Quantitative and Qualitative LC-MS/MS Analysis for Drug Discovery, Research and Development

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The former post
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Research Fellow & Scientific Adviser
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2004-2008
Director & Executive Officer
JCL Bioassay Corporation
1972-2004
Research Fellow
Biopharmaceutical and Pharmacokinetic Research Laboratories
Fujisawa Pharmaceutical Co.,LTD.

1972, Director Ryuichi Kato（Fujisawa Pharmaceutical Co.,Ltd.) scouted me as a Scientist of Mass Spectrometry and Chemistry of ^{14}C-labeled Compounds for Drug Discovery, Research and Development.

①1969 Hitachi RMU-7 (Osaka University)

②1972 S-LKB9000 GC-MS (Fujisawa)

③1972 Hitachi RMU-6M GC-MS (Fujisawa)

④1975 JEOL DX200 (Fujisawa)

⑤1977 F-M 4000 GC-MS (Fujisawa)

⑥1980 F-M 4500 GC-MS (Fujisawa)

⑦1985 F-M SSQ70 (Fujisawa)

⑧1987 F-M TSQ700 (Fujisawa)

⑨ **1990 F-M TSQ700 LC-MS** (Fujisawa)

⑩1993 F-M TSQ7000 LC-MS (Fujisawa)

⑪1998 F-M LCQ LC-MS (Fujisawa)

⑫1999 API3000(2), API2000(3) (Fujisawa)

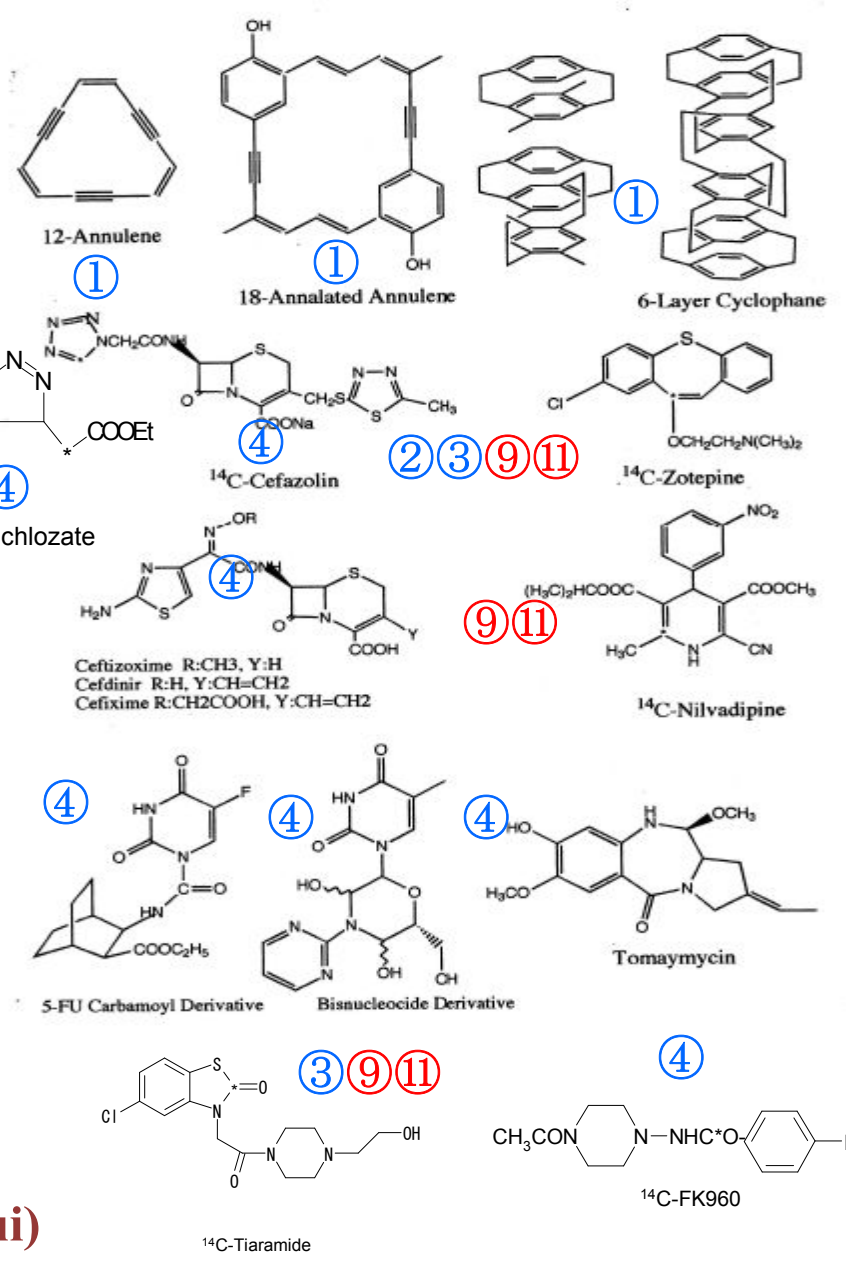
⑬2000 F LCQ Deca (3)LC-MS (Fujisawa)

⑭2001 T Quantam LC-MS (Fujisawa)

⑮2003 API4000(3) (Fujisawa)

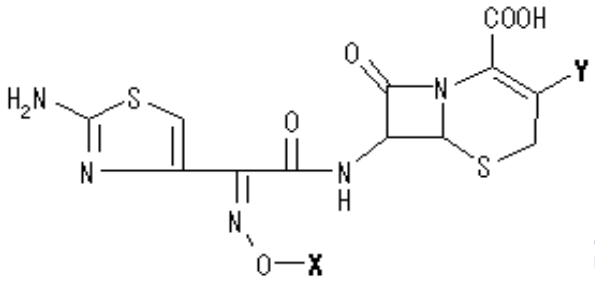
⑯2004 LTQ-FTICR MS (2), API5000(6) (JCL)

⑰2008 T LTQ Orbitrap, API5500QTrap (Sekisui)

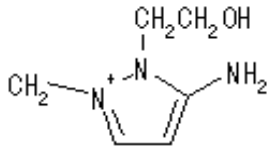


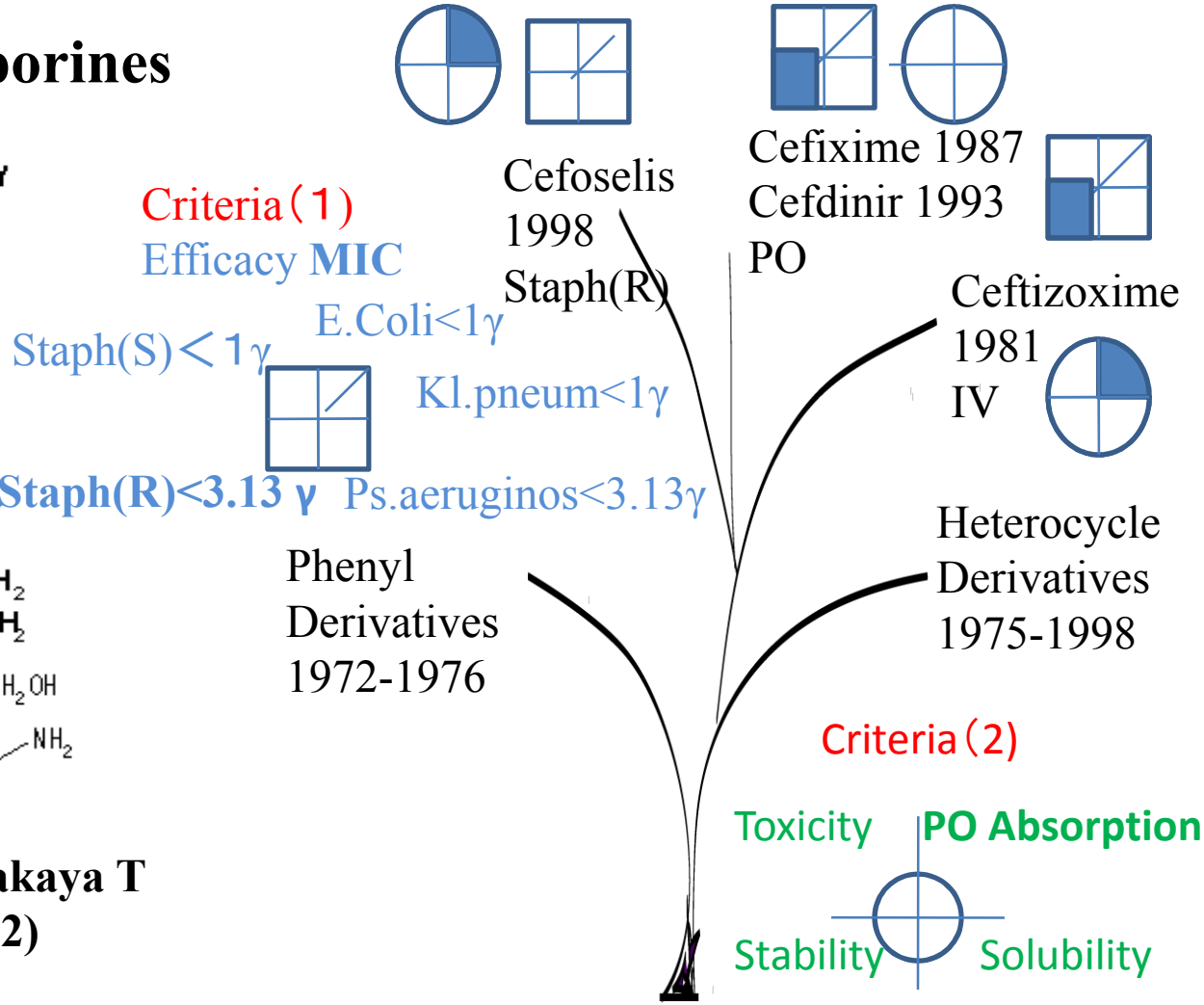
1975-1998,
The innovative drug discovery of cepharosporines

Synthesis of Cephalosporines



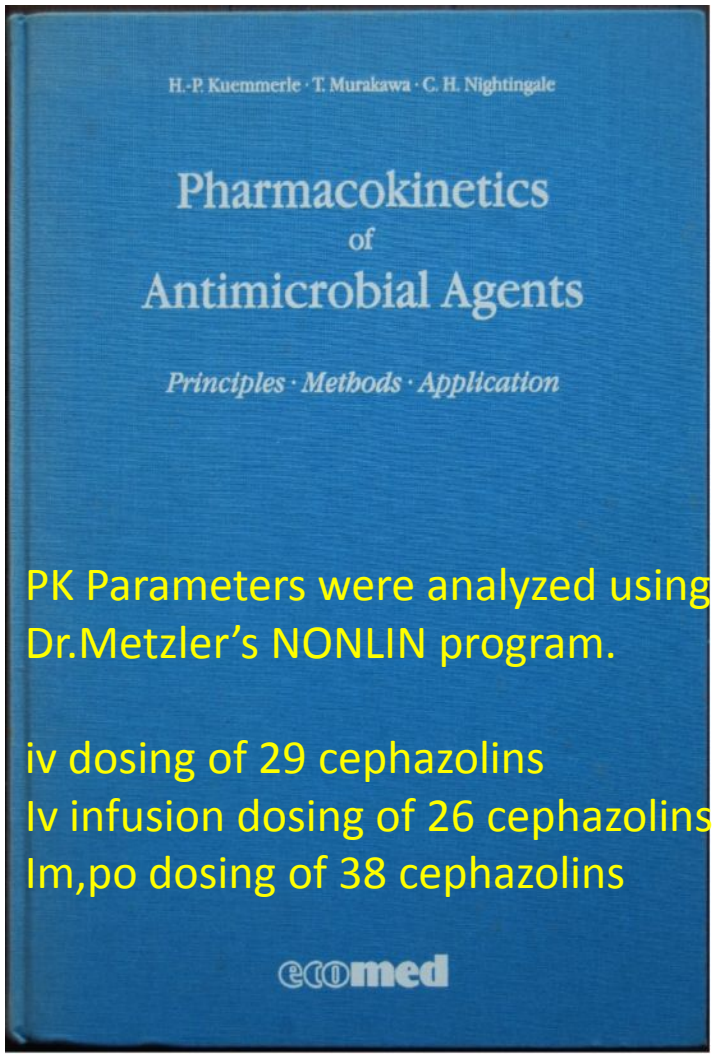
	X	Y
Ceftizoxime	CH_3	H
Cefixime	CH_2COOH	$CH=CH_2$
Cefdinir	H	$CH=CH_2$
Cefoselis	CH_3	





Tozuka Z.,Takasugi H., and Takaya T
***J. Antibiotics* 35: 585-588 (1982)**
***J. Antibiotics* 36: 36-41 (1983)**
Tozuka Z, Takasugi H, Chiba T, and Takaya T, US Patent 4,263,291, 1981 (April 3, 1979) GB 78-26806（19780413), Jpn.Kokai Tokkyo Koho (JP-77-110970), Brit.UK.Patent(GB 78-26806)GB 76-42057(19761008), FR 84-16694 (19841031), EP 86-101938 (19860215)

1975-1998, The innovative PK/PD study of cepharosporines



PK Parameters were analyzed using Dr.Metzler's NONLIN program.

iv dosing of 29 cephazolins
Iv infusion dosing of 26 cephazolins
Im,po dosing of 38 cephazolins

II – 2.1 Cephalosporins – Other β-Lactams – Parameters

By Z. Tozuka, Osaka

The serum data listed in this chapter was analysed and pharmacokinetic parameters were calculated. The results are summarized in Tables 1–6.

The pharmacokinetic parameters were analyzed using weighted nonlinear least squares regression. The analysis performed using Dr. Metzler's NONLIN program (The Upjohn Co., Kalamazoo, Mich. / USA). Observed concentration was used as the weighting factor.

In the case of the i.v. dosing of cephalosporins, the two-compartmental i.v. model provided the best fitting of the observed serum concentrations. The model was defined by the following equation:

$$C_t = A\,e^{-\alpha(t-T)} + B\,e^{-\beta(t-T)} \qquad 2.1$$

where C_t is the serum concentration at time t, T is the lag time, A and B are the coefficients of the distribution phase and the elimination phase, respectively, α and β are the rate constants of the distribution phase and the elimination phase, respectively. In the case of i.v. infusion dosing of cephalosporins, the two-compartmental i.v. infusion model provided the best fitting of the observed serum concentration. The model was defined by the following equations:

Infusion phase ($0 < t < T$)

$$C_t = A\,(1 - e^{-\alpha t})\,/\,(1 - e^{-\alpha T}) + B\,(1 - e^{-\beta t})\,/\,(1 - e^{-\beta T}) \qquad 2.2$$

Post-infusion phase ($t > T$)

$$C_t = A\,e^{-\alpha(t-T)} + B\,e^{-\beta(t-T)} \qquad 2.3$$

where T is the infusion time in hours. C_t, A, B, α and β were previously defined.

In the case of i.m. or p.o. dosing of cephalosporins, the two-compartmental model provided the best fitting of the observed serum concentration. The model was defined by the following equation:

$$C_t = A\,(e^{-\alpha(t-T)} - e^{-\beta(t-T)}) \qquad 2.4$$

where T is the lag time, α and β are the exponents of the absorption phase and the elimination phase, respectively.

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Z.Tozuka,
***Pharmacodynamics of Antimicrobial Agents*., Ecomed, 65-67, 69-91, 93-98, 107-108 (1993)**
Z. Tozuka and T. Murakawa,
***Antimicrobial Pharmacodynamics in Theory and Clinical Practice, Informa,* 129-146 (2007)**

Table 1: Pharmacokinetic data calculated from the serum concentration of cephalosporins in healthy subjects

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
cefamandole	1.0 g	i.m.	10	168.9	0.8088	0.905	1.320	0.8570	0.6191	57.98	20.11	1.047	0.996
cephapirin	1.0 g	i.m.	5	32.00	0.9390	0.389	17.31	0.7381	0.0400	32.22	25.69	0.567	0.829
cephalothin	0.5 g	i.m.	8	22.14	0.4765	0.352	15.62	1.455	0.0444	45.04	19.23	0.583	0.993
				52.14	0.4832	0.215	3.633	1.434	0.1908	93.35	33.17	0.856	1.000
cefotaxime	0.5 g	i.m.	6	21.91	0.6303	0.000	6.301	1.099	0.1066	31.38	15.68	0.398	0.990
cefotiam	0.5 g	i.m.	9	28.99	0.7830	0.000	3.719	0.986	0.1864	33.45	45.96	0.553	0.989
cefuroxime	0.5 g	i.m.	8	31.56	0.5633	0.334	18.07	1.255	0.0384	54.48	27.36	0.532	0.999
	1.0 g	i.m.	5										
cefmenoxime	0.5 g	i.m.	9	17.48	0.5366	0.000	3.201	1.292	0.2166	27.11	10.11	0.670	1.000
cefmetazole	0.5 g	i.m.	17	39.34	0.4743	0.112	12.07	1.465	0.0574	80.16	33.28	0.592	1.000
ceftizoxime	0.5 g	i.m.	29	24.67	0.4634	0.002	5.381	1.496	0.1288	48.65	17.89	0.300	1.000
cephacetrile	1.0 g	i.m.		46.37	0.7040	0.321	3.073	0.9846	0.2255	50.78	23.07	0.946	1.000
lenampicef	0.5 g	i.m.	4	34.04	0.2688	0.000	2.200	2.579	0.3151	111.2	22.30	1.089	0.988
ceftazidime	0.5 g	i.m.	3	40.34	0.4046	0.023	2.356	1.713	0.2967	92.43	25.10	0.931	1.000
cefazolin	0.5 g	i.m.	5	89.98	0.3830	0.000	1.859	1.810	0.3769	186.0	47.35	1.078	0.996
cefsulodin	0.5 g	i.m.	13	32.08	0.3792	0.079	1.399	1.828	0.2039	75.16	21.48	0.726	0.997
cefoxitin	0.5 g	i.m.	3	54.43	0.2607	0.000	1.941	2.659	0.3572	180.7	34.28	1.193	0.994
cefotetan	1.0 g	i.m.	18	108.5	0.2267	0.140	2.427	3.057	0.2855	405.3	71.97	1.218	0.999
cefroxadine	0.25 g	p.o.(F)	11	6.822	0.8712	0.333	1.094	0.7956	0.6337	15.94	5.70	1.355	0.949
	0.5 g	p.o.(F)	34	34.84	0.6534	0.199	1.497	1.061	0.4684	32.80	11.39	1.114	0.994
cefadroxil	0.25	p.o.(F)	10	12.17	0.5447	0.785	1.318	1.272	0.1306	28.05	8.42	1.243	0.902
	0.5 g	p.o.(F)	10	33.38	0.5035	0.333	1.908	1.375	0.3632	49.41	15.46	1.281	1.000
cephalexin	0.5 g	p.o.(F)	7	19.78	0.8589	0.950	17.84	0.7711	0.0389	28.90	16.63	1.127	0.933
	1.0 g	p.o.(F)	9										
cephradine	0.5 g	p.o.(F)	21	41.30	0.9618	0.299	2.125	0.7207	0.3262	32.51	11.78	0.981	1.000
	1.0 g	p.o.(F)	9	80.0	1.821	0.206	1.494	0.6787	0.4640	61.94	27.82	1.011	0.999
cefatrizine	0.5 g	p.o.(F)	8	167.3	0.7411	0.221	0.8022	0.9353	0.8641	17.19	4.853	0.989	1.000
cefaclor	0.5 g	p.o.(F)	20	6.749	0.9101	0.674	15.66	0.7616	0.0442	6.98	3.353	0.867	0.966

Table 1: Pharmacokinetic data calculated from the serum concentration of cephalosporins in healthy subjects (continued)

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
cefdinir	0.1 g	p.o.(F)	12	3.793	0.1480	0.393	1.954	4.917	0.3548	38.13	4.381	2.944	0.981
	0.2 g	p.o.(F)	9	28.22	0.3953	0.6205	0.9869	1.776	0.7023	45.72	9.301	2.175	0.995
cefpodoxime proxetil	0.25 g	p.o.(F)	6	15.63	0.3609	0.758	2.401	1.236	0.2888	20.94	7.627	1.344	0.968
	0.5 g	p.o.(F)	11	29.37	0.4483	0.320	1.155	1.573	0.6003	41.11	10.01	1.868	0.943
cefuroxime axetil	0.25 g	p.o.(F)	6	14.21	0.6083	0.245	1.282	1.139	0.1405	12.28	3.810	1.351	0.880
	0.25 g	p.o.(M)	6	23.70	0.65 7	0.746	1.358	1.064	0.1103	18.92	6.265	1.783	0.928
fusidic acid	0.5 g	p.o.(M)	20	10.80	1.2 9	0.419	1.457	0.5685	0.4758	2.673	1.307	1.168	0.996
lenampicillin	0.2 g	p.o.(M)	82	12.42	0.9498	0.563	1.595	0.7398	0.4343	5.291	2.343	1.346	0.982
	0.4 g	p.o.(M)	20	10.03	0.6537	0.584	2.298	1.080	0.3017	11.41	4.438	1.351	0.994
cefpodoxime proxetil	0.1 g	p.o.(F)	6	6.562	0.3624	0.446	0.6374	1.912	1.087	7.811	1.585	2.496	0.993
pivoxil	0.2 g	p.o.(F)	6	5.047	0.1477	0.286	0.7144	4.693	0.9702	27.10	2.655	3.067	0.733
cefteram pivoxil	0.25 g	p.o.(M)	17	40.80	0.4070	0.429	0.5259	1.703	1.318	22.21	3.759	2.581	0.987
	0.5 g	p.o.(M)	15	7.849	0.8697	0.726	1.767	7.970	0.3923	85.80	6.383	2.518	0.998
cefditoren pivoxil	0.1 g	p.o.(F)	6	11.39	0.4688	1.470	0.4688	1.475	1.147	5.451	1.062	3.343	0.984
	0.2 g	p.o.(F)	6	19.24	0.3283	0.767	0.3982	2.138	1.777	10.58	1.371	3.387	0.971
cefteram pivoxil	0.1 g	p.o.(F)	12	3.006	0.9210	0.000	0.3924	7.066	1.766	7.692	0.4758	4.710	0.957
	0.2 g	p.o.(F)	12	49.97	0.3341	0.000	0.3710	2.075	1.868	14.89	1.927	3.639	0.950
cefteram pivoxil	0.1	p.o.(M)	8	6.062	0.2585	0.632	0.4348	2.489	1.594	5.244	0.4681	3.482	0.892
	0.2	p.o.(M)	6	3.900	0.2564	0.631	1.155	2.421	0.5501	10.55	1.89	2.205	0.899

Table 2: Pharmacokinetic data calculated from the serum concentration of new β-lactams in healthy subjects

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
azthreonam	1.0 g	i.m.	9	68.47	0.2377	0.000	3.601	2.111	0.1925	189.9	48.96	0.722	0.998
neuroneam	1.0 g	i.m.	16	43.85	0.1810	0.111	5.476	2.094	0.1266	124.5	34.40	0.676	1.000

Z.Tozuka,
***Pharmacodynamics of Antimicrobial Agents*., Ecomed, 65-67, 69-91, 93-98, 107-108 (1993)**

Table 1: Pharmacokinetic data calculated from the serum concentration of cephalosporins in healthy subjects

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
cefamandole	1.0 g	i.m.	10	168.9	0.8088	0.005	1.320	0.8570	0.6191	57.98	20.12	1.047	0.996
cephapirin	1.0 g	i.m.	5	31.00	0.9390	0.389	17.31	0.7381	0.0400	32.22	25.80	0.567	0.839
cephalexine	0.5 g	i.m.	8	21.14	0.4765	0.352	15.62	1.455	0.0444	45.04	19.23	0.583	0.993
				51.14	0.4832	0.215	5.633	1.434	0.1908	93.55	33.7	0.856	1.000
cefotaxime	0.5 g	i.m.	6	21.91	0.6305	0.000	6.301	1.099	0.1066	31.38	15.41	0.398	0.993
cefotiam	0.5 g	i.m.	9	26.99	0.7038	0.000	3.719	0.986	0.1864	33.45	45.55	0.553	0.989
cefuroxime	0.3 g	i.m.	8	37.36	0.3813	0.334	18.07	1.235	0.0384	54.48	27.48	0.532	0.999
	1.0 g	i.m.	7										
cefmetazole	0.5 g	i.m.	9	17.48	0.3366	0.000	3.201	1.292	0.2166	27.31	10.35	0.670	1.000
cefmetazole	0.5 g	i.m.	17	39.34	0.4743	0.112	12.07	1.465	0.0574	80.16	33.34	0.392	1.000
cefuroxime	0.3 g	i.m.	29	24.67	0.4634	0.002	5.381	1.496	0.1288	48.63	17.59	0.500	1.000
cephacetrile	1.0 g	i.m.		46.37	0.7040	0.323	3.073	1.9846	0.2255	91.78	23.67	0.946	1.000
latamoxef	0.5 g	i.m.	4	34.04	0.2688	0.000	2.200	2.579	0.3151	111.2	22.41	1.089	0.988
cefmetazole	0.5 g	i.m.	3	48.34	0.4046	0.023	2.336	1.715	0.2967	82.43	23.11	0.931	1.000
cefazolin	0.5 g	i.m.	3	89.98	0.3830	0.000	1.835	1.810	0.3769	386.0	47.5	1.078	0.996
cefadroxil	0.5 g	i.m.	11	32.08	0.3792	0.079	3.399	1.828	0.2039	75.16	25.24	0.726	0.997
cefmenoxime	0.5 g	i.m.	3	81.42	0.2697	0.000	1.841	2.570	0.3771	180.7	34.11	1.195	0.994
cefotetan	1.0 g	i.m.	10	301.3	0.2267	0.340	2.427	3.057	0.2855	405.3	71.37	1.218	0.999
cefmenoxime	0.25 g	p.o.(F)	15	61.32	0.8712	0.333	1.094	0.7956	0.6337	15.94	5.30	1.353	0.849
	0.5 g	p.o.(F)	34	31.84	0.6534	0.199	1.697	1.061	0.4084	32.80	11.79	1.114	0.994
cefadroxil	0.25	p.o.(F)	10	12.17	0.3447	0.785	5.308	1.272	0.1306	20.05	8.12	1.243	0.902
	0.5 g	p.o.(F)	10	35.38	0.3059	0.533	1.908	1.375	0.3632	49.41	15.14	1.281	1.000
cephalexin	0.5 g	p.o.(F)	7	19.78	0.8989	0.910	17.84	0.7711	0.0389	20.90	16.35	1.127	0.955
	1.0 g	p.o.(F)	9										
cephradine	0.5 g	p.o.(F)	25	48.30	0.9618	0.299	2.125	0.7207	0.3262	32.31	11.74	0.981	1.000
	1.0 g	p.o.(F)	9	200.0	1.021	0.206	1.494	0.6787	0.4640	61.94	27.81	1.011	0.999
cefaclor	0.5 g	p.o.(F)	8	167.3	0.7411	0.221	0.8022	0.9353	0.8641	17.19	4.873	0.989	1.000
cefaclor	0.5 g	p.o.(F)	20	6.749	0.9101	0.674	11.44	0.7616	0.0482	6.98	5.353	0.867	0.966

Table 1: Pharmacokinetic data calculated from the serum concentration of cephalosporins in healthy subjects (continued)

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
ceftibuten	0.1 g	p.o.(F)	12	5.795	0.1480	0.593	1.954	4.917	0.3548	38.13	4.381	2.044	0.983
	0.2 g	p.o.(F)	9	28.22	0.2001	0.6285	0.5869	3.776	0.7023	43.72	9.301	2.175	0.995
cefpodoxime	0.25 g	p.o.(F)	6	15.65	0.5699	0.758	2.400	1.216	0.1888	20.84	7.627	1.544	0.968
	0.5 g	p.o.(F)	11	29.27	0.44.3	0.520	1.135	1.571	0.1003	41.31	10.01	1.868	0.945
cefuroxime axetil	0.25 g	p.o.(F)	6	14.21	0.6083	0.245	1.282	1.139	0.1403	12.28	3.810	1.351	0.980
	0.25 g	p.o.(M)	6	23.70	0.6517	0.746	1.338	1.064	0.5105	18.92	6.265	1.787	0.928
fenem	0.1 g	p.o.(M)	20	20.00	1.289	0.419	1.457	0.5685	0.4758	2.673	1.307	1.165	0.996
hexetil	0.2 g	p.o.(M)	92	12.42	0.9498	0.543	1.595	0.7398	0.4345	5.291	2.343	1.346	0.982
	0.4 g	p.o.(M)	20	10.61	0.6387	0.584	2.298	1.090	0.3017	11.41	4.438	1.358	0.994
cefpodoxime proxetil	0.1 g	p.o.(F)	6	6.562	0.3624	0.446	0.6374	1.912	1.087	7.811	1.343	2.499	0.993
	0.2 g	p.o.(F)	6	5.047	0.1477	0.286	0.7144	4.693	0.9702	27.10	2.651	3.067	0.711
cefuroxime axetil	0.25 g	p.o.(M)	17	40.00	0.4070	0.429	0.5219	1.705	1.318	32.21	3.759	2.581	0.987
	0.5 g	p.o.(M)	15	7.849	0.8497	0.726	1.767	7.970	0.3923	85.80	6.385	2.515	0.998
cefdinir	0.1 g	p.o.(F)	6	11.39	0.4888	1.470	0.4688	1.479	1.147	5.451	1.062	3.345	0.984
	0.2 g	p.o.(F)	6	19.24	0.3513	0.767	0.3982	2.358	1.777	10.28	1.371	3.387	0.971
cefixime	0.1 g	p.o.(F)	12	1.006	0.9810	0.800	0.3924	7.066	1.766	7.692	0.4754	4.718	0.957
	0.2 g	p.o.(F)	12	49.97	0.3641	0.800	0.3710	2.073	1.868	14.89	1.927	3.639	0.950
cefteram pivoxil	C.1	p.o.(M)	6	4.062	0.2785	0.632	0.4348	2.489	1.594	5.244	0.6683	3.482	0.892
	C.2	p.o.(M)	8	3.900	0.2864	0.631	1.195	2.421	0.5801	10.33	1.89	2.213	0.899

Table 2: Pharmacokinetic data calculated from the serum concentration of new β-lactams in healthy subjects

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h⁻¹)	LAG-T (h)	β (h⁻¹)	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
azthreonam	1.0 g	i.m.	9	68.47	0.2277	0.000	3.603	2.115	0.1925	189.9	48.96	0.732	0.999
carumonam	1.0 g	i.m.	16	41.81	0.1318	0.111	5.476	2.094	0.1266	124.5	34.40	0.636	1.000

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***Pharmacodynamics of Antimicrobial Agents*., Ecomed, 65-67, 69-91, 93-98, 107-108 (1993)**

Table 3: Pharmacokinetic data calculated from the serum concentration of parenteral cephalosporins

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h^{-1})	LAG-T (h)	β (h^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
cefamandole	1000 mg	0.33h. i.v. Inf.	10	91.06	4.802	10.64	1.146	0.1443	0.6047	63.14	87.15	1.000	1.000
cefoxitin	1000 mg	1h. i.v. Inf.	14	299.0	15.67	37.49	0.7304	0.0442	0.9480	70.41	45.48	1.000	1.000
flomoxef	1000 mg	1h. i.v. Inf.	25	344.5	18.82	36.54	0.7816	0.0370	0.8868	65.88	44.18	1.000	1.000
cefotiam	1000 mg	1h. i.v. Inf.	16	591.6	15.60	32.89	0.5240	0.0442	1.323	100.8	63.14	1.000	1.000
cefmetazole	1000 mg	1h. i.v. Inf.	17	117.7	3.273	11.64	0.7185	0.2118	0.9647	82.79	58.10	1.000	1.000
cefuzonam	2000 mg	1h. i.v. Inf.	5	214.5	3.028	13.13	0.8508	0.2289	0.8264	110.6	89.98	1.000	1.000
cefmetazole	1000 mg	1h. i.v. Inf.	14	74.69	2.853	45.71	0.5410	0.2446	1.281	147.8	75.16	1.000	1.000
ceftizoxime	1000 mg	1h. i.v. Inf.	18	60.70	2.038	32.84	0.4622	0.3401	1.500	101.9	52.20	1.000	0.995
cephacetrile	1000 mg	0.5h. i.v. Inf.		92.58	1.825	9.13	0.2122	0.3799	3.267	93.85	69.90	0.500	1.000
latamoxef	1000 mg	1h. i.v. Inf.	19	77.60	1.686	55.29	0.2892	0.4112	2.397	161.1	66.43	1.000	1.000
sulbutamoxole	1000 mg	0.5h. i.v. Inf.	3	197.7	5.989	89.29	0.4237	0.1157	1.628	266.2	111.8	1.000	0.988
ceftazidime	1000 mg	1h. i.v. Inf.	7	113.8	1.890	31.78	0.3633	0.3667	1.908	147.3	77.40	1.000	0.975
cefpiramide	1000 mg	1h. i.v. Inf.	17	53.25	2.168	37.33	0.3952	0.3197	1.754	169.6	69.13	1.000	0.976
cefpirome	1000 mg	1h. i.v. Inf.	10	189.2	5.891	31.53	0.4367	0.1177	1.380	136.0	60.20	1.000	0.999
cefsulodin	1000 mg	1h. i.v. Inf.	8	55.45	8.000	44.92	0.4997	0.0866	1.415	98.49	42.43	1.000	1.000
cefoxitin	1000 mg	1h. i.v. Inf.	4	216.4	7.999	30.69	0.2922	0.0866	2.372	301.2	97.02	1.000	1.000
cefepime	1000 mg	1h. i.v. Inf.	6	85.48	2.029	46.87	0.4199	0.3416	1.651	113.7	74.86	1.000	0.997
cefoperazone	1000 mg	1h. i.v. Inf.	6	357.1	8.000	91.81	0.2523	0.0866	2.748	408.6	125.8	1.000	0.997
cefmenoxime	1000 mg	1h. i.v. Inf.	3	234.3	9.000	92.23	0.2988	0.0770	2.320	334.7	105.8	1.000	0.999
ceforanide	1000 mg	0.5h. i.v. Inf.	10	528.0	9.000	70.44	0.2222	0.0770	3.120	375.8	121.8	1.000	0.865
cefonicid	2000 mg	1h. i.v. Inf.	3	354.0	0.7431	109.9	0.1958	0.9328	3.614	780.4	208.7	1.000	0.999
cefpiramide	1000 mg	1h. i.v. Inf.	10	109.4	3.801	130.4	0.2364	0.2310	2.933	588.2	150.8	1.000	0.896
ceftriaxone	1000 mg	1h. i.v. Inf.	1	138.9	1.808	107.6	0.1152	0.3833	6.016	1005.0	161.2	1.000	0.893

Table 4: Pharmacokinetic data calculated from the serum concentration of new β-lactams in healthy subjects

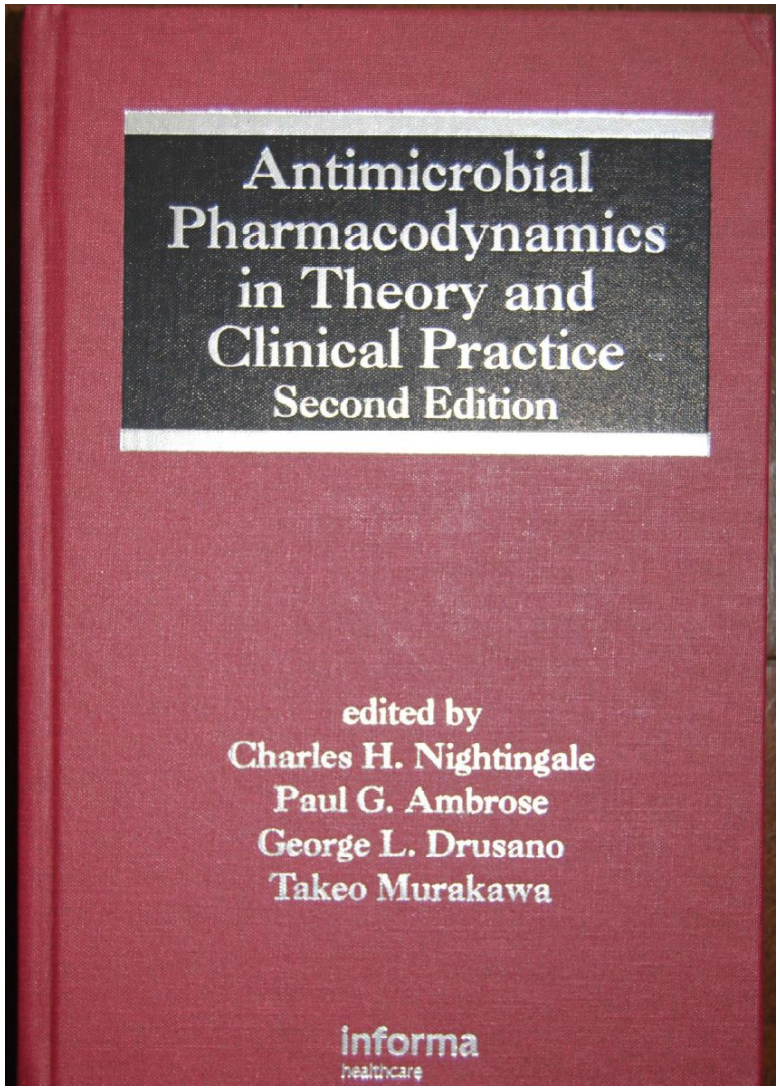
Compound	Dose	Route of Administration	n	A (μg/ml)	α (h^{-1})	LAG-T (h)	β (h^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	Corr
cefpirome	1000 mg	1h. i.v. Inf.	4	74.01	0.7691	51.16	0.7691	0.1807	1.9012	85.81	54.57	1.000	0.996
CS-533	1000 mg	1h. i.v. Inf.	5	71.27	1.430	18.54	0.5703	0.4849	1.203	82.03	52.01	1.000	0.985
	500 mg	1h. i.v. Inf.	5	36.48	2.866	15.87	0.6033	0.3355	1.149	44.06	27.42	1.000	1.000
	250 mg	1h. i.v. Inf.	5	17.64	2.750	11.67	0.7547	0.2520	0.9699	22.73	14.33	1.000	1.000
cefbuperazone	1000 mg	1h. i.v. Inf.	5	73.27	1.451	66.09	0.3852	0.4777	1.788	222.0	93.51	1.000	0.994
carumonam	1000 mg	1h. i.v. Inf.	33	78.79	1.958	57.02	0.4641	0.3504	1.493	320.0	64.18	1.000	1.000

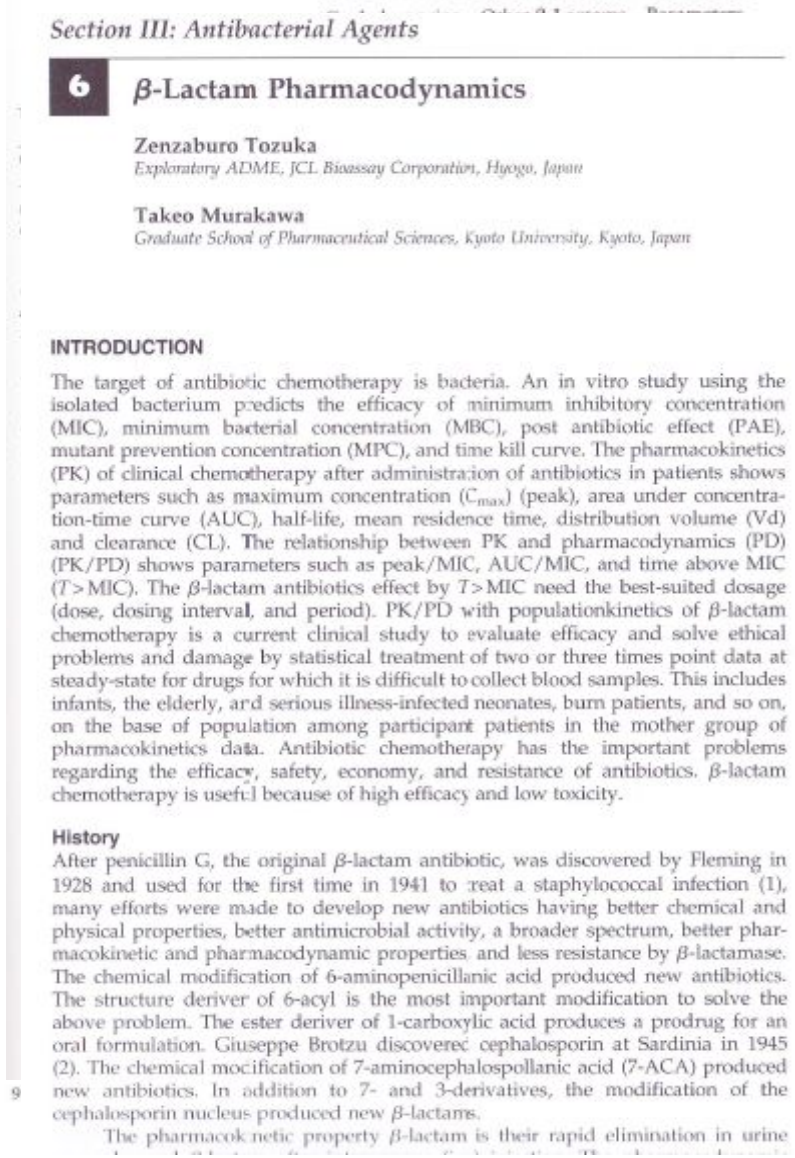
Table 5: Pharmacokinetic data calculated from the serum concentration of parenteral cephalosporins in healthy subjects

Compound	Dose	Route of Administration	n	A (μg/ml)	α (h^{-1})	LAG-T (h)	β (h^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0-\infty}$ (μg·h/ml)	Corr
cefodizime	1.0 g	i.v.	6	262.2	36.50	51.53	1.349	0.0477	0.5139	56.28	0.999
cephalothin	1.0 g	i.v.	6	163.7	5.887	27.64	1.473	0.0763	0.4704	36.89	1.000
cephapirin	1.0 g	i.v.	5	265.9	3.920	15.91	1.109	0.1771	0.6251	59.27	1.000
cefuzonam	1.0 g	i.v.	14	83.75	2.973	23.24	0.7410	0.2332	0.9554	59.53	1.000
flomoxef	1.0 g	i.v.	22	84.34	4.559	46.18	0.8781	0.1520	0.7893	79.08	1.000
cefotiam	0.5 g	i.v.	33	89.20	4.076	23.82	0.6854	0.1141	1.011	49.58	0.999
cefoxitin	1.0 g	i.v.	6	84.98	1.337	30.46	0.5872	0.2986	1.181	88.23	1.000
cefuzonam	1.0 g	i.v.	3	63.22	3.029	41.23	0.6846	0.2288	1.146	89.07	0.999
	1.5 g	i.v.	2	476.2	5.999	42.60	0.5905	0.0691	1.174	153.6	0.999
cefbuperazone	1.0 g	i.v.	37	126.3	4.096	21.75	0.7199	0.1235	0.9629	73.08	1.000
cefuzonam	1.0 g	i.v.	21	100.3	1.183	19.86	0.7660	0.2171	0.905	17.43	0.999
cefoxitin	1.0 g	i.v.	19	120.0	19.89	9.00	0.1370	0.0134	5.058	67.68	0.918
cefuzonam	1.0 g	i.v.	15	114.7	1.024	17.42	0.3263	2.124	2.124	164.7	1.000
latamoxef	1.0 g	i.v.	26	97.36	1.434	40.39	0.3810	0.2631	1.810	195.2	1.000
cefbuperazone	1.0 g	i.v.	6	82.54	1.293	79.19	0.3029	0.3028	1.378	193.5	1.000
cefsulodin	1.0 g	i.v.	24	75.38	1.947	44.21	0.3953	0.3582	1.754	171.0	1.000
cefpiramide	1.0 g	i.v.	18	83.16	1.042	37.32	0.3894	0.3398	1.780	136.4	1.000
cefsulodin	1.0 g	i.v.	6	113.3	3.674	86.84	0.3985	0.1887	1.740	246.2	0.998
cefpirome	1.0 g	i.v.	11	50.95	5.129	37.21	0.3963	0.3253	1.749	117.8	1.000

Z.Tozuka,
***Pharmacodynamics of Antimicrobial Agents*., Ecomed, 65-67, 69-91, 93-98, 107-108 (1993)**

1975-1998, The innovative PK/PD study of cepharosporines





Section III: Antibacterial Agents

6 β-Lactam Pharmacodynamics

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INTRODUCTION

The target of antibiotic chemotherapy is bacteria. An in vitro study using the isolated bacterium predicts the efficacy of minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC), post antibiotic effect (PAE), mutant prevention concentration (MPC), and time kill curve. The pharmacokinetics (PK) of clinical chemotherapy after administration of antibiotics in patients shows parameters such as maximum concentration (C_{max}) (peak), area under concentration-time curve (AUC), half-life, mean residence time, distribution volume (Vd) and clearance (CL). The relationship between PK and pharmacodynamics (PD) (PK/PD) shows parameters such as peak/MIC, AUC/MIC, and time above MIC ($T>MIC$). The β-lactam antibiotics effect by $T>MIC$ need the best-suited dosage (dose, dosing interval, and period). PK/PD with populationkinetics of β-lactam chemotherapy is a current clinical study to evaluate efficacy and solve ethical problems and damage by statistical treatment of two or three times point data at steady-state for drugs for which it is difficult to collect blood samples. This includes infants, the elderly, and serious illness-infected neonates, burn patients, and so on, on the base of population among participant patients in the mother group of pharmacokinetics data. Antibiotic chemotherapy has the important problems regarding the efficacy, safety, economy, and resistance of antibiotics. β-lactam chemotherapy is useful because of high efficacy and low toxicity.

History

After penicillin G, the original β-lactam antibiotic, was discovered by Fleming in 1928 and used for the first time in 1941 to treat a staphylococcal infection (1), many efforts were made to develop new antibiotics having better chemical and physical properties, better antimicrobial activity, a broader spectrum, better pharmacokinetic and pharmacodynamic properties and less resistance by β-lactamase. The chemical modification of 6-aminopenicillanic acid produced new antibiotics. The structure deriver of 6-acyl is the most important modification to solve the above problem. The ester deriver of 1-carboxylic acid produces a prodrug for an oral formulation. Giuseppe Brotzu discoverec cephalosporin at Sardinia in 1945 (2). The chemical mocification of 7-aminocephalospollanic acid (7-ACA) produced new antibiotics. In addition to 7- and 3-derivatives, the modification of the cephalosporin nucleus produced new β-lactams.

The pharmacokinetic property β-lactam is their rapid elimination in urine

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Z.Tozuka,
***Pharmacodynamics of Antimicrobial Agents*., Ecomed, 65-67, 69-91, 93-98, 107-108 (1993)**
Z. Tozuka and T. Murakawa,
***Antimicrobial Pharmacodynamics in Theory and Clinical Practice, Informa,* 129-146 (2007)**

6 β-Lactam Pharmacodynamics

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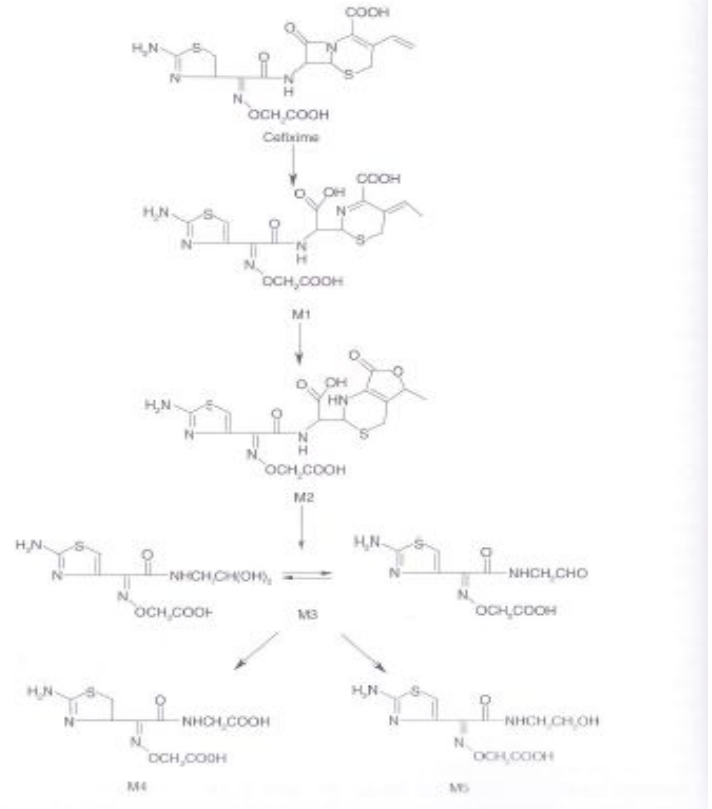
The pharmacok netic property β-lactam is their rapid elimination in urine

Intestinal β-lactamase metabolize oral β-lactam agents such as cefixime and the metabolites are absorbed into the body. The metabolite was studied by mass spectrometry as exemplified by the metabolism of cefixime (70). The structures of M4 and M5 were detected in biological samples such as serum, urine, and feces and identified by comparison to synthesized authentic samples. M3 was detected only in feces and is a key compound to study on the metabolism of cephalosporins, but it was difficult to determine the chemical structure because of a mixture between aldehyde and acetal. NMR spectra of the mixture showed a small amount of the aldehyde proton and the counter amount of the acetal proton at the field of the specific chemical shift. It was determined as dinitrophenylhydrazone of which secondary ion mass spectrometry SIMS showed m/z 467 as the quasi-molecular ion.



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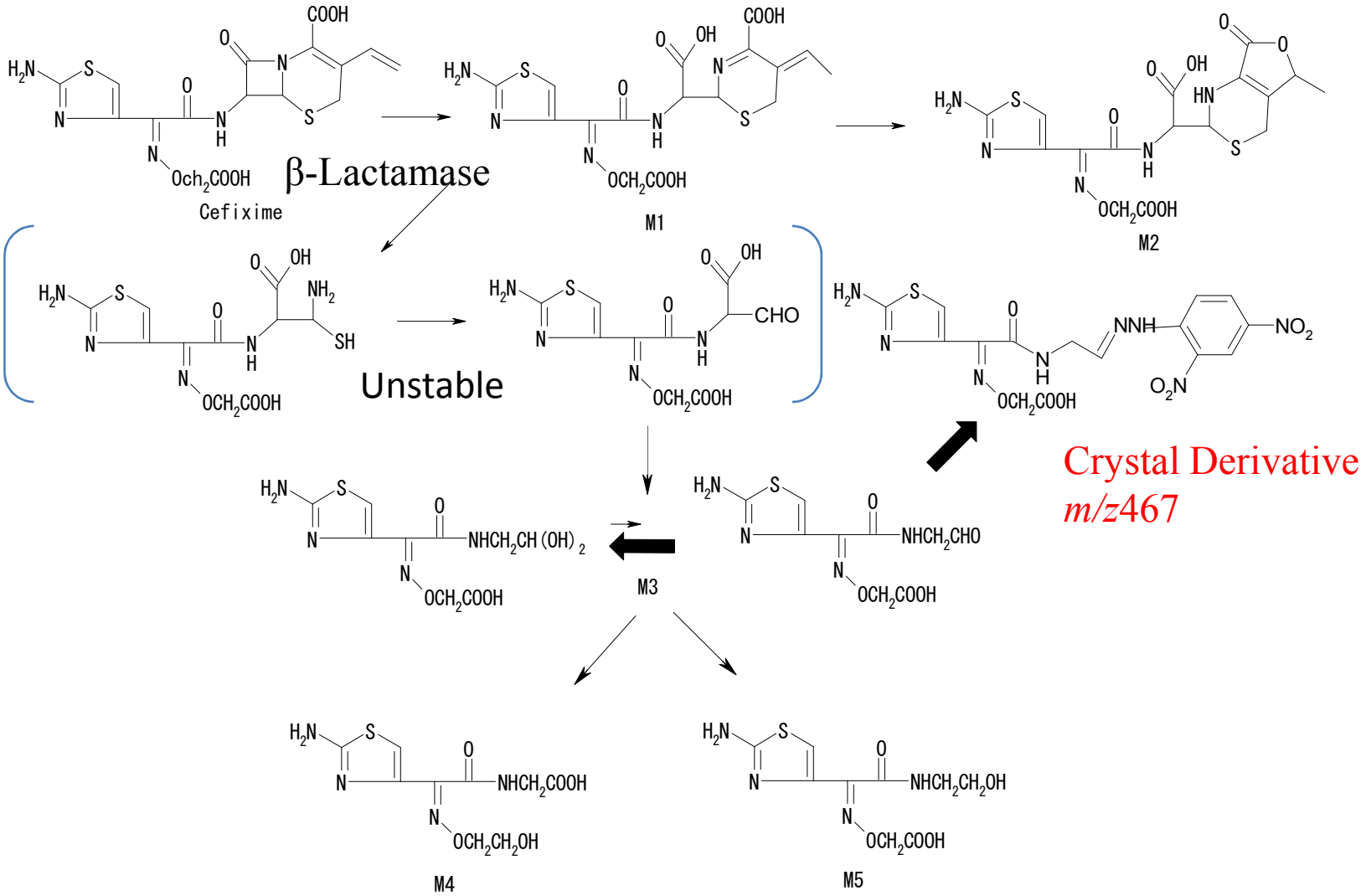
1983-1984,

The Innovative Structural Analysis of Cefixime Metabolites



Metabolism of Cefixime

Z.Tozuka, *Antimicrobial Pharmacodynamics in Theory and Clinical Practice,Informa,* 129-146 (2007), J Mass Spectrom 2003, 793-808.

1992-1995, Hot ADME Study of ^{14}C-Cefoselis

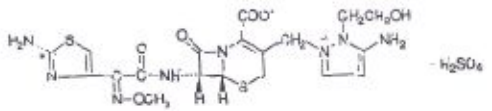


Fig. 1 Chemical structure of ^{14}C-FK037
Asterisk indicates position of ^{14}C label.

薬物動態, 10(1): 119-128 (1995).

FK037 の体内動態（第 1 報）：ラットおよびイヌに単回静脈内投与後の分布および排泄

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Disposition of FK037 (1): Distribution and Excretion of FK037 in Rats and Dogs after Single Intravenous Dosing

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Summary

Distribution and excretion of radioactivity were studied in male rats and male dogs after an intravenous dosing of ^{14}C-FK037 (20 mg/kg).

1. After dosing to rats, plasma and blood levels of radioactivity declined rapidly; the radioactivities at 4 hr after dosing were less than 1% of the concentration at 5 min. The unchanged FK037 in the plasma disappeared two-exponentially with the terminal half-life of 0.43 hr. The ratio of the unchanged FK037 to total radioactivity in the plasma was more than 95% between 5 and 30 min after dosing, and then decreased gradually. The highest levels of radioactivity at 5 min after dosing were detected in the kidney, followed by plasma > blood > lung > liver, heart, spleen > brain. The radioactivities in the tissues, except for the kidney, were not detectable at 24 hr after dosing, and that in the kidney decreased to less than 0.5% of the maximum values. Urinary and fecal excretion of radioactivity was 92.6 and 4.6%, respectively, during 72 hr after dosing. Urinary and biliary excretion of radioactivity in the bile duct-cannulated rats was 98.6 and 1.8%, respectively within 48 hr after administration. Urinary excretion of the unchanged FK037 was 95.4% during 48 hr, indicating that most of radioactivity in the urine was in the unchanged form.
2. After dosing to dogs, plasma and blood levels of radioactivity declined rapidly; the radioactivities at 8 hr after dosing were about 1 % of the concentration at 5 min. The unchanged FK037 in the plasma disappeared two-exponentially with the terminal half-life of 1.23 hr. The ratio of the unchanged FK037 to total radioactivity in the plasma was more than 97% between 5 min and 2 hr after dosing, and then decreased gradually. Urinary and fecal excretion of radioactivity was 99.8 and 2.4%, respectively, during 168 hr after dosing. Urinary excetion of the unchanged FK037 was 87.7% during 168 hr, indicating that most of radioactivity in the urine was the unchanged FK037.

Key words : FK037, Rat, Dog, Intravenous dose, Radioactivity, Unchanged drug

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薬物動態, 10(

FK037 の体内動態（第 2 報）：ラットにおける反復投与後の体内動態

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黒沢 敏*・二宮 真一*・塙 真也*・石崎 正男*

Disposition of FK037 (2): Distribution, Metabolism and Excretion of FK037 in Rats after Multiple Intravenous Dosing

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**Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ltd., Ibaraki*

Summary

The ^{14}C-labelled compound of FK037 (^{14}C-FK037), a new antibiotics, was intravenously administered in male rats at a dose of 20 mg/kg once a day for 14 days, and its distribution, metabolism and excretion were investigated.

1. The radioactivity in the blood collected at 8 hr after 1st, 2nd, 10th and 14th dosing was as follows n.d. (not detected), 0.24, 0.42 and 0.43 μg eq./ml, respectively and attained the steady state after the 10th dosing. The area under the blood concentration—time curves up to infinite ($AUC_{0-\infty}$, 22.6 μg eq.·hr/ml) after single intravenous administration was similar to $AUC_{0-24\,hr}$ after 7th and 14th dosing (19.2 and 24.4 μg eq.·hr/ml, respectively).
2. Tissue levels of radioactivity in most of tissues at 8 hr after each dosing increased according to the multiple intravenous dosing for 14 days. The radioactivity in the tissues such as kidney, skin, bladder, stomach, lung, brown fat, skeletal muscle, prostate gland and parotid gland at 8 hr after the final dosing was 3.1–5.5 times higher than those at 8 hr after the 1st dosing. The radioactivity in other tissues at 8 hr after the final dosing was 2 times higher than those at 8 hr after the 1st dosing. Tissue concentration of radioactivity at 24 hr after the final dosing was less than 4% of those at 5 min after the final dosing.
3. During the multiple intravenous dosing of ^{14}C-FK037, the ratios of unchanged FK037 and metabolites in plasma and urine were not changed. The most of radioactivity in plasma and urine consisted of the unchanged FK037.
4. During the multiple intravenous dosing of ^{14}C-FK037, the ratios of urinary and fecal excretion of radioactivity were not changed. The urinary excretion of radioactivity was 94.2 and 94.3% of cumulative doses at 24 and 120 hr after the final dosing, respectively.

Key words : FK037, Rat, Multiple doses, Radioactivity, Distribution, Accumulation, Metabolism, Excretion

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薬物動態, 10(1): 142-153 (1995).

FK037 の体内動態（第 3 報）：ラットにおける胎児移行および乳汁移行

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黒沢 敏*・二宮 真一*・塙 真也*

Disposition of FK037 (3): Transfer into the Fetus and Milk in Rats after Single Intravenous Dosing

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Summary

The ^{14}C-labelled compound of FK037, a new antibiotics, was administered intravenously at a dose of 20 mg/kg to pregnant and lactating rats to study its transfer into the fetus and milk, respectively.

1. The radioactivity levels in the fetus was only 1 % of that in the maternal plasma at 5 min after intravenous dosing to rats on day 13 of gestation and was not detected at 24 hr after dosing. The radioactivity in the fetus was less than 1 % of that in the maternal plasma at 5 min after intravenous dosing to rats on day 18 of gestation and declined to 19% of the maximum concentration at 24 hr after dosing. The whole body autoradiograms showed no radioactivity in the fetus after intravenous dosing to rats on day 13 and 18 of gestation.
2. The radioactivity in milk attained the maximum concentration of 0.93 μg eq./ml at one hour after intravenous dosing to lactating rats, then declined with a half life of 5.6 hr until 8 hr after dosing, and was not detected at 24 hr after dosing.

Key words : FK037, Rat, Intravenous dose, Fetus, Milk, Radioactivity

緒 言

FK037 は藤沢薬品工業株式会社で創製された注射用セファロスポリン剤で，好気性および嫌気性のグラム陽性菌，陰性菌に幅広い抗菌スペクトルと強い抗菌力を示し，ブドウ球菌および緑膿菌にも良好な活性を有している[1-3]．特にメチシリン高度耐性ブドウ球菌（H-MRSA）に対して既存のセフェム剤より強い抗菌力を示し[4]，さらにceftazidime 耐性の弱毒グラム陰性桿菌に対しても活性を有する[1-3]．本試験では，^{14}C 標識 FK037 を妊娠ラットあるいは哺育中雌性ラットに静脈内投与後の組織内濃度，全身オートラジオグラフィーによる放射能分布および乳汁中放射能

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Tozuka Z.et al.FK037 の体内動態、*薬物動態*, 10(1)、(1995)
(第1報) : 単回投与*薬物動態*, 10(1) P119-128, (1995) 、(第2報)反復投与 P129-141,（第3報) : 胎児移行および乳汁移行,*P*142-153, (1995)

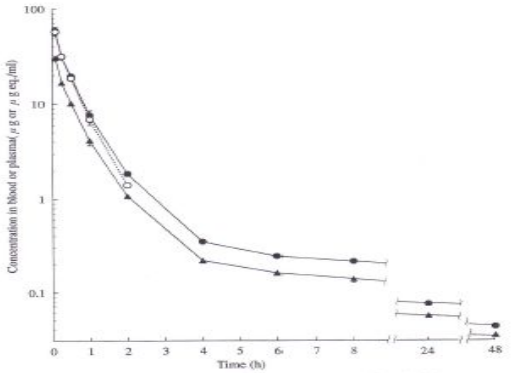

Fig. 2 Plasma (●) and blood (▲) concentrations of radioactivity and plasma concentration of unchanged FK037 (○) after an intravenous dosing of ^{14}C–FK037 to rats

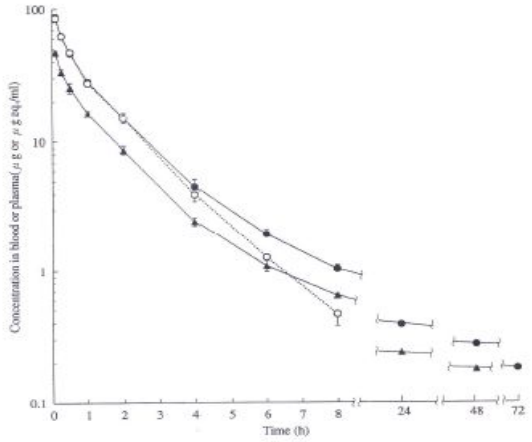

Fig. 3 Plasma (●) and blood (▲) concentrations of radioactivity and plasma concentration of unchanged FK037 (○) after an intravenous dosing of ^{14}C–FK037 to dogs

Table I Pharmacokinetic parameters of FK037 after intravenous dosing of ^{14}C–FK037 (20 mg/kg) to rats and dogs

Parameter	Rat	Dog
A (μg/ml)	43.1	54.4±3.2
B (μg/ml)	35.3	39.7±2.2
α (/hr)	6.21	2.34±0.26
β (/hr)	1.61	0.57±0.02
$T_{1/2}\alpha$ (hr)	0.11	0.30±0.03
$T_{1/2}\beta$ (hr)	0.43	1.23±0.04
$AUC_{0-\infty}$ (μg·hr/ml)	28.8	93.9±5.0
CL (ml/min/kg)	9.74	3.00±0.15
Vd_{ss} (ml/kg)	298	257±6

Results are mean values or mean values±S.E. for three animals.

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Table III Radioactivity concentration in milk and plasma after single intravenous administration of ^{14}C–FK037 to non-fasting lactating rats (dose: 20 mg/kg)

Time	Radioactivity concentration (μg eq. of free FK037/g or ml)	
	Milk	Plasma
5 min	0.17±0.06 (0.00)	59.34±1.17
1 hr	0.93±0.08 (0.07)	13.91±1.86
2	0.91±0.05 (0.25)	3.63±1.26
4	0.79±0.02 (1.72)	0.46±0.06
8	0.42±0.09 (1.62)	0.26±0.02
24	N.D.	0.12±0.02
48	N.D.	N.D.

Data are expressed as the mean values±S.E. of three animals.
Figures in parentheses are expressed as the ratio of concentration in milk relative to plasma.
N.D.: Not detected.

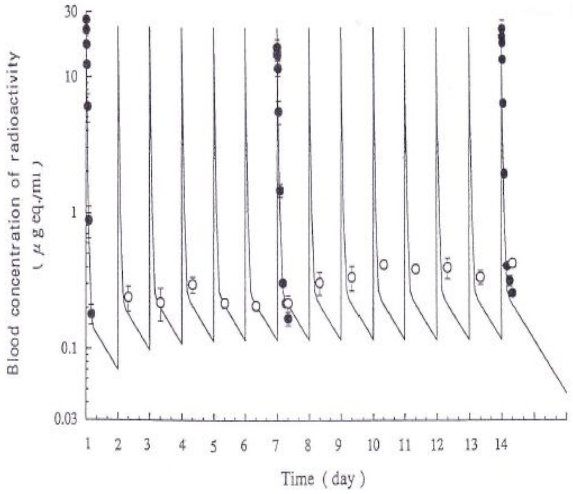

Fig. 7 Blood concentration of radioactivity during multiple intravenous administration of ^{14}C–FK037 for 14 days to male rats (○; at 8 hr after each dosing, ●; on 1st, 7th and 14th day) and its simulation curve calculated from pharmacokinetic parameters of single intravenous administration of ^{14}C–FK037 (dose 20 mg/kg/day, Mean±S.E., n=3)

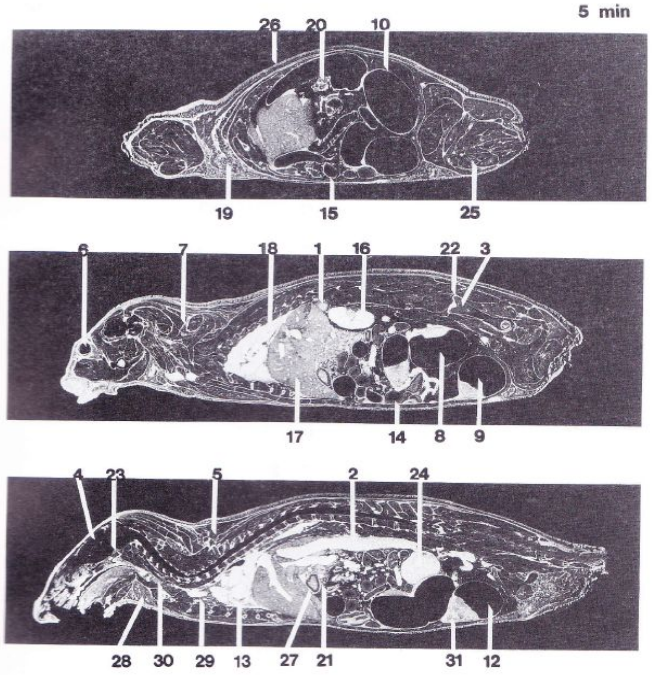

Fig. 5 Whole body autoradiograms at 5 min after single intravenous administration of ^{14}C–FK037 to a non-fasting rat on the 18th day of pregnancy (dose 20 mg/kg)

1. Adrenal gland
2. Blood
3. Bone marrow
4. Brain
5. Brown fat
6. Eyeball
7. Fascia
8. Fetal blood
9. Fetal liver
10. Fetal membrane
11. Fetal urine in bladder
12. Fetus
13. Heart
14. Intestinal contents
15. Intestine
16. Kidney
17. Liver
18. Lung
19. Mammary gland
20. Ovary
21. Pancreas
22. Periosteum
23. Pituitary gland
24. Placenta
25. Skeletal muscle
26. Skin
27. Stomach
28. Submaxillary gland
29. Thymus
30. Thyroid gland
31. Uterus
32. Urine in bladder

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1980 Paradigm Shift of Drug Discovery by Genetic Engineering

C-DNA Synthesis Project of Human Growth Hormone

Ikehara M, Otsuka E, Matsubara K, Fujino M, Marumoto K. Shin M, Tozuka Z. Nucleic Acids Research 11,192 (1982)

A new condensing reagent, 1-(2,4,6-triisopropylbenzenesulphonyl-5-(pyridin-2-yl)tetrazolide and its use in the synthesis of λ cro heptadecanucleotide binding on a polymer support

E.Ohstuka,Z.Tozuka, S.Iwai and M.Ikehara

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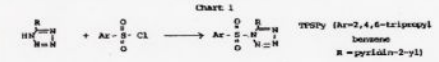
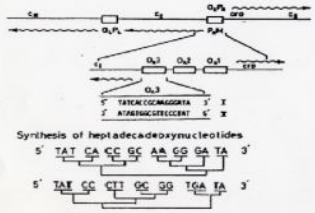
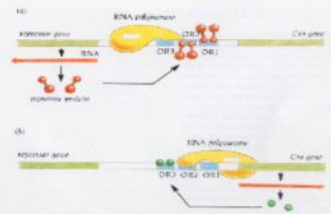
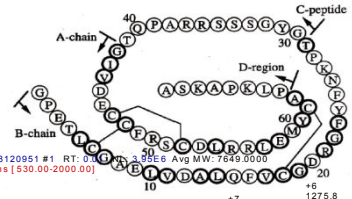


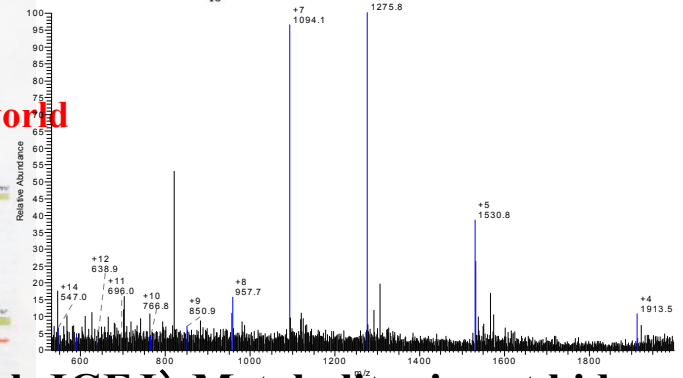
The First DNA Synthesis on Solid Phase in the world





1981-1999 The Research & Developmen of Somazone（rh-IGF I）By LC-MS





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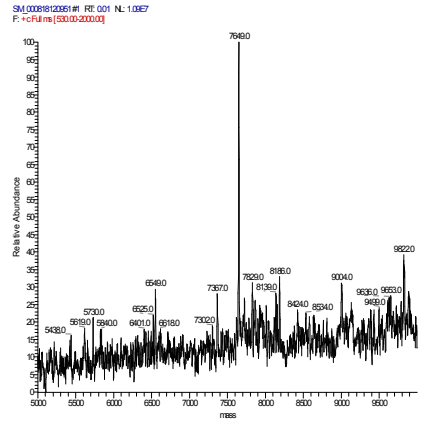
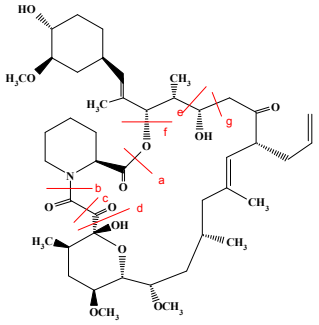


Structure Determination of Somazone（rh-IGF I）Metabolites in rat kidney lysosomes by LC-MS

Peak	Product	M W (observed)	M W (theoretical)	Structure
1	I	4900		
2	II	4882	4883	GIVDECCFRSCDLRRLEMC GPETLCGAELVDALQFVCGDRGKP
3	III-1	4203	4204	VDECCFRSCDLRRLEMYC TLCGAELVDALQFVCGDRG
	III-2	4487	4487	VDECCFRSCDLRRLEMYC GPETLCGAELVDALQFVCGDRG
	III-3	4615	4614	IVDECCFRSCDLRRLEMYCA GPETLCGAELVDALQFVCGDR
	III-4	4831	4832	GIVDECCFRSCDLRRLEMYC TLCGAELVDALQFVCGDRGFYF
4	IV-1	4316	4317	IVDECCFRSCDLRRLEMYC TLCGAELVDALQFVCGDRG
	IV-2	4430	4430	GIVDECCFRSCDLRRLEMYC TLCGAELVDALQFVCGDR
	IV-3	4599	4560	VDECCFRSCDLRRLEMYC GPETLCGAELVDALQFVCGDR
	IV-4	4953	4953	IVDECCFRSCDLRRLEMYC GPETLCGAELVDALQFVCGDRG
5	V	4567	4567	IVDECCFRSCDLRRLEMYCAPLK GPETLCGAELVDALQFVCGDR
6	VI	4600	4600	GIVDECCFRSCDLRRLEMYC GPETLCGAELVDALQFVCGDRG
7	VII	4728	4728	GIVDECCFRSCDLRRLEMYC GPETLCGAELVDALQFVCGDR
				GIVDECCFRSCDLRRLEMYCA GPETLCGAELVDALQFVCGDRG

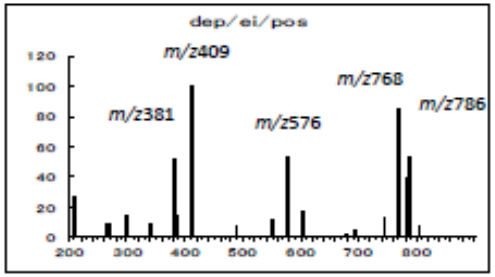
- Metabolism and degradation products of recombinant human insulin-like growth factor-I in lysosomes of rat kidney. Tanaka Y, Tamoto H, Tozuka Z, Sato A, Kimura T、*Xenobiotica*, 29 (3) 281-295 (1999)

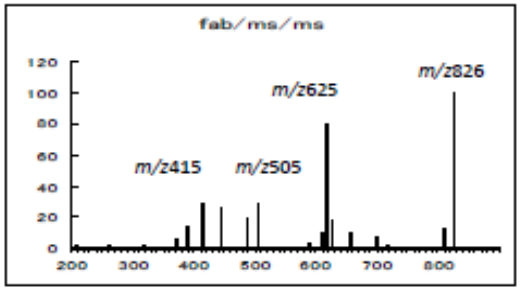
Qualitative LC-MS/MS Analysis of Tacrolims Metabolites

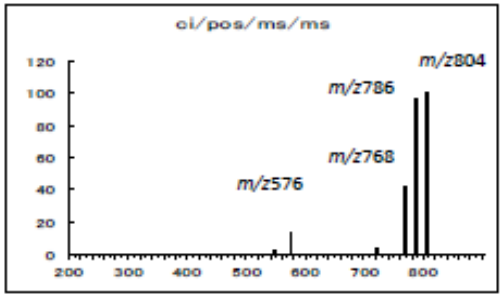


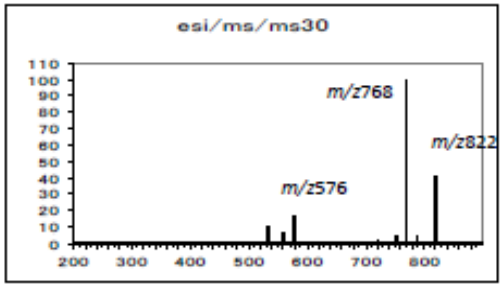
Tacrolims

Fragmentations of Tacrolimus by EI, CI ,ESI and FAB MS



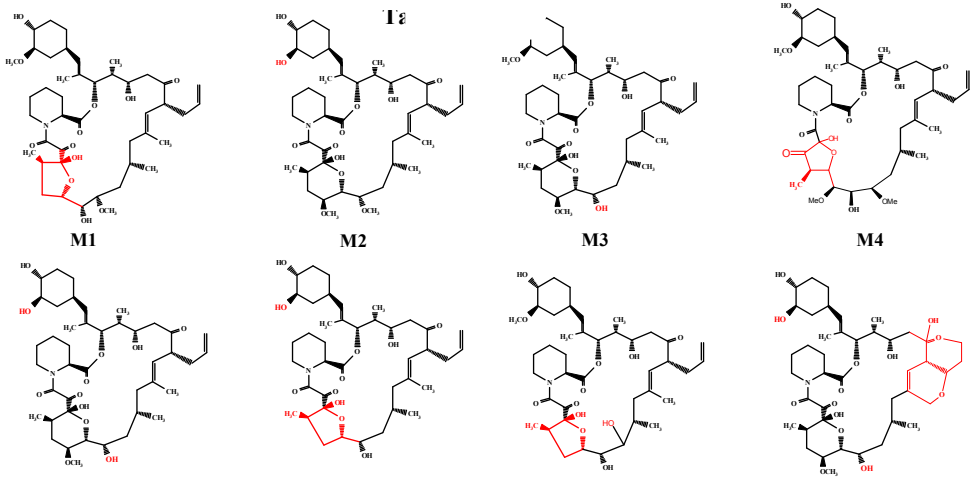






Fragmentation	EI/POS	CI/POS	CI/NEG	ESI/POS 30V	ESI/POS 80V	FAB
M^+ 803	804.5	804.1	803.3	821.8	821.7	826.4 (M+Na)
M-H2O 785	786.5	786.2	785.3		786.7	
M-2H2O 767	768.5	768.1			768.6	
a-b(111) 692						715.5 (692+Na)
a-b-H2O 674						697.5 (674+Na)
a-c(155) 658						
a-c-H2O 630						653.4 (630+Na)
a-d(183) 620						
a-d-H2O 602						625.2 (602+Na)
e-f(210) 593	593.5	594				616.1 (593+Na)
a-d-2H2O 584						607.5 (584+Na)
e-f-H2O 575	576.3	576.1			576.5	
		548.1				
						505.3
e-b(337) 466						487.2
e-c(365) 438						
e-c-H2O 420						443.3 (420+Na)
e-d(393) 410	409.3, 410.3	409				
e-d-H2O 392						415.3 (392+Na)
						387.4
e-d-CO 381	381.3					
f-g(565) 238		238.9	238.2			261.2 (238+Na)

Metabolites of Tacrolims



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Quantitative LC-MS/MS Analysis of Tacrolims in Human Blood

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INTRODUCTION

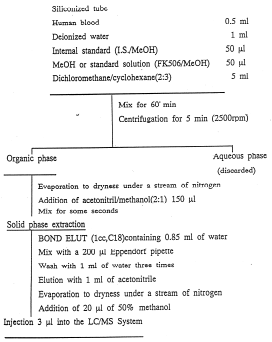
FK506, a maclolide antibiotics obtained from *Streptomyces tsukubaensis*, has strong immunosuppresive activity and is useful in organ transplantation. The specific high sensitive assay of FK506 by electrospray ionization (ESI) LC/MS was developed.

EXPERIMENTAL

1) Pretreatment

Whole blood aliquot spiked FK506 and I.S. was extracted by ether. The extract was reconstitute in 50% methanol. The flow chart for the sample treatment procedure is shown.

Schematic representation of sample extraction procedure for FK506 in human blood



2) Equipment

ESI LC MS system : LC system (Kontron data system, Pump 420, Auto-sampler 465), LC Column: LC Packing Accurate (µ-capillary Column C-18, 3 µ m, 0.32x150mm), Separator (switching the mobile phase to elute the biological dirty fraction out of LC MS system), and Finnigan Mat TSQ 700 ESI LC MS instrument)

2.1) HPLC mobile phase and sheath liquid

Some combination mobile phase system (organic solvent s with 5% acetic ammonium) were checked noise signal by ESI LC/MS). The high noise signals (HNS) were observed at *m/z* 50-900 in the high boiling point (b.p.) organic solvents. The low noise signal s (LNS) were observed *m/z* 300-500 in the low b.p. organic solvents. The no noise signal (NNS) was Observed in acetonitrile with 5% acetic ammonium (<75%) or acetonitrile with 5% formic acid (<75%). The average scan of HNS and LNS mobile phase showed some impurity peaks. To avoide the Precipitation of acetic ammonium salt, the mobile phase with aqueous acetonitrile and sheath liquid with 10mM acetic ammonium /isopropanol (70%) were Adopted. The flow rate of mobile phase Was controlled at 3-4 µ L/min by Accurate to obtain optical stability and high sensitivity of the assay. The flow rate of mobile phase was same value to select optical column. The flow rate of sheath liquid was optimized at 1 µ L/min.

Table 1 Noise level of HPLC mobile phase

HPLC mobile phase	Ratio	Noise
THF:5%AcONH4	20:80	HNS
DMSO:5%AcONH4	20:80	HNS
n-HexOH:5%AcONH4	Saturate	HNS
Dioxane:5%AcONH4	20:80	HNS
MeCN:5%AcONH4	10:90	NNS
MeCN:5%AcONH4	50:50	NNS
MeCN:5%AcONH4	75:25	LNS
HCO2H:MeCN:5%AcONH4	0.5:75:25	NNS
HCO2H:MeCN:5%AcONH4	5:75:25	LNS
HCO2H:MeCN:5%AcONH4	10:75:25	LNS
AcOH:MeCN:5%AcONH4	0.5:75:25	LNS
AcOH:MeCN:5%AcONH4	5:75:25	HNS
HCO2Et, MeCN:5%AcONH4	5:75:25	LNS
28%NH3, MeCN:5%AcONH4	0.1:75:25	NNS
28%NH3, MeCN:5%AcONH4	1:75:25	NNS
28%NH3, MeCN:5%AcONH4	1.5:75:25	LNS
MeOH:5%AcONH4	50:50	NNS
MeOH:5%AcONH4	75:25	LNS
EtOH:5%AcONH4	50:50	LNS
iPA:5%AcONH4	50:50	NNS

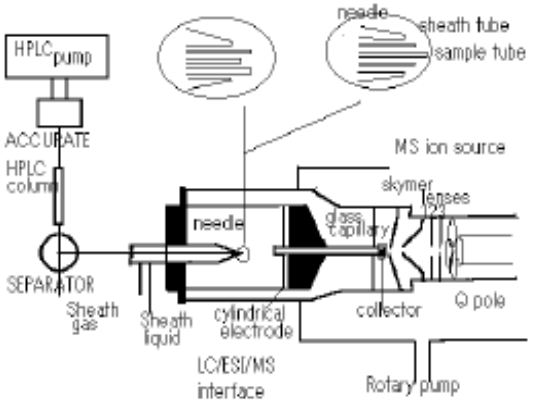


Figure 1 LC/ESI/MS SYSTEM

2.2) Needle distance

The sheath tube (middle) shoud projected about 2 mm from needle tube (outer).
The end of a sample tube (center) projected 0.5mm from a sheath tube (project position) Or was pulled in the sheath tuve (pulled in position).
The mixed vapor spray straightly from the sample tube of the glass capillary to the heated capillary (Needle distance 2.3cm) at project position and the peak signals were detected easily. At pulled in position (Needle distance 1.3 cm), the mobile phase was mixed with the sheath liquid enoughly and the mixed vapor was sprayed gently foggy to stbilize the ionization.

2.3) Ionization high voltage

The voltage was increased slowly until the intensity of the peak reached a maximum value and was stable (-3.55 kv at project position and -3.75 kv at pulled in position). At the optical voltage setting, the ion current was stable. A stable ion current was usually acheaved between 5x 10^{-8} A and 3 x 10^{-7} A .

2.4) Drying gas and sheath gas

The nitrogen gas (99.999%) was used as drying gas and sheath gas. The impure gave the noise. The bubble with nitrogen gas (99.999%) in the sheath liquid and mobile phase decreased the noise peaks. The drying gas was settled at 200 ℃, but the gas temperature was checked at 70 ℃ by a thermometer. The pressure of the sheath gas was optimized at 11 psi (project position) or 25-29 psi (pulled in position).

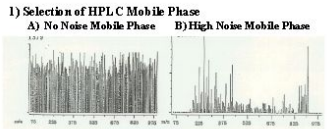
2.4) Ion affinity of FK506

FK506 has good affinity with K>Na>NH4>H ions to give the aduct ion peaks, but the high purified water for vascular injection and the siliconized glass tubes were used for assay.

RESULT and DISCUSSION

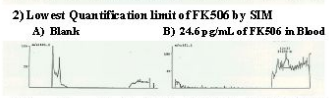
1) Mass chromatography and Specifisity

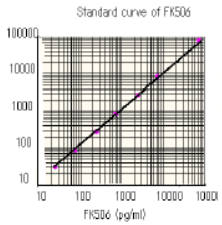
Mass chromatogram showed no interfere peak.

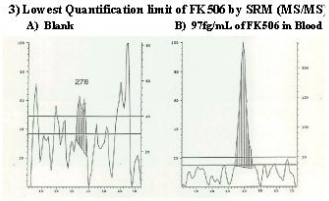


2) Standard curve

The relationship between the peal area ratio of FK506/I.S. and the concentration of FK506 in human blood was linear in the range from 25 pg/mL and 75 ng/mL. The limit of detection on culumn was 3.33 pg/mL. The limit of quantification for the blood assay was 25 pg/mL.







The accuracy and precision of the intra assay and the inter assay on 5 days were good.

Table 2 Intra-precision and accuracy of FK506 assay in human blood

Concentration spiked (ng/mL)	Mean concentration found (ng/mL)	SD	CV (%)	n	RE (%)
0.0246	0.0225	0.0021	9.5	5	-8.5
0.074	0.0749	0.0020	2.6	5	1.2
2.46	2.30	0.28	12.3	5	-6.5
74.0	71.3	6.1	8.6	5	-3.6

Table 3 Inter-precision and accuracy of FK506 assay in blood on 5 days

Concentration spiked (ng/mL)	Mean concentration found (ng/mL)	SD	CV (%)	n	RE (%)
0.74	0.737	0.057	7.7	5	-0.4
7.4	7.25	0.58	8.0	5	-2.0
74.0	73.9	1.4	1.8	5	-0.1

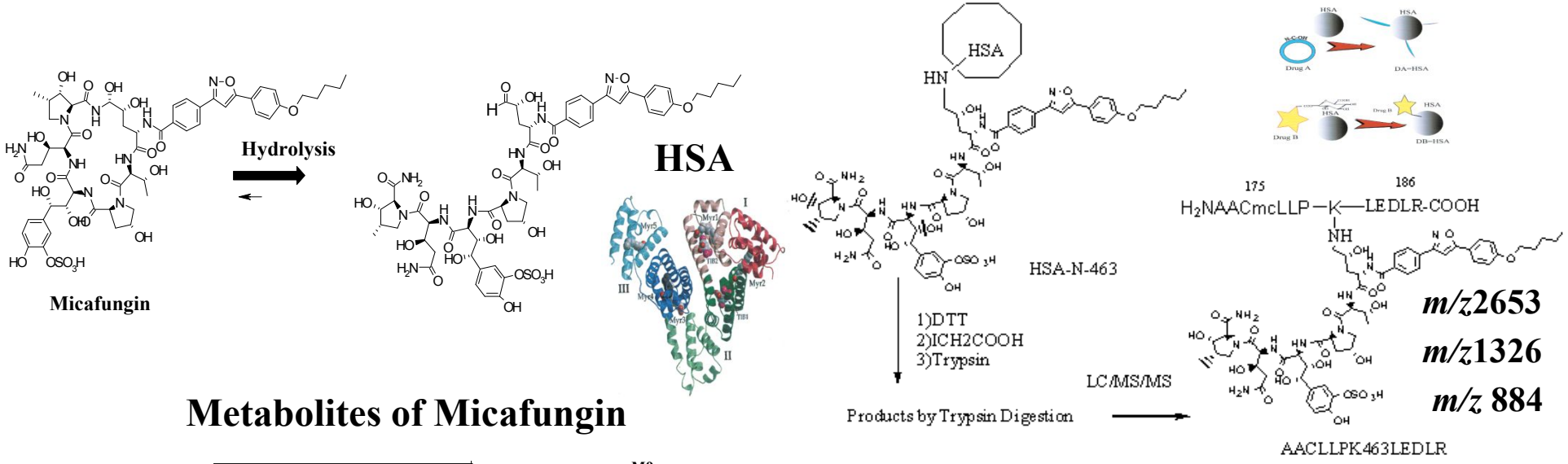
4)Stability and Sensitivity of ESI LC/MS

The novel quantification needed to establish stability and sensitivity of ESI LC/MS that was investigated by controlling HPLC mobile phase, sheath liquid, needle distance, ionozation voltage, drying gas and sheath gas.

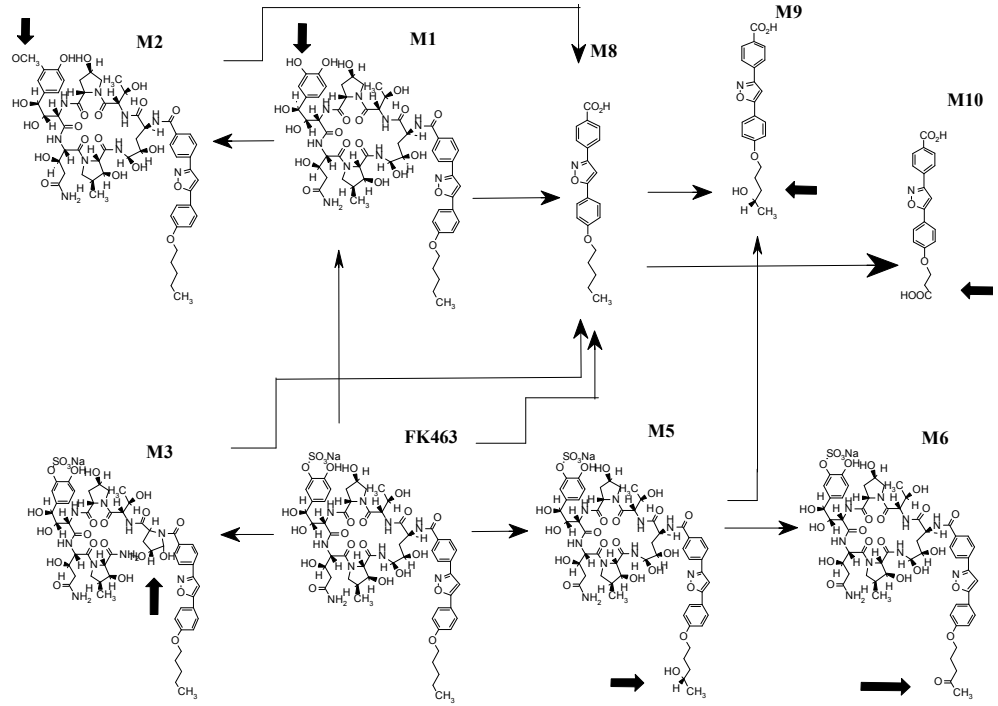
Proceedings of the Kyoto's 92, P430

International Conference on Biological Mass Spectrometry

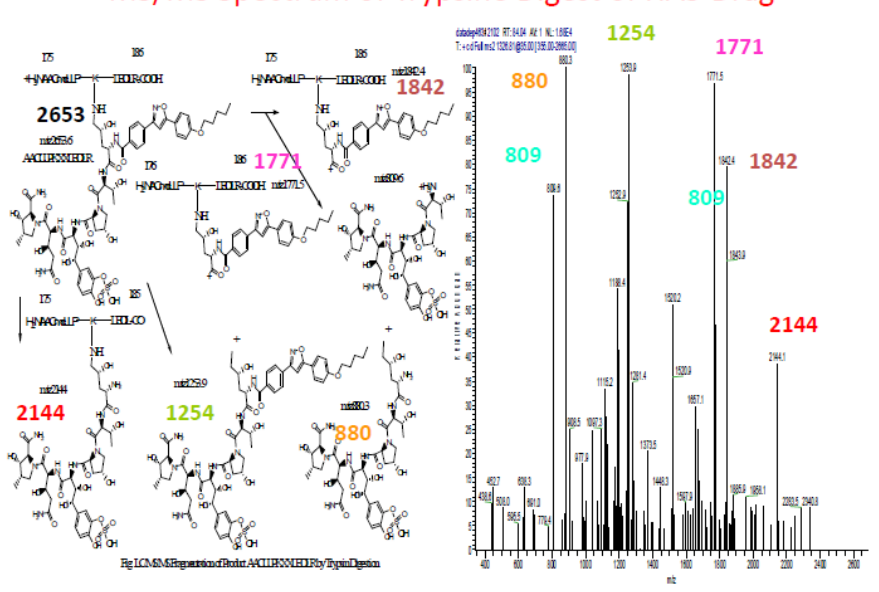
The Innovative Qualitative LC-MS/MS Analysis of Micafungin



Metabolites of Micafungin

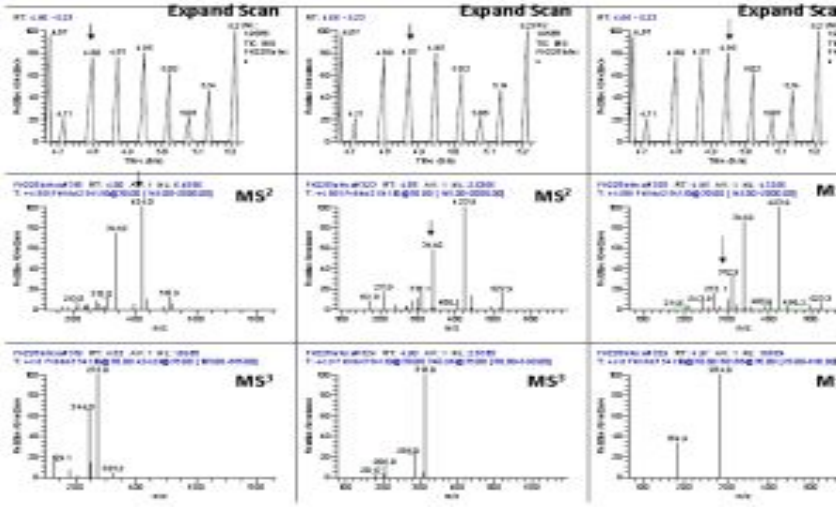


MS/MS Spectrum of Trypsine Digest of HAS-Drag

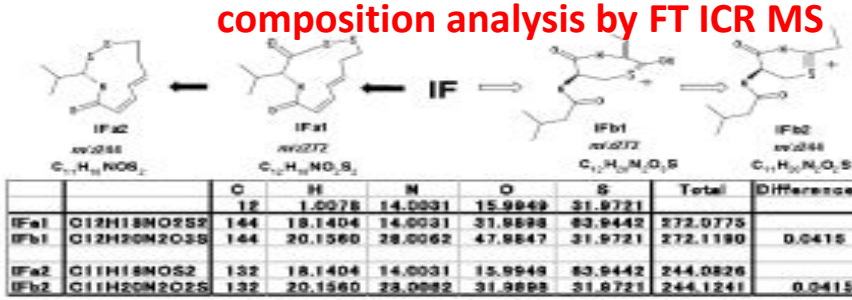


2003, The Innovative Qualitative LC-MS/MS Analysis of Isodack (FK228)

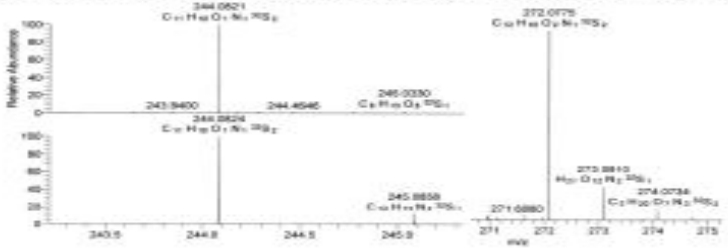
SRM data dependent exclusion MS^n measurement

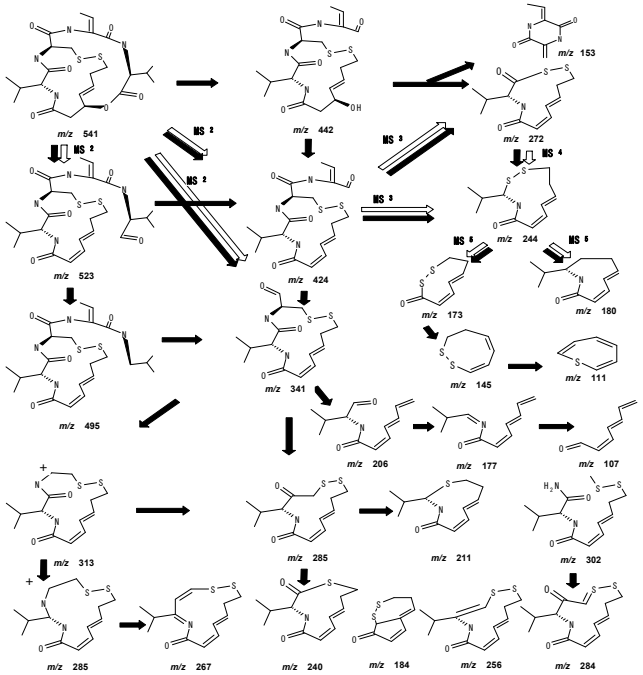


composition analysis by FT ICR MS



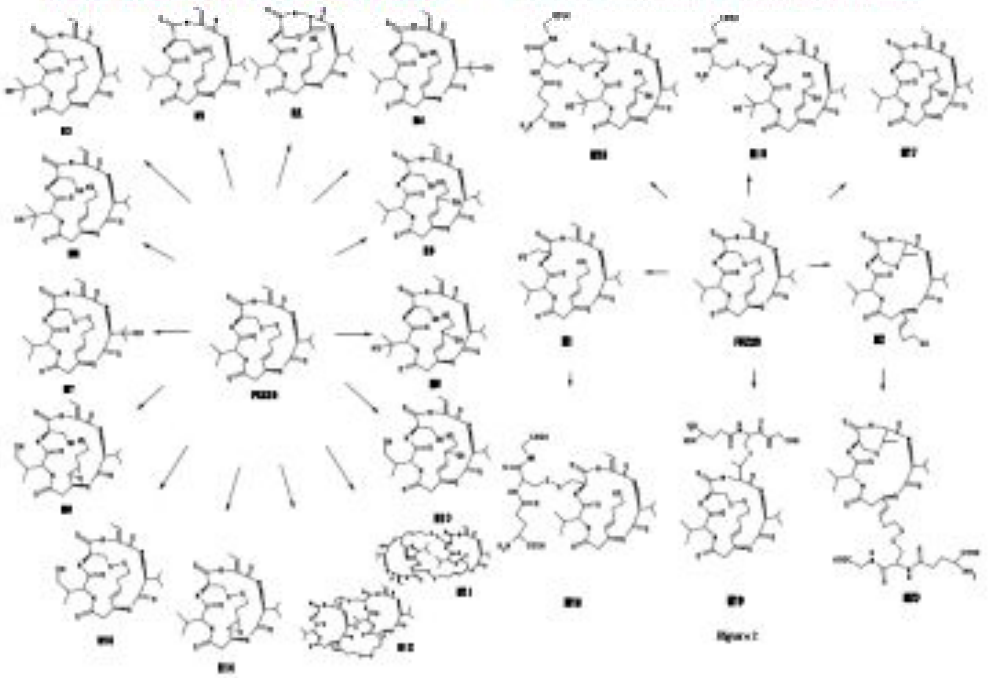
		C	H	N	O	S	Total	Difference	PPM
		12	1.0078	14.0031	15.9949	31.9721			
IFa1	C12H18NO2S2	144	18.1404	14.0031	31.9898	63.9442	272.0775		
IFb1	C12H20N2O3S	144	20.1560	28.0062	47.9847	31.9721	272.1190	0.0415	1.53
IFa2	C11H18NOS2	132	18.1404	14.0031	15.9949	63.9442	244.0826		
IFb2	C11H20N2O2S	132	20.1560	28.0062	31.9898	31.9721	244.1241	0.0415	1.70





In Vitro Metabolic Pathway of FK228

In Vivo Metabolic Pathway of FK228



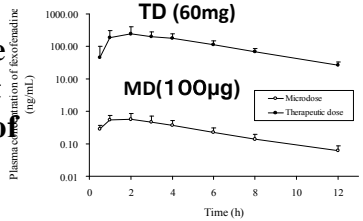
Tozuka Z, Strupat WM, Shiraga T, Ishimura R,Hashimoto T, *J Mass Spectrum Soc Jpn.* 53, 89-99 (2005).

2004-2007, Challenges to Microdose Clinical Study, Metabolome, Proteome and Biomarkers by High Resolution and Accuracy LTQ FT ICR MS

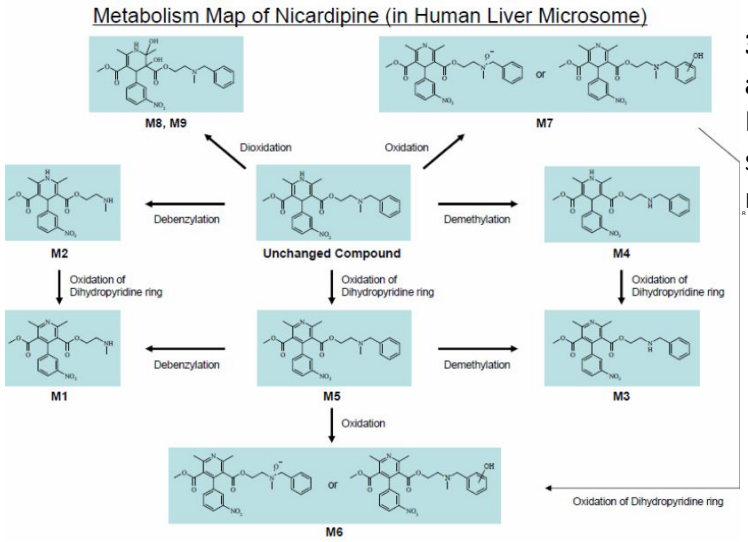
Z.Tozuka, S.Shioyama, N.Yamane, R.Goto, T.Takami, Y.Yamashita, K.Fujii, N.Inoue and K.Momiyama

JCL Bioassay Corporation

1) Challenge to observe PK linearity in the First MD/TD Clinical Study of Fexophenadine by LC-API5000 MS



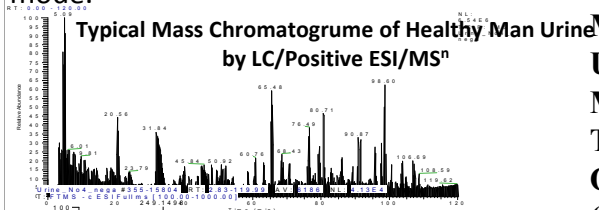
1) Challenge to observe the same metabolic patern in the 2nd MD/TD Clinical Study of Nicardipine by LTQ FT ICR MS.

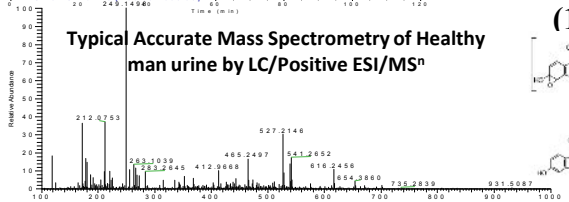


LTQ FT ICR MS

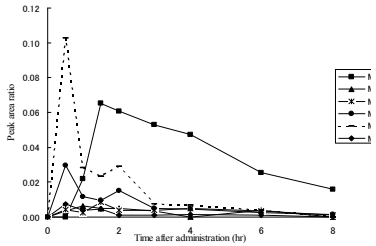
3) Challenge to observe Human Plasma and Urine Metabolome by LTQ FT ICR MS.

Number of metabolites observed in each subjectsby negative and positive ionization mode.

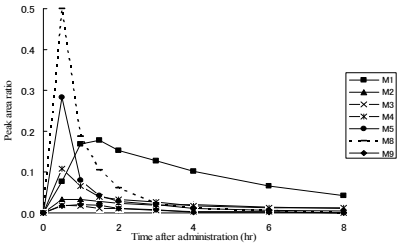


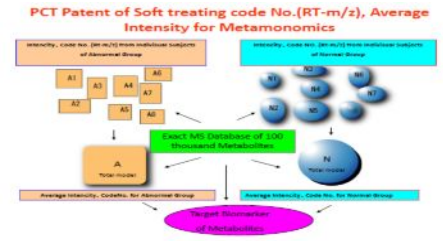


MD (100μg)



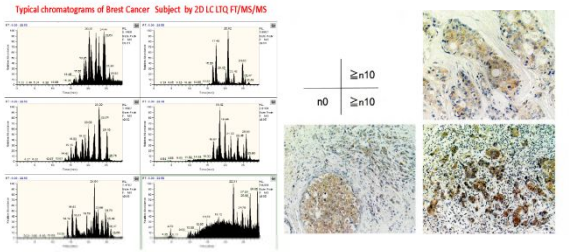
TD(20mg)



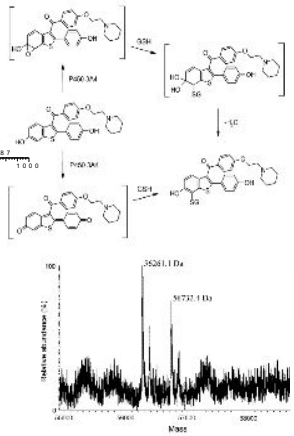


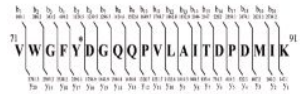
4) Challenge to determine the 12 biomarkers of breast cancer by shot gun analysis in combination with their antibody analysis using breast cancer tissues (international patent) and various target proteome analysis by FT ICR MS.

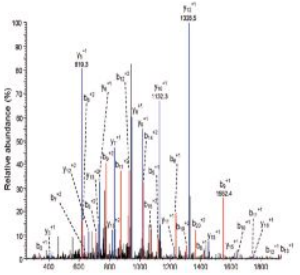
Tissue Stain with Specific Antibody



5) Identification of Cytochrome P450 3A4 Modification Site with Reactive Metabolite Using Linear Ion Trap-Fourier Transform Mass Spectrometry, Yukinaga H., Takami T., Shioyama S.,Tozuka Z., Masumoto H., Okazaki O., Sudo K.,*Chem.Res.Toxicol.*, 20 (10), 1373-1378 (2007).



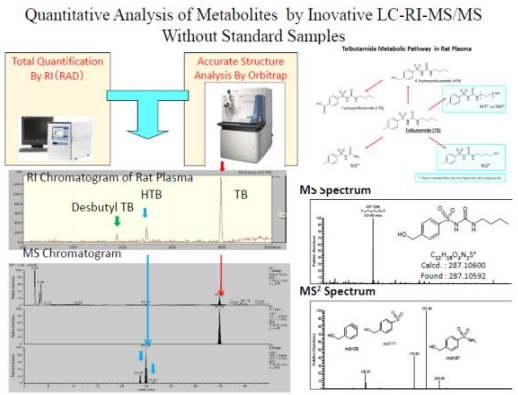


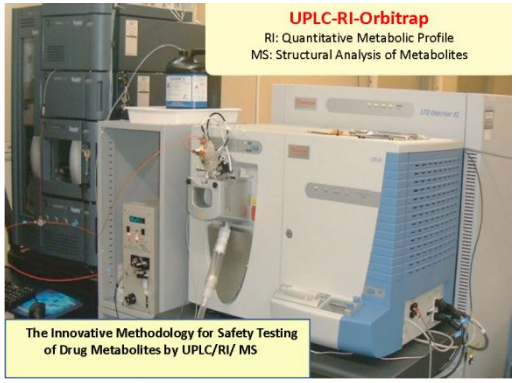


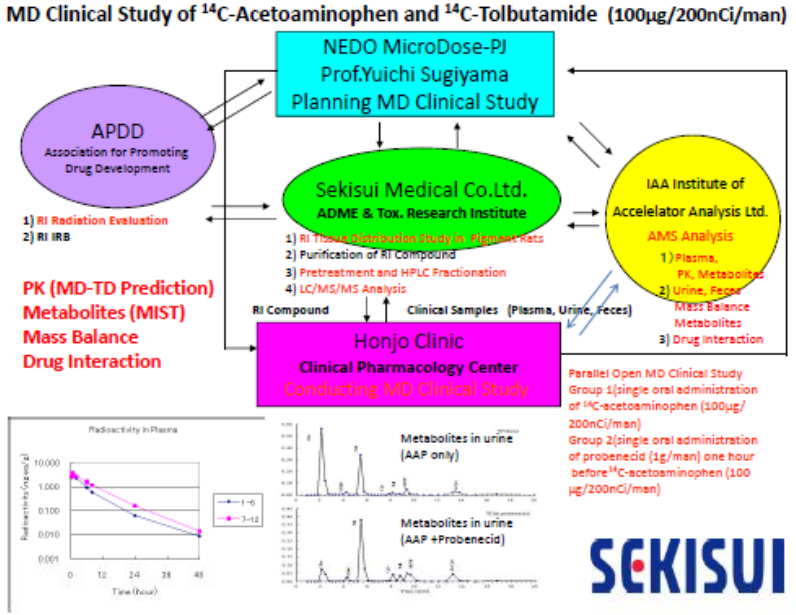
CYP3A4-Raloxifene active metabolites adduct

2008-2011, Innovative Strategy for MIST and Microdose Clinical Study by LC/RI/Orbitrap MS

Z.Tozuka, S. Aoyama, K.Nozawa, K.Fujii, S.Akita, T.Shiroshita, Furukawa, T.Ohara,Y. Adachi, S. Ninomiya
ADME/TOX Research Institute, Sekisui Medical Co., Ltd.







1) Calculation of response factor

$m_3 = I_3/R_3$ = 6000000 / 3000= 2000(count/dpm)

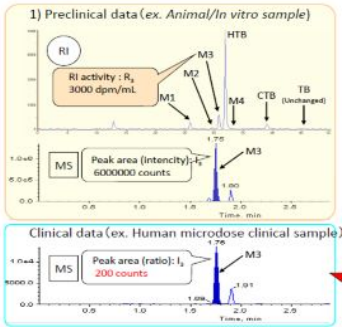
2) Convertion using responsfactor

M_3 (dpm) = I_3/ m_3

= 200/ 2000 = 0.1 dpm/mL

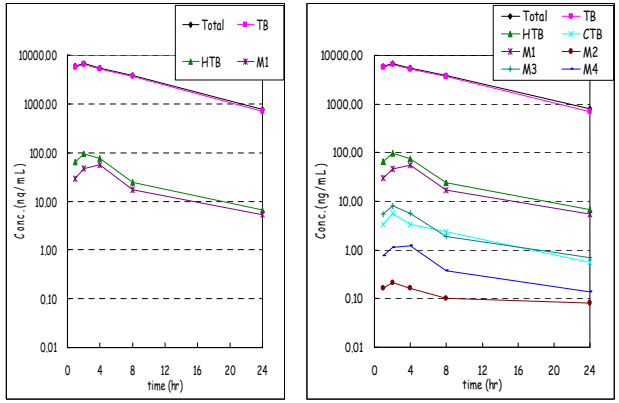
3) Convertion using RI specific activity of 14C–TB ; 100dpm/nmol=0.1dpm/pmol

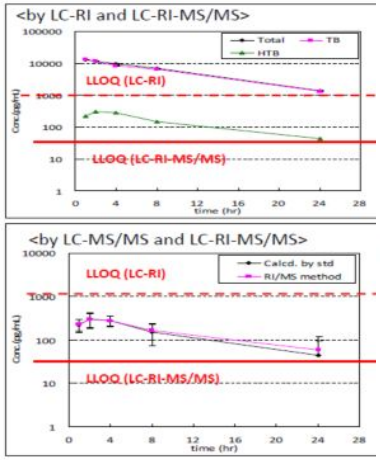
4)M3 concentration =1pmol/mL

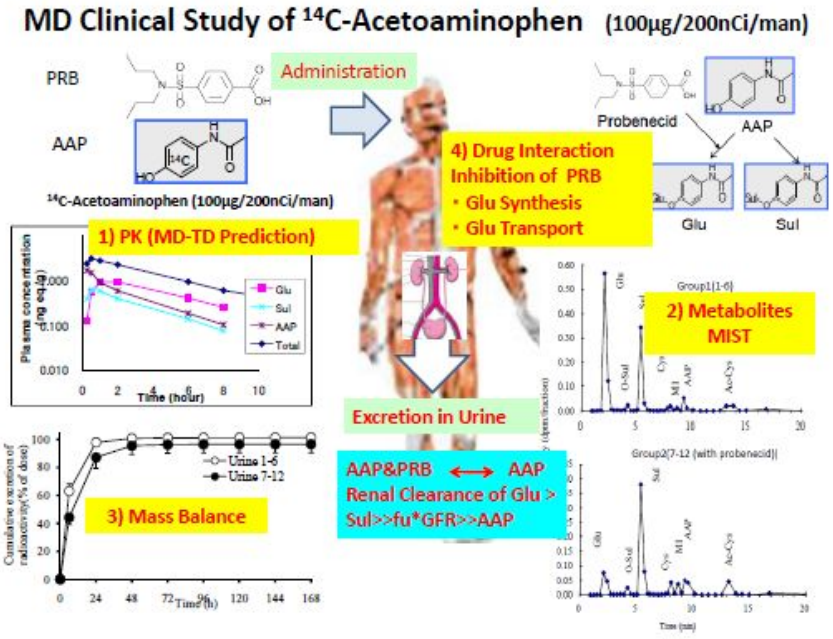


Plasma concentration time curve of Total RI, TB and TB Metabolites after MD (1.67μg/kg) & TD (1mg/kg) of ¹⁴C-TB by LC-RI and LC-RI -MS/ MS

TD <LC-RI analysis> <LC-RI-MS/MS analysis MD







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Kanazawa University : Akira Tsuji, Ikumi Tamai
Kitasato University : Yuji Kumagai
Wakayama Medical University: Yasushi Nakamura
Yokohama City University: Toshihiko Ikeda, Satoko Akashi, Mitsuo Takayama

All of coworkers

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JCL Bioassay Corporation : Kunio Momiyama (President), Noriko Inoue, Shohei Shioyama, Naoko Yamane, Rieko Goto, Tomonori Takami, Yasuhiro Yamashita, Kaori Fujii, All of JCL Bioassay Corporation)

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