Electronic Supplementary Material (ESI) for RSC Advances.

Development and validation of a sensitive LC-MS/MS method for Pioglitazone: Application towards pharmacokinetic and tissue distribution study in rats

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Table S-I: A staggering collection of blood and tissue samples for pharmacokinetic studies of PGZ in rats

Animals	0 hrs	0.5hrs	1hrs	3 hrs	5hrs	7 hrs	24 hrs
1	B	B	NC	B	NC	B	B
2	B	B	NC	B	NC	B	T
3	B	B	NC	B	NC	B	T
4	B	B	NC	B	NC	T	X
5	B	B	NC	T	NC	X	X
6	B	T	X	X	X	X	X
7	B	NC	B	NC	B	B	B
8	B	NC	B	NC	B	B	T
9	B	NC	B	NC	B	B	T
10	B	NC	B	NC	B	T	X
11	B	NC	B	NC	T	X	X
12	B	NC	T	X	X	X	X

B: blood**; T:** tissues; **NC**: no collection; X: None

Table S-II: Mobile phase compositions used for LC-MS/MS method development

S.No	Organic phase		Aqueous phase	Aqueous: organic Ratio	Inference
1.	Ammonium acetate	1mM	Acetonitrile	50:50 60:40 70:30 80:20 90:10 95:05	Peak tailing and Spilt peak
		2mM			
		3mM			
		4mM			
		5mM			
2.	Ammonium formate	1mM	Acetonitrile	50:50 60:40 70:30 80:20 90:10 95:05	Peak tailing and fronting
		2mM			
		3mM			
		4mM			
		5mM			
3.	Acetic acid	0.1% v/v	Acetonitrile	50:50 60:40 70:30 80:20 90:10 95:05	Spilt peak and Peak tailing
		0.5% v/v			
		1% v/v			
		2% v/v			
		3% v/v			
4.	Formic acid	0.1% v/v	Acetonitrile	50:50 60:40 70:30 80:20 90:10 95:05	Fronting Peak tailing Peak tailing Peak tailing Peak tailing Good peak

Table S-III: Comparison of various methods available for the estimation of Pioglitazone in biological matrices

Authors	Species and Biological matrix	Pharmacokinetics data				Various method	Method validation					Collision energy (eV)	Run time (min)	Retention Time (Min)	Ref.
		C_{max} (ng/ml)	$t_{1/2(hr)}$	T_{max} (hr)	AUC (0-24 hrs) ng/h/ml		Linearity range (ng/ml)	Precision (Intra & Inter day) %	Accuracy (Intra &inter day) %	LLOQ ng/ml	LOD ng/ml				
Gananadhamu et al.	RP	6062	4.9	1.5	55116	LC-ESI-MS/MS (SM-Sitagliptin)	8 - 1571	9.5-9.8 & 6.1-6.5	101.0-102.8 & 96.3-98.2	8.2	-	39	5	2.81	(1)
Elgawish et al.	RP	1920	10.8	1.0	18214	LC-MS/MS (SM-metformin)	1 - 2500	0.6-14.3 & 0.6-10.3	95.8–104.6 & 93.2-95.5	14.7	0.95	40	15	14.72	(2)
Sengupta et al.	RP	3870	-	3.6	4158	LC-MS/MS (SM-Telmisartan)	5–10000	3.1 -6.7 & 5.6 -8.1	94.2-106.1 & 92.3-98.9	5.00	-	40	-	-	(3)
Abdel-Ghany et al.	HP	1462	5.9	1.5	12178	LC-MS/MS (SM-Alogliptin)	25-2000	2.5 – 1.4 & 1.9 –0.5	99.4-100.4 & 94.6-97.4	25.0	-	40	-	-	(4)
Hess et al.	HP	-	-	-	-	LC-MS (SM-Hydroxy tolbutamide, Vildagliptin)	1-750	5.4 -10.1 & 8.9-13.0	96.4-100.5 & 94.9-98.6	25.0	-	43	-	-	(5)
Jagadeesh et al.	HP	709	-	4.5	20640	LC-MS (SM-Metformin)	15–2500	13.2-15.1 & 41.8-42.7	88.0-97.4 & 84.3-89.5	15.0	-	55	4	2.6	(6)
Zhang et al.	DP	2,640	5.8	0.9	7121.9	LC-MS/MS (SM-Metformin)	1-1000	9.6 & 6.5 & 9.7-6.8	90.7-101.6 & 89.6-94.3	1.0	0.50	31	8	6.1	(7)
Sengupta et al.	HP	-	-	-	-	LC-MS/MS (SM)-Glimepride	2–1000	2.5-2.7& 2.6-2.7	96.0–105.1 & 94.3-97.0	2.5	-	60	6	2.71	(3)
Khadiga et al.	HP	1847	8.8	3.7	26572	LC-MS/MS (SM-Aloglitpin)	10-3000	10-10.6 & 9.6-10	96.6-103.3 & 90.2-97.6	10	-	60	-	3.01	(8)
Current method	RP	495	5.6	1.0	1056	LC-MS/MS	1-500	6.0-8.1 & 7.5-9.8	93.2-97.6 & 95.8-98.7	1.0	0.50	30	4	2.45	--
	RA	247	4.38	1.0	666			5.2-7.0 & 6.0-8.0	93.7-98.3 & 91.9-						

									97.6						
	RH	125	5.16	3.0	780			6.1-8.2 & 6.8-8.9	88.8-92.8 & 87.7-90.9						
	RB	24.	4.37	5.0	194			6.2-10.1 & 9.5-11.6	89.9-91.0 & 87.6-90.0						
	RK	95	2.78	3.1	3969			6.7-8.4 &-7.4-10.1	95.0-96.8 & 94.9-96.0						
	RBr	39	2.75	3.1	166			6.64-8.51%&7.69-10.14%	93.4-95.6 & 91.7-95.0						

AUC – Area Under curve; LLOQ – Lower limit of Quantification; ESI- Electron Spray Ionization, SM- Simultaneous method, RP- Rat plasma, RA- Rat adipose tissue, RH- Rat heart, RB-Rat bone, RK- Rat kidney, RBr- Rat brain, HP- Human plasma, DP- Dog plasma.

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