Pre-Clinical Compartmental Pharmacokinetic Modeling of 2-[1-hexyloxyethyl]-2-Devinyl Pyropheophorbide-a (HPPH) a Photosensitizer in Rat Plasma by Validated HPLC Method

Kowthavarapu Venkata Krishna[a], Ranendra Narayana Saha[b], Anu Puri[c], Mathias Viard[d], Bruce A. Shapiro[c], Sunil Kumar Dubey[a]*

[a]*Department of Pharmacy, Birla Institute of Technology and Science, Pilani (BITS-PILANI), Pilani Campus, Rajasthan, INDIA*

[b]*Department of Biotechnology, Birla Institute of Technology and Science, Pilani (BITS-PILANI), Dubai Campus, Dubai - United Arab Emirates*

[c]*RNA Structure and Design Section, RNA Biology Laboratory (RBL), Center for Cancer Research, National Cancer Institute – Frederick, Frederick, MD, USA*

[d]*Basic Science Program, CIP, Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, MD, USA*

Supplementary Data

Table S1: Solid phase extraction method for HPPH

Procedure	Reagent	Flow rate (µL/min)	Volume (µL)	n
Conditioning	Methanol	4000	1000	2
Equilibration	Milli Q water	4000	1000	2
Loading	Sample	2000	600	1
Cartridge wash	1% Acetonitrile	2000	1000	1
Elution	Acetonitrile	2000	1000	2

n, number of times

Table S2: Optimization of Solid-Phase Extraction (SPE) method for sample preparation

Extracting technique	Extracting solvent	Sample volume (µL)	Volume of extraction solvent added (mL)	Vortex time (min)	Centrifugation [speed (rpm), time (min)]	% Recovery	Remarks
Protein Precipitation (PP)	Acetonitrile	200	3	5	6000, 15	42-48%	Poor recovery, interference of plasma was observed and inconsistent recovery
PP	Methanol	200	3	5	6000, 15	35-39%	Poor recovery, interference of plasma was observed and inconsistent recovery
Liquid-Liquid extraction (LLE)	Methylene chloride (DCM)	200	3	5	6000, 15	19-25 %	Very poor recovery and high interference of plasma was observed
LLE	n-hexane	200	3	5	6000, 15	29-35 %	Increase in the recovery of analyte was observed, but recovery was inconsistent and high plasma interference was observed
LLE	n-hexane: IPA (97:3 v/v)	200	3	5	6000, 15	35-39 %	Slight increase in the recovery of analyte was observed, but recovery was inconsistent and high plasma interference was observed
LLE	n-hexane: IPA (95:5 v/v)	200	3	5	6000, 15	37-41%	Similar recovery of analyte was observed, but recovery was inconsistent and high plasma interference was observed
Solid-Phase extraction (SPE)	Acetonitrile	200	2	2	--	59-64%	No interference of plasma proteins was observed but recovery was reduced
SPE	Methanol	200	2	2	--	51-55%	Consistent recovery was observed, no interference of plasma proteins was observed
SPE	Acetonitrile: 10 mM Ammonium formate (pH:4.2) (90:10 v/v)	200	2	2	--	57-61%	Slight decrease in the recovery of analyte was observed, no interference of plasma proteins was observed
Based on the above results, SPE was selected as the extracting technique and acetonitrile as solvent of choice for elution and sample volume were further optimized							
SPE	Acetonitrile	100	2	2	--	56-59%	LLOQ was high (in µg/mL)
		200	2	2	--	59-64%	LLOQ was improved (in ng/mL)
		300	2	2	--	**61-65%**	Consistent recovery and reproducible with no plasma interference, LOD was observed at 80 ng/mL, I.S. recovery was also good and consistent

Table S3: Regression parameters of the calibration curve generated for each weighting factor (w_i) and their respective sum of the relative errors (Σ%RE)

Model (w_i)	*b*	*a*	r^2	Σ%RE
Unweighted	0.000300	-0.060000	0.999200	28.756462
1/var	0.000296	-0.041152	0.998916	9.022589
$1/x^2$	0.000289	-0.038070	0.999981	0.256647
1/x	0.000297	-0.045214	0.999523	1.125505
$1/x^{1/2}$	0.000299	-0.050980	0.999641	8.632172
$1/y^2$	0.000283	-0.035209	0.996163	7.020774
1/y	0.000296	-0.043203	0.999422	3.842983
$1/y^{1/2}$	0.000298	-0.049315	0.999637	6.818966

*b,*slope; *a,*constant;r^2, regression co-efficient

Fig.S1 Schematic representation of a two-compartmental model