Title
Liquid-chromatographic determination of antiarrhythmic drugs

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Publication Date
1980

Peer reviewed|Thesis/dissertation

LIQUID-CHROMATOGRAPHIC DETERMINATION OF ANTIARRHYTHMIC DRUGS: PROCAINAMIDE, LIDOCAINE, QUINIDINE, DISOPYRAMIDE, AND PROPRANOLOL

by

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B.S., National Taiwan University 1976

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF CLINICAL LABORATORY SCIENCE

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

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ACKNOWLEDGEMENT

I would like to express my deepest appreciation to Dr. Pokar M. Kabra for his patient guidance and assistance.

I am also grateful to Dr. Laurence J. Marton and Dr. S. Nussenbaum for their reviewing this thesis.

ABSTRACT

A liquid chromatographic (LC) method for the analysis of antiarrhythmic drugs (procainamide, N-acetylprocainamide (NAPA), lidocaine, quinidine, disopyramide, N-desisopropyldisopyramide, and propranolol) in serum is described. The drugs, together with an internal standard, are extracted from 0.2 to 1.0 ml of serum, separated on an octyl bonded reversed-phase column using a mobile phase consisting of acetonitrile/phosphate buffer, and monitored by either ultraviolet or fluorescence spectrophotometry. The proposed method offers good reproducibility, sensitivity, linearity, and accuracy. Of more than 50 drugs and metabolites tested for possible interference, only diazepam, flurazepam, and the N-oxide metabolite of quinidine interfere with quinidine analysis, while meperidine co-elutes with disopyramide. However, diazepam and flurazepam do not interfere with quinidine analysis when fluorescence detection is utilized.

INTRODUCTION

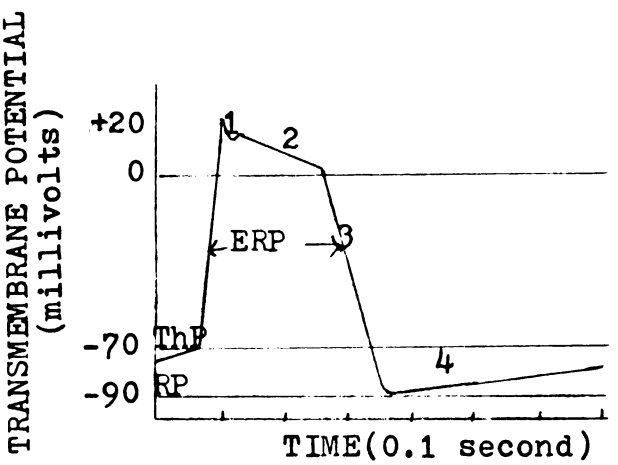
All cells are immersed in electrolyte solutions. Sodium is the major cation of the extracellular fluid as is potassium of the intracellular fluid. Active transport of sodium ions takes place in an outward direction while potassium is actively transported inwardly. The presence of sodium and potassium pumps and the nondiffusibility of these cations once inside the cell create an imbalance of charges and therefore an electrical potential across the cell membrane.

In normal resting nervous and muscular cells, the membrane potential (resting potential) inside the cells is approximately -80 to -85 millivolts.

Any stimulus that increases the permeability of the membrane to sodium is likely to elicit a sequence of rapid changes in membrane potential. These changes result in an action potential which includes two separate stages, depolarization and repolarization. Depolarization occurs when sodium ions enter the cell negating the normal resting potential. This is followed immediately by an increase in potassium permeability coupled with a simultaneous decrease of sodium permeability, and the normal resting membrane potential is restored during this repolarization stage. After repolarization potassium permeability decreases.

Action potentials can be propagated. Such transmission of the depolarization process occurs along nerve and muscle fibers. Cardiac muscle when excited generates a specific type of action potential. After an initial very large change in

membrane potential (spike potential), the membrane remains depolarized for 0.15 to 0.3 second to exhibit a plateau when recorded with microelectrodes, this is followed by abrupt repolarization. The cell is refractory to restimulation during the action potential period. An absolute refractory period occurs during early repolarization during which the membrane is not excitable. This is followed by an effective refractory period (ERP) during which the membrane is excitable, but the impulse does not propagate. Figure-1 illustrates the changes in the membrane potential during excitation of cardiac muscle measured by using microelectrodes.



ERP: effective refractory period

ThP: threshold potential

RP: resting potential

Fig. 1. Action Potential of Cardiac Muscle.

When an action potential propagates through a muscle fiber it causes diffusion of calcium ions into myofibrils and produces muscle contraction. Cardiac contraction is initiated by the spontaneous generation of an action potential in the pacemaker cells of the sino-atrial(S-A) node. The action potential travels rapidly through both atria and then to the

atrio-ventricular(A-V) node. The impulses from the atria are delayed in the A-V node before passing into the ventricle. The A-V bundle conducts the impulses from atria to the bundles of Purkinje fibers which conduct to all parts of the ventricle.

The automaticity of S-A node is also shared to a smaller extent by specialized atrial fibers adjacent to the S-A node, the fibers of the A-V node and Purkinje system. Under pathological conditions, the automaticity of any of these cardiac fibers may exceed that of the normal pacemaker cells, resulting in the establishment of ectopic foci. This is the basis of certain arrhythmias; alterations or abnormalities of normal cardiac rhythm.

Antiarrhythmic drugs can selectively suppress the automaticity at ectopic sites. Quinidine was first used for its antiarrhythmic effect in 1914 (1) and is still considered the prototype among antiarrhythmic drugs. Quinidine depresses the excitability of both the atrium and the ventricle to electrical stimulation. Automaticity of pacemaker cells is decreased and the conduction velocity of cardiac impulse is slowed. It also prolongs the action potential duration (APD) and increases the effective refractory period of the action potential. The increase of the ERP is relatively more pronounced than the increase of the APD, so the ratio of ERP/APD is increased.

In 1936, the antifibrillatory and antiarrhythmic effect of procaine was reported (2). However, this agent was clinically unsuitable because of its central nervous system-

stimulation and its degradation by enzymatic hydrolysis. On the other hand, procainamide, an amide derivative of procaine, is an effective antiarrhythmic drug which has negligible central nervous system effects and is stable to enzymatic hydrolysis. The pharmacological activity of procainamide is very similar to that of quinidine except for a difference in their potencies. The effective concentration of procainamide in isolated tissue preparations are approximately ten times as high as equipotent concentrations of quinidine.

Lidocaine, first synthesized by Lofgren and Lundqvist (3), is used as a local anesthetic. It is an effective antiarrhythmic agent used for ventricular arrhythmias. The electrophysiological effects of lidocaine on the heart are similar to those of quinidine, except that it may also shorten the action potential duration. Nevertheless, as for quinidine, the ERP/APD is increased.

Propranolol, a beta-adrenergic blocking agent, is most commonly used as an antiarrhythmic agent. Propranolol reduces heart rate, prolongs A-V conduction time and reduces contractility. It also has some direct "Quinidine-like" antiarrhythmic activity (4). It is especially useful in ventricular arrhythmias induced by digitalis intoxication. Disopyramide, a new antiarrhythmic agent (5),was introduced in the United States in 1977. It is similar to quinidine and procainamide in its antiarrhythmic activity.

The therapeutic blood concentrations, dosages and pharmacokinetic characteristics of these agents are illustrated in Table 1.

Table 1: Properties of the Antiarrhythmic Drugs (6, 7, 8, 9, and 10)

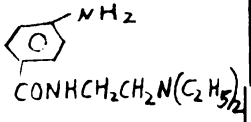
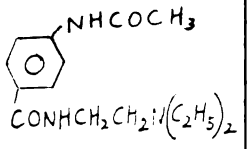
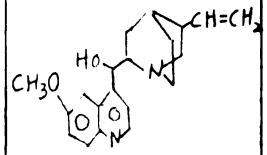
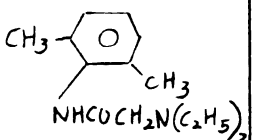
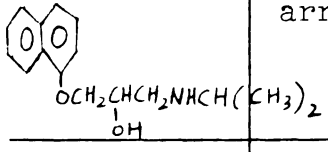
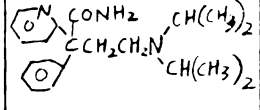
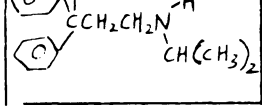
DRUG	USE	SERUM HALF LIFE(hours)	ROUTE	DOSE mg/kg/D	THERAPEUTIC RANGE(mg/L)	TOXIC LEVEL (mg/L)	VOLUME OF DISTRIBUTION (L/kg)
PROCAINAMIDE 	Prevention of premature ventricular beats, ventricular tachycardia	2 - 4	Oral I.V.	50	4 - 8	>16	2
Metabolite: N-ACETYL PROCAINAMIDE 		6 - 8		Serum concentration ranges from 60 to 180 % of procainamide.			
QUINIDINE 	Treatment of atrial fibrillation	5 - 12	Oral	10 - 20	2 - 5	>10	2.0 - 3.5
LIDOCAINE 	Treatment of ventricular tachycardia (acute myocardial infarction)	1 - 2	I.V. I.M.	30 - 40	1 - 5	>6	1.7

Table 1: (Cont.)

DRUG	USE	SERUM HALF LIFE(hours)	ROUTE	DOSE mg/kg/D	THERAPEUTIC RANGE(mg/L)	TOXIC LEVEL (mg/L)	VOLUME OF DISTRIBUTION (L/kg)
PROPRANOLOL 	Treatment of digitalis arrhythmias	2 - 6	Oral I.V.	1.1-1.7	50 - 100 ug/L	variable	2 - 4.6
DISOPYRAMIDE  Metabolite: N-DESISO PROPYL DISOPYRAMIDE 	Treatment of ventricular tachycardia	4 - 10	Oral I.V.	8.6	2 - 4	>7	0.8

METABOLISM AND BIOTRANSFORMATION

Procainamide is almost completely absorbed after oral administration and is rapidly distributed in body tissues (11). Peak plasma concentrations are generally reached within 1 to 2 hours, and about 15% of procainamide binds to plasma proteins. Procainamide is eliminated mainly via the kidney. Recovery of unchanged procainamide in the urine averages about 50%. N-acetylprocainamide (NAPA), the major metabolite formed during the pass of procainamide through the intestinal wall and/or liver, accounts for 15% of the urinary excretion of administered procainamide. The rate of acetylation is genetically determined (12). NAPA has comparable toxicity as well as therapeutic potency to procainamide (13). Its concentration in plasma ranges from 60 to 180% of procainamide. Therefore, it is important to measure both procainamide and NAPA concentrations as a guide to therapy.

Quinidine is fully absorbed from the gastro-intestinal tract, but the fraction of the oral dose which reaches the systemic circulation is approximately 75%. This reduction in bioavailability is due to first-pass hepatic drug removal (14). Quinidine is bound to plasma proteins to the extent of 70-95%. Fifteen to 40% of the quinidine is eliminated by renal excretion as intact drug, and 60 to 85% as various metabolites (7). At least 4 different metabolites of quinidine have been identified, including 3-hydroxyquinidine (3-OH), 2'-quinidinone (2'-OXO), O-desmethylquinidine (O-DMQ) and an N-oxide rearrangement product of quinidine (N-oxide). After correcting

for differences in protein binding, Drayer et al. (15) found the ratios of 3-OH/quinidine and 2'-OXO/quinidine in serum to be 0.61 ± 0.31 and 0.39 ± 0.44 respectively. Quinidine, 3-OH and 2'-OXO metabolites have equal antiarrhythmic potency (15). O-DMQ accounts for approximately 1 to 2% of the quinidine dose and is less active. The quinidine metabolites contribute significantly to the overall therapeutic and toxic effects of quinidine therapy.

The oral bioavailability of lidocaine is about 35%. With intravenous injection, multicompartment kinetics is observed (16). Lidocaine is metabolized predominantly by the liver. Excretion of unchanged lidocaine is a minor route of elimination. Two de-ethylation metabolites of lidocaine, monoethylglycylxylidide (MEGX) and glycylxylidide (GX), are found in significant concentrations in the blood of patients receiving lidocaine therapy (17). MEGX and GX have been shown to have antiarrhythmic activities of 83% and 10 to 26% respectively (18). Accumulation of metabolites during prolonged intravenous administration may account for the observed toxicity which occurs despite blood lidocaine concentrations which are within the therapeutic range.

Propranolol is completely absorbed after oral administration. Peak plasma concentrations are achieved in approximately 2 hours (19). The low bioavailability (about 36%) is due to avid hepatic extraction leading to presystemic first-pass elimination. Clearance is dependent mostly on the activity of hepatic blood flow (20). After I.V. administration,

most of the propranolol is excreted in the urine as metabolites which are formed in the liver. Four primary metabolic pathways have been described, O-dealkylation, side chain oxidation, glucuronic acid conjugation and ring oxidation. The inactive metabolite propranolol glucuronide accounts for 75% of the urinary excretion. 4-hydroxypropranolol (4-OH), an equipotent metabolite, is observed in plasma only after oral administration. The concentration of this metabolite is equal to that of parent drug (21).

The bioavailability of orally administered disopyramide phosphate is about 90%, but it is absorbed much more slowly. Peak serum concentrations are generally achieved between 2 to 3 hours after administration (22). About 55% of a disopyramide dose is excreted in the urine, and this excretion is independent of the urinary pH. About 25% appears in the urine as N-desisopropyldisopyramide, 10% as minor metabolites and the rest in the feces. N-desisopropyldisopyramide is only 25% as potent as the parent drug (23).

SERUM CONCENTRATION AS GUIDES TO THERAPY

For most drugs the relation between dosage and intensity of the pharmacological effect is unpredictable. An inter-patient variation in the dose-effect relationship may arise largely from genetically determined individual differences in the drug absorption, distribution, biotransformation and excretion. Other factors such as age, sex, effects of disease, and concomitant administration of other drugs also contribute to this variation.

The variations in the dose-effect relationship are reflected in a wide range of serum drug concentrations for the same prescribed dose in different individuals. For a number of drugs, the correlations between serum concentrations and clinical efficacy have been established. Determination of the serum concentration of these drugs can help in the individualization of drug dosage to achieve therapeutic efficacy without adverse toxicity.

The antiarrhythmic agents described above are widely used drugs. They all have narrow therapeutic ranges and adverse side effects. These antiarrhythmic drugs show good correlation between their serum concentrations and clinical effects, therefore, it is usually desirable to monitor serum concentrations of these drugs to serve as therapeutic guides.

HISTORY OF ANALYTICAL METHODS

A. Procainamide

1. Colorimetry

A colorimetric method developed by Bratton and Mark (24, 25) was based on coupling diazotized procainamide with N-(1-naphthyl)-ethylenediamine and measuring the resultant product at 550 nm. The sensitivity of this method was 5 to 40mg/L. The original method was modified by stabilizing the diazo intermediate to increase the sensitivity (26). These colorimetric methods were all interfered with by drugs containing a primary aromatic amino group. N-acetylprocainamide (NAPA) can only be measured following its hydrolysis to procainamide.

2. Fluorometry

Koch-Weser and Klein (27) measured the native fluorescence of procainamide at alkaline pH with an excitation wavelength of 295 nm and an emission wavelength of 360 nm. Interference by NAPA limits the specificity of the test. In another fluorometric method (28), fluorescence of NAPA was measured at acidic pH with excitation at 288 nm and emission at 341 nm. The acidic extract was subsequently alkalinized and fluorescence of procainamide was measured at excitation 298 nm and emission 354 nm. When procainamide and NAPA were present in the sample, they interfered with each other.

Derivatizing procainamide with fluorescamine and measuring the resultant fluorescence was reported by Sterling et al. (29). The method was nonspecific, since primary aromatic amino substituents could also react with fluorescamine to give fluorescent products.

3. Gas-Liquid Chromatography (GLC)

A GLC-flame ionization detection (GLC-FID) method for the analysis of procainamide was developed by Atkinson et al. (30). The method was nonlinear at low concentrations due to the adsorption of procainamide on the active sites of the stationary phase (3% phenyl methyl silicone (OV-17) on 80-100 mesh Chromosorb W-HP). A more polar stationary phase of 0.5% polyamide and 6% (3,3,3-trifluoropropyl) methyl silicone eliminated these adsorptive sites (29). Other stationary phases such as 10% phenyl methyl dimethyl silicone (OV-7) coated on 60-80 mesh Chrom Q also eliminated the adsorption problem (31). A simultaneous determination of procainamide and NAPA by GLC using 5% OV-17 on 100-120 mesh Chrom Q was presented (32). Most GLC methods were not sensitive at low concentrations, especially for NAPA.

4. Gas Chromatography-Mass Spectrometry (GC-MS)

Strong et al. (33) measured NAPA concentrations in plasma by electron impact quadrupole mass spectrometry using the selected ion monitoring mode. They also employed deuterated procainamide and NAPA as internal standards for pharmacokinetic studies (34). Although GC-MS overcame the inherent insensitivity of conventional GLC-FID techniques

for NAPA, the complexity and cost of the instrumentation limited its clinical utility.

5. Thin-layer Chromatography (TLC)

Reidenberg et al. (35) determined procainamide and NAPA by separation on silica gel TLC plates and spectrodensitometric scanning at 260 nm. This method yields a coefficient of variation (C.V.) of 9% for procainamide and 11% for NAPA.

6. Enzyme Multiplied Immunoassay Technique (EMIT^{R})

The two separate EMIT assays for procainamide and NAPA utilize identical protocals and principles. The EMIT methods are based on the principle of competitive binding of free and enzyme-labeled drug to antibody binding sites. Glucose-6-phosphate dehydrogenase was used to label the drug. The enzymatic activity is monitored at 340 nm due to conversion of NAD^{+} to NADH. The change at 340 nm is directly proportional to the concentration of the drug in serum. Comparisons of EMIT procainamide assay with fluorometric method, and of NAPA with LC or fluorometric methods showed good correlations (36, 37). These EMIT assays showed no significant cross-reactivity.

EMIT^{R} - Trade mark Syva Corp. - Palo Alto, CA

7. Liquid Chromatography (LC)

Adams et al. (38) reported a reversed-phase LC method for the simultaneous analysis of lidocaine and procainamide using procaine as an internal standard. Although charcoal

extraction of procainamide resulted in low recovery, detection at 206 nm overcame the problem of insensitivity. Several methods for the simultaneous analysis of procainamide and NAPA using reversed-phase LC were reported. Carr et al. (39) used N-formylprocainamide as an internal standard, 10% n-propanol in chloroform as an extraction solvent, and detection at 254 nm. A similar reversed-phase method by Rocco et al. (40) utilized N-propionyl procainamide as an internal standard, extraction of serum with methylene chloride, and detection at 280 nm. Shukur et al. (41) reported a reversed-phase LC method without an internal standard. Analytical recoveries of 55% and external standardization resulted in low precision. Adsorption chromatography was used by Dutcher and Strong (42) for simultaneous quantitation of procainamide and NAPA. After addition of p-nitro-N-(2-diethylaminoethyl) benzamide hydrochloride as an internal standard, serum was extracted with ethyl acetate, separated on a microparticulate silica column, and detected at 254 nm. One mL of plasma was required to achieve sufficient sensitivity for therapeutic monitoring. A similar adsorption assay by Butterfield et al. (43) resulted in sensitivities of 0.1mg/L for both procainamide and NAPA form 2mL of plasma. Gadalla et al. (44) deproteinized plasma with acetonitrile and injected the resulting supernatant on a cation-exchange column. Since solvent extraction or evaporation steps were not involved, the external standardization still offered acceptable precision, and as little as 20 uL of plasma was

sufficient for detection of 1 mg/L of procainamide and NAPA.

B. Quinidine

1. Fluorimetry

In 1943, Brodie and Udenfriend (45) reported the first fluorometric method for quinidine determination. Serum proteins were precipitated with metaphosphoric acid and the fluorescence of the filtrate was measured. Because the filtrate contained a mixture of fluorescent quinidine metabolites and dihydroquinidine, spurious high values were obtained. Extraction of plasma with ethylene dichloride followed by back extraction with trichloracetic acid was also interfered with by quinidine metabolites and dihydroquinidine (46). Cramer and Isaksson (47) developed a double extraction method using benzene followed by sulfuric acid back extraction prior to fluorescence determination at excitation wavelength 365 nm and emission wavelength 460 nm. Several modifications of the original method, such as the incorporation of 1% amyl alcohol in the benzene to improve specificity (48), and washing the benzene extract with alkali to reduce interference from metabolites were reported (49). Although good specificities were claimed by the authors of double extraction methods, they were subsequently found to be unsatisfactory. Problems of coextracting quinidine metabolites and dihydroquinidine, and background fluorescence from unknown constituents

in plasma were still present. The Cramer and Isaksson method lacked absolute specificity for quinidine even when an additional alkali clean-up was incorporated into the procedure.

2. Thin-Layer Chromatography (TLC)

Hartel and Korhonen (50) extracted quinidine, metabolites and dihydroquinidine from plasma and separated them on Kieselgel G TLC plates. The fluorescent quinidine band was eluted from the TLC plates, and the fluorescence measured. A similar method was developed by Ueda et al. (14) for the bioavailability study of quinidine. These methods are specific but time-consuming. A simplified method involving separation on silica gel TLC plates and direct scanning by a spectrodensitometer was also developed (51).

3. Gas-Liquid Chromatography (GLC)

Midha and Charette (52) reported a GLC method using flash-heater methylation, followed by separation on OV-7 on Chromosorb W, and FID detection of quinidine. The resolution between quinidine and the internal standard was poor, and extraneous peaks also appeared in the chromatogram. An on-column methylation and temperature programmed GC-FID determination using a stationary phase of 3% OV-17 on Chromosorb W-HP was reported (53). The co-elution of dihydroquinidine with quinidine lowered the specificity of this assay. A GLC analysis of underivatized quinidine on 3% methyl silicone gum OV-1 on Gas Chrom Q and nitrogen-phosphorus detection was presented (54). However, this method required acidic delipidation and sample preparation was complex and time-

consuming. Although the sensitivity limit of 0.5 mg/L was not adequate for pharmacokinetic studies, it was sufficient for the therapeutic monitoring of quinidine.

4. Gas Chromatography/Mass Spectrometry (GC-MS)

Huffman and Hignite (55) correlated the fluorescent methods of Brodie and of Kessler with a GC-MS method. The electron impact mass spectra were monitored throughout complete temperature-programmed GLC analysis. Selected ion monitoring of the base peaks of methylated quinidine and the internal standard were used for quantitation.

5. Enzyme Multiplied Immunoassay Technique (EMIT^R)

The EMIT^R assay system is now commercially available for the rapid measurement of quinidine. The protocol and principle are similar to the procainamide EMIT^R assay. Comparison with fluorometric and LC methods showed good correlations (56). However, dihydroquinidine has approximately equal cross-reactivity with the quinidine antibody.

6. Liquid Chromatography (LC)

Methods utilizing adsorption chromatography for the analysis of quinidine were reported. Peat and Jennison (57) used ether extraction followed by separation of quinidine and dihydroquinidine on a microparticulate silica column and detection at 280 nm. The incomplete resolution between quinidine and the internal standard made this procedure an unreliable method for quinidine quantitation. Another method utilizing ion-pair extraction and chromatography on a silica column was published by Sved et al. (58). The complex

back-extraction followed by external standardization resulted in poor precision. Reversed-phase LC (RPLC) is ideally suited for the determination of quinidine from biological fluids such as serum, saliva, and urine, which contain a number of polar compounds. Crouthamel et al. (59) extracted quinidine and dihydroquinidine at alkaline pH, eluted them from a C_{18} column followed by detection at 254 nm. A similar RPLC method was reported by Drayer et al.(60). They separated quinidine and the 3-OH metabolite on a C_{18} column followed by fluorometric detection, using 240 nm as the excitation wavelength and an emission cutoff filter KV418.Fifty uL of serum provided a sensitivity of 5 ug/L. Powers and Sadee (61) developed a simple and rapid method for the analysis of quinidine by precipitating the serum proteins, and injecting the serum supernatant.

A simultaneous assay of quinidine, dihydroquinidine and four metabolites (3-OH, 2'-OXO, O-DMQ and N-oxide) was reported by Guentert et al. (62). Normal phase LC chromatography with detection at 235 nm provided a sensitivity of 20 ug/L when 2 mL of plasma was extracted. This method was quite specific for quinidine.

C. Lidocaine

1. Colorimetry

The original method of Brodie et al. (63) was modified and used extensively in clinical laboratories (64). Lidocaine was extracted at alkaline pH, washed and reacted

with methyl orange. The colored product was measured at 540 nm. This method was simple but nonspecific, since the colored product was also formed with other amino-containing compounds, including the metabolites of lidocaine.

2. Gas-Liquid Chromatography (GLC)

GLC was extensively used for the analysis of lidocaine and its metabolites. Different derivitization,columns and detectors were used to simplify the GLC methods. Difazio and Brown (65) directly injected the chloroform extract of lidocaine, MEGX and GX on 10% vinyl methyl silicone UCW982 on Chromosorb W. They did not report on reproducibility, accuracy or interference. Another GLC-FID method utilizing heptafluorobutyryl derivatives of lidocaine and metabolites on a 4% XF112 column was reported by Keenaghan and Boyes (66). Benowitz and Rowland (67) used multiple ether extractions followed by chromatography on 3% OV-17 on Chromosorb W DMCS/AW HP. During the extraction, ether dissolved enough water to interfere with the GLC analysis. A temperature programmed GLC-FID method employed protein precipitation followed by carbon disulfide extraction and separation on 3% OV-17 on Gas Chrom Q (68). In some patient samples a large unknown peak eluted after the internal standard which could interfere with subsequent analyses.

A nitrogen-specific FID detector was used in a temperature gradient GLC method for the simultaneous determination of the acetylated derivatives of lidocaine, MEGX and GX using a 3% cyclohexane dimethanol succinate stationary

phase (69). Irgens (70) also utilized a N-specific detector to achieve sensitivity and selectivity with separation of underivatized lidocaine and MEGX on KOH-treated 2% polyethylene glycol. The linearity for lidocaine was only to 3 mg/L. Nation et al.(71) eliminated the derivatization by using KOH-treated 2% polyglycol (UCON 75-H-90000) on Gas Chrom Q to minimize the adsorption of the amines on the column. Rossel and Bogaert (72) used a capillary column packed with OV-17, and a nitrogen-phosphorus FID detector for the simultaneous measurement of lidocaine, MEGX and GX. The capillary column gave improved resolution and provided higher specificity. The N-P detector increased sensitivity with trifluoroacetic derivatives.

3. Gas Chromatography-Mass Spectrometry (GC-MS)

Strong and Atkinson (73) separated underivatized lidocaine, MEGX and GX on GLC and monitored them on a quadrupole mass spectrometer in the selected ion monitoring mode. Trimecaine was used as the internal standard. The ions had minimal intensities, thus the method lacked sensitivity.

4. Mass Spectrometry (MS)

An electron-impact mass spectrometric method was developed by Breck and Trager (74) to confirm the presence of lidocaine, MEGX and 2,6-dimethylaniline in human urine. Nelson et al. (75) quantitated lidocaine and several metabolites utilizing a direct insertion probe and chemical-ionization mass spectrometry. Deuterated analogs of the metabolites were used as the internal standards.

5. Enzyme Multiplied Immunoassay Technique (EMIT^R)(76)

Walberg (77) and Pape et al. (78) both reported good reproducibility and accuracy for lidocaine assay when compared to a GLC method. Adaption of the EMIT^R assay to the Gilford 3500 Analyzer increased the speed and versatility of this assay (79). Calibrators and patients' sera were sampled automatically by the analyzer. The EMIT^R method is quite specific and does not cross-react with lidocaine metabolites or other antiarrhythmic drugs.

6. Liquid Chromatography (LC)

A simultaneous reversed-phase LC (RPLC) method for the determination of lidocaine and procainamide was reported by Adams et al. (38). Charcoal extraction gave low recoveries. Narang et al. (80) analyzed lidocaine, MEGX and GX by RPLC with detection at 210 nm. They extracted the serum at alkaline pH with chloroform:hexane:isopropanol(60:30:10) and reported good recoveries. Direct injection of serum samples through a pre-column onto a C_{18} column was used by Wisnicki et al. (81) for the analysis of lidocaine, MEGX, and GX. The pre-column had to be replaced after every fifteen analyses to protect the analytical column. Without the usual concentration step, the method did not provide good sensitivity.

D. Propranolol

1. Fluorometry

The fluorescence method of Shand et al. (20) extracted 4 mL of plasma or urine at alkaline pH into heptane/

isoamyl alcohol followed by back extraction into HCl. Fluorescence of the acidic phase was measured at an excitation wavelength of 295 nm and an emission wavelength of 360 nm. Chidsey et al. (82) critically evaluated this method and found that it lacked specificity and had a high, variable blank due to interfering substances present in the plasma. This method was not sensitive enough to detect low concentrations of propranolol. Shand's method was later modified by Ambler et al. (83). They used amyl acetate for the extraction and induced fluorescence by citric acid. Sensitivity was increased about ten fold but the method still suffered from poor specificity. Kraml and Robinson (84) scanned the extract obtained by Shand's method from 250 to 500 nm. Interference from other drugs or contaminations could be recognized from the scan.

2. Gas-Liquid Chromatography (GLC)

Di Salle et al. (85) reported a GLC-electron capture detection (ECD) method for propranolol. The method utilized multiple extraction steps, followed by derivatization with heptafluorobutyric anhydride and injection on a 3% OV-17 on Chromosorb Q column. A similar GLC-ECD method (86) for the analysis of propranolol,propranolol glycol and N-desisopropyl propranolol utilized either a single extraction step with benzene, or followed this benzene extraction with back extraction into H_2SO_4, alkalinizing the acidic extract and reextracting with benzene. Trifluoroacetyl derivatives of the extract were then separated on 2% OV-17 on Chromosorb W.

The back extraction clean-up step was necessary to measure low propranolol concentration. Slow eluting contaminant peaks limited the frequency of analysis. Modification of the derivatization step using pentafluoro-propionic anhydride with pyridine as the catalyst was reported by Kates and Jones (87). Derivatives of propranolol were separated on 10% OV-1 on Gas Chrom Q and detected with ECD. Contaminating slow eluting substances were hydrolyzed before the injection. Due to removal by extraction or decomposition, 4-hydroxy propranolol (4-OH) could not be analyzed by this method. GLC methods provide better sensitivity and specificity than fluorometric methods, but they still require extensive sample manipulation and demanding chemical reaction which limit their usefulness in clinical laboratories.

3. Gas Chromatography-Mass Spectrometry (GC-MS)

Walle et al.(88) presented the first method for simultaneous determination of propranolol and 4-OH using GC-MS. The plasma samples were extracted at pH 9.6 with ethyl acetate after the addition of sodium bisulphite and an internal standard. The extract was derivatized with trifluoroacetic anhydride,separated on 10% OV-1 on Chromosorb W-HP, and quantitated by electron-impact MS in the selected ion monitoring mode. The addition of sodium bisulfite overcame the problem of stability and decomposition of 4-OH. Other approaches including extraction of 4-OH at its isoelectric point (pH 9.6) and separation on high liquid load stationary phases, also facilitated the quantitation of 4-OH.

4. Radioimmunoassay

A highly specific and sensitive method capable of selectively measuring the biological active l-form of propranolol was developed by Kawashima et al. (89). Antisera against isomeric forms of propranolol were produced by immunizing rabbits. Unknown serum was incubated with the antisera and the radioactivity of antibody-bound propranolol was counted. This method is capable of measuring metabolites of isomeric forms of propranolol, but it is seldom used because of the nonavailability of antisera commercially.

5. Liquid Chromatography (LC)

Krol et al. (90) suggested the use of a low excitation wavelength (250 nm) for fluorometric detection to enhance sensitivity by the liquid chromatographic methods. Complete details of the procedures were not published. Mason et al. (91) used a simple extraction followed by separation on cyanopropylsilane (CN) bonded column for propranolol and 4-OH. Two different detector settings were required- excitation at 220 nm and no emission filter for propranolol, excitation at 235 nm and a 370 Schoeffel emission filter for 4-OH. Hence, two injections were necessary to quantitate both components. The single extraction followed by fluorometric detection at low excitation wavelength resulted in a variable blank and high background noise. The CN column also required elution with larger volumes of organic solvents than required by reversed-phase columns.

A CN bonded RP column was used by Simon et al. (92) for the therapeutic monitoring of propranolol. Plasma was extracted at basic pH after the addition of pronethalol as an internal standard, and the residue was separated on a CN column by ion-pair chromatography with detection at excitation 210 nm and a 340 nm emission cutoff filter. A similar method for propranolol analysis by Nygard et al. (93) used CN RP without ion-pairing.

An alkyl phenyl column was used by Nation et al. (94) for the simultaneous quantitation of propranolol and 4-OH. 4-methylpropranolol hydrochloride was added to serum as internal standard, extracted at pH 9.5 with ethyl acetate and back extracted with pH 2.2 dilute sulphuric acid. Excitation at 205 nm and a KV340 emission filter was used to monitor fluorescence. Back extraction provided clean residues and a sensitivity of 5 ug/L using 200 uL of serum. However, the less-selective low wavelength excitation may cause potential interferences from endogenous or exogenous substances.

C_{18} columns were used by the following investigators: Wood et al. (95) studied the bioavailability of propranolol using a single extraction and detection with a 295 nm interference filter for excitation and a Kodak #34 filter for emission. Four mL of plasma was extracted to achieve a sensitivity of 5 ug/L. Simultaneous determination of propranolol and 4-OH was reported by Jatlow et al. (96), after back extraction, with fluorometric detection at excitation 285 nm and emission 350 nm. However, the recovery of 4-OH was

variable and losses too unpredictable to monitor the metabolite. Taburet et al. (97) employed ether extraction followed by ion-pair chromatography and two detection settings for propranolol and 4-OH. The low recovery by ether extraction was compensated for by using a large sample volume, to achieve good sensitivity. Simultaneous analysis of propranolol, 4-OH and propranolol glycol in plasma was achieved by Schneck et al. (98) using a method similar to Taburet's, except that only a single excitation wavelength of 310 nm was used. A higher excitation wavelength provided higher specificity but lower sensitivity. Pritchard et al. (99) described a method for the simultaneous determination of propranolol and various metabolites, their glucuronides and sulfate conjugates in human urine. Both basic and acidic extracts were analyzed using two different chromatographic conditions, but the same excitation wavelength of 295 nm. Since the clinical activity of all of these metabolites were not well defined, the utility of measuring them was questionable. However, the technique was useful for pharmacokinetic studies of propranolol metabolism.

E. Disopyramide

1. Fluorometry

Ranney et al. (22) developed a fluorescence method for the analysis of disopyramide in strong acid, with excitation of 275 nm and emission of 410 nm. This method was nonspecific because the major metabolite N-desisopropyl

disopyramide (MND) exhibited the same fluorescence characteristics. In addition, other components produced the fluorescense under experimental conditions, leading to variable blanks.

2. Spectrophotometry

Martin et al. (100) published a spectrophotometric method for monitoring disopyramide in plasma. A laborious extraction procedure involved extraction of 3 mL plasma at pH 7.5 with 30 mL dichloromethane, washing with 0.45N KOH, and finally extracting into 0.1N H_2SO_4. The absorbance of disopyramide was measured at 260 nm. MND interfered with this analysis.

3. Thin-Layer Chromatography

Disopyramide and MND were extracted from plasma by benzene, and spotted on silica gel TLC plates (101). After developing the plate, it was sprayed with sulfuric acid and the resultant fluorescence scanned with a densitometer (excitation 266 nm, emission 405 nm).

4. Gas-Liquid Chromatography (GLC)

Hutsell and Stachelski (102) analyzed disopyramide and the acetate derivative of MND by GLC-FID on 2.6% OV-17 on Chromosorb W-HP. Multiple extractions and time-consuming clean-up steps were necessary. Because MND-acetate had a longer retention time and a lower response than disopyramide, two different chromatographic conditions were required. The non-zero intercept of the calibration curve indicated decomposition of the drug on the column. By using a selective

nitrogen detector, Duchateau et al. (103) simplified the above procedure for disopyramide, but still had a non-zero intercept of the calibration curve. A GLC-FID method by Daniel et al. (104) chromatographed underivatized disopyramide on 1.5% methyl silicone gum (SE-30) on Chrom Q. A simple extraction allowed for external standardization. Another simple extraction and GLC-FID analysis of underivatized disopyramide was published by Ilett et al. (105). In addition to the problem of a non-zero intercept of the calibration curve, the day-to-day variation of the slope of the standard curve also limited the reproducibility of the method. Hayler and Flanagan (106) silanized a 3% OV-1 column to prevent on-column decomposition and the calibration curve was linear with a zero intercept. The simple extraction for a nonselective flame ionization detector might cause potential interferences from basic and neutral drugs.

Dodens and Forney (107) reported a GLC-FID method for disopyramide which included a back extraction procedure. The column was silanized and packed with 3% OV-17 on Gas Chrom Q. Diazepam interfered with disopyramide. A similar back extraction method for disopyramide was developed by Vasiliades et al. (108) utilizing a nitrogen detector. Diazepam still interfered with disopyramide and MND decomposed. To alleviate many of these problems, LC separation or GC-MS was proposed (109). Low recovery of MND was obtained when using the LC system, therefore, two determinations with different

sensitivity settings were required for disopyramide and MND. GC-MS in the selected ion monitoring mode eliminated interferences, but the complexity of the instrumentation limited its usefulness as an alternative choice.

5. Liquid Chromatography (LC)

Lima (110) used a simple extraction of disopyramide, MND and the internal standard p-chlorodisopyramide, followed by chromatographic separation on a CN column and detection at 254 nm. The method was rapid, specific and reproducible.

C_{18} reversed-phase columns were used in the following methods: Meffin et al. (111) determined disopyramide and MND using ion-pair LC with back extraction of plasma or diluted urine followed by detection at 254 nm. The method was not optimized for therapeutic and toxic concentrations of disopyramide. Direct injection of the sulfuric acid extract onto the reversed-phase column decomposed the packing more rapidly. Modification of the above extraction steps by alkalinizing the acidic phase and extracting it with organic solvent was reported by Draper et al. (112). A simple extraction of disopyramide and internal standard with LC separation and detection at 254 nm was reported by Broussard and Frings (113). The method suffered from unsatisfactory specificity due to many interferences.

F. Multiple Antiarrhythmic Drugs

1. Thin-Layer Chromatography (TLC)

The simultaneous determination of lidocaine,

procainamide, propranolol and quinidine by high performance thin-layer chromatography (HPTLC) was introduced by Lee et al. (114). The extracted drug residues were applied to silica gel HPTLC plates and chromatographed using a two step development. Lidocaine was scanned after the first solvent development, and the remaining drugs after the second development. This method had many drawbacks which limited its use in clinical laboratories. The extensive sample manipulation and two step development prolonged the chromatographic time. The accuracy was poor, and the method was not sensitive enough to cover the therapeutic range.

2. Liquid Chromatography (LC)

Lagerstrom et al. (115) reported a LC method for the analysis of disopyramide, lodicaine, procainamide and quinidine using liquid-solid adsorption chromatography. A single simplified sample preparation, but different analytical parameters including column packing size, mobile phase, and detection wavelength were required for each drug. A simultaneous analysis of disopyramide, lidocaine and quinidine was reported by Flood et al. (116). After addition of p-chlorodisopyramide as internal standard, serum was extracted at a basic pH, chromatographed on a C_{18} column and detected at 254 nm. This method was simple and sensitive.

This paper describes a method for the simultaneous analysis of the antiarrhythmic drugs: procainamide, NAPA, lidocaine, quinidine, disopyramide, N-desisopropyl disopyramide, and propranolol by gradient liquid chromatography. For laboratories without gradient capabilities, two simple

isocratic methods, which differ only in the composition of their mobile phases are presented. This method has several advantages over any existing methodology. It provides quantitation of 5 drugs and two metabolites. The sensitivity (10.0 ug/L for propranolol with U.V. detection, 1.0 ug/L for propranolol with fluorescence detection, and 0.1 mg/L for all other drugs.) allows for the measurement of both the parent drug and its metabolites at low concentrations. The linearity covers both the therapeutic and toxic levels of each drug. Good recoveries and excellent precision with the one step extraction procedure offer simplicity, accuracy and versatility for routine application in a clinical laboratory.

MATERIALS AND METHODS

Apparatus:

For all liquid chromatography (LC) either a Model Series 2 or a Series 1 liquid chromatograph equipped with a Model 204A fluorescence detector, a Model LC 100 temperature controlled oven (all from Perkin-Elmer Corp., Norwalk, CT 06856) was used. The reversed phase column "Ultrasphere octyl 5u" 25cm x 4.6mm (Altex Scientific, Inc., Berkeley, CA 94710) was mounted in the oven. The chromatograms were recorded either on a Honeywell Electronic Model 194 (Honeywell, Inc., Fort Washington, PA 19036) or on a Sigma 10 data system (Perkin-Elmer). The sample was injected into a Rheodyne 7105 valve (Rheodyne, Berkeley, CA 94710) mounted on the chromatograph.

Materials:

All chemicals used were of reagent grade. Acetonitrile, propanol-2, and methylene chloride, all distilled in glass, were obtained from Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442. Ethyl ether anhydrous, analytical reagent grade, was obtained from Mallinckrodt, Inc., St. Louis, MO 63147.

Carbonate buffer 1 mol/L, pH 10.8 was prepared by dissolving 10.6 g of sodium carbonate in 100 mL of water, and adjusting the pH to 10.8 with 1 mol/L sodium bicarbonate.

Polypropylene tubes: Nalgene^{R} 15 mL capacity were obtained from Fisher Scientific Co., Pittsburgh, PA 15219.

Drug Standards:

Procainamide HCL was obtained from Pfaltz and Bauer, Inc., Flushing, NY 11368, NAPA and N-propionyl procainamide from Pierce Chemical Co., Rockford, IL 61105, quinidine-HCL from K & K Laboratories, Inc., Plainview, NY 11803, lidocaine HCL-monohydrate and metabolites from Astra Pharmaceutical Products, Inc., Worcester, MA 01606, disopyramide, N-desisopropyl disopyramide, and p-chloro disopyramide from G.D. Searle & Co., Chicago, IL 60680, propranolol-HCL, and pronethalol-HCL from Ayerst Laboratories, Inc., New York City, NY 10017. Metabolites of quinidine and propranolol were a gift from Dr. Sidney Riegelman, School of Pharmacy, University of California, San Francisco, CA 94143.

Mobile Phase Buffers:

a) 25 mmol phosphate buffer of pH 3.4 was prepared by dissolving 3.4 g of KH_2PO_4 in water. The pH of the solution was adjusted to 3.0 with H_3PO_4 and diluted to the mark in a 1L volumetric flask with water.

b) 75 mmol phosphate buffer of pH 3.4 was prepared by dissolving 10.2 g of KH_2PO_4 in water. The pH of the solution was adjusted to 3.4 with H_3PO_4 and diluted to the mark in a 1L volumetric flask with water.

c) 100 mmol phosphate buffer of pH 3.4 was prepared by disdissolving 13.6 g of KH_2PO_4 in water. The pH was adjusted to 3.0 with H_3PO_4 and diluted to the mark in a 1L volumetric flask.

Chromatographic Mobile-Phases for Gradient Analysis:

a) 30% acetonitrile in 100 mmol phosphate buffer (pH 3.0) was prepared by diluting 300 mL of acetonitrile and 700 mL of 100 mmol phosphate buffer.

b) 7% acetonitrile in 25 mmol phosphate buffer (pH 3.0) was prepared by diluting 70 mL of acetonitrile and 930 mL of 25 mmol phosphate buffer.

Chromatographic Mobile-Phases for Isocratic Analysis:

a) 9.5% acetonitrile in 25 mmol phosphate buffer (pH 3.0), the mobile phase for the analysis of procainamide and NAPA, was prepared by diluting 95 mL of acetonitrile with 905 mL of 25 mmol phosphate buffer.

b) 25.5% acetonitrile in 75 mmol phosphate buffer (pH 3.4), the mobile phase for the isocratic analysis of lidocaine, quinidine, disopyramide, and propranolol, was prepared by diluting 255 mL of acetonitrile with 745 mL of 75 mmol phosphate buffer.

Drug Standards:

a) Reference Standard for Procainamide and NAPA: Dissolve 11.55 mg of procainamide-HCL, 10 mg of NAPA, and 10 mg of N-propionyl procainamide in 100 mL of methanol. This reference standard for procainamide, and NAPA analysis is stable for 6 months at $4^{\circ}C$.

b) Concentrated Internal Standard: Dissolve 10 mg of N-propionyl procainamide in 100 mL methanol. This internal standard for the analysis of procainamide

and NAPA is stable for 6 months at 4°C.

c) Working Internal Standard: Dilute 1 mL of concentrated internal standard to 10 mL with distilled water. This solution is stable for 3 months at 4°C.

d) Reference Standard for Lidocaine, Quinidine, Disopyramide, N-desisopropyldisopyramide, and Propranolol: Dissolve 10 mg each of disopyramide, N-desisopropyldisopyramide; 12.33 mg of lidocaine-HCL-H_2O; 11.70 mg of quinidine-HCL; 1.1 mg of propranolol-HCL, and 10 mg of p-chlorodisopyramide in 100 mL of 10% methanol. This solution is stable for 6 months at 4°C.

e) Concentrated Internal Standard for Ultraviolet Detection: Dissolve 10 mg of p-chlorodisopyramide in 100 mL of water. This solution is stable for 6 months at 4°C.

f) Working Internal Standard: Dilute 1 mL of concentrated internal standard to 10 mL with distilled water for the analysis of quinidine, disopyramide, and lidocaine. For propranolol analysis, dilute 1 mL of concentrated internal standard to 100 mL with distilled water. Both of these working internal standards are stable for 1 month at 4°C.

g) Reference Standard for Quinidine and Propranolol Analysis by Fluorescence Detection: Dissolve 5.85 mg of quinidine-HCL and 5 mg of pronethalol-HCL in 1L water. For the propranolol standard, dissolve 5.7 mg propranolol-HCL and 50 mg of pronethelol-HLC in 1L water.

h) Concentrated Internal Standard for Quinidine and Propranolol by Fluorescence Detection: Dissolve 10 mg of pronethalol-HCL in 100 mL of 0.1N HCL. This solution is stable at 4°C for 3 months.

i) Working Internal Standard (Fluorescence Detection): Dilute 1 ml of concentrated internal standard to 40 mL with distilled water for quinidine analysis. Dilute 1 mL of concentrated internal standard to 400 mL with distilled water for propranolol analysis.

Instrumental Conditions for Gradient Analysis:

Column flow rate: 2.0 mL/min; column temperature: 50°C; monitor column effluent at 225 nm.

Linear Gradient: Starting at 7% acetonitrile in 25 mmol phosphate buffer (pH 3.0), and ending with 30% acetonitrile in 100 mmol phosphate buffer (pH 3.0) in 10 minutes.

Instrumental Conditions for Isocratic Analyses:

a) Procainamide and NAPA Analysis: Column flow rate: 2.0 mL/min.; column temperature 40°C. Monitor column effluent at 280nm.

b) Lidocaine, Disopyramide, Quinidine and Propranolol Analysis: Column flow rate: 1.5 mL/min.; column temparature 40°C. Monitor column effluent at 216 nm.

For the fluorescence detection of quinidine and propranolol, set excitation wavelength at 290nm and emission wavelength at 350nm.

Procedure for Procainamide and NAPA Analysis:

Place 500 uL of serum, standard, and control into 12 mL glass centrifuge tubes. To each tube add 500 uL of working internal standard, 100 uL of 5 mol/L NaOH. Vortex each tube for 10s., then add 6 mL methylene chloride. Gently rock the tubes for 5 min. on a mechanical shaker. Centrifuge the tubes for 5 min., discard the upper aqueous and protein layer. Pour the organic phase into a clean 12 mL tube and evaporate the methylene chloride at 50°C with an air stream. Dissolve the residue in 100 uL of methanol and inject 10-20 uL into the chromatograph.

Procedure for Quinidine, Lidocaine, Disopyramide, and Propranolol Analysis by Ultraviolet Detection (U.V.):

Place 500 uL of standard, serum sample, and control into 12 mL glass centrifuge tubes (use 1 mL for propranolol). To each tube add 100 uL of 1 mol/L NaOH, add 500 uL of working internal standard. Vortex each tube for 10s., then add 6 mL methylene chloride. Shake the tubes for 5 min. and then centrifuge for 5 min. to separate the phases. Aspirate off the upper aqueous and protein layer and pour the methylene chloride layer into a clean 12 mL glass centrifuge tube (use polypropylene tubes for quinidine analysis to avoid glass adsorption). Evaporate the organic layer to dryness at 50°C under a gentle stream of air. Dissolve the residue 50 uL of methanol and inject 20 uL onto the chromotagraph (inject all of the sample for propranolol).

Procedure for Quinidine and Propranolol Analysis by Fluorescence Detection:

Place 200 uL (as little as 50 uL for pediatric sample) of serum sample, standard, or control into 12 mL glass centrifuge tubes. To each tube add 100 uL of 1 mol/L carbonate buffer (pH 10.8) and 200 uL of working internal standard. Vortex mix for 10 s., then add 8 mL of diethyl ether. Vortex mix the tubes for 30 s., centrifuge briefly at 200 g (2500 rpm) and freeze the aqueous layer (bottom layer) in acetone dry ice bath. Decant ether into a clean 12 mL glass tube (polypropylene tube for quinidine analysis) and evaporate at 50°C under a stream of air. Dissolve the residue in 100 uL of methanol and inject 20 uL onto the chromatograph.

Quantitation:

The reference drug standards were injected. Since the peaks were sharp and symmetrical, peak height measurement was adequate for quantitation. Relative retention time (RRT) was calculated as follows:

$$RRT = \frac{\text{Retention time of the drug from the injection point}}{\text{Retention time of the I.S. from the injection point}}$$

The RRT was used to identify unknown drugs.

A calibration curve was constructed for each drug, using the analyte/internal standard peak height ratio. The ratio for an unknown was converted to concentration by use of this calibration curve.

RESULTS AND DISCUSSION

Analytical Variables

A. Chromatography

Separation of procainamide, NAPA, quinidine, lidocaine, and disopyramide was initially tried using an octadecyl reversed-phase column. However, the components eluted too slowly even when a high concentration of acetonitrile was used. Quinidine could not be resolved from lidocaine. A CN column did not provide complete separation of all drugs. Many of the components were barely retained on this column and eluted in the void volume. With a C_2 reversed-phase column the five components were chromatographed in two isocratic runs. However, propranolol coeluted with quinidine. The octyl reversed-phase column was capable of separating five anti-arrhythmic drugs and two metabolites with gradient programming. For convenient routine laboratory use, two similar isocratic systems also separated the seven components. The octyl reversed-phase column eliminated the poor resolution and peak tailing associated with octadecyl columns. Using this column ion pairing or ion suppression were not necessary. An acetonitrile and phosphate buffer in the pH range of 3 to 3.4 gave optimum resolution and good symmetry for all of these antiarrhythmic agents.

B. Mobile Phase Selection

Phosphate was selected as the buffer because of its optical transparency at lower wavelengths. The pH range

of 3 to 3.4 was found to be optimal range for the separation of these drugs.

The ratio of 9.5/90.5 (acetonitrile/phosphate buffer) was optimum for the analysis of procainamide and NAPA. A 25 mmol phosphate buffer at pH 3.0 and oven temperature of 40°C were crucial for separation of acetaminophen and salicylate from procainamide.

The least amount of exogenous and endogenous interference from serum constituents was observed with this mobile phase. Fig. 2 illustrates a chromatogram for the procainamide and NAPA analysis.

Various ratios of acetonitrile/phosphate buffer (18.5/81.5, 27.7/72.3, 20/80, 22/78, 25/75, 27/73, 28/72, 29/71, and 25.5/74.5) were tried to choose the mobile phase for the isocratic analysis of lidocaine, quinidine, disopyramide and propranolol. A composition of 25.5/74.5 proved to be best. Separation of dihydroquinidine (a contaminant in the quinidine standard)from disopyramide and quinidine was possible with this mobile phase. Procainamide and NAPA were not resolved from the solvent front. Fig. 3 illustrates a chromatogram obtained under these conditions.

Gradient chromatography employing acetonitrile and a phosphate buffer provided baseline resolution for all seven antiarrythmic agents and two internal standards. The ten minute linear gradient was started at 7% acetonitrile and completed at 30% acetonitrile. A

chromatogram obtained under these conditions is illustrated in Fig. 4.

C. Column Temperature

Initial attempts to use the column at ambient temperature resulted in retention times which varied significantly as ambient temperature fluctuated. Operation at 40°C eliminated this problem, improved column efficiency and lowered back pressure. Equilibration time after interruption of flow was also reduced at elevated temperature. An oven temperature of 40°C eliminated the interferences from the procainamide assay and provided the best resolution for N-desisopropyl disopyramide, lidocaine and quinidine.

D. Detection

225 nm was used for the gradient chromatography of the aqueous standards of all components. Gradient chromatography of serum samples was not done.

For the isocratic system, procainamide and NAPA were first detected at 195 nm. However, interference from other absorbing compounds presented a problem; 280 nm was selected to provide a cleaner background and improved sensitivity.

Detection at 216 nm clearly provided adequate sensitivity for the therapeutic monitoring of propranolol and lidocaine. Although all of these antiarrythmic agents could be detected at 216 nm at low therapeutic concentrations, fluorescence detection provided even better

sensitivity for quinidine and propranolol and was useful for micro samples.

E. Sample Preparation

Direct protein precipitation with acetonitrile was tried for all the antiarrhythmic agents. Interferences from serum components made it undesirable. All of the drugs could be easily extracted from serum at alkaline pH (>9.5). A simple methylene chloride extraction was selective enough to provide a clean background and better sensitivity.

Recovery and Linearity

Drug-free serum was supplemented with known amounts of each drug and metabolite to achieve the concentration shown in Table 2. A known amount of internal standard was added to each sample and processed as described above. The recovery from spiked sera was linear for each drug at the concentrations shown in Figs. 5, 6. These concentration ranges correspond to low therapeutic through toxic.

An occasional variation in the recovery of quinidine was noticed when glass centrifuge tubes were utilized for the extraction of sera. This problem was alleviated by substituting polypropylene tube during the final evaporation step. Lidocaine recovery could be improved slightly by adding a small proportion of isopropanol (5%) to the extraction solvent. The recovery of pronethalol (internal standard for quinidine and

propranolol by fluorescence detection) was lower when methylene chloride and NaOH were used in the extraction. This problem was solved by substituting ether and a carbonate buffer.

Precision:

Precision of the assay was determined by repeated analyses of plasma samples containing known amounts of each drug. Analyses were performed as a batched run on a single day to determine within-day variation, and separately on consecutive days to assess day-to-day variation. Detailed precision data, obtained with spiked serum pools at two different concentrations, are listed in Table 3. For within-day precision, C.V. ranged from 1.5 to 8.6%, for day-to-day precision, C.V. ranged from 2.3 to 11.5%.

Sensitivity:

The lower limit of detection for each drug is listed in Table 4. At these lower concentrations the signal to noise ratio was greater than 3 for each of these drugs.

Background:

Several drug-free serum and plasma samples were processed as described above to ascertain the level of background peak interference at the elution times corresponding to those drugs of interest. The background calculated from these samples was negligible and did not interfere with any of the analyses.

Interference:

The drugs listed in Table 5 were evaluated as for their potential interference with the analysis of procainamide and NAPA. Pure drug solutions and serum standards were chromatographed individually and retention times of each noted. Acetaminophen and theophylline eluted at 3.0 min., after procainamide (2.7 min), and do not interfere with the analysis. None of the tested compounds interfered under the conditions of the analysis.

In a similar manner, drugs listed in Table 6 were evaluated for potential interference with the analysis of lidocaine, quinidine, disopyramide, and propranolol. Diazepam and flurazepam interfere with quinidine analysis by ultraviolet detection, but do not interfere with fluorescence detection. Meperidine elutes close to disopyramide and might interfere with its analysis when present in toxic concentrations.

Several of the matabolites listed in Table 7 were chromatographed and their retention times noted. The method could be used to monitor these metabolites for pharmacokinetic studies. The method was not optimized for the study of these matabolites. A recently isolated metabolite of quinidine (N-oxide or its rearranged product) was found to coelute with quinidine under the assay conditions. However, this metabolite could be separated from quinidine by employing 24% acetonitrile and pH 2.8 phosphate buffer. Concentrations of this

metabolite in clinical samples were found to be too insignificant to warrant a change in the procedure.

Lipemic, icteric, or hemolyzed sera did not interfere with the analysis.

Accuracy:

To assess the accuracy of the method, over 200 sera from subjects on antiarrhythmic drug therapy were compared with other reference methods. These samples were analyzed by gas-liquid chromatographic (31,71), liquid chromatographic (41,61), or immunoassay (56) methods. In general, the correlation was extremely good between different methods as shown in Table 8. The comparison parameters were calculated by these formulae:

$$\text{regression coefficient } (r) = \frac{N(\Sigma xy)(\Sigma x)(\Sigma y)}{\sqrt{[N\Sigma x^2-(\Sigma x)][N\Sigma x^2-(\Sigma y)^2]}}$$

x = the independent variable (previous method)

y = the dependent variable (proposed method)

$$\text{slope} = \frac{N\Sigma xy-\Sigma x\Sigma y}{N\Sigma x^2-(\Sigma x)^2}$$

$$\text{Y-intercept} = \frac{\Sigma y\Sigma x^2-\Sigma x\Sigma xy}{N\Sigma x^2-(\Sigma x)^2}$$

Figures 7-16 illustrate the scatter plots of comparisons constructed with values for reference method on X-axis and values for the proposed LC method on the Y-axis. Several chromatograms obtained utilizing the

proposed LC methods from patients receiving antiarrythmic drugs are illustrated in Figures 17-20.

CONCLUSIONS

The value of regular measurements of serum or plasma concentrations of antiarrhythmic drugs has been well established (117). The diversity of these agents pose special problems to the analyst; special columns have been recommended for gas chromatographic procedures; relatively large amount of samples are necessary; and, in general, different analytical procedures have been utilized for each individual drug. The data presented in this paper indicates the usefulness of a flexible liquid chromatographic system, which may use either gradient or isocratic elution and either UV or fluorescence detection, in the analysis of this class of drug in serum. Detection by absorption at 216 nm provides adequate sensitivity for the analysis of lidocaine and propranolol and fluorescence detection can provide the additional sensitivity in micro samples. The ability of this method to simultaneously analyze parent drugs and a number of their metabolites might prove quite useful if appropriate studies reveal pharmacologic activity for these metabolites.

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Table 2. Relative Recovery of Drugs from Plasma (n=3).

Drug	ADDED mg/L	RECOVERED mg/L	RECOVERY %
Procainamide	2.50	2.10	84
	5.00	4.15	83
	6.25	5.20	83
	12.50	10.30	82
	25.00	20.50	82
N-acetyl	2.50	2.03	81
procainamide	5.00	3.95	79
	6.25	5.25	84
	12.50	10.50	84
	25.00	21.00	84
Disopyramide	1.00	1.13	113
	2.50	2.75	110
	5.00	5.60	112
	10.00	11.10	111
	20.00	20.60	103
N-desisopropyl	1.00	1.08	108
disopyramide	2.50	2.68	107
	5.00	5.45	109
	10.00	10.70	107
	20.00	20.00	100
Lidocaine	1.00	0.76	76
	2.50	2.00	80
	5.00	4.15	83
	10.00	7.90	79

Table 2 (Cont.)

Quinidine (U.V.)	1.00	0.85	85
	2.50	2.15	86
	5.00	4.65	93
	10.00	8.90	89
	20.00	18.00	90
Quinidine (fluorescence)	1.00	0.96	96
	2.50	2.58	103
	5.00	5.10	102
	10.00	9.70	97
Propranolol (U.V.)	20 ug/L	20 ug/L	100
	50 ug/L	54 ug/L	108
	100 ug/L	112 ug/L	112
	200 ug/L	204 ug/L	102
Propranolol (fluorescence)	10 ug/L	9 ug/L	90
	20 ug/L	22 ug/L	110
	50 ug/L	52 ug/L	104
	100 ug/L	98 ug/L	98
	200 ug/L	200 ug/L	100

Table 3. Precision for Antiarrhythmic Drugs in Serum.

DRUG	WITHIN DAY			DAY-TO-DAY		
	Range, ± SD			Range, ± SD		
	mg/L	CV,%	n	mg/L	CV,%	n
Procainamide	5.0 ± 0.12	2.3	12	5.0 ± 0.22	4.3	20
	12.4 ± 0.19	1.5	12	12.0 ± 0.34	2.9	20
N-acetyl	4.8 ± 0.10	2.1	12	4.8 ± 0.16	3.4	20
procainamide	12.4 ± 0.23	1.9	12	12.4 ± 0.28	2.3	20
Disopyramide	2.4 ± 0.16	6.6	12	2.2 ± 0.22	10.0	17
	8.3 ± 0.20	2.4	12	8.2 ± 0.30	3.7	13
N-desisopropyl	2.3 ± 0.13	5.4	12	2.3 ± 0.23	10.0	17
disopyramide	8.7 ± 0.12	1.4	12	8.5 ± 0.25	3.0	13
Lidocaine	2.2 ± 0.19	8.6	12	2.4 ± 0.21	8.7	20
	9.8 ± 0.39	4.1	12	9.5 ± 0.7	7.2	19
Quinidine	2.3 ± 0.15	6.3	12	2.2 ± 0.23	10.0	10
(U.V.)	9.1 ± 0.42	4.3	12	9.9 ± 0.55	5.5	10
Quinidine	2.6 ± 0.16	5.9	12	1.7 ± 0.20	11.5	20
(fluorescence)	9.7 ± 0.31	3.2	12	4.1 ± 0.31	7.5	20
Propranolol	51 ± 3.5ug/L	6.9	13	55 ± 4.6ug/L	8.2	10
(U.V.)	203 ± 11ug/L	5.0	14	233 ± 13.1ug/L	5.8	18
Propranolol	38 ± 1.6ug/L	4.1	14	37 ± 2.4ug/L	6.5	20
(fluorescence)	69 ± 1.8ug/L	2.5	15	71 ± 3.4ug/L	4.7	20

Table 4. Sensitivity of Antiarrhythmic Drugs in Serum.

DRUGS	LOWER LIMIT OF DETECTION mg/L
Procainamide	0.1
N-acetyl procainamide	0.1
Disopyramide	0.1
N-desisopropyl disopyramide	0.1
Lidocaine	0.1
Quinidine (U.V.)	0.1
Quinidine (fluorescence)	0.1
Propranolol (U.V.)	10.0 ug/L
Propranolol (fluorescence)	1.0 ug/L

Table 5. Retention Times of some Drugs with Procainamide

Mobile Phase .

DRUG	RETENTION TIMES (min)
Procainamide	2.7
N-acetyl procainamide	4.2
I.S. N-propionyl procainamide	9.0
Acetaminophen	3.0
Theophylline	3.0
Caffeine	5.1
Primidone	14.0
Lidocaine	21.0
Salicylate	*N.D.
Butabarbital	*N.D.
Pentobarbital	*N.D.
Amobarbital	*N.D.
Phenobarbital	*N.D.
Phenytoin	*N.D.
Disopyramide	*N.D.
Quinidine	*N.D.
Propranolol	*N.D.

*N.D. - Not Detectable

Table 6. Retention Times of some Drugs.

DRUG	RETENTION TIMES (min)
Butabarbital	2.3
Theophylline	2.4
Phenylpropanolamine	2.4
Acetaminophen	2.8
Amphetamine	2.8
Caffeine	3.0
Methamphetamine	3.3
Diazepam	4.7
Flurazepam	4.8
Meperidine	6.4
Sodium salicylate	6.8
Methaqualone	2.6
Phenobarbital	8.3
Phenacetin	9.0
Methadone	10.6
Chlorodiazepoxide	15.2
Ethchlorvynol	*N.D.
N-desmethyldiazepam	*N.D.

*N.D. = Not Detectable

Table 6 (Cont.)

Nitrazepam	*N.D.
Secobarbital	*N.D.
Glutethimide	*N.D.
Digoxin	*N.D.
Propoxyphene	*N.D.
Fluphenazine	*N.D.
Phenytoin	*N.D.

*N.D. - Not Detectable

Table 7. Retention Times of Antiarrhythmic Drugs and Metabolites.

DRUG	RETENTION TIMES (min)
N-desisopropyl disopyramide	3.8
Lidocaine	4.1
Quinidine	4.9
Disopyramide	6.6
I.S. Pronethalol	7.0
Propranolol	12.0
I.S. p-chlorodisopyramide	13.5
Lidocaine Metabolites	
Glycylxylidide (GX)	2.5
Monoethylglycylxylidide (MEGX)	3.1
Methylethylglycylxylidide (EMGX)	3.4
Quinidine Metabolites	
3-OH quinidine	2.5
2' -quinidinone	3.0
N-oxide	4.9
Dihydroquinidine	6.2
Propranolol Metabolites	
4-OH propranolol	4.5
N-desisopropyl propranolol	6.0
α-naphthoxy lactic acid	12.5
Propranolol glycol	14.5
α-naphthol	*N.D.
α-naphthoxy acetic acid	*N.D.

*N.D. - Not Detectable

Table 8. Correlation of Proposed LC Method with Reference Methods.

DRUG	METHOD OF COMPARISON		PARAMETERS
	X	Y	
Procainamide	Reference LC	Proposed LC	N = 19
			r = 0.993
			Slope = 0.909
			Y-intercept = 0.386
	Gas chromatography	Proposed LC	N = 18
			r = 0.997
			Slope = 1.076
			Y-intercept = -0.153
N-acetyl procainamide	Reference LC	Proposed LC	N = 19
			r = 0.996
			Slope = 0.992
			Y-intercept =-0.271
Disopyramide	Gas chromatrography	Proposed LC	N = 14
			r = 0.993
			Slope = 1.138
			Y-intercept =-0.166

Table 8. (Cont.)

Lidocaine	Gas chromatography	Proposed LC	N = 17
			r = 0.971
			Slope = 0.932
			Y-intercept =-0.012
Quinidine	Reference LC	Proposed LC	N = 26
		with	r = 0.974
		fluorescence	Slope = 0.974
			Y-intercept = 0.275
Quinidine	Reference LC	Proposed LC	N = 15
		with U.V.	r = 0.975
			Slope = 1.054
			Y-intercept = 0.202
	EMIT(R)	Proposed LC	N = 20
		with	r = 0.962
		fluorescence	Slope = 0.921
			Y-intercept = 0.226

Table 8. (Cont.)

Quinidine	Proposed LC with fluorescence	Proposed LC with U.V.	N = 16 r = 0.973 Slope = 0.994 Y-intercept = 0.063
Propranolol	Proposed LC with fluorescence	Proposed LC with U.V.	N = 9 r = 0.996 Slope = 1.020 Y-intercept = 0.564

EMIT(R): Homogeneous enzyme immunoassay - Syva Company, Palo Alto, CA 94303

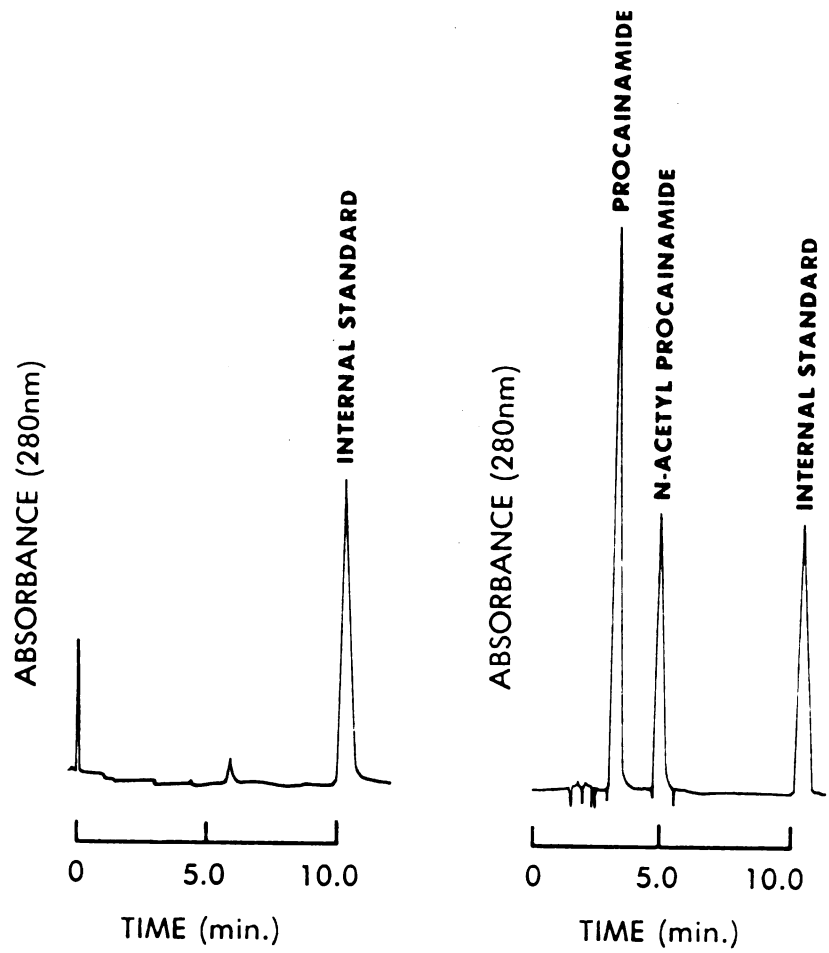

Fig.2. Left: Chromatogram of a drug-free serum with 10 mg of added internal standard per liter. Right: Chromatogram from a patient's serum containing 9.9 mg/L of procainamide and 8.4 mg/L of N-acetylprocainamide.

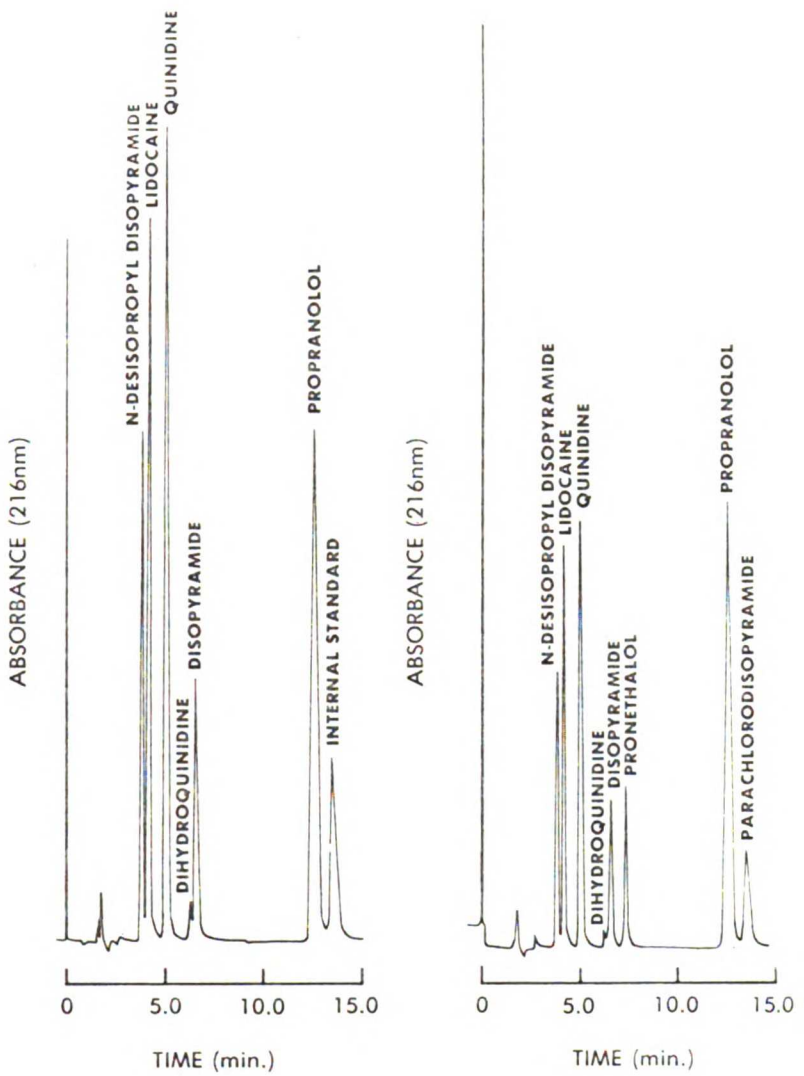

Fig.3. Left: Chromatogram of a standard mixture of drugs.

Right: Chromatogram of a standard mixture of drugs with pronethalol.

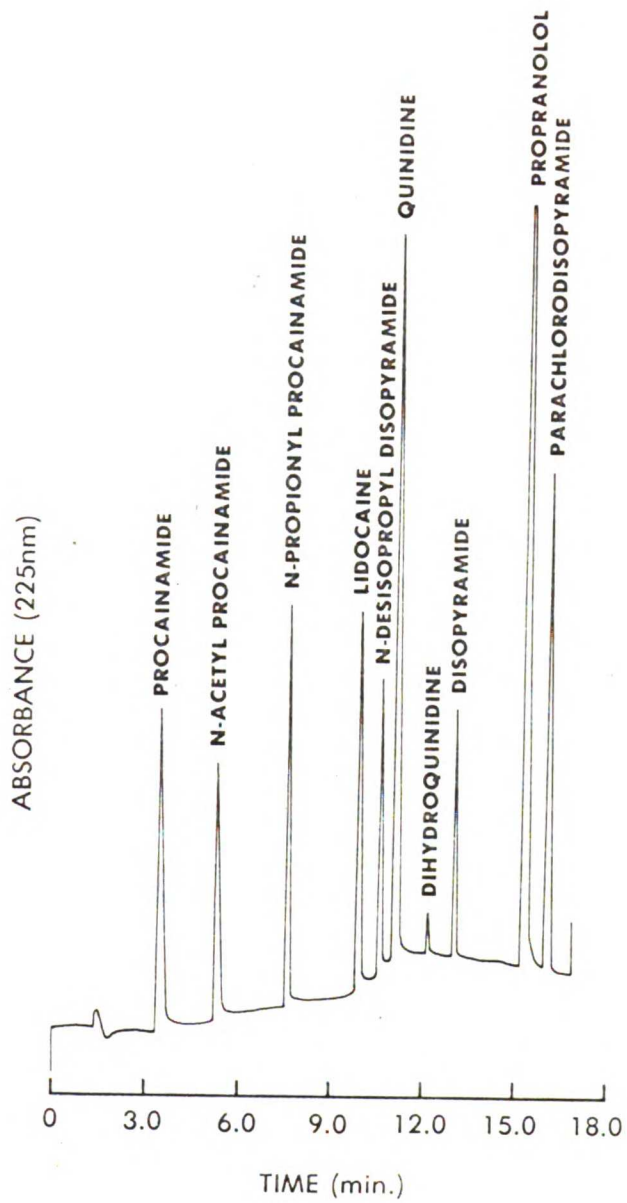

Fig.4. Chromatogram of a standard mixture of drugs run under gradient conditions.

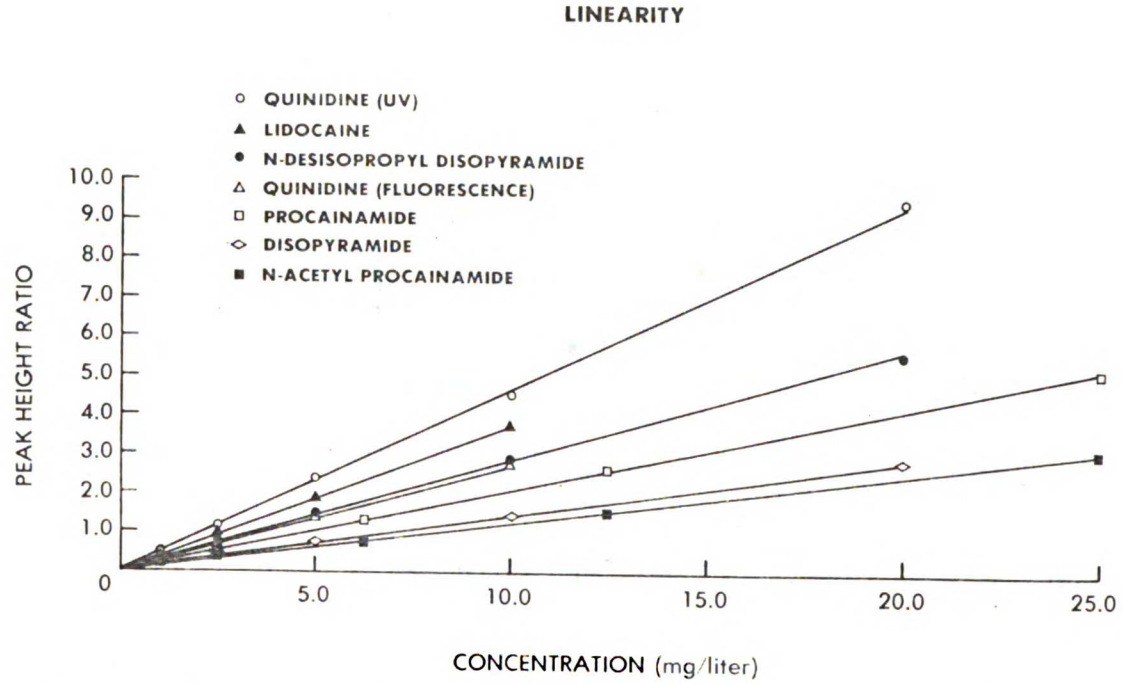

Fig.5. Peak-height ratios (drug / internal standard) for each drug plotted vs. concentration of each drug. Each point is the average of triplicate determinations.

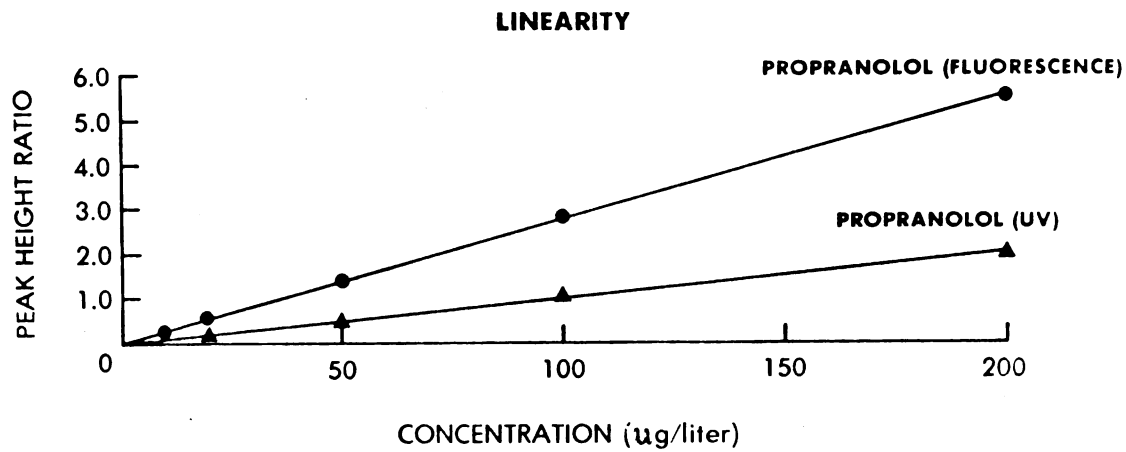

Fig.6. Peak-height ratio of propranolol / internal standard plotted vs. concentration of the drug.
Each point is the average of triplicate determinations.

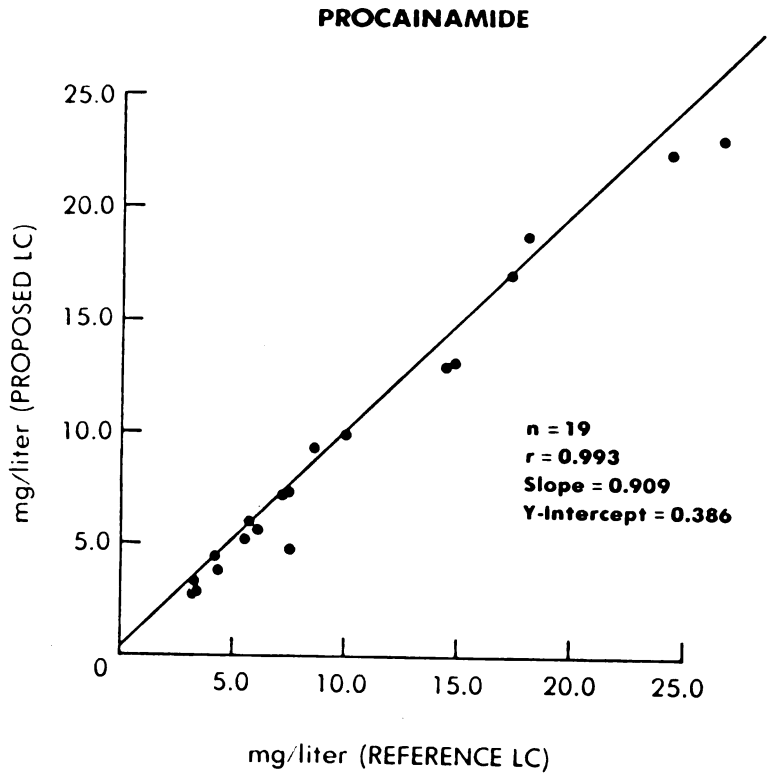

Fig.7. Procainamide analysis, proposed LC vs. reference LC.

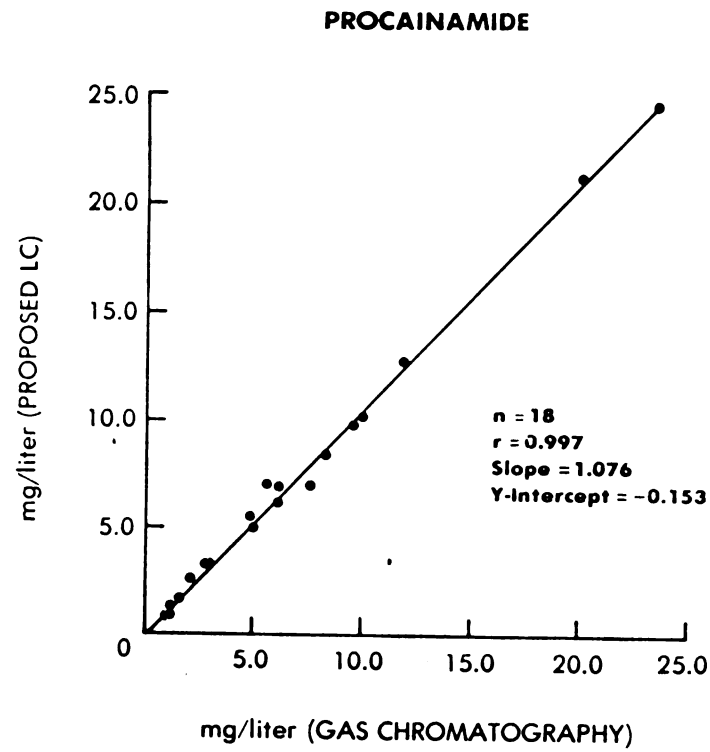

Fig.8. Procainamide analysis, proposed LC vs. GLC.

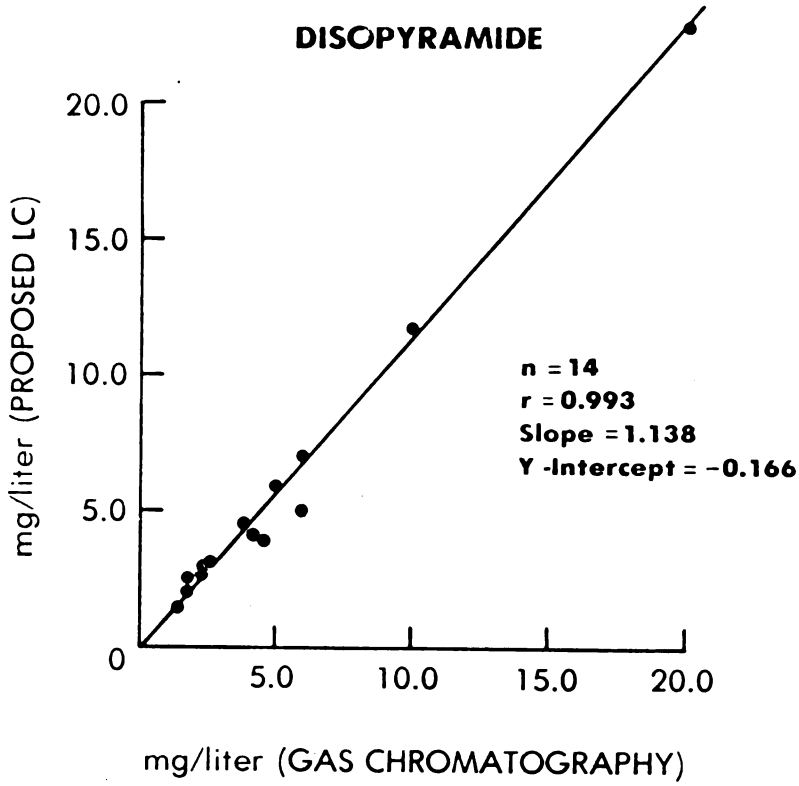

Fig. 9. Disopyramide analysis, proposed LC vs. GLC.

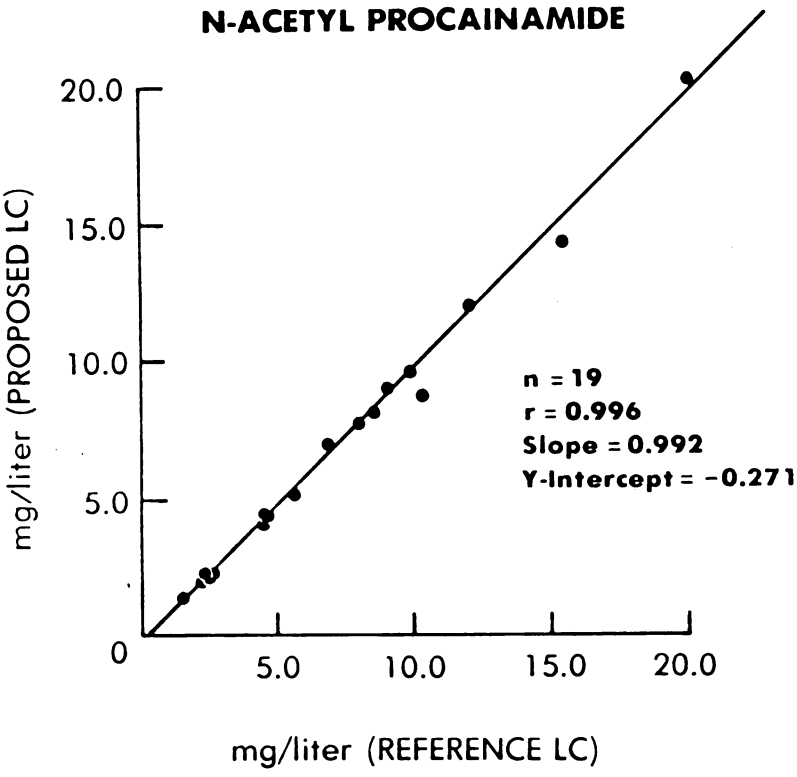

Fig.10. N-acetylprocainamide analysis, proposed LC vs. reference LC.

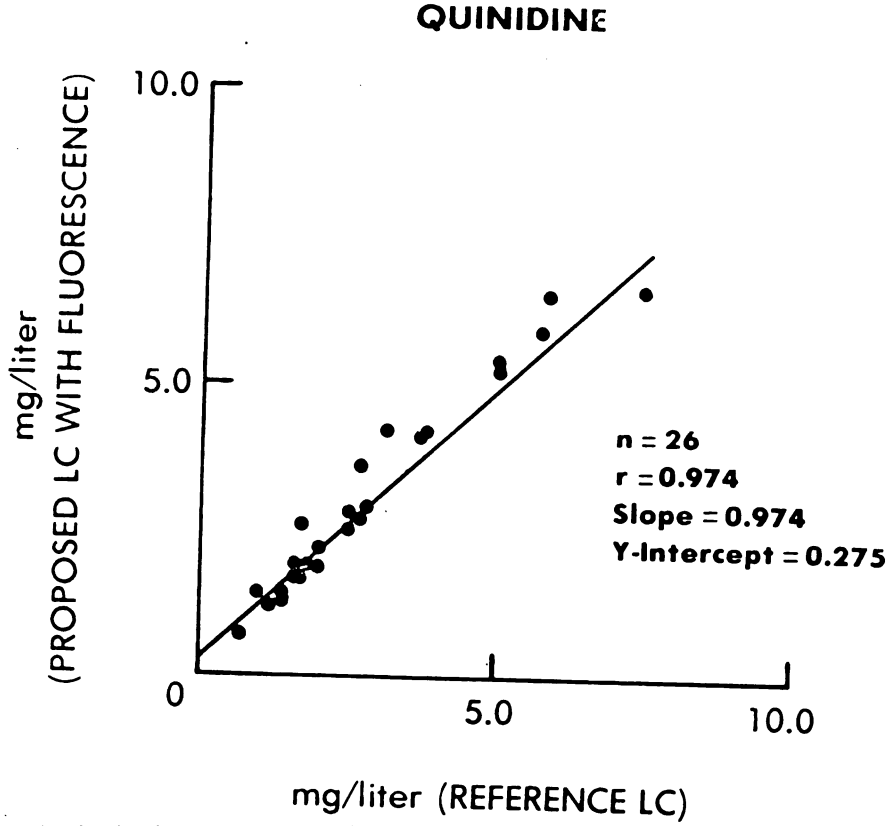

Fig.11. Quinidine analysis, proposed LC with fluorescence vs. reference LC.

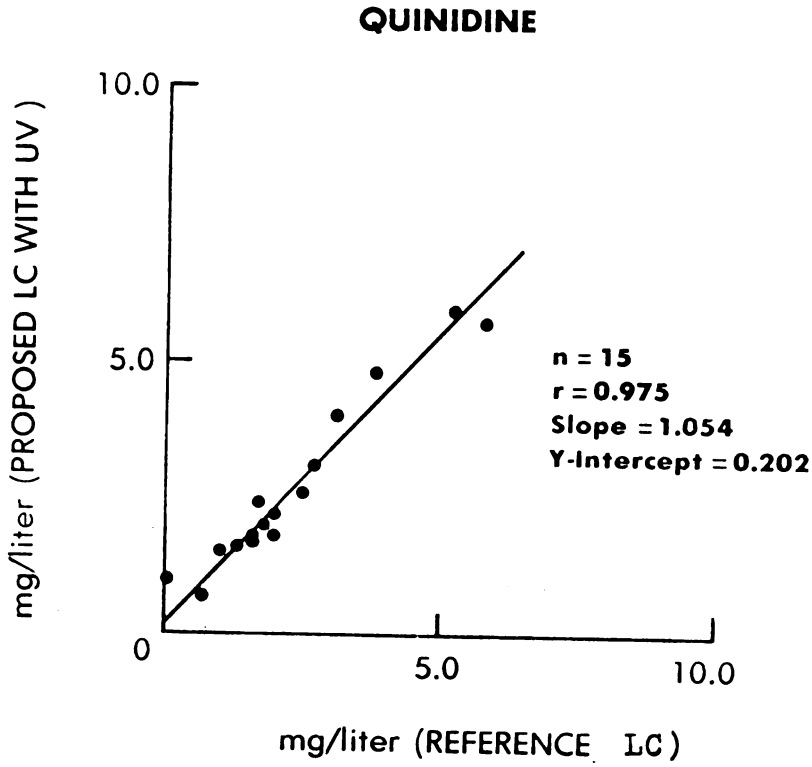

Fig.12. Quinidine analysis, proposed LC with U.V. vs. reference LC.

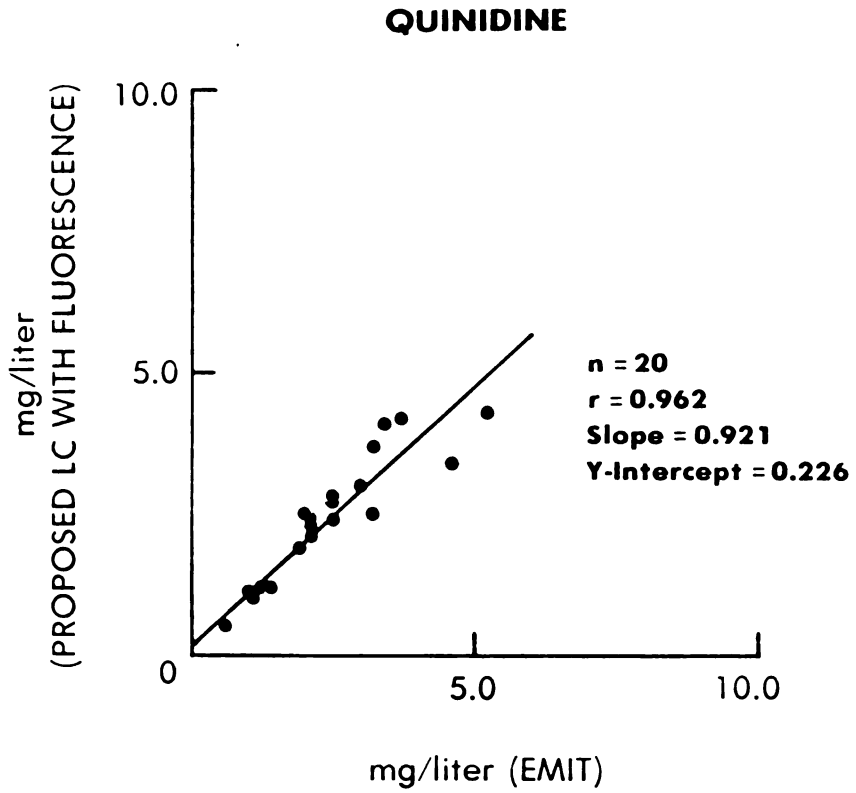

Fig.13. Quinidine analysis, proposed LC with fluorescence vs. EMIT^{R}.

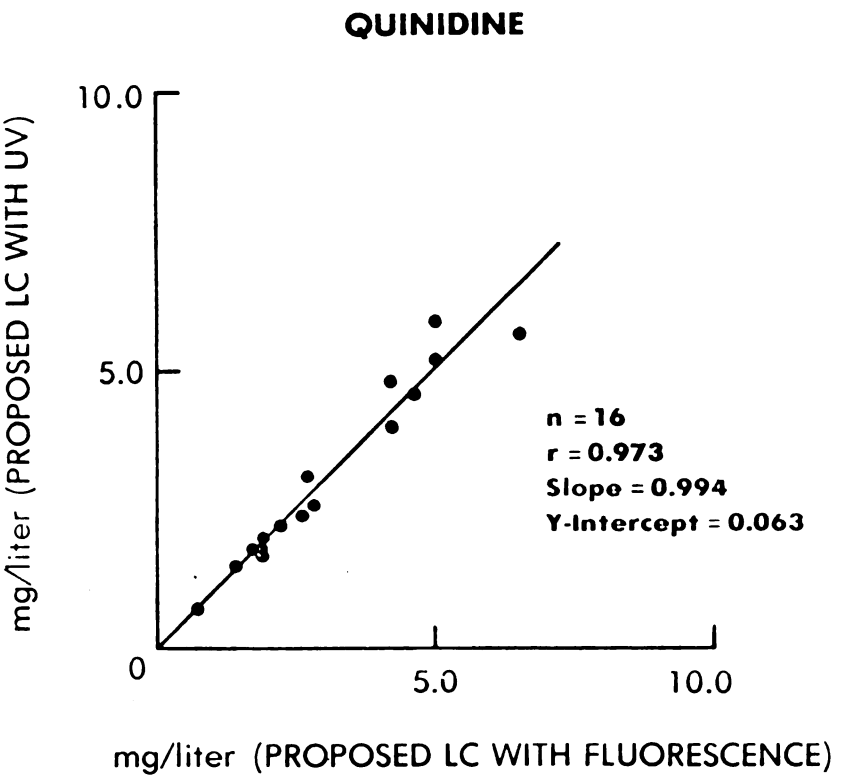

Fig.14. Quinidine analysis, proposed LC with U.V. vs. proposed LC with fluorescence.

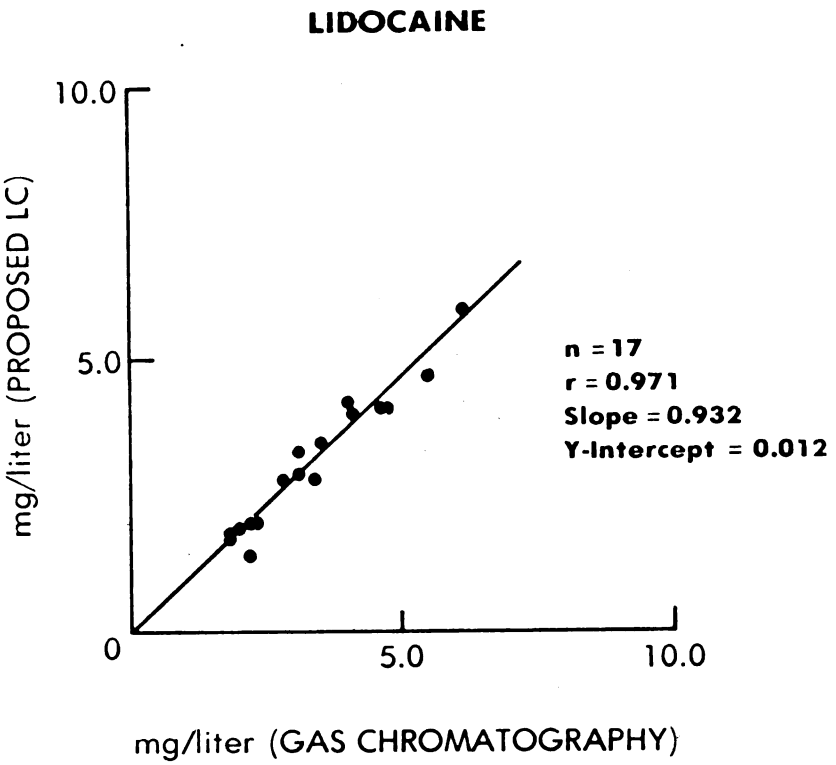

Fig.15. Lidocaine analysis, proposed LC vs. GLC.

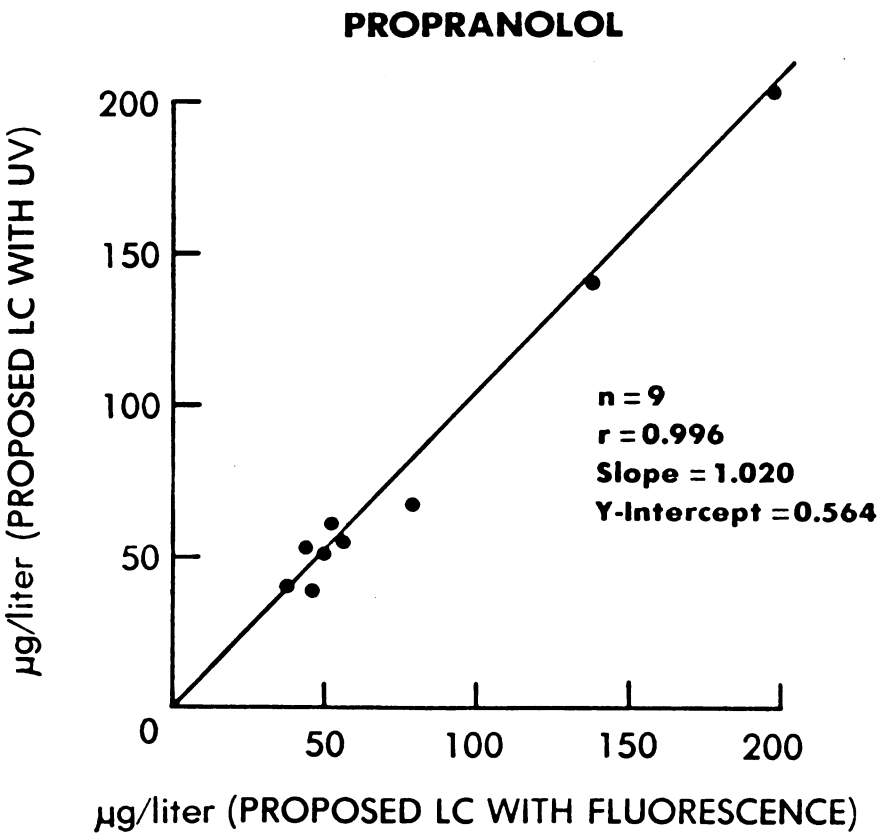

Fig.16. Propranolol analysis, proposed LC with U.V. vs. proposed LC with fluorescence.

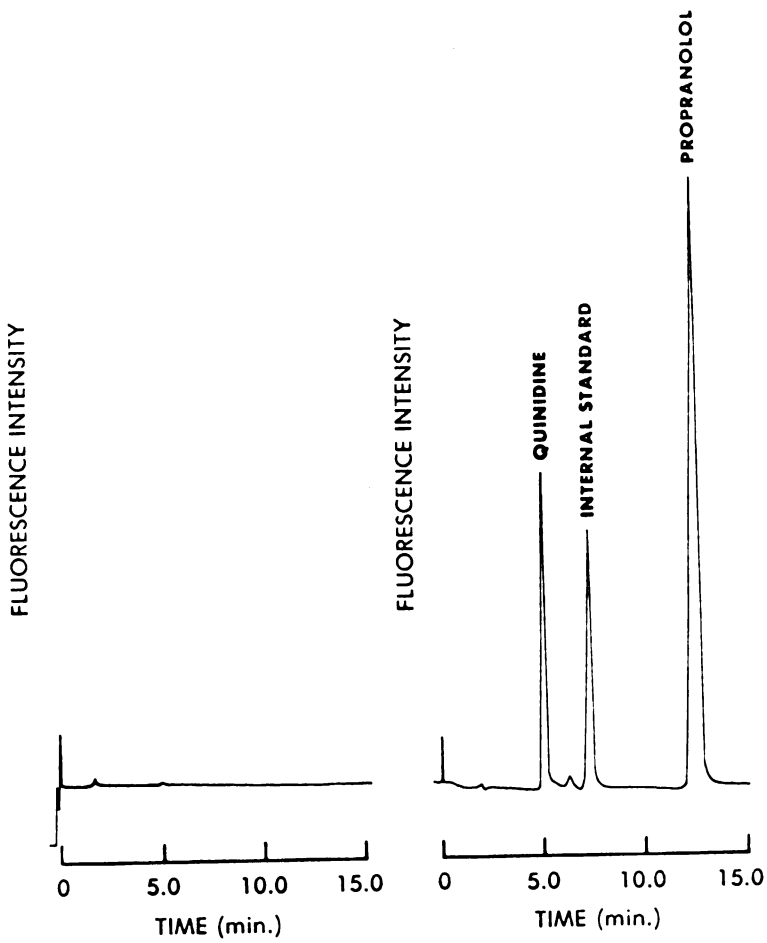

Fig.17. Left: Chromatogram of a drug-free serum obtained by fluorescence detection.
Right: Chromatogram of a standard mixture of quinidine, pronethalol (internal standard), and propranolol.

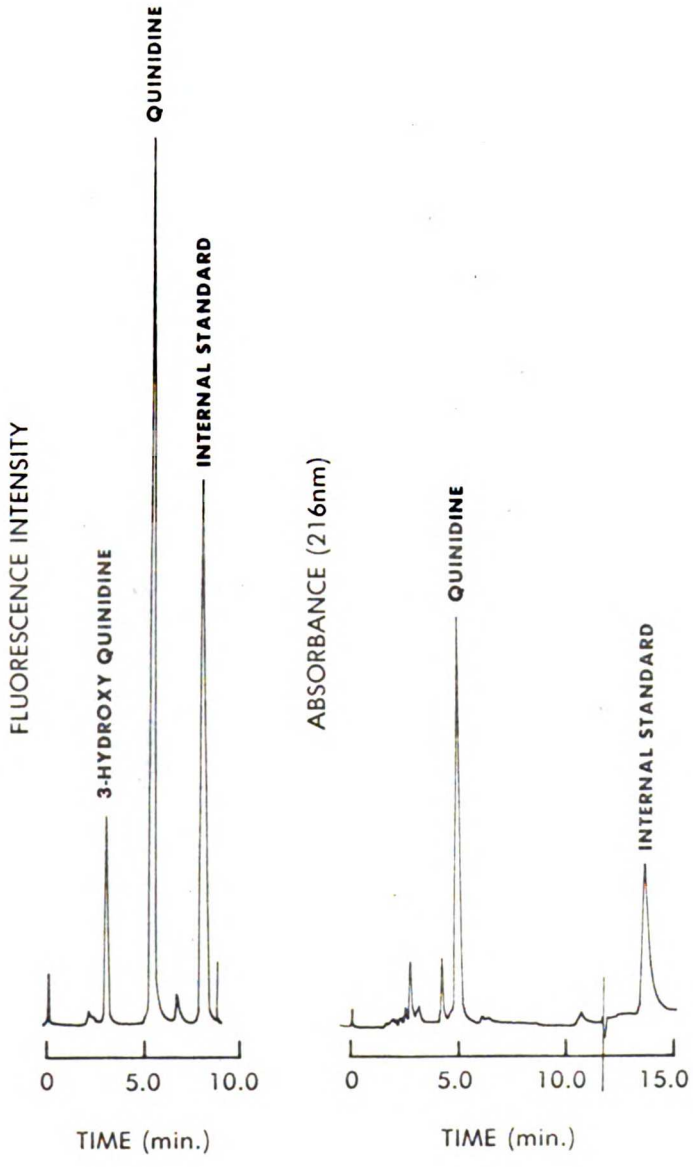

Fig.18. Left: Chromatogram from a patient's serum containing 5.0 mg/L of quinidine (fluorescence detection).

Right: Chromatogram from a patient's serum containing 5.7 mg/L of quinidine (U.V. detection).

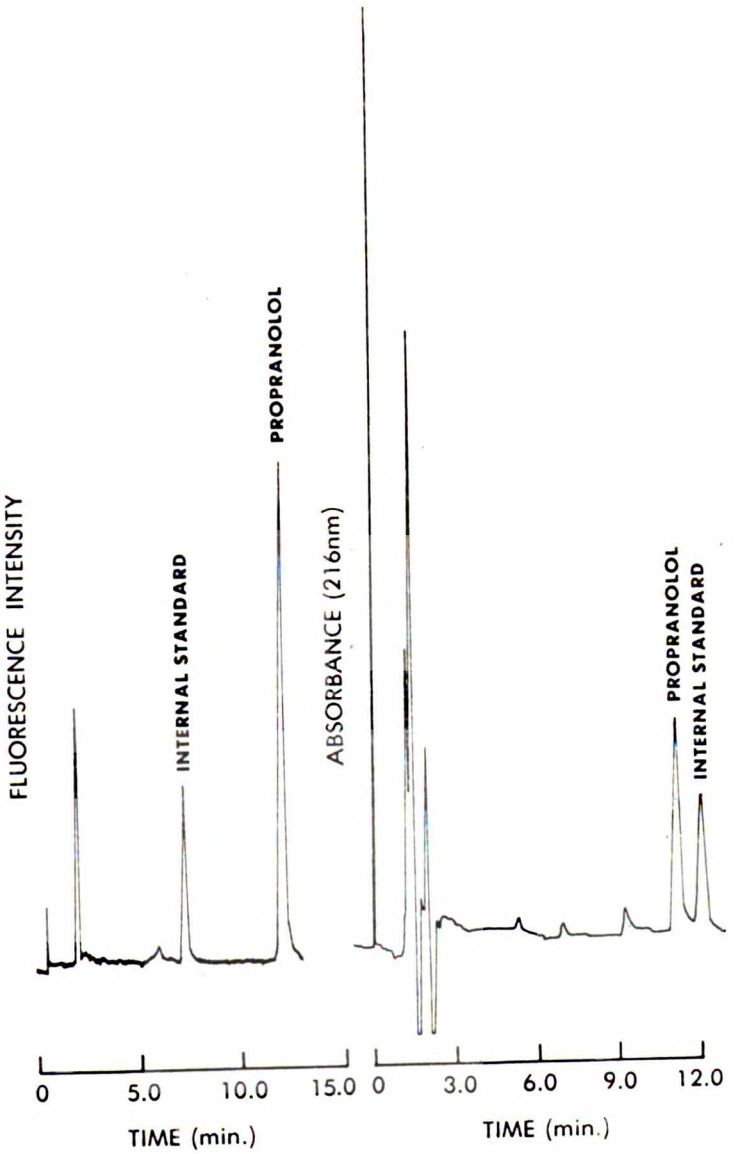

Fig.19. Left: Chromatogram from a patient's serum containing 100 ug/L of propranolol (fluorescence detection).

Right: Chromatogram from a patient's serum containing 140 ug/L of propranolol (U.V. detection).

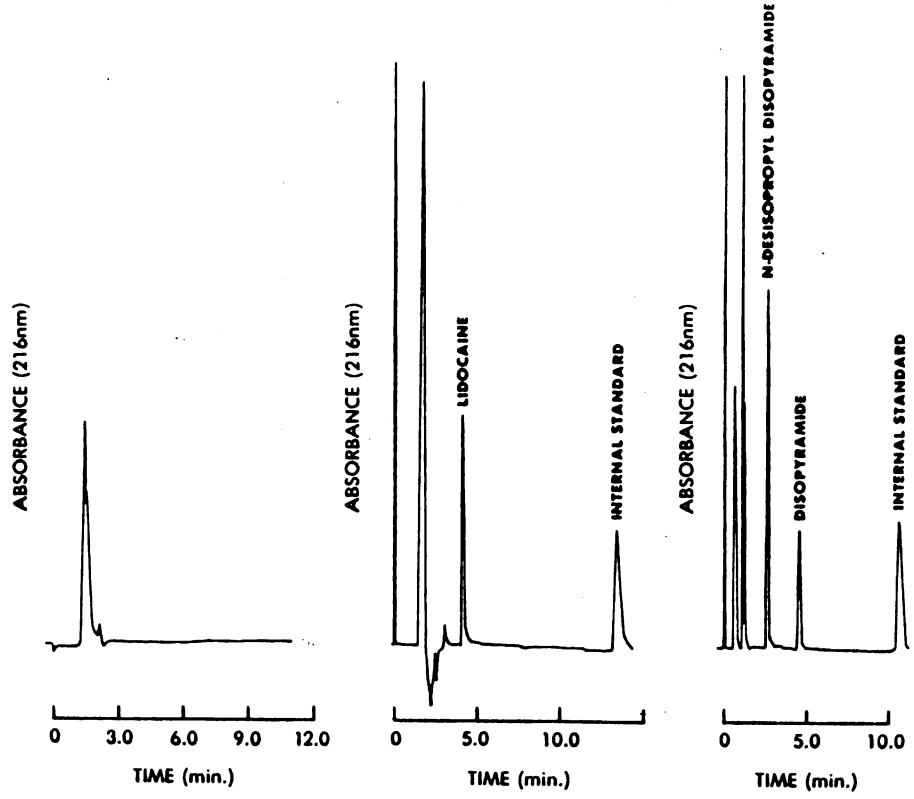

Fig.20. Left: Chromatogram of a drug-free serum.

Middle: Chromatogram from a patient's serum containing 4.7 mg/L of lidocaine.

Right: Chromatogram from a patient's serum containing 9.8 mg/L of N-desisopropyl disopyramide and 6.3 mg/L of disopyramide.