Published in final edited form as:

J Liq Chromatogr Relat Technol. 2018 ; 41(5): 232–238. doi:10.1080/10826076.2018.1436068.

Determination of the Transplacental Transfer of Paclitaxel and Antipyrine by High Performance Liquid Chromatography Coupled with Photodiode Array Detector

Shariq Ali^a, Norah Albekairi^b, Xiao-ming Wang^c, Svetlana Patrikeeva^c, Tatiana N. Nanovskaya^c, Mahmoud S. Ahmed^{a,c}, and Erik Rytting^{a,c}

^aDepartment of Pharmacology and Toxicology, The University of Texas Medical Branch, 301 University Blvd. Galveston, TX

^bCollege of Pharmacy, King Saud University, King Khalid Road, Riyadh, Saudi Arabia

^cDepartment of Obstetrics and Gynecology, The University of Texas Medical Branch, 301 University Blvd. Galveston, TX

Abstract

Ex vivo placental perfusion experiments are important in understanding the quantity and mechanisms of xenobiotic transport to the fetus during pregnancy. Our study demonstrates that paclitaxel and antipyrine concentrations in placental perfusion medium containing physiological concentrations of human serum albumin during pregnancy (30 mg/mL) can be quantified by RP-HPLC and UV detection. A liquid-liquid extraction method was developed for the quantification of paclitaxel and celecoxib (internal standard) from perfusion medium. Antipyrine, which is a necessary marker in placental perfusions for determining the validity of experiments and calculating the clearance index of xenobiotics, was also analyzed by HPLC and UV detection. Antipyrine concentrations were determined by HPLC after precipitating the perfusion medium in acetonitrile and separating the precipitated proteins by centrifugation. Concentrations were fitted to linear regressions with R^2 values approaching 1. Lower limits of detection for paclitaxel and antipyrine were 100 ng/mL and 200 ng/mL, respectively. Both methods demonstrated high intra-day and inter-day precision and trueness. Additionally, the use of these methods was demonstrated in a placental perfusion experiment using Taxol[®] (paclitaxel dissolved in Cremophor-EL). The fetal transfer rate of Taxol was 6.6% after 1 hour.

Keywords

paclitaxel; antipyrine; high performance liquid chromatography; photodiode array; placenta; pregnancy

1. Introduction

Breast cancer is one of the most common pregnancy-associated malignancies, occurring in 1 of every 3000 pregnancies. In recent years, it has been hypothesized that the prevalence of breast cancer in pregnancy is increasing because the risk of breast cancer increases with age, and pregnancy is being delayed to later years of life ¹. Physiological changes in breast tissue in pregnant patients may also mask changes that might be occurring due to breast cancer, and diagnostic procedures are limited due to the risk of fetal harm from large or multiple doses of ionizing radiation and the use of contrast agents. In addition to these challenges, treatment of breast cancer is particularly difficult because of the morbidity, including teratogenicity, of chemotherapeutics on the developing fetus ^{1,2}.

Paclitaxel is a chemotherapeutic compound that was originally isolated from the *Taxus brevifolia* tree. This compound (among others) is now used in a number of pharmaceutical formulations for the treatment of breast cancer that are both in preclinical development and clinical use ^{3,4}. In its most widely available formulation, Taxol® (paclitaxel dissolved in Cremophor-EL), it has been shown to cause fetal morbidity in the first trimester but be relatively safe in the second and third trimesters ⁵. During pregnancy, the placenta can facilitate the selective passage of some compounds, such as nutrients, to the fetal circulation while reducing the passage or causing the efflux of other compounds, including xenobiotics. Information regarding the transfer of compounds, including the mechanisms of efflux, can give insight into how they might affect fetal health. For instance, it has been shown that paclitaxel is a substrate of the efflux transporter P-glycoprotein, which is present in the placenta ^{6,7}. Cremophor-EL, the vehicle for paclitaxel delivery in the Taxol formulation, has been shown to affect the function of efflux transporters (P-gp) in the human intestine ⁷. To investigate the mechanisms of transplacental transfer of paclitaxel and what effect these mechanisms might have on fetal morbidity, it is essential to use various *in vitro* and *ex vivo* models. The *ex vivo* dually perfused human placental cotyledon is a reproducible, robust and informative model for examining the transplacental transfer of endogenous compounds and xenobiotics. In this model, antipyrine is typically administered along with the drug of interest because antipyrine freely diffuses across the placenta and has very little tissue accumulation. This allows for comparability between placentas and calculation of the clearance index of the drug ⁸. The investigation of the mechanisms of transport and uptake of these compounds, including interindividual differences, is essential in understanding factors of fetal risk. The ultimate goal from these types of studies is to create rational treatment strategies for patients suffering from breast cancer and other diseases during pregnancy. As such, accessible methods must be developed for determining concentrations of these compounds in model systems such as the *ex vivo* placental perfusion model.

Concentrations of various compounds and xenobiotics in perfusion medium—in addition to antipyrine—can be determined in a number of ways. For instance, radioactive compounds can be detected by beta counting using liquid scintillation analyzers ⁶. Reversed-phase high performance liquid chromatography can be coupled with a number of detectors, such as fluorescence detectors, photodiode array (PDA) detectors, and mass spectrometers (MS), depending on the compound of interest and concentration range ^{9,10}. One consideration regarding the detection of radiolabeled compounds is that, though highly sensitive, this

method is limited to which radiolabeled compounds are available commercially. Pharmaceutical formulations of those compounds in radiolabeled form are unlikely to be available. MS is highly sensitive, but owning and operating an HPLC-MS system is cost prohibitive for many labs. Fluorescence detectors are also quite sensitive, but they are dependent on the compound of interest being fluorescent, which is not always the case. However, PDA detectors have the advantage that they are less expensive and require only that the compound have ultraviolet absorption. The sensitivity of PDA detectors is less than that of fluorescence detectors and mass spectrometers, necessitating special sample preparation methods and chromatographic conditions to optimize detection ⁹.

Methods of determining paclitaxel and antipyrine concentrations in placental perfusion medium include both MS or radiolabeled compounds, both of which are cost-prohibitive ^{10,11}. There exist methods for the determination of paclitaxel in other media, including animal and human plasma, by HPLC using both PDA detectors and MS ^{12–14}. Though some of the available sample preparation methods include solid-phase extraction, preferred methods include liquid-liquid extraction or protein precipitation due to their lower cost and higher throughput. Here, we demonstrate for the first time a sensitive reversed-phase HPLC method for the quantification of both paclitaxel (liquid-liquid extraction) and antipyrine (protein precipitation) in M199 perfusion media containing 30 mg/mL human serum albumin by PDA detector. Albumin is often used in placental perfusions due to the effect of protein binding of drugs on their transplacental transfer ^{10,15}. Methods for the detection of paclitaxel in plasma by HPLC/PDA have also been shown; however, distinct advantages of our method over these methods include improved robustness (allowing concentration determination in the presence of blood accumulation seen in *ex vivo* perfusion studies), and a larger linear range¹⁶. The purpose of demonstrating these methods is to make accessible to other laboratories the tools they need to study paclitaxel transport and uptake mechanisms, and ultimately to further the development of rational treatment strategies for breast cancer in pregnancy. These methods bypass the need for solid-phase extraction, and mass spectrometry, and are applicable to pharmaceutical preparations of these compounds. These methods follow the FDA Bioanalytical method validation guidance for industry. To our knowledge, no currently published method allows sensitive detection by HPLC/PDA detector of paclitaxel and antipyrine in placental perfusion medium.

2. Experimental

2.1 Materials and instruments

Compounds used include paclitaxel (TSZ Scientific LLC, Framingham, Massachusetts), semisynthetic paclitaxel injection in Cremophor-EL (Mylan, Canonsburg, Pennsylvania), celecoxib (TSZ Scientific LLC), antipyrine (Sigma Aldrich, St. Louis, Missouri), gentamicin sulfate salt (Sigma Aldrich), sulfamethoxazole/trimethoprim (SICOR Pharmaceuticals, Inc., Irvine, California), and heparin sodium salt (Sigma Aldrich). Other perfusion medium components include dextran (Sigma Aldrich), human serum albumin fraction V (Calbiochem, La Jolla, California), and medium 199 (Sigma Aldrich). Other chemicals/reagents used include normal saline (0.9% sodium chloride, Nurse Assist, Inc., Fort Worth, Texas), chloroform (HPLC grade, Fisher Scientific, Waltham, Massachusetts), acetonitrile

(HPLC grade, Fisher Scientific), sodium bicarbonate (Fisher Scientific), and formic acid (Fisher Scientific). Deionized water was obtained from a Millipore Synergy UV Ultrapure Water System (Millipore, Billerica, Massachusetts).

The HPLC system used was comprised of a Waters 1525 Binary HPLC pump (Waters Corporation, Milford, Massachusetts), a Waters 2707 Autosampler (Waters Corporation), and a Waters 2998 Photodiode Array Detector (Waters Corporation). A C-18 column (150 mm x 4.6 mm, 5 μ m particle size, 100 Å pore size, AkzoNobel/Kromasil, Brewster, New York) was used for both methods and was kept at ambient temperature.

2.2 Methods

2.2.1. Paclitaxel and antipyrine sample preparation—Perfusion medium for method validation was prepared similarly to previously reported methods (4). Briefly, solid medium 199 with Earle's salts (11 mg/mL), dextran (3 mg/mL for maternal and 12 mg/mL for fetal medium), heparin (29 μ g/mL), gentamicin (33.3 μ g/mL), sulfamethoxazole (80 μ g/mL), trimethoprim (16 μ g/mL), and albumin (30 mg/mL) were dissolved in deionized water. The pH was then adjusted to 7.4 by the addition of sodium bicarbonate. Perfusion medium was stored at 4°C and used within 1 week. Fetal perfusion medium was used for method validation. Studies involving perfused media were conducted using fetal medium perfused in a term placenta and collected at 30 and 120 minutes of perfusion.

Perfusion medium for validation of the paclitaxel method was prepared by spiking paclitaxel (dissolved at 2 mg/mL in acetonitrile) into perfusion medium to concentrations of 100–30,000 ng/mL. Samples for HPLC analysis were then prepared by liquid-liquid extraction. A 250 μ L sample of perfusion medium at each concentration was placed in a glass test tube, to which a 25 μ L solution of celecoxib internal standard solution in acetonitrile was added (final concentration of 5 μ g/mL). To this solution, 3 mL of chloroform was added. This mixture was shaken vigorously, and then the organic and aqueous phases were allowed to separate. From the organic phase, 2 mL were then placed in a glass vial and placed under a stream of nitrogen (ZipVap 24, Glas-Col, Terre Haute, Indiana) and allowed to dry. Once dry, the residue was reconstituted in 100 μ L of a solution of 60% acetonitrile and 40% aqueous formic acid (0.01% v/v) for injection into the HPLC system.

Perfusion medium containing antipyrine was prepared by spiking antipyrine (dissolved at 5 mg/mL in acetonitrile) into perfusion medium to concentrations of 200–25,000 ng/mL. Samples for HPLC analysis were prepared by adding 250 μ L of media into 1 mL of acetonitrile, precipitating the proteins. Insoluble components were pelleted by centrifugation at 15,000 x g for 15 minutes, and 200 μ L of the resulting supernatant was placed in an HPLC vial for analysis.

2.2.2. Chromatographic conditions—For the analysis of paclitaxel samples, 50 μ L of the reconstituted residue was injected into the HPLC system. The mobile phase (1 mL/min) began as an isocratic flow of 60% acetonitrile and 40% aqueous formic acid (0.01% v/v). At 7 minutes, flow was changed to 95% acetonitrile over 3 minutes, held for 3 minutes and then reverted to the initial mobile phase and allowed to baseline for the next injection (total run

time of 21 minutes). Paclitaxel and celecoxib were both detected at 230 nm by the PDA detector.

Antipyrine samples were analyzed by injection of 10 μ L of the prepared solution into the HPLC system described above. The mobile phase (1 mL/min) was an isocratic flow of 35% acetonitrile and 65% aqueous formic acid (0.01% v/v). Total run time was 10 minutes, and antipyrine concentrations were analyzed at 243 nm by PDA detector.

2.2.3. Transplacental transfer of Taxol across the dually perfused human placental lobule

—To test the HPLC method following validation, a perfusion of Taxol was conducted. Placentas were obtained at the John Sealy Hospital Labor and Delivery unit immediately after delivery from uncomplicated pregnancies free of group B streptococcal, HIV, or other infection. A fetal artery and vein supplying one lobule were catheterized using polytetrafluoroethylene tubing. Flow of the perfusion medium through the fetal side of the lobule was 3 mL/min. The maternal side of the same lobule was catheterized by placing tubing into the maternal blood space, and the flow rate was set at 12 mL/min. The fetal reservoir (150 mL) was bubbled with a gas mixture of 95% nitrogen and 5% carbon dioxide, while the maternal reservoir (250 mL) was bubbled with 95% oxygen and 5% carbon dioxide gas. Oxygen and pH were monitored in the maternal and fetal arteries and veins using a Siemens Rapidlab 248 blood gas analyzer (Siemens, Munich, Germany) to ensure adequate oxygen transfer prior to the introduction of antipyrine and paclitaxel in the maternal reservoir. Samples of 0.8 mL were taken from the maternal artery and fetal vein for the duration of the experiment. Each sample was processed and analyzed by HPLC for determination of both antipyrine and paclitaxel concentrations.

2.2.4. Calculations and statistics

—Peak areas were used for the construction of the calibration curve for antipyrine. Peak area ratio, calculated as the ratio of the peak area of paclitaxel to the peak area of celecoxib, was used for the calibration curve of paclitaxel. Calibration curves for antipyrine and paclitaxel were generated in SigmaPlot (version 12.0) using the average of six injections. Linear regressions were made using 1/X weighting to improve trueness at lower concentrations. The lower limit of detection was determined to be the concentration at which the peak area of the analyte was at least three times higher than the blank response at that retention time. Similarly, the lower limit of quantification was determined as the concentration at which the peak area of the analyte was ten times higher than the blank response. Intra-day precision for each day was calculated as the relative standard deviation of the peak areas or peak area ratios of six samples of three concentrations prepared on the same day. Inter-day precision was calculated as the relative standard deviation of peak areas or peak area ratios of 18 injections performed over three days. Recovery was calculated as the peak area of either antipyrine or paclitaxel after undergoing the sample preparation method, divided by the peak area of a standard solution in the initial mobile phase at the theoretical concentration of 100% recovery. Trueness was calculated as the average peak area or peak area ratio for six injections at three concentrations as a percent of the value for those concentrations given by the linear regression. For stability studies, percent change is defined as the difference in the measured concentration of the same sample at day 1 and day 8, divided by the measured concentration

at day 1. Fetal transfer rate was calculated as the concentration of antipyrine or paclitaxel present in the fetal vein as a percent of the initial concentration of that compound in the maternal artery.

3. Results

3.1. HPLC methods

The sample processing methods and chromatographic conditions gave low noise and consistent retention times for both drugs (Figure 1). Paclitaxel had a retention time of 4.4 minutes and celecoxib a retention time of 7.2 minutes when detected at 230 nm under the paclitaxel method chromatographic conditions. Importantly, we observed variability in chromatogram noise after multiple sample injections, likely due to the presence of solutes present in the prepared sample with a high affinity for the reversed-phase column and thus significantly longer retention time. This necessitated a wash step with 95% acetonitrile after the elution of both compounds to stabilize the baseline between samples.

Antipyrine, detected at 243 nm under the antipyrine method chromatographic conditions, had a retention time of 2.4 minutes. Both methods were tested over 9 concentrations. Linear regressions were generated using SigmaPlot with 1/X weighting (Supplementary figure 1, Table 1). The use of 1/X weighting allowed for improved trueness at lower concentrations while maintaining acceptable trueness at higher concentrations. The ultraviolet absorbance spectra of both paclitaxel and antipyrine shown by this method (Supplementary figure 2) agree with previously reported spectra of these compounds^{17,18}.

Both methods were selective and specific for their respective analytes, as determined by comparison of the chromatograms of blank media samples to samples spiked with analytes. Paclitaxel and its internal standard celecoxib were well resolved and exhibited no interference from media. Similarly, antipyrine was well resolved from media components (Figure 1).

3.2. Precision, trueness, recovery, and stability

Precision and trueness was determined at the limits of quantification, half-maximum concentration, and maximum concentration for both paclitaxel and antipyrine. Intra-day precision at each concentration was less than 10% for both drugs at each concentration. Inter-day precision was less than 10% for all antipyrine samples and less than 15% for all paclitaxel samples. Trueness across all concentrations tested for both compounds was close to 100%. Antipyrine recovery was close to 100% for all antipyrine samples. Recovery of paclitaxel was comparable across all concentrations, but it was somewhat lower than that of antipyrine (Table 2).

Recovery and intra-day precision was also tested for both compounds in fetal perfusion medium that had been perfused in a placenta without the addition of paclitaxel or antipyrine for 30 minutes and 120 minutes. Blank media samples and samples spiked with antipyrine and paclitaxel were processed and analyzed by HPLC. Intra-day precision changed slightly in these samples but did not exceed 15%. Recovery of antipyrine samples was similar in perfused media compared to pure perfusion media. Recovery of paclitaxel from perfused

media and pure perfusion media demonstrated slightly more variability than is seen for antipyrine (Table 3).

Determination of sample stability was determined based on typical storage conditions for perfusion samples. Samples of perfusion media containing both antipyrine and paclitaxel at three concentrations for each were analyzed by HPLC once on the day of preparation and again after one week of storage at 4°C. Concentrations were calculated based on the linear regressions generated for each drug. Over one week of storage, all samples exhibited minimal change from the original concentrations (Table 4).

3.3. Placental perfusion and analysis

The methods for detecting paclitaxel and antipyrine were used in a placental perfusion experiment in order to demonstrate the applicability of the method and to determine the fetal transfer rate of paclitaxel (chromatograms shown in supplementary figure 3). Initial paclitaxel concentration (20 µg/mL) was chosen based on plasma C_{max} of paclitaxel in patients¹⁹. The initial concentration of both antipyrine and paclitaxel matched the expected concentration in calibration standards. Oxygen transfer from maternal to fetal circulations was confirmed in each experiment by ensuring that the fetal vein concentration of dissolved oxygen was higher than that of the fetal artery. Antipyrine concentrations appeared to be equilibrating after one hour of perfusion. Paclitaxel fetal transfer rate, calculated as the concentration of paclitaxel in the fetal vein as a percent of the initial maternal concentration, was above the limit of quantification by 15 minutes and approached 7% by one hour (Figure 2).

4. Discussion

A number of methods have been used to detect paclitaxel and antipyrine in placental perfusion medium^{6,10}. The methods in this study are the first to our knowledge to utilize HPLC with UV absorption as a detection method in placental perfusion medium. This is especially challenging because of the concentration of albumin used in this model (30 mg/mL), which is meant to represent the physiological concentration of human serum albumin found in the blood during pregnancy. It is essential to use this concentration in placental perfusions involving protein-bound compounds, as it has been shown that differences in albumin concentration can change the transplacental transfer of such compounds^{10,15}.

For the examination of these methods, fetal perfusion medium was used because of its slightly higher dextran concentration. It is also anticipated in placental perfusion studies that the lowest concentrations of the compound of interest, as well as antipyrine, will be found in the fetal medium at the earliest time points. All components of the media besides dextran are identical between maternal and fetal medium. It was found that the methods demonstrated here are sensitive and specific enough for both compounds. In the case of antipyrine, there was a linear response between concentration and peak area when measured at 243 nm. The limit of quantification was found to be 500 ng/mL, which is sufficiently low for the purpose of placental perfusion studies, as antipyrine typically equilibrates quickly between the maternal and fetal reservoirs. As may be expected, the relative standard deviation between injections at 500 ng/mL was slightly higher than at higher concentrations, but both intra-day

and inter-day precision values were within an acceptable range at the concentrations tested. The paclitaxel method had a lower limit of quantification at 200 ng/mL, and was detectable down to 100 ng/mL. It is known that paclitaxel transfer across the placenta is limited, and thus a highly sensitive method is required for determining these concentrations. Celecoxib has been used previously as an internal standard for paclitaxel and was thus chosen for this study²⁰. This method had acceptable intra-day and inter-day precision and trueness. Supplementary figure 1 demonstrates that calibration curves of both methods are linear in the high and low concentration ranges. Important to note regarding the method for paclitaxel quantification is that other published methods exist. Many of the most sensitive methods for paclitaxel quantification in perfusion medium rely on mass spectrometry, increasing the difficulty with which these methods can be implemented^{10,14}. Methods using ultraviolet absorption as the detection method also exist, and with reasonable sensitivity, albeit in other biological matrices^{16,21}. This is the first report, to our knowledge, of paclitaxel quantification in placental perfusion medium using ultraviolet detection.

Sample stability for both antipyrine and paclitaxel detection was assessed by storing perfusion medium spiked with each drug at 4°C for one week, which was our maximum storage time for samples to be analyzed after a perfusion experiment. Of particular concern regarding sample storage is the hydrolysis of ester groups of paclitaxel, as has been known to occur²². Though this process is slow, especially at physiological pH, any potential changes in peak area must be minimized for accurate quantification of the analyte. One week after storage, drug concentrations at all concentrations had only slight changes, indicating that this storage method for samples would be adequate.

During the placental perfusion experiment, the media used to perfuse the placenta change due to contact with whole human tissue. In particular, blood accumulates in both maternal and fetal reservoirs. Because these changes may alter the recovery and precision of the detection methods, media perfused for 30 minutes and 120 minutes in a placenta was used to determine if there were any changes in recovery from the changing matrix. It appeared generally that detection of drugs in media that had been perfused was less precise than in pure perfusion media. Paclitaxel recovery had the most variability in recovery between pure perfusion medium and perfused medium at the lowest concentration. This is likely due to differences in the partitioning of paclitaxel between organic and aqueous phases upon liquid-liquid extraction. Antipyrine recovery also appeared to have the widest variability at the lowest concentration. Thus, the effect of the change of the matrix was observable, but did not appear to be detrimental to the precision of the method. These determinations demonstrate robustness not shown in prior methods of HPLA/PDA detection of paclitaxel in other biological media¹⁶.

The methods developed here were used in a placental perfusion experiment with Taxol to demonstrate their utility (Figure 2, Supplementary figure 3). Perfusion experiments are considered valid based on adequate antipyrine transfer and minimal leaking from the fetal circulation (less than 2 mL/hour). As can be seen in Figure 2, antipyrine concentrations in both the maternal and fetal reservoirs approach 50% near the end of the experiment. All antipyrine concentrations were above the limit of quantification. Paclitaxel had a much lower fetal transfer rate than antipyrine, though it also appeared to leave the maternal circulation

more rapidly than antipyrine. This is an indication that it likely has higher tissue accumulation compared to antipyrine. Paclitaxel is also known to be a substrate of P-glycoprotein, which is found in the placental trophoblast and limits the transfer of paclitaxel and other xenobiotics into the fetal circulation ⁶. Fetal paclitaxel concentrations at both 5 and 10 minutes were below 200 ng/mL but above 100 ng/mL, indicating that low levels of paclitaxel were present in the fetal circulation even after 5 minutes.

5. Conclusion

The methods demonstrated in this study provide sensitive and selective detection of paclitaxel and antipyrine in placental perfusion medium in concentrations similar to those found in breast cancer patients. Previous methods have shown sensitive detection of paclitaxel and antipyrine, but rely on the use of mass spectrometry. The purpose of the development of this method was to show that, with the correct chromatographic conditions and sample preparation, it is possible to analyze paclitaxel and antipyrine without the need for mass spectrometry, an inherently more expensive instrument that may not be accessible for use for many researchers. Plasma detection by HPLC/PDA of paclitaxel has been shown in human and animal models ^{16,21}. In a similar vein, we designed this project to make the model of *ex vivo* placental perfusions of paclitaxel easier to perform and analyze, with the advantage of being tailored specifically to *ex vivo* placental perfusion medium and tolerating changes in blood concentration across the concentrations assessed. Recovery was shown to be similar across all concentrations for both compounds, with slight variability observed in 30 and 120 minute perfused media. To our knowledge this is the first method to use HPLC and PDA detection for quantification of paclitaxel in *ex vivo* placental perfusion medium containing physiological concentrations of albumin. The determination of the mechanisms of transplacental transfer of paclitaxel is necessary in understanding toxicities of the drug and developing new treatment strategies for malignancies such as breast cancer that arise in pregnancy. We anticipate that the development of this method will make the analysis of paclitaxel transfer in these experiments or other similar experiments more accessible to researchers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by a John Sealy Memorial Endowment for Biomedical Research Pilot Innovation Grant and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (U54HD047891).

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Figure 1.

Chromatograms of paclitaxel and antipyrine, analyzed by RP-HPLC using a PDA detector. (A) Paclitaxel (4.4 minutes) and celecoxib (7.2 minutes) were detected at 230 nm. (B) Antipyrine (2.4 minutes) was detected at 243 nm. (C) Paclitaxel lower limit of quantification and blank media sample shown for comparison. (D) Antipyrine lower limit of quantification and blank media sample shown for comparison.

Figure 2.

Placental perfusion of Taxol and antipyrine. Concentrations are given as a percent of the initial maternal concentration, i.e., 20,000 ng/mL paclitaxel and 20,000 ng/mL antipyrine.

‡Concentration of paclitaxel at these time points was below the lower limit of quantification but above the lower limit of detection.

Table I.

HPLC method regression characteristics

	Slope	Y-intercept	R ² value	LLOD (ng/mL)	LLOQ (ng/mL)
Paclitaxel	0.0716	0.0023	0.9961	100	200
Antipyrine	5741.0286	-668.9761	0.9989	200	500

Table II.

Precision, trueness, and recovery

	Concentration (ng/mL)	Intra-day precision			Inter-day precision	Recovery	Trueness
		Day 1	Day 2	Day 3			
Paclitaxel	200	7.2%	3.7%	8.9%	10.7%	58.9 ± 4.7%	101.1%
	10000	2.9%	2.8%	1.5%	5.7%	60.5 ± 6.1%	102.3%
	20000	2.9%	3.1%	2.8%	13.2%	65.6 ± 1.4%	105.7%
Antipyrine	500	8.9%	9.1%	7.3%	9.7%	95.1 ± 7.0%	98.2%
	10000	1.9%	2.4%	4.1%	6.2%	103.5 ± 4.3%	100.1%
	20000	5.1%	2.7%	0.6%	5.4%	99.5 ± 0.6%	97.9%

Table III.

Recovery of compounds in perfused media

	Spiked Concentration (ng/mL)	Pure perfusion medium		30 minute perfused medium		120 minute perfused medium	
		Precision	Recovery	Precision	Recovery	Precision	Recovery
Paclitaxel	200	7.2%	58.9 ± 4.7%	2.8%	45.3 ± 1.5%	7.1%	48.3 ± 2.6%
	10000	2.9%	60.5 ± 6.1%	3.2%	57.4 ± 2.0%	5.6%	69.2 ± 2.2%
	20000	2.9%	65.6 ± 1.4%	13.0%	54.2 ± 7.1%	5.5%	66.9 ± 2.5%
Antipyrine	500	8.9%	95.1 ± 7.0%	12.4%	99.0 ± 5.4%	10.0%	104.5 ± 9.9%
	10000	1.9%	103.5 ± 4.3%	2.1%	97.6 ± 2.1%	3.2%	106.1 ± 3.4%
	20000	5.1%	99.5 ± 0.6%	1.6%	96.6 ± 1.6%	0.5%	99.9 ± 0.5%

Table IV.

Stability of samples at 4°C

	Concentration (ng/mL)		Percent change
	Day 1	Day 8	
Paclitaxel	173	177	2.5%
	13752	13976	1.6%
	24827	24312	2.1%
Antipyrine	517	519	0.5%
	8780	8697	1.0%
	17602	17494	0.6%