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## **Accelerator Mass Spectrometry Allows for Cellular Quantification of Doxorubicin at Femtomolar Concentrations**

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## Abstract

Accelerator mass spectrometry (AMS) is a highly sensitive analytical methodology used to quantify the content of radioisotopes, such as  $^{14}\text{C}$ , in a sample. The primary goals of this work were to demonstrate the utility of AMS in determining cellular [ $^{14}\text{C}$ ]doxorubicin (DOX) concentrations and to develop a sensitive assay that is superior to high performance liquid chromatography (HPLC) for the quantification of DOX at the tumor level. In order to validate the superior sensitivity of AMS versus HPLC with fluorescence detection, we performed three studies comparing the cellular accumulation of DOX: one *in vitro* cell line study, and two *in vivo* xenograft mouse studies. Using AMS, we quantified cellular DOX content up to 4 hours following *in vitro* exposure at concentrations ranging from 0.2 pg/ml (345 fM) to 2  $\mu\text{g/ml}$  (3.45  $\mu\text{M}$ ) [ $^{14}\text{C}$ ]DOX. The results of this study show that, compared to standard fluorescence-based HPLC, the AMS method was over five orders of magnitude more sensitive. Two *in vivo* studies compared the sensitivity of AMS to HPLC using a nude mouse xenograft model in which breast cancer cells were implanted subcutaneously. After sufficiently large tumors formed, DOX was administered intravenously at two dose levels. Additionally, we tested the AMS method in a nude mouse xenograft model of multidrug resistance (MDR) in which each mouse was implanted with both wild type and MDR+ cells on opposite flanks. The results of the second and third studies showed that DOX concentrations were significantly higher in the wild type tumors compared to the MDR+ tumors, consistent with the MDR model. The extreme sensitivity of AMS should facilitate similar studies in humans to establish target site drug delivery and to potentially determine the optimal treatment dose and regimen.

## Introduction

The aim of this work is to demonstrate accelerator mass spectrometry (AMS) as a highly sensitive methodology for quantifying drug concentrations in tumor cells, with the ultimate goal of using this methodology to quantify drug delivery to target tissues in humans. Development of a sensitive and quantitative method of determining drug concentrations in tumors using aspirated cells or needle biopsies would therefore be clinically useful for guiding cancer therapy on an individual basis.

Current approaches to obtaining pharmacokinetic data in tumors include non-invasive techniques such as positron emission tomography (PET) [12,19-20] and nuclear magnetic resonance (NMR) [17]. Naturally,  $^{19}\text{F}$  NMR requires either that a fluorine atom already be an integral part of the drug of interest, e.g. 5-fluorouracil, or the incorporation of fluorine into the drug molecule. This method, while highly useful, is also compromised by lack of sensitivity, with a detection limit in the range of 0.01 to 0.1 mmol for fluorinated drugs. Deuterium ( $^2\text{H}$ ) NMR has also been used, but it has an effective sensitivity about 10- to 100-fold less than  $^{19}\text{F}$  NMR [13]. Positron emission tomography is a powerful imaging tool for the quantification of positron emitting radiotracers and can provide very detailed spatiotemporal data without biopsy sampling. Several labeled substrates for MDR-related drug transporters have been synthesized for imaging of the MDR phenotype in tumors [12,19-20]. However, PET's main limitations are the short half-life of the isotopes used and the specialized equipment and syntheses required [22].

Other more sensitive methods have been developed which require tissue collection for analysis ex-vivo. These include HPLC and capillary electrophoresis with either fluorescence or UV

detection, as well as mass spectrometry and enzyme immunoassays [5-7,11]. The most widely used methodology for the quantification of DOX is HPLC with fluorescence detection. A method has been developed for the determination of DOX and its metabolite doxorubicinol in plasma, with lower limits of quantification of 1.0 (1,724 pM) and 0.50 ng/ml (862 pM) plasma, respectively [6]. The main drawback of these methods is that they require a relatively large amount of material for analysis and are therefore not sufficiently sensitive for quantification of DOX in aspirated cells or needle biopsies. Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF), one of the most sensitive analytical methods developed to date, has demonstrated sensitivity similar to the above methods when analyzing patient plasma [10,16,18] or tumors [21]. However, much greater sensitivity, in the zeptomole ( $10^{-21}$  mole) range, has been reported using CE-LIF for the determination of DOX and metabolites in cell extracts and subcellular fractions [1-2].

In contrast to these more traditional techniques for drug quantification, AMS is a relatively new biomedical technology for quantification of isotope-labeled compounds in biological samples with attomole ( $10^{-18}$  mole) or greater sensitivity and high precision. The high sensitivity of AMS translates to the use of low chemical and radioisotope doses and relatively small sample sizes, which enables tracer studies to be performed safely in humans, using exposures which are environmentally or therapeutically relevant, whilst generating little radioactive waste. Most biomedical AMS studies completed to date have employed  $^{14}\text{C}$  as the radiolabel, although the capability exists for detecting other isotopes including  $^3\text{H}$ ,  $^{26}\text{Al}$ ,  $^{41}\text{Ca}$ ,  $^{10}\text{Be}$ ,  $^{36}\text{Cl}$ ,  $^{59}\text{Ni}$ ,  $^{63}\text{Ni}$  and  $^{129}\text{I}$  [23].

The goal of this work was to demonstrate that AMS has sensitivity advantages over HPLC with traditional fluorescence detection and offers equivalent sensitivity to the most sensitive CE-LIF methods for the quantification of DOX in biological samples.

## **Materials and Methods**

### Chemicals

[<sup>14</sup>C]Doxorubicin hydrochloride (55 mCi/mmol) was obtained from Amersham Pharmacia Biotech UK Limited. If radio or chemical purity was less than 98%, it was purified by HPLC prior to use. Conditions were 1 ml/min flow rate using a mobile phase consisting of 50% acetonitrile/35% water/15% 0.1 M phosphoric acid (v/v) using a C18 column and UV absorbance monitoring at 487 nm. The retention time of DOX was approximately 8 minutes. Doxorubicin hydrochloride, which was used as an HPLC standard, for the radiolabeled DOX, and also in the medium of MDA-MB-231-A1 cells, was obtained from Adria Laboratories (Columbus, OH). Trypsin (2.5%) was obtained from Invitrogen (Carlsbad, CA), and powdered PBS and EDTA were purchased from Sigma Chemical Company (St. Louis, MO). Doxorubicin HCl (mw 580.0) and daunorubicin HCl (DNR; mw 564.0) used in the HPLC analysis of DOX in the cells and tissues were purchased from Sigma. All reagents (chloroform, methanol, water, acetonitrile) were purchased from Fisher Scientific (Pittsburgh, PA) and were of HPLC grade.

### Laboratory Animals

The study was approved by the Institutional Animal Care and Use Committee at Lawrence Livermore National Laboratory in accordance with the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female, BalbC, Nu/Nu

athymic nude mice (4-6 weeks of age) were purchased from Simonsen Laboratories (Gilroy, CA) and were housed 5 per cage in negative airflow caging (Lab Products one-cage micro-isolator system) on autoclaved wood chip bedding. Cages and water bottles were autoclaved prior to use. Mice were fed autoclaved 18% protein diet (Harlan, Indianapolis, IN) and sterile filtered acidified water *ad libitum*.

### Cell Culture

Wild type MDA-MB-231, obtained from the American Type Culture Collection (Manassas, VA), and multidrug resistant MDA-MB-231-A1, obtained from the late Dr. William McGuire, estrogen receptor-negative human breast cancer cells were grown under normal conditions (37°C, 5% CO<sub>2</sub>/95% air) in improved minimum essential medium (IMEM) (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) in 75 cm<sup>2</sup> filter-capped flasks (Corning, Corning, NY). In addition, the medium of the MDA-MB-231-A1 cells contained 2 µg/ml DOX to maintain selective pressure. Cells were sub-cultured on a weekly basis. At each passage, the spent medium was aspirated, followed by the addition of warm (37°C) 0.25% trypsin solution (80% 1X PBS, 10% 10 mM EDTA, 10% 10X trypsin). Flasks remained at room temperature while the cells detached, after which the appropriate medium, warmed to 37°C, was added to the flasks. Aliquots of the cell slurry were then added to new flasks containing the appropriate medium. One passage prior to an experiment, the selective pressure on the MDA-MB-231-A1 cells was removed.

### Time Course of Cellular DOX Accumulation in Cell Culture

To assess the kinetics of cellular accumulation, MDA-MB-231 or MDA-MB-231-A1 cells were



exposed to [<sup>14</sup>C]DOX (specific activity 0.000171 mCi/mmol) at a concentration of 2 µg/ml (3.45 µM). Cells were plated in T-75 flasks, three flasks for each time-point and cell line. Cells were plated at a density of 50,000 cells/ml and allowed to grow to near confluency in a total volume of 15 ml medium. One milliliter of a 16X stock solution of [<sup>14</sup>C]DOX, prepared in sterile 0.9% sodium chloride, was then added to each flask. Cells were incubated for 10 minutes, 30 minutes, 2 hours or 4 hours. One additional flask of each cell line was used as a negative control and was spiked with 1 ml of a 0.9% sodium chloride solution. Following the exposure, the culture medium was aspirated, and the cells were washed with 5 ml of ice-cold (4°C) PBS. The rinse was then aspirated and 3 ml of fresh ice-cold PBS were added. The cells were removed from the flasks by scraping, and the resulting cell suspensions were transferred to clean glass tubes. Each tube was gently vortexed and a 0.2-ml aliquot was removed for counting. A Coulter Model ZM counter (Beckman-Coulter; Miami, FL) was used for cell counts. The cell samples were split equally to produce replicates for both AMS and HPLC analysis.

#### Dose-response of Cellular DOX Accumulation in Cell Culture

MDA-MB-231 or MDA-MB-231-A1 cells were exposed to [<sup>14</sup>C]DOX for 4 hours at concentrations of 0 pg/ml, 0.14 pg/ml (0.24 pM), 0.47 pg/ml (0.81 pM), 5 pg/ml (8.62 pM), 100 pg/ml (172.4 pM), 1 ng/ml (1.72 nM) and 2 µg/ml (3.45 µM) (specific activities from 0.00012 to 53 mCi/mmol). Cells were plated in T-150 flasks, one flask for each concentration and cell line. Cells were plated at a density of 100,000/ml and allowed to grow to near confluency in a total volume of 25 ml medium. One milliliter of a 26X stock solution of [<sup>14</sup>C]DOX was then added to each flask. Cells were incubated for 4 hours, harvested and counted. Each cell sample was then split into six replicates to produce three samples for AMS and three for HPLC analysis.

## Cellular DOX Accumulation in a Xenograft Model

### *Dose-response for DOX accumulation in MDA-MB-231 tumor cells*

Female, athymic, BalbC Nu/Nu nude mice (4-6 weeks of age) were implanted with MDA-MB-231 cells. Using a 1-ml tuberculin syringe fitted with a 21-gauge needle, each mouse was subcutaneously inoculated with approximately 10.6 million cells (injection volume 200  $\mu$ l) on the right front flank.

Three weeks later, the animals were systematically divided into 2 groups (8 animals per group) which exhibited equal mean body weights. The groups were randomly assigned to the treatments. One additional group of 4 animals received no DOX and acted as controls. [ $^{14}$ C]DOX was administered intravenously via a tail vein using a 28-gauge needle. A dose of 0.1 or 1 mg/kg body-weight [ $^{14}$ C]DOX contained in a volume of 50  $\mu$ l was administered to each mouse. The specific activities were 0.1057 and 0.0106 mCi/mmol for the 0.1 and 1 mg/kg body-weight doses, respectively. Two hours after dosing, each mouse was euthanized by CO<sub>2</sub> asphyxiation. Blood was collected by cardiac puncture into tubes containing heparin, as anticoagulant, and centrifuged at 3000xg for 10 minutes, to separate the plasma. Liver and tumors were excised promptly, weighed, and then snap-frozen in liquid nitrogen. All samples were stored at  $-80^{\circ}$ C until analysis. Samples were analyzed by AMS and HPLC to determine the tissue drug levels, which were used as the basis for assessing whether the drug was sufficiently delivered.

### *Comparison of DOX accumulation in MDA-MB-231 and MDA-MB-231-A1 tumor cells*

Female, athymic, BalbC Nu/Nu nude mice (4-6 weeks of age) were implanted with MDA-MB-231 and MDA-MB-231-A1 cells on opposite flanks. Each mouse was subcutaneously inoculated with approximately 2.88 million cells/200  $\mu$ l (MDA-MB-231) and 3.43 million cells/200  $\mu$ l (MDA-MB-231-A1). Two weeks later, the animals were injected intravenously via tail vein as described above with 1 mg/kg body-weight [ $^{14}\text{C}$ ]DOX (specific activity 0.0192 mCi/mmol). Two animals were used as controls and received sterile 0.9% saline only. Two hours later, tumors were removed, weighed, and snap frozen in liquid nitrogen until analysis by AMS.

### AMS Analysis

Cell samples (each between 107,000-3,010,000 cells) and plasma (10  $\mu$ l) were added to quartz tubes and up to 1 mg of tributyrin carrier was added to provide sufficient total carbon for AMS. Five to 10 mg of liver or tumor from each animal was analyzed without the addition of carrier. Each tumor sample was homogenized prior to analysis to establish a homogeneous distribution of DOX and its metabolites. Samples were prepared for AMS analysis and the  $^{14}\text{C}/^{13}\text{C}$  ratios measured as previously described [13-14,23]. The ratios were converted to ng DOX/ $10^6$  cells or ng DOX/g tissue following the subtraction of the  $^{14}\text{C}$  contribution from any added tributyrin and control samples. AMS measures only the amount of  $^{14}\text{C}$  in a sample as an isotope ratio and provides no structural information, and thus no distinction between DOX metabolites and parent compound within a sample can be made.

## HPLC Analysis

### *Chromatographic Conditions*

The HPLC system consisted of a Beckman (Fullerton, CA) Model 320 gradient liquid chromatograph, a Model 420 controller, and two Model 110A pumps. The system was equipped with a Beckman (Fullerton, CA) 5- $\mu$ m reverse phase C18 Ultrasphere ODS 4.6 x 250 mm column, 100- $\mu$ l injection loop, and a Rheodyne (Rohnert Park, CA) injector. The column was maintained at room temperature (20-25°C) for all analyses. DOX and internal standard (IS) were detected with a Linear Instruments Model LC305 fluorescence detector (Thermo Separation Products, Inc., San Jose, CA) set at an excitation wavelength of 470 nm and an emission wavelength of 550 nm. A Hewlett-Packard (Corvallis, OR) Model 3394 integrator was used for recording retention times and peak heights. The mobile phase consisted of acetonitrile (ACN)/water/0.1 M H<sub>3</sub>PO<sub>4</sub> 50/35/15 (v/v) and was degassed under vacuum for approximately 20 minutes daily prior to column equilibration. Before sample analysis each day, the column was allowed to equilibrate for approximately 45 minutes at a flow rate of 1.0 ml/min. Upon completion of daily sample analyses, the column was washed sequentially with ACN/water 50/50 (v/v) and methanol/water 50/50 (v/v) for 20 minutes each.

### *Sample Preparation, Extraction, and Analysis*

Prior to extraction, cell samples from the DOX cellular accumulation studies were spiked with 20  $\mu$ l of a 100- $\mu$ g/ml solution of DNR in saline (delivering 2  $\mu$ g DNR) as internal standard. The tumor and liver samples from the xenograft study were weighed on an analytical balance, spiked with IS, and then homogenized prior to extraction. To extract, 9.0 ml of a chloroform/methanol solution (80/20, v/v) were added to all samples, which were contained in glass 16 x 125 mm

tubes (Corning). All samples were vortexed for 1 minute and then centrifuged at approximately 1,500xg for 15 minutes. The organic layer of each sample was removed by pipette, placed in a clean 16 x 125 mm tube, and then evaporated to dryness under a gentle stream of nitrogen in an analytical evaporator at 37°C. Samples were stored at -20°C until they could be analyzed by HPLC.

Calibration standard stock solutions were prepared in normal saline (0.9% NaCl) at the following concentrations (10X): 25; 50; 100; 200; 400; 800; and 1000 ng/ml. Calibration standards for analysis were prepared by spiking 100 µl of each stock solution into 1.0 ml aliquots of plasma. After spiking with IS, extraction was carried out as described above. When not in use, all standard solutions were kept frozen at -20°C in the dark.

To analyze, samples and standards were reconstituted in 200 µl mobile phase, centrifuged at 10,000xg for 1 minute to remove particulates, and then injected onto the HPLC column. Samples were eluted isocratically at a flow rate of 1.0 ml/minute. Retention times and peak heights were recorded. Final sample concentrations of DOX were calculated based on the standard calibration curve and expressed in ng/10<sup>6</sup> cells or ng/g tissue as appropriate.

### Statistical Analyses

Statistical analyses, including t-tests, were performed using Microsoft Excel.

## Results

### Time Course of Cellular DOX Accumulation in Cell Culture

Doxorubicin concentrations in both MDA-MB-231 and MDA-MB-231-A1 cells in culture following exposure to [<sup>14</sup>C]DOX at 2 µg/ml (3.45 µM) for 10 minutes to 4 hours are plotted in Figure 1. At the earliest time points, there was no significant difference in DOX concentrations between the two cell lines. However, by 2 hours, concentrations were significantly higher (P=0.001) in the MDA-MB-231 cells and continued to increase at the 4-hour time point relative to the MDA-MB-231-A1 cells (P=0.000047). At the 4 hour time point, DOX concentrations were 4.7-fold higher in the MDA-MB-231 cells relative to the MDA-MB-231-A1 cells.

### Dose-response of Cellular DOX Accumulation in Cell Culture

Doxorubicin concentrations in MDA-MB-231 and MDA-MB-231-A1 cells in culture following exposure to [<sup>14</sup>C]DOX for 4 hours at doses in the range 0.14 pg/ml (0.24 pM) to 2 µg/ml (3.45 µM) are plotted in Figure 2. The dose response appears to be linear over a seven orders of magnitude dynamic range, and at all doses, DOX concentrations in the MDA-MB-231 cells were significantly higher than in the MDA-MB-231-A1 cells (P≤0.05). However, as the DOX dose was reduced, the difference between the cell lines became less pronounced (2.4-fold difference at 2 µg/ml versus 1.1-fold difference at 0.14 pg/ml).

### Cellular DOX Accumulation in a Xenograft Model

#### *Dose-response for DOX accumulation in MDA-MB-231 tumor cells*

The average weight of the MDA-MB-231 tumors in the mice was  $1.7 \pm 0.97$  grams. The average concentrations of DOX, quantified by AMS, in the tumors, liver and plasma for both the 0.1 and

1 mg/kg body-weight doses are shown in Table 1. In all tissue types, a clear increase in DOX concentrations was detected with increasing dose. The highest concentration was observed in the liver, followed by the tumor tissue, and then plasma. For comparison, HPLC with fluorescence detection was used to analyze the liver and tumor tissues. The HPLC data is compared in Table 2 to that obtained following [<sup>14</sup>C]DOX analysis by AMS in the same tissues. Plasma levels were well below the quantitative limit of the HPLC methodology (25 ng DOX/g tissue) and were not attempted. In all cases, the amounts of [<sup>14</sup>C]DOX in the tissues measured by AMS were well above the controls (the signal to noise ratio was on average 39:1 for liver and 10:1 for tumor tissue). The HPLC with fluorescence detection technique was limited in sensitivity and in many cases was unable to detect DOX. In those samples where DOX was quantifiable by both HPLC and AMS, AMS detected on average approximately 4-fold higher concentrations of <sup>14</sup>C DOX.

#### *Comparison of DOX Accumulation in MDA-MB-231 and MDA-MB-231-A1 Tumor Cells*

Seven animals that grew both MDA-MB-231 and MDA-MB-231-A1 tumors were dosed with [<sup>14</sup>C]DOX. Average tumor weights were  $0.093 \pm 0.041$  and  $0.27 \pm 0.11$ g for MDA-MB-231 and MDA-MB-231-A1 cells, respectively. The amounts of DOX in the tumors, quantified by AMS, are plotted in Figure 3. The mean concentrations of DOX in the MDA-MB-231 and MDA-MB-231-A1 tumors were  $333.5 \pm 86.2$  and  $230.1 \pm 65.8$  ng Dox/g tissue, respectively. The DOX concentrations were significantly higher in the MDA-MB-231 tumors (P=0.01337). Due to the small size of the tumors, there was insufficient tissue to allow for analysis by HPLC.

## Discussion

The relatively low assay sensitivity of traditional analytical methods has limited the clinical value of pharmacokinetic studies in cancer therapeutics. Traditionally, drug levels are measured in plasma or serum and non-target tissues. While there is a correlation between toxicity and plasma pharmacokinetics for at least one cancer chemotherapeutic agent, methotrexate, predicting response and toxicity and determining effective dosing of chemotherapeutic agents based upon plasma or serum pharmacokinetic data has proven to be inconclusive at best. Ideally, drug concentrations should be determined within the target tissue to more accurately assess delivery. The development of new analytical techniques such as AMS that offer greatly enhanced sensitivity over traditional HPLC has now given us the opportunity to begin serious study of tissue-specific drug delivery and kinetics.

For the purpose of demonstrating the utility of AMS, we elected to use a well-described *in vitro* breast cancer model of drug resistance. The model as previously described [3,25] uses the multi-drug resistant human breast cancer cell line MDA-MB-231-A1, which has been previously used to demonstrate the accumulation defect once drug resistance has developed to agents such as doxorubicin [3]. The AMS technique showed the difference in DOX accumulation over time between wild type and MDR+ breast cancer cells exposed to a fixed concentration of DOX *in vitro* in a manner consistent with the previously published results [3,25]. This result was similar to that obtained using HPLC with fluorescence detection. When DOX exposure concentrations were reduced to levels that could not be detected by HPLC, the AMS technique still showed a significantly higher accumulation of DOX by the wild type cells at all concentrations, although the difference was less marked at the lower concentrations. Thus, the AMS technique proved to



be over five orders of magnitude more sensitive than our HPLC method with fluorescence detection, while still being able to demonstrate the accumulation defect.

When DOX accumulation was examined *in vivo*, AMS showed a clear difference in accumulation between a low and higher dose of DOX in wild type breast cancer xenografts. This result was in agreement with HPLC data, although concentrations determined by AMS were generally higher. Possible explanations for the higher concentrations determined by AMS are that AMS is unable to distinguish parent drug from metabolites, unlike HPLC, and that organic extraction from tissue, as used for the HPLC method, is not very efficient. In nude mice bearing both wild type and MDR+ breast cancer xenografts, AMS showed a significantly higher accumulation of DOX in the wild type tumors. The demonstration of the accumulation defect by AMS *in vivo* is significant. With the sensitivity advantages of AMS over other techniques, it may now be possible to determine adequate drug delivery to the target or to identify those patients who may not benefit from standard chemotherapy regimens due to the presence of *de novo* drug resistance.

As stated previously, sensitivity in the zeptomole range has been reported using capillary electrophoresis with laser-induced fluorescence to detect metabolites of doxorubicin in subcellular fractions [1-2]. If this method can be successfully applied to the analysis of biological specimens with the same level of sensitivity as has been demonstrated *in vitro*, and at least one article suggests that this may be possible [21], it would represent an enormous advancement over current bioanalytical methods for fluorescent agents, similar to the AMS method described here. However, one disadvantage of using AMS compared to CE-LIF

methods is that metabolites cannot be distinguished from the parent compound, as mentioned above. Nevertheless, AMS is a novel analytical technique with extreme sensitivity similar to the most sensitive CE-LIF methods available and is another potentially valuable tool for biomedical research. Additional studies need to be performed to determine the clinical value of this new and interesting analytical method.

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**Table 1.** Doxorubicin concentrations in plasma and in liver and tumor tissue from female, athymic, BalbC Nu/Nu nude mice, measured by AMS, two hours following injection with either 0.1 or 1 mg/kg [<sup>14</sup>C]DOX (N=8).

<u>Dose (mg/kg)</u>	<u>Average Tissue Concentration (±SD*)</u>		
	<u>Plasma (ng/ml)</u>	<u>Tumor (ng/g)</u>	<u>Liver (ng/g)</u>
0.1	3.5 ± 1.6	18.8 ± 11.0	357.7 ± 110.8
1.0	40.0 ± 9.6	370.6 ± 78.6	4660.2 ± 572.4

\*SD= standard deviation

**Table 2.** A comparison of DOX concentrations in liver and tumor tissue quantified by AMS versus analysis by HPLC with fluorescence detection (N=8).

<u>Dose (mg/kg)</u>	<u>Average Tissue Concentration (±SD*) (ng/g)</u>			
	<u>Liver (AMS)</u>	<u>Liver (HPLC)</u>	<u>Tumor (AMS)</u>	<u>Tumor (HPLC)</u>
0.1	357.7 ± 110.8	51.0 ± 44.5	18.8 ± 11.0	No detection
1.0	4660.2 ± 572.4	1434.0 ± 244.1	370.6 ± 78.6	50.8 ± 43.6

\*SD= standard deviation

## Figure Legends

1. Doxorubicin concentrations in MDA-MB-231 (■) and MDA-MB-231-A1 (▲) cells following incubation with [ $^{14}\text{C}$ ]DOX for up to 4 hours measured by AMS. Error bars represent the standard deviation of 3 replicate analyses per time point.
2. Doxorubicin concentrations in MDA-MB-231 (●) and MDA-MB-231-A1 (▲) cells measured by AMS following incubation with [ $^{14}\text{C}$ ]DOX at concentrations of 0.2 pg/ml to 2  $\mu\text{g/ml}$  for 4 hours. Error bars represent the standard deviation of 3 replicate analyses per dose point.
3. Doxorubicin concentrations in MDA-MB-231 (solid bars) and MDA-MB-231-A1 (open bars) tumors from 7 female, athymic, BalbC Nu/Nu nude mice, measured by AMS, 2 hours following injection with 1 mg/kg body-weight [ $^{14}\text{C}$ ]DOX. Error bars represent the AMS measurement errors for each sample.

Figure 1.

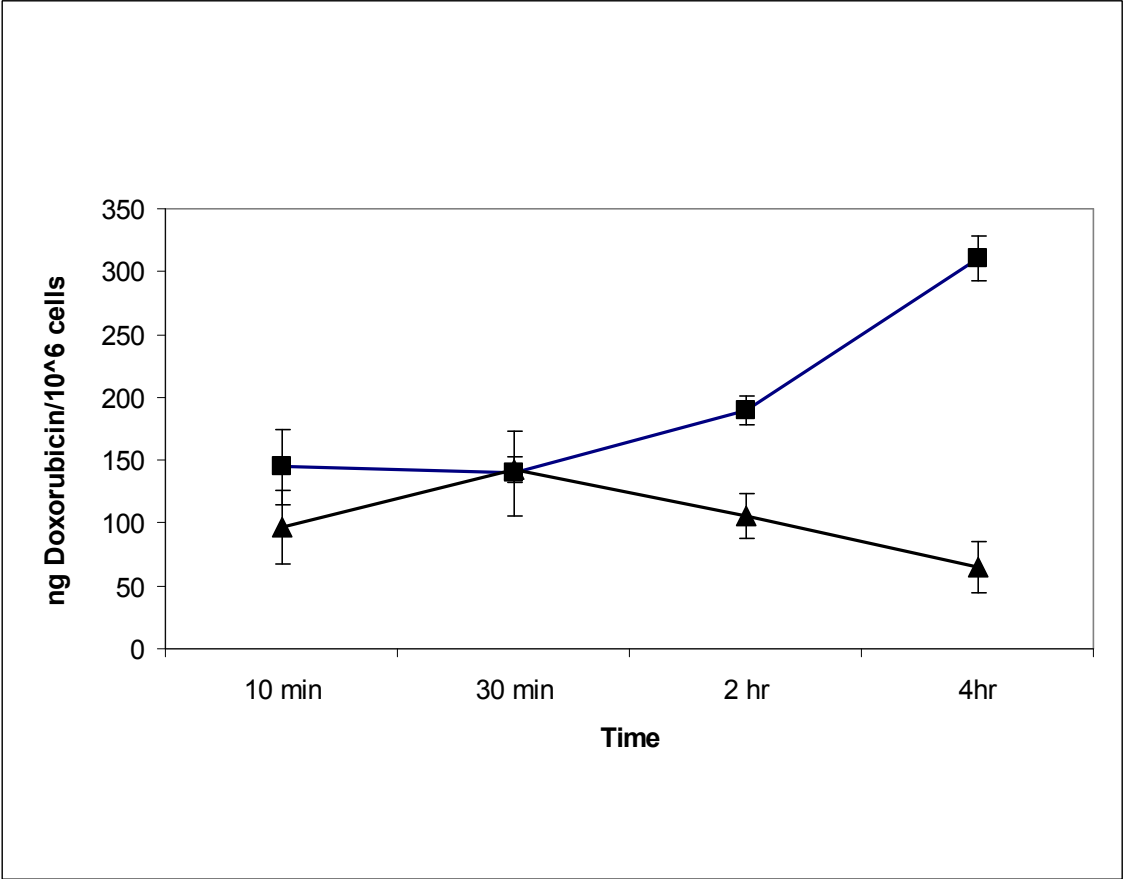




Figure 2.

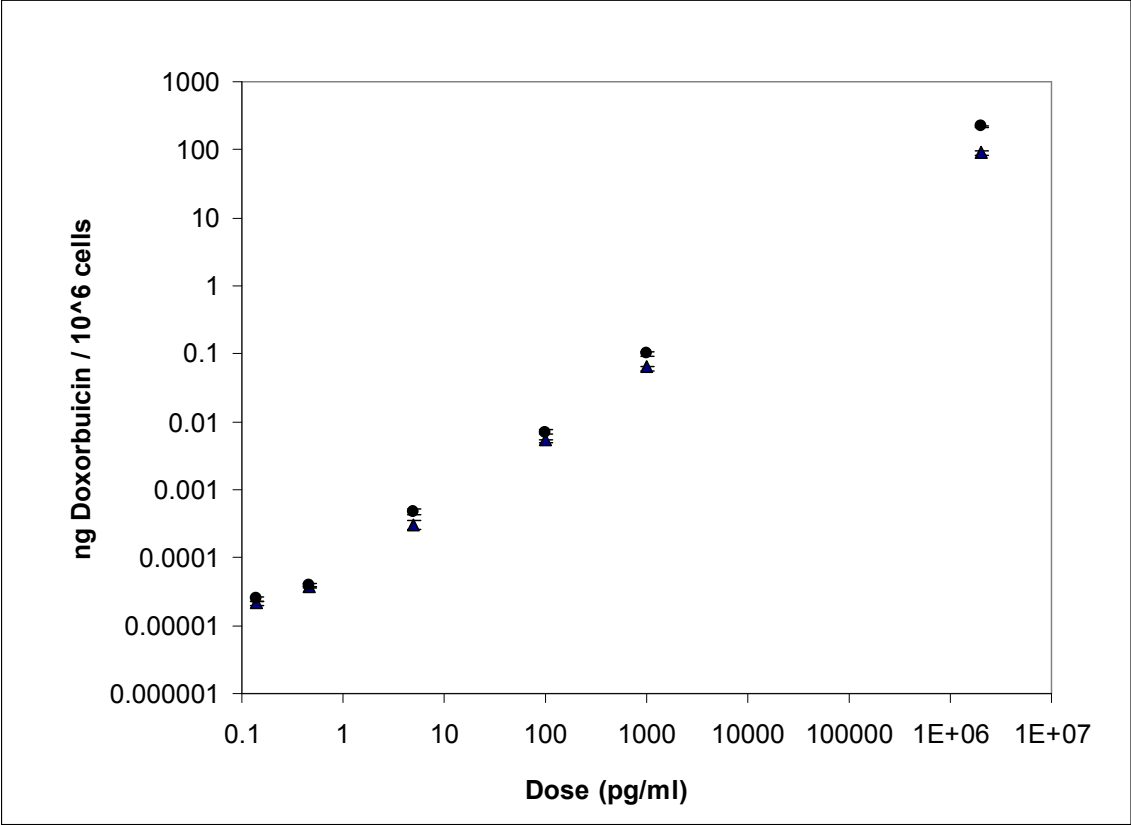


Figure 3.

