

Pharmacokinetic simulations for forensic toxicological evaluation and expertise

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1. Introduction

The accused in criminal cases or in cases of driving under the influence of medical drugs often claims to have taken certain doses of drugs. These statements are usually understated or exaggerated to suit individual needs. The forensic toxicologist has the task to judge this statement on the basis of a toxicological analysis result from only a single blood sample. To do this he must estimate the concentration of the drug according to the stated dose and the time interval before blood sampling. This time course of blood concentrations in the body is described by pharmacokinetic equations, which comprise different properties of a specific drug. The basic principle consists of absorption of the drug into the body and excretion of the drug out of the body (this is reduced to the absorption into blood and elimination from blood).

The absorption of an orally applied drug into the blood usually follows an exponential function. The time interval, during which the amount of drug in the intestine is halved is called the half-life of absorption or $t_{1/2 A}$. The time interval, during which the concentration in the blood is halved is called half-life of elimination or $t_{1/2 E}$ (also an exponential function). Three factors influence the process of absorption of an orally applied drug. First, a given dose is not absorbed entirely, and second, a part of the absorbed drug is metabolized by the liver before it can reach the blood (first-pass effect). Thus the fraction of a given dose of drug, which finally reaches the blood, is called bioavailability or f with unit percent. Bioavailability is the comparison of an orally applied dose to the same dose applied intravenously. A third factor is, that the drug stays not only in the blood but is distributed throughout the body. A correction factor for this distribution between blood and body is called distribution volume or V_d . It is usually given in liter per kilogram of body weight.

With these parameters (f , $t_{1/2 A}$, $t_{1/2 E}$, V_d) the time course of blood concentrations can be simulated using the pharmacokinetic equation (1). The K_a and K_e values result from conversion of $t_{1/2 A}$ and $t_{1/2 E}$ ($K = \ln(2)/t_{1/2}$). The calculation of this equation with a pocket calculator is a great effort, therefore a macro package was developed ("AutoKinetic", [4]), which is an easy to use Microsoft Excel[®]-based pharmacokinetic simulation program.

$$C = \frac{f \cdot D \cdot K_a}{V_d \cdot (K_a - K_e)} \cdot (e^{-K_e t} - e^{-K_a t}) \quad (1)$$

2. Concept of "AutoKinetic"

Microsoft Excel[®] is a well known spreadsheet program which allows very flexible data handling and includes a complete programming language (Visual Basic for Applications, VBA). "AutoKinetic" in the current version provides spreadsheets as user interface and a set of command buttons (Figure 1), which execute the actual simulation program and some further features, all written in VBA. For a pharmacokinetic simulation the essential pharmacokinetic parameters of a drug must be entered: body weight (BW, case dependent), bioavailability (f), half-life of absorption ($t_{1/2 A}$) or alternatively the time of the maximal

blood concentration (t_{max}), the distribution volume (V_d), the elimination half-life ($t_{1/2 E}$), the start time and end time of the simulation, the time interval between simulated data points and finally the dose of the drug. On pressing the "Calculate" button (pocket calculator icon) the program performs the simulation and enters the time and concentration data points in the spreadsheet and automatically generates (or updates) a diagram of the data. A special feature of the program is the possibility to freely define application of multiple doses.

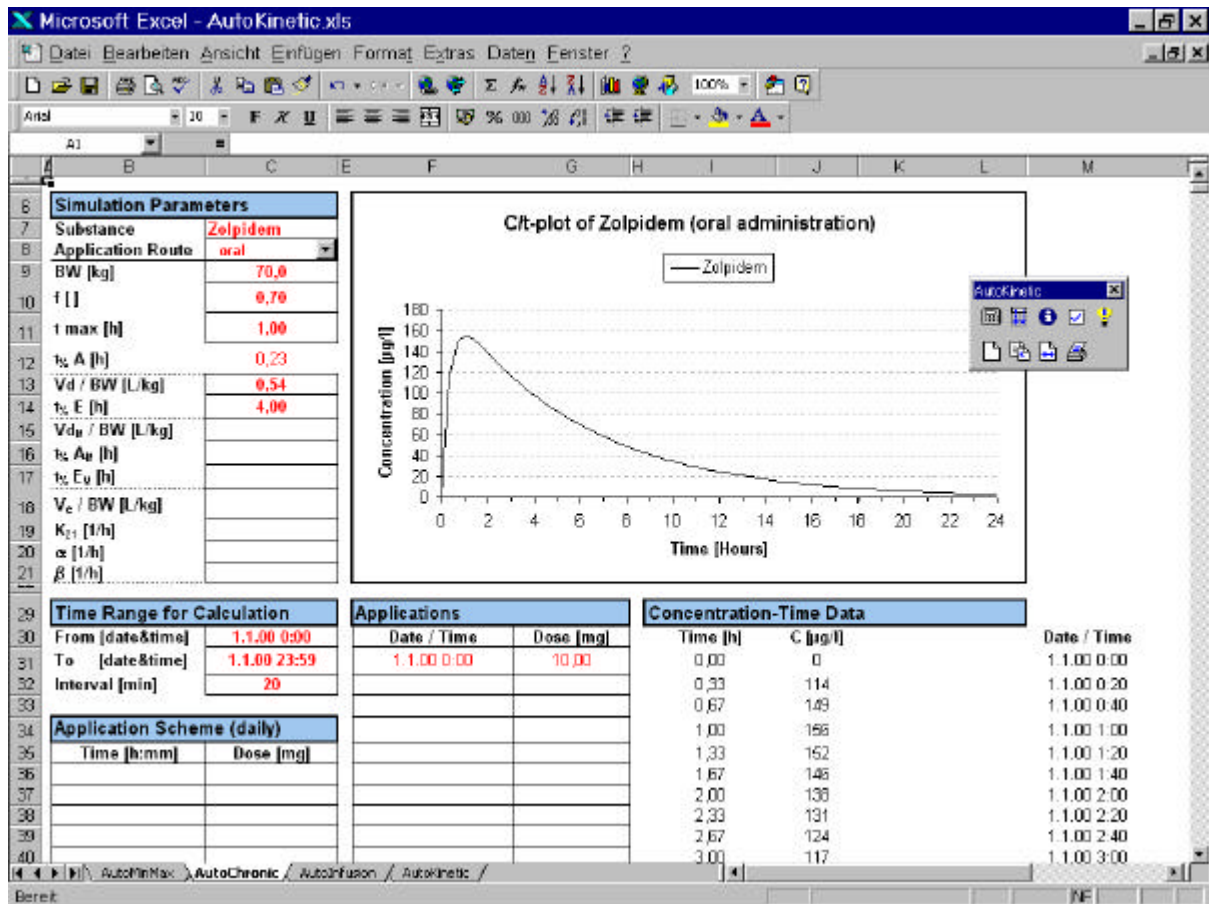


Figure 1: Screenshot of the spreadsheet "AutoChronic" with all essential user interface elements and a sample simulation.

3. Gathering pharmacokinetic parameters

The parameters needed for simulation must be gathered from literature. This can be specific scientific reports on the pharmacokinetic properties of a certain drug, but there are also other sources of information. From my knowledge there is only one compendium, that contains lots of medical drugs and usually provides all necessary pharmacokinetic data (along with further details): the German "Fachinformation" [1]. In most cases f , $t_{1/2 E}$, V_d and t_{max} are given. It is not necessary to know the $t_{1/2 A}$, which is given only for a few drugs, as from the t_{max} the necessary $t_{1/2 A}$ can be estimated. This is accomplished using an iterative algorithm, which is implemented in "AutoKinetic". V_d is in some cases also not available, but in these cases other data is available (the clearance or a dose and corresponding AUC) from which the V_d can be estimated (a feature also provided by the program).

Other literature may also be helpful. The Baselt/Cravey [2] contains t_{max} , $t_{1/2 E}$ and V_d information but lacks the very important bioavailability. An advantage is, that it provides specific data on half-lives, C_{max} and t_{max} values from different scientific studies. Another

very valuable source of pharmacokinetic data is the compilation of Haen/Forth [3], but only very few substances are assembled yet.

A bit of a thought should be given to the unit of the calculation result, which is amount of drug per volume. The unit of the volume is linked to the unit of the distribution volume which on the other hand derives from the concentration unit used in the original pharmacokinetic study of a certain drug. As concentrations are usually determined in mg per liter of serum/plasma, the resulting concentrations from the pharmacokinetic simulation do therefore also refer to serum/plasma and can not be taken as whole blood concentrations (if not specified otherwise). Another aspect is the unit of the amount of drug. This is linked to the unit of the calculated dose. If a dose of a drug would be defined in mmol, the unit of the simulated concentration would be mmol/l. If a 10 mg dose of morphine sulfate pentahydrate would be given, the simulation result would be mg morphine sulfate pentahydrate per liter of serum/plasma. As concentrations from toxicological analyses are usually mg of free drug per liter of serum/plasma, the dose must be corrected for the difference before calculation (weight ratio of e.g. morphine base and morphine salt).

4. Strategy for pharmacokinetic evaluation of a forensic case

The first task is to compile a validated set of pharmacokinetic parameters with which realistic results can be obtained. The problem lies in most cases in a broad range of interindividual differences (especially $t_{1/2 E}$ and V_d). For first estimation of the biologic diversity, it might be helpful to compile a set of parameters, with which minimal blood concentrations are obtained and another set of parameters, with which maximal blood concentrations are obtained. Minimal blood concentrations result from low f , long $t_{1/2 A}$ and t_{max} , large V_d and short $t_{1/2 E}$ (for maximal concentrations vice versa.) The most realistic parameters lie somewhere in between, as demonstrated in Figure 2. As in the literature extremes may be given (e.g. long elimination half-lives in patients with hepatic disorders), the average of a given range is usually not appropriate. It would be better to choose parameters on the basis of specific (and coherent) study results.

Selection of parameters should be directed to yield realistic simulation results. For this purpose at least one certain example from literature should be used as template (e.g. from bioavailability studies). If the simulation result (C_{max}) of a single dose application matches reported concentrations, another example should be tried which should be a chronic administration. In this example attention should also be directed towards a possible cumulation of the drug. If these simulation results also agree with reported concentrations, the selected parameters can be accepted and be applied to the forensic case (two examples for the described validation procedure are given later).

For simulations according to statements of accused in forensic cases the time range should start with the date/time of the first dose and end at the date/time of blood sampling. The body weight of the subject should be taken but all other parameters should be used unchanged. In case that the simulation result differs markedly from the measured concentration (which is, from my experience, mostly the case), the next step would be to check, if a parameter has to be adjusted to specific circumstances of the individual case. This is often true for the distribution volume, which is usually based on studies with young and healthy men. In older or obese persons the distribution volume might need an increase while in case of emaciated persons it might need a decrease. If still a marked difference is observed, the statements of the accused do not sufficiently explain the analytical result and can be judged as false.

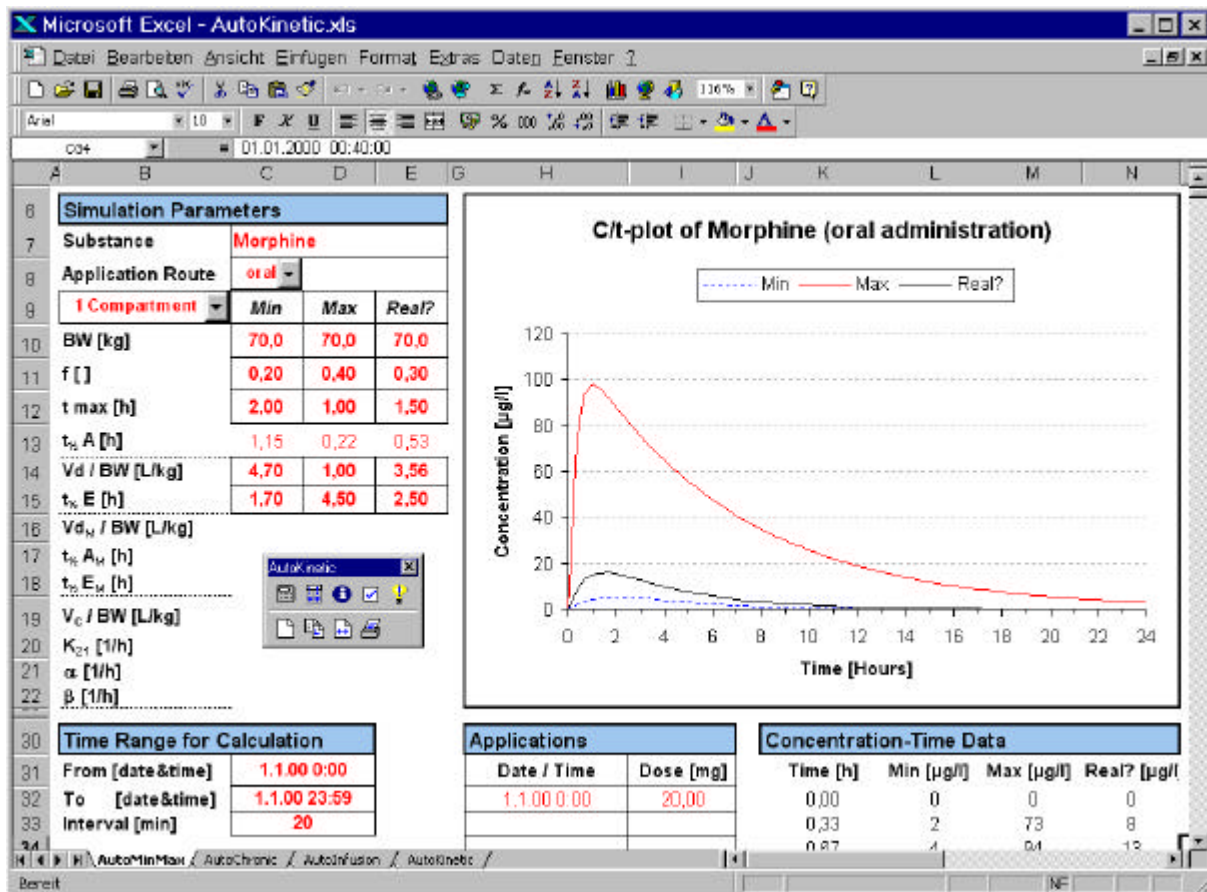


Figure 2: Screenshot of the spreadsheet "AutoMinMax" with an example of minimized and maximized blood concentrations and a set for acceptable realistic parameters.

In most cases the measured concentrations are much higher than the calculated values and it should be tried to estimate a minimum single dose with which the concentration can be reached. Therefore simulations with increasing single doses are carried out where the start time lies t_{max} hours before the time of blood sampling. Hereby the maximum blood concentration is reached at the time of blood sampling. A complementary approach is to simulate a chronic application with which the measured concentration can be explained. All these results can finally be incorporated into the forensic toxicological expertise.

It is of great importance, that during all simulations the toxicologist should be aware of the limitations of the model used. In most cases a fast distribution phase with at least a second distribution compartment is to be expected. The differences between 1 compartment model (CM1) and 2 compartment model (CM2) are, that in the CM2 the maximal concentration (C_{max}) is higher than in CM1 and that later concentrations are lower. However, a rough estimation may be possible with the CM1. Though "AutoKinetic" provides the possibility to perform simulations with the CM2, the necessary parameters are not always accessible (especially not for older substances). One should also be aware, that the chosen parameters may have a large interindividual variability and other modifying effects like enhanced or decreased metabolism might be of relevance. It should also be mentioned, that in intoxication cases serious deviations can be expected. A forensic toxicological expertise should always be presented with such reservations and caution.

5. Proposed structure of a forensic toxicological expertise on pharmacokinetic simulations.

The simulation model must be comprehensibly explained. The source of data must be given and the biologic variation of each pharmacokinetic parameter must be explained. The basis of the parameter selection should be discussed and it must be demonstrated, that the selected parameters are effective in the simulation of a single and multiple doses of the drug. Also the limitations of the model should be explained. The results of the case related simulation must be discussed (e.g. adaptation of parameters, if reasonable) and potential single and multiple doses should be offered, with which the measured concentration can be explained.

The most severe limitation of this kind of simulation is, that in an individual case generalized parameters are used which might be invalid under certain circumstances, e.g. comedication with inhibitors of hepatic metabolism or hepatic disorders may prolong the elimination half-life and lead to wrong simulation results. Therefore it is important to present all evaluation results with due reservation.

Example 1: Simulation of zolpidem concentrations after oral application

Used literature: Fachinformation Stilnox and the monograph Zolpidem from Haen/Forth: Wirkstoffprofile für die Arzneimitteltherapie.

Pharmacokinetic parameters of zolpidem: f is given as 70%, V_d is given as 0.54 l/kg and 0.34 l/kg in older persons, $t_{1/2 E}$ is given as 2.4 h and $t_{1/2 A}$ can be estimated from the given t_{max} of 0.5-3 h and $t_{1/2 E}$ of 2.4 h yielding 0.11-1.81 h.

As Stilnox contains a 10 mg dose of zolpidem as tartrate salt, the calculated concentrations must be corrected (10 mg zolpidem tartrate contain 8.03 mg of free base). A simulation of an oral dose of 10 mg yields C_{max} values from 58 to 118 $\mu\text{g/l}$ (Figure 3). In Haen/Forth the zolpidem plasma concentration after a single 10 mg zolpidem dose is reported as 139 $\mu\text{g/l}$, which is near to the calculated 118 $\mu\text{g/l}$ (using a t_{max} of 0.5 h). The same calculation for a single 20 mg dose yields similar results: calculated C_{max} is 236 $\mu\text{g/l}$, given C_{max} is 260 $\mu\text{g/l}$. When using a t_{max} of 1 hour (reported for the 10 mg single dose) the C_{max} values are a bit lower.

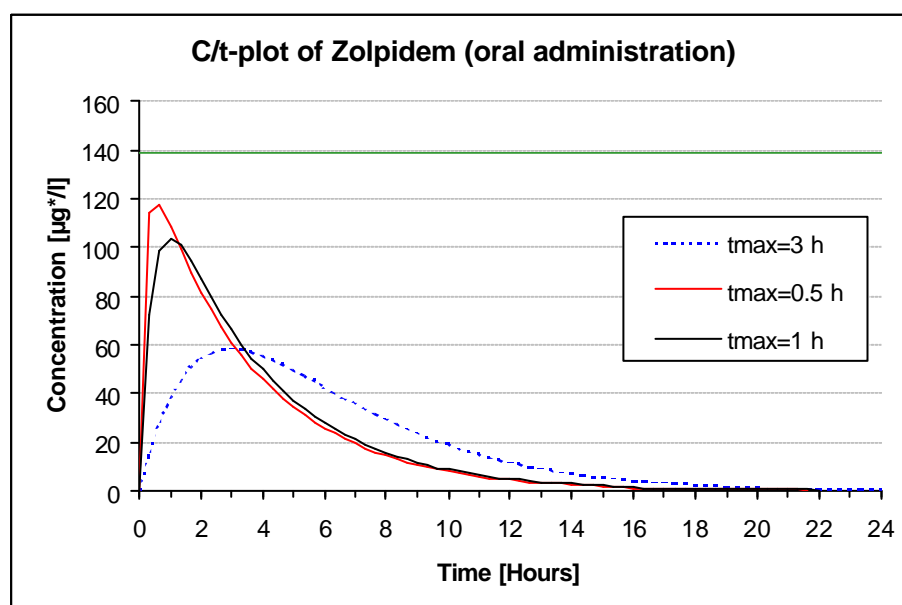


Figure 3: Simulation of a single 10 mg dose of zolpidem tartrate. The reported C_{max} is indicated by a horizontal line.

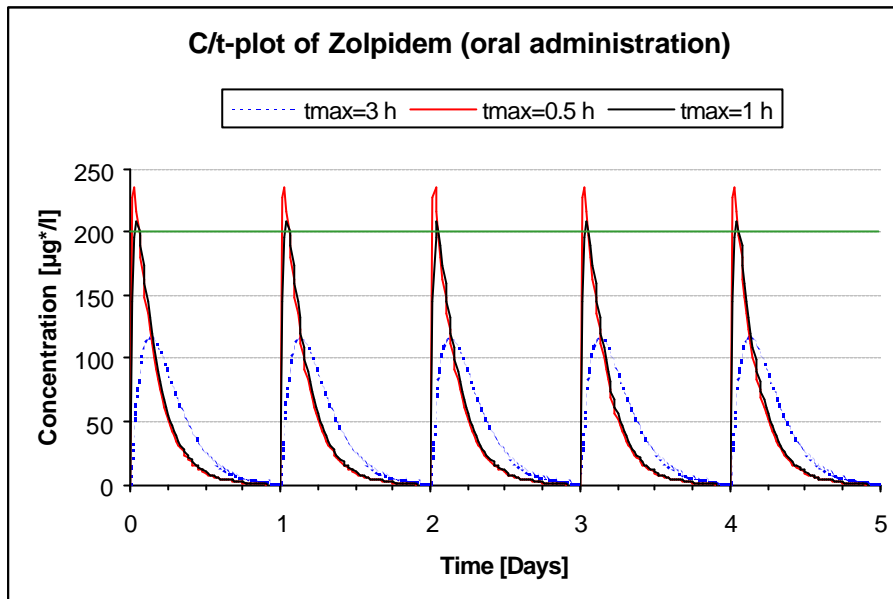


Figure 4: Simulation of a chronic application of 20 mg zolpidem tartrate over five days. The reported C_{max} is indicated by a horizontal line.

The selected simulation parameters yield zolpidem concentrations, that resemble those reported for single and multiple dose applications. Therefore this model can be used for the simulation in a forensic case of suspected driving under the influence of drugs: a 49 year old man (80 kg, 184 cm) claimed to have taken one tablet Stilnox at 17:30. The blood sample was taken at 18:45 and the zolpidem concentration was determined as 160 µg/l.

For simulation the parameters are used as above ($f=0.7$, $t_{max} 1\text{ h}$, $V_d 0.54\text{ l/kg}$, $t_{1/2 E} 2.4\text{ h}$) and the individual body weight of 80 kg is used. As the man is not young and a different V_d is given for older persons (Fachinformation Stilnox: 0.34 l/kg) this value is also used for comparison (Figure 5). Fazit: the simulation results are in rough agreement with the statement of the man.

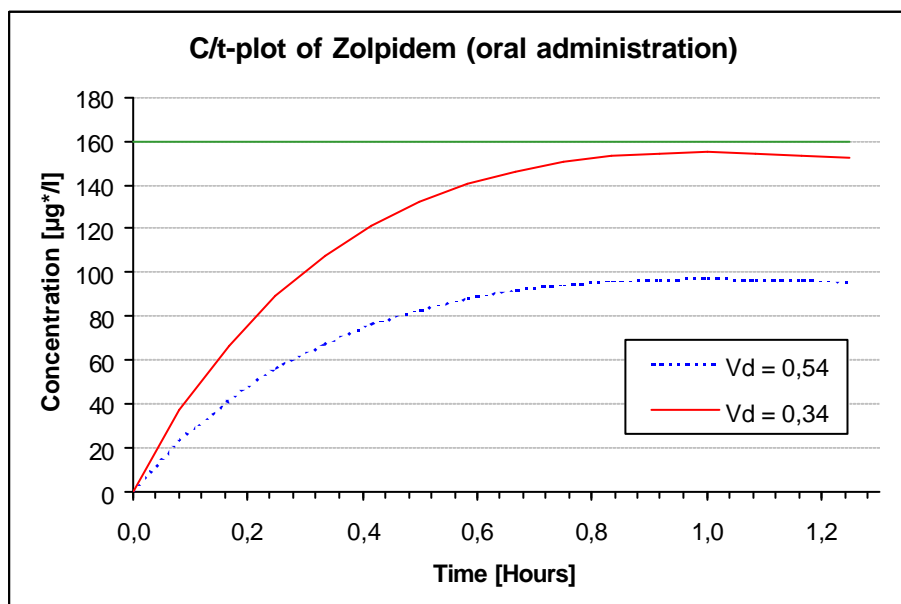


Figure 5: Simulation of the intake of one tablet Stilnox in a forensic case. The measured value of 160 µg/l is indicated by a horizontal line.

In another forensic case of suspected driving under the influence of drugs, a 60 year old woman (70 kg) claimed to have taken 4 tablets Stilnox at 0:00 and another 2 tablets at 2:00. At 11:50 a blood sample was collected and zolpidem was determined as 1700 $\mu\text{g}/\text{l}$. This can be simulated using the selected parameters. The V_d of 0.34 l/kg for older persons is used from which higher concentrations result, which is in favor of the woman. As can be seen clearly from the simulation diagram (Figure 6), the statement of the woman is not at all sufficient to explain the high value measured. For the forensic toxicologist two questions arise: what would be the minimal dose to yield the measured concentrations and what dose would be necessary for a chronic intake?

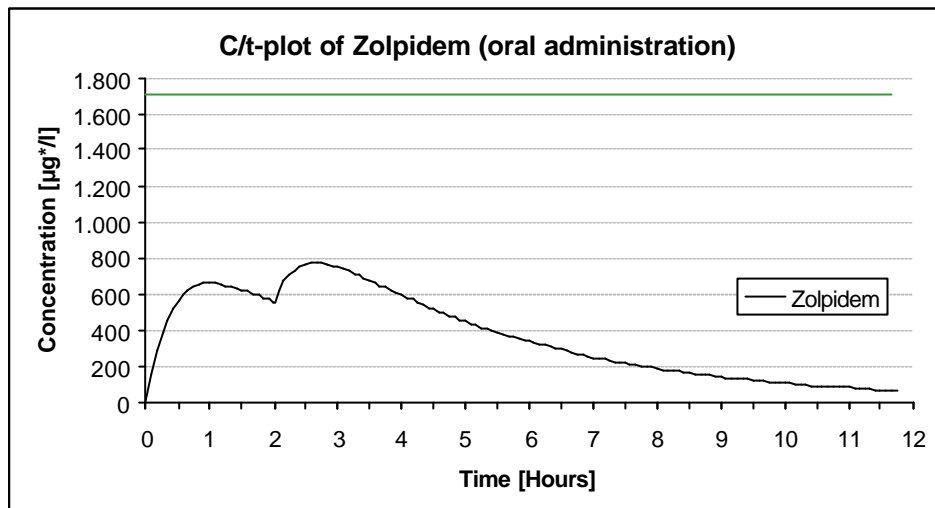


Figure 6: Simulation of the intake of 4 tablets Stilnox at 0:00 and additional 2 tablets two hours later. Blood sample was collected 11.75 hours later and contained 1700 $\mu\text{g}/\text{l}$ zolpidem (indicated by horizontal line).

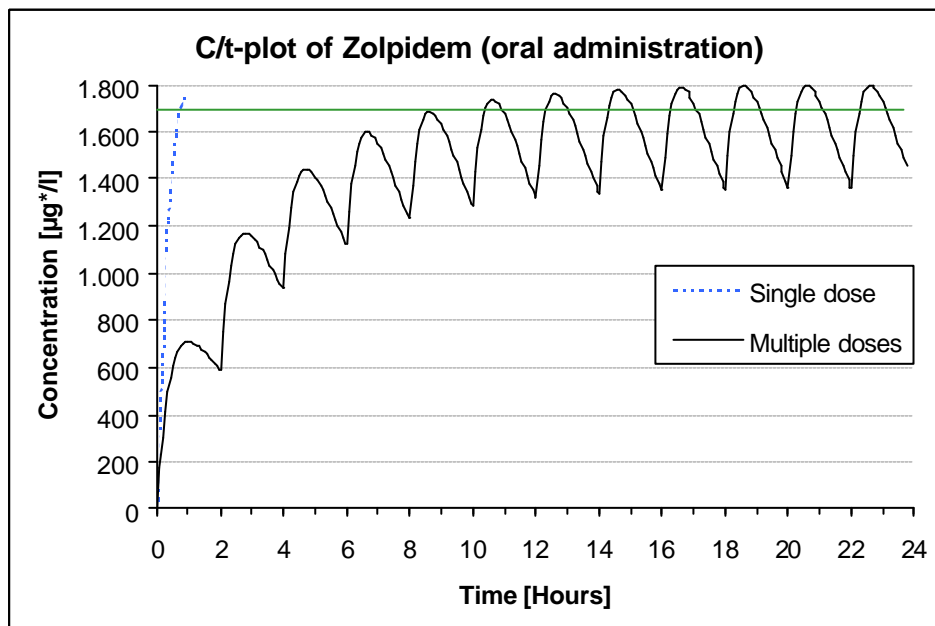


Figure 7: Simulation of the intake of a single overdose of 10 tablets Stilnox and of the chronic intake of 4 tablets Stilnox every 2 hours. The measured concentration is indicated by a horizontal line.

For solving the first question, the dose must hypothetically be applied t_{max} hours prior to blood sampling (i.e. 10:50 for a t_{max} of 1 hour) to reach maximal concentrations. By increasing the dose and recalculation, a single (over)dose of 10 tablets Stilnox (corresponding to 100 mg zolpidem tartrate) yields a concentration of 1761 $\mu\text{g/l}$ (Figure 7). However, the intake of a single overdose is not very likely in the actual case. For simulation of a chronic intake a time range of two days is sufficient, as the maximal cumulation is reached soon. By varying the daily dosing scheme up to 40 mg every two hours the simulated concentrations lie within a range of 1300 and 1800 $\mu\text{g/l}$ (Figure 7).

The case can be finally judged insofar, that the statement of the woman is insufficient and an overdose of 10 tablets Stilnox or a minimum of five repeated 40 mg doses in 9 hours are necessary to reach the measured concentration of 1700 $\mu\text{g/l}$.

Example 2: Simulation of nortilidine concentrations after oral application of tilidine hydrochloride solution

Used literature: Fachinformation "Tilimerck 50 mg/-Lösung" and "Valoron N retard".

In this example, not the parent compound is the target, but its active metabolite nortilidine. It can be assumed, that tilidine is almost completely converted to nortilidine in the first pass of the liver, therefore the simulation is based on the application of tilidine hydrochloride but the resulting concentrations are of nortilidine. Pharmacokinetic parameters for nortilidine are: $t_{1/2 E}$ is 3-5 h, f is 98% (referring to nortilidine from oral and i.v. application of tilidine), $t_{1/2 A}$ can be estimated from the given t_{max} of 0.86 h (solution) and an average $t_{1/2 E}$ of 4 h yielding 0.18 h. V_d can be estimated from the given AUC of 1026 $\mu\text{g/l}\cdot\text{h}$ resulting from a dose of 83.8 mg nortilidine (100 mg tilidine hydrochloride), the average $t_{1/2 E}$ of 4 h and a hypothetical body weight of 75 kg (male volunteers) yielding 6.15 l/kg. The applied dose of *tilidine hydrochloride* (MW 309 g/mol) must be adjusted in order to get *nortilidine* concentrations (MW 259 g/mol). A simulation of an oral application of 100 mg tilidine hydrochloride yields C_{max} values from 146 to 156 $\mu\text{g/l}$ (Figure 8), which is near to the reported 160 – 180 $\mu\text{g/l}$.

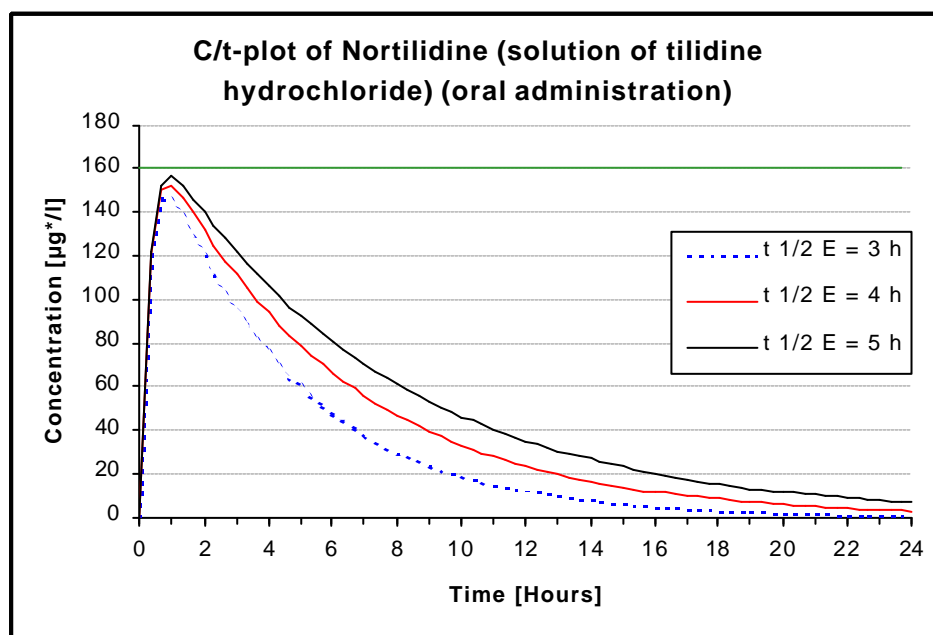


Figure 8: Simulation of nortilidine concentrations after oral application of 100 mg tilidine hydrochloride in solution. The elimination half-life is varied (3–5 h).

In the Fachinformation for Valoron N retard an example is given for a chronic application of four times 50 mg of tilidine hydrochloride solution per day. The simulation with the above selected parameters and elimination half-lives of 3 and 4 hours yields Figure 9. The result is very similar to the figure in the Fachinformation Valoron N retard, especially with the $t_{1/2 E}$ of 3 h (which can be estimated from that figure).

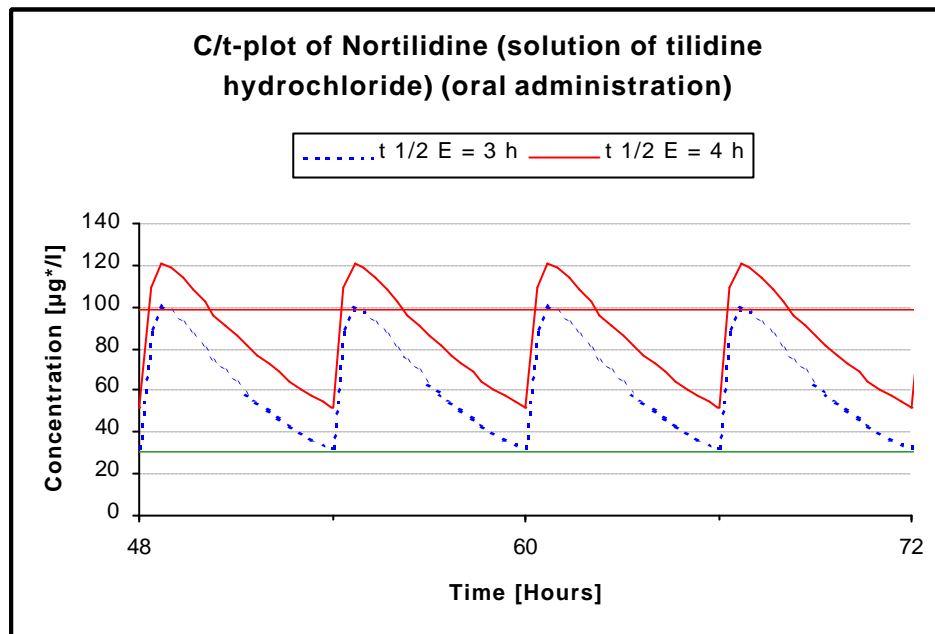


Figure 9: Simulation of nortilidine concentrations after repeated oral applications of 50 mg tilidine hydrochloride in solution (four times per day).

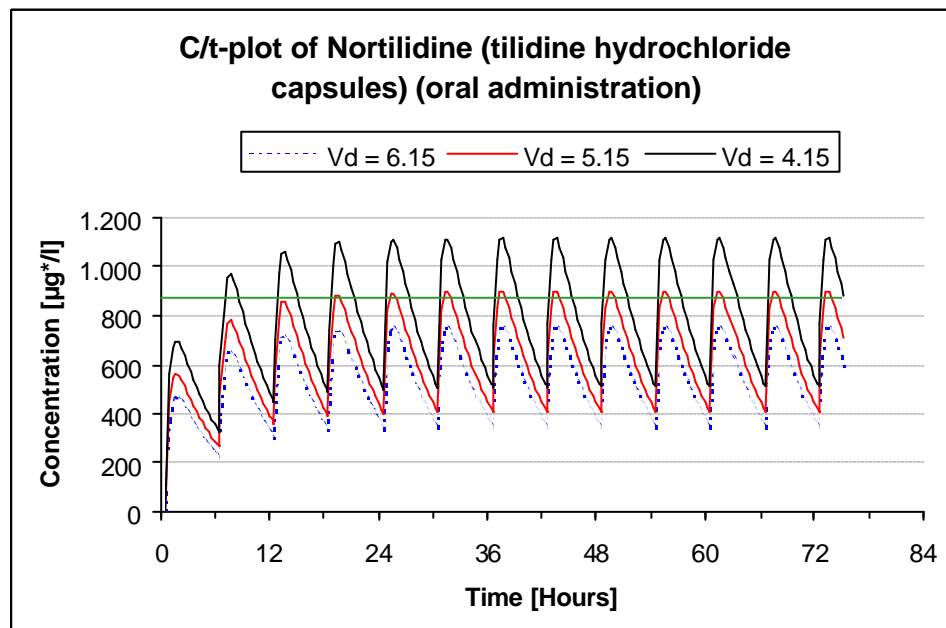


Figure 10: Simulation of nortilidine concentrations after repeated oral application of 300 mg tilidine hydrochloride capsules (four times per day).

The selected simulation parameters yield nortilidine concentrations, that resemble those reported for single and multiple dose applications of a solution of tilidine hydrochloride. This

model can be used for the simulation in a forensic case of suspected driving under the influence of drugs: a 24 year old man (69 kg, 184 cm) claimed to take 25 capsules Valoron per day, the last six at 23:30. The blood sample was taken at 2:15 the next day and the nortilidine concentration was determined as 870 µg/l.

For simulation the parameter $t_{1/2 A}$ needs adjustment for capsules (t_{max} 1.22 h \Rightarrow $t_{1/2 A}$ 0.3 h) and the individual body weight of 69 kg is used. The dosing scheme is defined as four doses of 300 mg tilidine hydrochloride capsules (24 capsules per day), the last at 23:30. As the man has a weight of only 69 kg with 184 cm height, a smaller distribution volume is probable (e.g. 5.15 or 4.15 l/kg instead of 6.15 l/kg). The simulation time range is extended over four days to allow accumulation of multiple doses and the end is the time of blood sampling. The simulation result (Figure 10) with default parameters lies markedly below the measured value but in the present case the individual circumstances should be considered by decreasing the distribution volume. Fazit: these results may confirm the statement of the man.

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