International Journal of Pharmaceutics, 39 (1987) 235–242
Elsevier

IJP 01331

Kinetics of indomethacin release from suppositories. In vitro–in vivo correlation

J.-M. Aiache *, M. Islasse *, E. Beyssac *, S. Aiache *, R. Renoux * and J.-P. Kantelip **

* *Laboratoire de Biopharmacie, Faculté de Pharmacie, B.P. 38, Clermont-Ferrand (France)*
and ** *Laboratoire de Pharmacologie, Faculté de Médecine et INSERM U 195, B.P. 38, Clermont-Ferrand (France)*

(Received 5 February, 1987)
(Modified version received 1 May 1987)
(Accepted 20 May 1987)

Key words: Suppository; Indomethacin; Kinetic release in vitro; Through-flow cell; Bioavailability; Correlation in vitro–in vivo

Summary

The 'in vitro' dissolution kinetics of two suppository formulations containing indomethacin were compared and a correlation with the 'in vitro' kinetics was established. The device used 'in vitro' was a special through flow cell. A mean dissolution curve was obtained for each dosage form, one containing a hydrophilic excipient (PEG), the other, a fatty base. A relative bioavailability study was then carried out on 12 healthy volunteers. Thus, from the pharmacokinetic parameters obtained, the simulation of plasma concentration values was made possible from the amounts of drug released and dissolved during the dissolution test. With the simulation programme used it was possible to predict the plasma level values and the results showed a good superimposition of the experimental plasma concentration curve validating the 'in vitro' study method of drug release from suppositories.

Introduction

The technical and economic problems of bioavailability studies have prompted research into the design of 'in vitro' tests capable of predicting the fate 'in vivo' of drugs released from dosage forms.

The work reported here, aimed to compare by means of a through flow cell, the dissolution kinetics of indomethacin from two different formulations of suppositories.

Using the results of a relative bioavailability study carried out in healthy volunteers with these two dosage forms, an 'in vitro–in vivo' correlation was then established.

Correspondence: J.M. Aiache, Laboratoire de Biopharmacie, Faculté de Pharmacie, B.P. 38, F-63001 Clermont-Ferrand Cédex, France.

Materials and Methods

'In vitro' study (through flow cell method)

Numerous devices have been proposed for the 'in vitro' study of suppositories (Puech et al., 1977; Besnard, 1985). Some are designed to measure the liquefaction time of the suppository while others allow the release kinetics of the drug to be followed (Carp et al. 1980; Desgardins et al., 1983). Among the latter is the through flow cell for suppositories of Langenbucher which we have chosen for the present study * (Langenbucher, 1983). The apparatus is shown in Figures 1–3. It

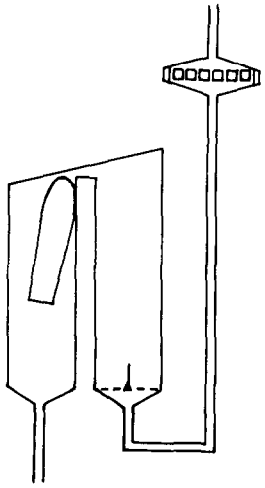

Fig. 1. *Suppository through flow cell principle.*

consists of 3 transparent parts which fit into each other. The lower part is made up of two adjacent vertical chambers which join at their top. The suppository is placed in the first chamber and is subjected to an up-flow of liquid which fills this first chamber and then spills over into the second one. The middle part has a convex cavity designed to collect softened lipidic excipients which float on the aqueous dissolution medium. This part is

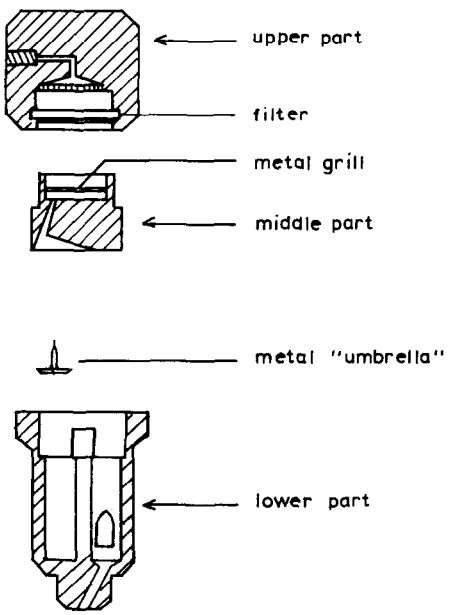

Fig. 2. Suppository through flow cell parts.

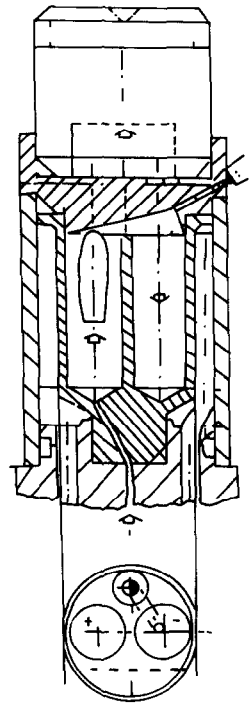

Fig. 3. Diagram of the through flow cell set up for an experiment.

also fitted with a metal grill which serves as a rough filter. The upper part holds paper or glass fibre filter. A small metal 'umbrella' can be placed in the second chamber to collect any large particles shed from the suppository and so avoid obstruction of flow.

The complete test set-up is shown Fig. 4. The dissolution medium (phosphate buffer pH 7.2) contained in the flask (A) is continuously stirred with a magnetic stirrer (B). The liquid is sucked up with a peristaltic pump (C) and discharged into a spectrophotometer cell (D). The optical density

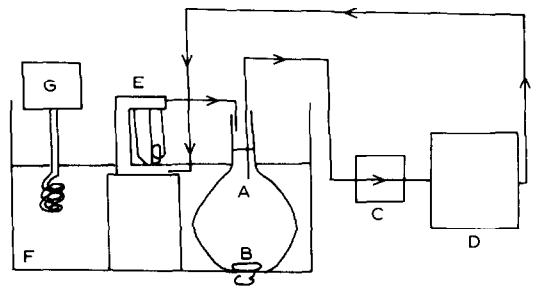

Fig. 4. Complete test set-up.

TABLE 1

Formulas of forms A and B

Form A	Form B
Indomethacin 50 or 100 mg	Indomethacin 50 or 100 mg
Suppocire AP (Gattefosse laboratories, Lyon, France)	Excipients, sufficient to produce a suppository of 1.63 g (butylated hydroxy-anisole, butylated hydroxy-toluene, EDTA, sodium chloride, glycerin, poly-oxyethylene glycol 4000 and 6000)

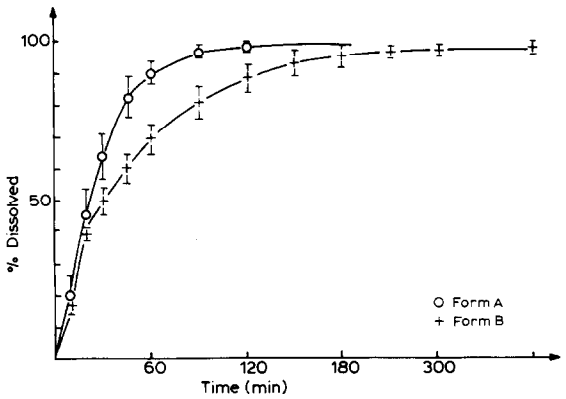

Fig. 5. Mean dissolution curves for forms A and B.

of the liquid is measured at 318 nm, one of the UV absorption maxima of indomethacin. The liquid then flows up into the through flow cell (E) and then, out into the flask which is maintained in a water-bath (F) heated to 37 °C by means of a heating element (G).

Two suppositories containing 100 mg of indomethacin were successively studied. The first form A, had a lipidic excipient (semi-synthetic glycerides of hydrophilic controlled properties *, and the second, form B, a water-soluble excipient (PEG) (Table 1).

* Marketed by Gattefossé S.A., Lyon, France.

Six kinetics were performed and an average dissolution time course established. The mean figures obtained for forms A and B are collected in Tables 2 and 3 and their dissolution profiles are shown in Fig. 5.

'In vivo' study

Experimental procedure

A relative bioavailability study was carried out on 12 healthy male volunteers aged 22–31 years (mean 25 years) and weighing between 68–80 kg (mean 74 kg). The subjects had normal hepatic and renal functions and normal hematological

TABLE 2

Percentage dissolved vs time for form A

	t (min)												
	5	10	20	30	45	60	90	120	150	180	210	240	300
$\bar{M}$	6.6	16.7	41.5	50.24	60.68	69.19	81.28	88.93	93.5	95.97	97.64	98.35	98.81
σ	2.74	2.41	5.15	4.13	4.29	4.69	5.53	4.66	4.14	4.06	2.57	2.08	1.87
C.V. %	41.59	14.45	12.42	8.22	7.07	6.77	6.8	5.24	4.43	4.23	2.63	2.11	1.9

$\bar{M}$, mean; σ, standard deviation; C.V., coefficient of variation.

TABLE 3

Percentage dissolved vs time for form B

	t (min)											
	5	10	20	30	45	60	90	120	150	180	210	240
$\bar{M}$	6.1	19.9	45.47	63.89	82.57	90.73	91.17	99.24	99.24	99.24	99.24	99.24
σ	2.4	6.88	8.22	7.65	6.53	3.67	1.7	1.54	1.54	1.54	1.54	1.54
C.V. %	39.32	34.55	18.08	11.97	7.91	4.04	1.75	1.55	1.55	1.55	1.55	1.55

profiles. None was taking any drug or alcohol. Each gave his written consent to take part in the study which was approved by an ethical committee.

Each subject attended on two separate occasions at least a week apart and received the two suppository formulations in a predetermined random order.

Blood samples were withdrawn from a cannula inserted into a forearm vein at the following times: 0.25, 0.5, 0.75, 1, 1.5., 2, 3, 4, 5, 6, 7, 8, and 10 h. Blood samples were collected in heparin tubes and the plasma separated and stored at $-30\,^{\circ}C$ until analysed for indomethacin.

Analytical procedure

Indomethacin was measured using a high-pressure liquid chromatographic technique with UV detection (Fredj et al., 1983; Ritschel, 1976).

Analysis was performed with a Waters apparatus consisting of a M6000A pump, a U_6K injector, a UV vis M440 detector fitted with a 254 nm filter (sensitivity 0.02 AUFS), an Omniscribe chart recorder (chart speed 0.25 mm/min), a Delsi Icap 50 integrator and a μBondapak C_{18} column (10 μm, 30 cm, 4 mm inside diameter) and a precolumn. The mobile phase consisted of acetonitrile (51%); water (0.003%) and acetic acid (49%). Flow rate was set at 1.7 ml/min and pressure at 1500 psi.

The sample work up was as indicated in Fig. 6.

A calibration curve was obtained by adding supplementary indomethacin to the plasma (at time t_0) in a mixture containing 50% of methanol and water, and working up as described. The regression line for the area ratio:

$$\frac{\text{indomethacin}}{\text{internal standard}}$$

according to concentration is defined by the equation:

$$y = 0.140x + 0.0003$$

(regression coefficient $r = 0.0991$)

The coefficient of variation obtained for the determination of plasma concentrations was less than 1%. The lower limit of detection was 0.02 μg of drug when 1 ml of plasma was used.

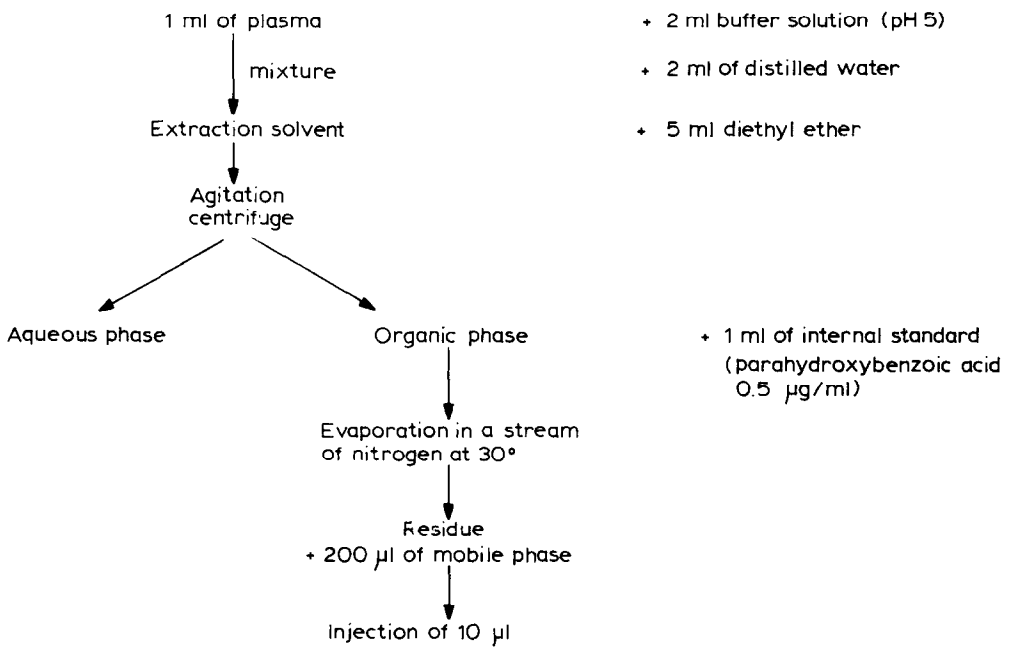

Fig. 6. Indomethacin dosage.

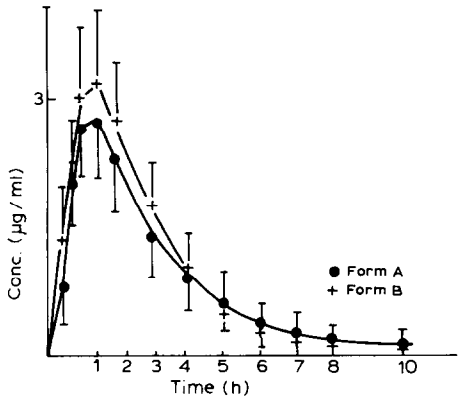

Fig. 7. Mean plasma concentration curves for forms A and B.

Data analysis

The pharmacokinetics parameters after rectal administration of indomethacin were determined using an ANAPHARM programme (LeBlanc and Dumas, 1983; LeBlanc and Aiache, 1984) adapted to an APPLE II microcomputer and adjusted with a nunlin program (Koeppe, 1980). Determination of areas under the plasma concentration curves between 0 and 8 h in both studies was performed by the trapezoidal rule. Peak concentrations [C_{max}] and their corresponding peak time [T_{max}] were extracted from a table of plasma concentration. Comparison of values for [t_{max}] between different modes of treatment was achieved using the Wilcoxon test (1947) for paired differences. Comparison of [C_{max}] values was carried out by analysis of variance of means for smallest significant differences.

The mean profiles for indomethacin in plasma for the two treatments are shown in Fig. 7. The pharmacokinetic parameters calculated from the observed plasma drug concentration are given in Table 4.

TABLE 4

Pharmacokinetic parameters

S	F	t_{lag} (h)	A mg/liter	K_a h^{-1}	$t_{1/2}$ K_a (h)	B mg/liter	K_e h^{-1}	$t_{1/2}$ K_e (h)	AUC_0^∞ mg · h/liter
1	A	0.29	6.41	2.61	0.26	3.28	0.309	2.24	8.953
	B	0.26	3.6	2.9	0.24	3.63	0.44	1.57	7.66
2	A	0.33	7.078	1.858	0.37	7.072	0.73	0.95	6.55
	B	0.21	9.802	1.921	0.36	9.736	0.663	1.05	11.43
3	A	0.3	11.76	2.03	0.34	11.7	0.89	0.78	8.22
	B	0.45	4.98	3.42	0.2	4.99	0.44	1.57	10.91
4	A	0.43	3.921	2.415	0.29	3.954	0.305	2.27	12.05
	B	0.32	7.34	1.607	0.43	7.319	0.485	1.43	11.58
5	A	0.29	5.864	1.526	0.45	5.874	0.3	2.31	17.25
	B	0.26	5.088	2.507	0.28	5.102	0.338	2.05	14.09
6	A	0.26	4.425	3.551	0.20	4.428	0.348	1.99	11.86
	B	0.24	8.3	2.078	0.33	8.214	0.787	0.88	6.71
7	A	0.44	6.135	1.746	0.40	6.1	0.688	1.01	5.86
	B	0.31	7.038	1.345	0.52	7.022	0.501	1.38	8.96
8	A	0.23	2.4	2.64	0.26	2.4	0.35	1.98	6.56
	B	0.04	4.14	1.73	0.4	4.14	0.39	1.78	8.42
9	A	0.28	2.536	2.341	0.3	2.527	0.295	2.35	8.16
	B	0.31	5.547	1.767	0.39	5.514	0.58	1.19	6.93
10	A	0.29	3.002	4.813	0.14	2.963	0.327	2.12	8.42
	B	0.27	5.184	2.177	0.32	5.208	0.321	2.16	14.52
11	A	0.23	3.159	2.728	0.25	3.164	0.304	2.28	9.48
	B	0.21	5.991	2.612	0.27	6.051	0.511	1.36	10.73
12	A	0.31	8.901	1.429	0.48	8.919	0.689	1.01	8.18
	B	0.29	6.991	1.95	0.36	7.02	0.728	0.95	6.45

S, subject; F, form; t_{lag}, lag time; A, macroconstant; k_a, absorption constant, $t_{1/2}K_a$, absorption half-life; B, macroconstant; K_e, elimination constant; $t_{1/2}K_e$, elimination half-life; AUC_0^∞, area under curve from 0 to infinity.

Results

From the different results obtained it seems that the bioavailability of these two dosage forms is similar. An analysis of variance carried out on the C_{max}, T_{max}, and AUC from 0 to 12 h and 0 to infinity, failed to show any significant difference between the two dosage forms ($P = 0.05$). A confidence interval determination according to Westlake (1972) for AUC and C_{max}, confirmed the bioequivalence of both dosage forms.

'In vitro–in vivo' correlations

The correlation tests used involved the simulation of plasma concentrations of a drug released from a drug dosage form, whit the pharmacokinetic parameters of a subject with regard to this drug and the release parameters as obtained from the 'in vitro' dissolution test.

The simulation program used predicts these values via integration of a system of differential equations describing the exchanges between the different compartments. This special model is presented in Fig. 8, and the details of calculations given in previous papers (Aiache et al., 1984).

For each suppository, the pharmacokinetic parameters used were calculated with the mean values obtained from the subjects of the bioavailability study.

TABLE 5

Experimental and simulated concentrations for form A

t (h)	Real conc. (μg/ml)	Simulated conc. (μg/ml)
0.5	0.8	1.24
1	2.6	2.37
1.5	2.7	2.74
2	2.3	2.48
3	1.41	1.63
4	0.94	1.02
5	0.59	0.64
6	0.37	0.4
7	0.26	0.25
8	0.20	0.15
10	0.15	0.06

TABLE 6

Experimental and simulated concentrations for form B

t (h)	Real conc. (μg/ml)	Simulated conc. (μg/ml)
0.5	1.3	1.56
1	2.94	3.24
1.5	3.1	3.16
2	2.66	2.63
3	1.7	1.62
4	0.98	0.97
5	0.52	0.57
6	0.29	0.34
7	0.2	0.2
8	0.15	0.12
10	0.1	0.04

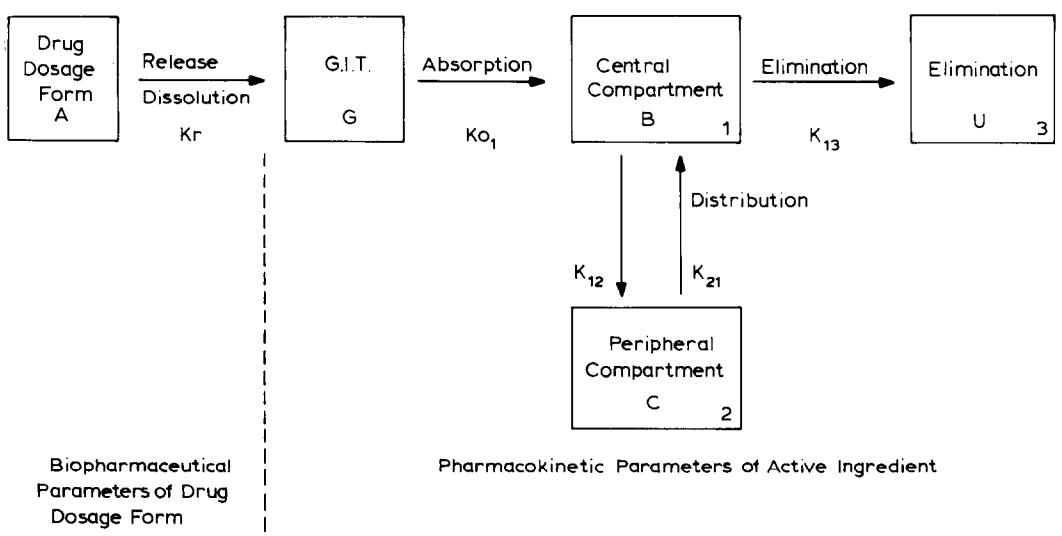

Fig. 8. Pharmacokinetic model used for 'in vitro–in vivo' simulation.

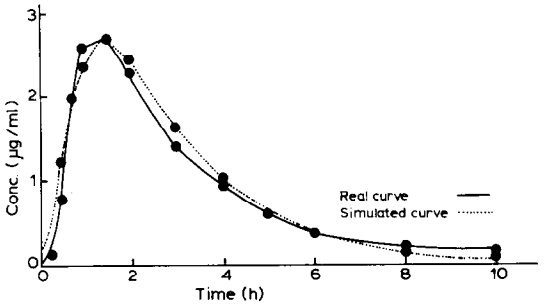

Fig. 9. Real and simulated curves for form A.

Correlation for form A

The equation which describes the mean plasma concentrations is as follows:

$$C_t = B_e^{-k'_e} - A_e^{-ka'}$$

where $k_e = 0.46 \pm 0.22\ h^{-1}$; $k_a = 2.47 \pm 0.94\ h^{-1}$; the area under the curve calculated from 0 to infinity is AUC_0^∞ 9.29 ± 3.14 mg.h/liter.

The absolute bioavailability factor f given in the literature for indomethacin administered rectally is 0.8. Hence the distribution volume can be calculated by the following equation:

$$V_d = \frac{f \cdot \text{dose}}{k_e \cdot AUC_0^\infty} = 18{,}72 \text{ liter}$$

($V_d = 18.72$ liters)

The simulated values were obtained by entering the pharmacokinetic parameters (k_a, k_e, $f \cdot$ dose and V_d) and the cumulated dissolved percentages into the program. Table 5 summarizes the results obtained and Fig. 9 shows the real and simulated curves, which are practically superimposable. A *t*-test of Student fails to reveal any difference between these two curves.

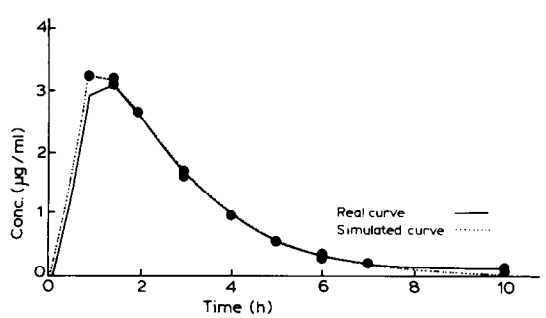

Fig. 10. Real and simulated curves for form B.

Correlation for form B

As for form A, the equation which describes the mean plasma concentration is:

$$C_t = B_e^{-k'_e} - A_e^{-ka'}$$

where $k_a = 2.16 \pm 0.57\ h^{-1}$; $k_e = 0.51 \pm 0.15\ h^{-1}$; the area under the curve is AUC_0^∞ 9.86 ± 2.77 mg.h/liter; $V_d = 15.9$ liter.

Table 6 summarizes the results obtained and Fig. 10 shows the real and simulated curves for form B. A *t*-test of Student failed to reveal any difference between these two curves.

Discussion

Many devices have been described for the study of drug release form suppositories, but the results did not allow the simulation of plasma concentration to be achieved.

The use of a through flow cell for tablets or capsules had yet allowed good correlations 'in vitro–in vivo' to be established. The extension of the use of the through flow cell for suppositories led to the obtention of the same correlation.

Conclusion

Development and quality control of drug dosage forms demand numerous 'in vitro' and more importantly 'in vivo' experiments, the cost of which are becoming increasingly onerous.

The design of simple 'in vitro' methods which allow plasma level curves to be obtained by simulation from pharmacokinetic parameters thus represents an important step forward.

In this work, 'in vitro/in vivo' correlations for indomethacin suppositories are obtained that should help to optimize the formulation of this drug and provide a basis for quality control procedures.

Acknowledgement

The authors are grateful to Martin laboratories (France) for the supply of suppositories.

References

Aiache, J-M., Turlier, M. and Tanguy, A., Pharmacokinetic data processed by microcomputer for the formulation of optimal drug dosage form. *Eur. J. Drug Metab. Pharmacokinet.,* 9 (1984) 89–109.

Besnard, E., *Méthode d'étude "in vitro" de la libération du principle actif à partir de suppositoires.* Thèse de Diplôme d'Etat de Docteur en Pharmacie, Paris, 1985.

Carp, G.B., Chemtob, C. and Chaumeil, J.C., Les méthodes d'étude "in vitro" des suppositoires et des capsules molles rectales. *Sci. Tech. Pharm.,* 9 (1980) 341–347.

Desgardins, S., Zuber, M. and Chemtob, C., Mise en pratique de l'essai de désagrégation de la Pharmacopée Française Xe édition pour suppositoires et ovules. *Sci. Tech. Pharm.,* 12 (1983) 437–440.

Fredj, G., Farinotti, R., Hakkon, F., Astier, A. and Dauphin, A., Biodisponibilité de l'indométacine par voie orale et par voie rectale, *J. Pharm. Belg.,* 38 (1983) 105–108.

Koeppe, P., A program for non-linear regression analysis to be used on desk top computers. *Computer Progr. Biomed.,* 12 (1980) 121–128.

Langenbucher, F., "In vitro" drug release from suppositories: flow-through method. In B. Glas and C.J. de Blaey (Eds.), *Rectal Therapy, Proceedings of the Symposium on the Advantages and Problems Encountered in Rectal Therapy*, Saint Remy de Provence, 1983, pp. 103–104.

LeBlanc, P.P. and Dumas, J., Calcul des valeurs initiales des paramètres pharmacocinétiques à l'aide d'un calculateur programmable. *Thérapie,* 38 (1983) 21.

LeBlanc, P.P. and Aiache, J-M., Estimation de la bioéquivalence à partir de paramètres pharmacocinétiques et pharmacodynamiques. J-M. Aiache and J. Hirtz (Eds.), *Proceedings of the Second Congress of Biopharmaceutics and Pharmacokinetics, Vol. 1,* Université de Clermont-Ferrand I, 1984, pp. 597–605.

Puech, A., Lasserre, Y. and Jacob, M., Cinétique de libération "in vitro" de principes actifs à partir de suppositoires: étude d'un nouvel appareillage. *Trav. Soc. Pharm. Montpellier,* 37 (1977) 165–172.

Ritschel, W.A., *Handbook of Basic Pharmacokinetics,* Hamilton, 1976.

Westlake, W.J., Use of confidence intervals in analysis of comparative bioavailability trials. *J. Pharm. Sci.,* 61 (1972) 1340–1341.

Wilcoxon, F., *Biometrics*, 3 (1947) 119–125.