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RP-HPLC method for quantification of cefotaxime sodium by using design of experiment, a green analytical approach: Analytical method development, validation, and application

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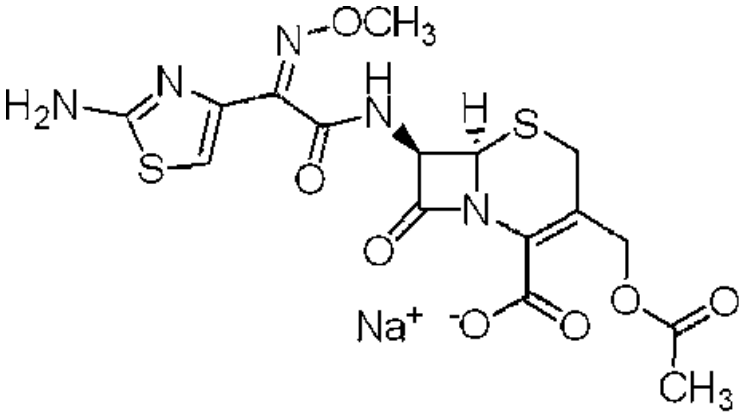
SUPPLEMENTARY FILE



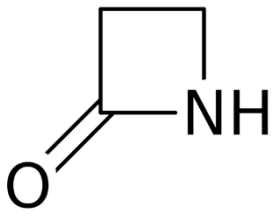
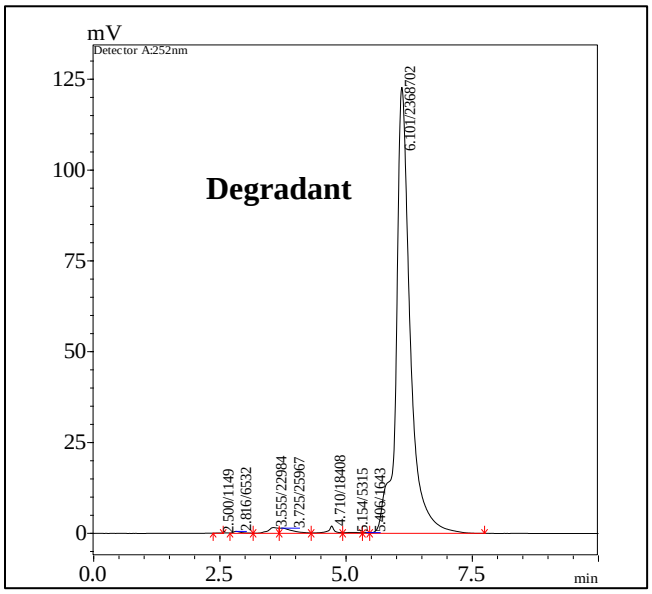
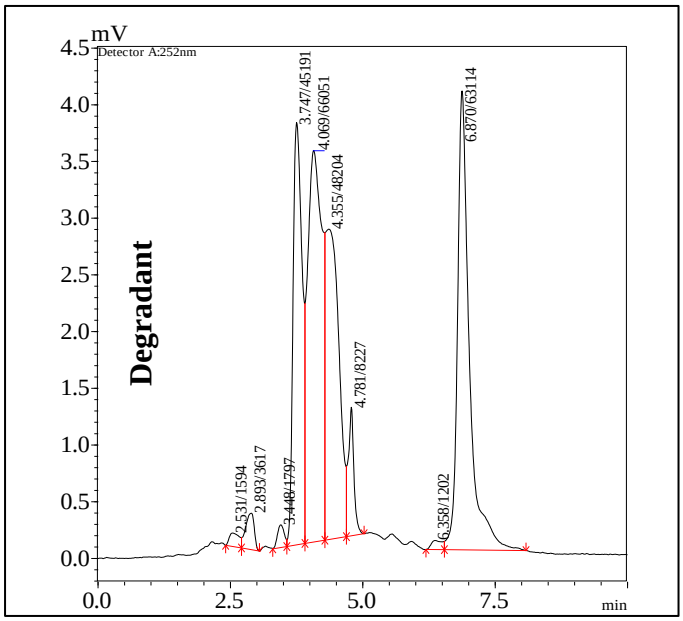


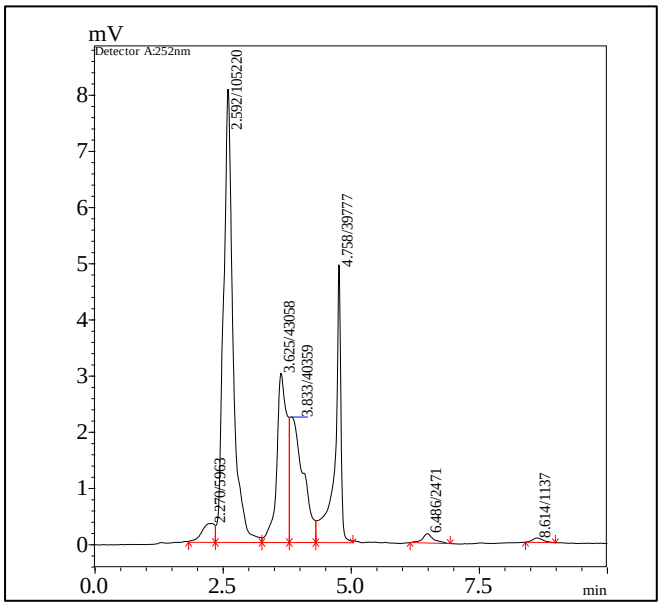
Fig. S1. Chemical structure of Cefotaxime sodium (CTX) and β-lactam ring respectively



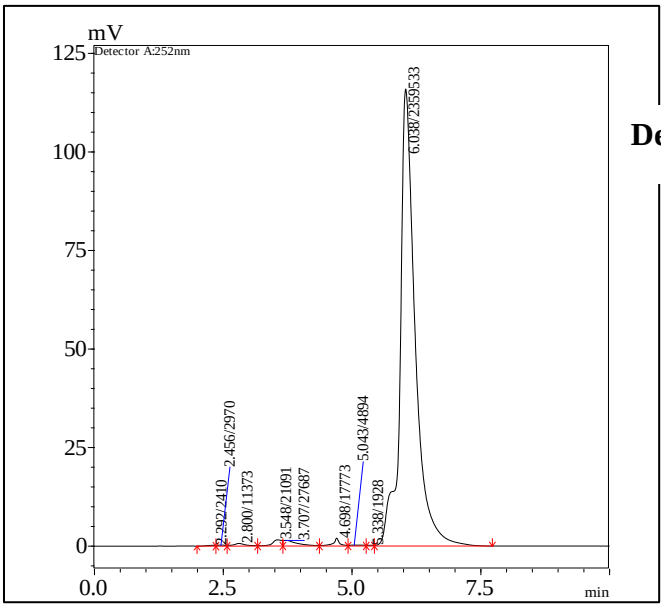
a) Acid Hydrolysis



b) Oxidative degradation



c) Heating hydrolysis



d) Base degradation

Fig. S2. Illustrates the chromatograms observed after the forced degradation a) acid hydrolysis, b) Oxidative degradation c) Heating degradation and d) base hydrolysis

Table S1. Full factorial design with the recorded response values

S.no	Flow rate (ml/min)	Mobile phase ratio (buffer %)	Column oven tempearture (°C)	Inj volume(µl)	Peak area	Tailing factor	Retention time	Theoritical plates	Desirability
1	0.9	89	26	18	211598	2.406	4.277	2709.83	1.000
2	0.7	81	26	18	161150	1.484	7.094	20342.7	1.000
3	0.7	89	26	18	176704	1.486	5.539	6171.98	1.000
4	0.7	81	26	22	154364	1.39	7.086	21191.2	1.000
5	0.7	89	26	18	158410	1.711	5.526	7699.26	1.000
6	0.7	89	23	18	10306	1.274	6.311	2572.41	1.000
7	0.9	81	26	22	203386	1.138	4.299	3125.04	1.000
8	0.9	81	26	18	205543	1.113	4.297	3565.67	1.000
9	0.7	81	26	18	160426	1.466	5.529	8699.43	1.000
10	0.7	81	23	18	242384	1.605	5.554	7484.52	1.000
11	0.9	81	23	18	216215	1.083	4.32	3896.22	1.000
12	0.9	89	23	22	161712	1.964	4.315	3088.08	1.000
13	0.7	81	23	22	348407	1.858	7.142	4413.94	1.000
14	0.9	81	26	22	172666	1.616	4.289	3884.79	1.000
15	0.9	89	23	18	3557	0.874	4.83	3520.18	1.000
16	0.9	81	23	18	234467	2.704	5.433	1602.09	1.000
17	0.9	81	23	22	200355	1.07	4.35	3224.89	1.000
18	0.7	89	23	22	202569	1.528	5.561	8520.96	1.000
19	0.7	81	23	22	300138	1.813	7.198	5137.12	1.000
20	0.9	89	23	18	184511	1.107	4.338	3063.65	1.000
21	0.9	81	26	18	202394	1.401	4.306	3728.51	1.000
22	0.7	89	26	22	173331	1.579	5.523	10299.3	1.000
23	0.7	81	23	18	213011	1.603	5.588	5923.72	1.000
24	0.9	89	26	22	4213	1.487	4.741	2776.65	1.000
25	0.9	89	23	22	4769	0.808	4.88	4442.17	1.000
26	0.9	89	26	18	5802	1.227	4.754	3171.99	1.000
27	0.7	89	23	18	8678	1.614	6.295	1233.79	1.000
28	0.7	89	23	22	336689	1.73	7.538	1625.04	1.000
29	0.9	81	23	22	226719	2.274	4.919	6018.81	1.000
30	0.9	89	26	22	9129	1.987	4.691	1135.48	1.000
31	0.7	89	26	22	356076	2.226	3.53	362.603	1.000
32	0.7	81	26	22	412110	1.051	6.354	2280.54	1.000

Table S2. The suggested solutions for numerical optimization of the proposed HPLC method

S.no	Flow rate (ml/min)	Mobile phase ratio (buffer %)	Column oven tempearture (°C)	Inj volume(µl)	Peak area	Tailing factor	Retention time	Theoritical plates	Desirability
1	0.772	80.026	27.442	21.206	274697.63	1.012	5.545	9894.504	1.000
2	0.797	81.591	28.642	21.055	237307.13	1.045	4.92	9473.466	1.000
3	0.794	80.02	28.245	20.875	256381.35	0.887	5.098	9798.288	1.000
4	0.801	80.156	27.658	20.658	247549.27	1.006	5.056	9100.405	1.000
5	0.796	81.879	29.145	21.364	238764.59	1.026	4.844	9766.002	1.000
6	0.788	80.471	28.298	18.886	222083.45	1.038	5.187	9859.941	1.000
7	0.798	80.869	28.617	20.247	234612.53	0.967	4.95	9677.574	1.000
8	0.798	80.422	28.514	18.242	211966.43	0.993	4.993	9755.197	1.000
9	0.797	81.321	29.105	18.171	199669.72	1.035	4.89	9912.002	1.000
10	0.814	80.152	27.423	21.929	254688.73	1.009	4.903	8539.973	1.000
11	0.811	80.689	27.946	20.113	229213.62	1.027	4.845	8857.452	1.000
12	0.811	80.165	27.885	18.515	218217.94	1.007	4.838	8948.46	1.000
13	0.792	80.864	28.712	19.598	227535.82	0.986	5.033	9905.505	1.000
14	0.792	80.782	28.355	19.333	224409.47	1.036	5.09	9679.376	1.000
15	0.794	82.048	29.438	20.417	224235.43	1.051	4.83	9974.348	1.000
16	0.788	80.612	28.264	21.801	267628.36	0.924	5.144	9784.602	1.000
17	0.779	81.004	27.888	21.982	273654.25	1.018	5.29	9641.945	1.000
18	0.799	81.368	28.589	20.273	227875.71	1.039	4.911	9452.776	1.000
19	0.808	80.319	27.654	19.555	228921.72	1.048	4.945	8848.467	1.000
20	0.8	80.884	28.312	20.557	237282.67	0.999	4.951	9372.3	1.000
21	0.803	80.964	28.589	18.299	205410.19	1.027	4.856	9471.842	1.000
22	0.796	81.67	28.599	21.609	245311.54	1.046	4.936	9450.055	1.000
23	0.775	80.44	27.351	21.934	281676.74	1.03	5.46	9569.337	1.000
24	0.807	80.531	28.258	18.748	216272.9	0.996	4.845	9240.162	1.000
25	0.788	81.247	28.407	20.932	246288.36	1.031	5.105	9684.335	1.000
26	0.801	81.31	28.745	19.868	222310.21	1.02	4.868	9542.813	1.000
27	0.798	81.174	28.482	19.983	227011.4	1.036	4.958	9479.217	1.000
28	0.799	80.527	28.341	19.309	225268.05	0.99	4.979	9544.312	1.000
29	0.801	81.132	28.789	18.947	212172.63	1.013	4.867	9633.426	1.000
30	0.813	80.71	27.691	21.865	247059.28	1.034	4.871	8600.607	1.000
31	0.796	81.039	28.309	20.708	239736.37	1.019	5.004	9432.89	1.000
32	0.783	80.486	28.045	21.931	275641.4	0.93	5.258	9829.318	1.000
33	0.787	80.091	27.55	20.935	260354.1	1.013	5.301	9491.764	1.000
34	0.774	80.687	27.912	21.286	268056.82	1.01	5.4	9922.348	1.000
35	0.795	80.141	27.623	20.635	250501.43	1.015	5.157	9265.101	1.000
36	0.81	80.313	27.695	20.119	234751.19	1.024	4.904	8809.022	1.000
37	0.805	80.072	28.463	18.479	219167.3	0.919	4.868	9605.303	1.000
38	0.803	80.508	28.233	20.046	233928.47	0.973	4.927	9350.462	1.000

39	0.81	80	27.293	21.872	258808.87	1.013	4.976	8594.738	1.000
40	0.795	80.916	28.856	19.293	221481.27	0.979	4.971	9921.321	1.000
41	0.803	81.474	28.724	19.948	220300.51	1.041	4.833	9417.819	1.000
42	0.775	80.267	27.214	21.99	285058.14	1.029	5.501	9554.267	1.000
43	0.788	80.88	28.372	19.848	232918.27	1.033	5.148	9783.798	1.000
44	0.787	80.961	28.535	21.755	263782.03	0.937	5.101	9893.871	1.000
45	0.803	80.625	28.024	19.022	219210.16	1.048	4.949	9155.012	1.000
46	0.8	81.051	28.981	20.385	232814.91	0.936	4.85	9811.651	1.000
47	0.806	80.015	27.536	21.727	260392.98	0.975	5.012	8919.81	1.000
48	0.779	81.313	28.504	20.612	245494.61	1.048	5.22	9971.801	1.000
49	0.798	81.461	29.178	21.998	251579.8	0.934	4.835	9884.279	1.000
50	0.816	80.003	27.451	18.744	222907.62	1.05	4.847	8544.978	1.000
51	0.812	80.138	27.278	21.973	256788.01	1.029	4.951	8489.437	1.000
52	0.79	80.714	28.661	21.931	267381.11	0.875	5.059	9993.975	1.000
53	0.793	80.06	28.041	18.718	223706	1.023	5.147	9657.31	1.000
54	0.794	80.554	28.444	18.921	220497.45	1.005	5.052	9752.541	1.000
55	0.814	80.23	27.477	19.853	231629.18	1.051	4.875	8551.484	1.000
56	0.806	80.11	27.76	20.902	248542.56	0.973	4.965	9037.738	1.000
57	0.782	80.628	27.835	20.903	256666.38	1.034	5.311	9666.378	1.000
58	0.805	80.529	27.838	19.749	229114.5	1.041	4.946	8983.03	1.000
59	0.794	81.259	28.676	20.056	228912.84	1.026	4.989	9703.353	1.000
60	0.805	81.13	28.433	20.078	225262	1.023	4.846	9240.38	1.000
61	0.795	80.858	28.411	21.149	249258.21	0.965	5.014	9603.732	1.000
62	0.796	80.815	28.909	20.937	246478.73	0.896	4.942	9969.83	1.000
63	0.808	80.018	27.75	18.585	221239.06	1.024	4.926	9000.199	1.000
64	0.786	81.305	28.669	19.957	230439.58	1.049	5.109	9895.096	1.000
65	0.806	80.521	28.053	18.6	214801.99	1.034	4.891	9115.343	1.000
66	0.782	81.27	28.589	21.767	264705.72	0.974	5.138	9967.756	1.000
67	0.8	80.872	28.643	18.947	215734.09	1	4.914	9637.04	1.000
68	0.806	80.096	28.527	19.316	228926.36	0.884	4.835	9600.677	1.000
69	0.796	81.43	29.227	18.031	196331.05	1.042	4.878	9976.811	1.000
70	0.797	80.733	28.77	20.745	243775.02	0.909	4.943	9853.314	1.000
71	0.773	80.301	27.577	21.214	271652.84	1.02	5.499	9873.982	1.000
72	0.802	80.357	27.563	21.552	256327.35	1.015	5.051	8941.347	1.000
73	0.797	80.427	28.271	18.03	208857.8	1.042	5.041	9599.409	1.000

Table S3. Point correction

Response	CI low 95%*	TI low 95% for 99% population*	Predicted mean	Observed mean	CI high 95%	TI high 95% for 99% population
Peak Area	111100	-140051	186584	1661150	262068	513220
Tailing factor	1.227	-0.052	1.552	1.484	1.878	3.158
Retention time	5.828	3.851	6.462	7.094	7.097	9.073
Theoretical plate	7236.54	-5765.8	105393.7	20342.7	13482.8	26845.2

Table S4: Detailed description of GAPI parameters for proposed and reported method for cefotaxime sodium

Number	Category	Reported HPLC Method	Proposed HPLC Method
	Sample preparations		
1	**Collection**	Offline (Red)	Offline (Red)
2	**Preservation**	None (Green)	None (Green)
3	**Transport**	Required (Yellow)	None (green)
4	**Storage**	None (Green)	None (Green)
5	**5- Type of method: direct or indirect**	Simple procedure (Yellow)	Simple procedure (Yellow)
6	**Scale of extraction**	Micro-extraction (Yellow)	Micro-extraction (Yellow)
7	**Solvents/reagents Used**	Non- green solvents/reagents (Red)	Non- green solvents/reagents (Red)
8	**Additional treatments**	None (Green)	None (Green)
	Reagents and solvents		
9	**Amount**	10-100 mL (10-100 g) (Yellow)	<10 mL (<10 g) (Green)
10	**Health hazard**	NFPA=2, Moderate toxicity (Yellow)	NFPA=2, Moderate toxicity (Yellow)
11	**Safety hazard**	NFPA=3; high flammability (Yellow)	NFPA=3; high flammability (Yellow)
	Instrumentation		
12	**Energy**	≤0.1 kWh per sample (Green)	≤0.1 kWh per sample (Green)
13	**Occupational hazard**	Emission of vapours to the atmosphere (Red)	Hermetic sealing of Analytical process(green)
14	**Waste**	>10 mL (<10 g) (Red)	1-10 mL (1-10 g) (Yellow)
15	**Waste treatment**	No treatment (Red)	No treatment (Red)
	ADITIONAL MARK: QUANTIFICATION		
	Circle in the middle of GAPI: *Procedure for qualification and quantification*		