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# A new similarity method for assessment of pharmacokinetic interaction between flucloxacillin and midazolam

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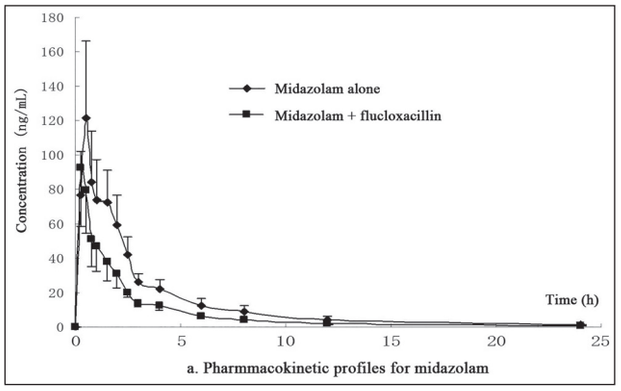
The purpose of this study was to develop a new similarity method to assess the drug-drug interaction between midazolam and flucloxacillin. Total quantum statistical moment (TQSM) of pharmacokinetic profiles were expressed by parameters $AUC_T$, $MRT_T$, $VRT_T$ et al. Statistical moment similarity (*SMS*) expressions were deduced to evaluate the similarity of the converted pharmacokinetic profiles. A trial of the pharmacokinetic interaction between midazolam and flucloxacillin by the *SMS* method was conducted. For midazolam, total quantum *SMS* ($SMS_T$) was 0.9582; total deviation was 0.0525; total variable probability was 4.18 %; total confidence of probability β was 97.17 % under significance level 0.05; $AUC_{0-\infty}$ were 334.3±334.1 ng•h•mL$^{-1}$ (after administration of midazolam alone) and 206.9±172.2 ng•h•mL$^{-1}$ (after co-administration of midazolam and flucloxacillin), respectively. While, for 1'-hydroxy midazolam, $SMS_T$ was 0.6920; total deviation was 0.3960; total variable probability was 30.80 %; total confidence of probability β was 94.10 % under significance level 0.05; $AUC_{0-\infty}$ were 1364±810.7 ng•h•mL$^{-1}$ (after administration of midazolam alone) and 1637±632.6 ng•h•mL$^{-1}$ (after co-administration of midazolam and flucloxacillin), respectively. These results revealed that flucloxacillin might have weak pharmacokinetic interactions on midazolam metabolized into 1'-hydroxy midazolam, indicating that there was weak induction to CYP3A by flucloxacillin and that there was at least 30.80 % of metabolic behaviour in change with bioavailability decreased by 38.11 % that took effect to flucloxacillin metabolism for liver injury in CPY3A4 poor metabolic polymorphisms. *SMS* can be an optional method applied to characterize and analyze pharmacokinetic profiles.

## 1. Introduction

Pharmacokinetics play a significantly important role in guiding the safety and efficacy of chemical drugs (Rottschäfer et al. 2018). At present, a series of pharmacokinetic parameters (Peters et al. 2008; Lyons et al. 2015) have always been applied to evaluate *in vivo* absorption, distribution, metabolism, and excretion of a drug. Generally, parameters like *AUC* (area under the concentration time curve), $C_{max}$ (the maximum observed plasma concentration), *CL* (clearance), $t_{1/2}$ (the half-life) et al (Dautzenberg et al. 2007; Darwish et al. 2008) are applied to the evaluation. It is worth noting that these parameters are interfered easily by biological individual factors and different data processing methods. Moreover, the above-mentioned parameters reflect only partial properties of pharmacokinetic profiles. Meanwhile, there is no appropriate method to evaluate whether pharmacokinetic behaviors of two pharmacokinetic profiles would be similar or different. Therefore, it is necessary to establish a stable, effective and simple method to assess the similarity or difference among these pharmacokinetic profiles.

Statistical moment model, describing the characteristics of time courses of plasma concentration (area, mean residence time, and variance of residence time), can be obtained by the integral (Yamaoka et al. 1978) for pharmacokinetic profiles. These pharmacokinetic profiles of individual drugs can be combined into a total pharmacokinetic profile represented by total quantum statistical moment (TQSM) parameters including total quantum zero moment ($AUC_T$), total quantum first moment ($MRT_T$), total quantum second moment ($VRT_T$) (He et al. 2008; Zhang et al. 2018). Furthermore, parameter *MRT* and *VRT* can be converted into a normal probability density function (NPDF). Consequently, the overlapped area of two NPDF curves can be defined as statistical moment similarity (*SMS*), by which the similarity and the variability of the two integral curves can be quantitatively analyzed (Roy et al. 2017; Zhang et al. 2015). In this paper, we deduced the mathematical expressions of TQSM parameters and *SMS* for individual pharmacokinetic profiles or total pharmacokinetic profiles.

Flucloxacillin, a semi-synthetic isoxazolyl penicillin, is active against many Gram-positive bacteria including penicillinase-producing staphylococci and streptococci. Clinically, flucloxacillin remains the predominantly prescribed antistaphylococcal oral antibiotic widely used for the treatment of skin and soft tissue infections and respiratory tract infections (Maier-Salamon et al. 2017). Clinical findings indicated that co-administration of flucloxacillin with cyclosporine might reduce the plasma concentrations of cyclosporine. Incubation of human LS 180 colorectal adenocarcinoma cells with flucloxacillin resulted in an induction of MDR1 as well as CYP3A4 mRNA (Huwyler et al. 2006). Lactate dehydrogenase was released in primary cultures of human hepatocytes, metabolized in biliary epithelial cells by the supernatant of human liver microsomes, recombined in human CYP3A4 liver microsomes preincubated with flucloxacillin, and then the indirect cytotoxicity was produced (Takai et al. 2015). These experiments *in vitro* indicated that the cytotoxicity in hepatic or biliary cells potentially induced by flucloxacillin were dose-dependent (Zhang



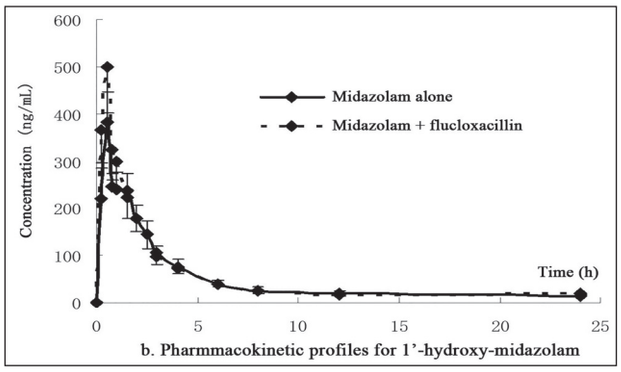




Fig. 1: Pharmacokinetic profiles for midazolam and 1'-hydroxy midazolam. This figure showed the average and standard deviation pharmacokinetic profiles of midazolam and 1'-hydroxy midazolam for 12 subjects, as a) midazolam pharmacokinetic profile, as b) 1'-hydroxy midazolam pharmacokinetic profile.

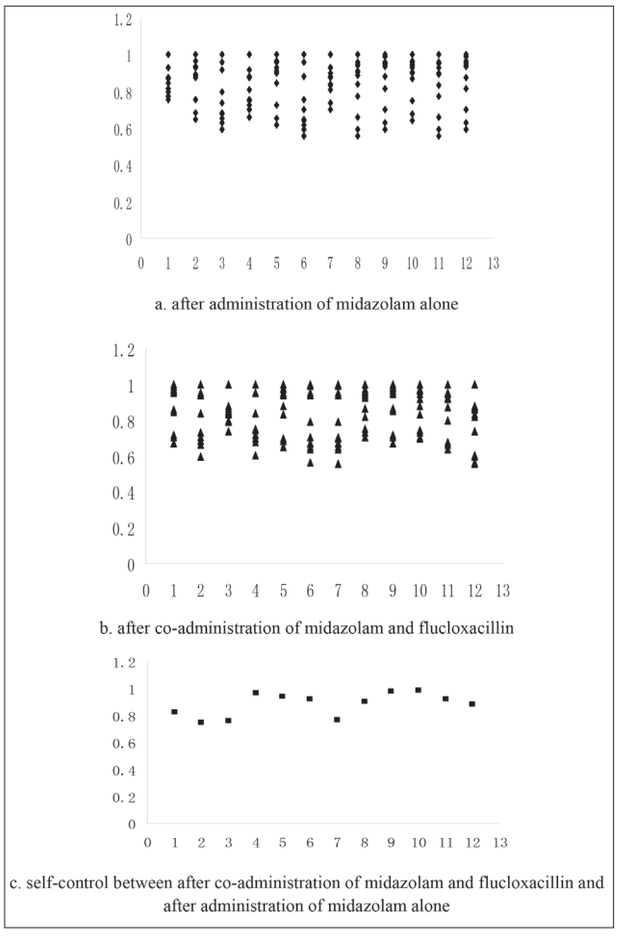



Fig. 2: Scatterplot of bilateral statistical moment similarity for midazolam. Scatterplot of bilateral statistical moment similarity was drew out 12 section bilateral statistical moment similarities of 1'-hydroxy midazolam by flucloxacillin with any subject to the others as a) after administration of midazolam alone, as b) after co-administration of midazolam and flucloxacillin, as c) self-control similarities between after administration of midazolam alone and after co-administration of midazolam and flucloxacillin.

2007). Midazolam, indicated for sedation, is a well-established probe drug for human intestinal and hepatic CYP3A activity with relatively low intra-individual pharmacokinetic variability. Midazolam is absorbed rapidly and completely following oral administration. More than 99 % of a midazolam dose is eliminated by metabolism, mainly via 1'-hydroxylation (60–80 %) and 4-hydroxylation (3 %), followed by glucuronidation. The hydroxylation is carried out nonspecifically by both CYP3A4 and CYP3A5 *in vitro* and *in vivo*. Meanwhile, a wide range of midazolam doses can be used to reliably quantify the sum of hepatic CYP3A4 and CYP3A5 activity and to quantify the combined activities of intestinal CYP3A4 and CYP3A5 (Fuhr et al. 2018). Therefore, in this paper, the pharmacokinetic interaction between flucloxacillin and midazolam by the *SMS* method established was studied to explore induction to CYP3A4 by flucloxacillin.

## 2. Investigations, results and discussion

### *2.1. Conventional evaluation by pharmacokinetics parameters*

According to conventional pharmacokinetic analyses, $AUC_{0\text{-}\infty}$, $C_{max}$, $t_{1/2}$, $K_{3\text{-}24}$, $CL_{0\text{-}\infty}$ et al. have been utilized to evaluate their coincidences in pharmacokinetics profiles. The mean (SD)

**Table 1: Classical pharmacokinetic parameters of midazolam and 1'-hydroxy midazolam ($\overline{X} \pm SD$, n=12)**

| Drugs and treatment groups | Pharmacokinetic parameters | | | | | |
|---|---|---|---|---|---|---|
| | $AUC_{0\text{-}\infty}$ ng·h·mL$^{-1}$ | $C_{max}$ ng·mL$^{-1}$ | $t_{max}$ h | $K_{3\text{-}24}$ h$^{-1}$ | $CL_{0\text{-}\infty}$ mL·h$^{-1}$·Kg$^{-1}$ | $V_{0\text{-}\infty}$ mL·Kg$^{-1}$ |
| **Midazolam** | | | | | | |
| **Midazolam alone** | 334.3±348.9 | 127.5±154.8 | 0.9989±1.258 | 0.1616±0.04084 | 250.8±229.2 | 928.9±1110 |
| **Midazolam + flucloxacillin** | 206.9±179.8 | 102.3±109.6 | 0.7125±0.9751 | 0.1622±0.04080 | 206.6±234.2 | 680.5±1081 |
| **Deviation ( %)*** | -38.11 % | -19.75 % | -28.68 % | +0.3531 % | -17.54 % | -26.74 % |
| **1'-hydroxy midazolam** | | | | | | |
| **Midazolam alone** | 1364±810.7 | 402.7±232.1 | 2.833 ±0.444 | 0.100 ±0.049 | 75.72±41.16 | 818.4±463.8 |
| **Midazolam + flucloxacillin** | 1637±632.6 | 547.0±310.3 | 2.700 ±0.450 | 0.081 ±0.039 | 48.68±17.76 | 940.0±1001 |
| **Deviation ( %)*** | +20.01 % | +35.83 % | -4.750 % | -19.00 % | -35.70 % | +15.15 % |

*Deviation mean the difference between "Midazolam alone" and "Midazolam + flucloxacillin", divided by "Midazolam alone".

**Table 2: Bilateral statistical moment similarities of pharmacokinetic profiles for midazolam**

| Midazolam alone / Midazolam + flucloxacillin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.8260 | 0.7028 | 0.8447 | 0.7172 | 0.9748 | 0.6733 | 0.6718 | 0.9608 | 0.9876 | 0.9664 | 0.9511 | 0.8561 |
| **2** | 0.8769 | 0.7510 | 0.8372 | 0.9501 | 0.6842 | 0.9413 | 0.9357 | 0.7338 | 0.7030 | 0.7330 | 0.6631 | 0.5949 |
| **3** | 0.7985 | 0.6837 | 0.7643 | 0.8377 | 0.8296 | 0.7928 | 0.7895 | 0.8662 | 0.8485 | 0.8775 | 0.7978 | 0.7390 |
| **4** | 0.8760 | 0.7568 | 0.9199 | 0.9694 | 0.6966 | 0.9513 | 0.9491 | 0.7512 | 0.7158 | 0.7462 | 0.6800 | 0.6011 |
| **5** | 0.8430 | 0.9654 | 0.6531 | 0.7247 | 0.9408 | 0.6534 | 0.6517 | 0.9358 | 0.9790 | 0.9460 | 0.9529 | 0.8809 |
| **6** | 0.7589 | 0.6469 | 0.9576 | 0.8791 | 0.6172 | 0.9204 | 0.9937 | 0.7064 | 0.6719 | 0.7014 | 0.6374 | 0.5616 |
| **7** | 0.9273 | 0.9281 | 0.7386 | 0.8117 | 0.8975 | 0.6997 | 0.7694 | 0.7051 | 0.6702 | 0.6997 | 0.6364 | 0.5596 |
| **8** | 0.7742 | 0.8851 | 0.5954 | 0.6619 | 0.9127 | 0.5609 | 0.8388 | 0.9056 | 0.9548 | 0.9740 | 0.9219 | 0.8185 |
| **9** | 0.8173 | 0.9340 | 0.6314 | 0.7010 | 0.9614 | 0.5959 | 0.8790 | 0.9504 | 0.9797 | 0.9670 | 0.9431 | 0.8624 |
| **10** | 0.8675 | 0.9634 | 0.6776 | 0.7492 | 0.9585 | 0.6405 | 0.9319 | 0.9037 | 0.9466 | 0.9882 | 0.9185 | 0.8327 |
| **11** | 0.7757 | 0.8947 | 0.5941 | 0.6617 | 0.9286 | 0.5599 | 0.8344 | 0.9579 | 0.9541 | 0.9013 | 0.9238 | 0.8695 |
| **12** | 0.8169 | 0.9363 | 0.6304 | 0.7002 | 0.9678 | 0.5950 | 0.8765 | 0.9444 | 0.9898 | 0.9437 | 0.9567 | 0.8825 |

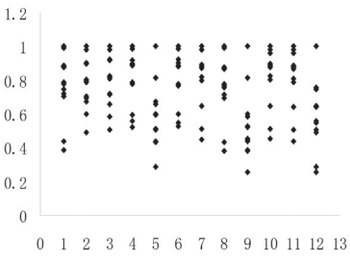
**Table 3: Bilateral statistical moment similarities of pharmacokinetic profiles for 1'-hydroxy midazolam**

| Midazolam alone / Midazolam + flucloxacillin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.3625 | 0.2274 | 0.3008 | 0.3450 | 0.6522 | 0.1551 | 0.2457 | 0.2048 | 0.2960 | 0.2752 | 0.2957 | 0.5739 |
| **2** | 0.7045 | 0.9798 | 0.8331 | 0.7354 | 0.3808 | 0.7729 | 0.9489 | 0.9069 | 0.8213 | 0.8832 | 0.8407 | 0.2802 |
| **3** | 0.7220 | 0.9791 | 0.8343 | 0.8898 | 0.4937 | 0.6239 | 0.8793 | 0.7631 | 0.9422 | 0.9461 | 0.9853 | 0.3729 |
| **4** | 0.7879 | 0.8983 | 0.9190 | 0.8085 | 0.5688 | 0.5377 | 0.7827 | 0.6637 | 0.9041 | 0.8470 | 0.8878 | 0.4513 |
| **5** | 0.4366 | 0.6754 | 0.6583 | 0.5935 | 0.2110 | 0.2622 | 0.4111 | 0.3400 | 0.4945 | 0.4567 | 0.4879 | 0.8201 |
| **6** | 0.7751 | 0.9026 | 0.9229 | 0.9849 | 0.6020 | 0.7054 | 0.7253 | 0.8419 | 0.6108 | 0.6661 | 0.6291 | 0.1831 |
| **7** | 0.8821 | 0.7977 | 0.8176 | 0.8947 | 0.5101 | 0.8838 | 0.8665 | 0.8607 | 0.8712 | 0.9318 | 0.8884 | 0.3074 |
| **8** | 0.9903 | 0.6964 | 0.7137 | 0.7788 | 0.4306 | 0.7661 | 0.8725 | 0.6493 | 0.7400 | 0.8052 | 0.7670 | 0.2401 |
| **9** | 0.3880 | 0.6021 | 0.5848 | 0.5205 | 0.8142 | 0.5254 | 0.4461 | 0.3830 | 0.4490 | 0.9291 | 0.9528 | 0.3868 |
| **10** | 0.8856 | 0.8046 | 0.8242 | 0.8982 | 0.5138 | 0.8857 | 0.9809 | 0.8760 | 0.4523 | 0.9379 | 0.9562 | 0.3450 |
| **11** | 0.8748 | 0.7902 | 0.8103 | 0.8891 | 0.5069 | 0.8804 | 0.9811 | 0.8654 | 0.4401 | 0.9620 | 0.9356 | 0.3709 |
| **12** | 0.7457 | 0.4900 | 0.5042 | 0.5602 | 0.2875 | 0.5501 | 0.6459 | 0.7540 | 0.2535 | 0.6453 | 0.6441 | 0.6780 |

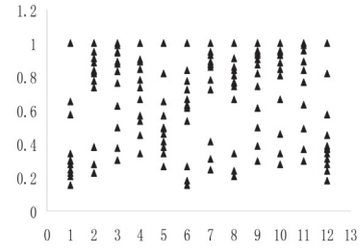
plasma concentration-time profiles of midazolam and 1'-hydroxy midazolam for twelve subjects are shown in Fig. 1, and the classical pharmacokinetic parameters are summarized in Table 1. After ANOVA test to analyze parameters $AUC_{0\text{-}\infty}$, $C_{max}$, $t_{max}$, $K_{3\text{-}24}$, $CL_{0\text{-}\infty}$, $V_{0\text{-}\infty}$ for midazolam and 1'-hydroxy midazolam, there were no statistically significant differences for any pharmacokinetic parameters ($p$ ranged from 0.137-0.978) observed after co-administration of midazolam and flucloxacillin compared with that after administration of midazolam alone. This meant that there were no pharmacokinetic interactions on statistics for midazolam and 1'-hydroxy midazolam by flucloxacillin. Numerically, however, the $AUC_{0\text{-}\infty}$, $C_{max}$, $t_{max}$, $CL_{0\text{-}\infty}$, $V_{0\text{-}\infty}$ of midazolam respectively decreased by 38.11 %, 19.75 %, 28.68 %, 17.54 %, 26.74 %, and the $K_{3\text{-}24}$ increased by 0.3531 %. Meanwhile, the $AUC_{0\text{-}\infty}$ and $C_{max}$ of 1'-hydroxy midazolam respectively increased by 20.01 % and 35.83 %, and the $t_{max}$, $K_{3\text{-}24}$, $CL_{0\text{-}\infty}$, $V_{0\text{-}\infty}$ respectively decreased by 4.750 %, 19.00 %, 35.70 % and 15.15 %, which suggested that there was possibly weak induction effect of flucloxacillin on midazolam from the numerical variation. For there was no reliable judgment, therefore, *SMS* method was applied to obtain the similarities of these pharmacokinetic profiles thereby to assess the pharmacokinetic interactions.

### *2.2. Integral evaluation by statistical moment similarity*

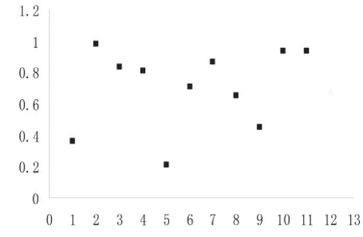
By statistical moment calculation, pharmacokinetic profiles were converted into SM and TQSM according to Eqs. (1)-(28), and SM parameters and TQSM parameters for pharmacokinetic profiles for midazolam and 1'-hydroxy midazolam were obtained. These parameters are shown in Supplementary Table 2 and Supplementary Table 3, respectively. The similarities between pharmacokinetic profiles after co-administration of midazolam and flucloxacillin and that



a. after administration of midazolam alone



b. after co-administration of midazolam and flucloxacillin



c. self-control between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone

Fig. 3: Scatterplot of bilateral statistical moment similarity for 1'-hydroxy midazolam by flucloxacillin. Scatterplot of bilateral statistical moment similarity was drew out 12 section bilateral statistical moment similarities of 1'-hydroxy midazolam by flucloxacillin with any subject to the others as a) after administration of midazolam alone, as b) after co-administration of midazolam and flucloxacillin, as c) self-control similarities between after administration of midazolam alone and after co-administration of midazolam and flucloxacillin.

after administration of midazolam alone are summarized in Table 2 for midazolam and Table 3 for 1'-hydroxy midazolam. In Table 2 and Table 3, the data in the down left corner less than diagonal indicate the bilateral statistical moment similarities after administration of midazolam alone, and the contralateral data up diagonal indicated the bilateral statistical moment similarities after co-administration of midazolam and flucloxacillin. The data on diagonal represent the similarities of the same subject after co-administration of midazolam and flucloxacillin and after administration of midazolam alone. From these data, all the average statistical moment parameters of midazolam and 1'-hydroxy midazolam had no significant difference between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone. The bilateral statistical moment similarities for midazolam ranged from 0.5599 to 0.9898 after administration of midazolam alone, and from 0.5616 to 0.9937 after co-administration of midazolam and flucloxacillin, while self-control bilateral statistical moment similarities ranged from 0.7510 to 0.9882, among which all the similarities were higher than the negative judgement critical value 0.008, and also higher than the positive judgement critical value 0.803 for one part within spacious interval. Consistently for 1'-hydroxy midazolam, the similarity ranges were located within 0.3880, 0.1551, 0.2110 for the minimum and 0.9903, 0.9853, 0.9798 for the maximum after administration of midazolam alone, after co-administration of midazolam and flucloxacillin and on self-control respectively, which also displayed out that higher than the positive critical value within spacious intervals. These results apparently revealed that flucloxacillin might have no significant pharmacokinetic interactions on midazolam metabolized into 1'-hydroxy midazolam on statistics.

### *2.3. Comparison between conventional method and SMS method*

In this paper, linear and nonlinear pharmacokinetic parameters were translated into SM and TQSM parameters (Kakutani et al. 1992; Mayer et al. 1998; Dingemanse et al. 2007), and further converted into normal distribution or standard normal distribution functions, and then *SMS* model, including *SMS*, deviation, variable probability, confidence of probability, first and second moment of a TPP, were developed. Furthermore, the model was verified by the pharmacokinetic interaction between midazolam and flucloxacillin to explore induction to CYP3A by flucloxacillin.

According to the parameters in Table 1, Supplementary Table 2-3 and the statistical analysis of classical pharmacokinetic parameters and SM parameters, it can be concluded that there were no significant effects on midazolam metabolized into 1'-hydroxy midazolam by flucloxacillin. However, on base of the numerical variation of $AUC_{0-\infty}$, $C_{max}$, $t_{max}$, $K_{3\text{-}24}$, $CL_{0-\infty}$, $V_{0-\infty}$, there were still weak pharmacokinetic interactions between midazolam and flucloxacillin within spacious interval with respect to the positive critical probability and the negative critical probability. These parameters were translated into bilateral pharmacokinetic similarities shown in Tables 2 and 3 and illustrated in Figs. 2 and 3. Then bilateral statistical moment similarities between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone were statistically analyzed. For midazolam, only the pharmacokinetic interactions by flucloxacillin took place in subject 3 on statistics ($p$<0.05), while weak interactions took place in subjects 6 and 12 ($p$<0.10), whereas for 1'-hydroxy midazolam these interactions existed in subjects 1, 6, 9 ($p$<0.01) and 12 ($p$<0.05). In contrast, the mean similarities for midazolam and its metabolite 1'-hydroxy midazolam after administration of midazolam alone were in consistent with after co-administration of midazolam and flucloxacillin. The results are displayed on the scatterplot of bilateral statistical moment similarity (Figs. 1 and 2). From Fig. 1, these data points were scattered in well-distributed from similarity about 0.6 to 1 by the comparison of after co-administration of midazolam and flucloxacillin and after administration of midazolam alone. From Fig. 2, while the data points were bad-distributed from less than 0.2 to 1 by the comparison of after co-administration of midazolam and flucloxacillin and after administration of midazolam alone. The mean *SMS* for midazolam between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone was 0.8851±0.0866; deviation was 0.1450; variability probability was 22.49 %; confidence of probability β was 96.53 % under size test *1*-α 0.95 by Eqs. (26)-(28) or looking up Supplementary Table 1b. $AUC_{0-\infty}$ of midazolam were 334.3±348.9 ng•h•mL$^{-1}$ after administration of midazolam alone and 206.9±179.8 ng•h•mL$^{-1}$ after co-administration of midazolam and flucloxacillin. For TPP of midazolam , however, total quantum *SMS* ($SMS_T$) between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone were 0.9582; total deviation was 0.0525; total variable probability was 4.18 %; total confidence of probability was β 97.17 % under size test *1*-α 0.95, respectively. Meanwhile, for 1'-hydroxy midazolam between after co-administration of midazolam and flucloxacillin and after administration of midazolam alone, the mean *SMS* was 0.7015±0.2462; deviation was 0.3830, variable probability was 29.85 %, confidence of probability β 94.25 % under size test *1*-α 0.95, respectively. $AUC_{0-\infty}$ of 1'-hydroxy midazolam were 1364±810.7 ng•h•mL$^{-1}$ after administration of midazolam alone and 1637±632.6 ng•h•mL$^{-1}$ after co-administration of midazolam and flucloxacillin, respectively. For a TPP 1'-hydroxy midazolam, however, $SMS_T$ was 0.6920; the total deviation was 0.396; total variable probability was 30.80 %; total confidence of probability was β 94.10 % with *1*-α 0.95, respectively. As for $AUC_{0-\infty}$ resulted from quantitative changes and

**Table 4: Statistical moment similarity of pharmacokinetic parameters of midazolam and 1'-hydroxy midazolam (, *n*=12)**

| | Similarities | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | |
| | | | | | | | midazolam | | | | | | |
| **Midazolam alone** | 0.8302 ±0.0503 | 0.8610 ±1074 | 0.7164 ±0.1197 | 0.7675 ±0.0869 | 0.8573 ±0.1250 | 0.6829 ±0.1253 | 0.8512 ±0.0735 | 0.8169 ±0.1402 | 0.8510 ±0.1368 | 0.8622 ±0.1118 | 0.8199 ±0.1432 | 0.8507 ±0.1373 | 0.8139 ±0.1309 |
| **Midazolam + flucloxacillin** | 0.8461 ±0.1314 | 0.7708 ±0.1245 | 0.8237 ±0.0402 | 0.7815 ±0.1221 | 0.8350 ±0.1368 | 0.7531 ±0.1456 | 0.7511▲ ±0.1452 | 0.8480 ±0.1083 | 0.8457 ±0.1314 | 0.8511 ±0.1126 | 0.8156 ±0.1357 | 0.7433▲ ±0.1358 | 0.8054 ±0.1273 |
| **Self-control** | 0.8260 | 0.7510 | 0.7643 | 0.9694 | 0.9408 | 0.9204 | 0.7694 | 0.9056 | 0.9797 | 0.9882 | 0.9238 | 0.8825 | 0.8851 ±0.08657 |
| ***SMS*$_T$** | | | | | | | 0.9582 | | | | | | |
| | | | | | | | 1'-hydroxy midazolam | | | | | | |
| **Midazolam alone** | 0.7448 ±0.1766 | 0.7583 ±0.1362 | 0.7687 ±0.1411 | 0.7932 ±0.1546 | 0.5481 ±0.1357 | 0.7890 ±0.1532 | 0.7921 ±0.1722 | 0.7388 ±0.1765 | 0.4918 ±0.1390 | 0.7935 ±0.1695 | 0.7859 ±0.1708 | 0.5528 ±0.1566 | 0.7131 ±0.1923 |
| **Midazolam + flucloxacillin** | 0.3247** ±0.1527 | 0.6937 ±0.2642 | 0.7300 ±0.2440 | 0.6921 ±0.1936 | 0.4880 ±0.1533 | 0.5462** ±0.2383 | 0.7139 ±0.2623 | 0.6485 ±0.2585 | 0.7226** ±0.2384 | 0.7311 ±0.2559 | 0.7329 ±0.2482 | 0.3938* ±0.1759 | 0.6181 ±0.2591 |
| **Self-control** | 0.3625 | 0.9798 | 0.8343 | 0.8085 | 0.2110 | 0.7054 | 0.8665 | 0.6493 | 0.4490 | 0.9379 | 0.9356 | 0.6780 | 0.7015 ±0.2462 |
| ***SMS*$_T$** | | | | | | | 0.6920 | | | | | | |

▲p<0.10; *p<0.05; **p<0.01.

*SMS* from qualitative changes, the comparison of critical value of total confidence of probability β 95% with *1*-α 0.95 were shown in Supplementary Table 1b. There were no significant differences in pharmacokinetic interactions (> positive critical value), i.e. the pharmacokinetic profiles for midazolam were similar with that for 1'-hydroxy midazolam on statistics, indicating that the integral analyses were coincident with the conventional analyses.

In a word, from conventional pharmacokinetic method, it might be concluded that there were no pharmacokinetic interactions of midazolam metabolized into 1'-hydroxy midazolam by flucloxacillin, while from biological individual analyzed by *SMS*, the pharmacokinetic interactions might exist. While by *SMS* method, the TQSM of 12 subjects can be precisely obtained, and $SMS_T$ were 0.9582 for midazolam and 0.6920 for 1'-hydroxy midazolam. Meanwhile, the accurate interaction intensities can be quantitatively analyzed, as $AUC_{0\text{-}\infty}$ for midazolam decreased by 38.11 % and for 1'-hydroxy midazolam increased by 20.01 %, which suggested that flucloxacillin has weak induction to CYP3A consistent with literature reported (Maier-Salamon et al. 2017; Huwyler et al. 2007). From Tables 1-4 and Supplementary Tables 2-3, compared with *SMS* method (Tables 2-4), the conventional methods were non-systematic, inconvenient and easily overlooked the special-sensitive-individual interaction information. In comparison of the average *SMS* and total quantum *SMS* for TPP, for midazolam the average $SMS_u$ was less than $SMS_T$. For 1'-hydroxy midazolam, however, the result was reverse. Because the results for parameter $SMS_T$ were not simply summation of individual pharmacokinetic profiles but the results from weighted calculation. *SMS* can be turned into other parameters, which can be utilized conveniently to evaluate the differences or similarities for multiple pharmacokinetic profiles under ascertainment of the critical values for the actual requirements.

### *2.4. Pharmacokinetic interaction information for the induction to CYP3A*

Flucloxacillin, an isoxazolyl-penicillin, caused cholestasis and biliary epithelium injury. One explanation of the mechanism was the observed drug-drug interaction effects in clinical and *in vitro* that flucloxacillin induced the expression of MDR1 and CYP3A4 in dose-dependent manner (Huwyler et al. 2007). At high concentrations, flucloxacillin activated the human Pregnane-X-Receptor (PXR), and led to a dose-dependent induction of MDR1 as well as of CYP3A4 mRNA to generate metabolites (Takai et al. 2015; Zhang et al. 2007). Among these metabolites, 5'-hydroxymethyl flucloxacillin was one of major metabolites, and on account that it may induce cytotoxicity in susceptible biliary epithelial cells. These metabolic events might contribute to the pathogenesis of drug-induced cholangiopathies (Fernández-Murga et al. 2018). Although taking into consideration the results in this paper, it can be concluded that there were no significant pharmacokinetic interactions between midazolam and flucloxacillin on statistics. In other words, flucloxacillin did not induce MDR1 and CYP3A4, and not generate metabolites accounting for hepatocytotoxicity, however in practice the trends and risks to cytotoxicity existed. $SMS_T$ were 0.9582 for midazolam and 0.6920 for 1'-hydroxy midazolam, while quantitative interaction intensities as $AUC_{0\text{-}\infty}$ decreased by 38.11 % for midazolam and increased by 20.01 % for 1'-hydroxy midazolam. The above data suggested that 95.82 % similar pharmacokinetic behaviour, and only 38.11 % bioavailability decreased for midazolam remained, while 69.20 % of similar pharmacokinetic behaviour and 20.01 % bioavailability in change for 1'-hydroxy midazolam remained. There was at least 30.80 % metabolic behavior changing with 38.11 % metabolic bioavailability decreased, which took effect to the metabolism of flucloxacillin accounting for liver injury in CPY3A4 poor metabolic polymorphisms. At the same time, when drug-drug interaction observed were within interval overflowing positive or negative critical values, it was vitally important to choose which data analytical methods applied to make adequate conclusion, especially on insufficient subject population. If conventional analytical method was selected, single parameter was only given on statistics with missing the important individual different information. If TQSM parameters were applied, the accurate conclusion would be made with TQSM parameters to prevent such cases as the pharmacokinetic interactions of midazolam with flucloxacillin in this paper.

Pharmacokinetics are widely used to evaluate drug bioequivalence (Purohit et al. 2018), to guide drug preparation for pharmacists, and to direct the safety and efficacy of medicals for clinicians. Generally, the evaluation and analytical methods for pharmacokinetic profiles play a vitally important role to make correct judgement and make ideal conclusion. When classical pharmacokinetic parameters applied, such as *AUC*, $C_{max}$, *CL*, $t_{1/2}$ et al, to evaluate

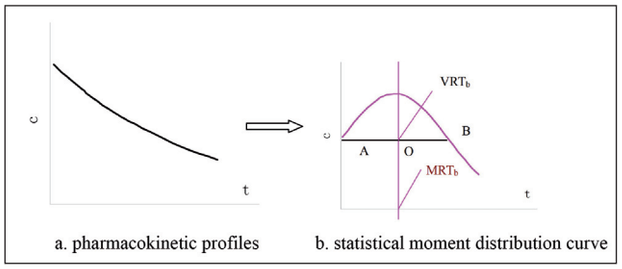



Fig. 4: Statistical moment parameter converted from pharmacokinetic profiles. The modelling process was a translation deal from pharmacokinetic profile (a) to statistical moment distribution curve (b) which includes mainly three parameters as the curve integral along time from time 0 to time infinite (*AUC*); the mean residence time (*MRT*), statistical moment center; the variance (*VRT*), a square of distance between two inflection points of statistical moment distribution curve.

pharmacokinetic behavior, it was easy to make mistakes or lose important information (Hohmann et al. 2016). Meanwhile, the single parameter was interfered easily by experimental conditions and data processing methods. While *SMS* model can integrate pharmacokinetics parameters *MRT* and *VRT* to establish a stable, effective, simple analytical method reflecting precise pharmacokinetic interaction information. However, *SMS* only uses *MRT* and *VRT*, which are time-based pharmacokinetic parameters, and this does not include volume based parameters, such as *CL* and *V*, which is still not very satisfactory to make this similarity model useful.

### *2.5. Conclusion*

In conclusion, pharmacokinetic profiles have been characterized and analyzed by *SMS* method under ascertainment of the critical values for the practical requirements. There was weak induction to CYP3A by flucloxacillin that there was at least 30.80 % metabolic behaviors changed with 38.11 % metabolic bio-availability decrease, which took effect to the metabolism of flucloxacillin accounting for liver injury in CPY3A4 poor metabolic polymorphisms. Based on the study described in this paper, pharmacokinetic/pharmacodynamic model evaluation through *SMS* for single drug or multiple drugs combination systems can be further characterized. However, a more thorough investigation of its properties is needed to determine if *SMS* method is statistically equivalent or superior to the standard statistical tests between two pharmacokinetic parameters.

## 3. Experimental

### *3.1. Statistical moment similarity*

Statistical moment theory is a non-compartmental analysis method. Almost simultaneously, Yamaoka et al. (1978), Cutler (1978), Van Rossum (1978), Benet and Galeazzi (1979), and Von Hattingberg and Brockmeier (1979) recommended the mean residence time (*MRT*) or mean time (*MT*) as an useful characteristic parameter for complex pharmacokinetic systems. Afterwards, Riegelman (1980) introduced the dissolution, release, absorption inside body of drugs with different dosage forms and further elucidated the concept of statistical moment in pharmacokinetics (Martinez et al. 1998; Chan et al 1982; Yamaoka et al. 1978).

#### *3.1.1. Statistical moment for individual drug*

Statistical moment parameters for individual drug include zero moment, first moment and second moment. Primary concept of statistical moment for individual drug is illustrated in Fig. 4. From Fig. 4, the modelling process from pharmacokinetic profile to distribution curve of statistical moment and three main parameters *AUC* (the area under plasma concentration-time curve), *MRT* (the mean residence time), *VRT* (the variance of mean residence time) are visually represented.

*Zero moment.* It is defined as the *AUC*, the integral for concentration-time curve from time 0 to time infinite, and can be calculated by Eq. (1).

$$AUC = \int_0^\infty c dt \tag{1}$$

So there is Eq. (2) for drugs fitting linear model.

$$AUC = \int_0^\infty \sum_{i=1}^{r} M_i e^{-\alpha_i t} dt = \sum_{i=1}^{r} \frac{M_i}{\alpha_i} \tag{2}$$

where, $M_i$ is the constant before the $i^{th}$ exponential item, $\alpha_i$ is the power of the $i^{th}$ exponential item in $r$ compartmental model.

For drugs fitting nonlinear model, Eq. (3) can be obtained.

$$AUC = \frac{k_m c_0}{V_m} + \frac{c_0^2}{2V_m} \tag{3}$$

where, $c_0$ is the initial concentration, $K_m$ and $V_m$ are Michaelis-Menten constants.

*First moment.* It is defined as *MRT* and can be calculated by Eq. (4).

$$MRT = \frac{\int_0^\infty tcdt}{\int_0^\infty cdt} = \frac{AUMC}{AUC} \tag{4}$$

where, *AUMC* is the integral for the product of drug concentration with time-time curve from time 0 to time infinite.

For drugs fitting the $r$ compartment linear model, there is Eq. (5) for *MRT*.

$$MRT = \frac{\sum_{i=1}^{r} \frac{M_i}{\alpha_i^2}}{\sum_{i=1}^{r} \frac{M_i}{\alpha_i}} \tag{5}$$

For drugs fitting nonlinear Michaelis- Menten model, the following Eq. (6) is given.

$$MRT = \frac{2c_0^2 + 9k_m c_0 + 12k_m^2}{V_m(12k_m + 6c_0)} \tag{6}$$

*Second moment.* It is defined as the variance of mean residence time (*VRT*) and can be calculated by Eq. (7).

$$VRT = \frac{\int_0^\infty (t - MRT)^2 cdt}{\int_0^\infty cdt} = \frac{\int_0^\infty t^2 cdt}{\int_0^\infty cdt} - MRT^2 = \frac{AUVC}{AUC} - \left(\frac{AUMC}{AUC}\right)^2 \tag{7}$$

where *AUVC* is the integral for the product of drug concentration with time squared-time curve from time 0 to time infinite.

Similarly, for linear $r$ compartmental model, the calculation of *VRT* is presented by Eq. (8).

$$VRT = 2\frac{\sum_{i=1}^{r} \frac{M_i}{\alpha_i^3}}{\sum_{i=1}^{r} \frac{M_i}{\alpha_i}} - MRT^2 \tag{8}$$

Eq. (9) is for the nonlinear Michaelis- Menten model.

$$VRT = \frac{2c_0^4 + 85k_m^2 c_0^2 + 20k_m c_0^3 + 180k_m^3 c_0 + 144k_m^4}{36V_m^2(2k_m + c_0)^2} \tag{9}$$

#### *3.1.2. Total quantum statistical moment for a total pharmacokinetic profile (TPP)*

Supposing that a total pharmacokinetic profile (TPP) are constituted of $n$ kinds of pharmacokinetic profiles. According to statistical moment theory, the total quantum zero, first, second moment for a TPP can be defined as follows.

*Total quantum zero moment for a TPP ($AUC_T$).* $AUC_T$ can be defined as the area under concentration-time curve from time 0 to time infinite for a TPP and can be calculated by Eq. (10).

$$AUC_T = \sum_{i=1}^{n} AUC_i \tag{10}$$

*Total quantum first moment for a TPP ($MRT_T$).* $MRT_T$ is namely to the residence time on average or mean residence time for a TPP composed of individual pharmacokinetic profiles, and can be calculated by Eq. (11).

$$MRT_T = \frac{\sum_{i=1}^{n} MRT_i \cdot AUC_i}{\sum_{i=1}^{n} AUC_i} \tag{11}$$

*Total quantum second moment for a TPP ($VRT_T$).* $VRT_T$ is defined as the variance of mean residence time for a TPP and can be expressed by Eq. (12).

$$VRT_T = \frac{\sum_{i=1}^{n} \left((MRT_i^2 + VRT_i) \cdot AUC_i\right)}{\sum_{i=1}^{n} AUC_i} - MRT_T^2 \tag{12}$$

It can be seen from Eq. (12) that $VRT_T$ is the variance of $MRT_T$ for a TPP, and can be used to describe the dispersion degree of these individual pharmacokinetic profiles.

#### *3.1.4. SMS for pharmacokinetic profiles*

The two statistical moment (SM) parameters, *MRT* and *VRT*, illustrating the dispersion degree of a pharmacokinetic profile, can be converted into a normal probability density function (Bland 2015; Gifuni et al. 2015). Equation (13) can be obtained according to the concept of normal distribution.

$$F(t) = \int_{-\infty}^{+\infty} \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{(t - \bar{t})^2}{2\sigma^2}\right) dt \quad (-\infty < t < +\infty) \tag{13}$$

where, $t$ is the pharmacokinetic time; $\bar{t}$ is *MRT*, $\sigma^2$ is *VRT*. Supposing that the first moments for two pharmacokinetic profiles respectively are $\bar{t}_A$ and $\bar{t}_B$, and second

moments respectively are $\sigma_a^2$ and $\sigma_b^2$. $t_1$ and $t_2$ are represented as the intersections of the two normal distribution curves and can be obtained by solving Eq. (14).

$$\frac{1}{\sqrt{2\pi}\sigma_a}\exp\left(-\frac{(t-\bar{t}_a)^2}{2\sigma_a^2}\right)=\frac{1}{\sqrt{2\pi}\sigma_b}\exp\left(-\frac{(t-\bar{t}_b)^2}{2\sigma_b^2}\right) \quad (-\infty<t<+\infty) \tag{14}$$

By regulating Eq. (14), Eq. (15) can be obtained.

$$(\sigma_b^2-\sigma_a^2)t^2-2(\bar{t}_a\sigma_b^2-\bar{t}_b\sigma_a^2)t+(\sigma_a^2\sigma_b^2\ln\frac{\sigma_b^2}{\sigma_a^2}+\bar{t}_a^2\sigma_b^2-\bar{t}_b^2\sigma_a^2)=0 \tag{15}$$

Finally, $t_1$ and $t_2$ are given in Eq. (16).

$$t_{1(2)}=\frac{\bar{t}_a\sigma_b^2-\bar{t}_b\sigma_a^2\pm\sqrt{(\bar{t}_a\sigma_b^2-\bar{t}_b\sigma_a^2)-(\sigma_b^2-\sigma_a^2)(\sigma_a^2\sigma_b^2\ln\frac{\sigma_b^2}{\sigma_a^2}+\bar{t}_a^2\sigma_b^2-\bar{t}_b^2\sigma_a^2)}}{\sigma_b^2-\sigma_a^2} \tag{16}$$

SM similarity (*SMS*) of the two normal distribution curves can be defined as the overlapped area for two probability density function curves surrounding with *t-axis* (Fig. 5), and can be calculated by Eq. (17).

$$SMS=1-\left|\int_{t_1}^{t_2}\frac{1}{\sqrt{2\pi}\sigma_a}\exp\left(-\frac{(t-\bar{t}_a)^2}{2\sigma_a^2}\right)dt-\int_{t_1}^{t_2}\frac{1}{\sqrt{2\pi}\sigma_b}\exp\left(-\frac{(t-\bar{t}_b)^2}{2\sigma_b^2}\right)dt\right| \quad (-\infty<t<+\infty) \tag{17}$$

There are three status as follows.

- When $\sigma_a=\sigma_b$, $\bar{t}_a>\bar{t}_b$, there only exist one intersection $t_1$ of two probability density function (Fig. 5a), and Eq. (18) can be given.

$$t_1=\frac{\bar{t}_a+\bar{t}_b}{2} \tag{18}$$

Then, *SMS* can be calculated by Eq. (19).

$$SMS=1-\left|\int_{-\infty}^{t_1}\frac{1}{\sqrt{2\pi}\sigma_a}\exp\left(-\frac{(t-\bar{t}_a)^2}{2\sigma_a^2}\right)dt-\int_{t_1}^{+\infty}\frac{1}{\sqrt{2\pi}\sigma_b}\exp\left(-\frac{(t-\bar{t}_b)^2}{2\sigma_b^2}\right)dt\right| \quad (-\infty<t<+\infty) \tag{19}$$

- When $\sigma_a=\sigma_b$, $\bar{t}_a=\bar{t}_b$, the two normal distribution curves are overlapped completely (Fig. 5b), and the *SMS* is 1.
- When $\sigma_a\neq\sigma_b$, $\bar{t}_a\neq\bar{t}_b$, and $\sigma_a>\sigma_b$, there existed two intersections $t_1$ and $t_2$ of two probability density function (Fig. 5c). And the *SMS* can be calculated by Eq. (17).

### *3.1.5. Standard SMS, deviation, variable probability, positive or negative judgement and critical values for standard normal distribution*

*Standard SMS.* According to the properties of normal distribution (Gifuni et al. 2015), any normal distribution function can be converted into standard type by replacing $\frac{(t-\bar{t})^2}{\sigma^2}$ with $x^2$. Hence, *SMS* also can be turned into standard *SMS* expressed by $SMS_u$ and Eq. (20) can be obtained.

$$SMS_u=1-\left|\int_{x_1}^{x_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx-\int_{x_1}^{x_2}\frac{1}{\sqrt{2\pi}\frac{\sigma_b}{\sigma_a}}\exp\left(-\frac{\left(x-(\frac{\bar{t}_b-\bar{t}_a}{\sigma_a})\right)^2}{2\frac{\sigma_b^2}{\sigma_a^2}}\right)dx\right| \quad (-\infty<x<+\infty) \tag{20}$$

and the two solutions of $x$ are given in Eq. (21).

$$x_{1(2)}=\frac{t_{1(2)}-\bar{t}_a}{\sigma_a} \tag{21}$$

Similarly, $\frac{\left(x-(\frac{\bar{t}_b-\bar{t}_a}{\sigma_a})\right)^2}{\frac{\sigma_b^2}{\sigma_a^2}}$ is replaced by $y^2$, the complete standard type is shown in Eq. (22).

$$SMS_u=1-\left|\int_{x_1}^{x_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx-\int_{y_1}^{y_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{y^2}{2}\right)dy\right| \quad (-\infty<x,y<+\infty) \tag{22}$$

Then, the two solutions of $y$ are given in Eq. (23).

$$y_{1(2)}=x_{1(2)}\frac{\sigma_a}{\sigma_b}-\frac{\bar{t}_b-\bar{t}_a}{\sigma_a} \tag{23}$$

When $\bar{t}_a$, $\bar{t}_b$, $\sigma_a$ and $\sigma_b$ are given, $t_{1(2)}$, $x_{1(2)}$, $y_{1(2)}$ can be calculated correspondingly by Eqs. (16), (21), (23), then the $SMS_u$ value can be calculated by Eq. (22).

*Deviation.* Owing to the conversion of NPDFs into standard NPDFs, the absolute differences of the two converted standard NPDFs can be computed in Eq. (24).

$$\int_{-D}^{D}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{u^2}{2}\right)du=\left|\int_{x_1}^{x_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx-\int_{y_1}^{y_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{y^2}{2}\right)dy\right| \quad (-\infty<x,y,u<+\infty) \tag{24}$$

where, $D$ is defined as the deviation representing the deviate degree of the two converted standard NPDFs. The more $D$ increase, the more the overlapped area decrease oppositely, the same to $SMS_u$ correspondingly. When $x$, $y$ and $u$ are united as one variable $x$, Eq. (24) is represented as follows.

$$\left|\int_{x_1}^{x_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx-\int_{y_1}^{y_2}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{y^2}{2}\right)dy\right|=\int_{D}^{D}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx \quad (-\infty<x<+\infty) \tag{25}$$

By substituting Eq. (25) into Eq. (22), Eq. (26) can be obtained. When $SMS_u$ is given, $D$ can be calculated out.

$$\int_{-\infty}^{-D}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx=\frac{S_u}{2} \tag{26}$$

*Variable probability.* Similarly, the mean variability within interval (*-D*, *+D*) of the two standard NPDF can be defined as the variable probability. From Eq. (22), the relationship between the overlapped area of the two standard NPDFs and *SMS* can be correspondingly turned into the exploration of absolute difference of the two standard NPDFs, one associated with $x$ ($x_{1(2)}$, $x$=0, σ=1), another associated with $y$ ($y_{1(2)}$, $y$=0, σ=1). Therefore, the variable probability of two standard NPDFs within the interval (*-D*, *+D*) is inversely proportional to the overlapped area of the two standard NPDFs and $SMS_u$. Thus, variable probability pertinently expressed by $p_V$ is given in Eq. (27).

$$p_V=\int_{-D}^{D}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx \quad (-\infty<D<+\infty) \tag{27}$$

*Positive or negative judgement.* Similar with the power of a hypothesis testing on statistics, when a positive judgement whether the two NPDFs belong to a same population under test size $u_\alpha$ is made, the probability of type-Π error (β error) not rejecting actual null hypothesis is given as β expressed by Equation (28), and also represented the probability without being different populations, i.e. being a same population. Correspondingly, α is defined as confidence of probability of type-I error (α error) rejecting actual null hypothesis, and decide a negative judgement on being a same population, so-called the power of test. Therefore, confidence of probability *1*-β can be calculated by Eq. (28).

$$1-\beta=\int_{-\infty}^{D-u_\infty}\frac{1}{\sqrt{2\pi}}\exp\left(-\frac{x^2}{2}\right)dx \quad (-\infty<D<+\infty) \tag{28}$$

*Critical values for standard SMS.* Based on Eqs. (20)-(28), under test size $u_\alpha$, these parameters, $SMS_u$, *D*, $p_V$, 1-β, can be calculated for negative and for positive judgement, and finally their critical values can also be ascertained, by which it is convenient to make judgement on similar or different for these pharmacokinetic profiles.

When *D*=1.96, $SMS_u$ would be 0.05 correspondingly, the variable probability $p_V$ is 95 %, while the confidence of probability *1*-β varied with the confidence coefficient α. If a negative judgement was made that the two pharmacokinetic profiles were different, then Supplementary Table 1a should be adopted. When α value is 0.05, *1*-β is 0.5, i.e. there is 50 % confidence of probability to make a judgement on 95 % of two samples are different, commonly it can be considered as a critical value to a negative conclusion that two samples are from different populations, as the routine requirement that *1*-β value is more than 0.75, and $SMS_u$ is less than 0.008 (α=0.05, significance level) or 0.001 (α=0.01). If a positive judgement was made that the two pharmacokinetic profiles were similar, Supplementary Table 1b should be adopted. When *1*-α is valued 0.95, β 0.95, $SMS_u$ 0.803, i.e. there is 95 % confidence of probability to make a judgement on 95 % of two samples are similar, commonly it can be considered as a critical value to a positive conclusion that two samples were from a same population, as the routine requirement that *1*-α value was less than 0.95, β value *is* more than 0.90, $SMS_u$ is more than 0.803, then there is 90 % confidence of probability to make a judgement on 95 % of two samples are similar. Within 25 %-75 % confidence of probability, a precise judgement would be made that two samples are from a same population, meanwhile the risk of these conclusions can also be estimated.

## *3.2. Experimental design*

### *3.2.1. Study drug and apparatus*

Standard substances midazolam hydrochloride (Sigma-UC429), 1'-hydroxy midazolam (Sigma-UC430) and paracetamol sulfate potassium salt (Sigma-UC448) were respectively dissolved in methanol to produce a 2.5, 5.0, 50 $\mu$g•mL$^{-1}$ stock solution which were then stored at +4 °C until analysis. Midazolam tablets (Shanghai Roche pharmaceutical L. M.) and flucloxacillin tablets (Guangzhou pharmacy valley biology medicine L. C.) were administered at ambient temperature according to the experimental protocol.

### *3.2.2. Study subjects and trial protocol*

Twelve healthy Chinese male volunteers (mean age 25.3±2.1 years, mean weight 60.4±5.1 kg) were enrolled in the study. The study protocol was approved by the Ethical Committee of Xiangya School of Medicine of Central South University and all subjects had been gave written informed consent for participating in the study. All subjects were in good health indicated by medical history, routine physical examination, and biochemical testing and they were nonsmokers and ate a normal diet. All subjects were asked to abstain from alcohol, caffeine, and grapefruit juice for a week before the study. After 12 h fast, subjects received a single oral dose of midazolam (7.5 mg) in the morning on Day 1. From Day 2 to Day 8, they received flucloxacillin 2.0 g each day (500 mg every 6 h per day). On Day 9, subjects received a single oral dose of midazolam (7.5 mg) and a single oral dose of flucloxacillin (500 mg) concomitant. Blood samples (2 mL each) were immediately collected on Day 1 and 9 prior to dosing (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after dosing. The blood samples were stored at -20 °C prior to pharmacokinetic analysis.

### *3.2.3. Analytic technique*

Midazolam and metabolite 1'-hydroxy midazolam were determined by the modification High Performance Liquid Chromatography (HPLC) method described by Zhu et al. (2001). After the addition of 100 $\mu$L of 50 $\mu$g•L$^{-1}$ paracetamol as internal standard and 1 mL of buffer glycine (0.75 mol•L$^{-1}$, pH 9), 1 mL of plasma was extracted with 4 mL diethyl ether. The organic phase was evaporated to dryness, the residue was dissolved in 50 $\mu$L of mobile phase, and 20 $\mu$L was injected into HPLC for analyzing. Midazolam and 1'-hydroxy midazolam were separated on a C18 column (4.6 mm × 250 mm, 5 $\mu$m; Grace Davison Discovery Sciences™). The composition of the mobile phase was 32 % acetonitrile/3 % methanol/65 % 0.1 mol•L$^{-1}$ buffer acetate (vol/vol/vol) (pH 4.34). The flow rate through the column at 35 °C was 1.0 mL•min$^{-1}$. Midazolam and 1'-hydroxy midazolam were monitored by ultraviolet absorbance at 254 nm. The chromatographic peaks for midazolam and 1'-hydroxy midazolam were completely separated. The within-day and between-day coefficients of variation for each compound of the assay were <10 %.

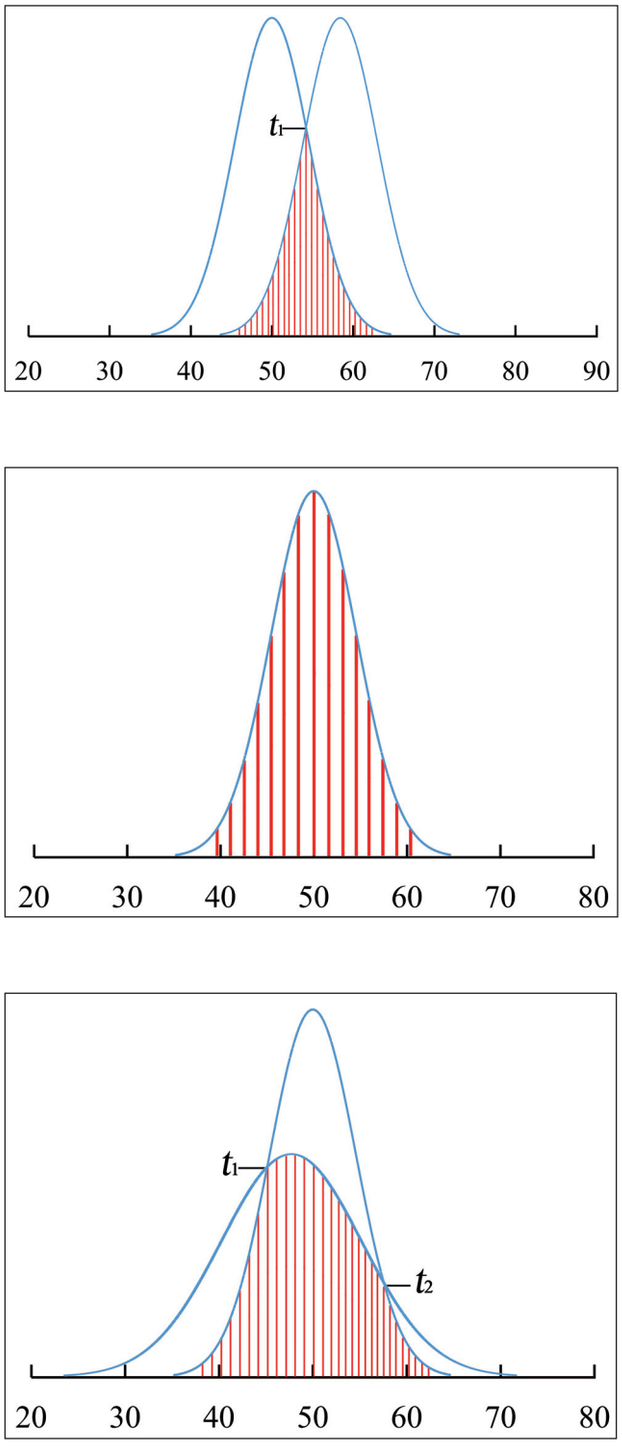



Fig. 5: Intersections and similarity for two probability density function in conversion of their pharmacokinetic curves. Statistical moment similarity was defined as area overlapped by two normal distribution probability density function curve, illustrated out solidus shadow parts with three situation: a. one intersection when equal deviation; b. complete overlap when equal mean and deviation; c. two intersections when different mean and deviation.

### *3.3. Pharmacokinetic analysis*

Pharmacokinetic parameters for midazolam and 1'-hydroxy midazolam were estimated using conventional pharmacokinetic method, and these pharmacokinetic profiles were analyzed with the DAS computer programs (State Food and Drug Administration, P. R. China). At the same time, these profiles were also analyzed with *SMS* method established in this paper according to Eqs. (1)-(28). The $C_{max}$ value at $t_{max}$ (the time to $C_{max}$) were estimated as the actually-determined concentrations after the beginning of 15 min of the administration, and the $K$ value estimated as the regression-estimated logarithm of concentrations versus time from time 3 h to time 24 h. The $AUC_{0-\infty}$, $AUMC_{0-\infty}$ and $AUVC_{0-\infty}$ were calculated by estimating plasma concentration profiles from time 0 to time 24 h according to the linear trapezoidal rule and extrapolated from time 24 h to time infinite by reference (Barnett et al. 2014) with Microsoft Excel 2007. The pharmacokinetic parameters $AUC_{0-\infty}$, $C_{max}$, $t_{1/2}$, $K_{3\text{-}24}$, $CL_{0-\infty}$ of midazolam and 1'-hydroxy midazolam were transformed using a natural logarithmic transformation and analyzed using ANOVA thereby to assess the effect of flucloxacillin on the pharmacokinetics of midazolam and 1'-hydroxy midazolam. The deviations of pharmacokinetic parameters of midazolam and 1'-hydroxy midazolam were calculated by the differences of the values (after co-administration of midazolam and flucloxacillin) subtracting the values (after administration of midazolam alone), further divided by the values (after administration of midazolam alone).

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