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# Direct (Non-Chromatographic) Quantification of Drugs and Their Metabolites from Human Plasma Utilizing Chemical Ionization Mass Spectrometry and Stable Isotope Labeling: Quinidine and Lidocaine†

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**Abstract**— Preliminary results are reported for the quantitative determination of the antiarrhythmic agents quinidine and lidocaine in human plasma by combined isobutane chemical ionization mass spectrometry and stable isotope labeling. The concentration of monoethylglycinexylidide, a known metabolite of lidocaine, was also determined using this method. In the procedure used, stable isotope analogs of the materials to be determined were added to serial plasma samples. The plasma was then made basic and extracted with benzene. The residue after evaporation of the solvent was placed directly into the mass spectrometer via the direct insertion probe and the spectrum determined. In this manner the above named compounds could readily be quantitated without recourse to either derivitization or further purification.

## Introduction

IN THE last ten years mass spectrometry has become one of the key analytical techniques for the qualitative determination of drugs and drug metabolites from biological fluids.[1,2] Often these studies have combined mass spectrometry with gas chromatography (g.c.-m.s.).[3,4] Typically in this situation the mass spectrometer serves merely as a sophisticated detector for the gas chromatograph, and is often used to either monitor a single ion (mass fragmentography) or several ions (multiple ion detection) in the gas chromatograph's effluent.[5,6] While most drugs and drug metabolites can be analyzed by mass spectrometry without recourse to derivitization, derivitization is sometimes mandatory to achieve the volatility necessary for g.c. In addition, a mass spectral analysis requires seconds, whereas a chromatographic analysis often requires many minutes.

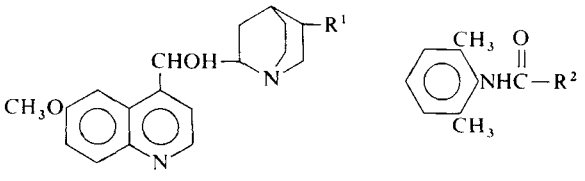
We would like to report preliminary results for the quantitative determination from human plasma of quinidine (**1**), dihydroquinidine (**2**) (hydroquinidine), lidocaine (**3**) and the lidocaine biotransformation products monoethylglycinexylidide (**4**) (MEGX) and glycinexylidide (**5**) (GX) by direct isobutane chemical ionization mass spectral analysis of the benzene extract of basified plasma. This method offers an alternate approach to bioassays currently being carried out by both 'classical' techniques (u.v., fluorescence, chromatography, etc.) and the now popular g.c.-m.s. techniques, especially mass fragmentography and multiple ion detection.[4–9]

Mass spectral drug analysis without prior recourse to either chromatography or derivatization has been previously suggested and demonstrated by Fales and Milne. These workers used the technique to successfully analyze the stomach content from drug overdose patients.[10] In this method the organic phase extract of the biological fluid was evaporated and the residue was placed directly into the mass spectrometer's ion source via the direct insertion probe. A successful analysis relies on both the fact that a drug or drug metabolite is typically present in a much greater concentration in the extract than the endogenous biochemicals, and also on the simple mass spectra obtained using isobutane chemical ionization. In this regard, a g.c.-m.s. system using methane chemical ionization mass spectrometry (c.i.m.s.) for drug analysis has recently been described.[11]

To quantitate the $[MH]^+$ peak height data obtained from the isobutane c.i. mass spectra, stable isotope analogs (**6**, **7**, **8** and **9**) of compounds **2**, **3**, **4** and **5** were synthesized. Compound **6** also served as the internal

† Abbreviations: MEGX = monoethylglycinexylidide; GX = glycinexylidide.

standard for **1**. This was thought to be appropriate since **1** and **2** are known to have similar properties, so much so that separation of one from the other is difficult and they are often found together in commercial pharmaceutical preparations.[12] A dihydro analog of carbamazepine, a drug like quinidine having a reducible isolated double bond, has been successfully used as an internal standard for mass fragmentographic analysis.[13]





**1** $R^1 = CH{=}CH_2$

**2** $R^1 = CH_2{-}CH_3$

**3** $R^2 = CH_2N(CH_2CH_3)_2$

**4** $R^2 = CH_2NHCH_2CH_3$

**5** $R^2 = CH_2NH_2$

**6** $R^1 = CHD{-}CH_2D$

**7** $R^2 = CH_2N(CD_2CH_3)_2$

**8** $R^2 = CH_2NHCH_2CD_3$

**9** $R^2 = CD_2NH_2$

The addition of a known amount of stable isotope analog of the compound to be analyzed to the biological fluid prior to extraction serves to convert the mass spectral peak height information obtained into absolute concentrations. This is accomplished by comparing the $[MH]^+$ ions generated in the mass spectrum from the compound to be quantitated to the corresponding ion given by its stable isotope analog. This same procedure is standard analytical practice in many bioassays, except that the internal standard used is radioactive and the detection is achieved by a scintillation counter. The use of stable isotope analogs in mass spectral assays has recently become quite popular.[4,7]

## Experimental

All melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected.

Mass spectra were obtained on an A.E.I. MS-9 double focusing mass spectrometer modified for chemical ionization. Isobutane c.i. mass spectra were obtained using a specially constructed ion source. The ion exit hole was 0.020 in while the electron entrance hole was 0.013 in. The electron gun voltage was set at 510 V, the ion repeller was variable between 0.0 and 1.0 V while the source and analyzer ion gauges read $1.8 \times 10^{-4}$ Torr and $7.6 \times 10^{-6}$ Torr, respectively. The source ion chamber pressure was 0.5 Torr as read some distance from the ion chamber by a McLeod gauge calibrated against published isobutane ion intensity pressure data. Both sample and reagent gas were introduced via a specially designed direct insertion probe. All mass spectra were run at an accelerating voltage of 8 kV at a mass resolution of $m/\Delta m = 3500$ (5% valley definition).

### SYNTHESIS

*3-(1,2 Dideuterioethyl)ruban-9-ol, dihydroquinidine-$d_2$* (**6**)

500 mg of quinidine sulfate (Merck) dissolved in 100 ml of 95% EtOH was reduced for 15 min with $D_2$ gas in a low pressure Parr hydrogenator in the presence of 50 mg of 5% rhodium on charcoal (K and K Laboratories, Inc.). The residue after filtration and evaporation of solvent was recrystallized twice from water, m.p. 165 to 167 °C, yield 394 mg.

An initial c.i. mass spectral analysis of the quinidine sulfate used in the reduction showed: quinidine 75.8%, dihydroquinidine 24.2%. After reduction a c.i. mass spectral analysis showed: quinidine 1.6% (*m/e* 325), quinidine-$d_1$ 1.8% (*m/e* 326), dihydroquinidine 17.6% (*m/e* 327), dihydroquinidine-$d_1$ 12.6% (*m/e* 328), dihydroquinidine-$d_2$ 28.0% (*m/e* 329), dihydroquinidine-$d_3$ 17.4% (*m/e* 330) and dihydroquinidine-$d_4$ 14.8% (*m/e* 331) dihydroquinidine-$d_5$ 4.7% (*m/e* 332) and dihydroquinidine-$d_6$ 1.4% (*m/e* 333).

A solution was prepared containing 2.10 μg/ml ($H_2O$) of reduced quinidine with the above isotopic distribution to serve as the source of the internal standard used in the assay. For the sake of simplicity this reduced quinidine will be referred to as dihydroquinidine-$d_2$.

*ω-[α,α-$d_4$-Diethylamino]-2,6-dimethylacetanilide, lidocaine-$d_4$* (**7**)

This compound was synthesized by the method of Lofgren[14] using α,α-$d_4$-diethylamine. The α,α-$d_4$-diethylamine was prepared by catalytic hydrogenation with $D_2$ gas of a mixture of acetonitrile and acetic anhydride to yield *N*-ethylacetamide-$d_2$, which was reduced with aluminum deuteride ($AlD_3$) to the $d_4$-amine. Complete details of the synthesis will be published at a later time. Lidocaine-$d_4$ had a m.p. 65 to 67 °C after recrystallization (3 times) from petroleum ether.

A c.i. mass spectral analysis showed the following isotopic abundances: lidocaine-$d_4$ 71.5% (*m/e* 239), lidocaine-$d_3$ 19.0% (*m/e* 238), lidocaine-$d_2$ 4.4% (*m/e* 237), lidocaine-$d_1$ 5.1% (*m/e* 236), lidocaine 0% (*m/e* 235). An e.i. mass spectrum showed essentially the same isotopic distribution after correcting for [M − 1] abundances. In addition there was negligible change in the c.i. abundances from spectrum to spectrum.

*ω-[β-$d_3$-Ethylamino]-2,6-dimethylacetanilide monoethylglycinexylidide-$d_3$* (MEGX-$d_3$) (**8**)

This compound was synthesized in the same manner as lidocaine-$d_4$ using β-$d_3$-ethylamine synthesized according to the procedure of Ross *et al.*[15] MEGX-$d_3$ had a m.p. 49 to 51 °C after recrystallization from hexane.

A c.i. mass spectral analysis showed the following isotopic abundances: MEGX-$d_3$ 66.0% (*m/e* 210),

MEGX-$d_2$ 22.4% (*m/e* 209), MEGX-$d_1$ 8.0% (*m/e* 208), MEGX 3.6% (*m/e* 207).

*α-$d_2$-ω-Amino-2,6-dimethylacetanilide glycinexylidede-$d_2$* (GX-$d_2$) (**9**)

This compound (m.p. 79 to 81 °C) was synthesized by 4 exchanges of the methylene hydrogens of the protio compound in $D_2O$-dioxane mixtures (90 °C) using anhydrous $K_2CO_3$. C.i.m.s. showed the following isotopic abundances: GX-$d_2$ 99.0% (*m/e* 181), GX-$d_1$ 1.0% (*m/e* 180), GX 0% (*m/e* 179).

## ISOLATION AND ASSAY PROCEDURE

### Quinidine

Serial plasma samples were obtained from three patients A, B and C, who had received a 15 mg/kg oral dose of quinidine gluconate (Lilly). Mass spectral analysis of this quinidine preparation showed 92.2% quinidine and 7.8% dihydroquinidine. To 1 ml of each plasma sample in a 20 ml pyrex culture tube fitted with a teflon cap was added 2 ml of the internal standard solution. This was followed by the addition of 1 ml of 0.1N NaOH and 5 ml of benezene. The tubes were then vigorously shaken by hand for 5 min and afterwards centrifuged at 800 × g. The benzene layer was removed and evaporated at 20 °C in a rotary evaporator. The residue was dissolved in 0.05 ml of benzene and the solution placed on the ceramic tip of the direct insertion probe. After solvent evaporation the probe was inserted into the mass spectrometer and the isobutane c.i. mass spectrum was obtained at 160 to 170 °C.

To 1 ml of pooled human serum was added known amounts of quinidine sulfate (Merck) to give concentrations of 0, 1, 3, 5 and 7 μg of quinidine (dihydroquinidine 24.2%, quinidine 75.8%) per ml and also 2 ml of internal standard solution (2.10 μg/ml) which contained 0.588 μg/ml (28%) quinidine-$d_2$. Two more 3 μg/ml standards and one other 0 μg/ml standard were also prepared and all eight samples subjected to the isolation procedure described above. The results of this analysis are given in Table 1.

TABLE 1. Analysis of the eight samples

| Total known concentrations of **1** and **2** in quinidine sulfate (Merck) | Analyzed concentration of both **1** and **2** (% difference) | Analyzed concentration of **1** (% quinidine) | Analyzed concentration of **2** (% dihydro-quinidine) |
|---|---|---|---|
| 0 μg/ml | <.02 | --- | --- |
| 0 μg/ml | <.02 | --- | --- |
| 1 μg/ml | 0.97 | 0.72 | 0.25 |
| | (3%) | (74%) | (26%) |
| 3 μg/ml | 2.98 | 2.17 | 0.81 |
| | (0.6%) | (73%) | (27%) |
| 3 μg/ml | 2.86 | 2.11 | 0.77 |
| | (4.7%) | (73%) | (27%) |
| 3 μg/ml | 2.83 | 2.09 | 0.74 |
| | (5.7%) | (74%) | (26%) |
| 5 μg/ml | 4.97 | 3.62 | 1.35 |
| | (0.6%) | (73%) | (27%) |
| 7 μg/ml | 6.73 | 4.91 | 1.82 |
| | (4%) | (73%) | (27%) |

### Lidocaine

Two healthy male volunteers (D, E) received a 250 mg oral dose of lidocaine hydrochloride monohydrate (xylocaine); a third (F) received 202 mg of lidocaine as the free base. Immediately prior to dosing, a 15 ml blood sample was drawn into a heparinized tube. Successive blood samples were taken 30, 60, 90 and 180 min following administration of the drug. Plasma was immediately separated by centrifugation at 800 × g for 15 min. To 2 ml samples of each plasma fraction was added 2 ml × 1.01 μg/ml of lidocaine-$d_4$, 1 ml × 1.00 μg/ml of MEGX-$d_3$ and 0.5 ml × 1.0 μg/ml of GX-$d_2$. To the 5.5 ml of solution was added 0.5 ml 5N NaOH and the basified mixture extracted with 10 ml benzene in 30 ml pyrex culture tubes fitted with teflon caps. The sample was then centrifuged at 800 × g for 5 min, the benzene layer removed and rotary evaporated to dryness at 20 °C. The residue was dissolved in 0.05 ml of ethanol and placed on the ceramic tip of the direct insertion probe. The probe was inserted into the mass spectrometer and the c.i. mass spectrum was determined at 120 °C.

Previous work has shown that extraction and mass spectral response to a compound and a deuterated analog of that compound are equivalent.[8,9] To test this under the c.i. conditions employed, lidocaine and lidocaine-$d_4$ (1 μg/ml), and MEGX and MEGX-$d_2$ (0.5 μg/ml) were added to 2 ml blank plasma and extracted as described. The c.i. mass spectral analysis of the nondeuterated standards based on the deuterated internal standards, showed good agreement between known and calculated concentrations, the largest variance being 2.2% for lidocaine vs lidocaine-$d_4$.

## CALCULATIONS

Peak heights were measured using a caliper. Standard procedure was followed in subtracting isotope contributions from the preceding two peaks.[16] The contributions of undeuterated or unreacted ionic species present in the reference solution (internal standard) were then subtracted. A comparison of the now corrected peak heights of labeled and unlabeled compound was made and the concentration of drug or metabolite initially present in the plasma was determined.

## MASS SPECTRA

The methane c.i. mass spectrum of quinidine has been published previously.[17] The isobutane c.i. mass spectrum is similar except that *m/e* 136 is insignificant (<1%). The $[MH]^+$ ion at *m/e* 325 of quinidine and its $^{13}C$ satellite represent greater than 98% of the total ion current (t.i.c.). The dihydroquinidine isobutane mass spectrum is identical except, of course, that $[MH]^+$ is at *m/e* 327. For lidocaine $[MH]^+$ = 84.8% t.i.c., *m/e* 164 is 15.2% t.i.c. The percentages for MEGX and GX

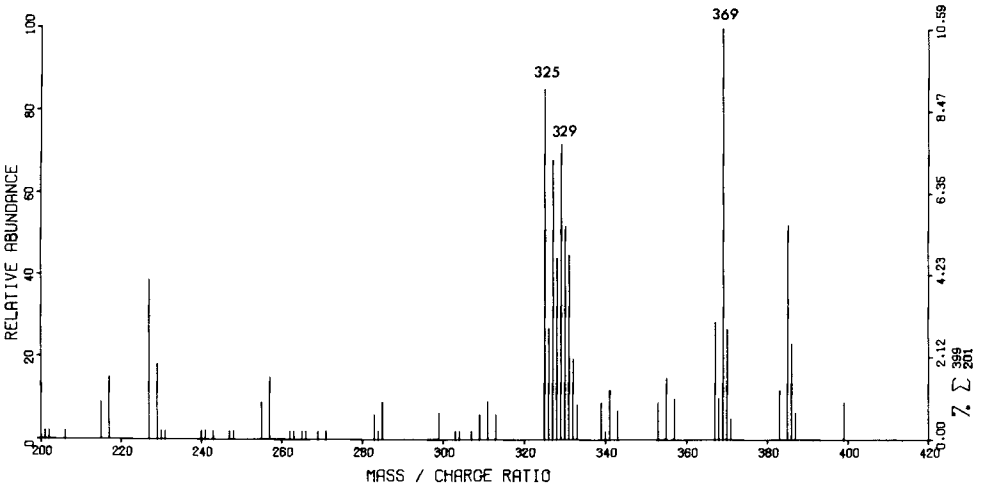



FIG. 1. Lowest galvanometer trace of isobutane c.i. mass spectrum of subject A's 180 min plasma sample (benzene extract) after administration of 15 mg/kg quinidine gluconate (Lilly). Ion at *m/e* 325 represents quinidine present in plasma, *m/e* 329 represents 1.17 μg of dihydroquinidine-$d_2$ internal standard, *m/e* 327 is dihydroquinidine present in both administered quinidine (7.8%) and the internal standard (17.6%). Base peak of the spectrum is at *m/e* 369 and presumably arises from loss of water from the $[MH]^+$ ion of cholesterol.

are 96.5% t.i.c. for $[MH]^+$, 3.5% for *m/e* 164 and 96.8% t.i.c. for $[MH]^+$, 3.2 t.i.c. for *m/e* 164, respectively. The nature of the ion at *m/e* 164 in the lidocaine, MEGX, and GX c.i. mass spectra is currently under investigation. The presence of the ion is a function solely of the geometry of the ion source. If the 0.020 in ion exit hole is replaced by a 0.125 in × 0.001 in ion exit slit this peak vanishes.

## Results

### Quinidine

Up to the time of this communication there was no specific analytical method for the determination of the extensively used orally active antiarrhythmic drug quinidine. There are presently two commonly used methods for the quantification of this drug from plasma.[18,19] Both assays utilize a nonspecific fluorescence technique. Since these assays are nonspecific in their measurement, both methods may include in their measurement inactive as well as active products of quinidine metabolism. To further complicate matters, the administered drug may itself be a mixture of both quinidine and dihydroquinidine depending on the commercial source of the drug.[12]

The practical implications of these analytical considerations are obvious. It is difficult to define an effective range of plasma concentrations for quinidine unless the method of determination is specified. Thus, Resnekou *et al.*[20] reported that the effective therapeutic range of plasma levels is 1 to 4 μg/ml utilizing the method of Cramer and Isaakson[19] and 3 to 7 μg/ml for the method of Hamfeldt and Malers.[18] Thus, it would appear that depending on the degree of specificity, a given method would yield a correspondingly different range of effective levels. Indeed, even an estimation of quinidine half-life is dependent on the species measured by a particular assay, since it cannot be assumed that the drug and its metabolites have identical pharmacokinetic properties.

An actual mass spectrum for subject A for the 180 min plasma sample can be seen in Fig. 1. At this concentration (1.47 μg/ml) the series of peaks due to quinidine either as administered drug or internal standard is prominent, overshadowed in the mass spectrum only by the ion at *m/e* 369 (cholesterol, $[MH^+ - H_2O]$). The unique isotopic distribution for the dihydroquinidine-$d_2$ internal standard combined with the peaks from the analyzed quinidine and dihydroquinidine serves as a flag to immediately mark the desired region in the mass spectrum, so as to facilitate counting of the spectrum, although this flag does make the calculations more tedious.

The plasma levels for three patients A, B, C as determined by c.i.m.s. are plotted in Fig. 2. Such a limited

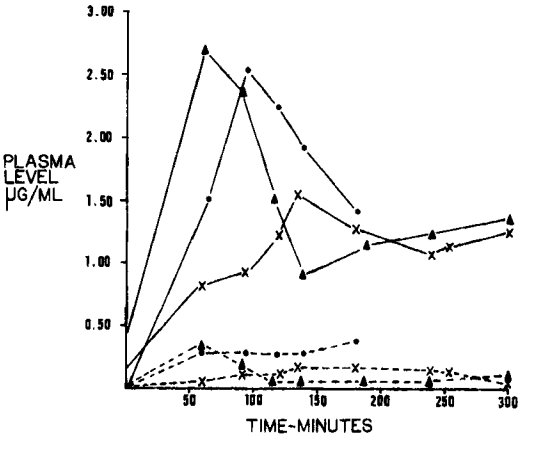



FIG. 2. Patient plasma levels of quinidine and dihydroquinidine after drug administration. Two of the patients show residual quinidine levels from previous administration of the drug. ▲, Patient A; ●, patient B; ×, patient C; broken lines, dihydroquinidine; full lines, quinidine.

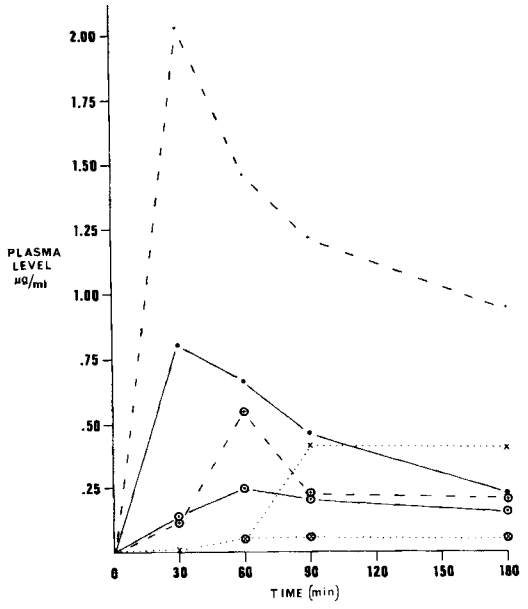



FIG. 3. Plasma levels of lidocaine and MEGX in two male human subjects, (D, E) after oral administration of 250 mg of lidocaine hydrochloride monohydrate and subject F after oral administration of 202 mg of lidocaine as the free base. + – – – + Subject D, lidocaine; ⊕ – – – ⊕ subject D, MEGX. • —• subject E, lidocaine; ⊙ — ⊙ subject E, MEGX. × ···· × subject F, lidocaine; ⊗ ···· ⊗ subject F, MEGX.

sampling of subjects precludes any pharmacological interpretation. However, as expected, the plasma levels of quinidine obtained by this method were consistently lower than those obtained from a fluorescence assay performed on the same sample.†

Lidocaine

Lidocaine is an agent widely used in the treatment of ventricular arrhythmias. Recently, Strong and Atkinson,[21] have used g.c.-mass fragmentography to detect lidocaine (**3**) and two of its de-ethylated metabolites, MEGX (**4**) and GX (**5**) in the plasma of patients receiving intravenous infusions of the drug.

Using deuterated internal standards and the c.i. technique described in the Experimental section, we have been able to directly detect and quantitate lidocaine and MEGX in the plasma of two male volunteers (D, E) after a single oral dose of 250 mg lidocaine hydrochloride monohydrate, and of one male volunteer (F) after a single oral dose of 202 mg of lidocaine as the free base. The range of detection was from 5 ng to 4 μg for lidocaine and 0.1 μg to 1 μg for MEGX, illustrating the great dynamic range of the direct c.i.m.s. technique. Although the GX-$d_2$ (**9**) internal standard was readily seen in all spectra ($[MH]^+$ ($d_2$) = 181) at the 0.25 μg level, no corresponding peaks above background could be found at 179, the $[MH]^+$ for GX (**5**).

Figure 3 shows the results of this preliminary study. Although subjects D and E are of similar weight and physical make-up, there was a great difference in peak plasma levels of lidocaine and MEGX. From the work of Boyes *et al.*[22] and Adjepon-Yamoah and Prescott,[23] subject D has higher plasma levels than are normally found after oral administration. An added 'subjective' note is that D was the only volunteer to feel 'lightheaded' during the first 60 min of the experiment.

The peak plasma levels and time course of both lidocaine and MEGX in subject E are very similar to those encountered following 500 mg and 400 mg oral doses of lidocaine hydrochloride monohydrate. Note that subject F, who received a molar equivalent of lidocaine as the free base, showed a lag period in reaching substantial concentrations of the drug, probably due to slower absorption of the free base. Unfortunately, the peak plasma concentration probably occurred between the 90 and 180 min sampling period.

As with the previously mentioned quinidine determinations, no statistical information can be derived from this small sampling.

The normalized c.i. mass spectrum of the 60 min plasma sample of subject E can be seen in Fig. 4.

## Discussion

We feel that the method described above could have clinical significance in the analysis of patients receiving not only quinidine and lidocaine, but other drugs as well. An academic drawback of this technique is its inability to provide information necessary to differentiate structural isomers of a metabolic product. This, however, may not be significant in clinical analysis if one is interested only in absolute amounts of monohydroxylated, dihydroxylated, reduced, unchanged drug, etc. An advantage is its potential speed and simplicity. Even for assays carried out with the presently used cumbersome direct insertion probe and allowing for sample application, solvent evaporation etc., a turnaround time per sample of under 5 min is easily met. A properly designed direct insertion probe could considerably shorten this time. The minimum time possible for analysis will certainly be shorter than any analysis utilizing chromatography and certainly equal to that necessary for a fluorescence or u.v. analysis. This method also has the crucial advantage of specificity over any spectrophotometric techniques, since it is the drug itself which is measured. The advantage of speed and simplicity is further accentuated since more than one drug or drug metabolite can be determined in the same analysis. In a future publication we will report the simultaneous determination of lidocaine and six of its metabolites from the urine of subjects D, E, F whose plasma data are reported in this communication.

Also of significance is that theoretically most clinically useful drugs can be analyzed in this fashion. Moreover, many drugs which cannot be analyzed by g.c. even with extensive derivitization can be analyzed by this method, e.g. we know of no g.c. assay for quinidine.

† Levy, R. H. Personal communication.

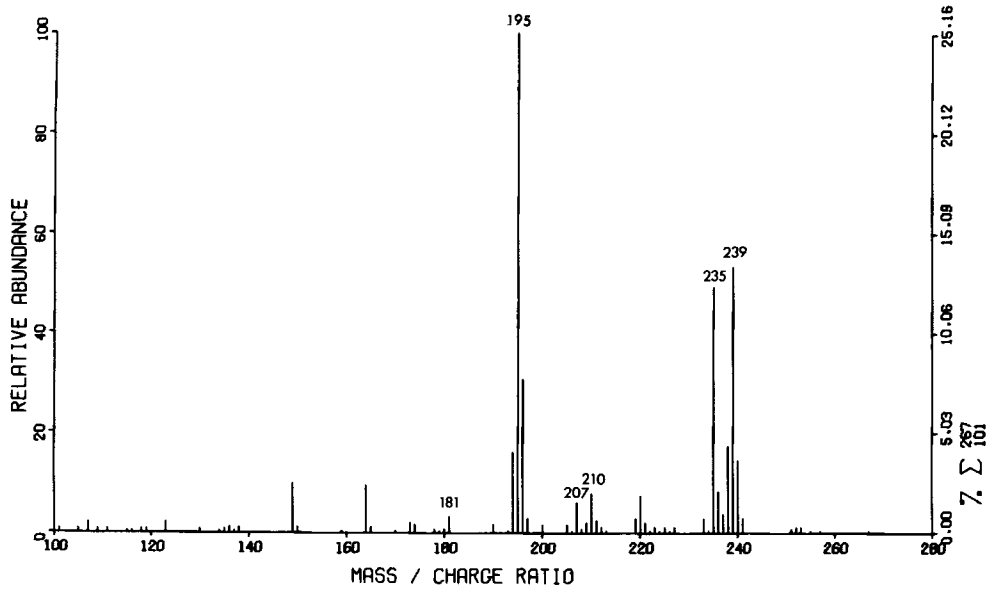



FIG. 4. Galvanometer ( × 3) trace of isobutane c.i. mass spectrum of subject E 60 min plasma sample (benzene extract) after oral administration of 250 mg lidocaine hydrochloride monohydrate. Ion at *m/e* 239 represents lidocaine-$d_4$ internal standard, *m/e* 235 represents lidocaine in plasma, *m/e* 210 represents MEGX-$d_3$ internal standard, *m/e* 207 represents MEGX in plasma. The large *m/e* 195 represents caffeine and was the largest ion found in the zero-time blood sample as well.

General application of this technique sometimes requires the use of computer analysis at a condition of moderate mass resolution. This approach is under active investigation in this laboratory and is necessary in two situations: when the drug or drug metabolite to be analyzed has an $[MH]^+$ *m/e* value equal to that of a normally occurring chemical present in large amounts in the extract of the biological fluid e.g. cholesterol, caffeine, cotinine, fatty acids, etc., or when the drug or drug metabolite is present in such a small quantity that its $[MH]^+$ ion is not considerably larger than background ions.

## ACKNOWLEDGMENTS

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