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SIMULTANEOUS QUANTITATION OF DISOPYRAMIDE AND ITS MONO-DEALKYLATED METABOLITE IN HUMAN PLASMA BY FUSED-SILICA CAPILLARY GAS CHROMATOGRAPHY USING NITROGEN—PHOSPHORUS SPECIFIC DETECTION

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SUMMARY

A nitrogen-specific detector gas—liquid chromatographic assay method is reported which provides improved selectivity and sensitivity for disopyramide and its mono-N-dealkylated metabolite using a crosslinked fused-silica capillary column. The quantitation of disopyramide and mono-N-dealkylated disopyramide was accomplished by injecting trifluoroacetic anhydride-treated samples containing derivatized internal standard *p*-chlorodisopyramide, into a gas chromatograph equipped with a nitrogen—phosphorus detector and an automatic liquid sampler. A 25 m × 0.31 mm crosslinked, 5% phenylmethyl silicone-coated fused-silica column was utilized and samples were injected using the splitless injection mode.

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Linearity was observed in the range 0.05—5.00 μg/ml for disopyramide and 0.02—3.00 μg/ml for the mono-N-dealkylated metabolite. The coefficient of variation was found to be within 10% for both compounds in the concentration range studied.

INTRODUCTION

Disopyramide, 4-(diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide, is an antiarrhythmic agent which shares a number of pharmacologic properties with quinidine and procainamide [1—9]. In patients with normal renal function, 50—60% of disopyramide is excreted unchanged in the urine with the remainder being excreted in urine and feces as the mono-N-dealkylated metabolite [4-(isopropylamino)-2-(2-pyridyl)-2-phenylbutyramide, MND] [10, 11]. Animal studies suggest that the antiarrhythmic potency of MND is about 80% that of disopyramide while the anticholinergic side effects are approximately 24-fold greater than that associated with the parent drug [12].

A number of analytical methods have been reported for the simultaneous measurement of disopyramide and MND in biological fluids including gas—liquid chromatography (GLC) [11, 13—23] and high-performance liquid chromatography (HPLC) [24—36] and a stable isotope dilution technique using gas chromatography—mass spectrometry (GC—MS) [37]. All GLC methods reported so far for disopyramide make use of conventional packed columns. The HPLC methods are convenient but sensitivity limitations necessitate injection of the entire extracted sample volume into the chromatograph.

The lack of adequately sensitive and selective assay methods has limited the disopyramide plasma protein binding determination to in-vitro binding studies [9, 38, 39]. In almost all cases the unbound percentage values are indirectly calculated from the individual binding curves rather than from measurements of binding to the proteins in plasma that was obtained from patients to whom drug had been administered [9, 38, 39].

The aim of the present report is to describe a sensitive and reproducible fused-silica capillary GCL—nitrogen—phosphorus detection (NPD) assay for the simultaneous quantitation of disopyramide and MND in human plasma in the presence of various antiarrhythmics and for use in binding studies.

Materials

Disopyramide, 4-(diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide (lot No. SC-7031), disopyramide phosphate (Norpace, lot No. SC-13957), mono-N-dealkyl disopyramide (lot No. ADAMEKCD3-185A) and *p*-chlorodisopyramide (lot No. SC-13957) were supplied by G.D. Searle of Canada (Oakville, Ontario, Canada). Trifluoroacetic anhydride (TFAA) was purchased from Pierce (Rockford, IL, U.S.A.). Toluene (distilled-in-glass) was purchased from Caledon Labs. (Georgetown, Ontario, Canada). Solutions of 1 *M* sodium hydroxide and 0.1 *M* hydrochloric acid were prepared from ACS reagent-grade chemicals (American Scientific and Chemical, Seattle, WA, U.S.A.). Deionised and distilled water was used in the preparation of stock solutions and throughout the analysis.

Instrumentation

A Model 5830A Hewlett-Packard (H-P) gas chromatograph equipped with a nitrogen—phosphorus selective detector and a Model 18835B capillary inlet system was used for all analyses. A Model 18850A H-P integrator system was used for peak area integration and quantitation. Capillary GLC electron impact mass spectrometry (EI-MS) and chemical ionisation mass spectrometry (CI-MS) were carried out utilizing GC—MS H-P Models 5970A and 5987A, respectively.

Chromatographic conditions

A 25 m × 0.31 mm I.D. crosslinked fused-silica capillary column (5% phenylmethyl silicone, ultra No. 2, film thickness 0.17 μm, siloxane-deactivated (Hewlett-Packard, Avondale, PA, U.S.A.) was used for all plasma analyses. The splitless injection mode employing a silanized fused-silica insert (78 mm × 2 mm I.D.) was used, with a 2-μl sample being injected. The operating conditions for routine analysis were: injection port temperature, 200°C; oven temperature 1, 160°C, programming rate, 5°C/min, oven temperature 2, 195°C; nitrogen—phosphorus detector temperature, 300°C; helium was used as a carrier at a flow-rate of 1 ml/min and make-up gas at a flow-rate of 30 ml/min; septum purge flow-rate was 3 ml/min; hydrogen—air flow-rate ratio was 3:50 (ml/min).

Stock solutions

Disopyramide phosphate (5 μg/ml, equivalent to base) was dissolved in distilled water; mono-N-dealkylated disopyramide (MND) (3 μg/ml) and the internal standard, *p*-chlorodisopyramide (PC-Dis) (5.5 μg/ml) were dissolved in 0.1 *M* hydrochloric acid. The solutions were stored at 4°C, following preparation, for up to two months.

Extraction and derivatization procedure

A 0.5-ml sample of blank human plasma was spiked with a volume (0.1, 0.2, 0.4, 0.6, 0.8 or 1.0 ml) of the prepared stock solutions of disopyramide (5 μg/ml) and MND (3 μg/ml). To this mixture, 0.5 ml of PC-Dis (5.5 μg/ml) and 0.5 ml of 1 *M* sodium hydroxide (pH 12) were added. The aqueous phase was adjusted to a total volume of 3.5 ml with distilled water. Toluene (6 ml) was added and the aqueous phase was extracted by shaking for 20 min on a rotary shaker (Labquake Tube Shaker, Model 415-110; Labindustries, Berkeley, CA, U.S.A.). After centrifugation for 10 min, 5 ml of the organic phase was removed and dried under a gentle stream of nitrogen in a 40°C water bath. The residue was reconstituted to a volume of 0.5 ml with toluene and 150 μl of trifluoroacetic anhydride (TFAA) were added. The sample was vortexed for 10 sec and incubated in an oven at 55°C for 45 min. The excess TFAA was removed by evaporating the sample under a gentle stream of nitrogen in a 40°C water bath. The residue was reconstituted with 100 μl of toluene and 2-μl aliquots were used for capillary GLC—NPD analysis. A similar extraction and derivatization procedure was carried out for diluted stock solutions of disopyramide (0.54 μg/ml), MND (0.23 μg/ml) and PC-Dis (0.55 μg/ml).

Quantitative analysis

A 2-μl aliquot of the toluene solution containing TFAA-treated disopyramide, MND and PC-Dis was injected into a gas chromatograph equipped with an automatic liquid sampler (H-P Model 7671A). Calibration curves for disopyramide and MND were constructed by plotting the area ratios against the known concentrations of disopyramide and MND. The calibration curves thus obtained were used for the estimation of the unknown concentration of disopyramide and MND in biological samples.

GLC—electron impact mass spectrometry

A computerized gas chromatograph—electron impact mass spectrometer H-P Model 5970A, equipped with a 12 m × 0.27 mm I.D. fused-silica column coated with methylsilicone fluid and a mass selective detector was used to study the fragmentation pattern of untreated and TFAA-treated samples of disopyramide, MND and PC-Dis. The following splitless, capillary GLC conditions were used: oven temperature 1, 225°C; time 1, 0.5 min; rate, 15°C/min; oven temperature 2, 250°C; time 2, 2.0 min; injection port temperature, 250°C; helium (carrier gas) flow-rate, 1.0 ml/min. For the mass spectrometer, the ionization beam energy was 70 eV, the electron multiplier voltage, 1400 V and the interface temperature was 280°C.

Chemical ionization mass spectrometry

An H-P Model 5987A gas chromatograph—mass spectrometer was used to determine the molecular ion of the parent, untreated compounds, as well as the TFAA-treated compounds. Samples were injected using the splitless mode and a linear scanning method was used. The instrument was equipped with a 12 m × 0.27 mm I.D. crosslinked methyl silicone fused-silica column. Methane was used as an ionizing gas and the following mass spectrometer conditions were used: injection port temperature, 250°C; interface oven temperature, 275°C; GC interface probe temperature, 275°C; ion source temperature, 100°C; oven temperature 1, 100°C; rate, 30°C/min; oven temperature 2, 260°C; multiplier voltage, 2007 V, emission current, 300 μA.

RESULTS

Representative GLC chromatograms from the extracts of blank plasma and spiked plasma are shown in Fig. 1. No extraneous (interfering) peaks from endogenous plasma constituents are apparent in the plasma extracts (Fig. 1A). Peaks with retention times (R_t) of 10.10 and 12.91 min are the dehydrated i.e. nitrile) forms of disopyramide and PC-Dis, respectively, and the peak at 10.59 min represents the TFAA derivative of the dehydrated form of MND. Baseline resolution is achieved between all peaks.

Fig. 2 represents the chromatogram of TFAA-treated mixture containing various antiarrhythmic drugs. All of the chromatographic peaks were resolved under the following splitless GLC conditions; oven temperature 1, 160°C; oven temperature programming rate, 5°C/min; oven temperature 2, 270°C; time 2, 25 min; injection temperature, 260°C; nitrogen—phosphorus detector temperature, 300°C; carrier gas (helium) flow-rate, 1.2 ml/min; make-up gas

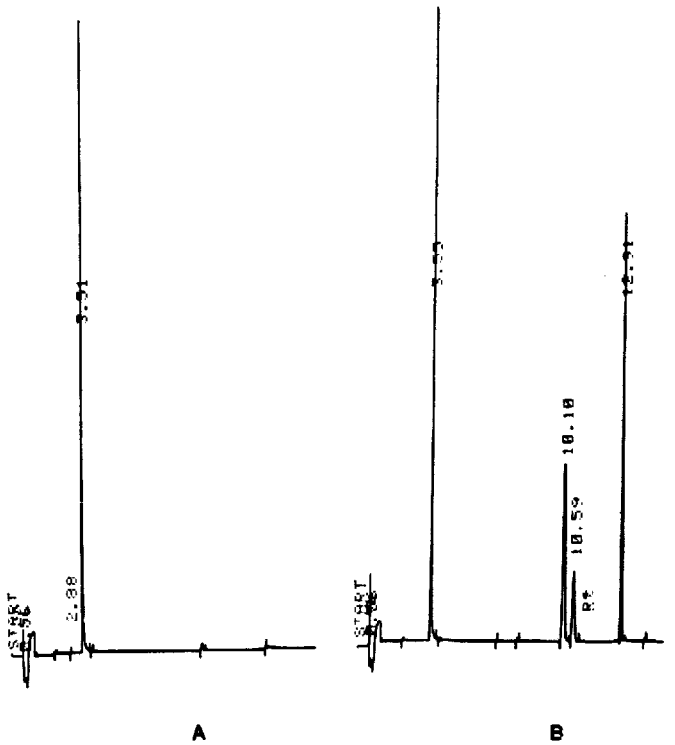


Fig. 1. Representative capillary gas—liquid chromatograms obtained from (A) blank and (B) spiked plasma extracts; the spiked sample before extraction contained disopyramide (R_t, 10.10 min), 1.0 ng/μl; MND (R_t, 10.59 min), 0.6 ng/μl and PC-Dis (R_t, 12.91 min), 2.7 ng/μl. Attenuation, 128; voltage 16 V; nitrogen—phosphorus detector collector was in use for ca. 200 h.

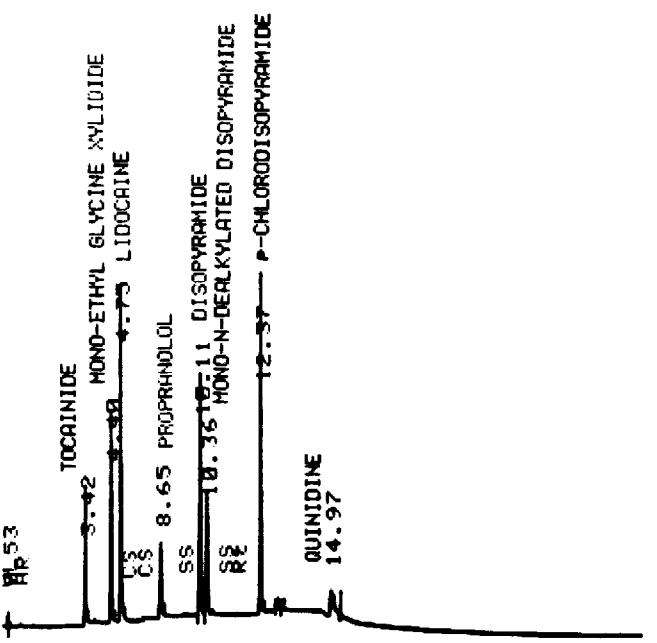


Fig. 2. Representative capillary GLC peaks of TFAA-treated antiarrhythmic agents.

(helium) flow-rate, 27 ml/min; hydrogen—air flow-rate ratio 3:50. Total analysis run time was 15 min.

Chromatographic responses for disopyramide and MND were linear in the range studied (0.05—5.00 μg/ml for disopyramide and 0.02—3.00 μg/ml for MND). The calibration curves were obtained by analyzing blank plasma samples

TABLE I

CALIBRATION CURVE OF DISOPYRAMIDE AND MND (HIGHER CONCENTRATION RANGE)

Statistics: linear regression lines for disopyramide; $y = (0.4689)x - 0.0463$; $r^2 = 0.999$; for MND; $y = (0.3061)x - 0.0157$; $r^2 = 0.996$.

Concentration of disopyramide (μg/ml)	Concentration of MND (μg/ml)	Area ratio disopyramide/PC-Dis range*	Area ratio MND/PC-Dis range*
0.5	0.31	0.206—0.219 (1.02)**	0.085—0.091 (4.98)**
1.0	0.61	0.408—0.421	0.175—0.182
1.99	1.23	0.879—0.891	0.357—0.368
2.99	1.84	1.235—1.398	0.485—0.549
3.98	2.45	1.748—1.880	0.668—0.751
4.98	3.07	2.12 —2.380 (2.53)**	0.902—0.983 (6.94)**

*Number of samples, $n = 2$—5 (one injection for each sample).
**$n = 5$, the numbers in the parentheses show the coefficient of variation (C.V., %).

TABLE II

CALIBRATION CURVE OF DISOPYRAMIDE AND MND (LOWER CONCENTRATION RANGE)

Statistics: linear regression lines for disopyramide; $y = (3.964)x + 0.001$; $r^2 = 0.980$; for MND; $y = (3.323)x - 0.025$; $r^2 = 0.986$.

Concentration of disopyramide (μg/ml)	Concentration of MND (μg/ml)	Area ratio* disopyramide/PC-Dis		Area ratio* MND/PC-Dis	
		Mean ± S.D.**	C.V.***	Mean ± S.D.	C.V.
0.054	0.023	0.229 ± 0.013	5.6	0.069 ± 0.007	10.2
0.109	0.046	0.454 ± 0.026	5.7	0.129 ± 0.010	8.1
0.217	0.092	0.877 ± 0.031	3.5	0.269 ± 0.026	9.5
0.326	0.139	1.160 ± 0.093	8.0	0.399 ± 0.033	8.2
0.435	0.185	1.811 ± 0.170	9.4	0.621 ± 0.054	8.7

*Number of samples, $n = 4$ (two injections for each sample).
**Mean ± one standard deviation.
***Coefficient of variation (%).

spiked with varying amounts of disopyramide and MND (Tables I and II). The best fit through the data points was obtained from linear regression analysis (Tables I and II). The coefficient of determination, r^2 was > 0.98 for all regression lines.

Reaction time

The optimum reaction time was evaluated by incubating samples containing equivalent amounts of disopyramide, MND, PC-Dis and TFAA for various times at 55°C. The peak areas of the dehydrated forms of disopyramide, PC-Dis and the TFAA derivative of the dehydrated form of MND were found to not change significantly over an incubation period of 2 h.

Excess TFAA was removed according to the method of Walle and Ehrsson [40], whereby the reaction mixture was evaporated on a water bath at 40°C under a gentle stream of nitrogen.

Extractability

Calibration curves for a serial dilution of the free base forms of disopyramide and MND in toluene were prepared. Following extraction of known quantities (Table III) of disopyramide and MND from human plasma, the recovery in the organic phase (toluene) was determined by using the free base calibration curves. The average percentages of disopyramide and MND extracted by toluene were 96% and 87%, respectively (Table III).

TABLE III

EXTRACTABILITY OF DISOPYRAMIDE AND MONO-N-DEALKYLATED METABOLITE

Disopyramide added (μg/ml)	Disopyramide measured (μg/ml) (mean ± S.D.)	Percent recovery* (mean ± S.D.)	MND added (μg/ml)	MND measured (μg/ml) (mean ± S.D.)	Percent recovery* (mean ± S.D.)
0.50	0.53 ± 0.01	106.1 ± 1.96	0.31	0.32 ± 0.01	104.2 ± 1.90
1.00	0.94 ± 0.02	94.08 ± 1.78	0.61	0.55 ± 0.01	90.3 ± 1.10
1.99	1.88 ± 0.02	94.55 ± 0.92	1.23	1.04 ± 0.02	84.4 ± 1.68
2.99	2.75 ± 0.23	92.08 ± 7.81	1.84	1.44 ± 0.11	78.2 ± 6.50
3.98	3.75 ± 0.19	94.25 ± 4.89	2.45	1.96 ± 0.11	79.9 ± 7.28
4.98	4.75 ± 0.23	95.34 ± 4.65	3.07	2.58 ± 0.17	83.5 ± 5.84

*Number of samples, $n = 3$.

Structural confirmation of compounds

Characterization of the structures of untreated and TFAA-treated compounds (disopyramide, MND and PC-Dis) was obtained using both EI and CI mass spectra. The total ion current mass chromatograms of disopyramide and PC-Dis under both ionization modes yielded single peaks, thus indicating the purity of these compounds. Fig. 3 shows the CI-MS results of TFAA-treated disopyramide and PC-Dis. In Fig. 4 can be seen the EI total ion current chromatogram of the underivatized MND. The MS data (both EI and CI) of untreated MND and its degradation products are shown in Fig. 5. Fig. 6 illustrates the MS data (EI and CI) of TFAA-treated MND (nitrile and amide forms).

DISCUSSION

The application of capillary column GLC in the analysis of biological

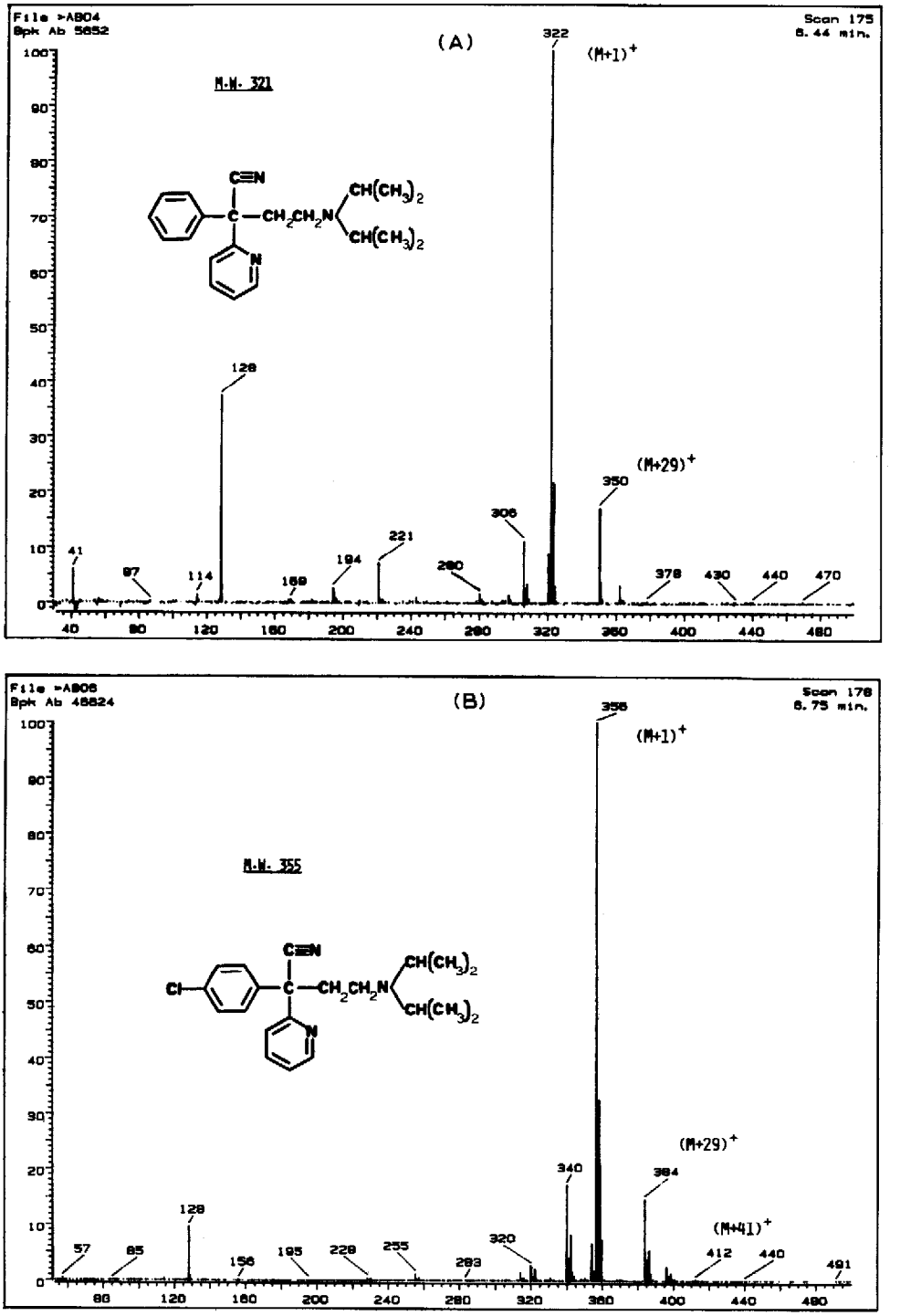


Fig. 3. Capillary GC mass spectral results. (A) CI (positive) mass spectrum of TFAA-treated disopyramide; (B) CI (positive) mass spectrum of TFAA-treated PC-Dis.

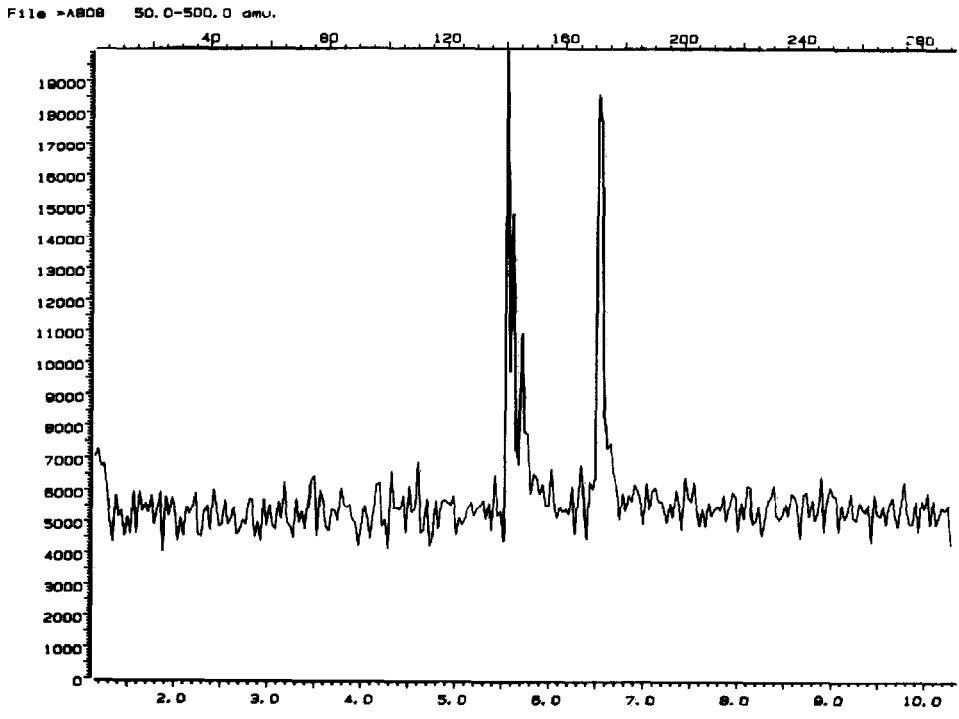
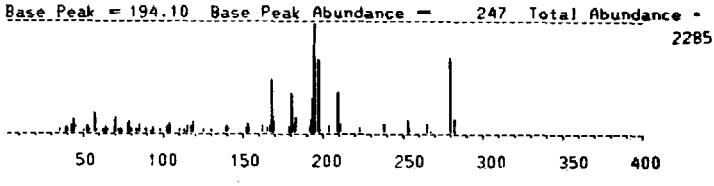
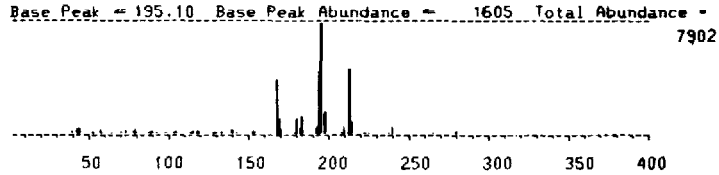


Fig. 4. Total ion current chromatogram of untreated MND.

(A)



(B)



PROMINENT FRAGMENT IONS :

MASS (m/e) :	195	212	194	167	196	182	213	239	43	44	280
ABUNDANCE(%) :	100	57	54	49	18	16	10	6	5	5	3

Fig. 5.

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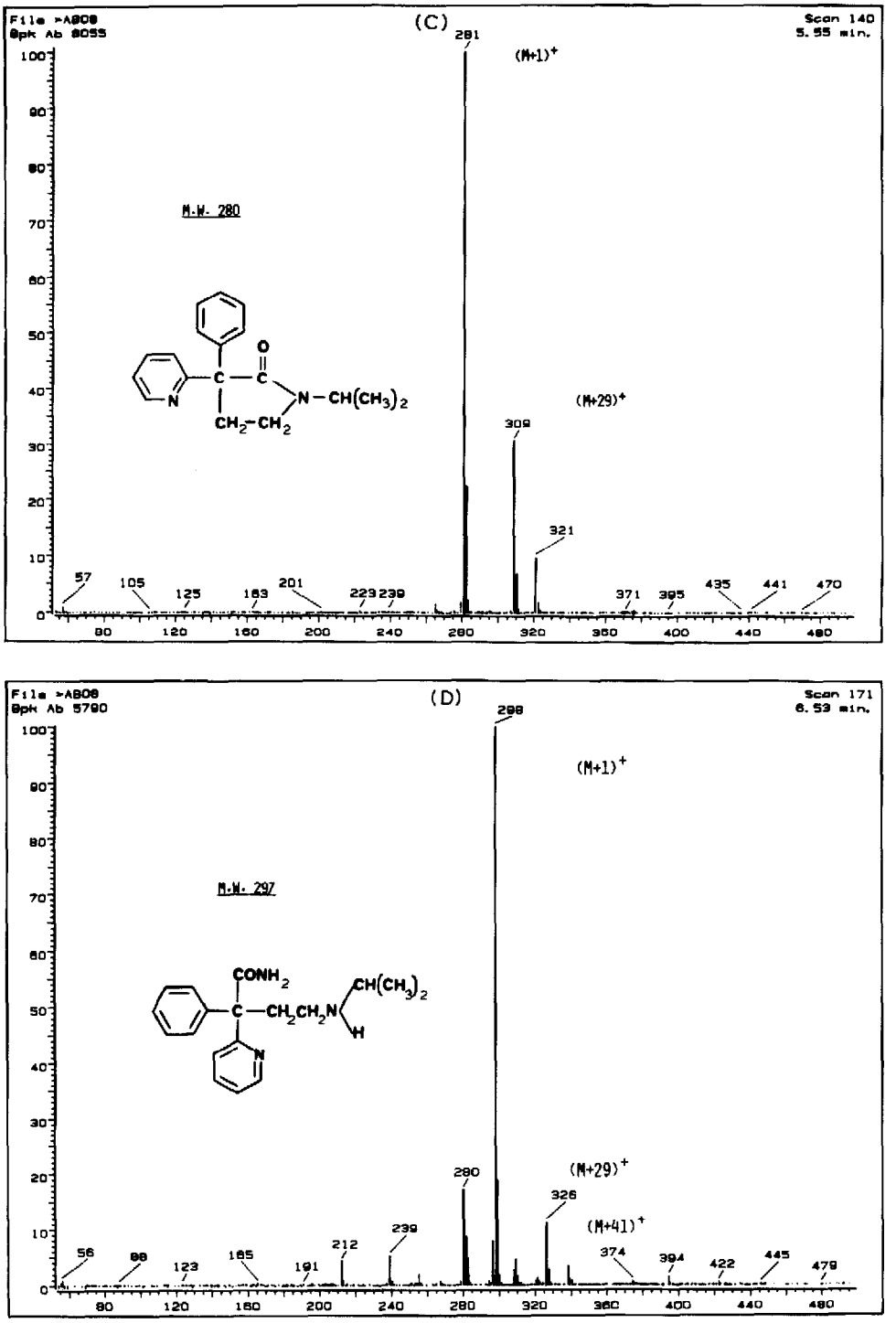
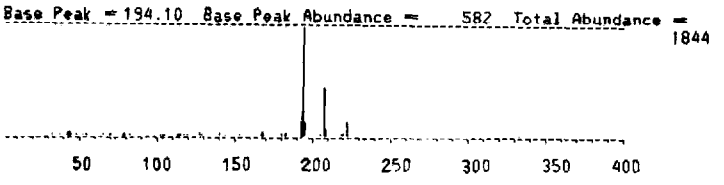


Fig. 5. Capillary GC mass spectral results. (A) EI mass spectrum and prominent fragment ions of degradation product of untreated MND; (B) EI mass spectrum and prominent fragment ions of intact MND; (C) CI mass spectrum of degradation product of untreated MND; (D) CI mass spectrum of intact MND.

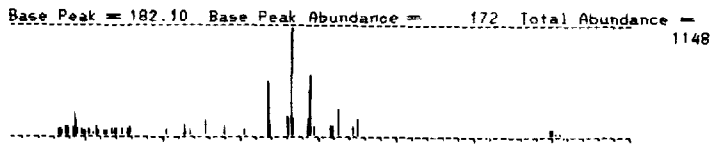
(A)



PROMINENT FRAGMENT IONS :

MASS (m/e) :	194	207	193	221	192	167	43	41	375
ABUNDANCE(%) :	100	45	21	13	13	5	5	4	1.2

(B)



PROMINENT FRAGMENT IONS :

MASS (m/e) :	182	195	167	194	212	43	225	193	44	69
ABUNDANCE(%) :	100	55	51	42	26	20	16	16	14	6

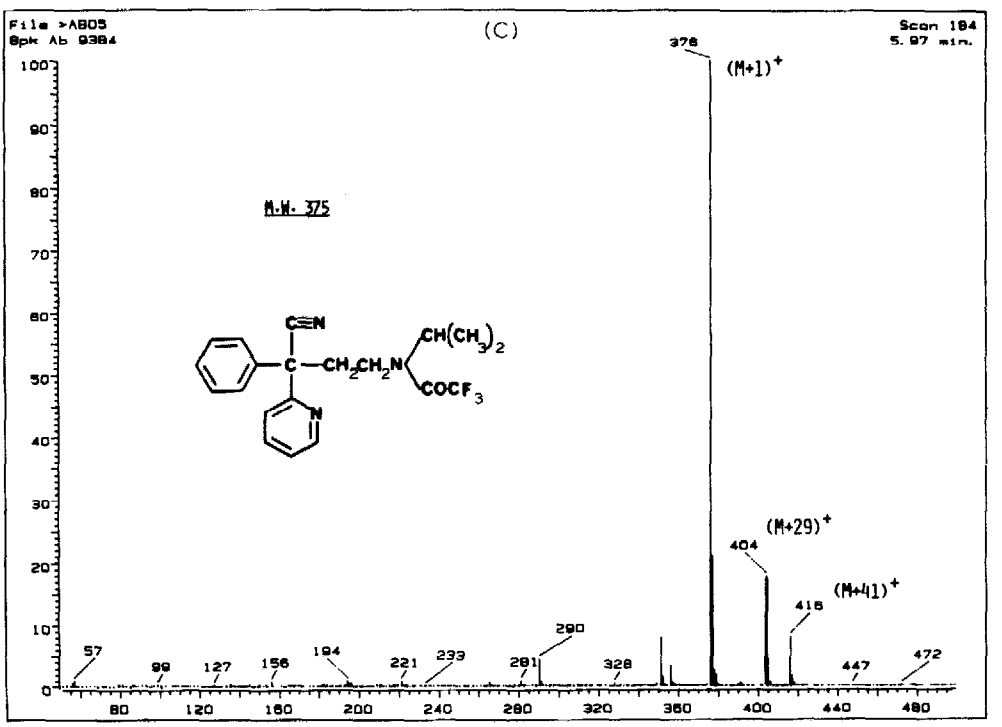


Fig. 6.

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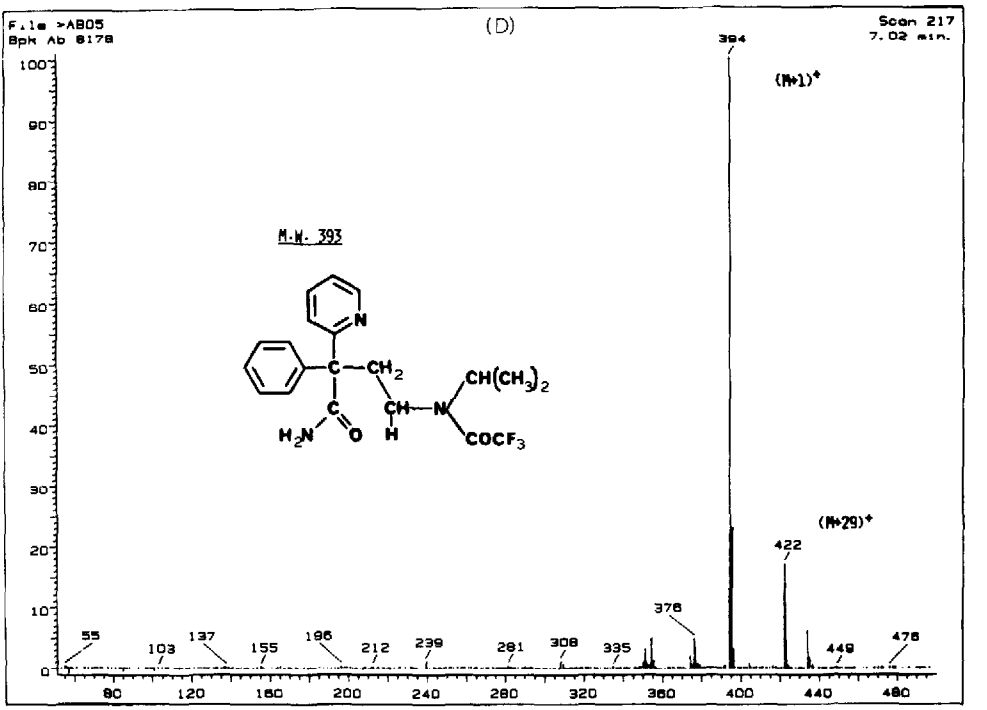

Fig. 6. Capillary GC—MS results. (A) EI mass spectrum and prominent fragment ions of TFAA-treated MND (derivatized nitrile form); (B) EI mass spectrum and prominent fragment ions of TFAA-derivatized MND (amide form); (C) CI mass spectrum of TFAA-treated MND (derivatized nitrile form); (D) CI mass spectrum of TFAA-derivatized MND (amide form).

samples has proliferated in recent literature. This technique has been applied in metabolic profiling of normal and diseased conditions [41—43], the measurement of drug concentration in human urine and plasma [43—45] and in pharmacokinetic drug studies in man [46]. With the advent of inert fused-silica capillary columns in 1979, more reliable and reproducible analysis of individualized drugs (acidic, basic, neutral, polar) became possible [47].

Before a pharmacokinetic study of disopyramide in coronary care patients could be undertaken, an analytical procedure with high selectivity and sensitivity was required to permit measurement of drug and metabolite in buffer (dialysate), plasma and serum.

A packed column GLC—flame ionization detection assay method developed for use in our laboratory using the technique of Hutsell and Stachelski [15] demonstrated potential for interference from other drugs and from endogenous substances in the plasma. Furthermore, the chromatographic peaks noted for disopyramide, MND and *p*-chlorodisopyramide exhibited excessive peak tailing and generally unsatisfactory symmetry using packed-column technology. Since the flame ionization detection measurement technique was generally insufficiently sensitive to permit measurement of free drug concentrations for disopyramide and MND during in vivo protein binding studies (0.10 μg/ml concentrations in 0.40 ml plasma volume), an attempt was made to develop a specific NPD assay method using splitless capillary GC.

A 25-m polysiloxane-deactivated, open-tubular fused-silica column with a crosslinked 5% phenylmethyl silicone phase was used for the development of a selective and sensitive assay for disopyramide and MND.

A complete baseline resolution between disopyramide and MND was obtained as illustrated in Fig. 1B and no interference from endogenous plasma components was observed (Fig. 1A) following a simple and single extraction step. The chromatographic peaks of disopyramide and MND under identical conditions were well separated (resolved) from several antiarrhythmic drugs, viz., tocainide, mono-ethyl glycine xylidide, lidocaine, propranolol and quinidine. The analysis time of the present assay is relatively short and replicate injections of plasma extracts every 15 min are possible without interference from plasma components. A series of samples containing 5 μg/ml of disopyramide, 3 μg/ml of MND and 4 μg/ml of PC-Dis were incubated at 55°C for periods ranging from 0—120 min to determine the optimum time required for reaction of the employed substances with TFAA. No apparent differences were observed in the areas for the three substances over the entire incubation time range; it would appear that the reaction was rapid. A 30-min reaction time was subsequently chosen to ensure complete dehydration of disopyramide, MND and PC-Dis as well as the derivatization reaction for MND. Excess TFAA was used (150 μl) to ensure completeness of the reactions.

Excess TFAA reagent was removed by evaporation rather than by the hydrolysis technique [40]. The hydrolysis of excess derivatizing reagent (heptafluorobutyric anhydride) and subsequent neutralization with an excess of ammonia has been reported to be a better technique [48]. However, the use of a small volume of toluene (100 μl) for reconstitution of dried sample extract did not permit an efficient separation of the organic layer from the aqueous phase during the hydrolysis procedure. Evaporation of the incubation mixture was carried out under a gentle steam of nitrogen, using a water bath at 40°C to prevent evaporation of the volatile TFAA derivatives. The TFAA-treated samples were found to be stable for at least ten days when stored at —4°C with repeat injections showing no significant decline in peak areas over the stored time period.

Toluene was found to be an efficient extraction solvent (Table III) with a recovery of 96.1% and 87.1% for disopyramide and MND respectively, over the entire concentration range of disopyramide (0.50—5 μg/ml) and MND (0.30—3 μg/ml). A single extraction step was sufficient for the quantitation of the compounds of interest without any interference from plasma constituents.

The present analytical technique has been found to show good linearity (Tables I and II) over the entire concentration range of disopyramide (0.05—5 μg/ml) and MND (0.02—3 μg/ml). These samples have been characterized by replicate calibration curves having a coefficient of determination (r^2) of at least 0.98. The reproducibility of the assay method is demonstrated in Tables I and II. The method is highly reliable and reproducible with a coefficient of variation within 10% for both compounds.

Identification of compounds and their derivatives by MS

Hutsell and Stachelski [15] in 1975 and subsequently other workers [21, 22] have reported that the mono-N-dealkylated metabolite of disopyramide

results in three unresolved chromatographic peaks when injected into a gas–liquid chromatograph equipped with a 3% OV-17 packed glass column. Unfortunately, no report has appeared in the literature regarding the identity of these peaks. A similar pattern of at least three peaks was observed when MND was injected into a gas chromatograph—mass spectrometer equipped with a 12-m fused-silica column coated with methyl silicone.

Previous studies have shown that acetylation of the secondary amino group of MND with acetic anhydride results in a single chromatographic peak [15, 21, 22]. However, recent findings in our laboratory have shown two limitations to this approach: (1) acetylation of the secondary amino group with acetic anhydride is not complete under usual conditions and (2) acetylated MND is associated with extensive peak tailing on columns conventionally used in disopyramide analysis, viz., 3% OV-17 and 5% phenylmethyl silicone.

Trifluoroacetic anhydride, a commonly used acylating agent, is more volatile than acetic anhydride. In addition to its acylating properties, TFAA has been reported to dehydrate aliphatic amide groups to form nitrile groups [49]. Gal et al. [23] in 1980 used TFAA as a dehydrating agent to accomplish such a conversion with disopyramide to improve its chromatographic properties. The EI-MS fragmentation of both disopyramide and PC-Dis in the present study followed a pattern similar to that reported by previous workers [23, 37]. The dehydrated derivatives (nitriles) obtained upon TFAA treatment of disopyramide and PC-Dis exhibited shorter retention times than the corresponding untreated amides. The prominent fragment ions for the nitrile derivative of disopyramide were at *m/e* 194, 221, 128, 114, 193, 43, 44, 72, 70, 195 (in order of decreasing intensity). The fragment ions at *m/e* 128,

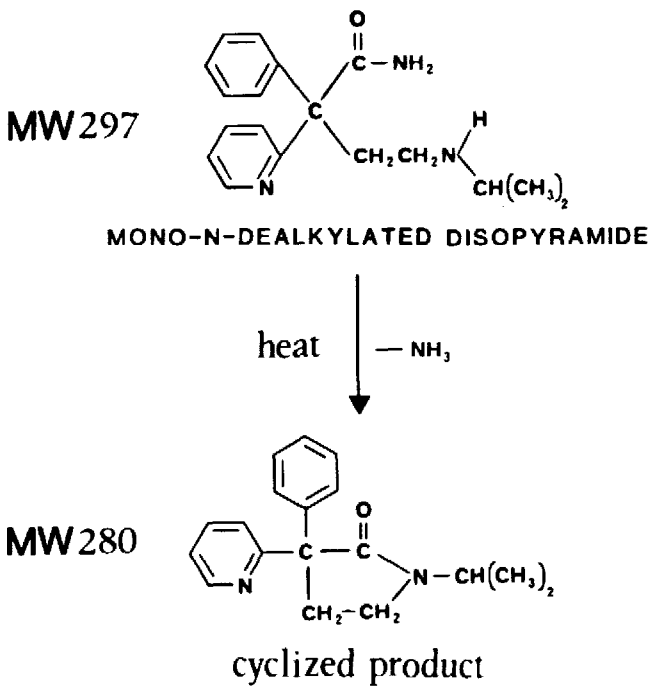

Fig. 7. Proposed degradation reaction of untreated MND at elevated temperature.

114, 72 and 42 were also present in the EI-MS profile of the nitrile derivative of PC-Dis. In addition, the latter contained ion pairs *m/e* 255:257, 228:230 and 229:231 in a 3:1 ratio, thus indirectly supporting the postulated structures reported for fragment ions of the nitrile derivative of disopyramide by Gal et al. [23].

Further evidence for dehydration of disopyramide and PC-Dis was obtained from the presence of pseudomolecular ions $(M+1)^+$ in the CI-MS mode (Fig. 3A and B). In addition to the molecular ions, characteristic ions of $(M+29)^+$ and $(M+41)^+$ were also found when methane was used as an ionizing gas.

Following the injection of untreated MND onto the GC—MS system, the total ion current chromatogram (Fig. 4) showed three unresolved MND degradation products from 5.5 to 5.8 min in addition to the peak for authentic MND at 6.5 min. It is proposed that untreated MND is unstable when exposed to high temperatures encountered in the injection port of the gas chromatograph. A comparison of the EI and CI spectra (Fig. 5A and C) of the three

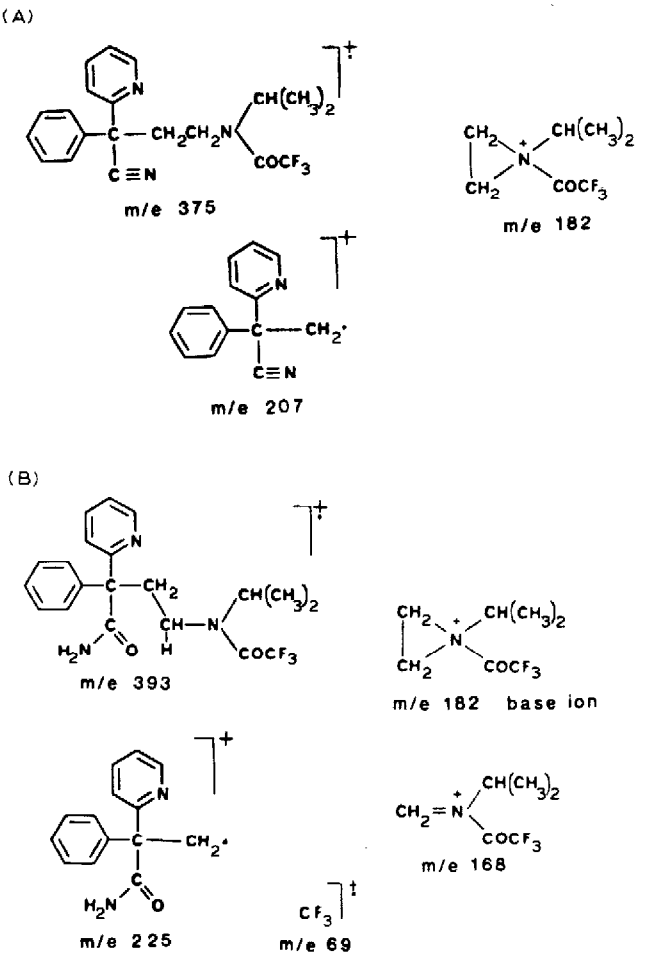

Fig. 8. Proposed prominent fragments based on the EI-MS of TFAA-treated MND. (A) dehydrated (nitrile) and trifluoroacetylated MND; (B) trifluoroacetylated MND (amide form).

peaks from 5.5 to 5.8 min, all show identical spectra. It appears that at least one of the degradation products may be a cyclized compound (Fig. 7) which could arise from MND through a loss of ammonia. This proposed structure is based largely on the presence of a strong molecular ion at *m/e* 280 in the EI spectrum and a corresponding pseudomolecular ion of *m/e* 281 in the CI spectrum. Confirmation of the cyclic structure requires further investigation. The fragment ions of MND in the EI mode (Fig. 5B) were similar to those reported by Haskins et al. [37]. The CI-MS profile (Fig. 5D) showing a pseudomolecular ion at *m/e* 298 confirms the identity of the MND chromatographic peak in Fig. 4.

Preliminary studies in our laboratory showed the presence of two peaks for TFAA (100 μl) treated MND. The EI and CI-MS data (Fig. 6A and C) indicated that the peak having the shortest retention time was the dehydrated form of the MND TFAA derivative whereas the later peak was that of the TFAA derivative of MND with the intact amide group. The EI- and CI-MS data of the amide are shown in Fig. 6B and D. Excess TFAA (150 μl), as used in the present assay, completely dehydrated the amide functional group of MND along with the complete acylation of the secondary amine group of MND. Structures for the characteristic fragment ions in the EI mass spectra of the two peaks are shown in Fig. 8A and B. Other ions present in the derivatized form of MND such as *m/e* 195, 194, 212 and 167 are also common to underivatized disopyramide [23, 37] or underivatized MND [37].

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