Supporting Information

J. Chem. Metrol. **15:2 (2021) 124-134**

Simultaneous estimation of Pyridoxine HCl and FMOC-Leucine using derivative and chromatographic approach in pharmaceutical dosage form

Chaitali Patel[1], Akhil Nagar[2*], Pritam Jain[2], Samadhan Shinde [2], Ruchita Bardiya[2] and Atul Shirkhedkar[2]

[1]Research Associate, Omgene Life Sciences Pvt. Ltd., Vadodara, Gujarat, India 390001

[2]R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule, Maharashtra, India 425405

Table of Contents **Page**

Table S1: Analysis of marketed preparation

Formulation	Drug name	Actual amt. (mg/mL) (n=6)	Amt. found (mg/mL) (n=6)	% Assay
UV SPECTROSCOPY				
kg low Tablet	Pyridoxine HCl	7.5	7.39	98.6%
	Leucine	550	545.6	99.2%
HPLC				
kg low Tablet	Pyridoxine HCl	7.5	7.40	98.7%
	Leucine	550	546.1	99.36%

Table S2: Combined repeatability (intraday precision) parameters for Pyridoxine HCl and FMOC-Leu

UV			HPLC			
			Pyridoxine HCl		FMOC-Leu	
Concentration (6:43.9 µg/mL)	Pyridoxine HCl at 299nm	FMOC-Leu at 251nm	Conc (µg/mL)	Peak Area	Conc (µg/mL)	Peak Area
1	0.0178	0.08061	6	264260	43.9	648760
2	0.0175	0.08053	6	263371	43.9	646002
3	0.0177	0.08042	6	26472	43.9	648587
4	0.0177	0.08102	6	263418	43.9	645621
5	0.0179	0.08057	6	264352	43.9	648401
6	0.0176	0.08063	6	264620	43.9	648633
Mean	0.0177	0.08063		264082.2		647667.3
SD	0.000141	0.000205		546.359		1447.156
%RSD	0.798	0.254		0.2068		0.2234

Drug	Conc.	Peak Area			Mean	SD	%RSD
		SET 1	SET 2	SET 3			
Pyridoxine HCl	4	210142	210130	263389	1734.0	1734.0	0.336
	6	263418	263405	312751	1741.8	1741.8	0.270
	8	312776	312763	516585	5785.3	5785.3	0.76
FMOC-Leu	29.3	516574	516570	645633	1734.087	1734.087	0.336
	43.6	645621	645610	763238	1741.876	1741.876	0.270
	58.6	312776	763254	763238	5785.340	5785.340	0.761

Table S3: Combined inter precision parameters in HPLC **(A)** and UV spectroscopy **(B)** of Pyridoxine and FMOC-Leu

FMOC-Leu						
Conc. (µg/mg)	**Day-1**	**Day-2**	**Day-3**	**Mean**	**SD**	**%RSD**
29.3	0.0588	0.0586	0.0583	0.0586	0.0026	0.4543
43.9	0.0806	0.0805	0.0802	0.0804	0.0001	0.2451
58.6	0.1016	0.1006	0.101	0.1013	0.0006	0.5956
Pyridoxine HCl						
4	-0.0140	-0.0138	-0.0140	-0.0139	0.0001	0.8287
6	-0.0176	-0.0176	-0.0179	-0.0177	0.0001	0.8597
8	-0.0220	-0.0221	-0.0223	-0.0221	0.0001	0.6901

Drug	**Conc.**	**Peak Area**			**Mean**	**SD**	**%RSD**
		Day-1	**Day-2**	**Day-3**			
Pyridoxine HCl	4	210142	205130	211126	208799.3	3215.598	1.540
	6	263418	262405	265589	263804	1626.718	0.616
	8	312776	304273	308534	308527.7	4251.504	1.377
FMOC-Leu	29.3	516574	509550	514585	513569.7	3620.403	0.704
	43.6	645621	649610	635633	643621.3	7199.87	1.008
	58.6	763263	753254	757228	757918.3	5039.063	0.664

Table S4: Combined parameters of different analyst in UV spectroscopy **(A)** and HPLC **(B)** of Pyridoxine HCl and FMOC-Leu

Pyridoxine HCl					
Conc. (µg/mg)	**Analyst 1**	**Analyst 2**	**Mean**	**SD**	**% RSD**
29.3	-0.0140	-0.0143	-0.01415	0.0002	1.499
43.9	-0.0180	-0.0180	-0.0179	0.0001	0.790
58.6	-0.0223	-0.0223	-0.0221	0.0002	0.957
FMOC-Leu					
Conc. (µg/mg)	**Analyst 1**	**Analyst 2**	**Mean**	**SD**	**% RSD**
29.3	0.05885	0.05916	0.0591	0.0004	0.741
43.9	0.08102	0.08197	0.0814	0.0006	0.824
58.6	0.10166	0.10203	0.1018	0.0002	0.256

Drug	**Conc.**	**Peak Area**		**Mean**	**SD**	**%RSD**
		Analyst 1	**Analyst 2**			
Pyridoxine HCl	4	210142	206130	208136	2836.912	1.36009
	6	263418	261405	262411.5	1423.406	0.542433
	8	312776	308273	310524.5	3184.102	1.025395
FMOC-Leu	29.3	516574	507550	512062	6380.932	1.246125
	43.6	645621	647610	646615.5	1406.435	0.217507
	58.6	763263	759254	761258.5	2834.791	0.372382

Table S5: Validation parameter as Différent Instrument in UV spectroscopy

Pyridoxine HCl					
Conc. (µg/mg	**Instrument 1**	**Instrument 2**	**Mean**	**SD**	**%RSD**
4	-0.0140	-0.0138	-0.0139	0.0004	1.017
6	-0.0178	-0.0175	-0.0176	0.0002	1.201
8	-0.0220	-0.0217	-0.0218	0.0002	0.970
FMOC-Leu					
Conc. (µg/mg	**Instrument 1**	**Instrument 2**	**Mean**	**SD**	**%RSD**
29.3	0.05885	0.055987	0.0593	0.0007	1.215
43.9	0.08102	0.08078	0.0809	0.0001	0.209
58.6	0.10166	0.10203	0.1018	0.0002	0.222

Table S6: Accuracy parameter of UV and HPLC

Drug	Level	Amount of sample (ppm)	Amount of std. (ppm)	Total Amount (ppm)	Amount found (ppm)		% Recovery	
					HPLC	UV	HPLC	UV
Pyridoxine HCl	50%	0.2	1	1.2	1.18	1.17	98.3%	98%
	100%		2	2.2	2.16	2.17	98%	98.7%
	150%		3	3.2	3.15	3.16	98.6%	98.9%
FMOC-Leu	50%	14.6	21.9	21.9	21.82	21.79	99.6%	99.4%
	100%		29.2	29.2	29.13	29.10	99.7%	99.6%
	150%		36.5	36.5	36.41	36.38	99.8%	99.7%

Table S7: Limit of Detection (LoD) And Limit of Quantification (LoQ) parameters

Parameter	**UV Spectroscopy**		**HPLC**	
Drug	FMOC-Leu at 251nm	Pyridoxine HCl at 299nm	Pyridoxine HCl	FMOC-Leu
SD of the Y-intercepts of the 3 calibration curve	0.000173	0.000153	355.8881	5730.898
Mean slop of the 3 calibration curve	0.0015	0.002	24130.33	8102.4
LOD (µg/mL)	0.380	0.252	0.04867	2.334119
Drug	FMOC-LEU at 251nm	Pyridoxine HCl at 299nm	Pyridoxine HCl	FMOC-LEU
SD of the Y-intercepts of the 3 calibration curve	0.000173	0.000153	16846.86	7828.86
Mean slop of the 3 calibration curve	0.0015	0.002	14840.96	8956.32
LOQ (µg/mL)	1.153	0.762	0.147486	7.07387

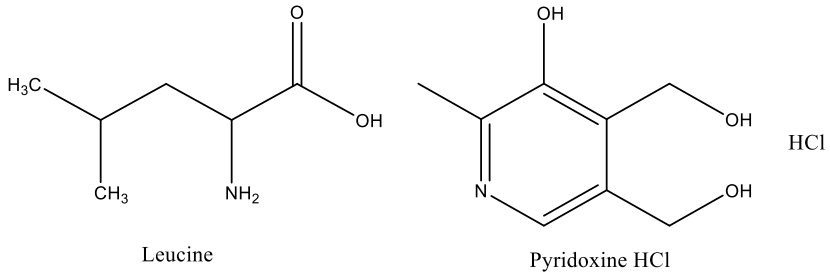


Figure S1 : Structure of Leucine and Pyridoxine HCl

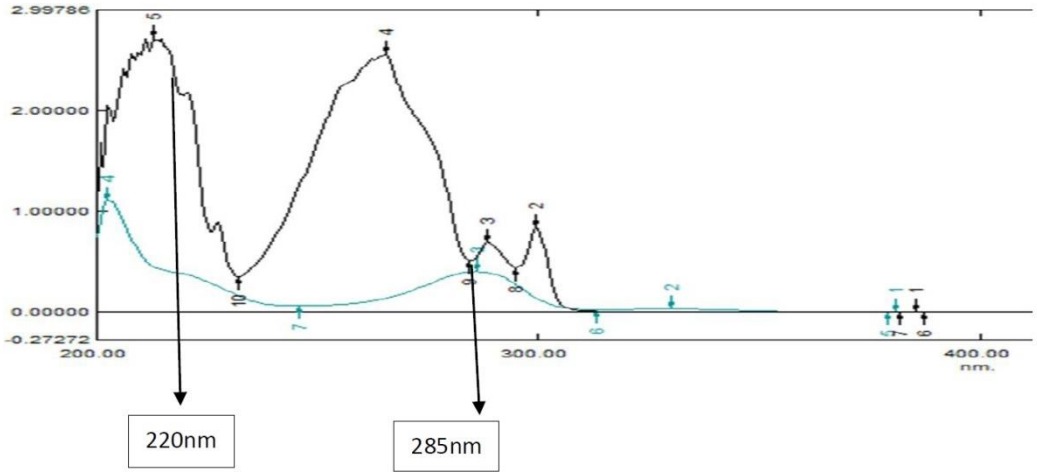
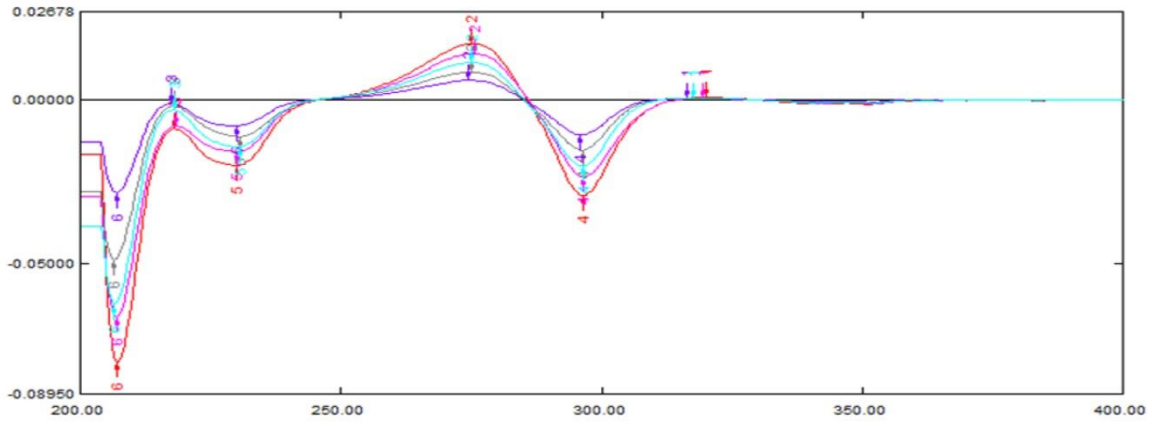
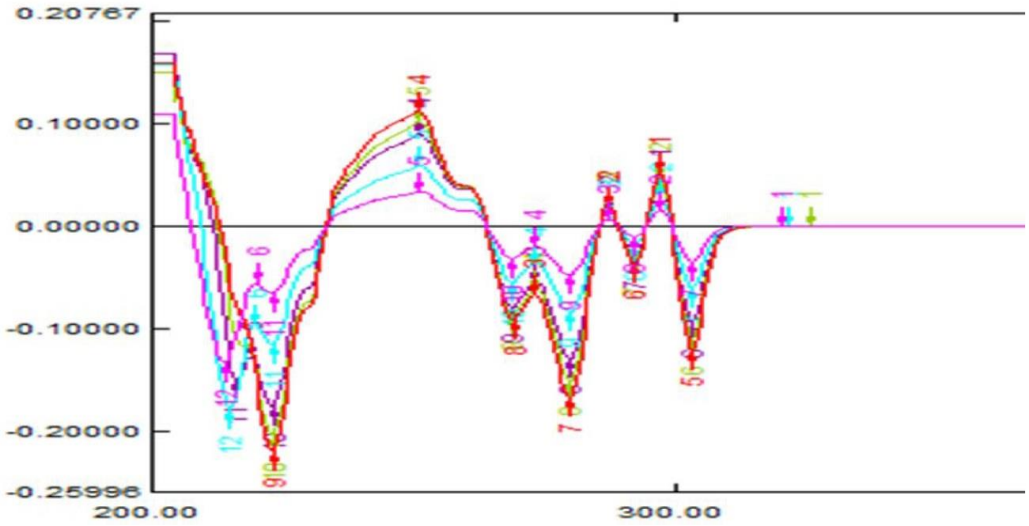


Figure S2: Overlay spectra of FMOC-LEU and Pyridoxine HCl for detection of wavelength



(A)



(B)

(A) - Pyridoxine HCl at 299 nm
(B) – FMOC-Leu at 251 nm

Figure S3: Overlay spectra at different concentration

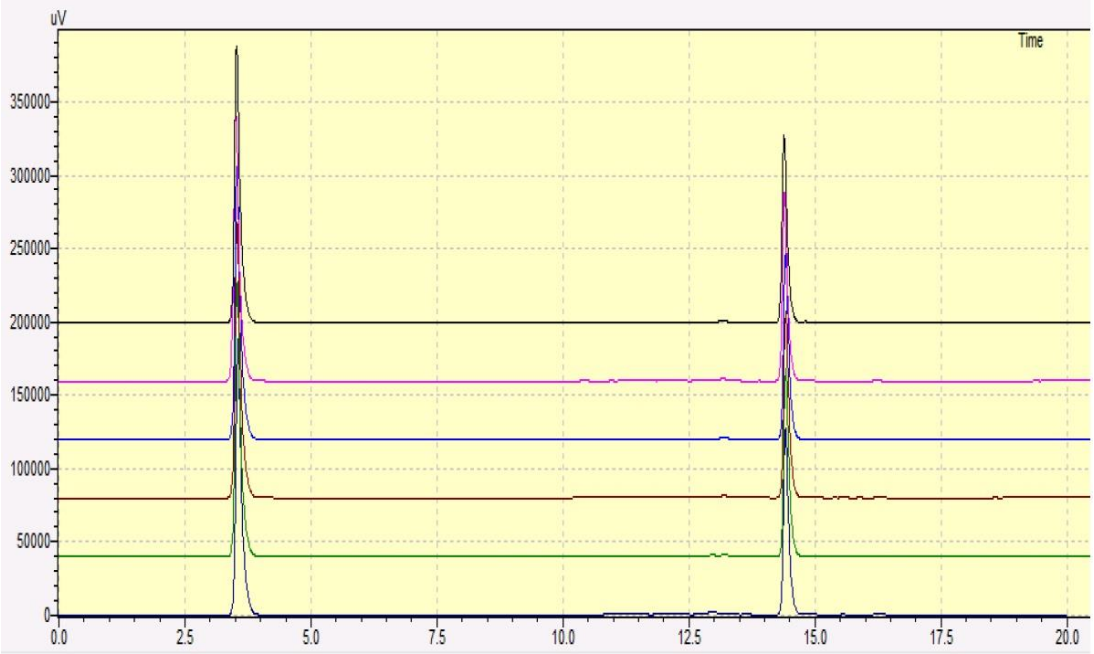
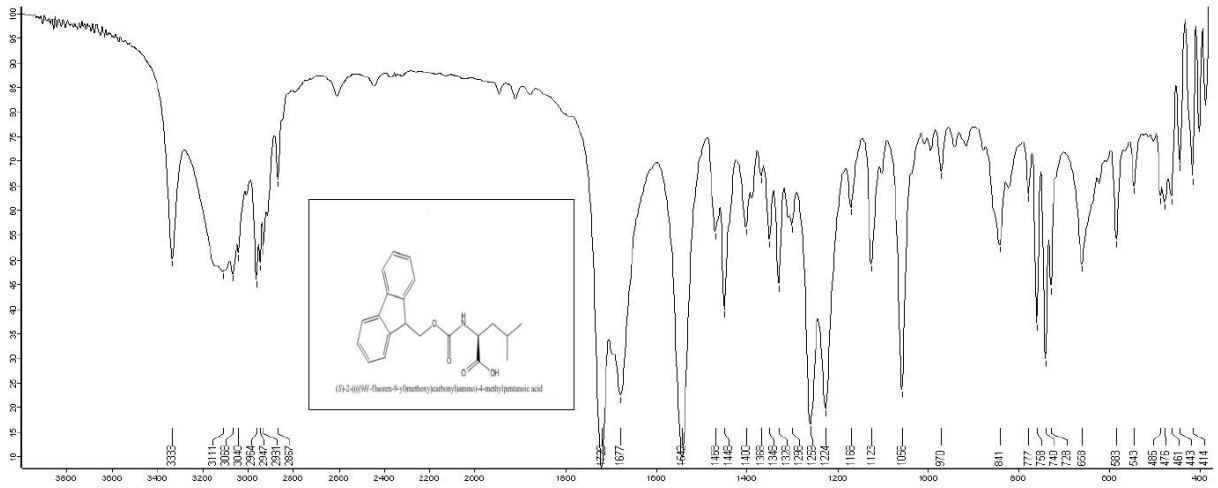
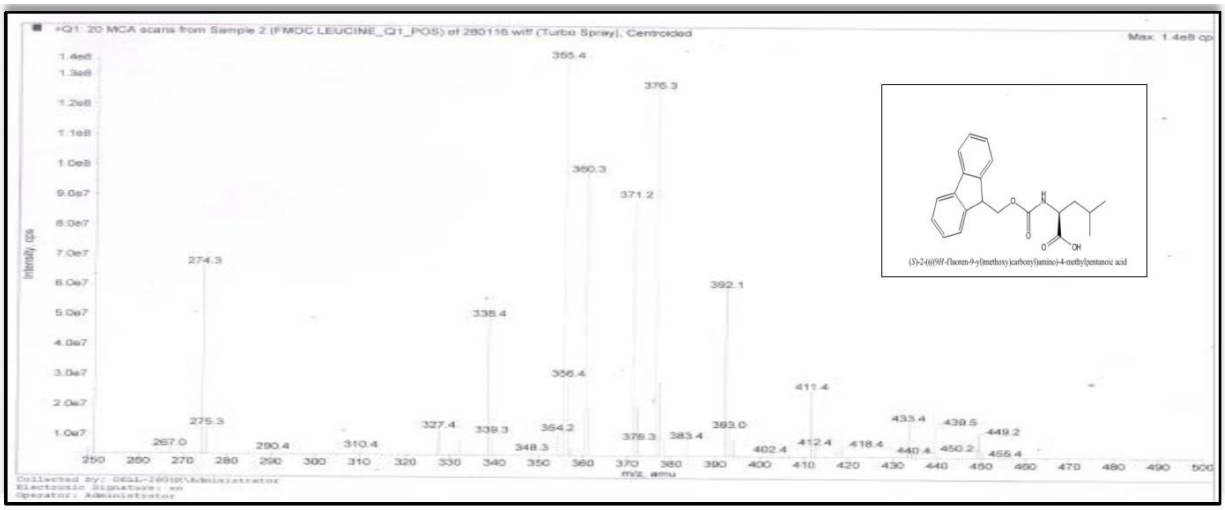


Figure S4: Overlay chromatogram of system suitability of Pyridoxine HCl and FMOC-Leu

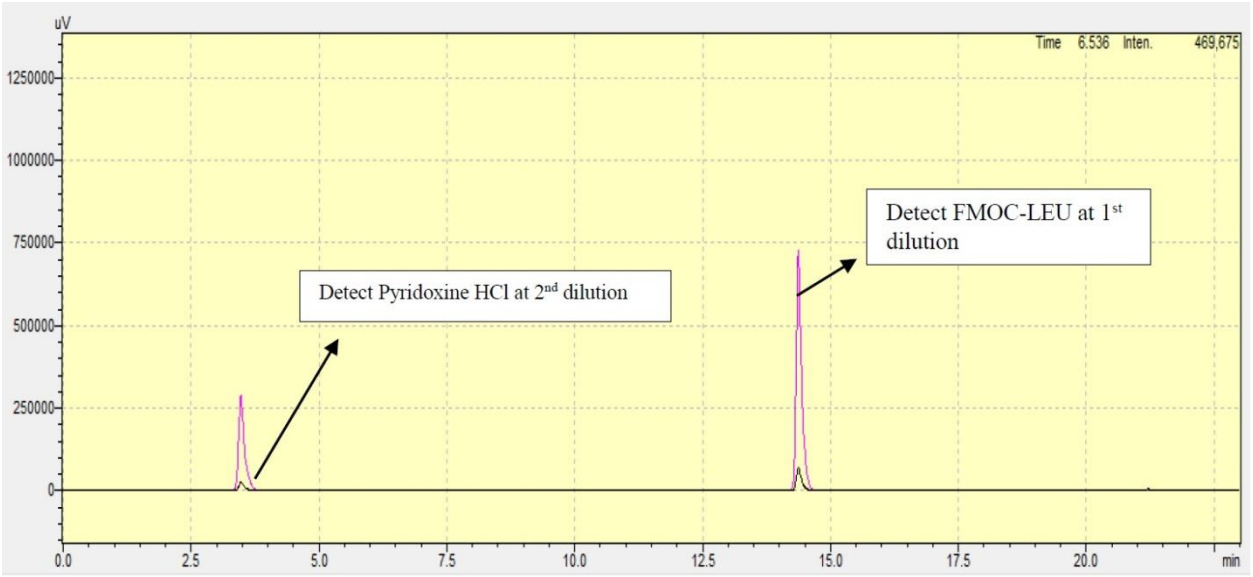


(A)

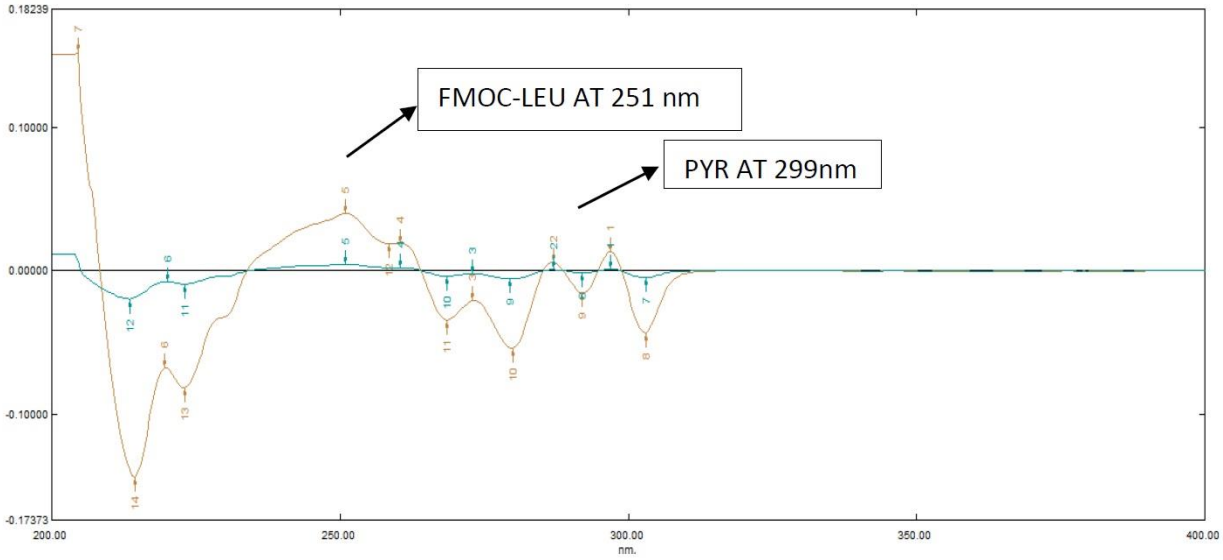


(B)

Figure S5: IR **(A)** and FTIR **(B)** spectra of FMOC-Leu



(A)

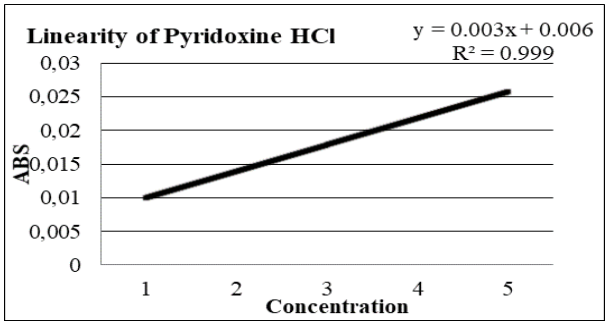


(B)

(A)- HPLC at 258 nm

(B)- UV at 299 nm

Figure S6: Analysis of marketed formulation



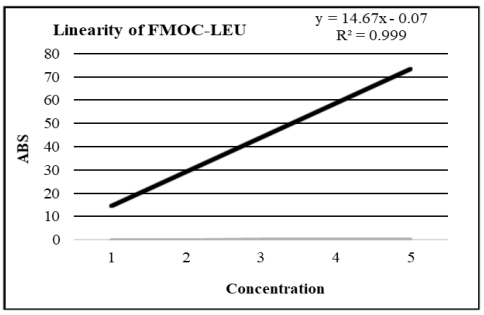
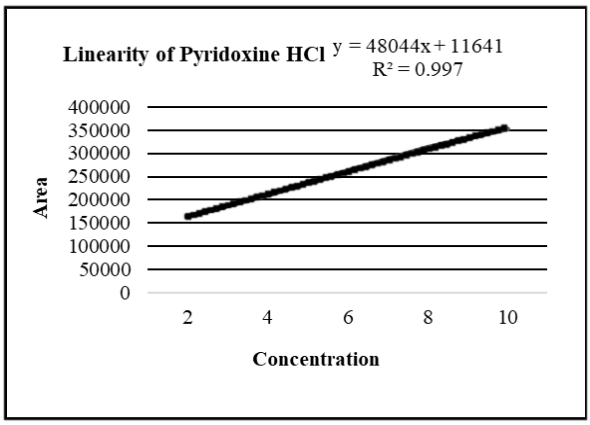


Figure S7: (UV Spectroscopy) **-** Linearity graph of Pyridoxine HCl and FMOC-Leu



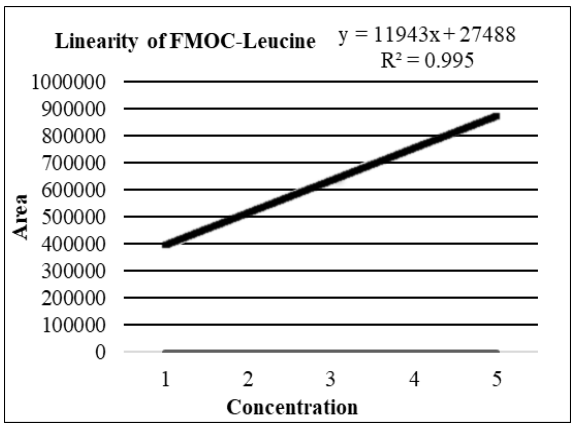


Figure S8 :(HPLC) **-** Linearity graph of Pyridoxine HCl and FMOC-Leu