In vivo Drug-Drug Interaction Study Between Ketotifen Fumarate And Amoxicillin Trihydrate Along With Their IR Studies

Mohammed AktarSayeed

Professor, Department of Pharmacy, International Islamic University Chittagong
Chittagong-4318, Bangladesh. Phone: 0088-01554310162,
e-mail: sayeed_ustc@yahoo.com

Abstract

The aim of the present study was to find out *in vitro* IR studies and *in vivo* interaction between ketotifen fumarate and amoxicillin trihydrate. By using Infrared spectroscopy study, the extra peaks were found out. Thirty five peaks were observed in the infrared spectrum of ketotifen fumarate. Similarly forty seven and thirty one extra peaks were located in the infrared spectrum when ketotifen & amoxicillin in aqueous and chloroform extract respectively. One and six extra peaks were found in the infrared spectrum of ketotifen& amoxicillin in aqueous and chloroform extracts respectively compared to the infrared spectrum of ketotifen. At time 30, 60, 120 and 180 minutes, the multiple comparison tables showed there is a significance difference in absorbance but the results which are obtained are statistically significant. The mean difference obtained from post hoc tests, we can draw a conclusion that the result is significant.

Key words: Infrared spectrum, *In vivo* study, Ketotifen fumarate, Amoxicillin trihydrate

.

Introduction

Ketotifen, is benzocycloheptathiophene derivative that has been shown to anti-histaminic and anti-anaphylactic properties. [1] In oral dosage form, ketotifen is most commonly used to prevent asthma attacks or anaphylaxis, as well as various mast cell, allergic-type disorders.[2-6] Common seasonal allergies can be prevented by ketotifen. Eye itchiness and irritation can also be prevented by it. The drug has not been studied in children under three. [2] The $t_{1/2}$ of ketotifen is 12 hours. [7] The drug may also help relieve the symptoms of irritable bowel syndrome.[8]Long term use of ketotifen shows some common side effects including drowsiness, weight gain and dry mouth. On the other hand amoxicillin is a moderate-spectrum, bacteriolytic, β-lactamantibiotic used to treat bacterial infections caused by susceptible microorganisms.

It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is one of the most common antibiotics prescribed for children. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, which are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin. For this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. This increases effectiveness by reducing its susceptibility to β-lactamase resistance. Amoxicillin is used in the treatment of a number of infections, including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, Urinary tract infections, *Salmonella* infections, Lyme disease, and chlamydia infections. [9] It is also used to prevent

bacterial endocarditis in high-risk people who are having dental work done, to prevent *Streptococcus pneumoniae* and other encapsulated bacterial infections in those without spleens, such as people with sickle-cell disease, and for both the prevention and the treatment of anthrax. [9]The United Kingdom recommends against its use for infectious endocarditis prophylaxis.
These recommendations have not appeared to have changed the rates ofinfection. [10]Amoxicillin and amoxicillin-clavulanate are recommended by guidelines as the first-choice drug for bacterial sinusitis, but most sinusitis is caused by viruses, for which amoxicillin and amoxicillin-clavulanate are ineffective. [11-12] Amoxicillin is occasionally used for the treatment of skin infections, such as acne vulgaris. [13] It is often an effective treatment for cases of acne vulgaris that have responded poorly to other antibiotics, such as doxycycline and minocycline. [14]

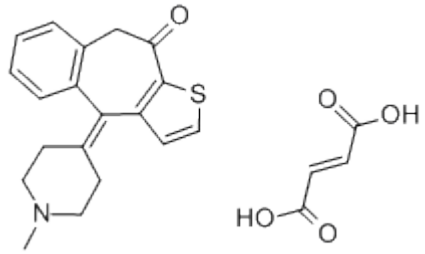


Figure 1: Structure of ketotifen fumarate

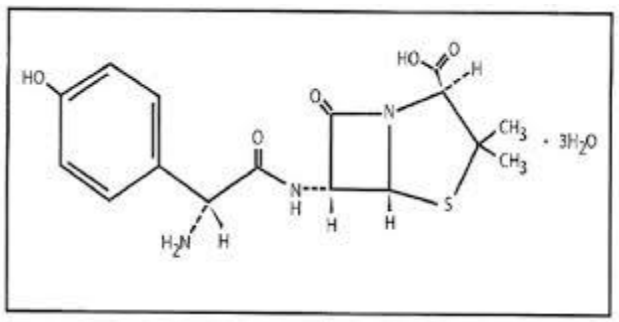


Figure 2: Structure of Amoxicillin trihydrate

Infrared Spectroscopy

Infrared photometry (IR photometry) deals with the infrared region of the electromagnetic spectrum. The light having a longer wavelength and lower frequency compared with visible light. It is based on light absorption. To identify the pure chemicals the IR photometry is the best method compared to the other spectroscopic techniques. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer.

The near-IR, 14000–4000 cm^{-1} (0.8–2.5 μm) is commonly used to detect harmonic vibrations. Whereas the mid-infrared, (2.5–25 μm) can be used to identify rotational-vibrational structure of the molecule. On the other hand the far-infrared, 400–10 cm^{-1} can be used to find out rotational energy of the molecule.Another important application of Infrared Spectroscopy is in the food industry to measure the concentration of various compounds in different food product. [16]

Materials and methods

Drugs and reagents used

The drugs ketotifen and amoxicillin trihydrate were collected from Beximco pharmaceuticals Limited, Dhaka, Bangladesh. For preparation of buffer solution sodium di hydrogen orthophosphate and disodium hydrogen orthophosphate, methanol, chloroform etc. analytical grade reagents were collected from Department of Pharmacy of IIUC that were purchased from Merck, Germany.

Instruments

UV Spectrophotometer (model number : UV- 1600, Shimadzu corporation, Japan), to maintain the specific pH of the solution a pH meter is used (Mettler Toledo, Switzerland),four digit
Analytical Balance (Model No. AL 204-S/01, Mettler Toledo, Switzerland), and a thermostatic water bath (Shimadzu, Japan) IR Affinity-1 A213747, Shimadzu Corporation, Japan were selected to perform the infrared spectroscopy study

Preparation of chloroform extracts for IR study

100 mg of pure powder of ketotifenfumarate was dissolved in water (10 ml distilled water) in a 50 ml beaker and similarly 100 mg of amoxicillin powder was dissolved in another beaker. Both solutions were mixed together in a 100 ml beaker with constant stirring. Then the mixture was transferred to the separating funnel and chloroform was added into it. Finally chloroform was evaporated and the precipitated solid drug product was analyzed by IR spectrophotometer.

Preparation of aqueous extracts for IR study

Another experiment for aqueous extracts were carried out by mixing both ketotifen and amoxicillin solutions and evaporated. The drug product obtained was also analyzed by IR spectrophotometer.

In vivo interaction study

Selection of Animal

Wister rats (140 grams to 200 grams) for both male and female were used to perform the test. The rats were keeping in dry environment and housed under normal conditions with normal diet and water up to the time of experiments. The rats were exposed to light and dark cycle (12 hours each) .The experimental animals were acclimatized to the laboratory environment one hour before the starting of the experiments.

Preparation of drug solution

20 ml of each drug solutions were prepared according to their corresponding doses.Ketotifenfumarate and amoxicillin trihydrate hydrochloride solutions were formulated in 5% and 0.5% tween-80 and carboxy methyl cellulose in milli-Q water.

Methodology

The rats were grouped into five groups. Freshly prepared ketotifen and amoxicillin trihydrate were administered as single dose to each group. The blood samples were

collected at 30, 60,120 and 180 minutes to compare the drug interaction between the group that took the single drug as well as mixtures (ketotifen& amoxicillin trihydrate)

Group I. Each rat received ketotifenfumarate (0.2 mg/kg),
Group II. Each ratreceived amoxicillin trihydrate,
Group III. They received ketotifen (0.2 mg/kg) & amoxicillin trihydrate.

Procedure

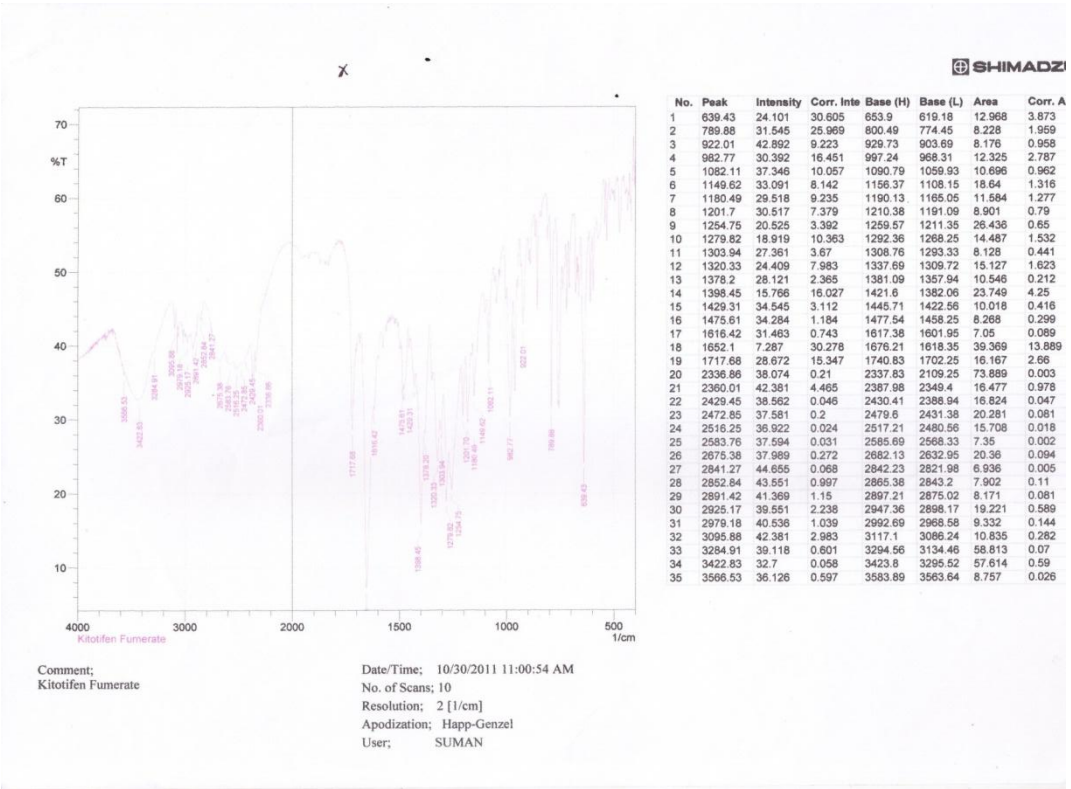
1. Five rats were separated into five baskets.
2. The standard drugs and mixtures were administered as per dose for 7 days.
3. The blood was collected from the hearts of the rats.
4. Serum was separated and heparin was added to it.
5. To precipitate the protein, 0.5 ml of serum and 1.5 ml of 0.9% NaCl solutions were taken in individual test tube.
6. The preparation was centrifuged at 4000 rpm for 20 minutes.
7. The supernatant fluid was separated and measured at 300 nm.

Statistical analysis

The results were expressed as mean ± SEM values for each experiment. Differences in mean values between different experimental groups were analyses by Dunnett ‘t’test. After analysis it was observed that the probability was found to be less than 0.05 ($p < 0.05$) which was significant. From the above observation it has been cleared that as the absorbance of drugs given together (ketotifen and amoxicillin trihydrate) at 1:1 complex indicates less than 0.05, so we can conclude that there is presence of drug interaction.

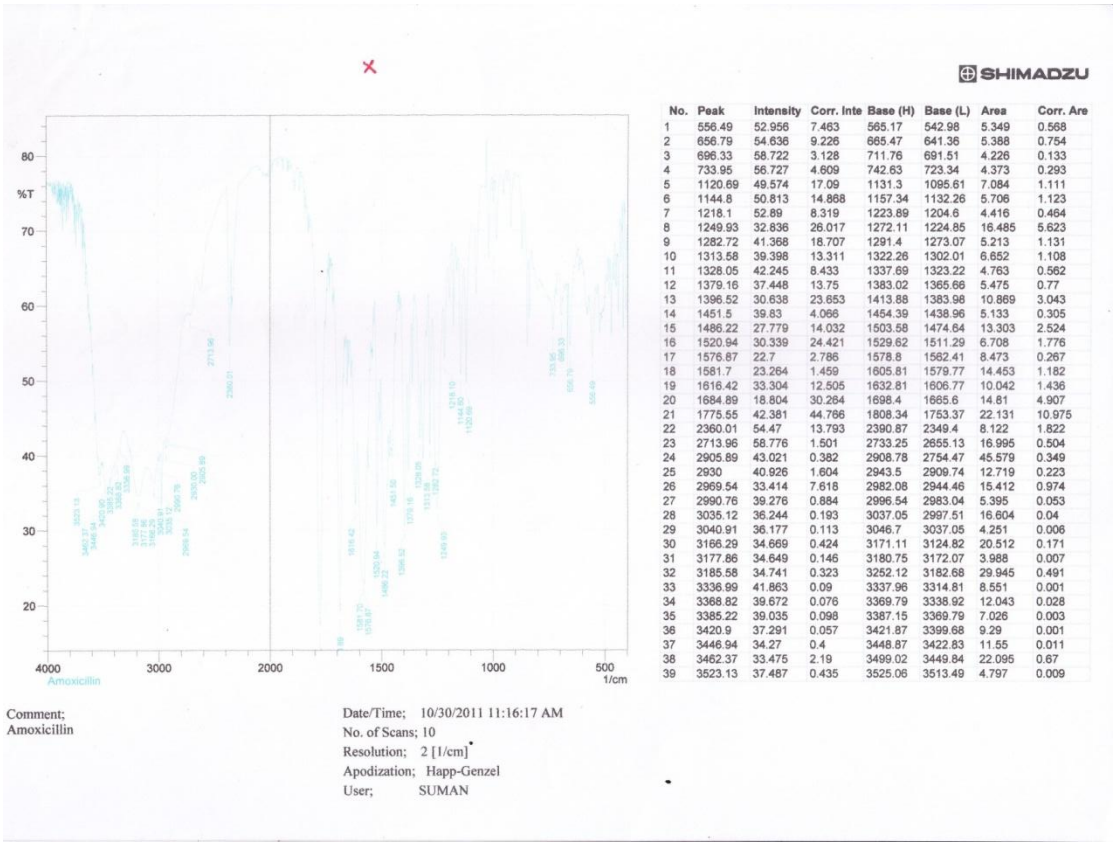
Results and Discussion

IR spectrum of ketotifen fumarate



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. A
1	639.43	24.101	30.605	653.9	619.18	12.968	3.873
2	789.88	31.545	25.969	800.49	774.45	8.228	1.959
3	922.01	42.892	9.223	929.73	903.69	8.176	0.958
4	982.77	30.392	16.451	997.24	968.31	12.325	2.787
5	1082.11	37.346	10.057	1090.79	1059.93	10.696	0.962
6	1149.62	33.091	8.142	1156.37	1108.15	18.64	1.316
7	1180.49	29.518	9.235	1190.13	1165.05	11.584	1.277
8	1201.7	30.517	7.379	1210.38	1191.09	8.901	0.79
9	1254.75	20.525	3.392	1259.57	1211.35	26.436	0.65
10	1279.82	18.919	10.363	1292.36	1268.25	14.487	1.532
11	1303.94	27.361	3.67	1308.76	1293.33	8.128	0.441
12	1320.33	24.409	7.983	1337.69	1309.72	15.127	1.623
13	1378.2	28.121	2.365	1381.09	1357.94	10.546	0.212
14	1398.45	15.766	16.027	1421.6	1382.06	23.749	4.25
15	1429.31	34.545	3.112	1445.71	1422.56	10.018	0.416
16	1475.61	34.284	1.184	1477.54	1458.25	8.268	0.299
17	1616.42	31.463	0.743	1617.38	1601.95	7.05	0.089
18	1652.1	7.287	30.278	1676.21	1618.35	39.369	13.889
19	1717.68	28.672	15.347	1740.83	1702.25	16.167	2.66
20	2336.86	38.074	0.21	2337.83	2109.25	73.889	0.003
21	2360.01	42.381	4.465	2387.98	2349.4	16.477	0.978
22	2429.45	38.562	0.046	2430.41	2388.94	16.824	0.047
23	2472.85	37.581	0.2	2479.6	2431.38	20.281	0.081
24	2516.25	36.922	0.024	2517.21	2480.56	15.708	0.018
25	2583.76	37.594	0.031	2585.69	2568.33	7.35	0.002
26	2675.38	37.989	0.272	2682.13	2632.95	20.36	0.094
27	2841.27	44.655	0.068	2842.23	2821.98	6.936	0.005
28	2852.84	43.551	0.997	2865.38	2843.2	7.902	0.11
29	2891.42	41.369	1.15	2897.21	2875.02	8.171	0.081
30	2925.17	39.551	2.238	2947.36	2898.17	19.221	0.589
31	2979.18	40.536	1.039	2992.69	2968.58	9.332	0.144
32	3095.88	42.381	2.983	3117.1	3086.24	10.835	0.282
33	3284.91	39.118	0.601	3294.56	3134.46	58.813	0.07
34	3422.83	32.7	0.058	3423.8	3295.52	57.614	0.59
35	3566.53	36.126	0.597	3583.89	3563.64	8.757	0.026

IR spectrum of amoxicillin trihydrate

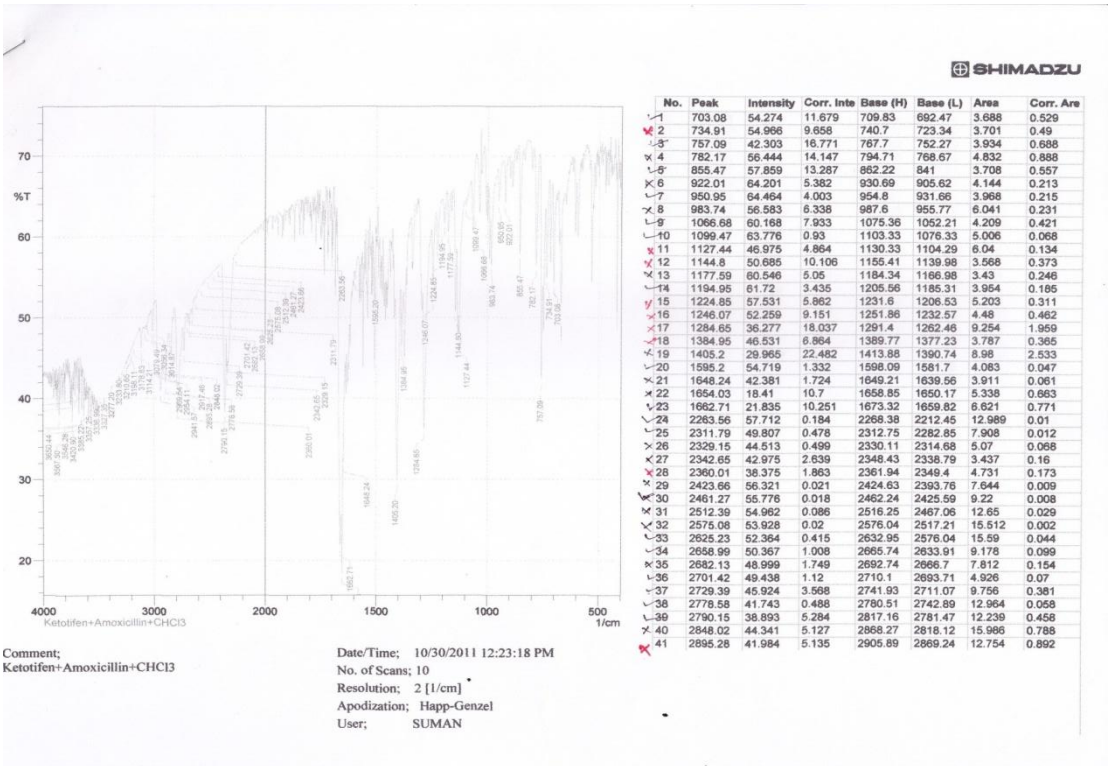


No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	556.49	52.956	7.463	565.17	542.98	5.349	0.568
2	656.79	54.636	9.226	665.47	641.36	5.388	0.754
3	696.33	58.722	3.128	711.76	691.51	4.226	0.133
4	733.95	56.727	4.609	742.63	723.34	4.373	0.293
5	1120.69	49.574	17.09	1131.3	1095.61	7.084	1.111
6	1144.8	50.813	14.868	1157.34	1132.26	5.706	1.123
7	1218.1	52.89	8.319	1223.89	1204.6	4.416	0.464
8	1249.93	32.836	26.017	1272.11	1224.85	16.485	5.623
9	1282.72	41.368	18.707	1291.4	1273.07	5.213	1.131
10	1313.58	39.398	13.311	1322.26	1302.01	6.652	1.108
11	1328.05	42.245	8.433	1337.69	1323.22	4.763	0.562
12	1379.16	37.448	13.75	1383.02	1365.66	5.475	0.77
13	1396.52	30.638	23.653	1413.88	1383.98	10.869	3.043
14	1451.5	39.83	4.066	1454.39	1438.96	5.133	0.305
15	1486.22	27.779	14.032	1503.58	1474.64	13.303	2.524
16	1520.94	30.339	24.421	1529.62	1511.29	6.708	1.776
17	1576.87	22.7	2.786	1578.8	1562.41	8.473	0.267
18	1581.7	23.264	1.459	1605.81	1579.77	14.453	1.182
19	1616.42	33.304	12.505	1632.81	1606.77	10.042	1.436
20	1684.89	18.804	30.264	1698.4	1665.6	14.81	4.907
21	1775.55	42.381	44.766	1808.34	1753.37	22.131	10.975
22	2360.01	54.47	13.793	2390.87	2349.4	8.122	1.822
23	2713.96	58.776	1.501	2733.25	2655.13	16.995	0.504
24	2905.89	43.021	0.382	2908.78	2754.47	45.579	0.349
25	2930	40.926	1.604	2943.5	2909.74	12.719	0.223
26	2969.54	33.414	7.618	2982.08	2944.46	15.412	0.974
27	2990.76	39.276	0.884	2996.54	2983.04	5.395	0.053
28	3035.12	36.244	0.193	3037.05	2997.51	16.604	0.04
29	3040.91	36.177	0.113	3046.7	3037.05	4.251	0.006
30	3166.29	34.669	0.424	3171.11	3124.82	20.512	0.171
31	3177.86	34.649	0.146	3180.75	3172.07	3.988	0.007
32	3185.58	34.741	0.323	3252.12	3182.68	29.945	0.491
33	3336.99	41.863	0.09	3337.96	3314.81	8.551	0.001
34	3368.82	39.672	0.076	3369.79	3338.92	12.043	0.028
35	3385.22	39.035	0.098	3387.15	3369.79	7.026	0.003
36	3420.9	37.291	0.057	3421.87	3399.68	9.29	0.001
37	3446.94	34.27	0.4	3448.87	3422.83	11.55	0.011
38	3462.37	33.475	2.19	3499.02	3449.84	22.095	0.67
39	3523.13	37.487	0.435	3525.06	3513.49	4.797	0.009

Comment;
Amoxicillin

Date/Time; 10/30/2011 11:16:17 AM
No. of Scans; 10
Resolution; 2 [1/cm]
Apodization; Happ-Genzel
User; SUMAN

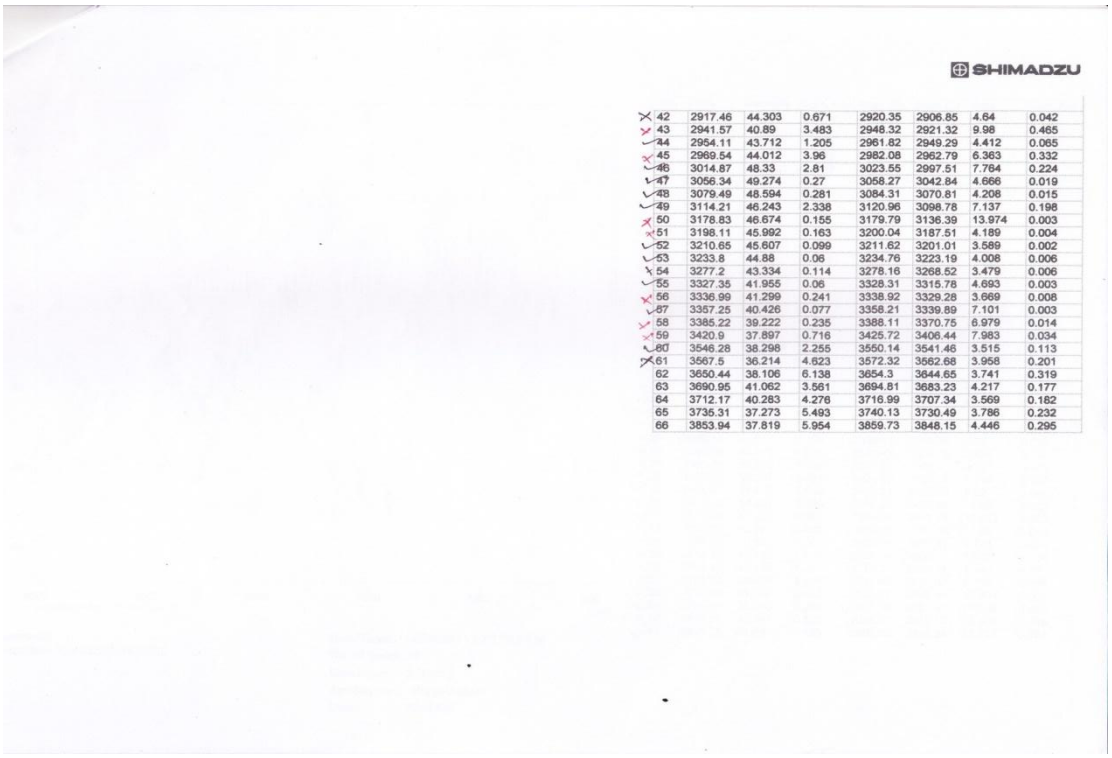
IR spectrum of ketotifenfumarate and amoxicillin trihydrate (chloroform extract)



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	703.08	54.274	11.679	709.83	692.47	3.688	0.529
2	734.91	54.966	9.658	740.7	723.34	3.701	0.49
3	757.09	42.303	16.771	767.7	752.27	3.934	0.688
4	782.17	56.444	14.147	794.71	768.67	4.832	0.888
5	855.47	57.859	13.287	862.22	841	3.708	0.557
6	922.01	64.201	5.382	930.69	905.62	4.144	0.213
7	950.95	64.464	4.003	954.8	931.66	3.968	0.215
8	983.74	56.583	6.338	987.6	955.77	6.041	0.231
9	1066.68	60.168	7.933	1075.36	1052.21	4.209	0.421
10	1099.47	63.776	0.93	1103.33	1076.33	5.006	0.068
11	1127.44	46.975	4.864	1130.33	1104.29	6.04	0.134
12	1144.8	50.685	10.106	1155.41	1139.98	3.568	0.373
13	1177.59	60.546	5.05	1184.34	1166.98	3.43	0.246
14	1194.95	61.72	3.435	1205.56	1185.31	3.954	0.186
15	1224.85	57.531	5.862	1231.6	1206.53	5.203	0.311
16	1246.07	52.259	9.151	1251.86	1232.57	4.48	0.462
17	1284.65	36.277	18.037	1291.4	1262.46	9.254	1.959
18	1384.95	46.531	6.864	1389.77	1377.23	3.787	0.365
19	1405.2	29.965	22.482	1413.88	1390.74	8.98	2.533
20	1595.2	54.719	1.332	1598.09	1581.7	4.083	0.047
21	1648.24	42.381	1.724	1649.21	1639.56	3.911	0.061
22	1654.03	18.41	10.7	1658.85	1650.17	5.338	0.663
23	1662.71	21.835	10.251	1673.32	1659.82	6.621	0.771
24	2263.56	57.712	0.184	2268.38	2212.45	12.989	0.01
25	2311.79	49.807	0.478	2312.75	2282.85	7.908	0.012
26	2329.15	44.513	0.499	2330.11	2314.68	5.07	0.008
27	2342.65	42.975	2.639	2348.43	2338.79	3.437	0.16
28	2360.01	38.375	1.863	2361.94	2349.4	4.731	0.173
29	2423.66	56.321	0.021	2424.63	2393.76	7.644	0.009
30	2461.27	55.776	0.018	2462.24	2425.59	9.22	0.008
31	2512.39	54.962	0.086	2516.25	2467.06	12.65	0.029
32	2575.08	53.928	0.02	2576.04	2517.21	15.512	0.002
33	2625.23	52.364	0.415	2632.95	2576.04	15.59	0.044
34	2658.99	50.367	1.008	2665.74	2633.91	9.178	0.099
35	2682.13	48.999	1.749	2692.74	2666.7	7.812	0.154
36	2701.42	49.438	1.12	2710.1	2693.71	4.926	0.07
37	2729.39	45.924	3.568	2741.93	2711.07	9.756	0.381
38	2778.58	41.743	0.488	2780.51	2742.89	12.964	0.058
39	2790.15	38.893	5.284	2817.16	2781.47	12.239	0.458
40	2848.02	44.341	5.127	2868.27	2818.12	15.986	0.788
41	2895.28	41.984	5.135	2905.89	2869.24	12.754	0.892

Comment;
Ketotifen+Amoxicillin+CHCl3

Date/Time; 10/30/2011 12:23:18 PM
No. of Scans; 10
Resolution; 2 [1/cm]
Apodization; Happ-Genzel
User; SUMAN


SHIMADZU

42	2917.46	44.303	0.671	2920.35	2906.85	4.64	0.042
43	2941.57	40.89	3.483	2948.32	2921.32	9.98	0.465
44	2954.11	43.712	1.205	2961.82	2949.29	4.412	0.065
45	2969.54	44.012	3.96	2982.08	2962.79	6.363	0.332
46	3014.87	48.33	2.81	3023.55	2997.51	7.764	0.224
47	3056.34	49.274	0.27	3058.27	3042.84	4.666	0.019
48	3079.49	48.594	0.281	3084.31	3070.81	4.208	0.015
49	3114.21	46.243	2.338	3120.96	3098.78	7.137	0.198
50	3178.83	46.674	0.155	3179.79	3136.39	13.974	0.003
51	3198.11	45.992	0.163	3200.04	3187.51	4.189	0.004
52	3210.65	45.607	0.099	3211.62	3201.01	3.589	0.002
53	3233.8	44.88	0.06	3234.76	3223.19	4.008	0.006
54	3277.2	43.334	0.114	3278.16	3268.52	3.479	0.006
55	3327.35	41.955	0.06	3328.31	3315.78	4.693	0.003
56	3336.99	41.299	0.241	3338.92	3329.28	3.669	0.008
57	3357.25	40.426	0.077	3358.21	3339.89	7.101	0.003
58	3385.22	39.222	0.235	3388.11	3370.75	6.979	0.014
59	3420.9	37.897	0.716	3425.72	3406.44	7.983	0.034
60	3546.28	38.298	2.255	3550.14	3541.46	3.515	0.113
61	3567.5	36.214	4.823	3572.32	3562.68	3.958	0.201
62	3650.44	38.106	6.138	3654.3	3644.65	3.741	0.319
63	3690.95	41.062	3.561	3694.81	3683.23	4.217	0.177
64	3712.17	40.283	4.276	3716.99	3707.34	3.569	0.182
65	3735.31	37.273	5.493	3740.13	3730.49	3.786	0.232
66	3853.94	37.819	5.954	3859.73	3848.15	4.446	0.295

: Interacted peaks for ketotifenfumarate and amoxicillin

Ketotifenfumarate Wave number (cm^{-1})	**Amoxicillin Wave number (cm^{-1})**	**Ketotifenfumarate and amoxicillin (Aqueous extract) Wave number (cm^{-1})**		**Ketotifenfumarate and amoxicillin (Chloroform extract) Wave number (cm^{-1})**	
639.43	556.49	649.07	2360.01	703.08	2917.46
789.88	656.79	720.44	2521.07	734.91	2941.57
922.01	696.33	736.84	2558.68	757.09	2954.11
982.77	733.95	757.09	2603.05	782.17	2969.54
1082.11	1120.69	790.85	2691.78	855.47	3014.87
1149.62	1144.8	840.04	2705.28	922.01	3056.34
1180.49	1218.1	853.54	2722.64	950.95	3079.49
1201.7	1249.93	964.45	2735.18	983.74	3114.21
1254.75	1282.72	987.6	2749.65	1066.68	3178.83
1279.82	1313.58	1038.71	2794.97	1099.47	3198.11
1303.94	1328.05	1083.08	2808.48	1127.44	3210.65
1320.33	1379.16	1098.51	2837.41	1144.8	3233.8
1378.2	1396.52	1129.37	2853.81	1177.59	3277.2
1398.45	1451.5	1151.55	2891.42	1194.95	3327.35
1429.31	1486.22	1174.7	2900.1	1224.85	3336.99
1475.61	1520.94	1244.14	2918.42	1246.07	3357.25
1616.42	1576.87	1280.54	2928.07	1284.65	3385.22
1652.1	1581.7	1281.75	2961.82	1384.95	3420.9
1717.68	1616.42	1329.98	3012.94	1405.2	3546.28
2336.86	1684.89	1339.62	3049.59	1595.2	3587.5
2360.01	1775.55	1354.09	3065.02	1648.24	3650.44
2429.45	2360.01	1362.77	3079.49	1654.03	3690.95
2472.85	2713.96	1386.88	3178.83	1662.71	3712.17
2516.25	2905.89	1404.24	3198.11	2263.56	3735.31

2583.76	2930	1448.6	3210.65	2311.79	3853.94
2675.38	2969.54	1457.28	3233.8	2329.15	
2841.27	2990.76	1490.07	3255.02	2342.65	
2852.84	3035.12	1507.43	3274.31	2360.01	
2891.42	3040.91	1559.51	3292.63	2423.66	
2925.17	3166.29	1576.87	3327.35	2461.27	
2979.18	3177.86	1595.2	3336.99	2512.39	
3096.88	3185.58	1602.91	3356.28	2575.08	
3284.91	3336.99	1609.67	3367.86	2625.23	
3422.83	3368.82	1617.38	3379.43	2658.99	
3566.53	3385.22	1623.17	3392.93	2682.13	
	3420.9	1635.71	3420.9	2701.42	
	3446.94	1667.28	3446.94	2729.39	
	3462.37	1653.07	3481.66	2778.58	
	3523.13	2311.79	3509.63	2790.15	
		2329.15	3545.32	2848.02	
		2342.65	3566.53	2895.28	

Discussion

Thirty five peaks were observed in the infrared spectrum of ketotifen fumarete. Similarly forty seven and thirty one extra peaks were located in the infrared spectrum of when ketotifen& amoxicillin in aqueous extract (82 peaks) and ketotifen & amoxicillin in chloroform extract (66 peaks). All these extra peaks are the indication of drug interactions between ketotifen fumarate and combination forms.

***In-vivo* drug interactions study of ketotifen fumarate and amoxicillin**

After completion of the experiment it is observed that the probability value less than 0.05 ($p<0.05$) was defined to be statistically significant when the drugs given together (ketotifenfumarate& amoxicillin trihydrate) at 1:1 mixture.

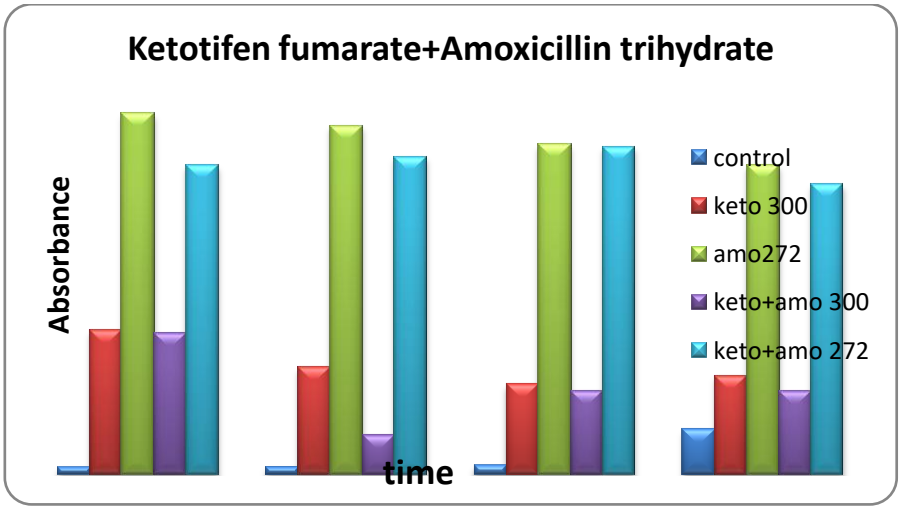


Figure 3: Graph for ketotifenfumarate& amoxicillin

Conclusion

Interaction of ketotifen with amoxicillindecreased the free drug concentration of both drugs which can result in decreased availability of the drugs at receptors. At the end of the research we can conclude that if both the amoxicillinand ketotifen administered at a time, the pharmacological response of any one may be decreased.

References

1. Martin U, Romer D (1978) “The pharmacological properties of a new, orally active anti- anaphylactic compound: Ketotifen, a benzocycloheptathiophene”. *ArzeneimForesch Drug Res*. 1978; **28** (7): 70-78.
2. Sokol K C, Amar N K, Starkey J, Grant J A (2013)"Ketotifen in the management of chronic urticaria: Resurrection of an old drug". *Annals of Allergy, Asthma & Immunology*.2013; **111** (6): 433–6.
3. Shawky R M, Seifeldin N S (2015) "The relation between antihistamine medication during early pregnancy & birth defects". *Egyptian Journal of Medical Human Genetics*. 2015; **16** (4): 287–90.
4. Torsten Z (2012) "A Summary of the New International EAACI/GA2LEN/EDF/ WAO Guidelines in Urticaria".*World Allergy Organization Journal*. 2012; **5** (1): 1–5.
5. Zuberbier T. et al (2009) "EAACI/GA²LEN/EDF/WAO guideline”: *Management of urticaria Allergy*. 2009; **64** (10): 1427–43.
6. Zhenhong L., Jocelyn C. (2015)“ Ketotifen: A Role in the Treatment of Idiopathic Anaphylaxis” *American Academy of Allergy*, Asthma & Immunology Annual Meeting. Houston.
7. Grahnen A, Lonnebo A, Beck O, Eckernas, S A, Dahlstrom B, Lindstrom B (1992) “Pharmacokinetics of ketotiffn after oral administration to healthy male subjects”.*Biopharmaceutics& Drug Disposition*.**13** (4): 255–62.
8. Klooker TK, Braak B, Koopman K E, Welting O, Wouters M M, Van Der Heide S, Schemann M, Bischoff S C, Wijngaard R M, Boeckxstaens G E (2010) “The mast cell stabiliserketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome”. *Gut*.**59** (9): 1213–21.
9. Amoxicillin, The American Society of Health-System Pharmacists, (2011).
10. Thornhill M H, Dayer M J, Forde J M, Corey G R, Chu V H, Couper DJ,Lockhar, PB,(2011) Impact of the NICE guideline recommending cessationof antibiotic prophylaxis for prevention of infective endocarditis before and afterstudy, *BMJ* (Clinical research ed.) , 342.
11. American Academy of Allergy, Asthma, and Immunology. (2012), Five ThingsPhysicians and Patients Should Question". Choosing Wisely: an initiative of theABIM Foundation.
12. Ahovuo A, Rautakorpi U M, Borisenko O V, Liira H , WilliamsJ W, Makela M. (2008), Antibiotics for acute maxillary sinusitis, *CochraneDatabase of Systematic Reviews*.
13. Sayeed M A, Sahaban M, KhalequeuzzamanM,Rafikul I, Rana S.(2013), In vitro study on interaction of ketotifenfumerate withamoxicillin trihydrate at different pH and are confirmed by IR spectroscopy,*ThePharma Innovation*, 1(11),20-28.
14. Cundiff, j.; Joe, S. 2007. "Amoxicillin-clavulanic acid-induced hepatitis".Amer.J. Otolaryngo, 28 (1): 28–30.

15. Sayeed M A, Hasan S M R, Rana S M (2011). “In vitro study on interaction ofketotifenfumerate with metformin hydrochloride”. *Latin American Journal of Pharmacy*.**30**, 189-192.
16 Villar A, Gorritxategi E, Aranzabe E, Fernandez S, Otaduy D, Fernandez L A (2012) “Low-cost visible–near infrared sensor for on-line monitoring of fat and fatty acids content during the manufacturing process of the milk”. *Food Chemistry*.**135** (4): 2756–2760.