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Short Communication

Determination of plasma propofol levels using gas chromatography–mass spectrometry with selected-ion monitoring

P. L. Stetson*

Department of Pharmacology, Upjohn Center for Clinical Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109 (USA)

E. F. Domino

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109 (USA)

J. R. Sneyd

Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI 48109 (USA)

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ABSTRACT

Propofol (2,6-diisopropylphenol, I.C.I. 35 868) is a rapid-acting, intravenous anesthetic agent recently introduced for the induction and maintenance of general anesthesia. This paper describes a gas chromatographic–mass spectrometric procedure using selected-ion monitoring for the determination of plasma propofol levels. The drug and the internal standard (thymol) were extracted from plasma into diethyl ether–pentane, and derivatized to their trimethylsilyl derivatives before analysis. The reproducibility of the daily standard curves had coefficients of variation ranging from 2.7% to 10.2%. The precision of the assay yielded a coefficient of variation ranging from 4.5% to 5.6%, and the concentration means for the seeded control samples were found to be within −1.6% to +0.6% of the theoretical values for propofol. No interfering peaks have been observed in application of this procedure to either normal volunteer or patient samples. The minimum detectable level under the conditions described was 0.20 ng propofol/ml plasma. This assay and a high-performance liquid chromatographic assay with fluorescence detection were both used to measure plasma propofol concentrations in 89 human plasma samples, and the correlation between the two methods was excellent.

INTRODUCTION

At present, propofol (2,6-diisopropylphenol) is a very popular intravenous (i.v.) agent for inducing anesthesia. Several quantitative high-performance liquid chromatographic (HPLC) methods using fluorescence [1–4], ultraviolet absorption [5–7], and electrochemical [8,9] detection have been described. These HPLC methods have many advantages, including rapidity, low cost,

* Corresponding author.

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and wide availability of HPLC equipment. Their detection limits are in the moderate to high ng/ml plasma levels.

The detailed pharmacokinetic and clinical studies of propofol administration would be facilitated by the availability of a more specific chemical assay with greater sensitivity (sub-ng/ml detection limits). This paper describes such a method, in which the trimethylsilyl (TMS) derivatives of propofol and the internal standard (thymol, I.S.) are separated by capillary column gas chromatography (GC), and detected using mass spectrometry (MS) with selected-ion monitoring (SIM) of the compound-specific electron-impact (EI) ionization products.

EXPERIMENTAL

Reagents

Propofol and diethyl ether (absolute, ACS) were purchased from Aldrich (Milwaukee, WI, USA). Thymol (5-methyl-2-isopropylphenol) and tetramethylammonium hydroxide pentahydrate were obtained from Sigma (St. Louis, MO, USA). Ammonium phosphate, monobasic (Baker "analyzed" reagent) was purchased from J. T. Baker (Phillipsburg, NJ, USA) and BSTFA [N,O-bis(trimethylsilyltrifluoroacetamide)] was purchased from Pierce (Rockford, IL, USA). The organic solvents and their commercial sources were: acetonitrile and isopropyl alcohol (HPLC grade) from EM Science (Gibbstown, NJ, USA); pentane from Burdick & Jackson Labs (Muskegon, MI, USA); and cyclohexane from Fisher (Far Lawn, NJ, USA).

Stock solutions

Both the propofol and the thymol stock solutions were prepared at 1.0 mg/ml in acetonitrile and stored at −30°C. Phosphate buffer (0.10 *M* ammonium dihydrogenphosphate, no pH adjustment) and the tetramethylammonium hydroxide (TMAH) solution (100 m*M* in isopropyl alcohol) were prepared and stored at 4°C. The diethyl ether–pentane (2:1, v/v) extraction mixture was prepared just prior to assay.

Standard solutions

Appropriate dilutions with acetonitrile of the propofol stock solution yielded propofol standard solutions 1 (200 μg/ml), 2 (20.0 μg/ml), 3 (2.0 μg/ml), and 4 (0.20 μg/ml). The thymol standard solution (20.0 μg/ml) was prepared by diluting the thymol stock solution with acetonitrile. All standard solutions were stored at −30°C until use.

Quality-control (QC) samples at two concentrations (10.0 and 1000 ng propofol/ml plasma) were prepared by adding 10.0 μl of propofol standard solution 2 or 20.0 μl of propofol stock solution to 20.0 ml of blank plasma, respectively. The seeded plasmas were mixed, and 1.0 ml aliquots were dispensed in 15-ml glass, screw-top tubes and kept frozen at −30°C until use.

GC–MS-SIM parameters

The instrument used in these studies was a Hewlett Packard Model 5987A gas chromatograph–mass spectrometer with EI ionization and SIM. Temperature zones were set as follows: ion source temperature, 200°C; analyzer temperature, 250°C; and transfer line temperature, 275°C. The electron energy was set at 70 eV, the emission current was set at 300 μA, and the electron multiplier voltage was 2300 V. The mass ions specific for the I.S., thymol-TMS (207.2 and 222.2 *m/z*), were monitored from 3.20 to 3.80 min, and those specific for propofol-TMS (235.2 and 250.2 *m/z*) were monitored from 3.80 to 4.50 min post injection.

Separation of the TMS derivatives of propofol and the I.S. was accomplished using a Hewlett Packard Ultra Performance fused-silica capillary column (25 m × 0.32 mm I.D.) coated with a crosslinked methyl silicone liquid phase (0.17 mm film thickness), GC conditions were: injection port temperature, 250°C; interface oven and GC–MS interface probe temperatures, 275°C; the GC column oven temperature was programmed from 80°C (0.10 min hold) at 30°C/min to 225°C (0.10 min hold) whereupon the sample run was completed. The carrier gas (helium) flow-rate was set at 1.0 ml/min. The total run time was 6.63 min.

Under these conditions, the retention times of

the IS-TMS and propofol-TMS derivatives were 3.55 and 4.15 min, respectively.

Sample preparation

To 1.0 ml of plasma were added 1.0 ml of phosphate buffer and 20.0 μl (400 ng) of thymol standard solution. The samples were vortex-mixed, and 3.0 ml of diethyl ether–pentane (2:1, v/v) was added. The tightly capped tubes were shaken vigorously for 10 min and then centrifuged at 2000 *g* for 10 min at 20°C. The ether–pentane phase (2.5 ml) was then transferred to conical glass, screw-capped reaction vials (3.0 ml) containing 20.0 μl of TMAH solution, and blown to dryness with a stream of nitrogen at room temperature. BSTFA (50–100 μl) was then added to each vial, and the vials were tightly capped, vortex-mixed, and incubated at 80°C for 15 min. After cooling to room temperature, the derivatized sample from each vial was transferred to autosampler vials for injection (1.0–2.0 μl) and GC–MS-SIM analysis.

Standard calibration curve

Blank plasma samples (1.0 ml) were spiked with the appropriate volumes (5.0 or 15.0 μl) of the propofol standard solution 1, 2, 3, or 4, resulting in calibration samples containing 1.0, 3.0, 10.0, 30.0, 100, 300, 1000, and 3000 ng propofol/ml plasma. Control samples containing no added propofol were also prepared. These calibration samples were then subjected to the sample preparation procedure described above. For statistical evaluation and validation of the procedure, the calibration samples were prepared in triplicate during each of three consecutive days. However, when assaying the propofol content in experimental (unknown) samples, each sample set was accompanied by a calibration curve run in duplicate.

Calculations

Calibration curves were constructed by plotting the ion abundance peak-height ratios (propofol/IS) as a function of the plasma propofol concentration. These data were then fitted to the ln-quadratic equation: $\ln(y) = b_0 + b_1 \ln(x) + b_2 [\ln(x)]^2$ with a least squares regression analysis. The propofol concentrations of unknown samples were calculated using the results of the regression analyses.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention times for the IS-TMS IS and propofol-TMS derivatives were 3.55 and 4.15 min, respectively. The EI mass spectra and molecular structures of IS-TMS and propofol-TMS are shown in Figs. 1a and b, respectively. The characteristic EI mass fragmentation pattern of TMS derivatives was observed, with mass ions of major abundance corresponding to the trimethylsilyl ion ($[TMS]^+$) at m/z 73, the molecular ion ($[M]^+$) at m/z 222 and 250 (for thymol-TMS and propofol-TMS, respectively), and, in each case, the mass ion of maximum abundance representing the loss of a methyl group ($[M - CH_3]^+$) at m/z = 207 and 235 (for thymol-TMS and propofol-TMS, respectively).

From the EI mass spectra shown in Fig. 1, it was apparent that, in order to maximize assay sensitivity and specificity for plasma propofol determination, one should selectively monitor during a retention time (t_R) window specific to each derivative, the mass ions corresponding to the molecular ion ($[M]^+$), and loss of a methyl group ($[M - CH_3]^+$). Since the retention times for thymol-TMS and propofol-TMS were 3.55 and 4.15 min, respectively, the thymol-TMS specific mass ions at m/z = 207.2 and 222.2 were exclusively monitored from 3.2 to 3.8 min, and the propofol-TMS specific mass ions at m/z 235.2 and 250.2 were monitored from 3.8 to 4.5 min.

Representative total ion chromatograms of propofol-seeded human blank plasma samples containing 0, 0.15, 1.00 and 400 ng propofol/ml plasma are shown in Figs. 2a–d. The inserts show the extracted ion chromatograms from t_R = 4.00 to 4.40 min, thus isolating the propofol-TMS peak in a chromatographic plot whose *y*-axis (ion abundance) scale is determined by the propofol-TMS peak alone. If the minimum detectable level is defined as three times the background signal noise, then from the insert of Fig. 2a, and as dem-

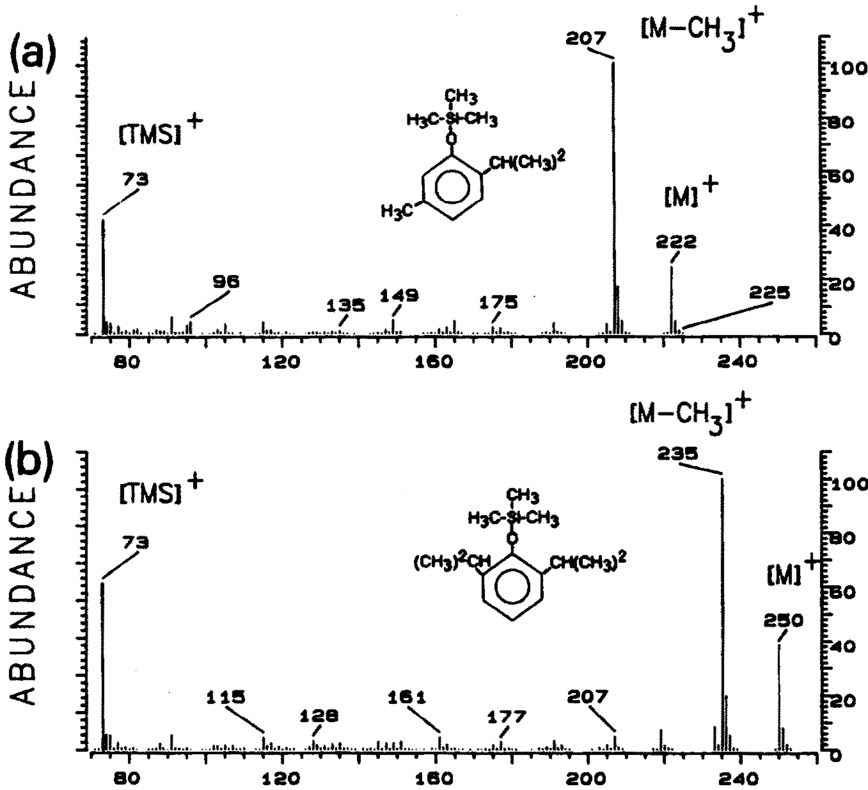

Fig. 1. Molecular structures and EI mass spectra of the trimethylsilyl derivatives of (a) the internal standard, thymol, and (b) propofol.

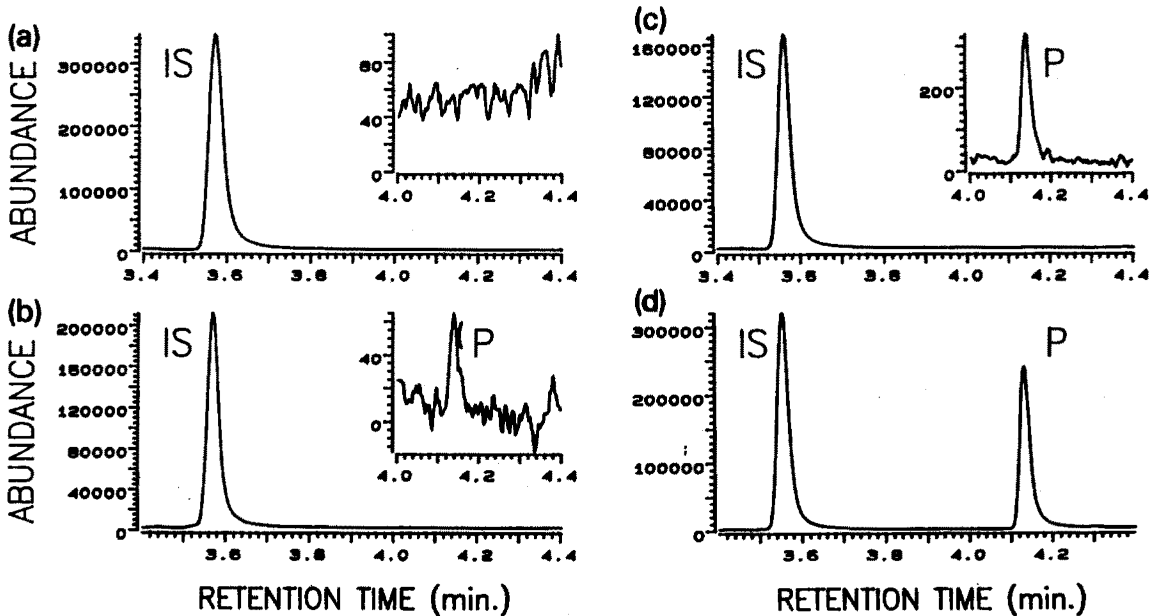

Fig. 2. Representative total ion chromatograms of propofol-seeded human blank plasma samples containing (a) 0.0, (b) 0.15, (c) 1.00, and (d) 400.0 ng propofol/ml plasma. The insets show the extracted ion chromatograms from 4.00 to 4.40 min, thus isolating the propofol-TMS region of the chromatogram on an expanded scale.

onstrated in Fig. 2b, one can estimate that under these conditions the minimum detectable level would be *ca.* 0.20 ng propofol/ml plasma. Thus, the assay procedure described here represents a ten-fold improvement in propofol assay sensitivity over previously published HPLC methods using fluorescence detection [2]. It should be noted, however, that the HPLC–fluorescence procedures for plasma propofol determination achieve their maximum sensitivity with injection of 50% of the total extracted propofol sample, whereas this method calls for injection of less than 5% of the extracted, derivatized propofol sample. Therefore, it would be safe to assume that additional increases in assay sensitivity can be achieved with only minor modifications of this GC–MS-SIM procedure.

Throughout our clinical studies, no interfering peaks were present in the hundreds of patient plasma samples assayed. The total analysis time required for each run was 6.63 min, and this laboratory has routinely prepared 50–100 samples (for overnight automated GC–MS-SIM analysis) during normal 8-h working day.

The validity of the assay procedure was established through a careful study of assay reproducibility, accuracy and precision. Triplicate sets of the calibration standards were assayed on each of three consecutive days. These peak-height ratio *vs.* concentration data were then fitted to the ln-quadratic equation (see Calculations, above) with a least-squares regression analysis. Table I gives the raw peak-height ratio data for the propofol analyses. Table II gives the back-calculated concentration values for these standards. As is evident from these data, the reproducibility of the daily standard curves had coefficients of variation (C.V.) that ranged between 2.67% (at 3000 ng/ml) and 10.23% (at 100 ng/ml). The results of the least-squares regression analyses are also summarized in Table II.

The accuracy and precision of the method were assessed by seeding QC samples at drug concentrations of 10 and 1000 ng propofol/ml. Tripli-

TABLE I

MEASURED PEAK-HEIGHT RATIOS FROM TRIPLICATE ANALYSES OF PROPOFOL CALIBRATION STANDARDS AND SEEDED CONTROL SAMPLES OVER A THREE-DAY PERIOD

Day	Peak-height ratio									
	Plasma propofol concentration (ng/ml)								Quality control plasma propofol conc. (ng/ml)	
	1.00	3.00	10.0	30.0	100	300	1000	2000	10.0	1000
1	0.002 33	0.007 07	0.020 66	0.060 30	0.163 89	0.528 21	1.681 69	5.519 59	0.019 32	1.700 85
	0.002 42	0.007 10	0.019 36	0.059 35	0.149 09	0.562 96	1.989 04	5.498 28	0.020 86	1.955 80
	0.002 21	0.006 86	0.021 98	0.057 80	0.193 77	0.567 09	2.150 84	5.616 25	0.021 71	1.884 92
2	0.002 13	0.007 00	0.020 87	0.058 10	0.213 64	0.592 79	2.134 05	6.403 51	0.021 48	1.885 14
	0.002 29	0.006 65	0.020 66	0.058 06	0.224 10	0.625 00	2.007 11	6.276 24	0.019 39	1.948 72
	0.002 04	0.006 54	0.021 64	0.057 32	0.186 40	0.560 19	1.860 80	6.315 79	0.020 58	2.056 66
3	0.002 35	0.006 89	0.023 07	0.056 36	0.201 63	0.612 44	1.925 41	5.889 47	0.020 43	1.842 59
	0.002 34	0.006 70	0.021 17	0.055 85	0.202 76	0.611 41	1.822 22	5.468 75	0.020 86	1.955 80
	0.002 19	0.006 28	0.020 89	0.053 97	0.189 03	0.584 94	1.744 29	5.485 56	0.021 71	1.723 68
Mean	0.002 26	0.006 79	0.021 14	0.057 46	0.191 59	0.582 78	1.923 94	5.830 38	0.020 70	1.883 80
S.D.	0.000 12	0.000 27	0.002 03	0.001 89	0.023 40	0.031 05	0.162 82	0.398 25	0.000 89	0.114 71
C.V. (%)	5.39	3.98	4.86	3.29	12.21	5.33	8.46	6.83	4.32	6.09

TABLE II

BACK-CALCULATED CONCENTRATIONS FOR PROPOFOL STANDARDS AND RESULTS OF LEAST-SQUARES REGRESSION ANALYSES FOR BEST FIT TO FUNCTION $Ln(y) = b_0 + b_1 Ln(x) + b_2 [Ln(x)]^2$

Theor. conc. (ng/ml)	Day 1		Day 2		Day 3		Back-calc. conc. (ng/ml)		
	Back-calc. conc. (ng/ml)	% of theor. conc.	Back-calc. conc. (ng/ml)	% of theor. conc.	Back-calc. conc. (ng/ml)	% of theor. conc.	Mean	S.D.	C.V. (%)
1.0	0.960	95.99	0.956	95.57	1.020	102.04			
	1.000	100.00	1.031	103.10	1.016	101.63	0.972	0.047	4.84
	0.906	90.57	0.912	01.17	0.946	94.63			
3.0	3.227	107.57	3.287	109.56	3.158	105.28			
	3.238	107.93	3.119	103.95	3.066	102.21	3.128	0.125	4.00
	3.124	104.14	3.063	102.11	2.865	95.50			
10.0	10.178	101.78	10.097	100.97	11.117	111.17			
	9.499	94.99	9.996	99.96	10.167	101.67	10.27	0.487	4.74
	10.871	108.71	10.482	104.82	10.030	100.30			
30.0	31.398	104.66	28.652	95.51	28.003	93.34			
	30.879	102.93	28.630	95.43	27.744	92.48	28.93	1.5265	5.28
	30.041	100.14	28.262	94.21	26.778	89.26			
100.0	88.225	88.23	106.55	106.55	103.683	103.68			
	80.065	80.07	111.78	111.78	104.277	104.28	98.81	10.11	10.23
	104.716	104.72	92.91	92.91	97.058	97.06			
300.0	289.406	96.47	295.383	98.46	321.647	107.22			
	308.551	102.85	311.346	103.78	321.100	107.03	304.9	14.26	4.68
	310.823	103.61	279.211	93.07	306.984	102.33			
1000	917.782	91.78	1049.79	104.98	1024.81	102.48			
	1083.05	108.31	988.245	98.82	969.446	96.95	1005.3	85.50	8.51
	1169.75	116.98	917.197	91.72	927.639	92.76			
3000	2937.84	97.93	3084.07	102.80	3150.32	105.01			
	2926.84	97.56	3024.19	100.81	2925.01	97.50	3001.4	80.16	2.67
	2987.74	99.59	3042.80	101.43	2934.02	97.80			
Mean % Theor. =		100.31		100.15		100.15			
S.D. =		7.807		5.591		5.451			
C.V. (%) =		7.8		5.6		5.4			
Coeff. b_0 =		−6.0235		−6.1086		−6.0728			
Coeff. b_1 =		0.9059		0.9589		0.9490			
Coeff. b_2 =		0.00781		0.00405		0.00311			
Correl. coeff. (r) =		0.9996		0.9998		0.9998			

cate QC samples were assayed on each of three consecutive days. Table III gives the results of this experiment. The precision of the assay was found to have C.V. ranging between 4.52% and 5.54%. The concentration means for the seeded control samples were found to be within +0.51% and −1.56% of the theoretical values.

The propofol levels in a total of 89 human (normal volunteer) plasma samples were assayed by both the GC–MS method described here and

TABLE III

PROPOFOL CONCENTRATIONS IN SEEDED CONTROL SAMPLES ASSAYED OVER A THREE DAY PERIOD

Day	Propofol concentration (ng/ml)	
	10.0	1000
1	9.478	928.11
	10.284	1065.2
	10.726	1027.2
2	10.400	929.02
	9.366	959.90
	9.957	1012.3
3	9.801	980.37
	10.014	1041.1
	10.435	916.59
Mean	10.051	984.42
S.D.	0.454	54.547
C.V. (%)	4.517	5.541
Difference from theory (%)	+0.51	−1.56

the HPLC–fluorescence method as described by Plummer [2]. The correlation between the two sets of data, over the observed range of plasma propofol concentrations from 0–2.00 μg/ml, was excellent. These data are graphically depicted in Fig. 3. Also shown in Fig. 3 are the derived linear regression parameters. The inset in Fig. 3 depicts an expanded-scale figure for the low-concentration data (0–0.250 μg/ml). The derived linear regression parameters for these low-concentration data again show excellent correlation between the methods but, in this case, a slightly greater slope (1.0553 *vs.* 0.9733), probably resulting from the GC–MS method's greater sensitivity and superior performance in the low-concentration range.

The reproducibility, accuracy and precision of the GC–MS-SIM method described here compare favorably with the HPLC–fluorescence methods previously published [1–4]. In addition, this method is no less rapid or simple to perform.

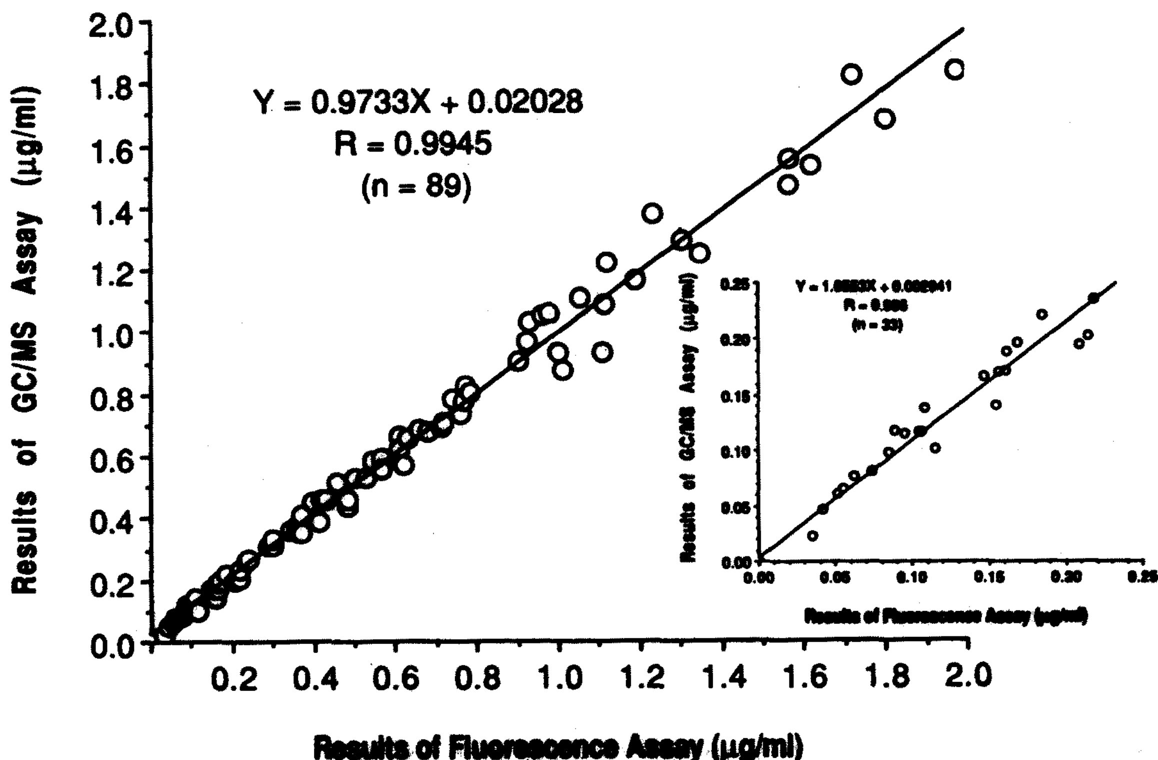

Fig. 3. Correlation between HPLC–fluorescence and GC–MS methods, 0.0–2.00 μg propofol/ml plasma (n = 89). The inset depicts the expanded-scale plot of the low-concentration range data. The derived regression parameters are also listed.

However, the increased specificity, selectivity, and sensitivity afforded by selectively monitoring compound-specific mass ions with the mass spectrometer results in considerable advantages over all the HPLC methods presently in the literature [1–9].

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