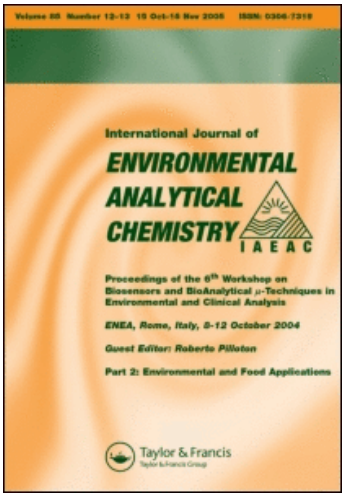

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QUANTITATIVE DETERMINATION OF OCTAMETHYLCYCLOTETRASILOXANE (D_4) IN EXTRACTS OF BIOLOGICAL MATRICES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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A method was developed and validated to measure octamethylcyclotetrasiloxane (D_4)† quantitatively by gas chromatography-mass spectrometry (GC-MS) at low level in extracts of several biological matrices that include plasma, liver, lung, feces and fat from rats. The key to the successful determination lay in the use of extracts dried with anhydrous magnesium sulfate. This was necessary in view of the propensity of the methyl siloxane based GC-stationary phase to generate D_4 by its reaction with water present in the extracts. To enable quantitation of D_4 at parts per billion (μg/L) levels, the base ion m/z 281 resulting from the loss of a methyl group from the parent molecule was selected for monitoring by SIM mode in GC-MS. The recovery of D_4 from any of the biological matrices was determined to be greater than 90% in three extractions. The D_4 response for the standards in GC-MS was linear ($R^2 > 0.9900$) and reproducible at concentrations ranging from 1–16,000 ng D_4/g solvent. Precision was less than 5%.

Keywords: Octamethylcyclotetrasiloxane; gas chromatography-mass spectrometry; quantitation; D_4; biological matrices

INTRODUCTION

Among the volatile silicones, octamethylcyclotetrasiloxane holds significant commercial importance. It is a colorless and odorless liquid of very limited aqueous solubility[1]. The majority of the D_4 produced is used as a site limited inter-

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† The abbreviation D_4 used for octamethylcyclotetrasiloxane is based on the General Electric's siloxane notation [D.T. Hurd, *J. Am. Chem. Soc.*, **68**, 364, 1946] according to which D= -$Me_2SiO_{2/2}$;

mediate for the production of polymeric dimethylsiloxanes. A significant portion of the remainder is used in personal care product formulations[2–4]. Besides personal care product applications, D_4 is also used in precision cleaning and as lubricants and penetrating oils. Several of the applications of D_4 stem from a variety of favorable physical characteristics such as adequate evaporation rate, low surface tension, lack of odor, and a high degree of compatibility with many formulation ingredients.

D_4 has been the subject of numerous toxicology studies. As part of the pharmacokinetic investigations of D_4, it was of importance to develop analytical methodology to quantitate D_4 following its extraction into an organic solvent from a variety of biological matrices. There are ample published articles dealing with the analysis of volatile silicones such as D_4. The samples in those investigations have originated from a variety of sources: air samples from clean room facilities[5]; air samples from indoors of homes and commercial buildings as well as outdoor environment[6–8]; sludge from biological waste water treatment process[9]; habitable environment of space shuttles[10]; biological tissues[11]; extracts of crimp vial caps[12]; bio-compatible medium containing siloxanes released from implants[13]. In general, the published works have neglected to raise an awareness of the potential for artifacts in siloxane analysis that could lead to systematic errors in analytical determinations. The present study describes in detail both the quantitation and recovery aspects of D_4 from a variety of biological matrices while giving due considerations to critical issues concerning the analysis.

EXPERIMENTAL

Materials

D_4 used in this work was a commercial Dow Corning® 244 fluid of purity of ~ 99% (by GC-MS). The reagent grade tetrahydrofuran (THF) used for extraction of D_4, and the anhydrous magnesium sulfate used for drying extracts were both purchased from Aldrich. The internal standard, tetrakis(trimethylsiloxy)silane (M_4Q) was purchased from Gelest and further distilled to obtain a material of purity ~99% (by GC-MS). AldricAlkamuls®EL-620/L used in the emulsion preparation was purchased from Rhone-Poulenc. Samples or reagents were mixed with either a VWR multitube vortex mixer (from Scientific Industries Inc., NY) or a horizontal platform shaker (from Eberbach Corporation, MI).

Exposure of animals to D_4

Animals administered D_4 in this study were male Fischer-344 rats, approximately 14 weeks of age at the time of dosing. The animals were purchased from Charles River Laboratories, Raleigh, NC. There were two groups of rats. Group 1 was comprised of three rats from which control tissues were collected. Group 2 was comprised of four rats. Each rat in group 2 was administered an intravenous dose of 80 mg/kg D_4 as an emulsion by tail vein injection. The intravenous dose was chosen because it provides rapid disposition of D_4 within the rat. For each dose, an aliquot (300–600 μL) of D_4 emulsion was drawn into a pre-weighed syringe. The syringe was re-weighed to determine the weight of the administered dose. The D_4 emulsion was prepared in the following manner: the formulation contained ethanol, Alkamuls EL-620/L and saline (0.9% w/v) in the proportion 1:1:7 by volume, respectively. The required amount of D_4 was dissolved in a known amount of ethanol. Alkamuls EL-620/L was added and the solution vortex mixed. Saline was added and the mixture vortex mixed to form an emulsion.

Two of the four rats in group 2 were sacrificed at 1hr post-dose and the remaining two were sacrificed at 24 hr post-dose. At each time point, the rats were anesthetized with methoxyflurane and euthanized by cardiac puncture. Blood was collected via the vena cava. For plasma, whole blood was transferred to a test tube containing heparin, centrifuged and the plasma that separated was removed.

Fat and liver samples were also collected from each animal. Fat was used as such for extraction. Liver was homogenized in saline using a Brinkman PT 10/35 Homogenizer. At each time point, feces was collected from below the cages of the sacrificed rats. Feces was also homogenized in saline as described above. Tissues and homogenates were stored in a freezer at −80 °C.

Calibration standards

Standards of D_4 in THF containing M_4Q as the internal standard (ISTD) were prepared by making appropriate dilutions of a known mass of $D_4$with THF containing ISTD. The standards were prepared at three concentration ranges: 1–20, 20–1000, and 1000–16000 ng/ml. An aliquot (250–1000μL) of each standard was placed in a 3ml conical vial and about 250 mg of anhydrous magnesium sulfate was added. The contents of the vial were vortex-mixed for about 15 seconds and allowed to stand at least four hours. After four hours or more, the vial was centrifuged for at least 20 minutes at approximately 2000 rpm. An aliquot of each dried standard was then placed in an autosampler vial insert and analyzed by GC-MS for D_4.

Extraction of biological matrices

The biological matrices collected during the in-life phase of the study and kept frozen at –80 °C were thawed on ice and extracted using THF following a published procedure[14] with slight modifications in that that the extraction solvent THF was fortified with M_4Q as an internal standard in an amount to achieve a known final concentration of approximately 320 µg/L. The THF extraction procedure is briefly summarized as follows: Aliquots of samples thawed on ice, ranging from 50–250 mg, were weighed into glass vials of 4 ml capacity and reagent grade tetrahydrofuran containing M_4Q (500–1000 µL) was added. The vials were tightly capped and the contents were first vortex-mixed at high speed settings for 4 min and then centrifuged at ~ 3000 rpm for 4 min. The clear top layer of THF was carefully removed by means of Pasteur pipettes and transferred into clean 7 ml capacity vials. Fresh THF containing M_4Q was added to the sample residues and the extraction procedure repeated as described. Three extractions were performed for each sample, and the extracts were combined. Separately, single extractions were also performed on aliquots to determine extraction efficiency in single vs triple extractions.

The combined extracts were weighed and then dried with anhydrous $MgSO_4$ as described above. Aliquots of the dried extracts were stored at –80 °C, if needed, until analysis. Extractions were performed on duplicate samples of each matrix.

Extractions of biological matrices collected from rats that were not exposed to D_4 were performed in an identical manner, and these served as control samples. If background levels of D_4 were found in control samples, the extracts were re-dried and re-analyzed by GC-MS. If re-drying three or more times did not lead to a blank control, the result found for the control sample was reported as detectable background of D_4.

GC-MS analysis

GC-MS was performed using a Hewlett Packard 5890 Series II or 6890 Gas Chromatograph, coupled with either an HP 5970, HP5972 or HP 5973 Mass Selective Detector. The GC-MS systems were also equipped with HP 7673 GC/SFC or 6890 Series injectors as well as electronic pressure control units. Both injection ports were equipped with Merlin Microseal Septum devices. Data analyses were performed using a Windows-based Chem Station. GC-MS conditions were as follows: The stationary phase of the GC glass capillary column is a cross-linked 5% phenyl methylsilicone of dimensions 30 m × 0.25 mm × 0.25 µm film thickness obtained from Hewlett Packard (HP-5MS). The GC oven was held for 2 min at an initial temperature of 70 °C and then heated at a

rate of 20°C/min to a temperature of 190°C. The heating was continued at a rate of 50 °C/min to a final temperature of 230 °C/min where it was held for a min. The total run time was 8.8 min. Helium was used as the carrier gas. The injection port and the detector temperatures were 250 and 280 °C, respectively. Two μL aliquots of samples were injected in a splitless mode. A single ion of m/z 281 was monitored for both $D_4$and M_4Q by SIM mode. The retention times for $D_4$and M_4Q under these experimental conditions centered around 4.37 and 6.08 min, respectively.

RESULTS AND DISCUSSION

It has been well established that octamethylcyclotetrasiloxane (D_4) can be extracted very efficiently (>90%) from a variety of biological matrices that include blood, plasma, lung, liver, feces, fat and urine[14] using tetrahydrofuran (THF) as the solvent. Since ^{14}C-labeled D_4 was used in the published procedure, recovery/quantitation was readily determined by radioactivity measurement using a liquid scintillation counter. Using a high pressure liquid chromatography system equipped with a radioisotope detector it was also demonstrated that the extraction process did not alter the structural integrity of D_4. However, D_4 can readily undergo equilibration reactions in presence of acids or bases to generate various cyclic and polymeric siloxanes[15]. Enzymatic interaction of D_4 is also quite well established[16]. Due to the potential for such chemical transformation, direct quantitation by radioactivity measurement cannot be relied upon to understand the kinetic behavior of parent D_4 in a test system. Hence developing an analytical method to quantify parent D_4 became essential. Since GC-MS systems are known to be quite sensitive and selective when used in selected ion monitoring mode and its utility to measure D_4 concentrations at nano gram levels has already been demonstrated[17], such a methodology was sought to address the current need.

As mentioned in the section under Methods, a single ion mass (*m/z* 281) was monitored for the analyte (D_4) as well as the internal standard (ISTD), tetrakis(trimethylsiloxy)silane (M_4Q). The selected ion chromatogram of a solvent (THF) blank, a standard in THF containing D_4 and ISTD, and for illustration, that of a THF extract of plasma from an animal dosed with D_4, are shown in Figure 1.

To demonstrate the validity of the method to quantitate D_4 in THF extracts, the following parameters were assessed: system suitability; specificity; linearity; carry-over; intra-assay precision and accuracy; inter-assay precision and accu-

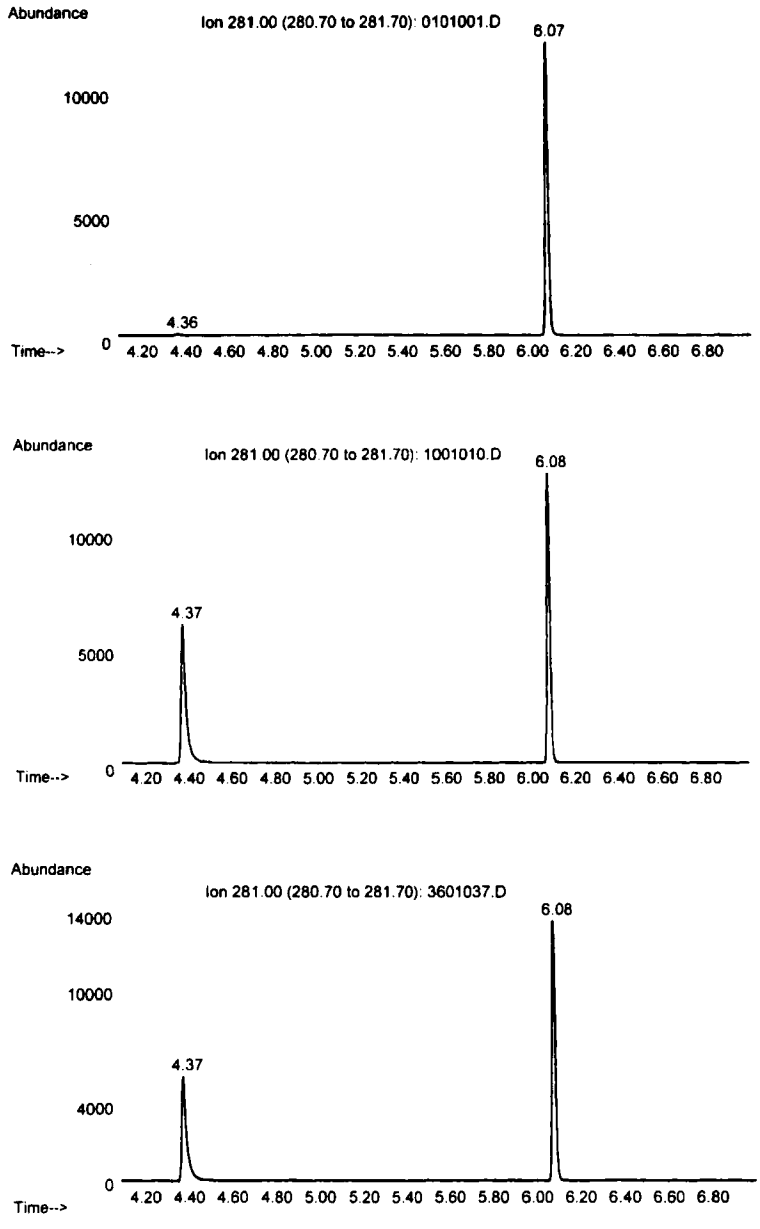

FIGURE 1 Gas chromatography-mass selective detector chromatogram (*m/z* 281) of a) solvent (THF) blank containing the internal standard M_4Q (~320 ng/g THF; 6.07 min); b) THF containing D_4(100 ng/g THF; 4.37 min) and M_4Q (~320 ng/g THF); and c) extract with THF containing M_4Q of plasma from an animal dosed with D_4

racy; and recovery. System suitability – the ability of the chromatographic method to separate the analyte peak from interfering peaks- was assessed using a solution of a single concentration of D_4 (100 µg/L) in THF containing M_4Q. Besides the m/z 281 expected for D_4, no other interfering peaks of similar mass were observed near the retention time of D_4. From the GC-MS response ratio of D_4 to M_4Q on triple injections (mean D_4:M_4Q = 52.89, Std. dev. 2.65), the coefficient of variation (CV) was determined to be 5.01%.

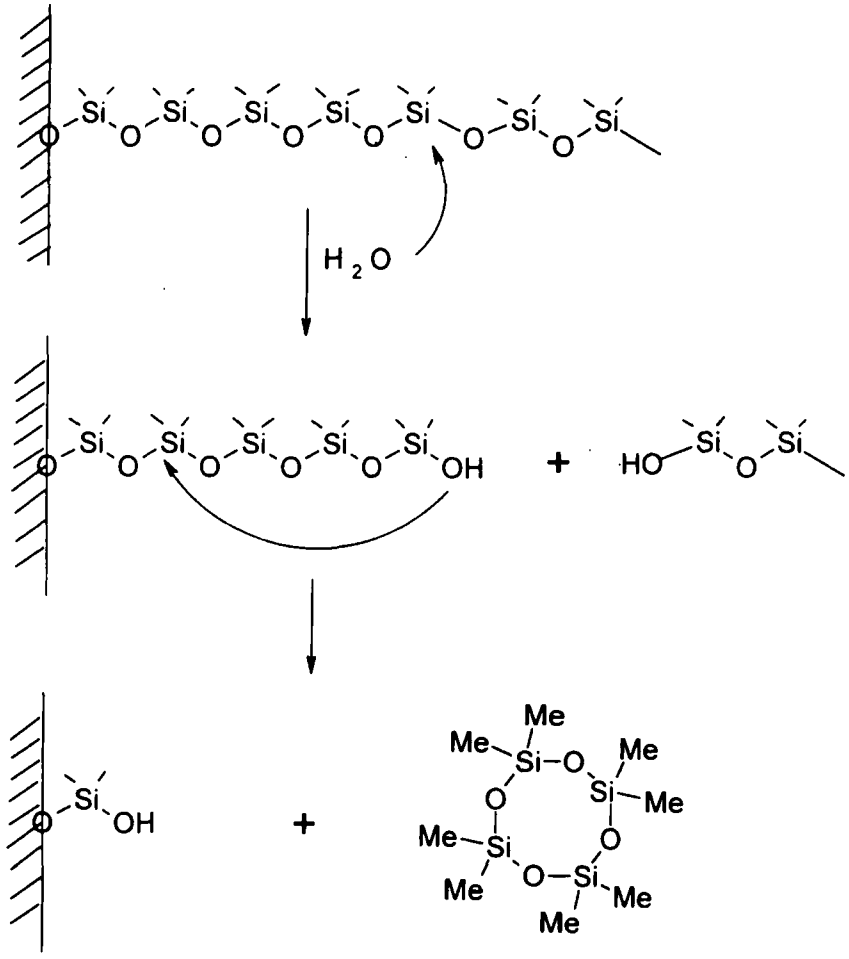

FIGURE 2 Generation of D_4 by interaction of water with PDMS stationary phase in GC

To demonstrate the specificity of this analytical method, three aliquots of both control blood and control plasma were extracted with THF. Internal standard was

added to the extracts to give a final ISTD concentration of 320 μg/L. The extracts were dried as described before and the extracts analyzed. Two control samples each from blood and plasma showed complete absence of D_4. Trace levels of background D_4 were detected only in one of the three control samples of blood and plasma. Occasional occurrence of such background level D_4. in blank runs can be attributed to residual water in the extract. It is hypothesized that water cleaves the Si-O bond of polydimethylsiloxane (PDMS) based GC stationary column. The resulting OH on the silicon atom then attack intramolecularly an appropriately placed silicon atom and releases a molecule of D_4 (Figure 2). The fact that residual water in samples generate D_4 can be seen from a comparison of the GC-MS runs (Figure 3) of the following set: a GC-MS run without injection, a run with reagent grade THF as purchased (and hence slightly wet) and a run with the same THF to which 10% water was deliberately added. Enhanced response with THF containing added water attest to the above hypothesis. High temperature low bleed rubber septa are known to contain PDMS and may also be a source of background D_4 in these type of analyses. To eliminate the rubber septum being a source of D_4, it was replaced with a Merlin-microseal septum. It is known that bleed signals consisting of cyclic siloxanes predominantly of cyclic trimer (hexamethylcyclotrisiloxane, D_3) and tetramer (D_4) arise from polysiloxane based stationary phases as a result of thermal and/or oxidative degradation[18]. It was proposed that the siloxane chains fold at high temperature bringing the reacting groups into closer proximity for reactions to occur forming the cyclic siloxanes. Bleed signals from rubber septum are also attributed to similar reactions. However, true column bleed does not generate peaks or humps. During bleeding, the degradation fragments are produced at an increased but constant rate causing base lines to rise. But the reaction of the column with water generated the cyclic siloxane D_4 as a distinct peak at a certain retention time.

Linearity for solvent standards was evaluated over three concentration ranges 1–20, 20–1000, and 1000–16,000 ng D_4/g THF that covered the expected range for the biological extracts. The correlation coefficient (R^2) for the linear regression analysis generated from the plot of D_4 concentration versus peak-area ratio of D_4:ISTD was equal to or greater than 0.990 in all cases. GC-MS data and standard curve calculations are shown in Table I. For the standards, with the exception of a very few, the D_4 concentrations calculated from the linear regression equation were within 5% the corresponding nominal values. For evaluation of inter-assay precision, the linearity of D_4 concentration versus peak area ratio of D_4:ISTD over low and high concentration ranges (1–20 & 1000–12000 μg/L respectively) was verified approximately 50 times. The data (Table II) suggested a highly reproducible inter-assay precision and accuracy. No carry-over was observed in the majority of runs. The stability of solvent standards stored at room temperature was demonstrated for 27 days (Table III).

TABLE I GC-MS Data of the Standards at Three Concentration Ranges Used in Generating the Calibration Curve

Standard	*D4 Area*	*M4Q Area*	*D4/M4Q*	*[D4] ng/g*	*Concentration Extrapolated From Regression*	*Percent Difference From Expected*
BLANK	0	161793	**0**	N.A.	N.A.	N.A.
Standard curve data for low concentration range						
$Standard_1$	0	154294	**0**	**1.09**	N.A.	N.A.
$Standard_2$	1103	152414	**0.0072**	**2.19**	2.42	10.50
$Standard_4$	2645	158028	**0.0167**	**4.34**	4.52	4.15
$Standard_6$	3881	154650	**0.0251**	**6.57**	6.38	2.89
$Standard_8$	5576	158675	**0.0351**	**8.70**	8.58	1.38
$Standard_{10}$	7303	160886	**0.0454**	**10.92**	10.86	0.55
$Standard_{12}$	8714	159585	**0.0546**	**12.82**	12.89	0.55
$Standard_{14}$	9829	156537	**0.0628**	**14.86**	14.70	1.08
$Standard_{16}$	11763	164185	**0.0716**	**17.02**	16.64	2.23
[a]$Standard_{18}$	15784	162445	**0.0972**	N.A.	N.A.	N.A.
$Standard_{20}$	15344	161794	**0.0948**	**21.34**	21.77	2. 01
Standard curve data for mid concentration range						
$Standard_{20}$	15344	161794	**0.0948**	**21.34**	17.55	17.76
$Standard_{50}$	49417	162440	**0.3042**	**53.10**	54.65	2.92
$Standard_{100}$	99977	165619	**0.6037**	**106.16**	107.70	1.45
$Standard_{200}$	201707	162851	**1.2386**	**219.75**	220.17	0.19
$Standard_{400}$	406847	164903	**2.4672**	**434.79**	437.82	0.70
$Standard_{600}$	601627	163987	**3.6687**	**651.86**	650.67	0.18
$Standard_{800}$	811021	167566	**4.8400**	**862.04**	858. 16	0.45
$Standard_{1000}$	1006688	164492	**6.1200**	**1082.64**	1084.91	0.21
Standard curve data for high concentration range						
$Standard_{1000}$	1006688	164492	**6.1200**	**1082.64**	933.63	13.76
$Standard_{2000}$	2118838	168178	**12.5988**	**2243.15**	2170.36	3.24
$Standard_{4000}$	4139087	166629	**24.8401**	**4445.97**	4507.11	1.38

Standard	*D4 Area*	*M4Q Area*	*D4/M4Q*	*[D4] ng/g*	*Concentration Extrapolated From Regression*	*Percent Difference From Expected*
$Standard_{6000}$	6297338	171939	**36.6254**	**6640.55**	6756.80	1.75
$Standard_{8000}$	8033294	168225	**47.7533**	**8781.22**	8881.01	1.14
$Standard_{10000}$	10326850	171429	**60.2398**	**11176.1**	11264.56	0.79
$Standard_{12000}$	12243173	173493	**70.5687**	**13205.2**	13236.24	0.24
Stock B	16276369	175216	**92.8932**	**17672.2**	17497.76	0.99
Blank	0	165938	**0.00**	N.A.	N.A.	N.A.

N.A. = Not Applicable.

a. Standard not used in calculation.

TABLE II Linear Regression Results from Standard Curve Analysis to Validate Inter-assay Precision and Accuracy

D_4 Concentration (Day)	*Linear Regression (R^2)*
1–20 μg/L, day 0	0.9918
1000–12,000 μg/L, day 0	0.9999
1–20 μg/L, day 2	0.9903
1000–12,000 μg/L, day 2	1.0000
1–20 μg/L, day 4	0.9935
1000–12,000 μg/L, day 4	0.9999

Efficiency of the recovery of D_4 from biological matrices using THF extraction has been assessed previously[14] and was determined to be >90%. In the present study, various biological matrices collected from rats that were dosed with D_4 were extracted repeatedly until no D_4 was detected in the extracts. The ratio of D_4: M_4Q (ISTD) for each extract was calculated, and applying the linear equation, the amount D_4 was obtained. The sum of the amount of D_4 from each extract was then used to determine the number of extractions required for 90% recovery. Results of recovery experiments are presented in Table IV. Based on the average of two trials, three extractions each with 0.5 ml THF provided 90% or greater extraction efficiency as well as a standard error of 2.1 or less in each biological matrix studied (plasma, liver, feces and fat). The results thus proved the precision, accuracy and recovery of the method.

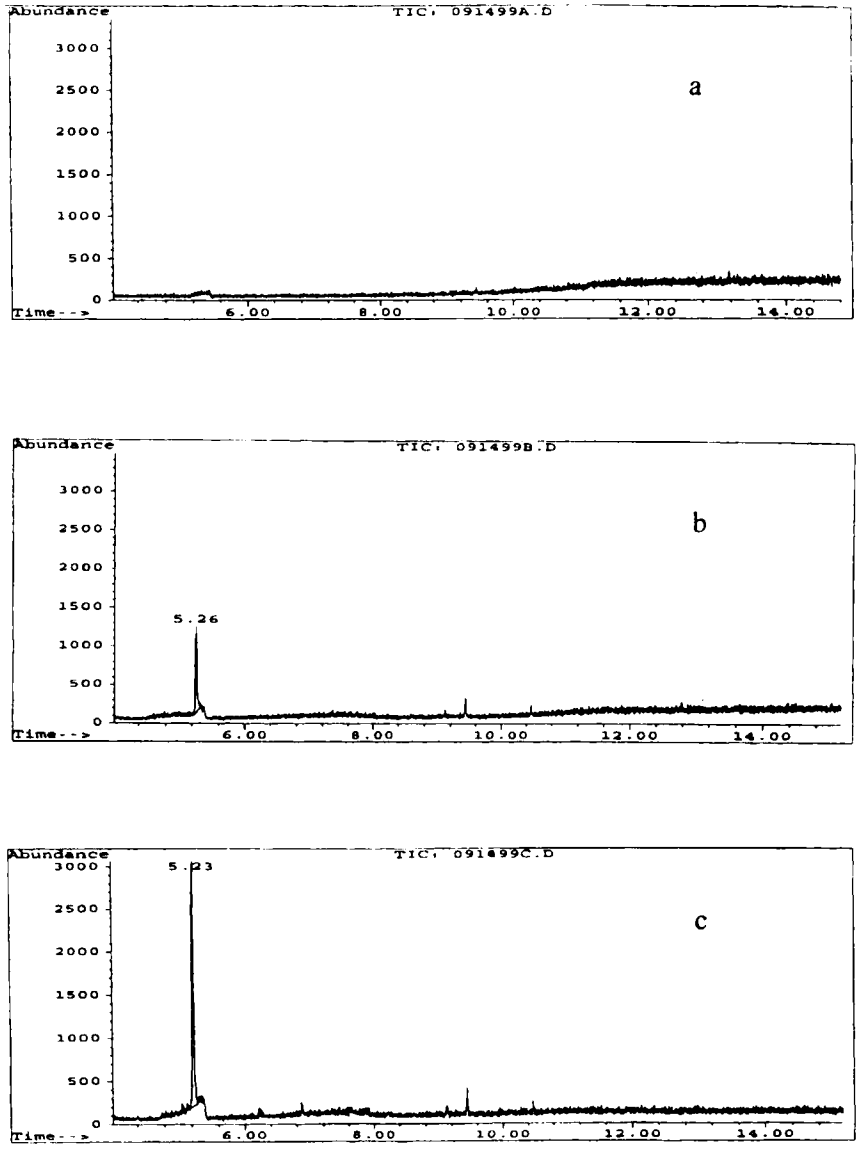

FIGURE 3 GC-MS of a) a dry run, b) 2 μL injection of undried reagent grade THF and c) 2 μL injection of reagent grade THF mixed with 10% water to show generation of D_4 (at retention times 5.23 & 5.26 min) by reaction of water with PDMS of the GC stationary phase (GC conditions: initial oven temperature of 70 °C held for 3 min, followed by heating at a rate of 20 °C/min to a final temperature of 210 °C)

TABLE III GC-MS Response Ratio of D_4:M_4Q showing the Stability of the Standards upto 27 days Period

Nominal D_4 Conc. ppb	*GC-MS Peak Area Ratio of D_4:M_4Q*				
	Day 0	*Day 3*	*Day 10*	*Day 17*	*Day 27*
1	0.0000	0.0000	0.0038	0.0043	0.0037
2	0.0072	0.0075	0.0084	0.0086	0.0078
4	0.0167	0.0156	0.0173	0.0176	0.0172
6	0.0251	0.0265	0.0269	0.0289	0.0263
8	0.0351	0.0344	0.0383	0.0380	0.0365
10	0.0454	0.0448	0.0461	0.0501	0.0464
12	0.0546	0.0574	0.0549	0.0576	0.0547
14	0.0628	0.0639	0.0671	0.0681	0.0673
16	0.0716	0.0735	0.0797	0.0781	0.0651
18	0.0972	0.0966	0.1054	0.1024	0.0976
20	0.0948	0.0957	0.0999	0.1002	0.0938
50	0.3042	0.2538	0.3028	0.3018	0.3002
100	0.6037	0.6018	0.5983	0.6011	0.5940
200	1.2386	1.2346	1.2300	1.2356	1.2177
400	2.4672	2.4293	2.4259	2.4296	2.4022
600	3.6687	3.6407	3.6428	3.6435	3.5902
800	4.8400	4.7904	4.8061	4.8040	4.7852
1000	6.1200	6.0539	6.0345	6.0395	5.9572
2000	12.5988	12.5013	12.6131	12.5140	12.3268
4000	24.8401	24.6618	24.5879	24.5820	24.1018
6000	36.6254	36.3308	36.2585	36.2385	34.0682
8000	47.7533	47.5521	47.4569	47.4728	46.8670
10000	60.2398	59.1836	59.3047	59.5483	58.7270
12000	70.5687	69.3509	69.3509	69.8550	68.3765
16000	92.8932	91.7305	91.2037	91.8159	89.6183

In preparing biological samples for the determination of D_4, there are two major concerns. Firstly, as mentioned earlier, there is a potential for D_4 to undergo chemical transformation assisted by enzymes while in contact with the

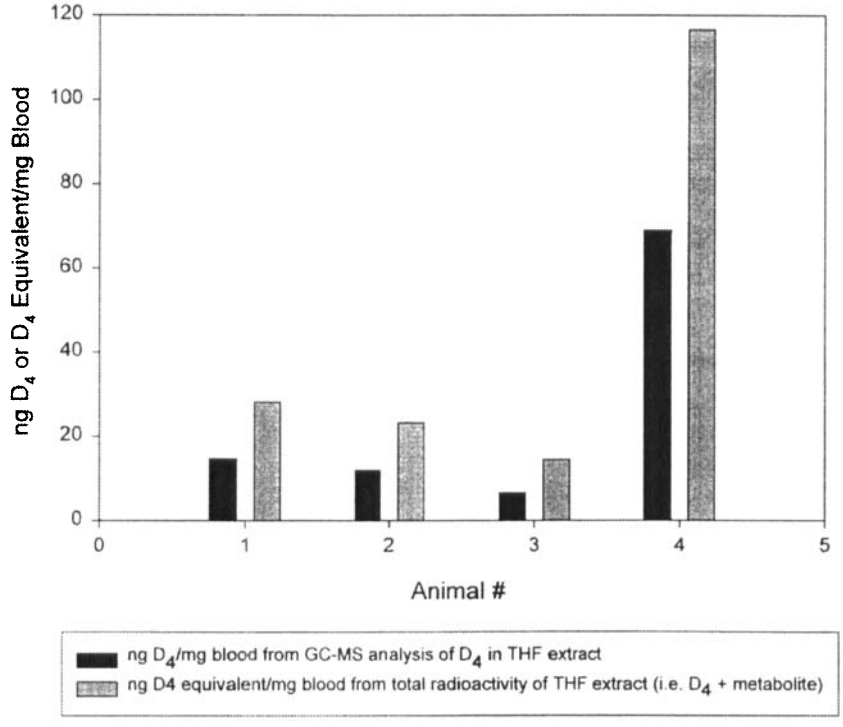

FIGURE 4 Amount of D_4 in relation to the total radioactivity (D_4 + Metabolites) in blood at various time points

matrices. Secondly, with a vapor pressure of 1 mm Hg at 25 °C [19], D_4 is volatile, and we find from our experience that if not handled very carefully significant losses occur. Hence it was of interest to investigate the stability/loss aspects of D_4 in biological samples. The matrices blood and plasma were chosen for this purpose. Control plasma and control blood were spiked with D_4 at two different concentrations (low: 50–190 μg/L; high 3,000–10,000 μg/L). In order to reduce potential loss of D_4 from evaporation, the spiked samples were kept cold on ice until the time of extraction. From an individual spiked sample, D_4 concentration was determined at 0,1,2,3, and 4 hr time points at room temperature as well as at approximately 37 °C. At the D_4 concentration of < 80 μg/L, no loss of D_4 was observed from blood at either room temperature or 37 °C. At higher concentration (~3000 μg/L) as much as 22% was lost even at room temperature. In plasma both at low (<200 μg/L) and high concentrations (10000 μg/L) 20 to 50 % losses were encountered at room temperature as well as at 37 °C. Therefore biological matrices must be extracted for D_4 evaluation as quickly as possible once thawed to ambient temperature.

TABLE IV Recovery of Octamethylcyclotetrasiloxane (D_4) from Biological Matrices

Single Extraction				*Triple Extraction*			
Animal #	*Matrix*	*% Recovery*		*Animal #*	*Matrix*	*% Recovery*	
		Trial 1	*Trial 2*			*Trial 1*	*Trial 2*
1	Plasma	84	85	1	Plasma	96	99
2	Plasma	84	84	2	Plasma	98	100
3	Plasma	72	86	3	Plasma	89	100
4	Plasma	87	85	4	Plasma	98	100
	Mean	***82***	***85***		***Mean***	***95***	***100***
	Std. dev.	***3.3***	***0.4***		***Std. dev.***	***2.1***	***0.3***
1	Liver	76	73	1	Liver	96	95
2	Liver	74	69	2	Liver	93	92
3	Liver	56	75	3	Liver	96	92
4	Liver	86	77	4	Liver	96	92
	Mean	**73**	**74**		***Mean***	**95**	**93**
	Std. dev.	**6.2**	**1.7**		***Std. dev.***	**0.8**	**0.5**
1	Fat	85	94	1	Fat	97	100
2	Fat	70	95	2	Fat	98	100
3	Fat	84	91	3	Fat	97	100
4	Fat	72	91	4	Fat	100	99
	Mean	**78**	**93**		***Mean***	**98**	**100**
	Std. dev.	**3.9**	**1**		***Std. dev.***	**0.7**	**0.3**
1	Feces	76	79	1	Feces	90	93
2	Feces	63	83	2	Feces	88	97
	Mean	**70**	**81**		***Mean***	**89**	**95**
	Std. dev.	**4.6**	**1.4**		***Std. dev.***	**0.7**	**1.4**

Application to determine D_4 to metabolites ratio in blood

Having established a validated procedure to quantitate D_4 in biological matrices, we proceeded to determine rate of D_4 bio-transformation as part of pharmacokinetic investigations. This required that D_4 amount in relation to the total amount

of metabolites generated be determined. Since it was quite convenient to determine accurately the total amount of metabolites in terms of radioactivity, rats were administered [^{14}C]-D_4 as an emulsion intravenously. Blood samples were collected at the following time points: 1,8,24 and 48 hrs post administration. A known amount of blood sample was subjected to THF extraction and the total radioactivity measured using a liquid scintillation counter. To verify that the metabolites were being extracted by THF with good recovery, an aliquot of the same blood was solubilized following a standard procedure[14] and the total radioactivity measured. These two measurements yielded identical results within experimental error suggesting essentially quantitative recovery. The radioactivity values were then converted to nanogram equivalent of D_4 /mg of blood based on the specific activity. The THF extract was also analyzed by the currently validated GC-MS method to determine the amount of parent D_4 from which the nanogram D_4/mg of blood was calculated. The data is summarized in Table V. It revealed that at 1 hr, only very little of D_4 and the metabolites entered into the blood stream. It reached a concentration at the 8hr time point and continually decreased thereafter. For the purpose of illustration, the amount of D_4 relative to the total radioactivity (i.e. amount of metabolites + D_4) was shown for the samples of 8 hr time point in Figure 4. The data showed that the parent D_4 makes up half the total material at all time points. Other applications of this methodology carried out in collaboration with Dow Corning have recently appeared in toxicological investigations concerned with D_4[20–21].

CONCLUSIONS

This analytical method using GC-MS can be applied with a high degree of confidence to quantitate D_4 in a variety of biological matrices following its extraction into tetrahydrofuran. The GC-MS method used here was shown to be both sensitive and selective. Determination of various analytical parameters such as system suitability, specificity, linearity of response, carry-over, inter and intra-assay precision clearly validated the utility of the method. For successful application of the method to determine D_4 at low levels, several key issues must be addressed. In the sample preparation, D_4 may be lost via volatilization, degradation catalyzed by enzymes or chemical transformation from exposure to acids or bases. Furthermore, the water present in the biological matrices, or in the reagents used can readily cause artifact peaks formed by the action of water on the silicone polymers used in chromatography columns and in septa. Published results for D_4 analysis often do not mention whether these issues were considered.

TABLE V Comparison of D_4 Concentration in blood at various time points obtained from GC-MS analysis for D_4 in THF Extracts to the total of amounts of D4+Metabolites obtained from RadioactivityMeasurements of THF extracts as well as whole blood

Sample ID	*1 hr*	*8 hr*	*24 hr*	*48 hr*
From GC-MS analysis				
		ng D_4 / mg blood		
1	0.0027	14.7043	0.0000	0.0000
2	0.0063	11.8987	0.1620	0.0000
3	0.0373	6.5157	0.4363	0.0000
4	0.0040	68.9947	0.0770	0.0000
From radioactivity measurement of THF Extract				
		ng equiv.of D_4 / mg blood		
1	0.4898	28.0980	0.5944	0.0892
2	0.6993	23.2436	1.2344	0.0128
3	0.7631	14.4503	0.4448	0.0137
4	0.6098	116.7386	0.4913	0.0141
From radioactivity measurement after solubilization				
		ng equiv.of D4 / mg blood		
1	0.5177	26.8723	0.6517	0.1135
2	0.7619	22.9165	1.3690	0.0549
3	0.8119	14.2204	0.4841	0.0698
4	0.6510	113.4010	0.5171	0.0481

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