Journal of Chromatography, 144 (1977) 223–230

CHROM. 10,389

SPECIFIC THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF QUINIDINE IN BIOLOGICAL FLUIDS

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(Received April 29th, 1977)

SUMMARY

A sensitive, accurate and specific spectrodensitometric method has been developed for the determination of quinidine in biological fluids. It involves extraction of quinidine, dihydroquinidine and metabolites, their separation on thin layers and quantitation of the corresponding spots by direct scanning in a densitometer at 278 nm. A linear relationship was obtained between the ratio of the peak area of an unknown sample to that of the standard and the concentration of the compounds at 0.4–4 μg/ml. The recovery from plasma was from 96 to 103% for quinidine and from 93.5 to 98.5% for dihydroquinidine. A comparison was made between this thin-layer chromatographic method and the fluorimetric assay frequently used for the determination of quinidine in plasma at present. The method is recommended for clinical assays and pharmacokinetics studies.

INTRODUCTION

Variations in the plasma levels after oral and intravenous administration of quinidine, an effective atrial and ventricular cardiac antiarrhythmic agent, have prompted the Food and Drug Administration to require evidence of drug bioavailability for all marketed oral quinidine pharmaceutical preparations[1].

The reasons for substantial differences in bioavailability of quinidine are not quite certain. Several studies have indicated that variations in both rate and the extent of quinidine absorption from the gastrointestinal tract may be responsible for the differences in quinidine plasma levels in subjects ingesting equivalent oral doses of this drug. Another contributing factor may be weaknesses in the analytical procedures used to evaluate quinidine plasma levels, many of which are neither specific nor sensitive enough for the low concentrations observed.

Most of the analytical methods make use of the ability of quinidine to fluoresce in acid and involve either precipitation of plasma proteins and direct fluorimetric determination of the filtrate[2,3] or extraction of the drug into an organic solvent and fluorimetric determination after acidification or transfer to sulfuric acid[4–7]. A photofluorimetric method of this type[8] is the most frequently used assay for the determination of quinidine in plasma at present. In this case the drug is extracted from plasma

with benzene and from the benzene into sulfuric acid. Fluorescence of the sulfuric acid solution is then measured[5]. Extraction of the drug from benzene into acid is said to separate quinidine from its more polar metabolites and other interfering substances from plasma[9]. In another similar spectrofluorimetric assay[10] the benzene extracts are washed with sodium hydroxide solution to remove some of the fluorescent metabolites. Consequently, the extraction methods produce lower results than the methods not using extraction, but they have greater specificity. None of these fluorimetric measurements, however, differentiates between quinidine and dihydroquinidine, a known impurity in medicinal grade quinidine.

More selective methods, including gas chromatography[11–13], high-speed liquid chromatography combined with thin-layer chromatography (TLC)[14] and chemical ionization mass spectrometry[15], have been used for determination of quinidine from pharmaceutical preparations and biological fluids, but most of these are time consuming procedures and require unusual instrumentation.

The use of TLC has been proposed and a method developed for the separation and quantitation of quinidine and other alkaloids in the extracts obtained from Cinchona bark on thin layers of silica gel using direct reflection and fluorescence measurements[16]. Other TLC methods have consisted of separation of quinidine on the plates, elution of the corresponding quinidine bands into a suitable solvent followed by measurements of the drug using an appropriate physicochemical assay procedure[17,18]. These methods require special care to avoid loss of the silica gel during transfer from the plates and to achieve complete extraction from the adsorbent. Direct quantitation of quinidine on chromatoplates has been reported for estimation of quinidine in serum[19]. Small volumes of deproteinated sera were spotted directly onto the chromatoplates and the fluorescence of the spots of quinidine was measured after development. In another TLC assay[20] the serum or plasma containing quinidine and salicylic acid was applied directly to silica gel layers without extraction, the proteins being precipitated on the plate with ethanol. The chromatograms were then developed and quantitated by fluorescence scanning. These TLC methods indicate the potential of TLC for quinidine measurements, but their failure to separate the drug and metabolites from serum proteins before applying to the plate constitutes a troublesome weakness, and their failure to demonstrate separation of dihydroquinidine and metabolites gives them no distinct advantage over other methods.

In this communication a method is described which is accurate for levels of quinidine in biological fluids found in single-dose studies. It involves extraction of quinidine, dihydroquinidine and metabolites, their separation on thin layers and quantitation of the corresponding spots by direct scanning in a densitometer. This procedure provides greater precision and sensitivity, its technique is simple and it is time saving. The major advantage is its specificity and the provision for simultaneous quantitation of quinidine, dihydroquinidine and metabolites on the same chromatoplate. The deficiency of the fluorimetric assay was demonstrated by chromatographic separation of the fluorescing components in the extracts obtained by the fluorimetric procedure[8] applied to samples of plasma spiked with quinidine sulfate, and to rabbit plasma and urine samples. Application of the TLC assay was demonstrated by measuring rabbit plasma and urine following an intravenous dose of quinidine gluconate.

EXPERIMENTAL

Materials and reagents

Silica gel 60 precoated plates 20 × 20 cm were used (E. Merck, Darmstadt, G.F.R.). A 50-μl Hamilton syringe was used for dissolving the evaporated extracted samples and 10-μl Hamilton syringes for spotting. The chromatograms were developed in glass tanks, 7 × 22 × 22 cm, containing 100 ml of solvent. The developing liquid was methanol–acetone (5:1).

All solvents were certified A.C.S. spectranalyzed grade.

Samples of quinidine sulfate and dihydroquinidine were received from Eli Lilly (Indianapolis, Ind., U.S.A.). Quinidine gluconate injections USP 80 mg/ml (Eli Lilly) were obtained commercially.

Authentic standard solutions of quinidine and dihydroquinidine were prepared from the stock solutions in chloroform containing 0.1 mg/ml, calculated as the free base.

Apparatus

Absorbance was measured with a UV–VIS-2 chromatograph scanner (Farrand, Valhalla, N.Y., U.S.A.) and recorded as a peak on a Farrand Model 100 strip chart recorder. Simultaneously, the area under each peak was computed and recorded with an integrator (CDS 101 Chromatography Data System, Varian, Palo Alto, Calif., U.S.A.).

Fluorescence was measured with an Aminco-Bowman spectrofluorimeter (American Instrument, Silver Spring, Md., U.S.A.).

Extraction procedure

The extraction of quinidine from plasma and urine was done in the following manner, which is similar to that applied to other basic drugs[21,22].

To 0.5–1.0 ml of plasma or urine in a 15-ml glass centrifuge tube is added 0.5 ml of 0.1 *N* sodium hydroxide and 4 ml of dichloromethane, and the mixture is mechanically shaken at room temperature for 15 min. The tube is then centrifuged for 10 min and the organic layer transferred to a second tube. The aqueous layer is re-extracted in the same manner with another 4 ml of dichloromethane and the combined organic layers are evaporated to dryness at 45° under a stream of nitrogen. The residue is dissolved in 50 μl of chloroform and an aliquot of 10 μl spotted on a TLC plate. On each plate three 10-μl spots of standard solutions are placed to serve as control for the particular plate. The plate is developed in the methanol–acetone solvent in a saturated tank. After the plate is developed, it is allowed to air dry and the absorbance intensity is measured at 278 nm in the spectrodensitometer.

The appropriate peak area corresponding to the quinidine in each unknown sample (A_u) is then divided by the area of the standard (A_s) on the same plate to obtain the ratio A_u/A_s.

Using standard solutions of quinidine and dihydroquinidine in chloroform, standard curves of concentration *vs.* area ratio were prepared and they were used for determination of quinidine (and if needed dihydroquinidine) in plasma and urine specimens.

Recovery

The recovery of quinidine and dihydroquinidine was determined by adding known amounts of the drugs to plasma (concentration 0.5–5 μg/ml) and comparing the absorbance peak areas obtained after extraction from these spiked plasma samples with the respective authentic standards scanned on the same plate.

Animal experiment

A 10-mg/kg dose of quinidine gluconate solution was injected over a period of 1 min into the ear vein of a New Zealand albino rabbit. Blood and urine specimens were collected and they were analyzed by the fluorimetric assay[8] and this TLC method.

Procedure for chromatographic analysis of the extracts from the fluorimetric method

Human plasma spiked with quinidine and collected rabbit plasma and urine samples were analyzed according to the fluorescence method[8]. After reading the fluorescence on the spectrofluorimeter, the 3-ml sulfuric acid extracts were made alkaline with sodium hydroxide and re-extracted with dichloromethane and chromatographed on a thin-layer plate as described in the above TLC procedure. The developed chromatoplate was observed under UV radiation and it was scanned with the spectrodensitometer.

RESULTS AND DISCUSSION

Standard curves for quinidine and dihydroquinidine are illustrated in Fig. 1. A linear relationship between the area ratios and quinidine and dihydroquinidine concentrations was observed in the range between 0.4 and 4 μg/ml. A greater dilution or a larger aliquot of the final solution may be readily used to bring the concentration of the drug in the sample applied to the plate within the desired range.

Recoveries of quinidine and dihydroquinidine from spiked human plasma samples were calculated by comparison of the areas under the peaks with those from standard solutions which had been spotted directly on the plates. These data, pre-

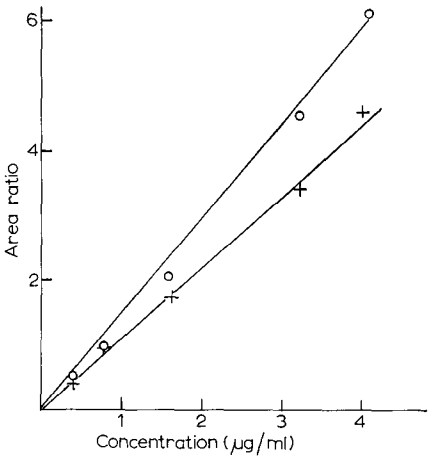

Fig. 1. Relationship of area ratio, A_u/A_s, to concentration, quinidine (○) and dihydroquinidine (×).

TABLE I

RECOVERY OF QUINIDINE AND DIHYDROQUINIDINE FROM SPIKED HUMAN PLASMA

Amounts added to plasma (μg/ml)		*Number of samples*	*Recovered (μg/ml)*		*Percent recovery ± coeff. variation*	
Quinidine	*Dihydro-quinidine*		*Quinidine*	*Dihydro-quinidine*	*Quinidine*	*Dihydro-quinidine*
0.5	—	5	0.49	—	97.5 ± 3.30	—
1.0	—	5	0.96	—	96.1 ± 2.40	—
2.0	—	5	2.06	—	103.4 ± 0.61	—
4.0	—	5	4.08	—	101.7 ± 9.70	—
5.0	—	5	5.00	—	100.3 ± 0.70	—
—	0.5	2	—	0.47	—	94.0 ± 0.00
—	1.0	2	—	0.94	—	93.9 ± 1.60
—	2.0	2	—	1.94	—	96.9 ± 4.00
—	4.0	2	—	3.81	—	93.5 ± 1.20
—	5.0	2	—	4.93	—	98.4 ± 0.20

sented in Table I, show recovery of 96–103% for quinidine and 93.5–98.5% for dihydroquinidine.

The effect of quinidine metabolites and dihydroquinidine on the fluorimetric assay was demonstrated on several samples of human plasma spiked with quinidine,

TABLE II

FRACTIONATION BY TLC OF THE EXTRACTS FROM THE FLUORIMETRIC ASSAY

Amount added to plasma (μg/ml)	*Per cent distribution of each fraction found by TLC in the assay extracts*			
	Quinidine	*Dihydroquinidine*	*Endogenous fluorescent compound*	*Metabolites*
Spiked human plasma				
0.5	20.8	0	79.2	0
1.0	39.4	8.6	52.0	0
2.0	54.1	13.5	32.4	0
4.0	65.4	15.6	19.0	0
5.0	67.6	15.5	16.9	0
Time after dosage (min)				
Rabbit plasma				
15	33.8	0	0	66.2
120	18.2	0	0	81.8
300	17.7	0	0	82.3
Rabbit urine				
15	100.0	0	0	0
30	70.8	0	0	29.2
60	100.0	0	0	0
90	86.7	5.7	0	7.6
120	61.0	24.6	0	14.4
180	43.9	6.0	0	50.1
240	40.8	9.2	0	50.0
300	35.9	16.1	0	48.0

and plasma and urine specimens collected after an intravenous injection of quinidine gluconate to a rabbit. These samples, measured by the fluorimetric method, were chromatographed and they usually contained several fluorescent compounds other than quinidine, including dihydroquinidine, metabolites and sometimes an endogenous compound extracted from human plasma. The results of these measurements are presented in Table II. It was noted that the amounts of the fluorescent compounds, other than quinidine, varied between different samples from the same animal. Such variations would constitute a substantial contribution to the difference commonly observed with the fluorimetric method.

Fig. 2 shows a typical recording of a plate scan from a sample of rabbit urine extract. When examined under UV radiation, quinidine (spot 5 with R_F 0.45), dihydroquinidine (spot 4 with R_F 0.26) and six additional fluorescent spots were observed on the developed chromatoplate.

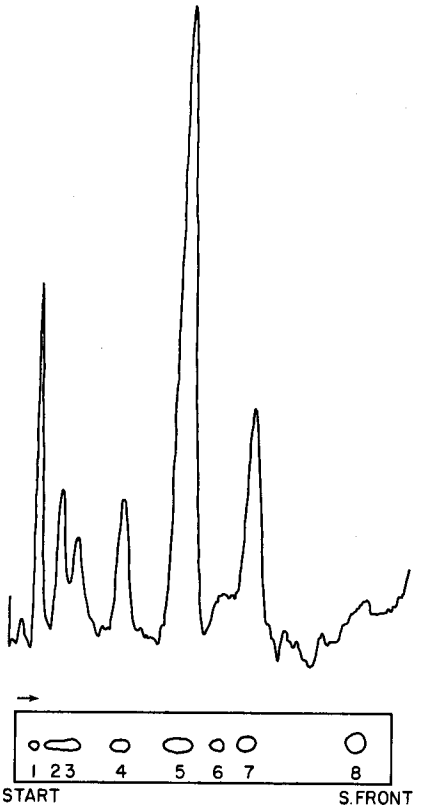

Fig. 2. Thin-layer chromatogram and densitometric scan of quinidine (5), dihydroquinidine (4) and metabolites (1, 2, 3, 6, 7, 8) isolated from rabbit urine.

The differences between the results obtained by the fluorimetric method and this TLC method are presented in Table III. Several plasma and urine samples from a rabbit, after quinidine gluconate injection, were measured by the two methods sepa-

TABLE III

COMPARISON OF THE RESULTS OF RABBIT PLASMA AND URINE SAMPLES USING THE FLUORIMETRIC AND TLC METHODS

Sample number	*Quinidine in plasma (μg/ml)*		*Quinidine in urine (μg/ml)*	
	Fluorimetric assay	*TLC*	*Fluorimetric assay*	*TLC*
1	0.15	0.10	0.23	0.22
2	0.08	0.05	0.44	0.30
3	0.04	0	0.22	0.20
4	—	—	1.32	1.02
5	—	—	2.06	1.20
6	—	—	3.40	1.52
7	—	—	2.60	1.20
8	—	—	3.00	1.05

rately. As expected, the fluorimetric method gave higher concentrations than the TLC assay, since it expresses all extractable fluorescing compounds as quinidine. In the TLC assay, metabolites and other fluorescing substances are separated from quinidine (Fig. 2).

Quinidine is metabolized in the organism and these metabolites are present in serum and urine in addition to unchanged quinidine (Table II). Quinidine metabolites show substantially less of the antiarrhythmic activity of quinidine and to control quinidine therapy the actual quinidine concentration must be determined, especially since the relative amount of quinidine metabolites in serum may vary considerably between individuals. The described TLC method is the most specific in this respect. It also enables the codetermination of dihydroquinidine, a compound said to have antiarrhythmic activity of the same order as that of quinidine or even greater and invariably present in the commercial quinidine preparations.

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