

## Supporting Information

**A sensitive and selective liquid chromatography mass spectrometry method for simultaneous estimation of anti-diabetic drugs inhibiting DPP-4 enzyme in human plasma. Overcoming challenges associated with low recovery and sensitivity.**

Shantikumar S, Prashanth B, Lingesh A, David Paul, Srinivas R, Satheesh Kumar N.

**\*Corresponding Author** : Dr. N. Satheesh Kumar, M. Pharm., Ph.D., Assistant Professor  
Department of Pharmaceutical Analysis National Institute of Pharmaceutical Education and  
Research [NIPER], Balanagar, Hyderabad-500037, India, E.mail: [satish@niperhyd.ac.in](mailto:satish@niperhyd.ac.in),  
Phone: +919652766320

Table S1. Study of recovery using diluents like 0.1% acetic acid, 0.1% formic acid, 0.1 % trifluoro acetic acid and acetonitrile for vildagliptin (VIL).

Diluent	Concentration of VIL (ng/mL)					
	Polypropylene			Glass		
	3	30	300	3	30	300
0.1 % acetic acid	2.3812	27.8445	273.1823	2.4599	24.7231	257.9664
	2.5134	25.9345	269.6732	2.3445	22.8824	260.9416
	2.2214	26.0132	276.9743	2.2959	23.4457	270.4295
Mean	2.3766	26.5933	273.2733	2.3667	23.6837	263.1125
STD	0.1350	1.0803	3.6508	0.0842	0.9431	6.5089
% Recovery	79.2222	88.6444	91.0911	78.8922	78.9457	87.7041
0.1 % trifluoro acetic acid	2.8001	26.42	254.7	2.8859	26.3829	244.4128
	1.9323	26.88	253.93	2.697	26.0909	250.3432
	2.8867	25.59	257.93	2.6812	27.667	243.2161
Mean	2.5366	26.2966	255.52	2.7547	26.7136	245.9907
STD	0.5269	0.6537	2.1223	0.1139	0.8385	3.8166
% Recovery	84.5555	87.6555	85.17333	91.8233	89.0453	81.9969
0.1% formic acid	3.3456	31.0539	274.5951	2.1151	27.0539	253.2488
	3.0327	29.0228	263.763	2.5669	27.0228	256.9274
	2.8489	28.2788	275.4254	2.0954	28.2788	255.3543
Mean	3.0790	29.4518	271.2612	2.2591	27.4518	255.1768
STD	0.2524	1.4364	6.5068	0.2667	0.7163	1.8457
% Recovery	102.3333	98.1727	90.4203	75.3044	91.5061	85.0589
Acetonitrile	2.8481	28.1641	289.7419	3.0964	26.3833	294.8317
	2.8742	25.952	292.644	2.54	26.0466	289.5476
	2.2224	27.3146	286.1049	2.4117	26.4224	286.1857
Mean	2.6482	27.1435	289.4969	2.6827	26.2841	290.1883
STD	0.3690	1.1159	3.2764	0.3639	0.2066	4.3584
% Recovery	88.2744	90.4785	96.4989	89.4233	87.6136	96.7294

Table S2. Study of recovery using diluents like 0.1% acetic acid, 0.1% formic acid, 0.1 % trifluoro acetic acid and acetonitrile for saxagliptin (SAX).

Diluent	Concentration of SAX (ng/mL)					
	Polypropylene			Glass		
	3	30	300	3	30	300
0.1 % acetic acid	2.5824	29.9767	294.8968	3.0582	27.9899	256.689
	3.4408	28.5057	277.2088	2.6442	28.5911	268.6166
	3.1369	28.827	282.7204	2.8648	26.6359	269.1789
Mean	3.0534	29.1031	284.942	2.8557	27.7389	264.8282
STD	0.4353	0.7734	9.0509	0.2071	1.0015	7.0543
% Recovery	101.7789	97.0104	94.9806	95.1911	92.4632	88.2761
0.1 % trifluoro acetic acid	3.3881	29.8432	279.6561	2.9482	24.242	271.3002
	3.301	28.8141	281.6229	3.5809	30.2454	264.7626
	3.3663	30.0003	280.0287	3.2546	29.1676	270.2334
Mean	3.3518	29.5525	280.4359	3.2612	27.885	268.7654
STD	0.0453	0.6443	1.0447	0.3164	3.2006	3.5073
% Recovery	111.7267	98.5084	93.47863	108.7078	92.95	89.5885
0.1% formic acid	3.7539	30.2995	288.4595	1.5315	30.2995	250.2344
	3.4047	32.2285	280.1223	1.9594	25.2285	252.721
	3.3706	31.925	278.8338	1.4344	24.925	256.9189
Mean	3.5097	31.4843	282.4719	1.6418	26.8177	253.2914
STD	0.2121	1.0373	5.2253	0.2793	3.0192	3.3786
% Recovery	116.9911	104.9478	94.1573	54.7256	89.3922	84.4305
Acetonitrile	3.1654	29.7398	313.7027	2.9364	29.643	319.9265
	3.2703	29.8306	307.7595	2.9942	29.2272	305.2905
	2.8606	29.7251	307.2654	3.175	29.7916	320.8583
Mean	3.0988	29.7652	309.5759	3.0352	29.5539	315.3584
STD	0.2128	0.0571	3.5825	0.1245	0.2926	8.7315
% Recovery	103.2922	99.2172	103.192	101.1733	98.5131	105.1195

Table S3. Study of recovery using diluents like 0.1% acetic acid, 0.1% formic acid, 0.1 % trifluoro acetic acid and acetonitrile for sitagliptin (SIT).

Diluent	Concentration (ng/mL)					
	Polypropylene			Glass		
	3	30	300	3	30	300
0.1 % acetic acid	2.159	26.5411	315.6356	2.6014	22.5908	271.9005
	2.7265	26.0369	313.2978	2.4298	21.9393	282.8101
	2.5557	25.9892	308.4133	2.4364	22.6802	278.0529
Mean	2.4804	26.1890	312.4489	2.4892	22.4034	277.5878
STD	0.2911	0.3058	3.6852	0.0972	0.4044	5.4696
% Recovery	82.68	87.2969	104.1496	82.9733	74.6781	92.5293
0.1 % trifluoro acetic acid	3.0901	28.3975	319.9387	3.0066	27.0002	288.8749
	3.419	28.3511	316.09	3.4081	26.4885	295.6252
	3.1552	28.3592	319.4784	3.0403	26.0575	292.0427
Mean	3.2214	28.3693	318.5024	3.1516	26.5154	292.1809
STD	0.1742	0.0247	2.1018	0.2227	0.4719	3.3773
% Recovery	107.3811	94.5642	106.1675	105.0556	88.3847	97.3936
0.1% formic acid	4.0292	37.3679	338.9608	2.3426	27.3679	292.9326
	3.11	30.7103	331.5842	2.6896	27.7103	288.9213
	3.7343	29.9262	333.5891	1.9711	27.9262	292.7856
Mean	3.6245	32.6681	334.7114	2.3344	27.6681	291.5465
STD	0.4693	4.0889	3.8142	0.3593	0.2815	2.2746
% Recovery	120.8167	108.8938	111.5705	77.8144	92.2271	97.1821
Acetonitrile	3.0225	30.2184	323.6499	2.5457	23.3935	311.8969
	3.1594	29.1035	315.9862	2.9215	23.8521	306.6095
	2.7683	28.008	309.5032	2.5213	23.3789	307.3906
Mean	2.9834	29.1099	316.3798	2.6628	23.5415	308.6323
STD	0.1985	1.1052	7.0815	0.2243	0.2691	2.8540
% Recovery	99.4467	97.0332	105.4599	88.7611	78.4717	102.8774

Table S4. Study of recovery using diluents like 0.1% acetic acid, 0.1% formic acid, 0.1 % trifluoro acetic acid and acetonitrile for linagliptin (LIN).

Diluent	Concentration (ng/mL)					
	Polypropylene			Glass		
	3	30	300	3	30	300
0.1 % acetic acid	3.0491	33.0267	263.3356	1.3028	18.7827	190.0781
	1.8034	30.1906	261.604	1.3912	17.4579	195.784
	2.4002	28.6165	254.765	1.0837	16.907	191.9911
Mean	2.4176	30.6113	259.9015	1.2592	17.7159	192.6177
STD	0.6230	2.2349	4.5318	0.1583	0.9641	2.9041
<b>% Recovery</b>	<b>80.5856</b>	<b>102.0376</b>	<b>86.6338</b>	<b>41.9744</b>	<b>59.0529</b>	<b>64.2059</b>
0.1 % trifluoro acetic acid	3.0216	30.9076	265.8895	3.0197	29.5096	247.0762
	2.7796	30.2271	268.8633	2.5733	29.0551	255.3517
	3.3127	30.5561	272.6607	2.6818	28.593	243.6446
Mean	3.0379	30.5636	269.1378	2.7582	29.0526	248.6908
STD	0.2669	0.3403	3.3939	0.2328	0.4583	6.0182
<b>% Recovery</b>	<b>101.2656</b>	<b>101.8787</b>	<b>89.7126</b>	<b>91.9422</b>	<b>96.8419</b>	<b>82.8969</b>
0.1% formic acid	3.3545	29.8018	295.838	0.4461	21.8018	217.6048
	2.7999	27.4061	274.0868	0.9307	22.4061	209.7765
	3.1314	28.0611	267.1174	1.1891	24.0611	214.3366
Mean	3.0953	28.423	279.0141	0.8553	22.7563	213.906
STD	0.2791	1.2382	14.9809	0.3771	1.1696	3.9319
<b>% Recovery</b>	<b>103.1756</b>	<b>94.7433</b>	<b>93.0046</b>	<b>28.51</b>	<b>75.8544</b>	<b>71.3019</b>
Acetonitrile	2.7077	31.0679	281.7317	1.3357	15.1977	208.8789
	2.4725	30.3544	255.1538	1.3689	15.3843	202.2313
	1.8912	31.0991	255.4979	1.0337	14.3935	206.1667
Mean	2.3571	30.8405	264.1278	1.2461	14.9918	205.759
STD	0.4203	0.4212	15.2464	0.1847	0.5265	3.3425

% Recovery	78.5711	102.8016	88.0426	41.5367	49.9728	68.5863
------------	---------	----------	---------	---------	---------	---------

Table S5. Study of recovery using diluents like 0.1% acetic acid, 0.1% formic acid, 0.1 % trifluoro acetic acid and acetonitrile for teneligliptin (TEN).

Diluent	Concentration (ng/mL)					
	Polypropylene			Glass		
	3	30	300	3	30	300
0.1 % acetic acid	2.7178	30.1208	276.9821	0	19.8503	237.7693
	1.7408	29.8476	282.0224	1.5712	20.5622	237.1028
	1.0692	27.9245	277.5815	2.7847	20.4921	240.2083
Mean	1.8426	29.2976	278.862	1.4519	20.3015	238.3601
STD	0.8290	1.1969	2.7533	1.3962	0.3924	1.6349
% Recovery	61.42	97.6588	92.954	48.3989	67.6718	79.4534
0.1 % trifluoro acetic acid	3.9684	27.2271	294.5008	2.8124	29.4448	272.0046
	3.9366	30.1836	294.534	1.9162	27.2953	278.1967
	2.9867	29.4444	294.6597	2.0406	28.4943	274.3448
Mean	3.6306	28.9517	294.5648	2.2564	28.4115	274.8487
STD	0.5578	1.5385	0.0838	0.4855	1.0771	3.1266
% Recovery	121.0189	96.5056	98.1883	75.2133	94.7049	91.6162
0.1% formic acid	3.0456	26.5678	295.401	0	17.4055	232.9455
	3.3059	28.7895	296.4169	0	18.9958	231.669
	0.371	26.5467	288.9763	0	0	236.2025
Mean	2.2408	27.3013	293.5981	0	12.1338	233.6057
STD	1.6245	1.2888	4.0347	0	10.5382	2.3377
% Recovery	74.6944	91.0044	97.8660	0	40.4458	77.8685
Acetonitrile	4.1125	32.3922	285.9734	1.8508	0	250.8775
	1.9001	29.6111	275.2096	0	16.9824	242.217
	0.8621	30.1461	275.8807	0.5562	0	233.6039
Mean	2.2916	30.7165	279.0212	0.8023	5.6608	242.2328

STD	1.6602	1.4757	6.0301	0.9496	9.8047	8.6368
% Recovery	76.3856	102.3882	93.0071	26.7444	18.8693	80.7443