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SIMULTANEOUS DETERMINATION OF ANTIARRHYTHMIA DRUGS BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A method is described for the determination of antiarrhythmia drugs in serum by high-performance thin-layer chromatography. Baseline separations are achieved for all the drugs and clozapine, an internal standard, in two developments with solvents of different polarity. Lidocaine and diphenylhydantoin are scanned at 220 nm after the first development. Procainamide, propranolol and quinidine are scanned at 290 nm after the second development. The relative standard deviation of the determination varies between 3 and 14% depending on the nature of the drug and its concentration.

INTRODUCTION

The antiarrhythmia drugs, lidocaine, procainamide, propranolol and quinidine are widely used in clinical practice for the treatment of cardiac disorders. Determination of the plasma concentration of these drugs enables the achievement of a "therapeutic plasma level" in a particular patient by modification of dose. Therapeutic, toxic and lethal concentrations of these drugs have been reported[1].

These drugs and their metabolites have been determined by several methods including colorimetric analysis[2], fluorimetric analysis[3,4,35] gas chromatography (GC)[5–12] GC–mass spectrometry[13], mass fragmentography[14], high-performance liquid chromatography[15–19], immunoassay[20], phosphoresence spectroscopy[21], thin-layer chromatography (TLC)[22–24] and ultraviolet (UV) spectroscopy[26–28]. A recent paper by Wesley-Hadzija and Mattocks[25] on the quantitation of quinidine in plasma or urine by TLC–densitometry shows good precision and accuracy. None of these methods has been used for the simultaneous determination of all four drugs. GC is the only method that has been used for the determination of each of the drugs individually. It would be of considerable advantage to a clinical laboratory to have a standard method that could be used for any of the four drugs singly or in combination.

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High-performance TLC (HPTLC) has recently been introduced[29–31]. This technique allows more rapid separations than can be achieved by conventional TLC. Advantages over most other methods are that several samples may be assayed simultaneously and that the detection method may be made sensitive to particular drugs by scanning at specific wavelengths corresponding to spectroscopic absorption maxima. We describe here the use of this technique for the simultaneous determination of the antiarrhythmia drugs discussed above. Diphenylhydantoin (Dilantin) is included as it may be conveniently determined in the same scheme as the other drugs. It is an antiepileptic drug that is occasionally used for cardiac disorders.

EXPERIMENTAL

Drug standards and reagents

Drug standards were provided as a gift by one of the authors (A. Karmen). Clozapine and other basic drugs were obtained from Dr. D. C. Fenimore of the Texas Research Institute for the Mental Sciences, Houston, Texas, U.S.A.

Certified ACS reagent grade benzene, ethyl acetate, methanol and pyridine were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.).

Sample preparation

A volume of 30 μl of a methanol solution of clozapine (12.5 μg/ml) was added (50-μl syringe) to a dry silylated 10-ml test tube equipped with a PTFE-lined screw cap. The methanol was removed under a stream of nitrogen while maintaining the tube at 45° in a Multi-Block Heater (Lab-Line Instr., Melrose Park, Ill., U.S.A.). A volume of 0.5 ml of serum (0.5-ml syringe) followed by 3 ml of benzene (5-ml graduated pipette) was added to the test tube which was then tightly closed and vortexed vigorously for one minute with a Vortex Genie Mixer (Scientific Products, McGaw Park, Ill., U.S.A.) and centrifuged for 1 min. A volume of 2.2 ml of the benzene solution was transferred into a silylated Reactivial (Pierce, Rockford, Ill., U.S.A.) and then the benzene removed under a stream of dry nitrogen while maintaining the Reactivial at 45° in the Multi-Block Heater.

The serum was then made basic by adding 200 μl of 0.5 *N* aqueous sodium hydroxide. A volume of 3 ml of benzene was added to the tube and the extraction and phase separation was repeated. A 2.4-ml volume of the benzene phase were transferred to the Reactivial and the benzene removed by the same procedure as above. The combined residue containing the antiarrhythmia drugs, was dissolved in 30 μl of benzene.

Chromatography

Chromatography was performed on 10 × 10 cm HPTLC plates coated with silica gel 60 F 254 (E. Merck, Darmstadt, G.F.R.). Two solvent systems were used. System I consisted of benzene–ethyl acetate–methanol (4:4:1) and system II of benzene–ethyl acetate–methanol–pyridine (4:2:3:3). In order to obtain a low background, it was found necessary to clean the plates by predevelopment in solvent I. The plates were dried in a stream of cool air and stored in a desiccator.

A volume of 1 μl of the benzene extract was applied to the HPTLC plate using a 1-μl Pt/Ir capillary (Antech, Bad Dürkheim, G.F.R.) attached to an EVA

Chrom-Applicator (W + W Electronic Sci. Instr., Basle, Switzerland). The spot diameter was held to less than 2 mm by drawing off solvent vapor with a glass tube attached to a vacuum line, held close to the surface of the plate.

Plates were developed in a 24 × 23 × 8 cm twin trough chamber (Camag, Muttenz, Switzerland)[32] lined on one side with an SP-240 saturation pad (Analtech, Newark, Del., U.S.A.). A volume of 25 ml of solvent system I was placed in one trough to allow saturation of the atmosphere. The HPTLC plate was placed in the second trough. Chromatography was initiated after 5 min by tilting the chamber to allow solvent to flow over the dividing ridge. After the first development which requires about 7 min for the solvent to travel about 5 cm from the origin, the plate is dried in a stream of cool air. Lidocaine and diphenylhydantoin are well resolved and are scanned at 220 nm with a Zeiss Model KM-3 Scanning Spectrophotometer (Carl Zeiss, New York, N.Y., U.S.A.). The plate is then developed with solvent system II using the same technique as for solvent system I. 13 min are required for the solvent front to travel a distance of 5 cm from the origin. The solvent is removed in a stream of warm air. Procainamide, propranolol, quinidine and clozapine are well resolved and are scanned at 290 nm.

Spectrophotometry

The spectrophotometer was operated in the reflectance mode for UV absorption. At 220 nm the slit width was set at 2.0 mm and the slit length at 3.5 mm. At 290 nm the slit width was set at 0.5 mm and the slit length at 3.5 mm. The spectrophotometer scanning speed was set at 50 mm/min. Integration was performed on a Spectraphysics Minigrator (SpectraPhysics, Santa Clara, Calif., U.S.A.).

RESULTS AND DISCUSSION

The concentration of the drugs in serum at the therapeutic level is in the order of μg/ml. Thus some form of preconcentration before chromatography is necessary. This is conveniently performed by extracting with benzene. Lidocain and diphenylhydantoin are extracted from neutral serum. Procainamide, propranolol, quinidine and clozapine are extracted from serum made basic with a small amount of aqueous sodium hydroxide. The benzene is evaporated after extraction leaving the drugs as a concentrate.

It was found possible to obtain a baseline separation for all the drugs and an internal standard, clozapine, using a two-step development procedure for which a total of about 30 min are required. The solvent system benzene–ethyl acetate–methanol (4:4:1) is moderately polar and yields a baseline separation of lidocaine and diphenylhydantoin (Fig. 1). The other drugs remain at the origin while clozapine migrates only a short distance. A small peak was found in most sera studied and was identified as caffeine. The plate was dried after the first development and lidocaine and diphenylhydantoin were determined using the scanning spectrophotometer.

The second development is performed with the solvent system benzene–ethyl acetate–methanol–pyridine (4:2:3:3) which is of greater polarity and yields a baseline separation of procainamide, propranolol, quinidine and the internal standard clozapine (Fig. 2). As with the first solvent system a small caffeine peak was found in most sera. Lidocaine and diphenylhydantoin migrate into the solvent front.

Fig. 1. Chromatogram of drugs separated using solvent system I. IS = internal standard; 1 = caffeine; 2 = lidocaine; 3 = diphenylhydantoin. A, serum blank; B, serum to which drugs have been added.

Fig. 2. Chromatogram of drugs separated using solvent system II. 1 = procainamide; 2 = propranolol; 3 = quinidine; 4 = caffeine; IS = internal standard. A, serum blank; B, serum to which drugs have been added.

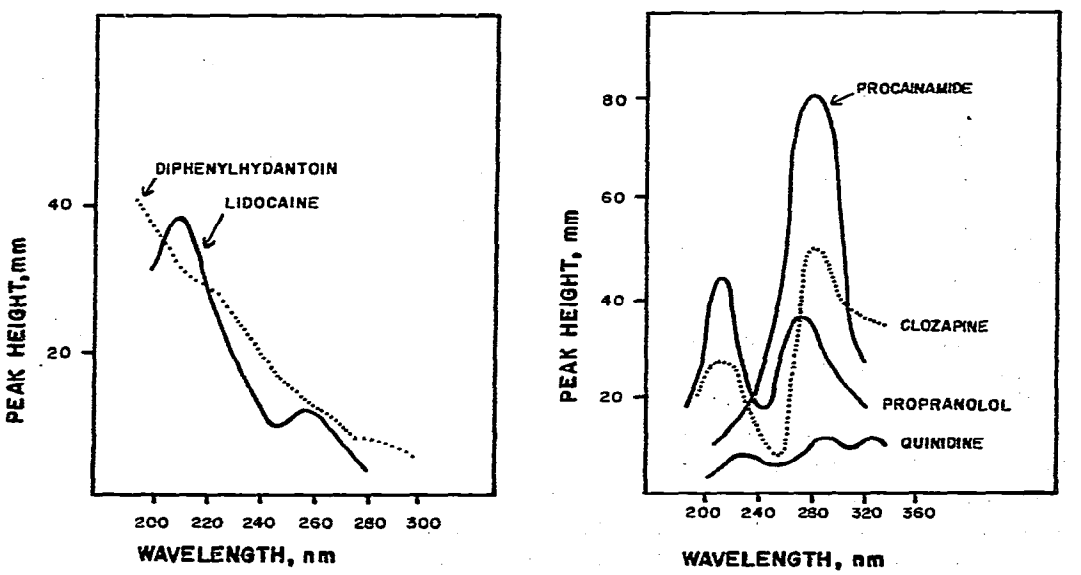

Fig. 3. UV absorption spectra of lidocaine and diphenylhydantoin determined by scanning a HPTLC plate in the reflectance mode.

Fig. 4. UV absorption spectra of procainamide, clozapine, propranolol and quinidine determined by scanning an HPTLC plate in the reflectance mode.

Figs. 3 and 4 show the absorption spectra of the various drugs, obtained by scanning the same spots at different wavelengths. The background noise became excessive below a wavelength of 220 nm. Thus diphenylhydantoin and lidocaine were scanned at 220 nm which is near the maximum absorbance for lidocaine. Procainamide, clozapine, propranolol and quinidine were scanned at 290 nm when a combination of these drugs is present in serum. However when only propranolol is present greater sensitivity is obtained at 220 nm. The spectrophotometer exhibits low sensitivity to quinidine. Fortunately the therapeutic level of quinidine (3–6 μg/ml) is high enough for this detection mode. If higher sensitivity is required the detection mode may be changed from UV absorption to fluoresence. The fluoresence intensity of quinidine may be increased by treating the separated spot with a drop of sulfuric acid–ether (5:95, v/v). The excitation and emission wavelengths are 360 nm and 455 nm, respectively. This lowers the detection limit for quinidine from 10 ng to 0.4 ng.

Calibration curves are shown in Fig. 5 which indicates the linear range for the drugs. The detection limit is about 10 ng except for propranolol and procainamide for which the limit is 2 ng. The therapeutic level for all these drugs is within the linear range. However in the case of propranolol the lower range of the therapeutic level (0.02–0.2 μg/ml) is below its detection limit. This is overcome by spotting 2–10 μl of extract instead of 1 μl.

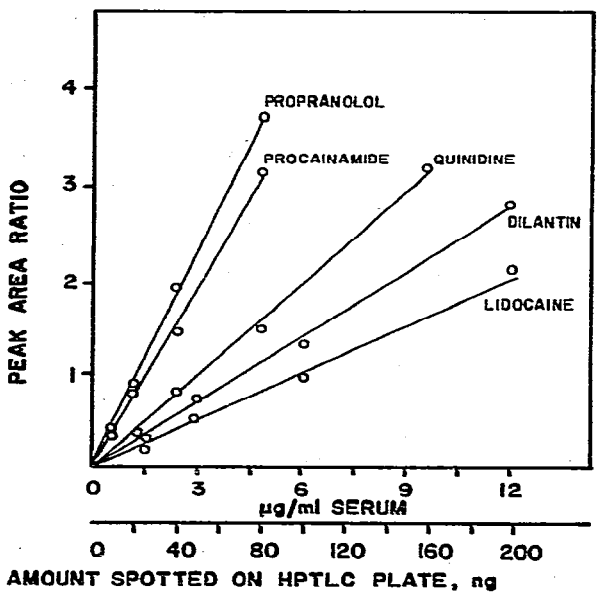

Fig. 5. Calibration curves of drugs extracted from blood serum and separated by HPTLC.

The reproducibility and recovery for the method are shown in Table I. Results were combined using the data pair technique[33]. The reproducibility is the result of individual determinations of five serum samples to which the mixture of drugs and internal standard were added. The reproducibility as measured by the relative standard deviation varies between 3% and 14%. A better reproducibility is obtained for each drug at a higher serum concentration.

TABLE I

REPRODUCIBILITY AND RECOVERY TEST FOR SPIKED SERUM ($n = 5$)

Drug	*Concentration (μg/ml)*	*Relative standard deviation (%)*	*Recovery (%)*
Dilantin	3	7.3	54.8
	12	5.2	72.5
Lidocaine	1.5	13.1	78.3
	6	6.8	81.1
Procainamide	1.2	7.9	46.5
	4.8	6.3	51.8
Propranolol	0.3	12.4	89.3
	1.2	7.4	93.5
Quinidine	2.4	6.6	97.5
	9.6	3.2	(103.5)

Recoveries were determined in the following way. A given amount of drug was added to serum and a value was determined after extraction and chromatography. This value was expressed as a percentage of the same amount of drug applied directly to the plate which was developed and dried before scanning. Recoveries vary between 46% for 1.2 μg/ml of procainamide and about 100% (considering the standard deviation for the method) for either 2.4 μg/ml or 9.6 μg/ml quinidine. For each drug a marginally higher recovery is obtained at a higher concentration. Both the reproducibilities and the recoveries may be considered satisfactory if the low serum concentration of the drugs is taken into consideration. The poor recovery of procainamide is compensated for by its high sensitivity when it is scanned at 290 nm. Diphenylhydantoin also exhibits a poor recovery but as it has a high therapeutic level its determination also presents no problem. The other drugs exhibit recoveries of greater than 80%. Recovery level must be considered in relating spectrophotometer reading to serum concentration. It may be possible to improve both the recovery and the reproducibility by including an appropriate carrier compound as has been used for other drug analyses[31]. The carrier must be chosen with care to avoid chromatographic interference.

Table II lists the values of quinidine and procainamide determined by both HPTLC and fluorimetry[3]. The HPTLC result for all but one of the sera is within about 11% of the fluorimetric result. The HPTLC values for all of the sera are lower than the fluorimetry values. It is interesting to note that two of the sera contained

TABLE II

COMPARISON OF THE DETERMINATION OF QUINIDINE AND PROCAINAMIDE IN SERUM BY HPTLC AND SPECTROFLUORIMETRIC ANALYSIS

Sample number	*Quinidine (μg/ml)*		*Procainamide (μg/ml)*	
	HPTLC	*Spectrofluorimetric analysis*	*HPTLC*	*Spectrofluorimetric analysis*
1	4.0	4.5	—	—
2	7.1	7.5	—	—
3	7.3	8.0	—	—
4	8.2	10.5	2.9	—
5	2.1	—	8.4	9

both quinidine and procainamide, with one of these drugs in very low concentration presumably as a carry over from previous treatment.

Table III includes some common drugs that could possibly interfere with the antiarrhythmia drugs discussed in this paper. The only drugs that would interfere are barbital, carbamazepine and ethosuximide. Procaine has nearly the same R_F value as clozapine but is not extracted when the serum is made basic.

TABLE III

R_F VALUES OF SOME COMMON BASIC DRUGS SCREENED FOR INTERFERENCE

Drug	*R_F value*	
	Solvent system I (at 220 nm)	*Solvent system II (at 290 nm)*
Acetophenazine	0.03	0.34
Barbital	0.49*	NR**
Butaperazine	0.03	0.29
Caffeine	0.27	0.69
Carbamazepine	0.32*	0.80
Clozapine	0.09	0.53
Diazepam	0.63	0.77
Diphenylhydantoin	0.52	NR
Ethosuximide	0.54*	NR
Glutethimide	0.66	NR
Lidocaine	0.34	NR
Nicotine	NR	NR
Phenobarbital	0.74	NR
Primidone	0.23	NR
Procaine	0.00	0.52*
Procainamide	0.00	0.16
Propranolol	0.00	0.23
Quinidine	0.03	0.41
Tridione	NR	NR
Thiothixine	0.06	0.29

* These drugs have R_F values that would interfere with the drugs discussed in this paper.
** NR corresponds to drugs that have no absorption at the wavelength scanned.

TABLE IV

A COMPARISON OF GAS CHROMATOGRAPHY AND HPTLC AS USED FOR THE DETERMINATION OF ANTIARRHYTHMIA DRUGS

Drug	*Gas chromatography*			*HPTLC*		
	Time required for extraction from serum (min) *	*Time required for analysis (min)*	*Relative standard deviation (%)*	*Time required for extraction from serum (min)*	*Time required for analysis (min)*	*Relative standard deviation (%)*
Diphenylhydantoin[34]	15	5	3.3			
Lidocaine[9]	35	15	3			
Procainamide[10]	100	10	5–10	20	30	3–14
Propranolol[11]	40	10	8–15			
Quinidine[12]	30	10	4.7			

* Extraction time estimated from authors' description.

Apart from HPTLC, GC is the only method that has been used to determine each of the drugs discussed here. Table IV compares these two techniques with respect to time required for sample preparation and analysis and with respect to precision as measured by relative standard deviation. The time requirements of HPTLC compare favorably to those of GC. Up to 16 samples can be run simultaneously by HPTLC whereas only one sample can be run at a time by GC. The precision of GC appears slightly better than that of HPTLC even though this can be overcome by applying the same sample several times to the plate and performing replicate determinations simultaneously. A major advantage of the HPTLC method is that it may be used routinely in a clinical laboratory for determining all of the drugs discussed here.

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