APPENDIX ONE

Table 9 Solubility of mefenamic acid esters **2** and **3** in various solvents

	Solubility (μg/ml)	
Solvent	Ester **2**	Ester **3**
Water	2.59	4.40
Ethanol	>2,000	>2,000
Propylene Glycol	>2,000	>2,000
PEG 300	>2,000	>2,000
0.01 N HCl (pH 2)	1.98	1748 ± 343
pH 4 acetate buffer	2.27	58.6 ± 15.6
pH 5 acetate buffer	4.89	6.28 ± 4.49
pH 6 acetate buffer	2.27	5.78 ± 1.87
pH 7.4 phosphate buffer	3.72	2.61 ± 2.10
pH 8 phosphate buffer	1.71	6.19 ± 2.64

Table 10 Rate constants and squared correlation coefficients of the time courses of mefenamic acid prodrugs in aqueous buffers

Compounds	pH 2		pH 5		pH 7.4	
	Rate constant (h^{-1})	R^2	Rate constant (h^{-1})	R^2	Rate constant (h^{-1})	R^2
2	0.135 ± 0.02	0.926 ± 0.097	0.134 ± 0.024	0.844 ± 0.056	0.088 ± 0.018	0.969 ± 0.012
3	*a*	*a*	*a*	*a*	0.150 ± 0.048	0.969 ± 0.036
6	0.045 ± 0.012	0.967 ± 0.022	0.023 ± 0.003	0.965 ± 0.014	0.169± 0.028	0.929 ± 0.023

a : No detectable degradation product of **3** were observed at pH 2 and 5.

Table 11 Rate constants and squared correlation coefficients of the time courses of mefenamic acid ester prodrugs in biological media

Compounds	Human plasma		Caco-2 homogenate		Rat liver homogenate	
	Rate constant (min^{-1})	R^2	Rate constant (min^{-1})	R^2	Rate constant (min^{-1})	R^2
1	0.015 ± 0.001	0.967± 0.009	0.012 ± 0.00	0.976 ± 0.01	0.024 ± 0.002	0.981 ± 0.007
2	0.045 ± 0.003	0.960 ± 0.027	0.137 ± 0.033	0.957 ± 0.031	0.037 ± 0.003	0.928± 0.029
3	0.012 ± 0.001	0.990 ± 0.006	$9.67 \pm 0.58 \times 10^{-4}$	0.974 ± 0.014	0.013 ± 0.002	0.972 ± 0.007
4	0.019 ± 0.002	0.989 ± 0.003	$5.48 \pm 0.72 \times 10^{-4}$	0.965 ± 0.025	$4.8 \pm 0.97 \times 10^{-4}$	0.970± 0.005
5	$2.3 \pm 0.11 \times 10^{-3}$	0.994 ± 0.003	$4.0 \pm 0.70 \times 10^{-4}$	0.967 ± 0.014	$1.49 \pm 0.16 \times 10^{-3}$	0.963 ± 0.026
6	N.D.	N.D.	0.088 ± 0.023	0.974 ± 0.007	N.D.	N.D.

N.D.: Not determined

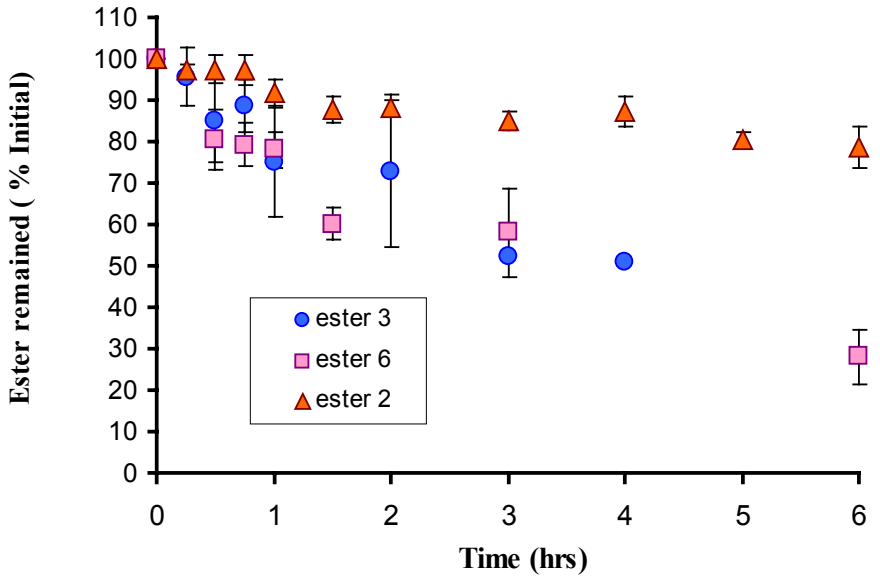


Figure 16 Hydrolysis profile of the esters **2**, **3**, and **6** of mefenamic acid in phosphate buffer pH 7.4 at 37°C

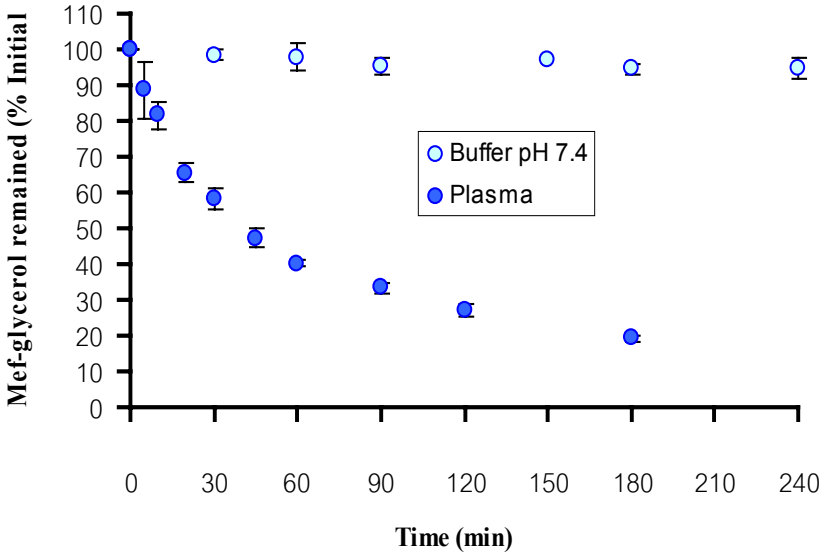


Figure 17 Hydrolysis of **1** in human plasma and phosphate buffer pH 7.4 at 37°C

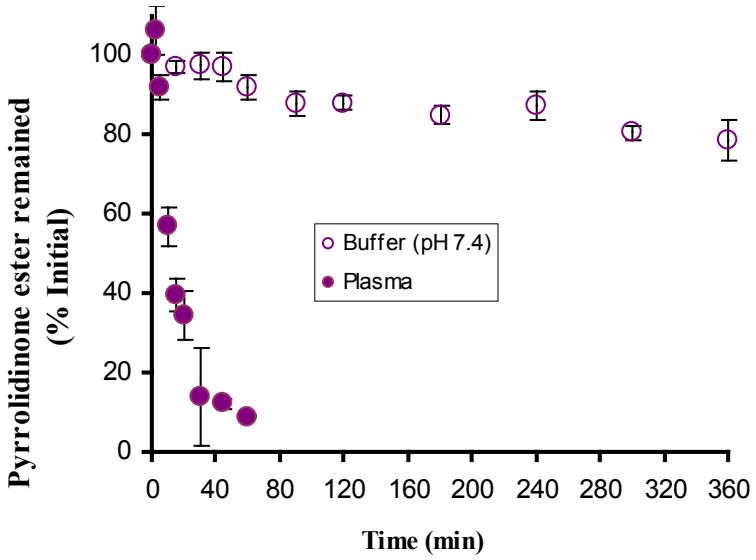


Figure 18 Hydrolysis of **2** in human plasma and phosphate buffer pH 7.4 at 37°C

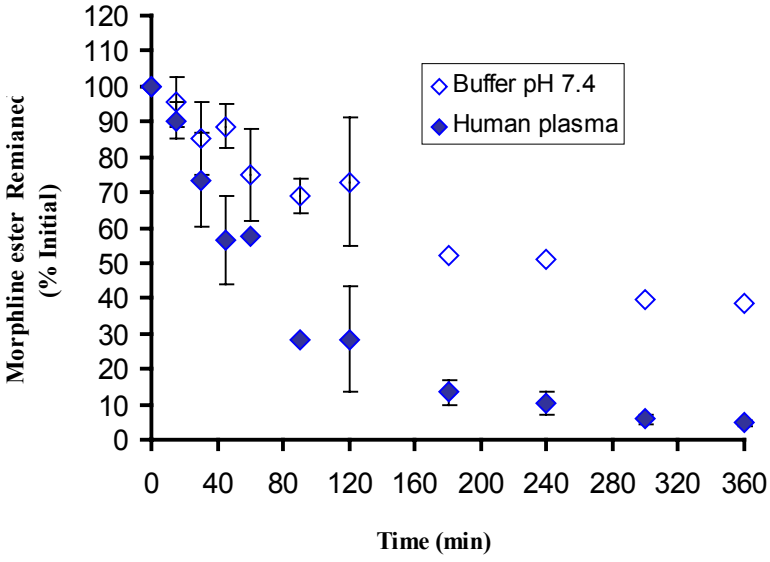


Figure 19 Hydrolysis of **3** in human plasma and phosphate buffer pH 7.4 at 37°C

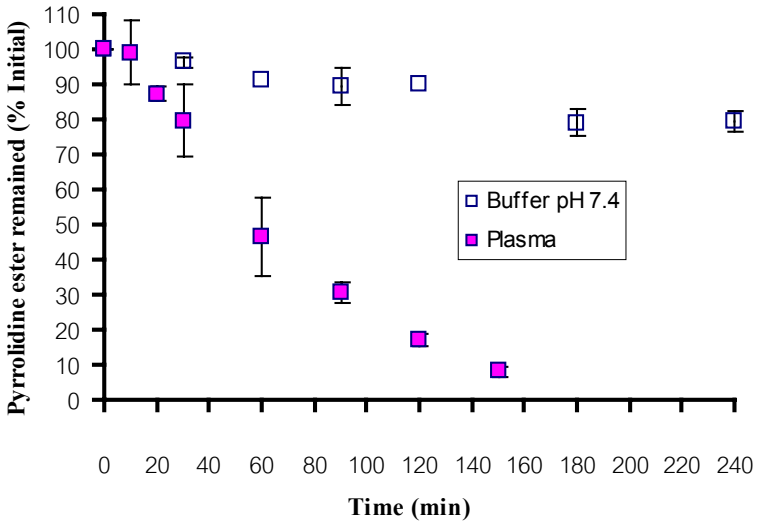


Figure 20 Hydrolysis of **4** in human plasma and phosphate buffer pH 7.4 at 37°C

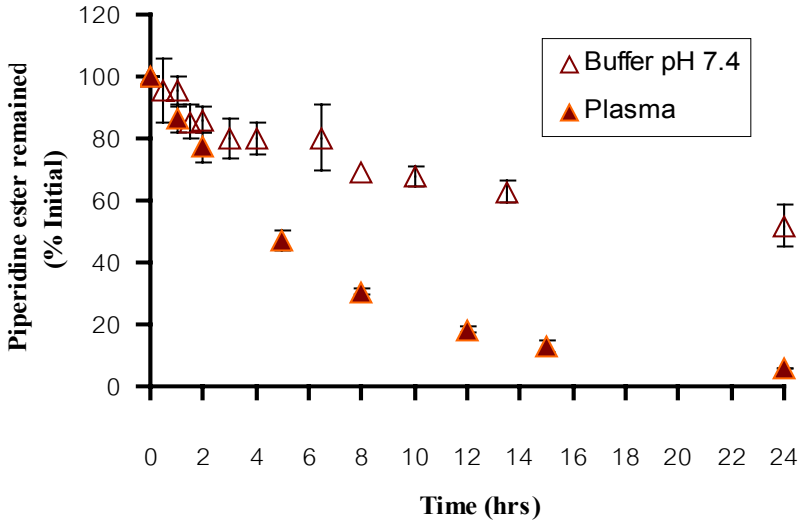


Figure 21 Hydrolysis of **5** in human plasma and phosphate buffer pH 7.4 at 37°C

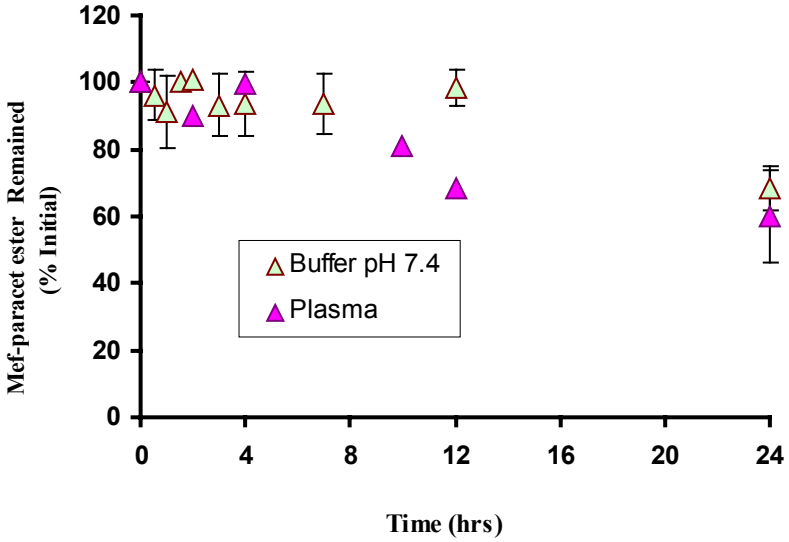


Figure 22 Hydrolysis of **6** in human plasma and phosphate buffer pH 7.4 at 37°C

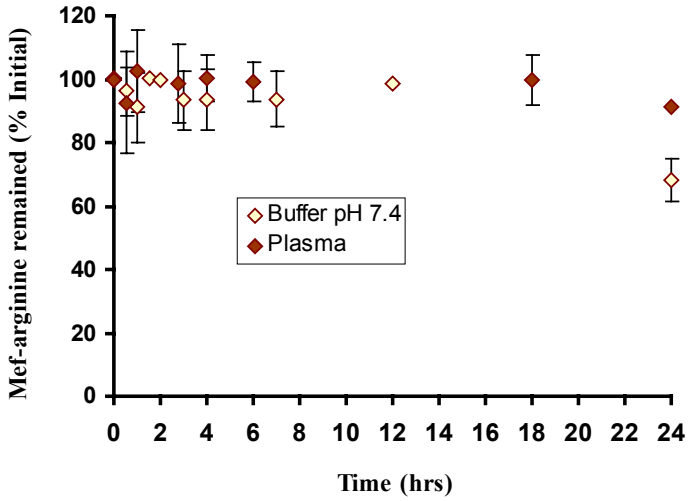


Figure 23 Hydrolysis of **7** in human plasma and phosphate buffer pH 7.4 at 37°C

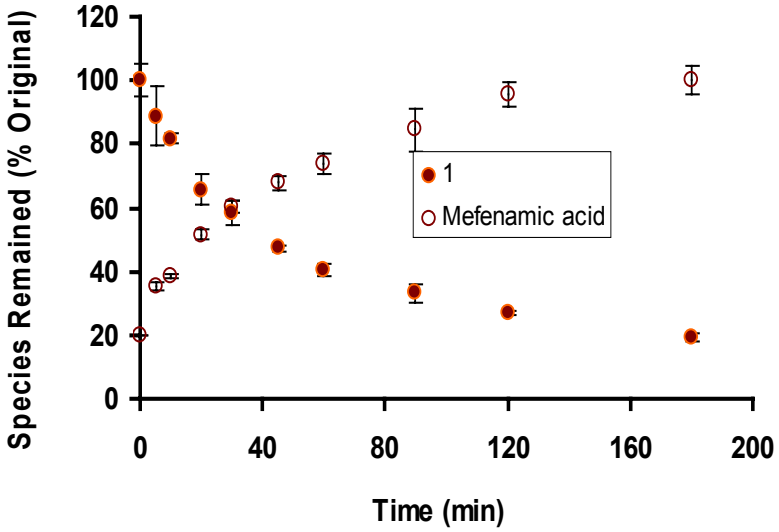


Figure 24 Degradation profiles of **1** in human plasma (pH 7.4, 37°C)

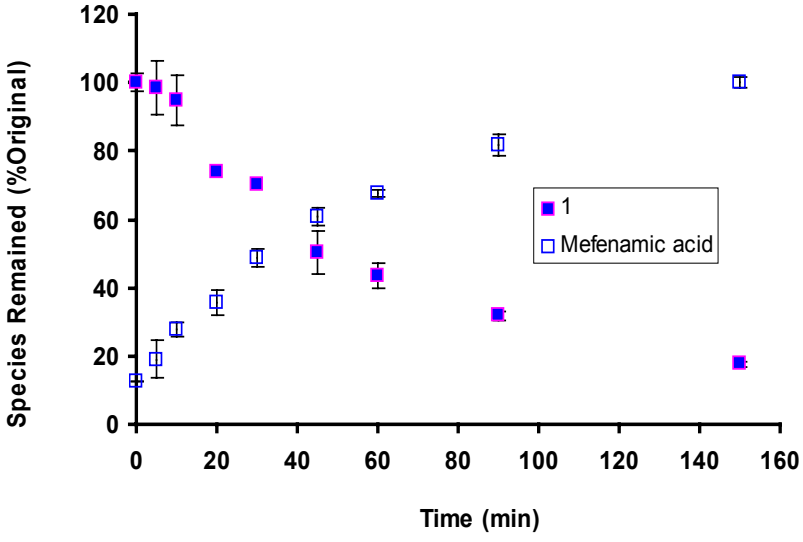


Figure 25 Degradation profiles of **1** in Caco-2 homogenate (pH 7.4, 37°C)

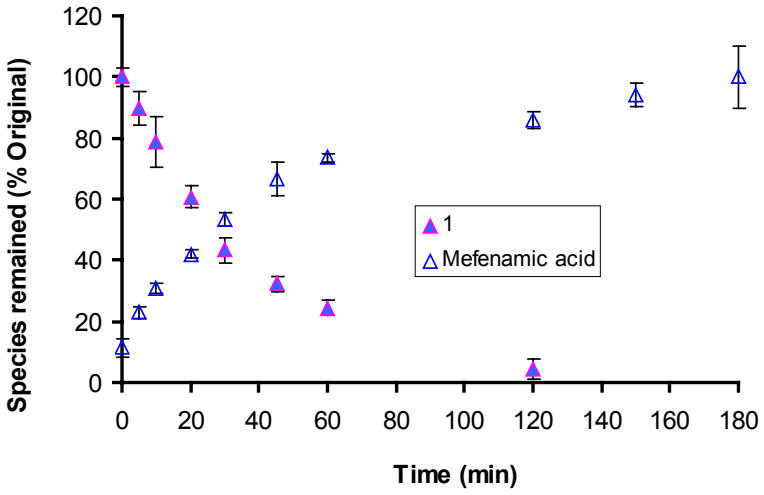


Figure 26 Hydrolysis of **1** in rat liver homogenate (pH 7.4, 37°C)

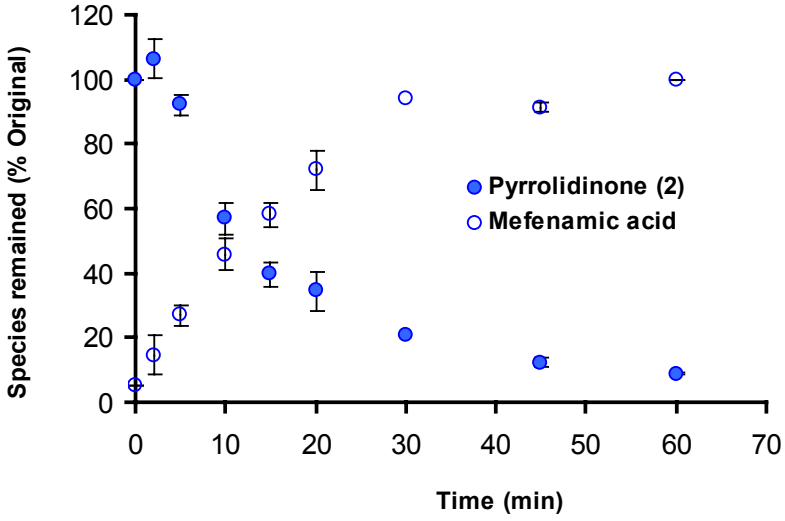


Figure 27 Degradation profiles of **2** in human plasma (pH 7.4, 37°C)

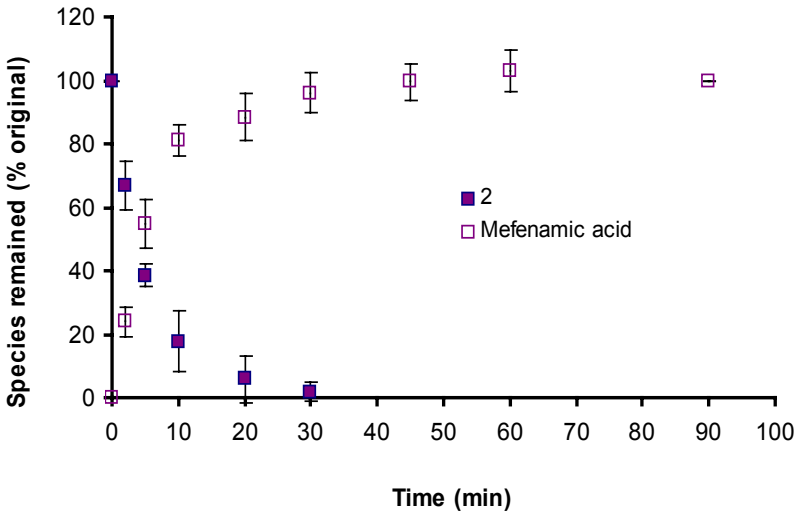


Figure 28 Degradation profiles of **2** in Caco-2 homogenate (pH 7.4, 37°C)

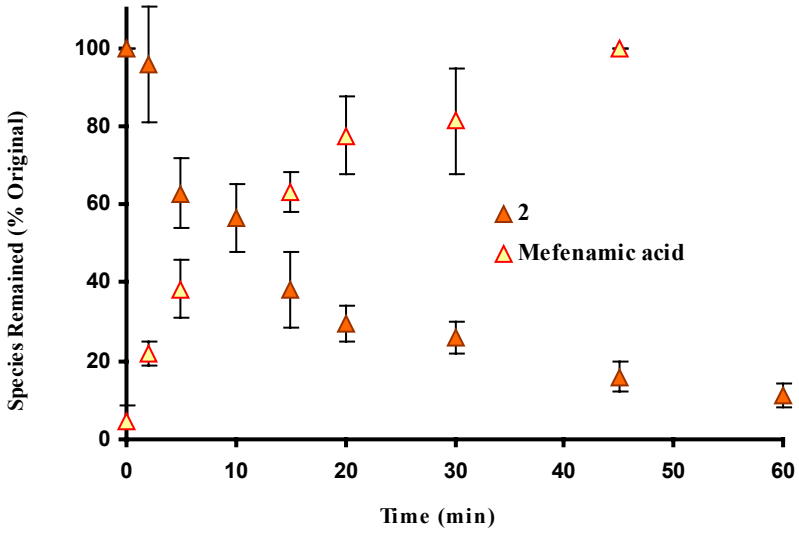


Figure 29 Degradation profiles of **2** in rat liver homogenate (pH 7.4, 37°C)

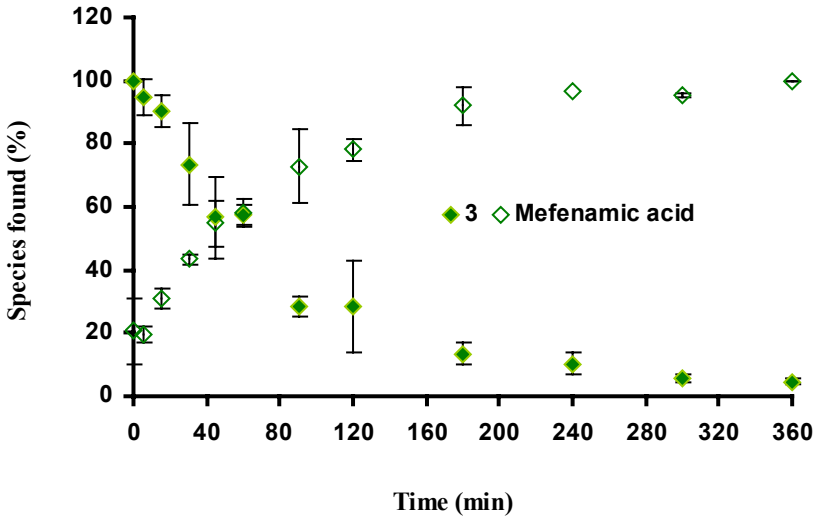


Figure 30 Degradation profile of **3** in human plasma (pH 7.4, 37°C)

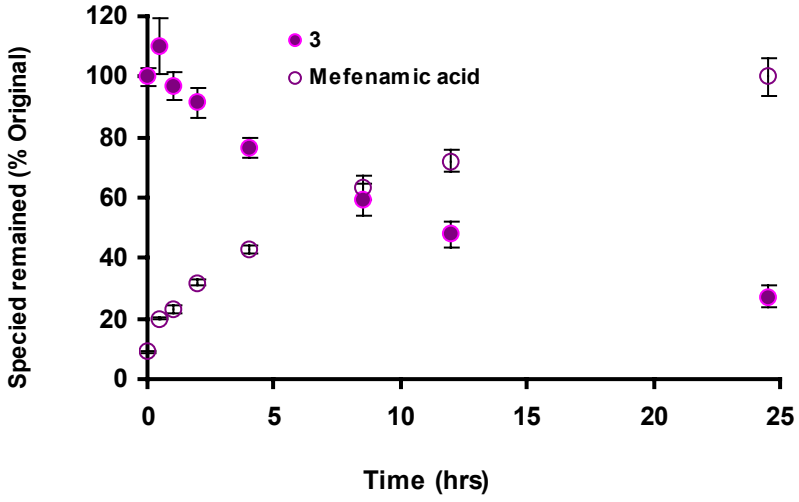


Figure 31 Degradation profile of **3** in Caco-2 homogenate (pH 7.4, 37°C)

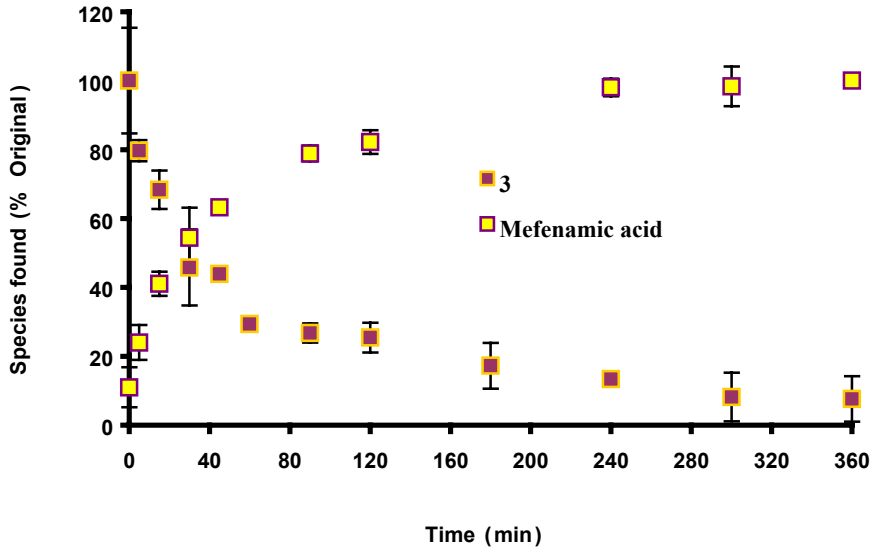


Figure 32 Degradation profile of **3** in rat liver homogenate (pH 7.4, 37°C)

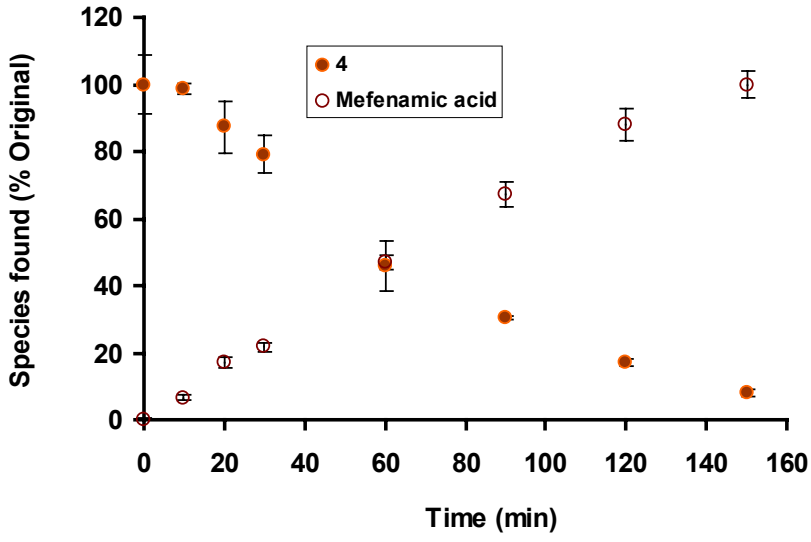


Figure 33 Degradation profile of **4** in human plasma (pH 7.4, 37°C)

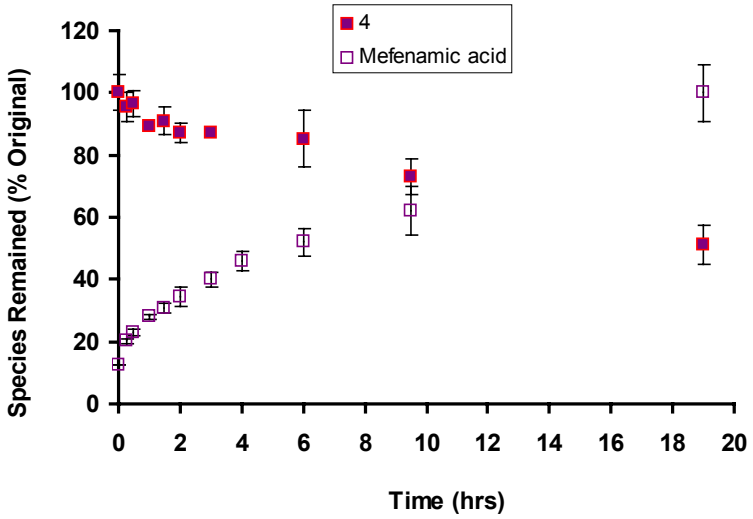


Figure 34 Degradation profile of **4** in Caco-2 homogenate (pH 7.4, 37°C)

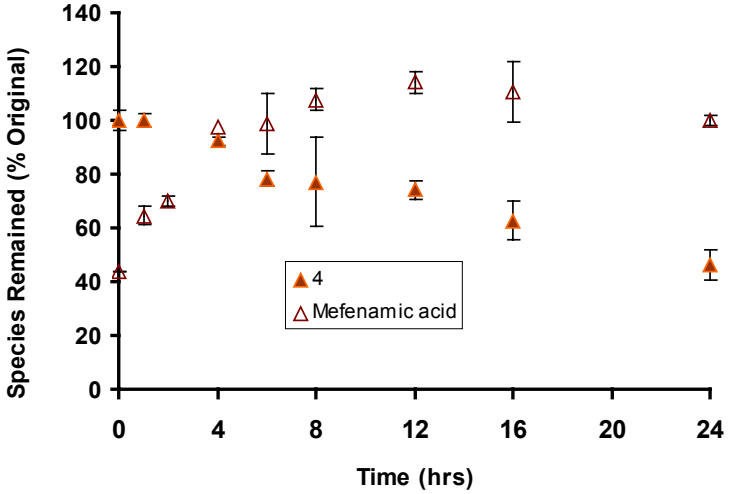


Figure 35 Degradation profile of **4** in rat liver homogenate (pH 7.4, 37°C)

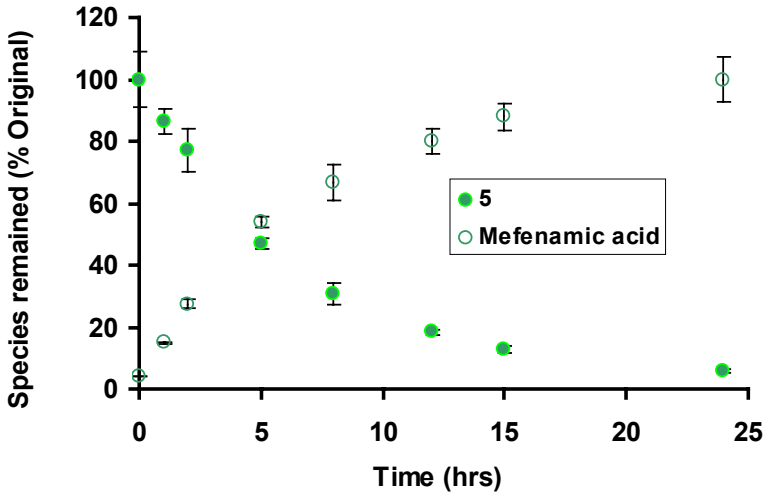


Figure 36 Degradation profile of **5** in human plasma (pH 7.4, 37°C)

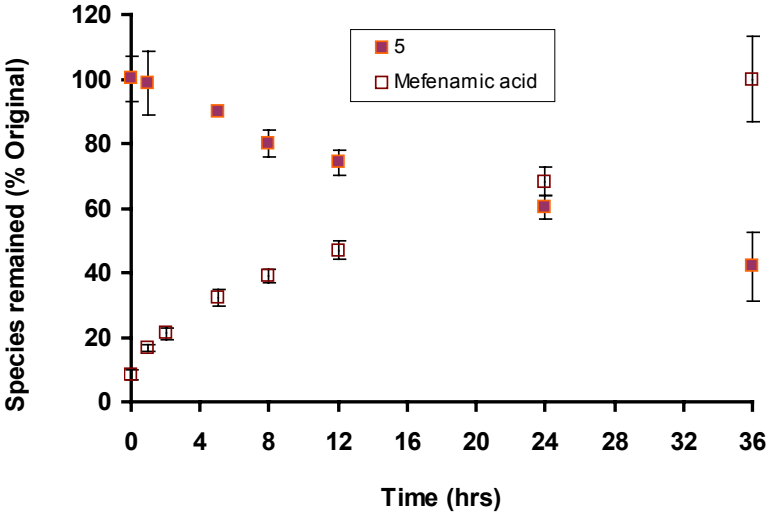


Figure 37 Degradation profile of **5** in Caco-2 homogenate (pH 7.4, 37°C)

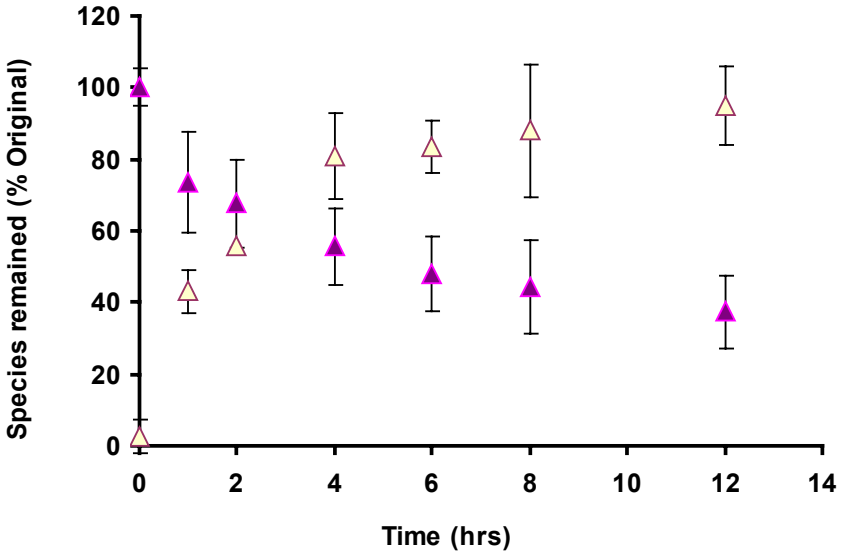


Figure 38 Degradation profile of **5** in rat liver homogenate (pH 7.4, 37°C)

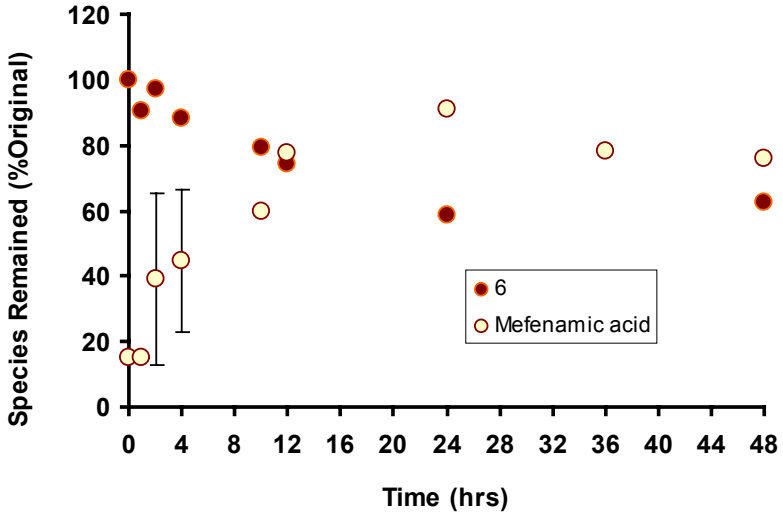


Figure 39 Degradation profile of **6** in human plasma (pH 7.4, 37°C)

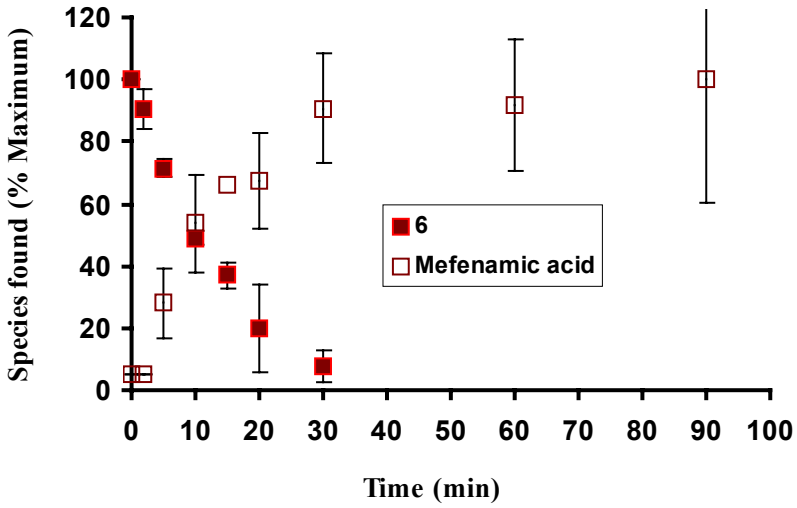


Figure 40 Degradation profile of **6** in Caco-2 homogenate (pH 7.4, 37°C)

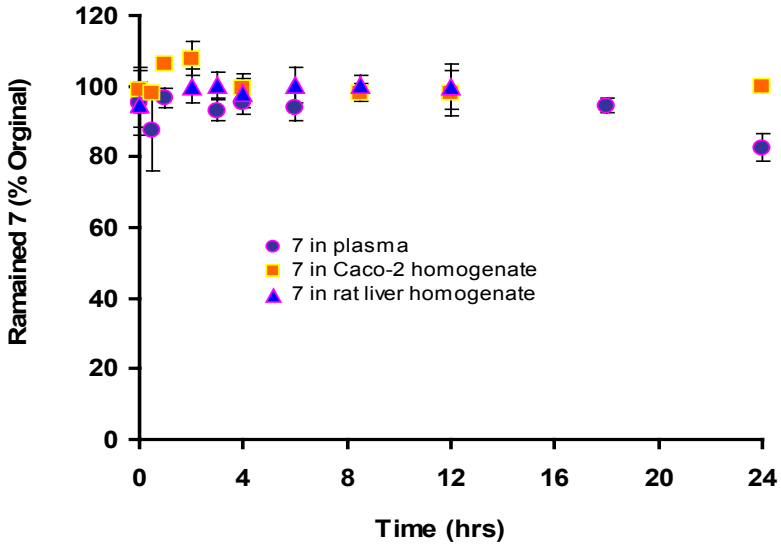


Figure 41 Time course of **7** in human plasma, Caco-2 homogenate, and rat liver homogenate (pH 7.4, 37°C)

APPENDIX TWO

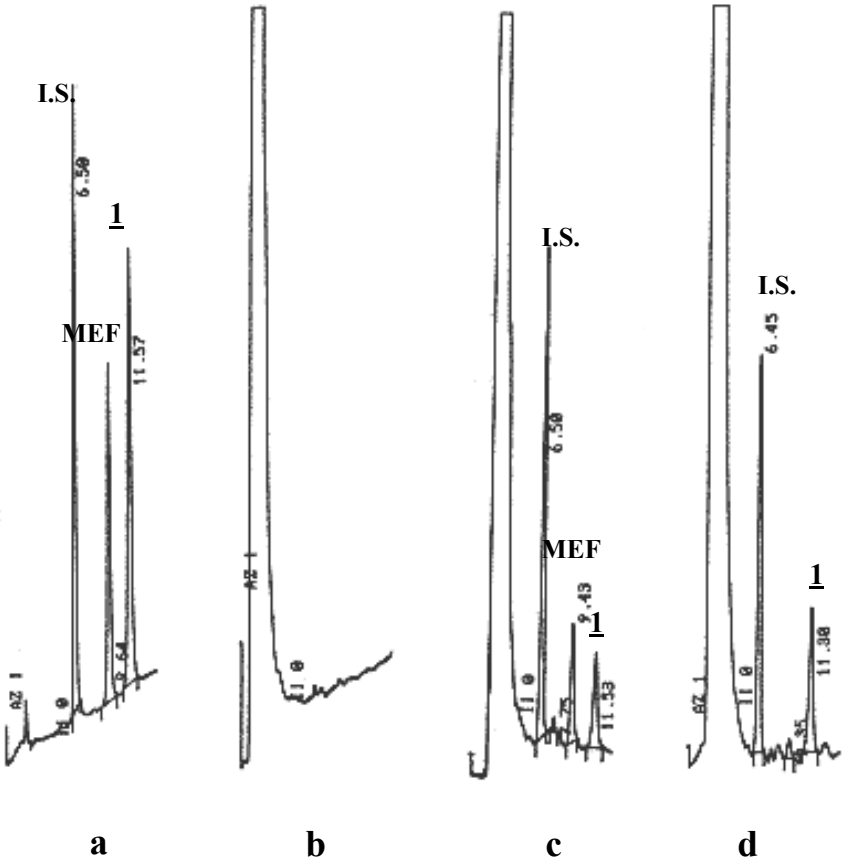


Figure 42 Representative chromatograms of degradation of **1** in rat liver homogenate. Mobile phase: Acetate buffer pH 5.0 (0.05M) and methanol (40:60), flow rate 1.0 ml/min. UV detected at 280 nm.
(a) Standard mixture of **1**, mefenamic acid, and diclofenac (internal standard, I.S.) yielded retention time of 11.5 min (**1**), 9.4 min (mefenamic acid, MEF), and 6.5 min (diclofenac); (b) Blank rat liver homogenate; (c) Rat liver homogenate containing 6.34 μM of **1**, 4.14 μM mefenamic acid, and diclofenac (I.S.); (d) Samples obtained at 150 min after incubating 10.9 μM of **1** in rat liver homogenate (pH 7.4, 37^{o}C).

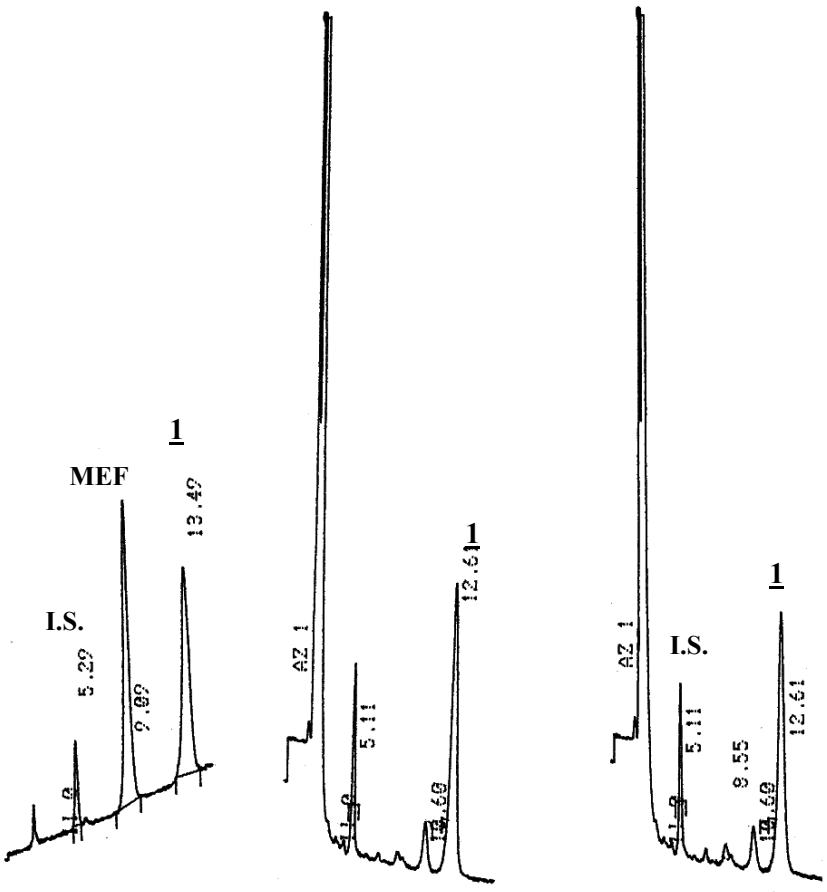


Figure 43 Representative chromatograms of transport of **1** across Caco-2 monolayer. Mobile phase: Acetate buffer pH 5.0 (0.05M) and methanol (40:60). UV detected at 280 nm. Transport was performed at 37°C.
(a) Standard mixture of **1**, mefenamic acid, and diclofenac (internal standard, I.S.) yielded retention time of 13.5 min (**1**), 9.09 min (mefenamic acid, MEF), and 5.29 min (diclofenac); (b) BL-AP transport, samples withdrawn at 90 min after incubating 26.3 μM of **1** with HBSS pH 7.4; (c)AP-BL transport, samples withdrawn at 90 min after incubating 18.7 μM of **1** with HBSS pH 6.5.

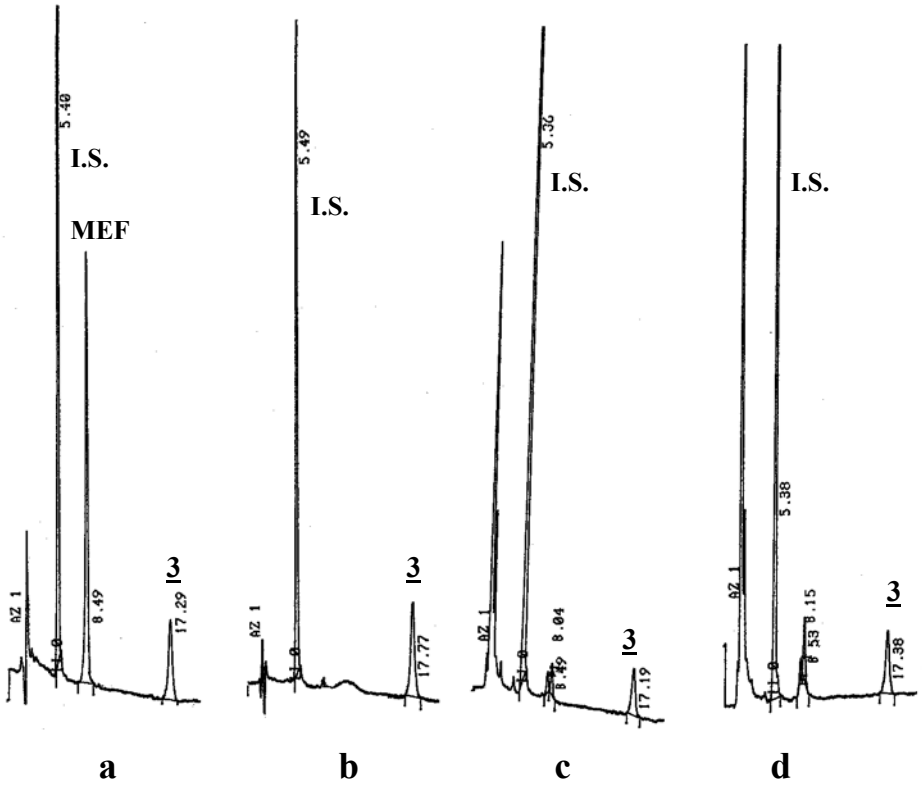
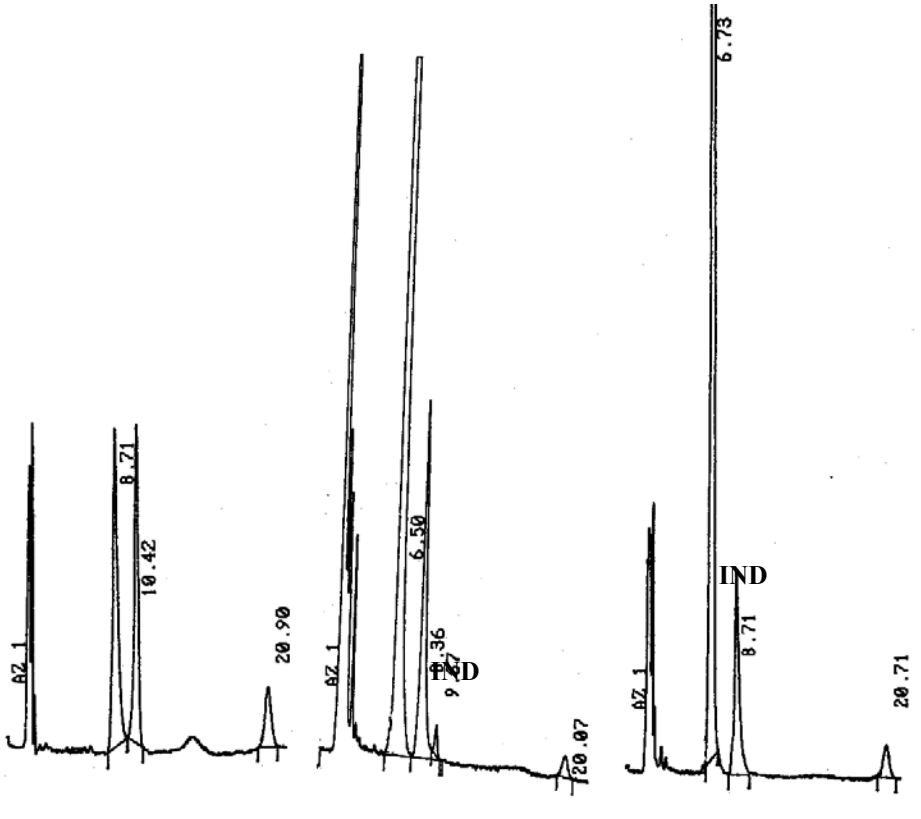
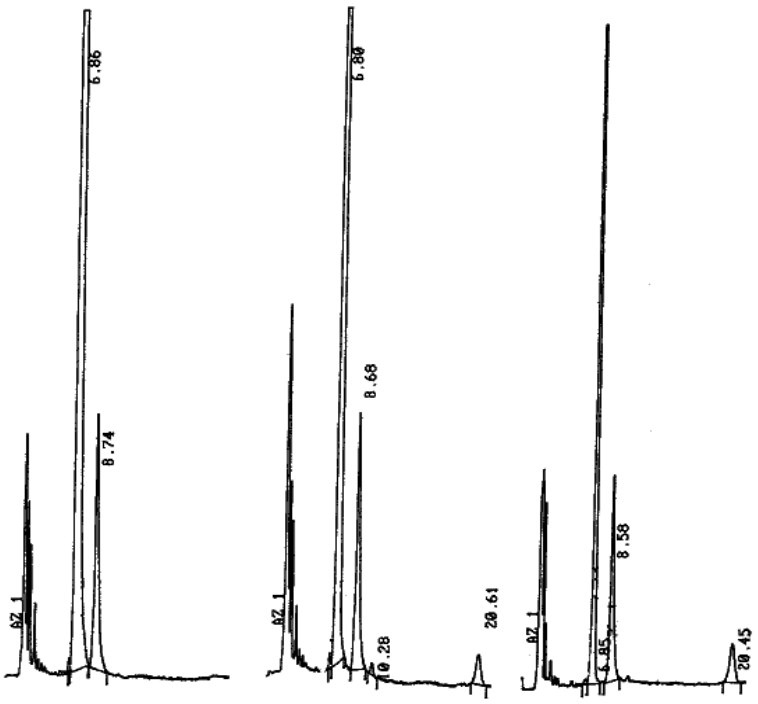


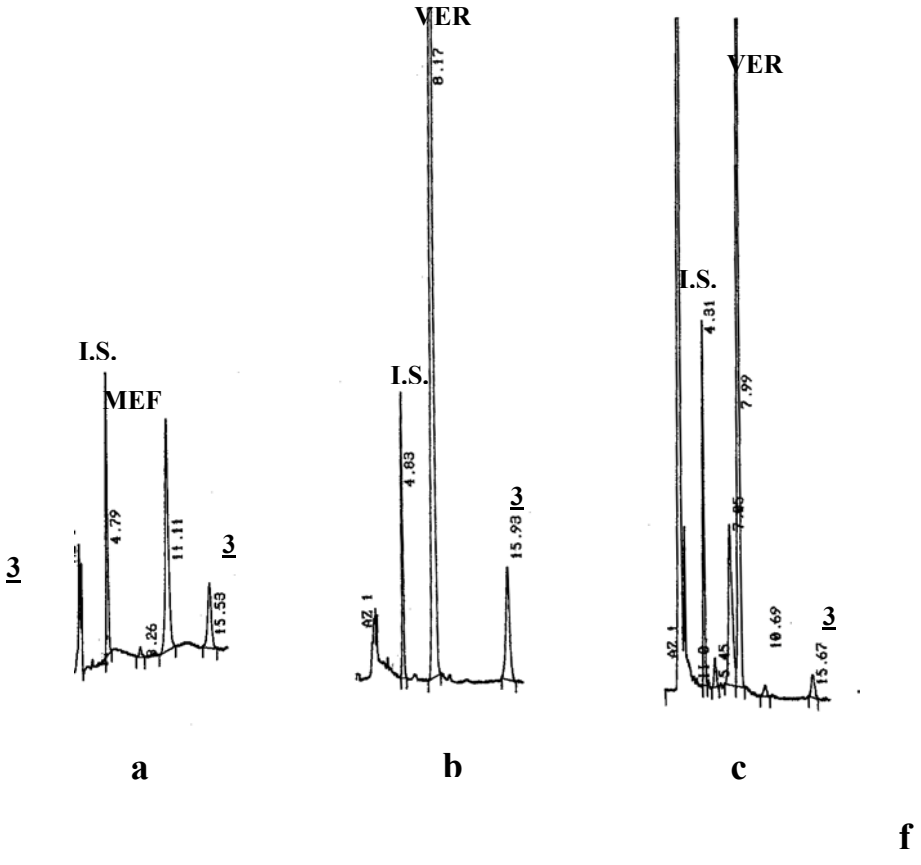
Figure 44 Representative chromatograms of transport (BL to AP) of **3** across Caco-2 monolayer. Mobile phase:Acetate buffer pH 4.5 (0.05M) and acetonitrile (55:45). UV detected at 280 nm. Transport was performed at 37°C. (a) HBSS containing 1.52 μM of **3**, 2.16 μM of mefenamic acid, and diclofenac (internal standard, I.S.) yielded retention time of 17.5 (**3**), 8.5 (mefenamic acid), and 5.4 min (diclofenac); (b) Initial loading containing 11.1 μM of **3** in HBSS pH 7.4; (c)-(d) Samples withdrawn at 90 and 150





IND **IND** **IND**

Figure 45 Representative chromatograms of transport of **3** with 100 μM indomethacin (IND). Mobile phase: Acetate buffer pH 4.5 (0.05M) and acetonitrile (55:45) UV detected at 280 nm. Transport was performed at 37°C. (a) Standard mixture of **3** (1.52 μM), mefenamic acid (2.16 μM), and dextromethorphan (I.S., 1.2μg) yielded retention time of 20.9 min (**3**), 10.4 min (mefenamic acid), and 8.71 min (I.S.) ; (b) AP-BL transport of **3,** samples withdrawn at 120 min after transport initiation. Indomethacin showed a distinct peak at 6.50 min; (c) AP-BL transport of **3**, samples at donor side 120 min after transport initiation; (d) Initial loaded **3** in HBSS pH 7.4; (e) BL-AP transport of **3,** samples withdrawn at 120 min after transport initiation. Dextromethorphan (internal standard) showed a peak at 8.68 min; (f) BL-AP transport of **3,** samples at donor side 120 min after transport initiation.



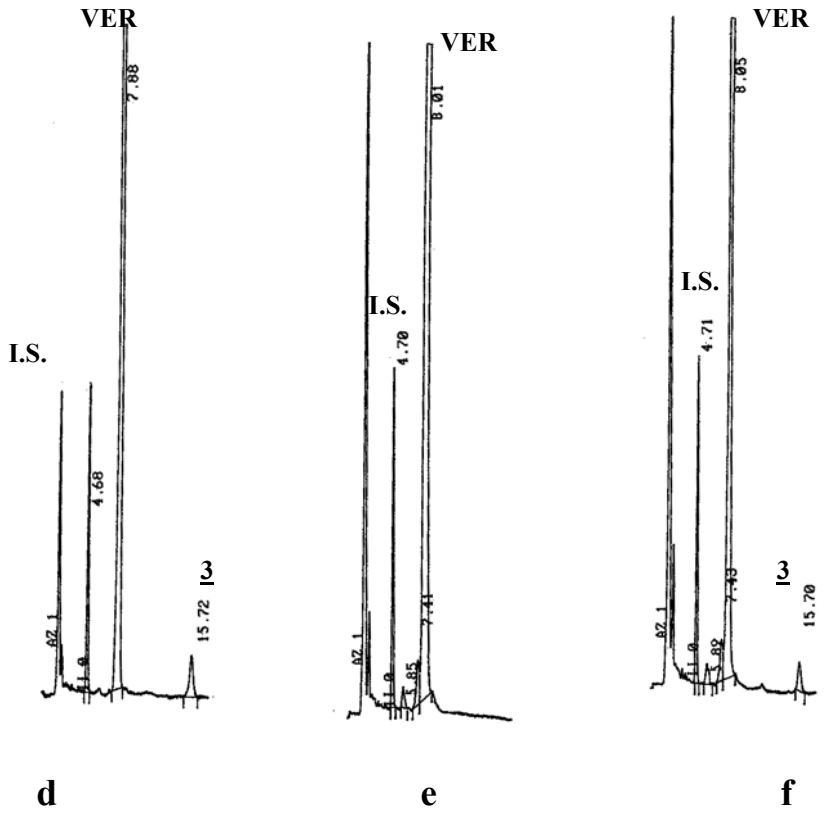


Figure 46 Representative chromatograms of transport of **3** with 100 μM verapamil. Mobile phase: phosphate buffer pH 4.35 (0.05M) and acetonitrile (55:45). UV detected at 280 nm. (a) Standard mixture of **3** (1.52 μM), mefenamic acid (2.16 μM), and naproxen (internal standard, 0.25μg) yielded retention time of 15.5 min (**3**), 11.1 min (mefenamic acid), and 4.79 min (naproxen); (b) Initial loaded **3** in HBSS pH 6.5. Verapamil showed a distinct peak at 8.17 min; (c) AP-BL transport of **3,** samples withdrawn at 90 min after transport initiation; (d) AP-BL transport of **3**, samples at donor side 20 min after transport initiation; (e) BL-AP transport of **3,** samples withdrawn from receiver compartment at 0 min after transport initiation; (f) BL-AP transport of **3,** samples withdrawn from receiver compartment at 90 min after transport initiation.

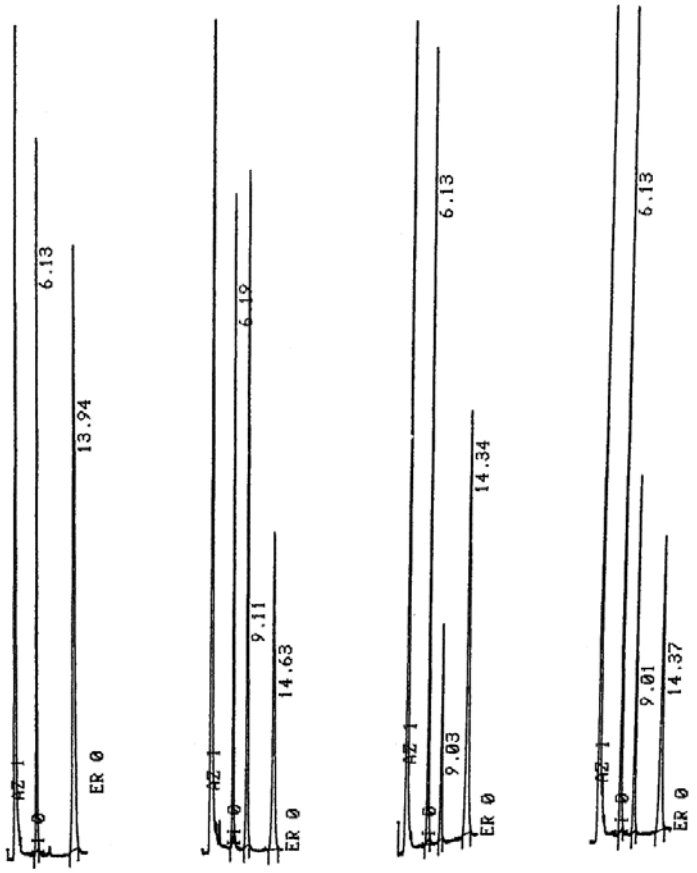


Figure 47 Representative chromatograms of hydrolysis of **4** in Caco-2 homogenate. Mobile phase: Acetate buffer pH 4.1 (0.05M) and acetonitrile (50:50), flow rate 1.0 ml/min. UV detected at 280 nm. (a) Caco-2 homogenate containing 50.87 μM of **4** at 37°C, retention time = 13.9 min. Diclofenac was used as internal standard (6.13 min); (b) Caco-2 homogenate containing **4** (29.9 μM), mefenamic acid (17.2 μM), and diclofenac, yielding retention times of 14.63 min (**4**), 9.11 min (mefenamic acid), and 6.19 min (diclofenac, I.S.); (c) Samples withdrawn after 9.5 hrs incubation; (d) Samples withdrawn at 20 hrs after incubation.

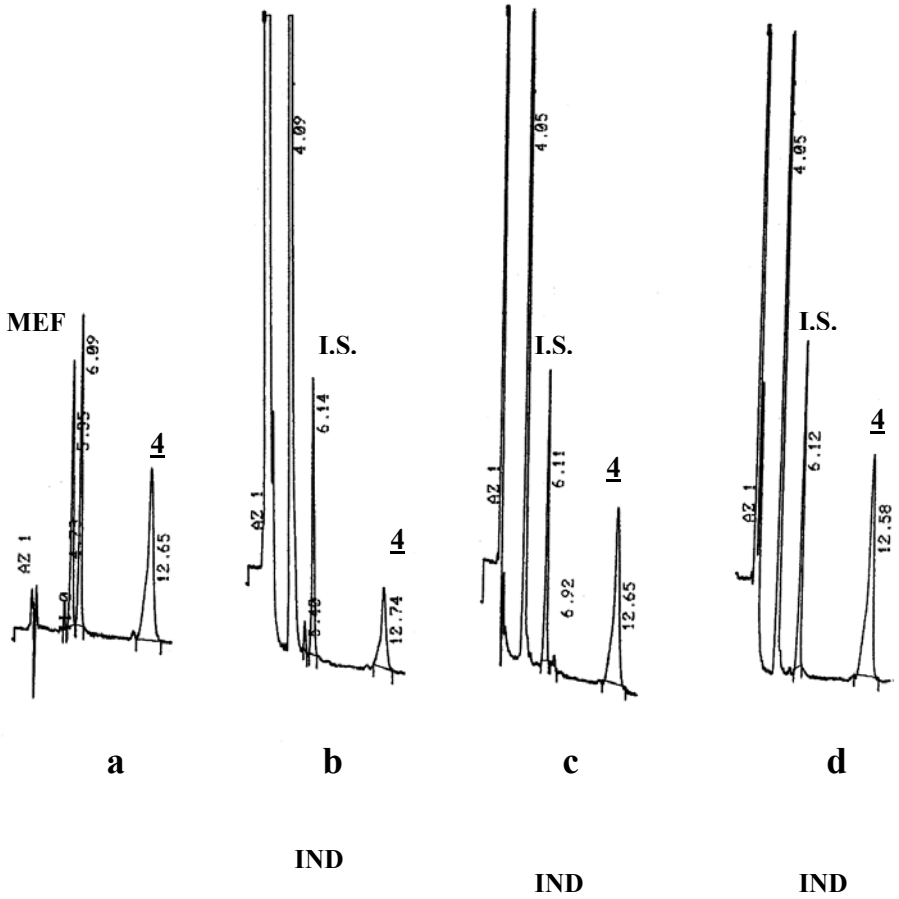


Figure 48 Representative chromatograms of transport of **4** with 100 μM indomethacin (IND). Mobile phase: Acetate buffer pH 4.1 (0.05M) and acetonitrile (50:50) UV detected at 280 nm. Transport was performed at 37°C. (a) Standard mixture of (2.95 μM), mefenamic acid (2.44 μM), and gemfibrosil (internal standard), yielding retention time of 12.65 min (**4**), 5.35 min (mefenamic acid), and 6.09 min (gemfibrosil); (b) AP-BL transport of **4,** samples withdrawn at 180 min after transport initiation. Indomethacin showed a distinct peak at 4.09 min; (c) AP-BL transport of **4**, samples at donor side 60 min after transport initiation; (d) BL-AP transport of **4,** samples withdrawn at 60 min after transport initiation. Gemfibrosil (I.S.) showed a peak at 6.12 min.

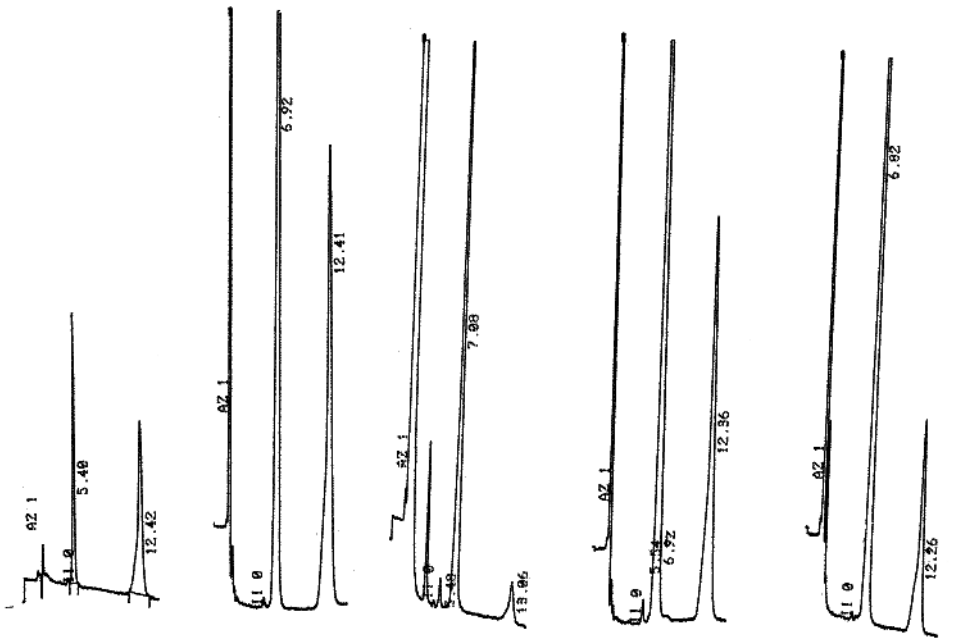


Figure 49 Representative chromatograms of transport of **4** with 100 μM verapamil (VER). Mobile phase: Acetate buffer pH 4.1 (0.05M) and acetonitrile (50:50). UV detected at 280 nm. (a) Standard mixture of **4** (2.95 μM), and mefenamic acid (MEF, 2.44 μM), yielding retention time of 12.4 min (**4**) and 5.40 min (MEF); (b) Initial loaded **4** in HBSS pH 6.5. Verapamil showed a distinct peak at 6.92 min; (c) AP-BL transport of **4**, samples withdrawn at 90 min after transport initiation; (d) Initial loaded **4** in HBSS pH 7.4. Verapamil showed a distinct peak at 6.92 min; (e) BL-AP transport of **4**, samples withdrawn from receiver compartment at 90 min after transport initiation.

a