org}/V_{aq}	C_{org}/C_{aq}
1	MIBK	1.33	1.93
1	ENBK	1.33	0.59
1	DPK	1.33	0.45
4	BzOH—Tol	0.25	0.43

The concentration of perchloric acid did not influence the extraction efficiency, provided it was kept above 0.5 *M*. Proper equilibration of the phases required several minutes of vigorous, vortex-type mixing; extractions using a tumbling mixer (Fisher RotoRack) required at least 20—30 min. In neither case was emulsification a problem and the phases were easily separated by centrifuging them briefly. A final wash of the extract with aqueous saturated potassium carbonate was required to remove dissolved perchloric acid which interfered with the chromatography. In earlier experiments dibasic potassium phosphate saturated with MIBK was used. However the saturated carbonate, by also removing most of the dissolved water, gave longer column life.

The recovery of quinidine with the final procedure was 94.2% (coefficient of variation (C.V.) 5.2%). A single extraction gave efficiencies of approx. 70%. While this could reduce the accuracy of the method, in clinical monitoring the resultant saving in time may warrant such a compromise.

Chromatography

In order to avoid the back-extraction of quinidine into an aqueous system, forward-phase chromatography was chosen. Of the mobile phases tried, methylene chloride—hexane (60:35) appeared optimal for separating dihydroquinidine from quinidine, while methanol was used to adjust the retention time. Small amounts of perchloric acid were included to induce fluorescence. The amount of perchloric acid was not critical for fluorescence, but had some influence on the retention times.

Fig. 1 shows chromatograms of plasma extracts. Fig. 1A was obtained by extracting blank plasma to which 1 μg quinidine sulphate standard per ml was added. Dihydroquinidine (peak 1, Fig. 1A; $k' = 4.0$) was estimated to be 9.3% by comparing peak areas. It is well separated from quinidine (peak 2, $k' = 5.0$). Fig. 1B represents the chromatogram of a plasma extract of a volunteer 30 h after ingestion of 200 mg quinidine sulphate. In addition to dihydroquinidine and quinidine, a third peak ($k' = 7.7$) is apparent; the identity of this peak has not been established. Fig. 1C, from a blank plasma, shows no interfering peaks with retention times similar to quinidine and dihydroquinidine.

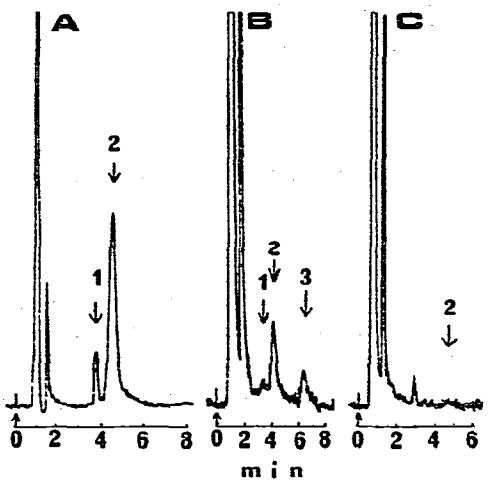

Fig. 1. Chromatographs of plasma extracts with and without quinidine. A, blank plasma spiked with 1 μg quinidine sulphate (containing 9.3% dihydroquinidine) per ml; B, plasma of a volunteer 30 h after the ingestion of 200 mg quinidine sulphate (containing dihydroquinidine) (estimated concentration: 79.3 ng/ml); C, blank plasma. Conditions: Flow-rate: A and C, 2 ml/min; B, 3 ml/min. Sensitivity, A, 50 nA full scale; B and C, 10 nA full scale. Peaks: 1 = dihydroquinidine; 2 = quinidine; 3 = unidentified.

To test for possible interferences, triamterene ($k' = 10.3$), 3-hydroxyquinidine ($k' = 12.1$) and 2′-quinidinone ($k' = 5.0$) have been chromatographed using the same system. Of these only 2′-quinidinone had a retention time identical to quinidine. Since, however, this metabolite contributed little to the measured in vivo plasma concentrations of quinidine (see below), no special attempts were made at this time to remove this interference.

Calibration curve

To blank plasma appropriate amounts of quinidine sulphate standard were added. These spiked standards were carried through the procedure and the peak heights obtained plotted against the concentrations. Table II summarizes the characteristics of the calibration curve. For concentrations of 0.05—2 μg/ml the C.V. values ranged from 3.75—6.94%. Good linearity was evidenced by the steady values for the peak height to concentration ratios. The correlation coefficient (r^2) for the 24 individual determinations was 0.998 and

TABLE II

CALIBRATION CURVE

Correlation: $n = 24$, $r^2 = 0.998$; Regression: slope = 0.0584 inches · μA f.s., intercept = 0.0008 inches · μA f.s. · μg^{-1} · ml.

Concentration (μg/ml plasma)	n	Mean peak height (inches · μA. f.s.)	ht*/C	S.D.	C.V. (%)
0.05	6	0.0030	0.060	0.0002	6.63
0.20	6	0.0132	0.066	0.0009	6.94
0.50	6	0.0301	0.060	0.0018	5.86
2.00	6	0.1174	0.059	0.0044	3.75

*Ratio of peak height to concentration.

the regression showed a negligible intercept (0.0008 inches · μA f.s.) compared to the slope (0.0584 inches · μA f.s. · μg^{-1} · ml).

Plasma concentrations in vivo

The validity of the method was tested by comparison of plasma profiles obtained by the described procedure with those obtained using a fluorometric procedure [8, 14] after administration of 200 mg of quinidine sulphate to four volunteers. Fig. 2 shows the profile for one volunteer from 0—30 h as

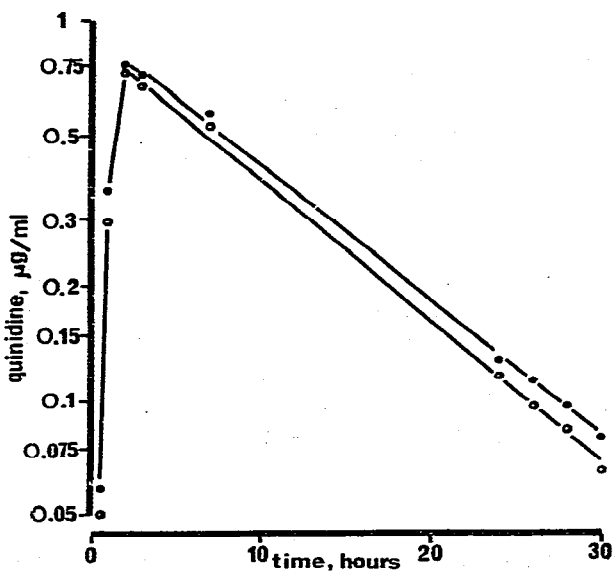

Fig. 2. Plasma profile of quinidine in a volunteer, estimated by fluorometry (•) and HPLC (○).

measured by both methods. Consistently lower values were found by HPLC, as expected, since dihydroquinidine was not measured. Similar results were obtained with the other three volunteers.

All plasma concentrations for the volunteers were 2—10% less by HPLC compared to fluorometry: mean for 1—7 h, 6.4% lower; the 24—30 h values, all in the 60—100 ng/ml range, were 15% lower.

The correlation between the chromatographic (ordinate) and fluorometric (abscissa) procedures was done on the data obtained on 37 random plasma samples, containing 50—1100 ng quinidine/ml. Least-square linear regression gave an intercept that was not significantly different from 0 (95% confidence interval: −9.44 ± 11.23). The regression line forced through the origin (r^2 = 0.999) had a slope of 0.95 ± 0.01 (95% confidence interval), significantly lower than unity, as expected.

Table III shows a comparison of the areas under the plasma concentration—time curve (AUC) for the four volunteers. All AUC values obtained by HPLC are 6—7% lower compared to fluorometry. The AUC for the means is 6.7% lower.

TABLE III

COMPARISON BETWEEN HPLC AND FLUOROMETRIC PROCEDURES FOR THE ESTIMATION OF AUC* FOR QUINIDINE

Volunteer No.	AUC ($\mu g \cdot h \cdot ml^{-1}$)		
	HPLC	Fluorometric	Diff. (%)**
1	10.03	10.77	−6.1
2	10.81	11.52	−6.2
3	9.86	10.54	−6.5
4	9.12	9.83	−7.3
Means***	9.85	10.56	−6.7

*Area under the plasma concentration—time curve, calculated by the trapezoidal rule.
**HPLC relative to fluorometry.
***AUC calculated from the mean plasma concentrations.

Since the values obtained by HPLC are within a few percent of the values obtained by fluorescence corrected for dihydroquinidine it would appear that 2'-quinidinone contributes little to the fluorescence of the quinidine peak. Whether this is because of a lower quantum efficiency at the wavelengths used, a relatively smaller importance in plasma as compared to urine or a combination of both cannot be established from the present study.

In conclusion, the present method can be used for the simultaneous assay of quinidine and dihydroquinidine in plasma. Its sensitivity makes it applicable to single-dose bioavailability studies in humans, while its speed would make it useful in clinical and toxicological monitoring of patients.

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