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Short communication

Determination of a new H^+–K^+ ATPase inhibitor (E3810) and its four metabolites in human plasma by high-performance liquid chromatography

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Abstract

A method for the simultaneous determination of E3810, 2-[{4-(3-methoxypropoxy)-3-methyl pyridine-2-yl}methyl sulfinyl]-1H-benzimidazole sodium salt and its four metabolites, demethylated-E3810 (DM), demethylated thioether-E3810 (DMTE), sulfone-E3810 (S), and thioether-E3810 (TE), in human plasma by high-performance liquid chromatography (HPLC) with UV absorbance detection has been established. The correlation coefficient for all the standard curves was 0.998 or greater. The quantitation limit was 5 ng/ml for E3810 and 20 ng/ml for each of its four metabolites. The recovery of E3810 and its four metabolites from human plasma was high, being greater than 80% when 100 ng of each substance was added per tube, except for DM (74.1%). The stability of E3810 and its four metabolites was evaluated and the following results were obtained: (1) when samples were centrifuged within 20 min after collection, there was no loss of E3810 or its metabolites; (2) when 100 μl of a 1% aqueous solution of diethylamine was added within 20 min after plasma isolation, there was no loss of E3810 or its metabolites; and (3) there were no stability problems during storage for a period of 10 months at −20°C.

1. Introduction

E3810, 2-[{4-(3-methoxypropoxy)-3-methyl pyridine-2-yl}methyl sulfinyl]-1H-benzimidazole sodium salt, is a newly synthesized compound which inhibits the action of H^+–K^+ ATPase in parietal cells [1–3], and is under development as an anti-ulcer agent superior to H_2-receptor antagonists. From the results of preclinical studies, E3810 is expected to have beneficial effects in the treatment of peptic ulcer [4].

Five metabolites are known: demethylated-E3810 (DM), demethylated thioether-E3810 (DMTE), sulfone-E3810 (S), thioether-E3810 (TE), and thioether carboxylic acid-E3810 (UM-2), shown in Fig. 1.

This paper describes a simple and selective HPLC method that separates E3810 and its 4 metabolites. This method has been used in several E3810 pharmacokinetic studies [5].

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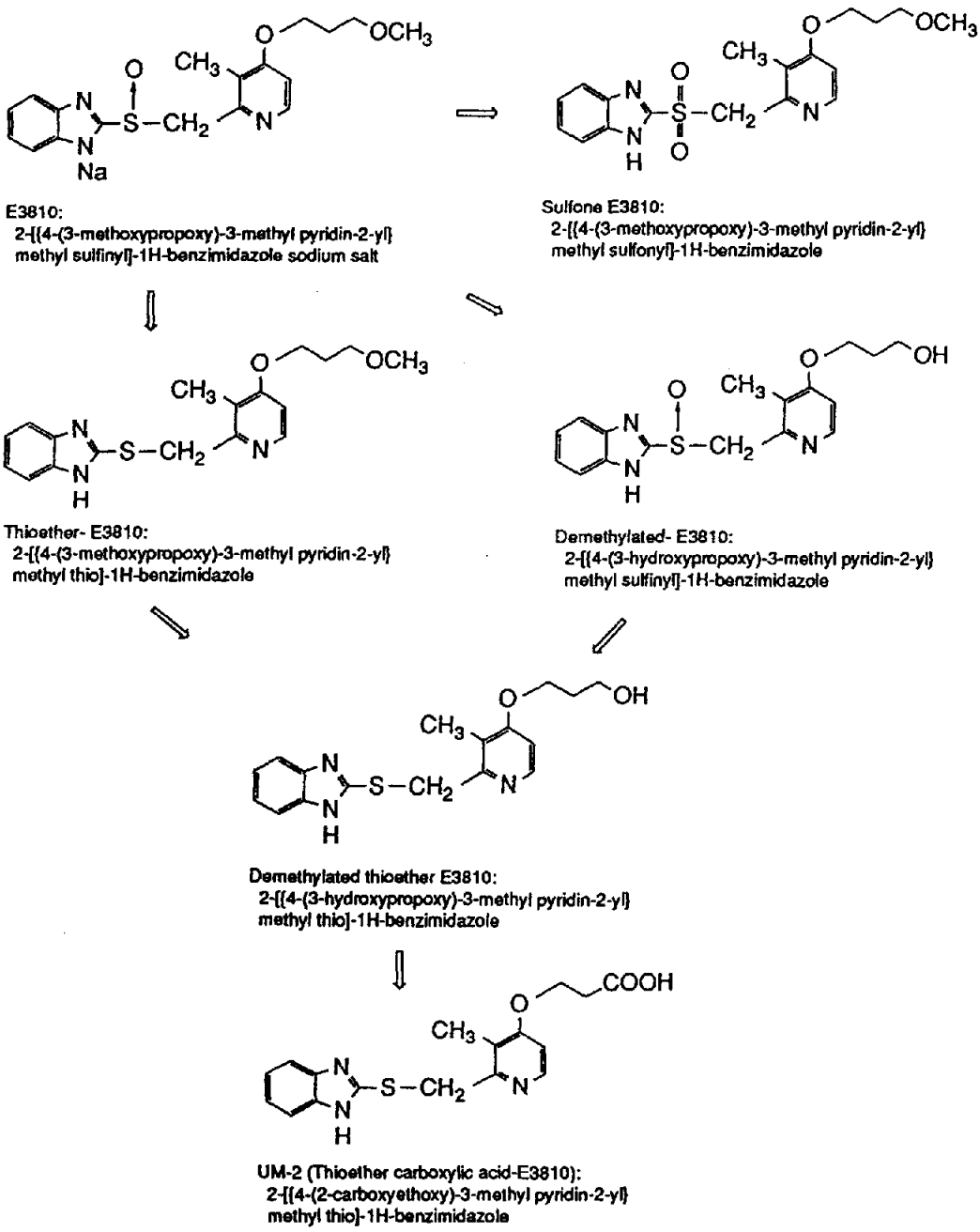
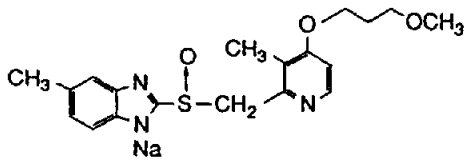


Fig. 1. Hypothesized metabolic pathway of E3810.

2. Experimental

2.1. Reagents and standards

Analytical grade reagents, methanol, diethylamine, glacial acetic acid, boric acid, sodium hydroxide, phosphoric acid, disodium hydrogen phosphate dodecahydrate, and potassium dihydrogen phosphate, and HPLC grade reagents acetonitrile and ethyl acetate, were used.



Internal standard (IS735):
5-methyl-2-{[4-(3-methoxypropoxy)-3-methyl pyridin-2-yl} methyl sulfinyl]-1H-benzimidazole sodium salt

Fig. 2. Chemical structure of the internal standard.

E3810, DM, DMTE, S, TE and internal standard IS735 were used. These standards were synthesized by Eisai. The chemical structure of the internal standard is shown in Fig. 2. The free form of DM in the hypothesized metabolic pathway shown in Fig. 1 is an N–H form. However, its stability was poor; the standard actually used for this measurement was its sodium salt.

2.2. Extraction procedure

A 1-ml volume of plasma was placed in a brown glass tube containing 100 μl of a 1% aqueous solution of diethylamine. To this solution, a 100-μl aliquot of 0.1% diethylamine in methanol and 1.0 ml of Britton–Robinson buffer, pH 10.38, and 4.0 ml of ethyl acetate were added. The mixture was shaken for 10 min and centrifuged for 5 min at 1500 *g*. This extraction procedure was repeated twice. The combined organic layers were transferred into a test tube and evaporated to dryness in a Dri-Block bath at 40°C under nitrogen. The residue was dissolved in 100 μl of 0.1% diethylamine in methanol and mixed for 1 min. A 30-μl aliquot was injected onto the column.

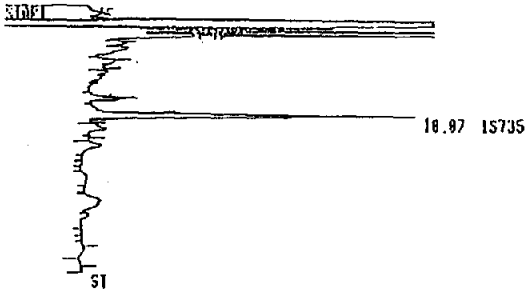


Fig. 3. Typical chromatogram of "blank" plasma containing 100 ng of IS735 (internal standard).

2.3. Apparatus

The HPLC system consisted of a Yanaco L-4000s system (Yanagimoto Co., Japan), a Waters 712 WISP (Milford, MA, USA), and a Waters Lambda-MAX 481 UV detector. The detector output was channeled to a Hewlett-Packard 3390A integrator (Avondale, PA, USA). An Iwaki V-S shaker (Tokyo, Japan), a Yamato Touch Mixer MT-31 vortex-mixer (Tokyo, Japan), a Kubota KS-5200C centrifuge (Tokyo, Japan) and a Scinics Dri-Block bath (Tokyo, Japan) were used to extract the analytes from plasma.

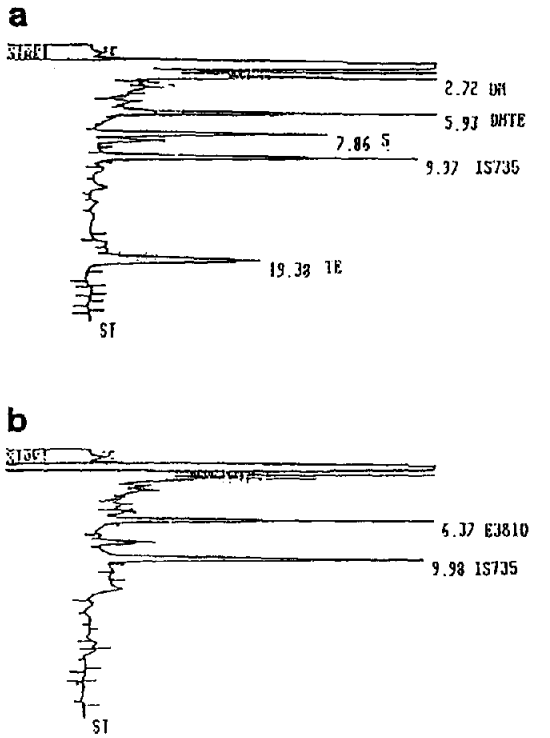


Fig. 4. (a) Typical chromatogram of plasma containing 50 ng/ml each of DM, DMTE, S, and TE, and 100 ng of IS735 (internal standard). (b) Typical chromatogram of plasma containing 50 ng/ml of E3810 and 100 ng of IS735 (internal standard).

Table 1
Extraction recoveries from human plasma ($n = 4$)

Compound		Recovery (%)					
		5 ng/ml	10 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml
E3810	Mean	112.4	106.1	97.9	89.2	96.2	85.8
	S.D.	7.3	3.2	6.6	0.5	2.5	3.6
	C.V. (%)	6.5	3.0	6.7	0.5	2.6	4.2
DM	Mean	158.0	104.3	77.7	74.1	74.8	73.4
	S.D.	25.4	2.7	13.2	4.3	3.5	1.8
	C.V. (%)	16.1	2.6	17.0	5.9	4.6	2.4
DMTE	Mean	95.4	89.5	96.2	94.4	96.8	92.3
	S.D.	14.4	4.7	5.4	3.2	0.7	4.4
	C.V. (%)	15.1	5.2	5.6	3.4	0.8	4.8
S	Mean	78.7	83.3	89.2	84.6	92.1	79.1
	S.D.	10.1	5.5	6.0	4.5	0.9	8.4
	C.V. (%)	12.9	6.6	6.8	5.4	1.0	10.7
TE	Mean	50.2	44.2	91.5	95.0	96.8	98.3
	S.D.	60.5	4.1	6.1	1.2	0.6	2.0
	C.V. (%)	120.6	9.2	6.6	1.2	0.7	2.1
IS735	Mean	–	–	–	98.5	–	–
	S.D.				0.8		
	C.V. (%)				0.8		

2.4. Chromatographic conditions

The mobile phase consisted of 280 ml of acetonitrile and 720 ml of 0.1 *M* phosphate buffer (pH 7.00). After mixing the pH of this solution was adjusted to 7.00 with phosphoric acid. The mobile phase was delivered at a flow-rate of 1.4 ml/min at 40°C. The column used was an Inertisil C_8 column (150 × 4.6 mm I.D., 5 μm particle size). The absorbance was measured at 288 nm.

2.5. Drug standards

Stock solutions of each compound were prepared in 0.1% diethylamine methanolic solvent and stored at −20°C for 10 months. Standards were prepared prior to use by spiking 1 ml of drug-free pooled human plasma with 100 μl of the appropriate dilution of stock solutions in methanol containing 0.1% diethylamine to provide plasma concentrations ranging from 5 to 500 ng/ml for E3810 and from 20 to 500 ng/ml for the metabolites. These standards were extracted as described above. Least-squares regression analysis of peak-height ratios (product/I.S.) of the standard versus their concentration was performed using an IBM 5550 (Tokyo, Japan). The concentrations of all compounds were calculated by comparison of their peak-height ratio with that of the I.S. using calibration curves.

2.6. Analytical variables

The extraction recoveries of E3810 and its metabolites were calculated by comparing the signal obtained following the injection of the dry residues of plasma samples spiked with 100 ng/ml E3810 or its metabolites. The intra-day validation was evaluated by determining coefficients of variation (C.V.) for E3810 and its metabolites at plasma concentrations of 5, 10, 50, 100, 200

and 400 ng/ml by replicate analyses of 4 plasma samples on the same day. The inter-day validation was evaluated by analysis of spiked control plasma samples stored at −20°C in 1.1-ml aliquots and tested each day. To study their stability, E3810 and its metabolites in human blood or plasma were measured after storage at room temperature within 30 minutes under physiological pH and after storage at −20°C throughout a 10-months period (intervals of 0, 2 weeks, 1, 3, and 10 months). Quadruplicate stored samples containing E3810 or its metabolites at a concentration of 250 ng/ml were analyzed and compared to the stock standards on each day of analysis. The compounds were certified to be stable at −20°C for 1 year.

3. Results and discussion

3.1. Specificity

The mean retention times for E3810, DM, DMTE, S, IS735 and TE were 6.5, 2.8, 6.0, 8.0, 10.1, and 19.7 min, respectively. No endogenous substances were found which co-eluted with or otherwise interfered with the detection of E3810 and its 4 metabolites. Typical chromatograms of blank plasma containing 100 ng of IS735 (Fig. 3) and of plasma containing 50 ng/ml each of E3810 and its 4 metabolites, with 100 ng of IS735 (Fig. 4), are shown.

3.2. Validation

A linear correlation was found for the peak-height ratio of E3810 and its metabolites to IS735. The concentration of the compounds was found to be in the range 5–400 ng/ml plasma. The correlation coefficient generally exceeded 0.996 and the intercept did not significantly differ from zero.

The recovery of E3810, its 4 metabolites and IS735 under the conditions described for this assay are given in Table 1. The mean recoveries of E3810, DM, DMTE, S, TE, and IS735 were 89.2, 74.1, 94.4, 84.6, 95.0 and 98.5%, respectively for the addition of 100 ng of the respective

Table 2
Intra-day validation of the method for the determination of E3810 and its metabolites

Compound		Peak-height ratio						Regression analysis		
		5 ng/ml	10 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml	S	IC	CC
E3810	Mean	0.106	0.187	0.825	1.574	3.196	6.229	0.0155	0.0399	0.99983
	S.D.	0.007	0.006	0.080	0.008	0.047	0.154	0.0004	0.0289	0.00010
	C.V. (%)	6.31	3.44	9.72	0.50	1.46	2.47	2.51		0.01
DM	Mean	0.187	0.270	1.073	2.170	4.352	8.542	0.0212	0.0561	0.99962
	S.D.	0.030	0.010	0.168	0.133	0.152	0.193	6.00E − 04	0.0755	0.00019
	C.V. (%)	16.09	3.81	15.63	6.14	3.49	2.25	2.72		0.02
DMTE	Mean	0.082	0.166	0.904	1.852	3.791	7.447	0.019	−0.0121	0.99989
	S.D.	0.012	0.008	0.027	0.055	0.077	0.276	0.001	0.0214	0.00008
	C.V. (%)	14.33	4.99	2.97	2.94	2.03	3.70	3.52		0.01
S	Mean	0.059	0.116	0.604	1.189	2.576	4.640	0.0117	0.0339	0.99754
	S.D.	0.007	0.008	0.032	0.064	0.048	0.421	0.0010	0.0651	0.00145
	C.V. (%)	11.72	7.23	5.37	5.43	1.88	9.07	8.66		0.15
TE	Mean	0.008	0.049	0.398	0.831	1.677	3.445	0.00869	−0.03991	0.99989
	S.D.	0.010	0.005	0.031	0.009	0.013	0.096	0.00026	0.02077	0.00012
	C.V. (%)	120.55	9.38	7.83	1.03	0.80	2.79	2.96		0.01

S: slope, IC: intercept, CC: correlation coefficient

Table 3
Inter-day validation of the method for determination of E3810 and its metabolites

Date	Run	Peak-height ratio						Regression analysis		
	No.	5 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	S	I	CC
E3810										
Jul./19/88	1	0.075	0.320	0.751	1.400	2.940	7.094	0.0142	0.0298	0.99987
Jul./26/88	2	0.084	0.317	0.731	1.403	2.964	6.950	0.0139	0.0486	0.99965
Aug./17/88	3	0.066	0.295	0.724	1.414	2.900	7.260	0.0145	−0.0094	0.99998
Aug./24/88	4	0.098	0.325	0.747	1.423	2.942	7.200	0.0144	0.0281	0.99995
Aug./29/88	5	0.081	0.319	0.765	1.403	2.966	7.365	0.0147	0.0038	0.99991
Aug./31/88	6	0.094	0.373	0.779	1.431	2.887	6.780	0.0134	0.0966	0.99973
Sep./ 5/88	7	0.095	0.310	0.738	1.390	2.790	6.571	0.0131	0.0762	0.99976
Sep./13/88	8	0.070	0.292	0.710	1.388	2.790	6.906	0.0138	0.0141	0.99999
Mean		0.083	0.319	0.743	1.407	2.897	7.016	0.0140	0.0360	0.99986
S.D.		0.012	0.025	0.022	0.015	0.072	0.265	0.0006	0.0361	0.00013
C.V. (%)		14.54	7.81	3.00	1.08	2.48	3.78	3.99		0.01
Demethylated-E3810										
Jul./19/88	1	0.217	0.534	1.078	1.969	4.163	9.817	0.0195	0.1257	0.99974
Jul./26/88	2	0.000	0.324	1.086	2.048	4.256	9.595	0.0193	0.0650	0.99869
Aug./17/88	3	0.000	0.550	1.198	2.191	4.345	10.638	0.0212	0.0606	0.99974
Aug./24/88	4	0.084	0.399	0.975	1.887	4.086	10.224	0.0205	−0.0523	0.99989
Aug./29/88	5	0.158	0.466	1.054	1.789	3.971	10.266	0.0205	−0.0331	0.99944
Aug./31/88	6	0.129	0.454	1.044	2.014	4.120	10.034	0.0200	0.0468	0.99995
Sep./ 5/88	7	0.144	0.443	1.013	1.957	3.875	9.376	0.0186	0.0832	0.99995
Sep./13/88	8	0.103	0.335	0.899	1.806	3.696	9.364	0.0188	−0.0370	0.99997
Mean		0.104	0.438	1.043	1.958	4.064	9.914	0.0200	0.0323	0.99967
S.D.		0.076	0.083	0.087	0.132	0.210	0.457	0.0010	0.0650	0.00043
C.V. (%)		72.37	18.89	8.38	6.75	5.17	4.61	4.58		0.04
Demethylated thioether-E3810										
Jul./19/88	1	0.087	0.366	0.927	1.754	3.665	8.948	0.0179	0.0126	0.99993
Jul./26/88	2	0.075	0.372	0.904	1.775	3.828	8.998	0.0181	0.0229	0.99961
Aug./17/88	3	0.078	0.378	0.952	1.881	3.836	9.539	0.0191	−0.0094	0.99999
Aug./24/88	4	0.135	0.445	0.997	1.889	3.830	9.286	0.0185	0.0693	0.99995
Aug./29/88	5	0.133	0.424	0.971	1.862	3.791	9.364	0.0187	0.0364	0.99998
Aug./31/88	6	0.115	0.408	0.943	1.819	3.679	8.660	0.0173	0.0877	0.99974
Sep./ 5/88	7	0.103	0.391	0.911	1.760	3.570	8.424	0.0168	0.0767	0.99975
Sep./13/88	8	0.113	0.417	0.969	1.896	3.727	9.259	0.0184	0.0410	0.99999
Mean		0.105	0.400	0.947	1.830	3.741	9.060	0.0181	0.0421	0.99987
S.D.		0.023	0.028	0.032	0.060	0.097	0.377	0.0008	0.0337	0.00015
C.V. (%)		22.23	6.98	3.37	3.28	2.60	4.16	4.19		0.01
Sulfone-E3810										
Jul./19/88	1	0.050	0.232	0.568	1.087	2.286	5.508	0.0110	0.0130	0.99986
Jul./26/88	2	0.050	0.226	0.557	1.116	2.360	5.505	0.0111	0.0240	0.99954
Aug./17/88	3	0.052	0.242	0.615	1.210	2.479	6.337	0.0127	−0.0295	0.99995
Aug./24/88	4	0.065	0.261	0.599	1.132	2.335	5.638	0.0112	0.0314	0.99990

(*Continued on p. 217*)

Table 3 *cont.*
Inter-day validation of the method for determination of E3810 and its metabolites

Date	Run	Peak-height ratio						Regression analysis		
	No.	5 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	S	I	CC
Aug./29/88	5	0.060	0.242	0.574	1.122	2.218	5.572	0.0111	0.0108	0.99999
Aug./31/88	6	0.054	0.250	0.571	1.109	2.279	5.346	0.0107	0.0442	0.99967
Sep./ 5/88	7	0.049	0.229	0.533	1.055	2.120	5.126	0.0102	0.0255	0.99991
Sep./13/88	8	0.059	0.242	0.571	1.108	2.235	5.493	0.0110	0.0191	0.99998
Mean		0.055	0.241	0.574	1.117	2.289	5.566	0.0111	0.0173	0.99985
S.D.		0.006	0.012	0.025	0.044	0.107	0.349	0.0007	0.0217	0.00016
C.V. (%)		10.60	4.80	4.34	3.97	4.67	6.28	6.37		0.02
Thioether-E3810										
Jul./19/88	1	0.043	0.186	0.420	0.842	1.712	4.146	0.00829	0.01638	0.99992
Jul./26/88	2	0.032	0.197	0.440	0.842	1.718	3.995	0.00797	0.04122	0.99954
Aug./17/88	3	0.033	0.204	0.452	0.861	1.768	4.338	0.00866	0.01245	0.99993
Aug./24/88	4	0.000	0.106	0.353	0.777	1.768	4.309	0.00878	−0.06140	0.99969
Aug./29/88	5	0.094	0.222	0.469	0.871	1.762	4.278	0.00847	0.04782	0.99996
Aug./31/88	6	0.045	0.159	0.390	0.761	1.556	3.719	0.00743	0.02130	0.99985
Sep./ 5/88	7	0.038	0.165	0.391	0.796	1.573	3.782	0.00756	0.02236	0.99986
Sep./13/88	8	0.062	0.138	0.436	0.835	1.699	4.189	0.00838	0.00417	0.99992
Mean		0.043	0.172	0.419	0.823	1.695	4.095	0.00819	0.01304	0.99983
S.D.		0.027	0.038	0.038	0.040	0.085	0.239	0.00050	0.03336	0.00015
C.V. (%)		61.98	22.04	9.16	4.88	5.00	5.83	6.05		0.01

Values used for calculations carried greater decimal place accuracy than those reported in the tables; apparent discrepancies are due to round-off error.

Table 4
Stability of E3810 and its 4 metabolites in blood at room temperature and at physiologic pH

Standing time (min)	Residual percentage (%)				
	DM	DMTE	E3810	S	TE
1	100.0	100.0	100.0	100.0	100.0
5	103.2	102.6	103.4	103.4	103.8
6	99.3	101.3	100.6	99.9	102.5
10	102.0	103.0	102.9	100.7	105.9
11	100.0	100.3	101.6	99.9	99.8
15	102.6	105.0	107.0	106.7	105.0
16	101.6	101.1	103.4	103.0	98.5
20	98.6	100.8	101.7	100.1	101.3
21	109.2	109.6	111.0	108.6	109.9
25	104.0	106.2	107.0	104.9	105.8
26	101.7	102.4	103.9	99.9	100.5
30	109.5	111.1	115.0	112.5	111.9

compounds per tube. The recovery of DM was lower than that of the other substances. This difference may be explained by the high hydrophilicity of DM. However, the recoveries of DM and TE on the addition of 5 or 10 ng/ml substance are overestimated or underestimated, owing to incorrect baseline drawing on the integrator caused by the interference of a small amount of endogenous substances with a retention time similar to those of DM and TE.

The reproducibility of the method was shown by the consistent slopes and intercepts obtained from curves generated intra-day and inter-day. The accuracy of the method was considered

Table 5
Stability of E3810 and its 4 metabolites in physiologic pH plasma at room temperature (250 ng added)

Compound	Run number	Residual percentage(%)			
		0 min	10 min	20 min	30 min
E3810	1	103.5	104.2	103.0	101.4
	2	98.8	99.5	98.4	100.5
	3	97.6	95.1	98.2	98.3
	Mean	100.0	99.6	99.8	100.1
	S.D.	3.1	4.5	2.7	1.6
	C.V. (%)	3.1	4.6	2.7	1.6
DM	1	108.6	106.3	104.2	103.5
	2	98.7	95.8	96.7	97.6
	3	92.7	90.3	88.4	93.2
	Mean	100.0	97.4	96.4	98.1
	S.D.	8.0	8.1	7.9	5.2
	C.V. (%)	8.0	8.3	8.2	5.3
DMTE	1	101.8	101.9	104.7	110.0
	2	100.3	97.0	103.7	93.0
	3	97.9	99.0	102.3	92.8
	Mean	100.0	99.3	103.6	98.6
	S.D.	2.0	2.5	1.2	9.9
	C.V. (%)	2.0	2.5	1.2	10.0
S	1	102.3	100.3	103.8	110.6
	2	100.0	98.1	112.1	95.2
	3	97.7	99.0	102.1	94.9
	Mean	100.0	99.1	106.0	100.2
	S.D.	2.3	1.1	5.4	9.0
	C.V. (%)	2.3	1.1	5.0	9.0
TE	1	99.0	97.8	101.4	111.1
	2	100.9	98.3	107.9	95.8
	3	100.0	102.9	106.1	96.1
	Mean	100.0	99.7	105.1	101.0
	S.D.	1.0	2.8	3.3	8.7
	C.V. (%)	1.0	2.8	3.2	8.6

acceptable by demonstrating that the deviation of the back-calculated concentrations from the true values was generally less than 10%.

The concentration range for which quantification is validated under the conditions described for this assay is 5 to 500 ng/ml. Data demonstrating both the intra-day and inter-day variability over the concentration range studied are

Table 6
Stability of E3810 and its 4 metabolites in frozen plasma

Sample	Run	Concentration(ng/ml)					Residual percentage (%)			
	No.	0	2 weeks	1 month	3 months	10 months	2 weeks	1 month	3 months	10 months
E3810	1	102.7	102.4	102.6	103.2	103.1	99.9	100.1	100.7	100.6
	2	102.8	105.0	101.4	102.0	101.9	102.4	98.9	99.5	99.4
	3	101.6	103.9	103.7	103.5	101.6	101.4	101.2	101.0	99.1
	4	102.7	104.4	103.8	101.2	107.1	101.9	101.3	98.7	104.5
	Mean	102.5	103.9	102.9	102.5	103.4	101.4	100.4	100.0	100.9
	S.D.	0.6	1.1	1.1	1.1	2.6	1.1	1.1	1.1	2.5
	C.V. (%)	0.5	1.1	1.1	1.1	2.5	1.1	1.1	1.1	2.5
DM	1	105.2	111.8	110.4	107.1	108.8	107.1	105.8	102.6	104.2
	2	104.5	109.4	108.0	106.2	103.1	104.8	103.5	101.8	98.7
	3	103.7	122.8	110.1	108.0	105.2	117.7	105.4	103.4	100.8
	4	104.4	104.1	105.9	107.2	104.9	99.8	101.5	102.7	100.5
	Mean	104.4	112.0	108.6	107.2	105.5	107.3	104.0	102.6	101.1
	S.D.	0.6	7.9	2.1	0.7	2.4	7.5	2.0	0.7	2.3
	C.V. (%)	0.6	7.0	1.9	0.7	2.3	7.0	1.9	0.7	2.3
DMTE	1	100.2	103.8	104.8	97.0	101.2	103.3	104.2	96.5	100.7
	2	100.3	105.6	102.6	96.4	99.4	105.1	102.1	95.9	98.9
	3	100.6	104.8	103.6	96.3	100.5	104.3	103.1	95.8	100.0
	4	101.1	104.4	105.8	94.3	99.4	103.9	105.3	93.9	98.9
	Mean	100.5	104.7	104.2	96.0	100.1	104.1	103.7	95.5	99.6
	S.D.	0.4	0.8	1.4	1.1	0.9	0.8	1.4	1.1	0.9
	C.V. (%)	0.4	0.7	1.3	1.2	0.9	0.7	1.3	1.2	0.9
S	1	102.4	104.0	102.7	96.7	101.9	101.6	100.3	94.4	99.5
	2	102.3	103.7	99.3	99.7	102.0	101.3	97.0	97.4	99.6
	3	100.6	103.4	101.7	99.6	105.1	101.0	99.3	97.2	102.6
	4	104.2	101.8	102.6	99.7	106.2	99.4	100.2	97.3	103.7
	Mean	102.4	103.2	101.6	98.9	103.8	100.8	99.2	96.6	101.3
	S.D.	1.5	1.0	1.6	1.5	2.2	1.0	1.5	1.5	2.1
	C.V. (%)	1.4	1.0	1.6	1.5	2.1	1.0	1.6	1.5	2.1
TE	1	101.4	105.5	102.1	97.2	108.5	103.9	100.6	95.8	106.9
	2	101.5	103.7	100.2	99.1	109.4	102.2	98.7	97.6	107.8
	3	99.3	102.7	101.6	100.2	106.1	101.2	100.1	98.7	104.5
	4	103.8	100.9	103.2	99.4	102.0	99.4	101.7	97.9	100.5
	Mean	101.5	103.2	101.8	99.0	106.5	101.7	100.3	97.5	104.9
	S.D.	1.9	1.9	1.2	1.3	3.3	1.9	1.2	1.3	3.3
	C.V. (%)	1.8	1.9	1.2	1.3	3.1	1.9	1.2	1.3	3.1

shown in Tables 2 and 3. These data show good precision for the intra-day and inter-day validation. The quantitation limits were determined as 5 ng/ml for E3810 and 20 ng/ml for each of its 4 metabolites.

Data on the stability of E3810 and its 4 metabolites are shown in Tables 4–6. The residual percentages of E3810 and its 4 metabolites in whole blood at room temperature and at physiologic pH ranged from 98.5 to 115.0%, and they showed no tendency to decrease or increase. When samples were centrifuged within 20 min after collection, there was no loss of E3810 or its metabolites. The residual percentages of E3810 and its 4 metabolites in plasma at physiologic pH at room temperature ranged from 96.4 to 106.0%, and they showed no tendency to decrease or increase. When 100 μl of 1% aqueous solution of diethylamine was added within 20 min after plasma isolation, there was no loss of E3810 or its metabolites. The residual percentages of E3810 and its 4 metabolites stored in plasma at −20°C for 10 months ranged from 99.6% to 104.9%, indicating that no stability problems occurred.

3.3. Pharmacokinetics

The mean plasma levels of E3810 after administration of 10–80 mg to healthy volunteers are shown in Fig. 5. The maximum E3810 plasma concentration of 0.406 μg/ml in the 20-mg dose (clinical dose) study was attained after 3.5 h. The terminal elimination half-life of E3810 was ca. 1.0 h. Plasma E3810 concentrations (quantitation limit: 5 ng/ml) could be determined up to at least 9 h after administration of a 20-mg dose. In addition, the metabolites TE and S could also be detected. These results indicate that the assay is sensitive and specific.

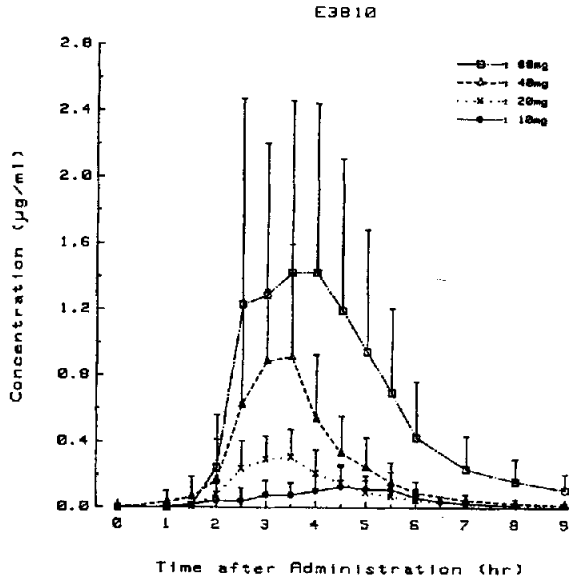


Fig. 5. Mean plasma levels of E 3810. Each point represents the mean ± S.D. of 4 (40-mg dose study) or 6 subjects. Results of subjects 7 and 8 in the 40-mg dose study were excluded from averaging since these two subjects had much lower plasma levels.

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