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GAS CHROMATOGRAPHY-MASS SPECTROMETRY MEASUREMENT OF XENON IN GAS-LOADED LIPOSOMES FOR NEUROPROTECTIVE APPLICATIONS¹

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Abstract

Rationale—We have produced a liposomal formulation of xenon (Xe-ELIP) as a neuroprotectant for inhibition of brain damage in stroke patients. This mandates development of a reliable assay to measure the amount of dissolved xenon released from Xe-ELIP in water and blood samples.

Methods—Gas chromatography-Mass Spectrometry (GC-MS) was used to quantify xenon gas released into the headspace of vials containing Xe-ELIP samples in water or blood. In order to determine blood concentration of xenon *in vivo* after Xe-ELIP administration, 6 mg Xe-ELIP lipid was infused intravenously into rats. Blood samples were drawn directly from a catheterized right carotid artery. After introduction of the samples, each vial was allowed to equilibrate to 37° C in a water bath, followed by 20 minutes of sonication prior to headspace sampling. Xenon concentrations were calculated from a gas dose-response curve and normalized using the published xenon water-gas solubility coefficient.

Results—The mean corrected percent of xenon from Xe-ELIP released into water was 3.87 ± 0.56% (SD, n = 8), corresponding to 19.3 ± 2.8 µl/mg lipid, which is consistent with previous independent Xe-ELIP measurements. The corresponding xenon content of Xe-ELIP in rat blood was 23.38 ± 7.36 µl/mg lipid (n = 8). Mean rat blood xenon concentration after IV administration of Xe-ELIP was 14 ± 10 µM, which is approximately 15% of the estimated neuroprotective level.

Conclusions—Using this approach, we have established a reproducible method for measuring dissolved xenon in fluids. These measurements have established that neuroprotective effects can be elicited by less than 20% of the calculated neuroprotective xenon blood concentration. More work will have to be done to establish the protective xenon pharmacokinetic range.

¹Non-standard abbreviations:

Xe-ELIP Xenon-loaded echogenic liposomes

NMDA N-methyl-D-aspartate

HS Headspace

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Keywords

xenon; neuroprotection; gas chromatography-mass spectrometry; headspace analysis; liposomes

1. Introduction

Xenon is a noble gas element that has long been known to have anesthetic properties, mainly due to its ability to inhibit N-methyl-D-aspartate (NMDA) receptors in the brain [1, 2]. As xenon has a low ionization potential, its electron shell can be polarized by surrounding molecules, enabling it to act as an inhibitor in the active sites of proteins such as serine proteases [3-6]. Xenon noncompetitive inhibition of the NMDA receptor is also responsible for the element's neuroprotective effects, in which Ca^{++} influx into neurons, inducing apoptotic mechanisms, is blocked [2, 7].

Numerous candidate neuroprotective agents have been tested for efficacy in acute ischemic stroke, but were shown to be ineffective in clinical trials [8-10]. During stroke, the cerebral ischemic area is composed of a zone of irreversibly damaged tissue known as the core and surrounding area of less severe and reversible ischemic tissue known as the penumbra [11]. If ischemia continues, the core expands into the penumbra, which leads to irreversible damage. One of the aims of neuroprotective therapies is to prevent irreversible neuronal damage in the ischemic penumbra before relieving vasospasm [12].

Noble gases such as xenon (Xe) are promising neuroprotective agents that have minimal adverse side effects, as these gaseous therapeutics have low blood-gas solubility, which is advantageous in terms of rapid inflow and washout [13]. Inhalation of 70% xenon provides a pharmacologic profile resembling that of low-affinity NMDA receptor antagonists with surprisingly few neurotoxic side effects [13-20]. Importantly, the noble gases easily cross the blood-brain barrier. Given by inhalation, however, high xenon concentrations (50-70%) are required for a demonstrable neurologic effect. This is difficult to achieve clinically, since high xenon concentrations in blood limit the fraction of inspired oxygen that is necessary for cell survival [15], restricting the use of xenon as a neuroprotectant by inhalation.

We have loaded xenon gas into an intrinsically echogenic liposomal (ELIP) formulation and achieved instantaneous ultrasound-triggered gas release. We demonstrated neuroprotection with extension of the therapeutic time-window for recombinant tissue plasminogen activator (rtPA) treatment by 5 hours in a rat thrombotic stroke model, as well as neuroprotective efficacy in a rat subarachnoid hemorrhagic stroke model [21, 22]. It has been estimated that the minimum neuroprotective blood xenon concentration in the local cerebral circulation is 0.1 mM [1, 7, 23]. The ability to routinely and accurately measure dissolved xenon levels in water and blood is necessary for quality control of Xe-ELIP preparations and pharmacokinetic characterization of Xe-ELIP administration for neuroprotection.

Dissolved xenon concentrations in fluids, including water, saline and blood, have been measured by radiotracer, mass spectrometric (MS) and nuclear magnetic resonance (NMR) methods [23-29]. Measuring Xe-133, a gamma (81 keV, 5.25 d $T_{1/2}$) and beta (346 keV max) emitter, by liquid scintillation counting, Ladefoged and Andersen determined an Ostwald

solubility coefficient of 0.0834 for water and 0.129 (average) for blood at 37°C [28]. Mass spectrometry is considered the gold standard for measuring the xenon content of gas mixtures [30]. Dhawan *et al.* [26] measured end-tidal xenon concentrations in air exhaled by anesthetized patients, using a mass spectrometer with a specially crafted inlet device.

Goto *et al.* [27] measured xenon in fluids, including blood, injected directly into a gas chromatograph interfaced with a mass spectrometer (GC-MS) and fitted with a 3 mm-diameter packed column containing a molecular sieve support. This method yielded Ostwald solubility coefficients of 0.096 for water and 0.115 for blood at 37°C, which have since been generally accepted as accurate [1]. Using this method, Nalos *et al.* [29] determined the steady-state systemic arterial xenon concentration during inhaled xenon anesthesia in pigs to be about 7.5% (75 µl/ml). Goto's method, however, is not suitable for routine determination of xenon concentrations in replicate fluid samples.

Quantitative magnetic resonance imaging (MRI) of hyperpolarized xenon-129 offers great advantages for xenon measurement in tissues and blood that are especially valuable for pharmacokinetic studies. Using this approach, Martin *et al.* [23] determined a peak gray matter xenon concentration of 0.036 mM in patients during anesthesia and estimated a tenfold greater arterial blood concentration. This method, however, requires specialized instrumentation and methodology utilizing a form of xenon that is even more expensive than the native isotope mixture. It is also not suitable for routine quality control measurements in replicate samples.

Recently, Thevis *et al.* [31] demonstrated the feasibility of using headspace analysis to measure xenon in human plasma samples by GC-MS. Headspace analysis utilizes sampling and GC-MS measurement of a gas or volatile compound in the gas phase above a solution of the solute at equilibrium between the two phases, dictated by the partition coefficient of that solute [32]. We report here the quantitation of dissolved xenon concentrations in water, reconstituted Xe-ELIP samples and rat blood from headspace sampling utilizing calculations based on published values of the appropriate Ostwald solubility coefficients. Using this method, we have also determined in initial pharmacokinetic investigations the carotid arterial concentration of dissolved xenon in rats injected with Xe-ELIP doses shown to be neuroprotective in previous studies.

2. Materials and methods

2.1. Preparation of Xenon-Loaded Echogenic Liposomes (Xe-ELIP)

Xe-ELIP were prepared as previously described [21]. Briefly, liposomes were composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC; Avanti Polar Lipids, Alabaster, Ala); 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC; Avanti Polar Lipids); and cholesterol (Sigma-Aldrich Chem., St Louis, Mo) at a molar ratio of 60:30:10. Five mg of lipids were mixed in chloroform and the solvent was evaporated under a stream of argon (Ar) in a 50°C water bath to form a thin film on the glass vial. The lipid film was placed under vacuum (100 µm Hg) for 4-6 hours for complete solvent removal.

The dried lipid film was hydrated with 0.32 M mannitol to a concentration of 10 mg lipid/ml, followed by sonication for 5 minutes. The sonicated suspension was transferred to a 2-mL glass vial with a cap fitted with a Teflon-rubber septum. Ten ml of a Xe/Ar (70/30%) (Concorde Specialty Gas Inc, Eatontown, NJ) mixture was injected into the glass vial through the septum with a 12-mL syringe attached to a 27G-½ inch needle. The pressurized liposomal dispersion was frozen at –70°C with dry ice for at least 30 minutes. The liposomal dispersion was allowed to thaw after the vial was depressurized by loosening the cap.

2.2. Instrument Configuration

The GC-MS system consisted of a HP 5973A mass spectrometer interfaced with a HP 6890 gas chromatograph, G1512 Controller, GC Injector (Tower and Tray), and an Edwards E2M2 Rough pump (Agilent Technologies, Santa Clara, CA). The instrument was controlled by a dedicated PC running G1701 DA software with monitor, Hub, and Cat-5 cables. Gases were separated with a Restek 30 m, 0.32 mm ID Rt-Msieve 5A fused silica PLOT molecular sieve column interfaced with the mass spectrometer through a 2.5 m particle trap (Restek Corp., Bellefonte, PA).

2.3. Xenon Gas Standardization

Measured volumes of pure xenon gas were removed from a small compressed gas cylinder (Matheson, Houston, TX) with a syringe and transferred to 2-ml glass sampling vials, sealed with Teflon-lined screw-top caps to produce vol/vol percent standards. Five microliter aliquots were manually injected into the GC. Peak areas (AUC) were determined in auto-integration mode and output traces were printed with an HP LaserJet P1606dn printer.

2.4. Quality Control

Replicate determinations were made using a custom fabricated 25% Xe/75% Ar ISO 13485 certified quality control standard obtained from Air Liquide Healthcare America Corp. (Plumsteadville, PA) and dilutions prepared from it. This QC standard was used to validate the MS-GC performance according to the IHC Q2A and Q2B Validation of Analytical Procedures. The validation involves measures of specificity, linearity, range, accuracy, precision, detection limit and quantitation limit.

2.5. Xenon-Water Determinations

Xenon gas was dissolved in water by a modification of the method of Goto *et al.* [27]. The setup, diagramed in Fig. 1, consisted of 30 ml of nanopure water (MilliQ system) stirred in a 50 ml Erlenmeyer flask maintained at 37°C in a water bath on a digital stirring hotplate (Fisher Isotemp, Fisher Scientific Co.). After fully charging the system with xenon gas, the loop, consisting of 1.2 mm ID Tygon tubing fed through a one-hole #2 rubber stopper, was closed. One end of the tubing was submerged in the water and a Masterflex C/L peristaltic pump (Cole-Parmer Instrument Co., Vernon Hills, IL) was used to circulate the gas through the water in a clockwise direction. A 5-ml plastic syringe with a blunt needle inserted into the water through the stopper hole between the tubing was used to sample 1 ml of water 1, 2, 3 and 4 hours after circulation commenced.

The water samples were transferred to sampling vials, which were equilibrated at 37°C for 30 minutes in a sonicating water bath, followed by an additional 20 minutes of sonication. Five microliters of headspace gas were removed for manual injection into the GC. The % xenon was calculated from the gas dose response curve and converted to % xenon in water assuming total re-equilibration with the headspace. Results were normalized to the published value for xenon-saturated water of 8.8% (water-gas partition coefficient = 0.096) at 37°C and 1 atm [27], generating an empirically determined recovery factor (to equate the GC response to the published xenon solubility in water) that was used to correct all subsequent headspace measurements.

2.6. Xenon-ELIP Measurements

Xe-ELIP samples (0.2 ml; 2 mg lipid) were diluted with water to a total volume of 1.0 ml in sampling vials and treated as for Xe-water; 5 µl of headspace gas was manually injected into the GC. The same procedure was repeated with Xe-ELIP samples diluted 40X (25 µl) with rat blood (975 µl) collected in a 1.5 ml Eppendorf microfuge tube containing 0.1 ml sodium heparin (1,000 USP units/ml; SAGENT Pharmaceuticals, Schaumburg, IL) per 1.0 ml blood collected, as would occur in the pharmacokinetics procedure described below, instead of water.

2.7. Pharmacokinetics

Sprague-Dawley male rats (260-280 g; Harlan Laboratories Inc, Indianapolis, IN) were housed and treated following a protocol approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at Houston (UTHealth). Various volumes (1.2-1.5 ml) of blood were collected from a carotid catheter coated with heparin into a tube containing a one-tenth volume of sodium heparin solution as described above during IV infusion of 0.6 ml Xe-ELIP into rats over a 6-minute period; 0.5 ml of the blood sample was transferred to a sampling vial, treated as described above, and 5 µl headspace gas was directly injected into the GC-MS.

2.8. Statistics

Data in comparative groups were subjected to normal variance analysis and compared for probability of population identity by Student's t-test using Microsoft Excel 2010 software.

3. Results

The molecular sieve column cleanly separated xenon from other gases (Fig. 2). Air components eluted at 2-2.5 minutes, while xenon eluted at 5.32 minutes. The mass spectrum for the xenon peak (Fig. 2, inset), with characteristic parent isotope/product fragment m/z around 132 and 66, was identified by the NIST MS library as xenon. The xenon gas standard curve, produced by manually injecting 5 µl of defined xenon gas mixtures in air is shown in Fig. 3. The relation yielded a linear regression curve of $y = 3.81 \times 10^7 \times - 2.23 \times 10^7$. Since the line essentially passes through the origin, which is not significantly different from replicate standard regression intercepts (varying up to $+1.0 \times 10^7$), the integrated area-under-the-curve (AUC) values of xenon peaks were divided by 3.81×10^7 to obtain the headspace xenon concentration for all subsequent analyses.

The ISO 13485 QC standard (25% Xe/75% Ar) eluted from the GC column slightly earlier than the standard, possibly due to near saturation of the column (Fig. 4), but exhibited the characteristic MS signature (Fig. 4, inset). The argon eluted from the column prior to 2 minutes and exhibited the characteristic isotope m/z of 40.1. There was air contamination, with m/z indicative of oxygen and nitrogen. The trace, therefore, satisfies the validation parameter of identification. The instrument discriminators were set to exclude responses from $m/z < 100$, so that xenon was the only analyte detected.

Dilutions of the QC standard fell around the xenon gas curve linear regression line (Fig. 3), demonstrating superimposability, a criterion of accuracy. The standard curve was linear through the range of standards (2.5-50% Xe; $r = 0.997$). Based on the standard error of the slope and the slope of the curve, the detection limit was found to be 0.022% Xe and the quantitation limit was 0.066% (Table 1). Extending the curve linearity to the latter value, the assay range, therefore, is at least 0.066 – 40%, since the 50% point, although close to the regression line, is not included in the regression analysis. Both repeatability (intra-assay variance) and intermediate precision (interassay variance) were generally less than 5% for the xenon gas standards, but higher for Xe-ELIP samples (Table 1; see below).

Xenon dissolved in 37°C water at equilibrium (attained after 2 hours of recirculation) under dynamic flow conditions was calculated as outlined in Table 2. Sonication maximized xenon recovery from water, which was 64.1% of what was expected from the published xenon water solubility. This empirically determined recovery factor, which was used to correct all subsequent headspace measurements, also served to normalize measurements to the published solubility, minimizing possible inaccuracies in the gas curve values.

The mean corrected percent of xenon from Xe-ELIP released into water was $3.87 \pm 0.56\%$ ($n = 8$), corresponding to $19.3 \pm 2.8 \mu\text{l/mg}$ lipid (Table 3), compared to a value of $15.0 \pm 2.3 \mu\text{l/mg}$ lipid determined by physical volumetric Xe-ELIP measurements [21]. The corresponding xenon content of Xe-ELIP in rat blood was 22.4 ± 6.3 (SD, $n = 8$) $\mu\text{l/mg}$ lipid (Table 3). The average carotid arterial blood xenon concentration after IV administration of Xe-ELIP to 3 rats was $0.036 \pm 0.026\%$, corresponding to $14.0 \pm 10.3 \mu\text{M}$ xenon (Table 4). While the headspace xenon measured in these samples was below the quantitation limit, it was above the detection limit.

4. Discussion

Xenon, as both an anesthetic and neuroprotective agent, has been administered clinically by inhalation. This method of delivery presents logistic and pharmacologic limitations. We have developed a novel xenon-loaded liposomal formulation (Xe-ELIP) that enables total xenon release in the carotid circulation for stroke applications by external ultrasound insonation after intravenous injection of the Xe-ELIP suspension. Using this approach, we have demonstrated neuroprotection, resulting in decreased brain infarction and improved behavioral scores in both ischemic and hemorrhagic rat stroke models [21, 22, 33]. Repetitive production of a xenon-containing therapeutic formulation involving quality assurance/quality control (QA/QC) procedures for preclinical development and clinical applications requires consistent, routine measurement of the element in the gas phase. In addition,

measurement of xenon in blood and biological tissue extracts is required for pharmacokinetic/pharmacodynamic (PK/PD) studies.

Until now, the method that best lends itself to such measurements is radiotracer assay with Xe-133, involving drawbacks associated with the use of radioactive materials. The most accurate method for measurement of xenon in the gas phase is GC-MS, but measurement of dissolved xenon in fluids presents special technical difficulties. Goto *et al.* [27] succeeded in this objective using an ingenious approach in which injected fluid was absorbed in a 3-mm packed column containing a molecular sieve support, allowing the gas to be carried through a more conventional GC column interfaced with a mass spectrometer. While sufficient to obtain relatively accurate estimates of Ostwald solubility coefficients for water, blood and several other fluids, the ability to reuse the packed columns is extremely limited and poses risks for maintenance of GC-MS integrity.

Recently, the feasibility of xenon GC-MS measurement in blood and other fluids by headspace analysis was demonstrated [31]. This group (Schaefer *et al.*) then used the method for quantitation of dissolved xenon in equine blood for detection of the gas as a doping agent in equine sports [34]. "Blood samples fortified with different Xenon concentrations" were used as standards, but the method of fortification and the means of assuring the accuracy of the xenon blood concentrations were not detailed. These investigators claimed that their assay had a detection limit of 0.25 μM with a GC injection volume of 10 μL .

Almost simultaneously, another group of investigators (Kwok *et al.*) reported a quantitative GC-MS headspace analytic method for assay of xenon and krypton in equine plasma for the same purpose [35]. They used a PLOT molecular sieve column for GC separation of gases, as we did. They employed the method of Goto [27] to saturate plasma with xenon and krypton, using the published solubility values to assign gas concentrations to standard plasma dilutions. This group reported a limit of detection (LOD) of 19 pM, about an order of magnitude lower than that achieved by Schaefer *et al.*, which was consistent with an injection volume of 100 μL .

The study reported here represents a systematic attempt to define the variables required for accurate, reproducible measurement of xenon in water, blood and other fluids. In addition, we have validated this method by the IHC Q2A criteria for analytic method validation adopted by the U.S. FDA. The critical feature of our approach is reliance on the published solubility coefficients of Goto *et al.*, which requires strict adherence to procedural methodology for equilibrium xenon solubilization that is effectively equivalent to what they employed.

GC-MS analysis of xenon-equilibrated water provided a recovery factor that could be used to normalize headspace xenon gas concentrations to the published solubility values. Thus, accuracy of the measured gas-phase concentrations is not critical, provided that the methodology, including the GC injection volume of 5 μL , is consistent. This methodology, consisting of equilibration and sonication of the sample at 37°C, was designed to optimize recovery of xenon from Xe-ELIP. Measured values of xenon concentrations in Xe-ELIP agreed well with those previously determined by entirely different means, lending additional

credence to the accuracy of the method. In addition, experiments in which we varied the volume of Xe-ELIP diluted to 1.0 ml (data not shown), from 0.2 ml to 0.5 ml, yielded the same ELIP xenon content (in $\mu\text{l}/\text{mg}$ lipid), indicating that the recovery factor did not vary with dissolved xenon concentration over the range that we measured. Assuming that the ELIP xenon content is the same in both water and blood, the recovery factor in blood would have to be 74.4% to account for the 16% discrepancy between the two values.

Our approach, which ultimately depends on gas phase standardization with utilization of a recovery factor to quantitate dissolved xenon levels in fluids, affords greater flexibility for measurement of a wide range of xenon levels in a variety of media. Other quantitative analytical methods for headspace GC-MS assay of dissolved xenon that have recently been developed are dedicated to one particular medium in a defined context. Our LOD of 0.022% dissolved xenon corresponds to about 5 μM in an analyzed 1.5 ml blood volume. The IHC Q2B method for determination of LOD that we employed corresponds to a 10:1 signal-to-noise ratio. However, a peak can be clearly detected and AUC measured at a 2:1 ratio. If this is the criterion employed by Schaefer *et al.* and Kwok *et al.*, then the quantitation limit of our method, taking into account differences in injection volumes, is comparable to theirs.

Based on a projected carotid artery xenon concentration of 0.5 mM after inhalation of 100% xenon [23] and rat studies indicating that a 19% inhaled xenon gas concentration would be sufficient to produce maximal neuroprotection in humans [7], it can be concluded that 0.1 mM xenon concentration in the carotid arterial circulation would be sufficient to produce optimal neuroprotection. We have shown that an IV infusion of 6 mg of Xe-ELIP lipid over a 6-minute period produces neuroprotective effects in rats, evidenced by significant brain infarct reduction and improved behavioral test scores after induced ischemic stroke [21, 22].

GC-MS measurement of carotid arterial blood xenon concentrations in 3 rats after intravenous Xe-ELIP infusion under the same conditions yielded an average value of 14 μM , less than 15% of the estimated neuroprotective level. If the blood recovery factor of 0.744 is used for this data, the xenon blood concentration is $11.6 \pm 8.6 \mu\text{M}$. Clearly, more work needs to be done to define the neuroprotective range of xenon blood levels and to delineate a dose-response relationship between these levels and a reproducible measure of neuroprotection. In addition, technical improvements are needed in PK/PD *in vivo* sampling techniques to raise the measured headspace xenon levels over the quantitation limit of the assay. These improvements will probably involve increases of GC injection volume within levels already shown to be acceptable by others, with restandardization or appropriate validation procedures.

This study demonstrates the ability of our methodology to accurately measure xenon concentrations in blood, allowing an assay to help determine blood concentrations vs. clinical efficacy. This methodology can be transitioned for measurement of blood concentrations of other bioactive noble gases that have potential to treat clinical disease states.

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Figure 1.

Schematic diagram of apparatus for determination of equilibrium xenon solubility in water at 37°C. Xenon gas was circulated through water on a digital hotplate in a closed system. Water aliquots were sampled with a syringe and transferred to vials for GC-MS analysis.

Figure 2.

GC-MS trace showing separation of air components from xenon and MS spectrum of the GC peak eluting at 5.323 minutes (inset).

Figure 3.

Xenon gas GC-MS dose-response curve. Gas samples (5 μ l) from mixtures of various proportions of xenon in air were manually injected. AUC for peaks were determined by auto-integration. Each point (filled circles) is the mean of 4-5 determinations $\pm$ SD; $r = 0.997$, $y = 38.1 \times - 22.3$. AUC of the certified gas standard (25% Xe/75% Ar; $n = 6$) and its dilutions (open circles) fall around the standard curve.

Figure 4.
GC-MS trace of the ISO 13485 QC standard, showing separation of argon and air components from xenon and MS spectra for all components (inset).

Table 1

IHC Q2A Quality Control Performance of the Xenon GC-MS Method

QC Parameter	Criterion	Result	Comment
Range	From Limit of Quantitation Through Linear Range	0.066-40%	
Precision: Repeatability (Intra-Assay Variance (Mean $\pm$ SD [n]))	AUC of 3 Standard Concentrations ($\times 10^{-6}$)	10%: 376.7 ± 15.3 [5] 20%: 729.3 ± 34.8 [5] 40%: 1446 ± 74.9 [4]	CV = 4.1-5.2%
	QC Std. (25% Xe)	$28.3 \pm 1.0\%$ [3]	CV = 3.5%
	Xe-ELIP in water (μl Xe/mg lipid)	$19.3 \pm 2.8\%$ [8]	CV = 14.5%
Precision: Intermediate Precision (Inter-Assay Variance (Mean $\pm$ SD [n]))	QC Std. (25% Xe)	$28.6 \pm 0.4\%$ [3]	CV = 1.4%
	Xe-ELIP in water (μl Xe/mg lipid)	$22.3 \pm 3.7\%$ [3]	CV = 16.6%
Detection Limit	SD of Response and Slope ($3.3\sigma/S$)	0.022% Xe	σ = SE slope
Quantitation Limit	SD of Response and Slope ($10\sigma/S$)	0.066% Xe	σ = SE slope

Table 2

Determination of the Xe-Water Recovery Factor.

	Calculation	Non-Sonicated (n = 3)	Sonicated (n = 3)
Liquid Volume (ml)		1.0	1.0
AUC ($\times 10^{-6}$) Mean $\pm$ SD		126.8 $\pm$ 8.5	196.0 $\pm$ 43.8
Headspace Xenon Percent (%)	AUC/3.81 $\times 10^7$	3.33	5.14
Micrograms (μg)	(HS vol)·(Xe atom wt)·(% Xe)/molar gas vol at 37°C [*]	172.2	266.3
Millimolar (mM)	HS Xe μ g/(HS vol)·(Xe atom wt)	1.30	2.02
% Xe in Water	(Xe air-water part coeff at 37°C)·(HS Xe μ g)·(molar gas vol at 37°C)/ Xe atom wt/liquid vol [*]	3.65	5.64
Recovery Factor	% Xe in water/Publ Xe water solubility	0.414	0.641

^{*} HS = headspace; normalized for μ g/g ml/l and ml/100ml (%).

Table 3

Xenon content of Xe-ELIP in Water and Rat Blood (n = 8).

	Xe-ELIP in Water		Xe-ELIP in Blood	
Liquid Volume (ml)	1.0		1.0	
	Mean	± SD	Mean	± SD
AUC ($\times 10^{-6}$)	86.2	12.5	11.9	3.3
Headspace Xenon Percent (%)	2.26	0.33	0.31	0.09
Micrograms (μg)	117.1	17.0	16.2	4.5
Millimolar (mM)	0.89	0.13	0.11	0.04
% Xe in Water or Blood	2.48	0.36	0.36	0.10
Corrected for Recovery *	3.87	0.56	0.56	0.16
μl Xe/mg Lipid [†]	19.3	2.8	22.4	6.3
μg Xe/ml Water or Blood [‡]	200.3	29.0	29.0	8.2
mM Xe in Water or Blood [‡]	1.52	0.22	0.22	0.06

* % Xe in Water or Blood/0.641

[†] 3.87 μl Xe/100 μl water (0.2 mg lipid) or 0.56 μl Xe/100 μl blood (0.025 mg lipid)[‡] Analogous calculations for headspace Xenon, but without volume constraint

Table 4

Xenon Blood Levels After Xe-ELIP Administration to Rats (n = 3).

	Mean	± SD
Liquid Volume (ml)	1.33	0.15
AUC ($\times 10^{-6}$)	1.36	0.64
Headspace Xenon Percent (%)	0.036	0.016
Micrograms (μg)	1.31	0.85
Millimolar (mM)	0.014	0.006
% Xe in Blood	0.023	0.017
Corrected for Recovery	0.036	0.026
$\mu\text{g Xe/ml Blood}$	1.85	1.36
mM Xe in Blood	0.014	0.010