Table I—Lidocaine GLC Assay Precision with Column Oven Temperature Programming

Actual Plasma Lidocaine Concentration, μg/ml	Measured Lidocaine Concentration, μg/ml
4.00	4.01 ± 0.13 (21)[a]
1.62	1.61 ± 0.04 (15)
0.16	0.17 ± 0.01 (15)
0.08	0.08 ± 0.01 (18)
0.04	0.04 ± 0.002 (14)

[a] Mean ± *SE* (*n*).

temperature programming modification, plasma lidocaine concentrations as low as 30 ng/ml can be measured. The retention times for lidocaine and mepivacaine (internal standard) are 6.0 and 7.1 min, respectively.

Calibration Curve—Calibration curves were constructed with the ratio of lidocaine–mepivacaine peak heights plotted along the ordinate and the lidocaine concentration plotted along the abscissa. Initially, curves were developed by spiking blank plasma with 0.03, 0.08, 0.16, 0.40, 0.80, 1.60, 4.0, and 8.0 μg of lidocaine/ml and analyzing the samples as described. Calibration data were fit to a single straight line using a least-squares regression[6] with $r = 0.994$. Despite the apparent fit, the calculated regression line did not coincide adequately with the observed peak height ratios for low lidocaine concentrations. Instead, the calibration curves were described best by two straight lines: one for lidocaine concentrations above 0.8 μg/ml and another for concentrations below 0.8 μg/ml.

The calculated correlation coefficients and equations were $r = 0.994$, ratio = 2.121 [lidocaine concentration (μg/ml)] − 1.04 for the upper range, and $r = 0.999$, ratio = 1.149 [lidocaine concentration (μg/ml)] + 0.05 for the lower range. Curves prepared from aqueous standards (same procedure but without the first two centrifugations) were the same as those using plasma standards; therefore, aqueous standards were used for daily calibration.

Precision—Assay precision was evaluated by analyzing multiple plasma samples spiked with 0.04, 0.08, 0.16, 1.62, or 4.00 μg of lidocaine/ml (Table I). Over this concentration range, assay precision was quite satisfactory.

Interference—Neither the monoethylglycinexylidide or glycinexylidide lidocaine metabolites nor any of the tested drugs produced interfering peaks or alterations in the lidocaine–mepivacaine (internal standard) peak height ratios with this method. Meperidine (1 μg/ml) generated a sharp peak with a peak height almost 75% as large as the internal standard, but the meperidine peak (retention time of 5.1 min) eluted before the lidocaine and mepivacaine peaks and did not interfere with the lidocaine assay.

Conclusions—The GLC lidocaine assay reported by Kline and Martin (10) is a simple and rapid method; however, the sensitivity is not adequate for many pharmacokinetic studies. Incorporating column oven temperature programming improved the assay sensitivity so that plasma lidocaine concentrations as low as 40 ng/ml could be measured readily. The modification retains the simplicity, speed, and sample capacity of the original method.

[6] TI Program ST 1-08, Texas Instruments Inc., Dallas, Tex.

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COMMUNICATIONS

Specific Radioimmunoassay for Flunitrazepam

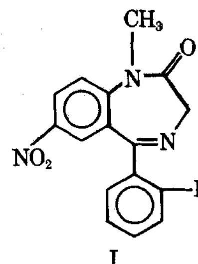
Keyphrases □ Flunitrazepam—quantitation by radioimmunoassay, compared with electron-capture GLC, human plasma □ Radioimmunoassay—quantitation of flunitrazepam, compared with electron-capture GLC, human plasma □ Hypnotic agents—flunitrazepam, quantitation by radioimmunoassay, human plasma

To the Editor:

Flunitrazepam[1] [5-(2-fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2*H*-1,4-benzodiazepin-2-one, I], a benzodiazepine derivative, is clinically effective as a hypnotic when administered orally in the 0.5–2-mg dose range and may be administered parenterally, usually in combination with other drugs, for the induction of anesthesia (1, 2). Flunitrazepam has been marketed for several years in Europe and is presently under clinical evaluation in the United States.

In humans, I is completely metabolized; apart from intact I, the major metabolites found in plasma are the *N*-desmethyl and 7-amino derivatives (3). However, intact I is primarily responsible for the hypnotic activity of the drug (3) and has a half-life of 20–36 hr on chronic administration (4, 5).

Electron-capture GLC has been the method of choice



I

[1] Rohypnol, Hoffmann–La Roche.

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for the determination of the low nanogram concentrations of I in plasma following administration of therapeutic doses to humans (6, 7). However, none of the reported procedures can approach the simplicity, sensitivity, and speed of radioimmunoassay. We now report a specific and sensitive radioimmunoassay for I that permits its quantitation directly in plasma or blood.

To obtain antibodies to I, an immunogen first was prepared by covalently coupling 3-hemisuccinyloxyflunitrazepam to bovine serum albumin using the mixed anhydride procedure of Erlanger *et al.* (8). The resulting conjugate consisted of 18 moles of the hapten coupled to 1 mole of albumin. Rabbits were immunized intradermally, and the antiserum with the highest titer (1:3000 dilution) of antibodies to I was used.

The radioligand used for the assay was [*methyl*-^{3}H]-flunitrazepam[2] with a specific activity of 87.5 Ci/mmole. Prior to use, radiolabeled I was purified by TLC on silica gel with chloroform–acetone (4:1) as the solvent system.

The radioimmunoassay was carried out in a manner identical to that described recently for diazepam (9). A logit–log calibration curve for I was linear from 15 to 1000 pg/tube; thus, a working limit of sensitivity of 0.15 ng/ml was obtained using 0.1 ml of plasma. This value represents about a fivefold increase in sensitivity compared to electron-capture GLC techniques. However, for routine analysis of plasma and blood from subjects receiving chronic therapeutic doses of I, such sensitivity is unnecessary and a 10-μl sample is more appropriate for analysis. The intra- and interassay coefficients of variation did not exceed 10% over a range of 1.65–10 ng of I/ml in a selection of random clinical samples.

The antiserum specificity was determined initially by its cross-reactivity with the known metabolites of I present in blood and with the benzodiazepine drugs, diazepam, *N*-desmethyldiazepam, flurazepam, and nitrazepam. For each compound tested, cross-reactivity was $<1\%$ relative to I (100%); this finding demonstrated that the 1-methyl, 7-nitro, and 2′-fluoro groups on the hapten were potent antigenic determinants and indicated that the antiserum was highly specific for I.

Further evidence for the specificity of the radioimmunoassay procedure was obtained by comparison with an electron-capture GLC method (7). The joint determinations for I in 20 plasma and 12 whole blood samples from subjects who received a 2-mg dose of the drug were subjected to linear regression analysis by a method that takes into account differences in the precision of both analytical procedures (10). The correlation coefficient, regression line slope, and y-intercept were 0.98, 0.93, and 0.13, respectively, over a range of 0.5–18 ng/ml. Furthermore, the two groups of data were not significantly different ($p > 0.05$) using a t test. These statistical parameters indicate that the radioimmunoassay and electron-capture GLC methods yield equivalent results.

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Calcium Binding by Arteriographic Contrast Media

Keyphrases □ Arteriographic contrast media—calcium binding, effect of additives in media on calcium binding *in vitro* □ Calcium—binding in plasma by arteriographic contrast media, effect of additives in media □ Diatrizoate meglumine sodium—arteriographic contrast medium, effect on calcium binding □ Binding—calcium to ionic arteriographic contrast media, effect of additives

To the Editor:

Selective coronary arteriography with common ionic contrast media is associated with a decrease of myocardial force, which was suggested to be due to decreased ionized calcium levels in the blood perfusing the myocardium (1, 2). This depressant action has been attributed exclusively to the potent calcium-binding ligands added to contrast media for anticoagulation, stabilization, and buffering (1–3). The calcium-binding properties of contrast media have not yet been reported.

To investigate the basis for the reduction of ionized calcium levels, fixed quantities of diluted contrast media were titrated with increasing amounts of calcium chloride *in vitro*. The following contrast media were evaluated: ioxaglate meglumine sodium[1] (59% sodium and methylglucamine *N*-(2-hydroxyethyl)-2,4,6-triiodo-5-{2-[2,4,6-triiodo - 3 - (*N* - methylacetamido) - 5 - (methylcarbonyl)-benzamido]acetamido} isophthalamic acid, I), lysine diatrizoate[2] [80% L-lysine 3,5-bis(acetamido)-2,4,6-triiodobenzoate, II], diatrizoate meglumine sodium[3] [76% sodium and methylglucamine 3,5-bis(acetamido)-2,4,6-triiodobenzoate, III], and diatrizoate meglumine sodium[4] with 0.32% sodium citrate and 0.04% edetate disodium [(ethylenedinitrilo)tetraacetic acid disodium salt] (IV).

[1] Hexabrix, Byk Gulden, Konstanz, West Germany.
[2] Peritrast-400, Dr. Franz Köhler Chemie, Alsbach, Bergstrasse, West Germany.
[3] Urografin-76, Schering AG, Berlin/Bergkamen, West Germany.
[4] Renografin-76, Squibb, Princeton, N.J.

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