Concomitant heart and peripheral blood determinations were performed on 40 fatal cases involving nordiazepam (20 cases) and bromazepam (20 cases). The heart blood concentration for the two drugs (588 ng/mL for nordiazepam and 802 ng/mL for bromazepam) does not differ from the corresponding peripheral blood concentration (587 ng/mL for nordiazepam and 883 ng/mL for bromazepam). The mean ratios for the heart and peripheral blood concentrations were 0.95 for nordiazepam and 0.86 for bromazepam. No postmortem redistribution was observed for these two benzodiazepines. The authors thus suggest that corresponding heart blood can be proposed in the quantitative analysis of these drugs when peripheral blood is unavailable. The present study also shows the stability of the two drugs after a year of storage.

Introduction

Postmortem forensic toxicology has disclosed many fatal poisonings each year in France, in which benzodiazepines are often involved. Nordiazepam (Nordaz®) and bromazepam (Lexomil®, Quietiline®) are both appertaining to the benzodiazepine drugs (Figure 1). Nordiazepam is a metabolite of several benzodiazepines such as clorazepate dipotassium (Tranxene®) (1), prazepam (Lysanxia®) (2), diazepam (Valium®) (3), and chlordiazepoxide (Librium®) (4). In vivo, clorazepate dipotassium and prazepam are unstable and rapidly converted to nordiazepam (1,2). The presence of nordiazepam in blood can be due to the absorption of nordiazepam itself and/or its precursors. The study of nordiazepam can include the treatment by the drug or by its precursors. Blood is essential for evaluating whether the deceased was under the influence of drugs at the time of death. Heart blood and peripheral blood are often used in analytical toxicology. Postmortem drug concentrations may vary according to the sampling site and the interval between death and specimen collection (5). These site and interval-dependent variations are called postmortem redistribution (5). Previous studies have demonstrated that for some, but not all, drugs, there are important differences in concentrations between peripheral and corresponding heart blood. The drug concentrations in the heart blood are often higher than in the peripheral blood (6) and do not reflect concentration at the time of death. The heart blood concentrations of certain drugs such...
as digoxine or tricyclic antidepressants are still greater than
those of the peripheral blood, even when both samples are si-
multaneously collected (7). Postmortem heart blood is a mi-
ture of cardiac, pulmonary, and liver blood and is
overwhelmingly influenced by redistribution from the largest
organs, the lungs and liver (8). The mechanisms involving
postmortem redistribution have been described by Hilberg and
co-workers (9–11) and have been the subject of a more ex-
tensive review (5). Drugs that undergo postmortem redistribu-
tion are typically lipophilic weakly basic compounds with a
relatively large volume of distribution or preferential binding to
the myocardium (5,11). The tricyclic antidepressants are
lipophilic drugs with a volume of distribution particularly ele-
vated, in mean of 10–42 L/kg (12) and have tendency to bind the
myocardium and other viscera. Bromazepam and nordiazepam
are both lipophilic compounds. However, the volume of distribu-
tion of each was not large, 0.9 L/kg for bromazepam (12) and
1.1–1.7 L/kg for nordiazepam (12). It seems that the distribu-
tion of the two drugs to the internal organs was unimportant.
The peripheral blood, particularly the femoral blood, is the
specimen of choice for quantitative analysis, because it is the
least susceptible to postmortem redistribution (7). In general,
the heart blood is reserved for qualitative analysis because it
may facilitate detection.

The present study was undertaken to follow-up a previous
observation concerning the postmortem redistribution phe-
nomenon of two benzodiazepines (nordiazepam and bro-
mazepam). The study is based on the comparison between
peripheral and heart blood, collected simultaneously from the
same subject. Without any antemortem specimen and seeing
that peripheral blood is a specimen of choice for quantitative
purposes, this will be considered as the reference.

We wanted to know if a cardiac blood sample could be sub-
stituted for peripheral blood when this is unavailable or scant
in the quantitative analysis of nordiazepam and bromazepam.
Our study was limited to these two main compounds. The
findings concerning their respective active metabolites, ox-
azepam (13), 3-hydroxybromazepam (14), and certain precu-
sors of nordiazepam, are not yet studied.

Materials and Methods

Population and protocol study

Blood samples for concomitant cardiac-peripheral blood
drug concentration determinations were obtained from 40
fatal cases involving nordiazepam or bromazepam. Twenty
cases for nordiazepam [14 men (M) and 6 women (W)] and 20
cases also for bromazepam [12 men (M) and 8 women (W)] en-
tered the trial. All fatal cases were from the southeast of France
and autopsied during 2005 and 2006 at the department of
Forensic Medicine, University of Aix-Marseille. All samples
taken for forensic toxicology were analyzed at the Toxicology
Laboratory of National Institute of Scientific Police (Marseille,
France). Each case was required by the study protocol to have
paired cardiac and peripheral blood samples available. These
samples were collected from the same subject (thus elimi-
nating interindividual variation due to sex, age, height, weight,
treatment) and simultaneously (thus reducing inherent vari-
ation time intervals between death and sampling).

Blood samples

The femoral venous and heart bloods were taken into
tubes containing sodium fluoride (1% NaF) to prevent micro-
bial degradation. The samples were stored at −10°C until analy-
sis. Some benzodiazepines show variable changes in the
immediate postmortem period. The nitrobenzodiazepines are
very unstable in postmortem blood (15). Diazepam and
temazepam may deteriorate in badly putrefying tissues (15). As
for nordiazepam and bromazepam, no information was avail-
able about the lability of the drugs in postmortem blood sam-
plies. The stability of the two drugs was tested under the
conserving condition in blank blood spiked with the analyzed
drugs. No degradation was observed in at least one year of
conservation.

Drug determination

Nordiazepam and bromazepam were determined in 1 mL of
blood submitted to routine drug analysis (16). Clotiazepam
was utilized as internal standard. After extraction by Toxi-
Tube A® (extraction tube), the extracts were injected into a
liquid chromatograph (Waters Alliance HPLC system, Mil-
ford, MA). The system consisted of a 2695 separation module
(integrated solvent and sample management platform), a 996
diode array detector, and Millenium 3.2 software for the in-
strument control and signal processing. A Symmetry C8
column (25 cm × 4.6-mm i.d., 5-µm particle size, 30°C tem-
perature) was eluted with a gradient of acetonitrile, methanol,
and 0.1 M ammonium acetate, delivered at a flow rate of 1
mL/min. The detector was monitored at 235 nm. The reten-
tion times of bromazepam, nordiazepam, and clotiazepam
were 13, 23.1, and 30.5 min, respectively. The UV spectrum of

Figure 2. Application of the routine method (16) for the analysis of ben-
zodiazepines. Chromatogram obtained from a blank blood (1 mL),
spiked with the studied drugs and their metabolites and precursors.
Peak identification: 1, 3-hydroxybromazepam (200 ng); 2, bromazepam
(200 ng); 3, oxazepam (2000 ng); 4, chlordiazepoxide (200 ng); 5, nor-
diazepam (200 ng); 6, diazepam (200 ng); and 7, clotiazepam, internal
standard (1000 ng).
the analyzed drugs was scanned between 200 and 350 nm. Nordiazepam and bromazepam are respectively metabolized to active metabolites oxazepam (13) and 3-hydroxybromazepam (14). The procedure proved selective as shown in the chromatogram of Figure 2, towards either of the metabolites, oxazepam (18.6 min), 3-hydroxybromazepam (9.5 min), and the precursors of nordiazepam like diazepam (26.4 min) and chlordiazepoxide (20.8 min). Other precursors of nordiazepam, clorazepate dipotassium and prazepam, are generally undetected in blood (1,2). For both bromazepam and nordiazepam, the method was linear up to 1000 ng/mL with a limit of quantification of 20 ng/mL. For levels higher than 1000 ng/mL, the analyzed sample should be diluted. Paired cardiac-peripheral blood samples were analyzed in duplicate in the same day, thus reducing inherent interday variation. Intra- and interday error percents of the method used were less than 10%.

### Statistical methods

Drug concentration in cardiac blood (CB) was compared with drug concentration in peripheral blood (PB), which was considered the reference. If CB is higher than PB (CB > PB), the postmortem redistribution occurs. If CB is lower than PB (CB < PB) or CB is similar to PB (CB = PB), the postmortem redistribution does not occur. When CB is lower than PB (CB < PB), other phenomena can be involved; the result will be discussed. Statistical analysis utilized paired Student’s t-test for raw CB–PB difference (n = 20), percent difference CB–PB(% (n = 20), and CB/PB ratio (n = 20). The relationship between CB and PB of each drug was established by a regression equation y = bx + a where y = PB and x = CB. The CB/PB ratio from each case is an intrasubject coefficient of postmortem redistribution for which 1 was considered as the referee of match.

### Table I. Blood Concentrations of Nordiazepam (ng/mL) From 20 Lethal Cases: Statistical Results

<table>
<thead>
<tr>
<th>Case</th>
<th>CB* n1 = 20</th>
<th>PB† n2 = 20</th>
<th>CB*–PB† n = 20</th>
<th>CB*–PB† (%)‡ n = 20</th>
<th>CB*/PB† n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>1420 ± 587 ± 153 ± 10.7 ± 1.12 ±</td>
<td></td>
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<tr>
<td>2 M</td>
<td>161 ± 197 ± –36 ± –22.4 ± 0.81 ±</td>
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<tr>
<td>3 M</td>
<td>125 ± 131 ± –6 ± –4.8 ± 0.95 ±</td>
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</tr>
<tr>
<td>4 M</td>
<td>152 ± 157 ± –5 ± –3.6 ± 0.96 ±</td>
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</tr>
<tr>
<td>5 M</td>
<td>118 ± 170 ± –51 ± –43.5 ± 0.69 ±</td>
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</tr>
<tr>
<td>6 M</td>
<td>93 ± 81 ± 11 ± 12.3 ± 1.14 ±</td>
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<tr>
<td>7 M</td>
<td>246 ± 333 ± –87 ± –35.1 ± 0.74 ±</td>
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<tr>
<td>8 M</td>
<td>328 ± 328 ± 0 ± 0 ± 1.0 ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 M</td>
<td>709 ± 695 ± 14 ± 1.9 ± 1.02 ±</td>
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<td></td>
</tr>
<tr>
<td>10 M</td>
<td>446 ± 390 ± 56 ± 12.5 ± 1.14 ±</td>
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<tr>
<td>11 M</td>
<td>2736 ± 2719 ± 17 ± 0.6 ± 1.01 ±</td>
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</tr>
<tr>
<td>12 M</td>
<td>334 ± 371 ± –37 ± –11.2 ± 0.89 ±</td>
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<tr>
<td>13 M</td>
<td>187 ± 198 ± –11 ± –5.9 ± 0.94 ±</td>
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<tr>
<td>14 M</td>
<td>892 ± 924 ± –32 ± –3.5 ± 0.96 ±</td>
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</tr>
<tr>
<td>15 W</td>
<td>2540 ± 2410 ± 129 ± 5.1 ± 1.05 ±</td>
<td></td>
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<tr>
<td>16 W</td>
<td>310 ± 347 ± –37 ± –11.8 ± 0.89 ±</td>
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<tr>
<td>17 W</td>
<td>99 ± 118 ± –19 ± –18.9 ± 0.84 ±</td>
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<tr>
<td>18 W</td>
<td>224 ± 233 ± –9 ± –4.1 ± 0.96 ±</td>
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<tr>
<td>19 W</td>
<td>259 ± 272 ± –13 ± –5.2 ± 0.95 ±</td>
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<tr>
<td>20 W</td>
<td>377 ± 403 ± –26 ± –6.9 ± 0.93 ±</td>
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</tr>
<tr>
<td>Mean</td>
<td>588 ± 587 ± 0.51 ± –6.68 ± 0.95 ± (SD) (771) (738) (56) (14.11) (0.12)</td>
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</tr>
</tbody>
</table>

Student’s t-test results comparing CB*–PB† ≠ 0, paired t-test comparing CB*–PB† ≠ 0, paired test comparing CB*/PB† ≠ 1, p(tss) < 0.05§, p < 0.968#, p < 0.0071§, p < 0.000028#, t = 0.04, t = –2.06, t = –1.93, df = 19, df = 19, df = 19.

* Cardiac blood concentration.
† Peripheral blood concentration.
‡ (CB*–PB†)/CB* × 100.
§ p(tss): threshold statistical significance.
# Significant difference, assuming the hypothesis of CB* = PB†.

### Table II. Blood Concentrations of Bromazepam (ng/mL) From 20 Lethal Cases: Statistical Results

<table>
<thead>
<tr>
<th>Case</th>
<th>CB* n1 = 20</th>
<th>PB† n2 = 20</th>
<th>CB*–PB† n = 20</th>
<th>CB*–PB† (%)‡ n = 20</th>
<th>CB*/PB† n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>441 ± 505 ± –63 ± –14.4 ± 0.87 ±</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 M</td>
<td>406 ± 592 ± –186 ± –45.7 ± 0.68 ±</td>
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</tr>
<tr>
<td>3 M</td>
<td>110 ± 118 ± –8 ± –7.4 ± 0.93 ±</td>
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</tr>
<tr>
<td>4 M</td>
<td>325 ± 311 ± 14 ± 4.4 ± 1.04 ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 M</td>
<td>61 ± 81 ± –20 ± –32 ± 0.75 ±</td>
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<td></td>
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</tr>
<tr>
<td>6 M</td>
<td>61 ± 90 ± –29 ± –48 ± 0.67 ±</td>
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<tr>
<td>7 M</td>
<td>61 ± 618 ± –6 ± –9 ± 0.99 ±</td>
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</tr>
<tr>
<td>8 M</td>
<td>453 ± 488 ± –35 ± –7.8 ± 0.92 ±</td>
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<tr>
<td>9 M</td>
<td>795 ± 880 ± –85 ± –10.6 ± 0.90 ±</td>
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</tr>
<tr>
<td>10 M</td>
<td>1902 ± 2159 ± –256 ± –13.5 ± 0.88 ±</td>
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</tr>
<tr>
<td>11 M</td>
<td>128 ± 158 ± –31 ± –24.1 ± 0.81 ±</td>
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</tr>
<tr>
<td>12 M</td>
<td>2524 ± 3050 ± –526 ± –20.8 ± 0.83 ±</td>
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</tr>
<tr>
<td>13 W</td>
<td>53 ± 81 ± –28 ± –53.6 ± 0.64 ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 W</td>
<td>1775 ± 1665 ± 110 ± 6.2 ± 1.06 ±</td>
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</tr>
<tr>
<td>15 W</td>
<td>79 ± 128 ± –49 ± –61.6 ± 0.61 ±</td>
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<tr>
<td>16 W</td>
<td>976 ± 1090 ± –114 ± –11.7 ± 0.89 ±</td>
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<tr>
<td>17 W</td>
<td>240 ± 254 ± –14 ± –6 ± 0.94 ±</td>
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</tr>
<tr>
<td>18 W</td>
<td>294 ± 317 ± –85 ± –28.7 ± 0.90 ±</td>
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</tr>
<tr>
<td>19 W</td>
<td>3312 ± 3570 ± –258 ± –7.8 ± 0.93 ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 W</td>
<td>1486 ± 1509 ± –22 ± –1.5 ± 0.99 ±</td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>802 ± 883 ± –84.6 ± –19.28 ± 0.86 ± (SD) (926) (1018) (136) (19.23) (0.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Student’s t-test results comparing CB*–PB† ≠ 0, paired test comparing CB*–PB† ≠ 0, paired test comparing CB*/PB† ≠ 1, p(tss) < 0.05§, p < 0.968#, p < 0.0071§, p < 0.000028#, t = –2.78, t = –4.37, t = –4.73, df = 19, df = 19, df = 19.

* Cardiac blood concentration.
† Peripheral blood concentration.
‡ (CB*–PB†)/CB* × 100.
§ p(tss): threshold statistical significance.
# Significant difference, assuming the hypothesis of CB* = PB†.
Results

The findings from the subjects for nordiazepam and bromazepam are listed in Tables I and II, respectively.

For nordiazepam, there is no significant difference between CB and PB for every test (p < 0.968, p < 0.055, and p < 0.07, respectively, Table I). Thus CB is similar to PB (PB = PB). No postmortem redistribution was observed.

For bromazepam, there is a significant difference between CB and PB for every test (p < 0.0071, p < 0.00001, and p < 0.000028, respectively, Table II). Nevertheless, the t value of each test was negative (t = –2.78, t = –4.37, t = –4.73, respectively). Thus, CB is lower than PB (CB < PB). No postmortem redistribution was observed. This result will be discussed.

Discussion

In both of these studies, an important interindividual variation in heart and femoral blood concentrations was noted. The standard deviation was higher than its average (Tables I and II). It is evident that this variation would be due to individual factors (sex, age, corporeal surface, treatment, and dosage) from the different subjects. However, the comparison of drug concentrations in heart and femoral blood inherent in each subject gave similar concentrations between these two specimens.

For nordiazepam, the result suggested that CB does not differ from PB (mean: 588 ± 587 ng/mL); no postmortem redistribution was observed.

For bromazepam, CB is lower than PB (mean: 802 < 883 ng/mL, percent difference = 9.2%); no postmortem redistribution occurred. This result (CB < PB) could be due to the instability of bromazepam in the cardiac blood specimen during the storage, where the drug is reduced. Nevertheless, this cannot be confirmed because of the percent difference CB–PB(% for bromazepam (in the range of –6.1% to 6.2%, Table II), may be considered to fall within assay error (10%) of the used method.

For nordiazepam, the blood levels encountered in therapy can be of a wider range, 20–800 ng/mL, and toxic levels ranged from 1500 to 2000 ng/mL (17). Based on these reference values, there are three cases (1, 11, and 15, Table I) that could be in the toxic range. The concentrations of the drug in the cardiac blood, 1420, 2736, and 2540 ng/mL, respectively, are higher than in peripheral blood, 1268, 2719, and 2410 ng/mL, respectively. The corresponding CB/PB ratios were higher than the unity: 1.12, 1.01, and 1.05, respectively (Table I). All cases (100%) reflected the phenomenon. It is possible that the redistribution phenomenon tended to be positive when blood concentrations of nordiazepam were higher than the therapeutic range. It would be useful to investigate more cases with higher concentrations of the drug.

For bromazepam, the toxic blood concentrations were, in general, observed at concentrations higher than 300 ng/mL (17). There are 12 cases of bromazepam (1, 2, 4, 7, 8, 9, 10, 12, 14, 16, 19, and 20, Table II) in the toxic range (17). Among these 12 cases, there are only 2 cases, 4 and 14 (16.7%), in which the cardiac blood concentrations were higher than peripheral blood concentrations (Table II).

There is a good linear relationship with a positive slope between CB and PB in the range of cardiac blood: 93–2736 ng/mL for nordiazepam (r = 0.998) (Table III) and 53–3312 ng/mL for bromazepam (r = 0.995) (Table III). The equations may be written in the form:

\[
\begin{align*}
PB &= 0.954 \times CB + 26.30 \text{ (nordiazepam)} \\
PB &= 1.08 \times CB + 6.58 \text{ (bromazepam)}
\end{align*}
\]

However, for bromazepam, the difference between these two specimens was statistically significant, but only slightly higher in favor of the femoral blood concentration. This small excess of drug concentration in the femoral blood (within 10%) could be considered to be due to an error in the method. Thus, the regression equation of bromazepam can be reliable to predict the femoral blood concentration, though the correlation was less good than that of nordiazepam.

In conclusion, no postmortem redistribution was observed for the two drugs. For nordiazepam, it could be that the phenomenon occurs at the toxic concentrations. For bromazepam, the slight difference in drug concentrations between these two specimens could be attributed to an error in the method used. It is obvious that the utility of cardiac blood in the measurement of blood levels of drugs cannot be satisfactorily resolved when only this specimen is available. We suggest that cardiac blood can be proposed when peripheral blood is unavailable or scant for the quantitative analysis of nordiazepam or bromazepam. Therefore, toxicologists need to be clear and cautious about this phenomenon. Investigation must be extended to a larger number of cases. The study also found that the concentration of the two drugs was stable after a year of storage.

References

2. A.C. Moffat, M.D. Osselton, and B. Widdop. Clarke’s Analysis of Drugs and Poisons in Pharmaceutica, Body Fluids and Post-

Table III. Pearson Correlation (r) and Slope (b) Values for Cardiac-Peripheral Blood Regression of Nordiazepam and Bromazepam

<table>
<thead>
<tr>
<th>Compound</th>
<th>y* = bx + a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordiazepam</td>
<td>y = 0.954x + 26.3, r = 0.998</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
</tr>
<tr>
<td>Bromazepam</td>
<td>y = 1.08x + 6.58, r = 0.995</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
</tr>
</tbody>
</table>

* y: peripheral blood concentration.
* x: cardiac blood concentration; 93–2736 ng/mL (nordiazepam) and 53–3312 ng/mL (bromazepam).


