Electronic Supplementary Material (ESI) for RSC Advances.

A Pre-Clinical Pharmacokinetic-Pharmacodynamic Modelling and Biodistribution Studies of Donepezil Hydrochloride by Validated HPLC Method

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Supplementary Data

Fig. S1

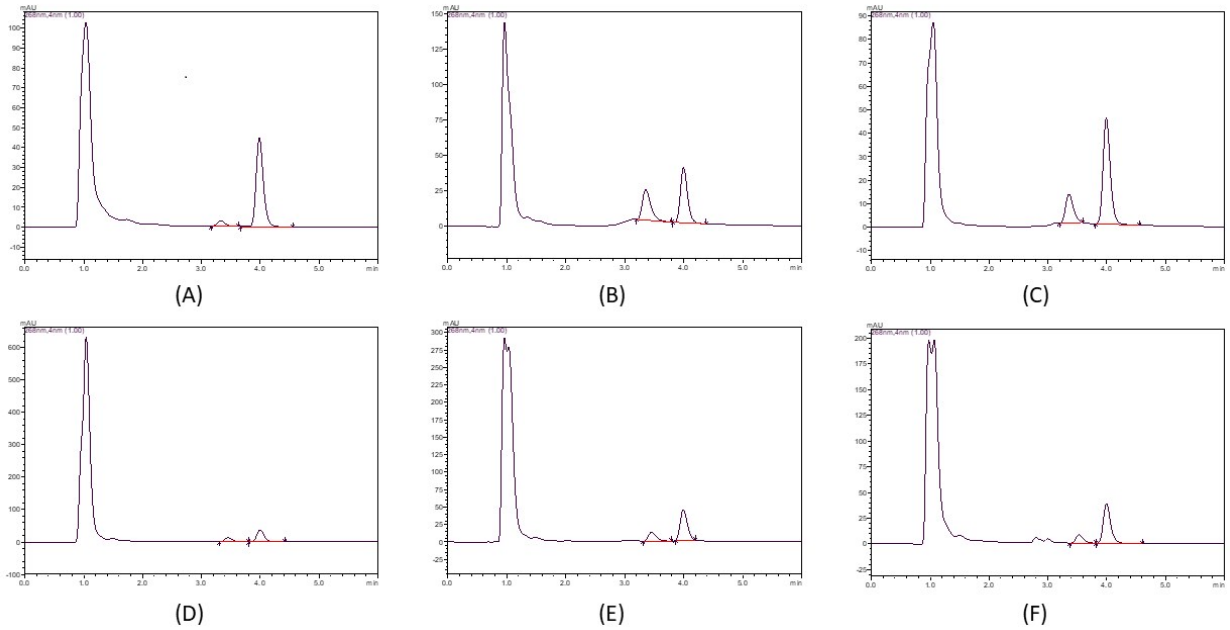

Fig. S1: Representative HPLC Chromatograms for (A) Brain (B) Spleen (C) Heart (D)Kidney (E) Lungs (F) Liver

Table S1

Solid phase extraction method for DNP

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% Methanol	2000	1000	1
Elution	Methanol	2000	1000	2

n, number of times

Table S2

Method optimization: system suitability parameters obtained upon performing deliberate variations in the chromatographic conditions.

Variations in Chromatographic condition	Effect on chromatographic parameters of analytes (n=6)								
	t_R (min)		Tailing factor (10%)		HETP		N		£Resolution
	DNP	IS	DNP	IS	DNP	IS	DNP	IS	
No variation (¥Optimized)	3.31 ± 0.01	3.99 ± 0.01	1.05 ± 0.06	1.14 ± 0.04	69.96 ± 6.44	30.20 ± 1.09	2431.83 ± 143.65	4993.86 ± 187.30	2.88 ± 0.09
Flow rate (0.8 mL/min)	4.25 ± 0.02	5.01 ± 0.01	1.22 ± 0.08	1.27 ± 0.01	75.22 ± 17.72	29.77 ± 0.27	2076.87 ± 175.78	5037.57 ± 46.35	2.30 ± 0.16
Flow rate (1.2 mL/min)	2.80 ± 0.02	3.32 ± 0.01	1.28 ± 0.10	1.19 ± 0.02	86.25 ± 12.71	38.15 ± 0.45	1528.87 ± 219.47	3931.61 ± 47.57	1.49 ± 0.32
Mobile phase composition (60:40 % v/v)	3.49 ± 0.01	4.43 ± 0.02	1.22 ± 0.17	1.18 ± 0.01	68.71 ± 6.57	31.49 ± 0.28	2198.00 ± 209.92	4763.50 ± 43.90	3.35 ± 0.12
Mobile phase composition (64:36 % v/v)	3.18 ± 0.02	3.71 ± 0.18	1.15 ± 0.07	1.18 ± 0.02	82.25 ± 20.70	36.42 ± 0.32	1275.77 ± 172.56	4118.09 ± 34.34	1.46 ± 0.34
Column temperature (35 °C)	3.55 ± 0.04	4.08 ± 0.04	1.31 ± 0.09	1.28 ± 0.04	56.01 ± 11.94	34.40 ± 0.32	2772.14 ± 88.70	4360.73 ± 41.14	2.02 ± 0.02
Column temperature (45 °C)	3.33 ± 0.08	3.90 ± 0.02	1.36 ± 0.07	1.29 ± 0.02	45.71 ± 1.04	32.97 ± 0.38	2793.95 ± 61.73	4549.15 ± 53.31	2.36 ± 0.06
Buffer (pH:4.4)	2.69 ± 0.12	3.99 ± 0.09	1.37 ± 0.09	1.24 ± 0.04	78.75 ± 2.39	33.44 ± 0.09	1921.48 ± 135.36	4485.23 ± 52.85	5.35 ± 0.08
Buffer (pH:8.4)	3.53 ± 0.16	3.97 ± 0.11	1.34 ± 0.14	1.27 ± 0.09	48.75 ± 2.87	30.50 ± 0.95	3079.95 ± 244.04	4965.65 ± 31.81	1.89 ± 0.18

¥Optimized; Flow rate-1 mL/min, Mobile phase composition (62:38 % v/v), Column temperature (40 °C), Buffer pH (6.4)
£Resolution; Resolution factor for DNP and IS peak
t_R, retention time; HETP, height equivalent to theoretical plate; N, number of theoretical plate

Table S3

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	5000, 15	35-42%	Poor recovery, interference of plasma was observed and inconsistent recovery
PP	Methanol	200	3	5	5000, 15	40-49%	Poor recovery, interference of plasma was observed and inconsistent recovery
Liquid-Liquid extraction (LLE)	Methylene chloride (DCM)	200	3	5	5000, 15	25-37 %	Very poor recovery and high interference of plasma was observed
LLE	n-hexane	200	3	5	5000, 15	64-72 %	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	5000, 15	65-79 %	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	5000, 15	68-78%	Similar recovery of analyte was observed, but recovery was inconsistent and high plasma interference was observed
Solid-Phase extraction (SPE)	Acetonitrile	200	2	2	--	49-64%	No interference of plasma proteins was observed but recovery was reduced
SPE	Methanol	200	2	2	--	70-78%	Consistent recovery was observed, no interference of plasma proteins was observed
SPE	Methanol: 10 mM Ammonium formate (pH:6.4) (90:10 v/v)	200	2	2	--	66-72%	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 methanol as solvent of choice for elution and other extraction conditions (sample volume) were further optimized						
SPE	Methanol	100	2	2	--	75-76 %	LLOQ was high (in µg/mL)
		200	2	2	--	77-79 %	LLOQ was improved (in ng/mL)

	300	2	2	--	**78-80%**	Consistent recovery and reproducible with no plasma interference, LOD was observed at 20 ng/mL, I.S. recovery was also good and consistent

Table S4

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	0.000400	-0.0114	0.9999	-5.6094
1/var	0.000383	-0.0082	0.9997	3.9284
$1/x^2$	0.000384	-0.0088	0.9999	0.5904
1/x	0.000382	-0.0083	0.9995	0.7194
$1/x^{1/2}$	0.000384	-0.0093	0.9999	4.1923
$1/y^2$	0.000380	-0.0081	0.9993	0.7303
1/y	0.000384	-0.0087	0.9999	1.4182
$1/y^{1/2}$	0.000384	-0.0092	0.9999	3.2725

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