Deterministic chaos and wash-out determination in crossover trials

Background: Key elements of crossover studies are the determination of the wash-out (clearance) period. The purpose of this work is an attempt to implement and justify the use of deterministic chaos theory for estimating the wash-out value and reference the obtained values to the currently applicable rules. **Results/methodology:** The elliptical model of drug wash-out was presented to prove the hypothesis. Pharmacokinetic profiles were used for the analysis, obtained from healthy subjects during 12 studies. **Conclusion:** On the grounds of these calculations it may be concluded that the pharmacokinetics of xenobiotics, such as drugs, is a product of deterministic chaos.

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Keywords: chaos • elliptical model • Feigenbaum • pharmacokinetics • wash-out

One of the key elements of design studies in clinical and preclinical phases is the determination of a wash-out (clearance) period. This parameter is common for all multidose, non-clinical studies and studies occurring in the crossover design [1]. In cases where the calculated wash-out is too long, the crossover study is not recommended. This applies to both classical and biotechnological drugs [2]. The problem of appropriately calculated wash-out period applies to design of bioequivalence studies and analysis of pharmacokinetic (PK), toxicokinetic (TK), pharmacodynamic (PD) and toxicodynamic (TD) parameters. In PK studies, the presence of detectable/residual drug from the first or preceding dose in the next dosing interval depends on having an appropriately calculated wash-out period. A similar situation occurs during the analysis of dose/biologic effect relationships. When wash-out intervals are appropriately calculated, the next period will be free of concentrations present from the initial drug administration carrying over to the second or subsequent doses. The designation of wash-out is essential in any crossover studies, such

as bioequivalence studies. At present, regulatory guidelines draw attention to the necessity of determining both the PK and PD wash-outs [3–5]. The most common method of calculating the wash-out is the method based on the iteration of the half-life of elimination ($t_{0.50\text{kel}}$). However, it has been proven that mean residence time (MRT) is also the basis for calculation of wash-out interval equal to $5 \times \text{MRT}$ [6]. In some regulatory documents the method of determining wash-out is still generally described as ‘period with adequate duration’ [7] or ‘sufficiently long’ [3]. The current requirements for the determination of wash-out are not fully harmonized. Thus, depending on the drug registration authority, there are different requirements for determining this period. In regulatory trials, depending on the authority, the acceptance interval for wash-out amounts to: $5 \times t_{0.50\text{kel}} > 5 \times t_{0.50\text{kel}}$, $\geq 6 \times t_{0.50\text{kel}}$, $\geq 7 \times t_{0.50\text{kel}}$ or $10 \times t_{0.50\text{kel}}$ [8–15]. Some documents describe the correct choice of wash-out [16,17], which relate to the drug concentration in the zero samples after crossing over the groups/cohorts. According to WHO guidelines, the presence of the drug

Tomasz Grabowski^{*1}, Shayne Cox Gad², Jerzy J Jaroszewski³ & Pieter JM Guelen⁴

¹Polpharma Biologics, Trzy lipy 3, 80–172 Gdańsk, Poland

²Gad Consulting Services, 102 Woodtrail Lane, Cary, NC 27518, USA

³Department of Pharmacology & Toxicology, Faculty of Veterinary Medicine, University of Warmia & Mazury in Olsztyn, Oczapowskiego 13, 10–718 Olsztyn, Poland

⁴Pharmacokinetic Consultancy Services, Voorstraat 11, 5371 KE Dieren, The Netherlands

*Author for correspondence:

Tel.: +48 78 58 75 021

Fax: +48 22 36 46 102

tomasz.grabowski@polpharma.com

in such samples is acceptable at the level of $\leq C_{\max} \times 0.05$, where $C_{\max}$ is the maximum concentration of drug obtained in the next study period [16]. The same rules also apply to PD wash-outs. The value of variability of individual points illustrating the dependency between concentration and time (C–T) should also be taken into account during the determination of the wash-out. The minimum and maximum range of wash-out in a human subject is defined between 7 and 28 days [16]. In preclinical studies, it is recommended that the wash-out period should not be long enough for changes in animal physiology to influence study outcomes [15]. This is particularly important in fast-growing animals. In biotechnological drugs, agencies indirectly define acceptable length of wash-out. It is concluded that crossover studies can be carried out in relation to a drug for which $t_{0.50\text{kel}} < 120 \text{ h}$ [2]. Due to the multiple regulatory requirements regarding the calculation of wash-out time, an attempt to identify a new method of determining a wash-out period was pursued.

PK and PD models are usually nonlinear, dynamic models. Mitchell Feigenbaum created a theory according to which all the equations of nonlinear dynamic processes have the same course [18]. A dynamic process can describe the value of the variables distributed in time. Growth of this process in time is dependent on certain laws and rules [19]. Generally, chaos is understood to be the observation of processes unstructured or lacking in logical order. However, it is currently known that chaotic processes can be characterized by specific rules [20]. Such deterministic chaos is a chaos characterized by phenomena subject to accidental, disordered behavior of systems subject to strict rules [21]. It has been shown that many of the processes related to the biology and physiology of living organisms (gastric myoelectrical activity, hormone interactions, levels of circulating neutrophils) are chaotic processes [19,21–24]. Evaluation of certain organs has demonstrated that the interval of the C–T has fractal characteristics [25–27]. It is also known that fractals (strange attractors) are typical attractors of chaotic systems [27]. This means that a system defined as chaotic can depend on the shape of drug PK.

This work attempts to implement and justify the use of a Feigenbaum number (δ) for estimating the wash-out value and reference the obtained values to the currently applicable rules. δ in the chaos analysis is some kind of equivalent value to golden ratio $\phi = 1.618$ in algebra, π value $\pi = 3.1415$ in geometry and the base of the natural logarithm $e = 2.718$ [22,24]. The study verified the hypothesis that if it is possible to demonstrate the relationship between ϕ , π and δ , it should also be possible to prove the relationship between ϕ , π and elimination kinetics and C–T characteristics.

Materials & methods

Clinical data

PK profiles used for the analysis were obtained from healthy subjects from 12 studies [28]. During these trials the concentrations of nine drugs (study: 1 – midazolam; 3 – enalapril; 5 – loperamide; 6 – glimepiride; 7 – simvastatin; 9 – phytomenadione; 10 – cinnarizine; 11 – haloperidol; 12 – cetirizine) and three metabolites (study: 2 – hydroxymidazolam; 4 – enalaprilat; 8 – simvastatin acid) were determined in the plasma. To test the hypotheses, groups of drugs with short elimination half-life – $t_{0.50\text{kel}} (<24 \text{ h})$ but within a broad range of arithmetic mean (M) and standard deviation (SD; study 3: $t_{0.50\text{kel}} = 1.51 [0.60] \text{ h}$; study 10: $t_{0.50\text{kel}} = 21.12 [15.95] \text{ h}$) and MRT (study 3: MRT = 1.62 [0.28] h; study 11: MRT = 14.72 [0.72] h) value were used. All studies involved single oral administration of the drug. In each case, sampling included taking the zero samples before the drug administration.

Pharmacokinetics & statistical analysis

Wash-out time analysis: noncompartmental modeling was used to estimate PK parameters. PK calculations were performed with the use of Phoenix^{TM} WinNonlin® 6.3 (Certara L.P.). The area under the concentration time curve from time 0 to the last concentration time point ($AUC_{0-\text{tlast}}$) and area under first moment of the curve from time 0 to the last measured concentration point ($AUMC_{0-\text{tlast}}$) were determined by the trapezoidal method. MRT was calculated based on $AUMC_{0-\text{tlast}}$ and $AUC_{0-\text{tlast}}$ value. The elimination rate constant (k_{el}) was determined by linear regression of the last three points on the concentration time (C–T) curve in all studies. For each study $t_{0.50\text{kel}}$ was calculated using formula: $t_{0.50\text{kel}} = 0.693/k_{\text{el}}$ and $t_{0.50\text{MRT}}$ calculated with the use of the equation $t_{0.50\text{MRT}} = 0.693/(1/\text{MRT})$. Wash-out time was calculated using different requirements: 5, 6, 7, $10 \times t_{0.50\text{kel}}$, $\text{MRT} \times 5$ and with application of Feigenbaum number ($t_{0.99} \approx \delta/k_{\text{el}}$). In order to calculate the wash-out formulas from Table 1 were used.

The differences in the values of calculated parameters were analyzed by means of student t-test. Differences that generated p-values < 0.05 were considered as statistically significant.

Golden ratio & C–T characteristics

One of the basic tools of the chaos theory is the fixed value of the logistic map point: $1-1/\lambda$, where λ means the attractor, which is the bifurcation parameter of the sequence. In PK the attractor's function, in reference to the analysis of half-life or MRT, is represented by the Euler's number (e). δ determined with a precision

Table 1. Method of half-life in elimination phase ($t_{0.50\text{kel}}$), mean residence time (MRT) and 99% wash-out determination using Feigenbaum number ($t_{0.99}$).

Initial concentration (%)	Logistic derivative	Equation	Wash-out period
50.00	$(1-1/e^{0.693}) \times 100 = 50.00$	$t_{0.50\text{kel}} = 0.693/k_{\text{el}}$	$t_{0.99} \approx 6.7 \times t_{0.50\text{kel}}$
63.21	$(1-1/e) \times 100 = 63.21$	$t_{0.63} = \text{MRT}$	$t_{0.99} \approx 6.8 \times t_{0.63}$
99.06	$(1-1/e^{4.669}) \times 100 = 99.06$	$t_{0.99} \approx 4.669/k_{\text{el}}$	–

0.693 = Ln2; e: Euler number (2.781); δ : Fengeibaum number (4.669); k_{el} : Elimination rate constant; $t_{0.63}$: Time after which drug concentration decreases to 63.21% the initial concentration; $t_{0.99}$: Time after which drug concentration decreases to 99.06% the initial concentration.

of three decimal places is 4.669. δ refers to the distance between two consecutive bifurcation points:

$$\delta \approx (n_{x+1} - n_{x+2}) / (n_x - n_{x+1})$$

where n_x, n_{x+1} are bifurcation points [29]. This expression is similar to $\phi = 1.618$, which is derived from the Fibonacci sequence [30]. Within this sequence:

$$\phi \approx n_x / n_{x+1}$$

where n_x, n_{x+1} are the next sequence points. To demonstrate the relationship between e, δ, ϕ and C–T sequences, an equation was derived based on earlier observations [31,32]:

$$\delta \approx 2 / (\text{Ln}2 / \phi) = 2 / (0.693 / \phi)$$

In addition, to demonstrate the relationship between δ, ϕ and the value of logistic derivative, being the basis of MRT, a new equation was introduced:

$$1 \approx \text{Ln}(\delta - 0.632 / 2.303) - \text{Ln}\phi$$

Both equations indicate that the relationship between δ and the values used for describing the sequences of drug concentration changes in blood (C–T) is not accidental. It is assumed that PK sequences expressed by successive concentrations C_n, C_{n+1}, C_{n+2} are in their course similar to values obtained in the Fibonacci sequence. If sum of the successive terms of the Fibonacci sequence is divided by ϕ , we will get the next expression expression. This means that in the Fibonacci sequence:

$$n_{x+1} = (n_x + n_{x+1}) / \phi$$

Using a similar conversion in relation to the concentrations (C) in data sequence illustrating the change of drug concentration, we obtain:

$$C_{n,\text{conv}} = (C_n + C_{n+1}) / \phi$$

Thus, it can be proven that the value of ϕ is closely related to the course of C–T. In this way, it can be shown indirectly that δ is not a random number, but refers to the specific of C–T courses.

π value & drug wash-out

δ in the form of many different approximations was

associated with the value π . One of the simplest is [33]:

$$\delta \approx 10 / (\pi - 1)$$

If δ is a value that can be applied to another mathematical constant, π , it means that the processes of elimination can be also applied to the number π . In the case of drug wash-out analysis, the relationship between wash-out length and the number π can also be proven. The elliptical model of drug wash-out (Figure 1) allows determination of the location of chaotic processes in PK and determines new value of wash-out in the crossover studies. In this model k_{el} and MRT plays a key role. The relaxation time (63.21%) of the dynamic system in PK is represented by MRT. To illustrate the relationship between δ, π and value of relaxation time: $(1 - 1/e) \approx 0.6321$

the following equation has been proposed:

$$\pi \approx 1 + \text{Ln}(0.6321^{-\delta})$$

Transforming EQUATION 9, we obtain direct representation of the relationship between δ, π, e and relaxation time (EQUATION 10):

$$0.6321 \approx 1 / (e^{\pi-1})^{1/\delta}$$

EQUATIONS 9 and 10 indicate the presence of relationship, which allows expression of drug elimination in a circular or elliptical model. The basis of this model characteristic is the number π . The π value is calculated using the formula:

$$\pi \approx C / d$$

where C it the circumference of a circle and d is its diameter. In PK, C can be assumed to be the process time that comes full circle, starting from zero concentration (t_0), followed by administration of drug dose. This dose is gradually eliminated to zero $\approx t_{0.99}$. The determinant of C is in this case δ/k_{el} or differently:

$$4.669 / k_{\text{el}}$$

The d value in such a model is $t_{0.50\text{kel}}$ and $t_{0.50\text{MRT}}$ designated not with k_{el} but with its equivalent in process typical for one compartmental model, which is $1/\text{MRT}$. In elliptical model both values are the basis to

Figure 1. Elliptical model of drug wash-out.

f : Polar flattening of ellipse: $f = [(r_2 - r_1)/r_2]$; $f = [(r_2 - r_1)/r_2]$;

r_1 : Minor radius of ellipse: $r_1 \approx t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}$

r_2 : Major radius of ellipse: $r_2 \approx t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}$

Sampling time: Time between $t_{0,EL}$ and $t_{last,El}$; $t_{0.50kel}$: Half-life in elimination

phase: $t_{0.50kel} = 0.693/k_{el}$; $t_{0.99}$: Time of wash-out: $t_{0.99} \approx 4.669/k_{el}$;

$t_{0,El}$: Position of the zero point on the ellipse: $t_{0,El} \approx [(f \times \pi)/360] \times [3/2 \times (r_1 + r_2)] - \sqrt{r_2^2 - r_1^2}$

$t_{last,El}$: Position of the last sampling point on the ellipse model: $t_{last,El} = t_{0,El} +$

t_{last} ; Wash-out: Time between $t_{last,El}$ and $t_{0.99}$.

determine the large

$$r_2 \approx t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}$$

and small

$$r_1 \approx t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}$$

radius of the ellipse. Consequently, in such a model,

$$MRT = r_1/(k_{el} \times r_2)$$

$t_{0.99}$ is the value approximately equal to the circumference of the designated ellipse. π value in such a model is illustrated by the equation:

Using EQUATION 7 we obtain:

$$\pi \approx (\delta/k_{el})/(t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} + t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}})$$

$$t_{0.50} \approx (\delta/k_{el})/2/\pi$$

or

$$t_{0.50} \approx t_{0.99}/2/\pi$$

Thus, the elliptical model of drugs' wash-out is based on the π value and the constant δ . Coordinates of the ellipse are in this model the half-life values $t_{0.50kel}$ and $t_{0.50MRT}$. Hence, the formula for the circumference of an ellipse is:

$$L_{El} \approx \pi \times [3/4 \times (r_1 + r_2) - \sqrt{r_1 \times r_2}]$$

where r_1 and r_2 are elliptical shafts, but

$$\pi \approx L_{El}/(r_1 + r_2)$$

Because the formula for the area of the ellipse is:

$$A_{El} \approx \pi \times r_1 \times r_2$$

consequently:

$$A_{El} \approx \pi \times t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} \times t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}$$

$$A_{El} \approx \pi \times (t_{0.50kel})^2 \quad \text{and} \quad A_{El} \approx (Ln2/10) \times t_{0.99}^2$$

$$L_{El} \approx \pi \times 3/4 \times [(t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} + t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}) - \sqrt{t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} \times t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}}]$$

$$\pi \approx L_{El}/[t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} + t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}]$$

$$\delta \approx 10/[L_{El}/[t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}} + t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}] - 1]$$

$$t_{0.50kel} \approx (L_{El}/\pi)^2$$

Polar flattening (f) is determined by the formula:

$$f = (r_1 - r_2)/r_1$$

which, in the case of the elliptical model (Figure 1)

means that:

$$f \approx [(t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}}) - (t_{0.50MRT} \times \sqrt{t_{0.50kel}/t_{0.50MRT}})]/(t_{0.50kel} \times \sqrt{t_{0.50kel}/t_{0.50MRT}})$$

With the rise of flattening $f \rightarrow 1$, ($t_{1/2MRT} < t_{1/2kel}$) or $f \rightarrow -1$, ($t_{1/2MRT} > t_{1/2kel}$). This means that the more the model is circular – one compartmental, ($t_{1/2MRT} = t_{1/2kel}$) the more f value approaches zero. To determine the location time of drug administration (t_0) on the circumference of the ellipse, the length of ellipse segment should be calculated using the formula:

$$L_{El\alpha} \approx [(\alpha \times \pi)/360] \times [3/2 \times (r_1 + r_2) - \sqrt{r_1 \times r_2}]$$

where α is the value of the angle between the radii limiting the beginning and the end of the analyzed section. If we base the coordinates of the time t_0 on the ellipse ($t_{0,El}$) and not on the angle, but in a proportional way of flattening of the ellipse (f) then, the distance $t_{0,El}$ from the point of crossing of the long axis of the ellipse with the circuit is equal to:

$$t_{0,El} \approx (L_{El}/4) - L_{Elf}$$

for L_{Elf} is equal to:

$$L_{Elf} \approx [(f \times 90 \times \pi)/360] \times [3/2 \times (r_1 + r_2) - \sqrt{r_1 \times r_2}]$$

Calculated this way, the value of the segment $L_{Elf} \approx MRT$ means that the section between the crossing of the long axis of the ellipse with the circuit and the point t_0 is equal to:

$$MRT \approx (L_{El} / 4) - t_{0,El}$$

or

$$t_{0,El} \approx (L_{El} / 4) - MRT$$

If the model is elliptical, the long radius is the derivative of $t_{0.50kel}$ and the short one $t_{0.50MRT}$ ($t_{0.50kel} \neq t_{0.50MRT}$), t_0 point is not the point of intersection of the long axis with the ellipse circuit. When $t_{0.50kel} > t_{0.50MRT}$, coordinates t_0 are shifted in relation to the intersection of the long axis with the ellipse circuit (anticlockwise shift). When $t_{0.50kel} < t_{0.50MRT}$, coordinates t_0 are also shifted in relation to the crossing point of the long axis with the circuit (clockwise shift).

Based on the performed analysis and characteristics of the elliptical model of drug wash-out, the new equation allows calculation of a sufficiently long wash-out and is proposed as follows:

$$t_{wash-out} \geq t_{last} + \{6.74 \times (t_{1/2kel} + |t_{1/2kel} - t_{1/2MRT}|)\}$$

The half-life in elimination phase predicted on the basis of the elliptical model ($t_{0.50kel, pred}$) was calculated using equation:

$$t_{0.50kel, pred} \approx \sqrt{[(t_{0.99} / \pi)^2] / 2}$$

Half-life in elimination phase calculated from MRT predicted on the basis of the elliptical model ($t_{0.50MRT, pred}$) was calculated using equation:

$$t_{0.50MRT, pred} \approx (t_{0.99} / 4) - t_{0,El}$$

Results

Values for wash-out period calculated in accordance with regulatory requirements are presented in **Table 2**. Wash-out value calculated as $t_{0.99}$ was set equal to $6.74 \times t_{0.50kel}$. $t_{0.99}$ in tested group of drugs did not differ significantly ($p > 0.05$) from the value designated as $7 \times t_{0.50kel}$. A significant difference was found for $t_{0.99}$ and $t_{wash-out}$ ($p < 0.05$). Moreover, $t_{wash-out}$ had a higher value of wash-out calculated as $10 \times t_{0.50kel}$ (25.55%; 8.55, RSD%) but difference was not statistically significant (**Table 2**). In order to demonstrate the similarity of C-T sequences to the Fibonacci sequence, the values of PK parameters of all studied drugs before and after conversion with ϕ were chosen for the exemplary analysis (**Table 3**). C_{max} , t_{max} , AUC_{last} and percent of prediction error after observed drug concentrations conversion ($PE\%_{\sum Cn, conv}$) were calculated. Only in studies 5, 6 and 12 $C_{max, conv}$ was different from C_{max} ($p < 0.05$). $t_{max, conv}$ was different from t_{max} ($p < 0.05$) only in studies 3, 4 and 5. In case of AUC_{last} , significant differences were noted only in study 9 and 12 ($p < 0.05$). Mean $PE\%_{\sum Cn, conv}$ from all studies was 14.55% (28.24, RSD%).

Parameters describing the elliptical model are presented in **Table 4** and an example application of this model is presented in **Figure 2**. It has been shown that the area of the ellipse (A_{El}) and the length of its circuit (L_{El}) designated in elliptical model (**Figure 1**) are proportional to t_{last} designated in *in vivo* studies ($R^2 = 0.6654$; $p < 0.05$; $R^2 = 0.8123$; $p < 0.05$). A correlation between r_1 , r_2 and $t_{wash-out}$ was shown and the coefficient of determinations were $R^2 = 0.8636$ ($p < 0.01$) and $R^2 = 0.9857$ ($p < 0.01$), respectively. A similar correlation between r_1 , r_2 and $t_{0.99}$ was found and the coefficient of determinations were $R^2 = 0.9200$ ($p < 0.01$) and $R^2 = 0.9619$ ($p < 0.01$), respectively.

The prediction error between $t_{0.50kel}$ observed and predicted ($t_{0.50kel, pred}$) on the basis of the elliptical model was 7.28% for all analyzed drugs. Mean prediction error between $t_{0.50MRT}$ observed and predicted ($t_{0.50MRT, pred}$) on the basis of the elliptical model was 15.52 (RSD% 36.43) for all analyzed drugs. In the elliptical model, it has been shown that if $r_1 = r_2$ or $t_{0.50kel} = t_{0.50MRT}$ or $k_{cl} = 1/MRT$ or $f = 1$, then π and δ values calculated based on elliptical model:

$$\pi \approx L_{El} / (r_1 + r_2) \quad \text{and} \quad \delta \approx 10 / (\pi - 1)$$

are equal 3.1415 and 4.669, respectively.

Currently, the formula most often used to calculate wash-out is $5 \times t_{0.50kel}$ or no fewer than 7 days. In all analyzed compounds, wash-out calculated with the use of new method was at least 2.25-times longer than wash-out equal to $5 \times t_{0.50kel}$. In all analyzed substances, wash-out equal to 7 days should be used because $5 \times t_{0.50kel} < 7$ days. However, with the use of the new method in compound number 10 wash-out was equal to 11.6 days. Hence, in that case at least 2 weeks of wash-out should be used. For compound number 4, wash-out was equal 8.5 days. Hence, in that case at least 2 weeks of wash-out should be applied.

Discussion

On the basis of the above calculations, it can be concluded that the PK of xenobiotics is product of a deterministic chaos. Thus, the possibility that exists to use δ for calculation of the xenobiotics' wash-out confirms, to some extent, this theory. **EQUATION 10** confirms the close relationship between MRT, the π value and Feigenbaum constants. A definition of MRT can be assumed as the time taken for the particular molecule to repeat passage through a defined space. This definition refers directly to the structure bifurcation diagrams and fractal structure of some organs [19,25,26,34–36].

An elliptical model of drug wash-out confirms the close relationship of PK parameters, such as $t_{0.50kel}$ and MRT with δ and π . Even with a certain error, the compatibility of δ and π calculated using the elliptical

Table 2. Values of mean residence time (MRT), elimination half-life ($t_{0.50kel}$), and wash-out periods based on regulatory requirements and Feigenbaum constant (δ).

Study	MRT (h) M (SD)	$t_{0.50kel}$ (h) M (SD)	t_{last} (h) M (SD)	Wash-out period (h)					$t_{0.99}$ (h)	$t_{wash-out}$ (h)
				$5 \times MRT$	$5 \times t_{0.50kel}$	$6 \times t_{0.50kel}$	$7 \times t_{0.50kel}$	$10 \times t_{0.50kel}$		
1	3.39 (0.72)	3.50 (1.81)	13.49 (2.80)	16.96	17.51	21.01	24.52	35.02	23.60	44.86
2	2.93 (0.83)	3.52 (2.52)	9.81 (2.69)	14.65	17.59	21.11	24.62	35.18	23.70	43.55
3	1.62 (0.28)	1.51 (0.60)	8.26 (2.11)	8.09	7.56	9.07	10.58	15.11	10.18	21.07
4	9.24 (1.75)	14.86 (4.01)	48.00 (0.00)	46.20	74.30	89.16	104.02	148.59	100.11	205.14
5	10.96 (0.39)	8.87 (0.77)	32.00 (0.00)	54.80	44.33	53.20	62.07	88.67	59.74	100.33
6	5.42 (0.68)	5.49 (1.34)	23.47 (2.00)	27.10	27.44	32.92	38.41	54.87	36.97	72.12
7	8.57 (1.99)	6.11 (2.26)	28.59 (2.52)	42.83	30.55	36.66	42.77	61.10	41.17	70.95
8	9.80 (1.80)	6.55 (2.63)	28.15 (4.09)	49.01	32.76	39.31	45.87	65.52	44.14	73.93
9	5.00 (0.06)	2.61 (0.24)	10.00 (0.00)	25.02	13.04	15.65	18.26	26.09	17.58	33.37
10	11.79 (5.27)	21.12 (15.95)	48.00 (17.69)	58.96	105.58	126.69	147.81	211.16	142.26	277.56
11	14.72 (0.72)	13.51 (1.27)	48.00 (0.00)	73.58	67.54	81.04	94.55	135.07	91.00	161.35
12	9.86 (0.72)	11.18 (1.76)	35.00 (3.31)	49.32	55.89	67.07	78.25	111.78	75.31	139.61
Min	1.62	1.51	8.26	8.09	7.56	9.07	10.58	15.11	10.18	21.07
Max	14.72	21.12	48.00	73.58	105.58	126.69	147.81	211.16	142.26	277.56
Mean	7.78	8.23	27.73	38.88	41.17	49.41	57.64	82.35	55.48	103.65
RSD%	49.74	68.61	52.47	49.74	68.61	68.61	68.61	68.61	68.61	72.06

1: Midazolam; 2: Hydroxyimidazolam; 3: Enalapril; 4: Enalaprilat; 5: Loperamide; 6: Glimepiride; 7: Simvastatine; 8: Simvastatine acid; 9: Phytomenadione; 10: Cinarizine; 11: Haloperidol; 12: Cetirizine.

kel: Elimination rate constant; M: Arithmetic mean; Max: Maximal value; Min: Minimal value; RSD%: Percent of relative standard deviation; SD: Standard deviation; $t_{0.99}$: Wash-out calculated using the equation: $t_{0.99} \approx 4.669/k_{el}$; t_{last} : Last observed concentration; $t_{wash-out}$: Wash-out calculated using the equation: $t_{wash-out} = t_{last} + \{6.74 \times (t_{1/2kel} + |t_{1/2MRT} - t_{1/2MRT}|)\}$

model of drug wash-out with the theoretical values was still shown to be valid. The adoption of such a model of drug wash-out from the body raises the question of the location of the next bifurcation points during the disposal of the drug in the body. The results of the analyses lead to the conclusion that the kinetics of changes in blood levels after a single dose of the drug is the result of chaotic processes. However, elimination kinetics excluding numerous exceptions caused by the redistribution processes is considered to be a predic and relatively easy to describe. It can be assumed that the development of chaotic processes progresses from the administration, so their elimination should be their

reflection. This is not the case, however, as the final phase of elimination is possible to define with simple equations. A possible explanation for this is that, when analyzing the kinetics of C–T changes in blood, we only characterize the elements of kinetics that are illustrated by concentration value in the area, in which the bifurcation number can be relatively small. Although blood is a complex and specific tissue, it can cause model ‘duality’ by transferring the drug to the cells or via blood or by binding to blood proteins.

The whole area of the body is much more complex because of the large number of organs, tissues, cells, organelles and membrane barriers cause difficulties in

Table 3. Arithmetic mean and standard deviations (SD) of selected pharmacokinetics parameters, before and after conversion by the golden ratio ($\varphi = 1.618$) value ($C_{n,conv} = (C_n = C_{n+1})/\varphi$).

Study	C_{max}	$C_{max, conv}$	t_{max}	$t_{max, conv}$	AUC_{last}	$AUC_{last, conv}$	PE% _{$\Sigma C_{n, conv}$}
1	87.22 (46.88)	93.38 (45.03)	0.84 (0.63)	0.72 (0.54)	216.25 (59.21)	235.78 (64.37)	11.07 (22.38)
2	37.46 (21.86)	40.52 (21.99)	0.77 (0.57)	0.70 (0.53)	82.22 (24.25)	85.82 (26.28)	9.28 (24.63)
3	135.01 (37.64)	154.47 (41.01)	0.88 (0.28)	0.70 (0.21)*	216.77 (70.04)	245.97 (80.36)	4.89 (22.76)
4	76.12 (24.94)	88.40 (26.89)	3.40 (0.96)	2.90 (0.69)*	602.35 (137.70)	650.01 (152.95)	17.70 (26.01)
5	11.06 (0.82)	13.19 (0.99)*	5.00 (0.00)	4.67 (0.47)*	167.33 (17.21)	174.93 (18.13)	18.83 (19.36)
6	488.29 (110.74)	553.15 (110.74)*	2.70 (0.35)	2.52 (0.35)	2187.80 (879.06)	2415.28 (758.44)	16.83 (28.31)
7	13576.43 (8984.23)	17194.33 (9364.94)	2.63 (2.69)	3.68 (6.57)	102,802.39 (56,160.95)	122,808.37 (64,837.09)	14.49 (22.99)
8	10365.06 (4517.13)	11745.26 (5322.86)	5.74 (2.98)	4.88 (2.49)	105,508.77 (58,272.49)	119,621.39 (66,155.86)	15.67 (23.92)
9	15.73 (1.15)	16.02 (1.36)	4.00 (0.00)	4.00 (0.00)	80.77 (7.12)	93.75 (8.54)*	18.23 (34.11)
10	35.29 (16.42)	41.53 (19.61)	2.19 (0.80)	4.83 (14.02)	293.82 (216.96)	339.38 (234.94)	12.92 (25.25)
11	12.73 (1.43)	13.77 (3.67)	5.00 (0.00)	4.58 (1.21)	231.25 (30.96)	221.07 (71.12)	18.54 (24.82)
12	233.96 (30.22)	273.46 (33.56)*	0.60 (0.20)	0.60 (0.20)	2000.99 (252.12)	2527.98* (279.70)	16.16 (23.38)

*Significant differences between pharmacokinetics parameters before and after conversion ($p < 0.05$).

1: Midazolam; 2: Hydroxymidazolam; 3: Enalapril; 4: Enalaprilat; 5: Loperamide; 6: Glimepiride; 7: Simvastatine; 8: Simvastatine acid; 9: Phytomenadione; 10: Cinarizine; 11: Haloperidol; 12: Cetirizine.

AUC_{last} : Observed area under the curve; $AUC_{last, conv}$: Observed AUC_{last} after conversion by φ value; C_{max} : Observed maximal concentration; $C_{max, conv}$: Observed C_{max} after conversion by φ value; PE% _{$\Sigma C_{n, conv}$} : Percent of prediction error after raw drug concentrations conversion, calculated using equation: $PE\%_{\Sigma C_{n, conv}} = [(C_{n, conv} - C_n)/C_{n, conv}] \times 100$; t_{max} : Observed time to reach C_{max} ; $t_{max, conv}$: Observed time to reach C_{max} after conversion by φ value.

defining dependency network. Drug molecules penetrating these spaces are subject to chemical and physical processes, and form a chaotic system as a result of various different interactions. Thus, the processes of drug elimination from the body are a way of transition from the unbalanced (antichaotic) arrangement of molecules (high polar fattening models) to limited and predic (circular models) processes (chaotic – homeostatic models), such as a late phase of elimination [37]. Such reasoning leads to the conclusion that the administration of the drug results in increased numbers of unbalanced processes, which at some stage revert to ‘structured’ processes, such as a late phase of elimination. This hypothesis supports the description of drug distribution in fractal compartments (strange attractors) and fractal fluctuations of dynamic processes (fractal-like kinetics) [34,35]. This applies both to the characteristics of the

fractal anatomy of the organs or tissues (e.g., hepatic sinusoidal network, vascular system, neural networks, convoluted surface of the brain, His-Purkinje network) and fractal characteristics of some processes (absorption, ion channel kinetics, distribution of blood flow through the blood vessels, atrial and ventricular fibrillation) [19,25,26,36]. An elliptical model provides, to some extent, confirmation of the hypothesis that homeostasis is a picture of deterministic chaos. This hypothesis seems to be true because only in the case of perfectly circular wash-out model (when $k_d = 1/MRT$), the value of δ and π are equal to the theoretical values. In the present consideration, the hypothetical ‘equilibrium point’ of chaotic processes is the MRT. If the above assumption is correct, it means that only a simultaneous analysis of MRT and $t_{0.50kel}$ ensures accurate determination of the wash-out period of the drug. This paper establishes that

Figure 2. An example of the application of the elliptical model based on observed concentration–time data of cinarizine.

t_{last} and MRT calculated values, see 2. MRT: Mean residence time; t_{last} : Time of last observed concentration; t_0 : Time of drug administration; $t_{0.99}$: Time of wash-out.

δ is associated with both MRT and $t_{0.50kel}$. It was shown that the wash-out value calculated on the basis of δ is related to MRT, $t_{0.50kel}$ of the drug and mathematical constants in a logical way. The study demonstrates that the ratio δ/k_{el} is consistent with the principle of the duration of wash-out equal to $7 \times t_{0.50kel}$ starting from t_{last} . The elliptical model has been tested on research planned in accordance with the requirements of regulatory bodies. As presented in this work, the elliptical model expresses the hypothetical process of drug wash-out based on the values of $t_{0.50kel}$ and $t_{0.50MRT}$. The differences between the two values are a result of the differences between k_{el} and $1/MRT$. Thus, to some extent, the model reflects the difference between single- and multicompartment models. The consequence of a distribution model with a more complex course, in the case of an elliptical model, is its flattening. The presence of such a flattening in the interval in which the researcher has observed data (range t_0 to t_{last}) is justified. However, the elliptical model is a symmetric model. This means that for drugs with a very complex kinetics of distribution in elliptical model the flattening increases. But, it is a symmetrical flattening that occurs in the ‘observed’ kinetics phase and also in the late phase of elimination, wherein the course is not known because it is outside the t_{last} . The interpretation of model flattening in the late elimination phase beyond t_{last} is difficult and at the present stage of research we can only propose a hypothesis that needs to be confirmed with further works. Many observations indicate that the kinetics of elimination phase beyond t_{last} is not always linear [38–41]. This means that despite the correct designation of wash-out in the crossover studies on clinical zero samples, the drug concentration is still reported to be consistent with calculations than expected from the calculations. Therefore, it might be assumed that k_{el}

in the elimination phase beyond t_{last} may have different values at different time intervals. Such an assumption would coincide with the elliptical model, which together with an increase in the difference between k_{el} and $1/MRT$ would qualify the study as more vulnerable to the effect of the lack of wash-out of the drug at the expected time of t_{last} .

Despite the application of the rules regarding the calculation of wash-out, in the zero samples (predose samples) and in the subsequent periods, drug concentration remains after the previous period. Some studies indicate that the value of the concentrations of predose samples (2×2 crossover studies) at the level of $> 0.5\% C_{max}$ still have no significant impact on results of crossover studies [42]. However, this verifies properly designated wash-out only with respect to drug concentration. It should be also considered very likely that the principle of $0.5\% C_{max}$ will not be used with reference to biotechnological drugs. Currently, none of the key regulatory guidelines propose separate rules for determining wash-out period for this specific group of drugs. Wash-out determination based on the analysis drug concentration, observed PD effects and dynamics of antidrug antibodies formation must be explained. Changing agency requirements and the evolution of research into new classes of drugs (e.g., biotechnological drugs) require a new perspective on the current rules for the proper determination of wash-out. Further research is also need for new challenges, such as proper determination of wash-out in the study due to the need of determining the period based on several end points (PK – drug concentration, PD – drug effect, TK – anti-drug antibodies) [2].

Based on the above analyses, the revision of determination of wash-out was proposed. The current, most common attitude is a determination of wash-out based on five-times value of $t_{1/2kel}$. However, it should be pointed out that, according to the assumptions of the elliptical model, the drugs with strongly expressed distribution require nonstandard approach to wash-out. Currently, the wash-out is determined based on the data concerning elimination phase. As was pointed out, the crucial element of drug kinetics is fractal characteristics of many physiological processes. A range of these processes is closely linked to the range of distribution that has not been considered so far in determination of drug wash-out. In view of meaningful distribution, some drugs can remain much longer in selected tissues, changing the view of final elimination phase for instance by double peak phenomenon or long persisting high drug concentration. In the case of such drugs, these effects are usually difficult to observe during chemical analyses because, apart from the time equal MRT, sampling in classic studies after

Table 4. Basic parameters of the elliptical model constructed for each analyzed drug.

Study	r_1 (h)	r_2 (h)	A_{El} (h)	L_{El} (h)	ϖ	δ	f (°)	$t_{0,El}$ (h)	$t_{last,El}$ (h)
1	2.87	4.27	38.51	22.65	3.1707	4.6068	29.59	1.86	15.35
2	2.67	4.63	38.86	23.35	3.1975	4.5506	38.06	2.47	12.27
3	1.30	1.75	7.17	9.65	3.1573	4.6353	23.23	0.62	8.88
4	9.75	22.63	693.32	105.90	3.2695	4.4063	51.21	15.06	63.06
5	8.21	9.58	246.87	55.93	3.1447	4.6627	12.90	2.00	34.00
6	4.54	6.63	94.55	35.39	3.1678	4.6131	28.39	2.79	26.26
7	6.02	6.20	117.23	38.38	3.1402	4.6725	2.56	0.27	28.86
8	6.67	6.44	134.81	41.16	3.1403	4.6723	-3.31	-0.38	27.77
9	3.01	2.26	21.37	16.63	3.1558	4.6387	-29.63	-1.37	8.63
10	13.14	33.94	1400.04	155.44	3.3017	4.3447	55.17	23.82	71.82
11	11.74	15.55	572.90	86.09	3.1554	4.6396	22.05	5.27	53.27
12	8.74	14.29	392.37	73.40	3.1863	4.5739	34.96	7.13	42.13
Min	1.30	1.75	7.17	9.65	3.1402	4.3447	-29.63	-1.37	8.63
Max	13.14	33.94	1400.04	155.44	3.3017	4.6725	55.17	23.82	71.82
Mean	6.56	10.68	313.17	55.33	3.1822	4.5847	22.10	4.96	32.69
RSD%	55.90	85.66	125.42	74.54	1.56	2.12	103.23	142.61	61.88

π : Pi value calculated using equation: $L_{El} \approx \pi \times [\frac{3}{4} \times (r_1 + r_2) - \sqrt{r_1 \times r_2}]$; δ : Feigenbaum constant calculated using equation: $\delta \approx 10/(\pi-1)$ where π is calculated from elliptical model; A_{El} : Area of ellipse calculated using equation: $A_{El} \approx \pi \times r_1 \times r_2$; f : Polar flattening of ellipse calculated in degrees (°) using equation: $f = [(r_2 - r_1)/r_2] \times 90$; L_{El} : Length of ellipse calculated using equation: $L_{El} \approx \pi \times [3/4 \times (r_1 + r_2) - \sqrt{r_1 \times r_2}]$; Mean: Arithmetic mean; Min: Minimal value; Max: Maximal value; r_1 : Minor radius of ellipse calculated using equation: $r_1 \approx t_{0.50MRT} \times \sqrt{t_{0.50kel} / t_{0.50MRT}}$; r_2 : Major radius of ellipse calculated using equation: $r_2 \approx t_{0.50kel} \times \sqrt{t_{0.50kel} / t_{0.50MRT}}$; RSD%: Percent of relative standard deviation; $t_{0,El}$: Position of the zero point on the ellipse calculated using equation: $t_{0,El} \approx [(f \times \pi) / 360] \times [\frac{3}{2} \times (r_1 + r_2)] - \sqrt{r_2 - r_1}$; $t_{last,El}$: Position of the last sampling point on the ellipse model calculated using equation: $t_{last,El} = t_{0,El} + t_{last}$.

single drug application is often very rare (24, 48, 96 h etc.). Many effects in this scheme of sampling escape, but the influence of carryover of the first administration on the concentration level after subsequent drug administration may be significant or at least difficult to predict. The presented study proposes the method of wash-out determination worked out on the grounds of the share analysis of processes defined as chaotic in drug elimination. Based on that, a new method of wash-out determination was proposed:

$$t_{wash-out} = t_{last} + \{6.74 \times (t_{1/2kel} + |t_{1/2kel} - t_{1/2MRT}|)\}$$

that subdivision is Feigenbaum constant. The wash-out periods calculated with the use of the proposed method are more than two-times longer for all analyzed drugs with reference to a rule $5 \times t_{0.50kel}$.

The presented calculation describes that δ is a value that can be used in the calculation of the wash-out of drugs and can be considered as a standard. It has been proven that the course of k_{el} and MRT reflect changes in drug concentration in the evaluated intervals are the result of deterministic chaos. This paper presents mathematical justification for the use of δ in order to

calculate the wash-out. It was also confirmed that the convergence of currently used assumptions based on the analysis of wash-out is a derivative of multiplicity $t_{0.50kel}$.

Conclusion

One of the key elements of design studies in the clinical and pre-clinical phases is a determination of a wash-out ('clearance') period. Many of the processes related to the biology and physiology of living organisms are chaotic processes. Certain organs has fractal characteristics. It is also known that fractals (strange attractors) are typical attractors of chaotic systems. This means that a system defined as chaotic can depend on the shape of drug pharmacokinetics. The processes of drug elimination from the body are thus a way of transition from the unbalanced (antichaotic) arrangement of molecules (high polar fattening models) to limited and predictable (circular models) processes (chaotic – homeostatic models) such as late phase of elimination. The administration of the drug results in increased numbers of unbalanced processes, which at some stage revert to 'structured' processes such as a late phase of elimination. The proposed elliptical model

allows, to some extent, confirmation of the hypothesis that homeostasis is a picture of deterministic chaos. The presented calculations prove that the Feigenbaum number is a value that can be used in the calculation of the wash-out of drugs.

Future perspective

The wash-out issue has an increasingly important role in PK studies, especially in regard to biological drugs. In such a case, wash-out optimization covers not only chemical wash-out (concentrations in blood plasma) but also PD and immunogenicity wash-out. Since the problem includes new areas of analysis, there is a need to revise the rules applied thus far. Optimal wash-out guarantees unbiased results in crossover studies, which are still one of the best methods for comparative analysis.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Based on the calculations, it can be concluded that the PK of xenobiotics, such as drugs, is a product of a deterministic chaos.
- Definition of mean residence time refers directly to the structure bifurcation diagrams and fractal structure of some organs.
- The processes of drug elimination from the body are a way of transitioning from the unbalanced (antichaotic) arrangement of molecules (high polar fattening models) to limited and predictable processes (chaotic – homeostatic models).
- The administration of drug results in an increased number of unbalanced processes, which at some stage revert to 'structured' processes.
- The presented calculations prove that the Feigenbaum constant is a value that can be used in the calculation of the wash-out of drugs.

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