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Note

An integrated approach to measurements of quinidine and metabolites in biological fluids

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Several procedures to quantitate quinidine in plasma by isocratic reversed-phase high-performance liquid chromatography (HPLC) have been described in the literature [1—6], but only a few of them allow simultaneous, separate quantitation of some of the known major metabolites [4—6]. We recently reported a method for determination of quinidine and its metabolites in urine by reversed-phase HPLC [7]. This analytical technique has now been modified and expanded so that one procedure allows determination in plasma or urine of quinidine, dihydroquinidine and the metabolites 3-hydroxyquinidine, an N-oxide of quinidine and 2′-quinidinone.

EXPERIMENTAL

Materials

The solvents used (acetonitrile and tetrahydrofuran, UV grade; all others, analytical grade) were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.) except dichloromethane and isopropanol, which were supplied by Mallinckrodt (St. Louis, MO, U.S.A.).

Dihydroquinidine-free quinidine was prepared from commercially available quinidine (J.T. Baker, Phillipsburg, NJ, U.S.A.) according to the method described by Thron and Dirscherl [8]. The quinidine metabolites 2′-quinidinone and 3-hydroxyquinidine were kindly supplied by Dr. Irwin Carroll, Triangle Research Institute, O-desmethylquinidine by Syva Labs. (Palo Alto, CA,

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U.S.A.) and the N-oxide was prepared as described elsewhere [9]. Pronethalol was obtained from ICI (Macclesfield, Great Britain).

A Varian Model 8500 high-performance liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) was used, equipped with either a Varian ultraviolet detector set at 230 nm or fluorescence detector (Schoeffel Instrument Corp., Westwood, NJ, U.S.A.), excitation at 245 nm, emission at 340 nm (cutoff filter) and an alkyl phenyl μBondapak column (particle size, 10 μm) of 30 cm × 3.9 mm I.D. (Waters Assoc., Milford, MA, U.S.A.). The mobile phase was 0.05 *M* phosphate buffer pH 4.75—acetonitrile—tetrahydrofuran (80:15:5, v/v) with a flow-rate of 1.5 ml/min.

Procedure

Plasma or urine (200 μl) is mixed with 200 μl or 400 μl respectively of an aqueous solution containing 200 μg/ml of pronethalol (internal standard). A 200-μl aliquot of 0.6 *M* borate buffer pH 9.0 is added followed by 10 ml of dichloromethane—isopropanol (4:1, v/v). After 1 min vortexing and centrifugation for 5 min at 540—1200 *g*, the organic layer is transferred to a separate test-tube and evaporated to dryness. The residue is reconstituted in 200 μl of eluent and a 50-μl aliquot injected onto the high-performance liquid chromatography column. Detection was achieved using the fluorescence detector for plasma extracts and the ultraviolet detector for urine extracts.

For low plasma levels of quinidine and metabolites the volume of plasma taken for assay and of buffer added can be increased from 200 μl to 1.0 ml.

RESULTS

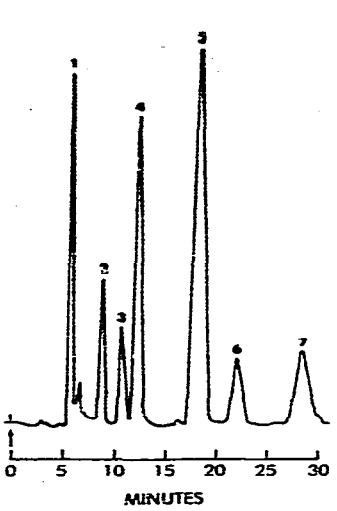
Fig. 1 shows a chromatogram from a plasma sample spiked with quinidine and metabolites. The retention times of 2'-quinidinone, 3-hydroxyquinidine, O-desmethylquinidine, pronethalol, quinidine, dihydroquinidine and an N-oxide of quinidine [9] are 5.7, 8.8, 10.7, 12.1, 18.1, 22.0 and 28.4 min respec-

TABLE I

PRECISION AND ACCURACY OF REVERSED-PHASE ASSAY OF SPIKED URINE SAMPLES

A 1-ml aliquot of urine was taken for assay.

	Spiked concn. (μg/ml)	Measured concn. (μg/ml)					Precision (C.V., %)	Bias (%)
		1	2	3	4	Mean		
Quinidine	0.570	0.565	0.557	0.567	0.596	0.571	3.0	+0.2
	3.80	3.80	3.61	4.09	3.59	3.77	6.1	−0.8
	11.40	12.50	11.07	11.75	12.17	11.87	5.2	+4.1
3-Hydroxyquinidine	0.374	0.388	0.394	0.389	0.398	0.392	1.2	+4.8
	0.934	0.921	0.942	0.938	0.902	0.926	2.0	−0.9
	2.24	2.31	2.27	2.23	2.29	2.28	1.5	+1.8
Quinidine-N-oxide	0.337	0.339	0.337	0.303	0.345	0.331	5.7	−1.8
	0.984	1.03	1.03	1.01	0.991	1.02	1.8	+3.7
	1.69	1.71	1.61	1.62	1.64	1.65	2.7	−2.4
2'-Quinidinone	0.302	0.303	0.301	0.303	0.309	0.304	1.1	+0.7
	0.503	0.494	0.511	0.497	0.489	0.498	1.9	−1.0
	1.21	1.20	1.18	1.17	1.19	1.19	1.1	−1.7



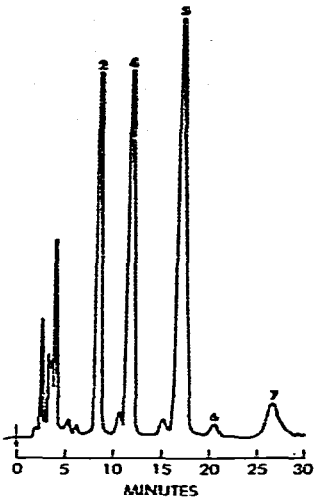


Fig. 1. Chromatogram of plasma spiked with quinidine and metabolites. Peaks: 1, 2'-quinidinone; 2, 3-hydroxyquinidine; 3, O-desmethylquinidine; 4, pronethalol (internal standard); 5, quinidine; 6, dihydroquinidine; 7, N-oxide of quinidine.

Fig. 2. Chromatogram of plasma sample from a patient after multiple doses of quinidine. Peaks: 2, 3-hydroxyquinidine; 4, pronethalol (internal standard); 5, quinidine; 6, dihydroquinidine; 7, N-oxide of quinidine.

tively. Fig. 2 shows a chromatogram of a plasma sample from a cardiac patient after multiple doses of quinidine. No interfering endogenous compounds could be detected in the blank plasma taken before the start of quinidine therapy in this patient. Not all metabolites are found in samples from each individual.

Results from accuracy studies with urine samples spiked at three different concentration levels unknown to the analyst are shown in Table I, where the precision of the assay for each of the compounds is expressed as a coefficient of variation (C.V.) after repeated measurement (n = 4) of the sample. The precision and accuracy are similar to that reported before for this assay using a different internal standard.

Plasma standard curves (Fig. 3) covering the concentration range of the samples to be analyzed were prepared by spiking blank plasma. They were linear for all the compounds, the coefficients of variation for concentration-normalized peak height ratios being 3.4, 7.8, 2.9 and 2.7% for quinidine, 2'-quinidinone, 3-hydroxyquinidine and the N-oxide respectively. Reproducibility and bias specifications for the plasma assay using fluorescence detection are given in Table II.

When taking 1 ml of sample the limit of detection in the assay described is 10 ng/ml for quinidine and 3-hydroxyquinidine, 15 ng/ml for the N-oxide and 20 ng/ml for 2'-quinidinone. The recently identified metabolite O-desmethylquinidine [10] could be detected in spiked plasma at levels exceeding 200 ng/ml.

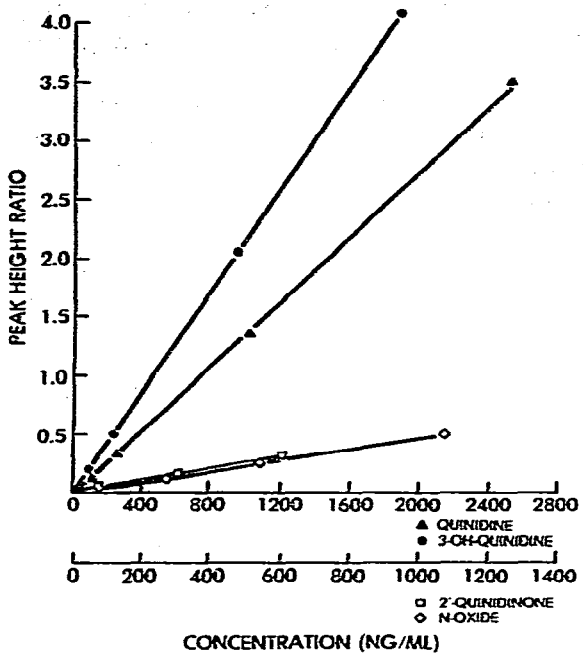


Fig. 3. Standard curves for quinidine and metabolites in plasma.

TABLE II

PRECISION AND ACCURACY OF REVERSED-PHASE ASSAY OF SPIKED PLASMA SAMPLES

A 200-μl aliquot of plasma was taken for assay.

	Spiked concn. (ng/ml)	Measured concn. (ng/ml)					Precision (C.V., %)	Bias (%)
		1	2	3	4	Mean		
Quinidine	285	285	284	281	278	282	1.1	−1.1
	980	981	994	970	968	978	1.2	−0.2
	1960	1870	1883	1851	1847	1863	0.9	−4.9
3-Hydroxyquinidine	93	93	94	93	92	93	0.9	0.0
	458	469	467	459	462	464	1.0	+1.3
	1220	1212	1218	1201	1156	1197	2.3	−1.9
Quinidine-N-oxide	105	101	110	101	108	105	4.5	0.0
	295	284	290	280	301	289	3.2	−2.0
	699	707	712	701	663	696	3.2	−0.4
2'-Quinidinone	74	73	74	71	76	74	2.8	0.0
	147	156	152	144	146	150	3.7	+2.0
	189	186	194	191	186	189	2.1	0.0

DISCUSSION

The procedure described above enables assay of both urine and plasma samples using the same extraction procedure and chromatographic system. Different detection methods are, however, required. Interfering endogenous compounds do not allow fluorescence detection of urine extracts without extensive work-up, but fortunately drug and metabolite levels in urine are high enough to be measured conveniently with ultraviolet detection. Plasma samples, in con-

trast, are often available in only limited volume or may contain levels in only the lower nanogram range. Fluorescence detection, with its greater sensitivity, can, however, be used for plasma extracts.

Several charges had to be made to our previously reported method to allow assay in both plasma and urine. The internal standard pronethalol has been adopted because it can be traced with both ultraviolet and fluorescence detectors. To achieve better separation between the compounds the pH of the eluent was adjusted to 4.75.

Adjustment of the plasma or urine pH to a value of 9 by addition of borate buffer is important to avoid the reduced extraction of O-desmethylquinidine at higher pH. Below pH 9 polar endogenous compounds are extracted and interfere with the 2'-quinidinone peak.

The above modifications to the pre-existing urine assay [7] thus allow analysis of either plasma or urine with a coefficient of variation of less than 6%. No appreciable bias is introduced (Tables I and II). All known quinidine metabolites can be separated (Fig. 1) and the assay is sufficiently sensitive for use in pharmacokinetic or clinical studies if quantitation of any of the metabolites other than O-desmethylquinidine is important. O-Desmethylquinidine appears in much lower concentration in plasma or urine than the other metabolites [11].

The superiority of assay procedures, where metabolites are separated from parent drug, over still widely used fluorescence techniques has been previously discussed [5, 7, 12, 13]. A lower but more accurate estimate of quinidine levels results. To gain full advantage of these more accurate levels, reassessment of the therapeutic range of quinidine plasma concentrations is necessary.

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