Concentration Ratios of Methamphetamine to Amphetamine in Blood Can Help to Distinguish Use of Methamphetamine from Various Mixtures of the Two Stimulants

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Using a forensic toxicology database, the authors investigated cases of driving under the influence of drugs (DUID) if methamphetamine (MA) was identified in the blood samples (N = 9,310). The concentrations of MA and amphetamine (AM) in blood were determined after liquid–liquid extraction by gas chromatography–mass spectrometry at limits of quantitation of 0.03 mg/L for both stimulants. In 814 cases, AM was negative in blood and MA was positive at mean (median) and highest concentrations of 0.19 mg/L (0.11 mg/L) and 3.4 mg/L, respectively. Both amines were present in blood in 8,496 cases at concentrations of 0.54 mg/L (0.35 mg/L) and 10.4 mg/L for AM and 0.41 mg/L (0.22 mg/L) and 5.6 mg/L for MA. However, the correlation between AM and MA was low and insignificant (r = −0.13) in the whole material. The coefficient of correlation increased to r = 0.41 (P < 0.001) when the MA/AM concentration ratio was >1. When MA/AM ratios were selected at intervals of 1.0 (e.g., >3.0 and <4.0 up to >9.0 and <10.0), the correlation between AM and MA was r = 0.99 (P < 0.001). Such cases represent the use of MA without contamination from AM, and the mean (median) and highest concentrations of this secondary amine in blood of DUID suspects were 0.72 mg/L (0.56 mg/L) and 4.2 mg/L, respectively.

Introduction

Amphetamine (AM) and methamphetamine (MA) are major drugs of abuse in Sweden, other Nordic countries and worldwide, and these central stimulant amines are frequently identified in blood samples from people arrested for driving under the influence of drugs (DUID) (1–3). MA is the preferred stimulant of abuse in Australia and throughout the United States, as verified by drug seizures, epidemiologic surveys and toxicology reports (4–6). This contrasts with the situation in Sweden and other Nordic countries, where AM is the major recreational drug of abuse (7, 8).

Experiments on the disposition and fate of AM and MA in the body verifies that 30–40% of the ingested dose is excreted unchanged in the urine (9, 10). Other studies show that approximately 5–7% of MA undergoes N-demethylation to AM as the primary metabolite, whereas AM is not metabolized into MA (11–13). Accordingly, after intake of pure MA, the concentration ratio of MA/AM should be greater than unity (13). The elimination half-lives of AM and MA from blood or plasma are relatively long (8–20 h), partly depending on urinary pH. Alkaline urine facilitates reabsorption of the free drug, which delays renal clearance and increases the elimination half-life from blood or plasma (14).

In toxicologic investigations, it is not always obvious whether a person has taken MA alone or taken a mixture of MA and AM or one of the stimulants containing the other as an impurity (1, 2). If AM is positive and MA is negative in blood, this offers compelling evidence for recreational use of AM, because the primary amine is not metabolized into MA. If the concentration of AM in blood is high and the concentration of MA is low, this suggests abuse of AM that might have contained MA as a minor impurity.

Blood samples containing roughly equal concentrations of the two amines suggest that a person had co-ingested the two stimulants or used them interchangeably. Cases with negative AM and positive MA in blood might mean very recent use of MA before much N-demethylation has occurred. Alternatively, residual amounts of MA might have been present in blood after the AM metabolite has decreased below the limit of quantitation (LOQ) of the analytical method, because the plasma elimination half-lives of both amines are approximately the same (15).

The aim of this study was to investigate the concentration relationships between AM and MA in blood samples from people arrested in Sweden for DUID and to present the correct descriptive statistics and relative frequency distribution of concentrations of MA without contamination from AM.

Materials and Methods

Drug-impaired drivers

Sweden has a population of approximately 9.3 million, and blood samples from people apprehended by the police for DUID are sent to one central laboratory for toxicological analysis. The analytical results, along with demographics of the offenders, are entered into a national forensic toxicology database (TOXBASE).

Blood from DUID suspects are received in 10 mL evacuated tubes containing a mixture of sodium fluoride and potassium oxalate as preservatives. The time between arrest and the taking of blood varies from case to case, but on the average is approximately 30–90 min after making an arrest.

This forensic database contained 9,310 cases with MA verified positive in blood. In 814 cases, AM was reported as negative (<0.03 mg/L). The remaining 8,496 cases contained both AM and MA verified as present in blood samples. Tens of thousands of DUID cases in Sweden contain AM as the only stimulant in blood, and these cases have been reported elsewhere (16).

Analysis of AM and ME in blood

Quantitative analysis of AM and MA in blood was conducted by liquid–liquid extraction with ethyl acetate after adding deuterium labeled internal standards and adjusting to an alkaline pH. Before analysis by gas chromatography–mass spectrometry (GC–MS), the trifluoroacetic acid (TFAA) derivatives of AM and

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MA were prepared. The quantifying ions for selected ion monitoring were \textit{m/z} 118 and 126 for AM and its \textit{d}_8\text{-analogue, and \textit{m/z} 154 and 161 for MA and its \textit{d}_8\text{-analogue\textsuperscript{[16]}}.}

Calibration curves were linear from 0.02 to 2 mg/L and the LOQ for both amines in blood in routine case work, such as DUdU, was 0.03 mg/L. If the concentration of AM or MA exceeded 2 mg/L, the analysis was repeated with a smaller volume of blood, after dilution with drug-free blood before the extraction and analysis\textsuperscript{[16]}.

### Statistical methods

Mean and medians were used as descriptive statistics for concentrations of MA and AM in blood. Because the frequency distributions of the concentrations were skewed, two medians were compared by the Mann–Whitney \textit{U} test. The ages of men and women were compared and contrasted by Student’s \textit{t}-test. The proportions of male to female users of central stimulants were evaluated by a chi-squared test. The strength of the correlation between AM and MA concentrations in blood was calculated and reported as the Pearson’s correlation coefficient.

### Results

#### Demographics of methamphetamine users

Table I presents mean age \[\pm\] standard deviation (SD]) and gender of MA users arrested in Sweden for DUdU, and these data verify a clear predominance of male offenders at 84–92\% (\(P<0.001\)). The bulk of all traffic delinquents were in their mid- to late-30s when both AM and MA were verified present in blood samples.

#### Positive MA and negative AM in blood

Table I shows that there were 814 cases in which AM was negative (\(<0.03\text{ mg/L}) and MA was positive in blood. The mean (median) and highest concentrations of MA in these AM negative cases were 0.19 mg/L (0.11 mg/L) and 3.4 mg/L (Table I).

#### Positive MA and AM in blood

In 8,496 cases, both AM and MA were positive (\(>0.03\text{ mg/L}) and descriptive statistics for the concentrations are reported in Table I. The median concentration of AM was higher (\(P<0.001\)) than that of MA (0.35 versus 0.22 mg/L), which is not compatible with intake of pure MA. The skewed nature and appreciable overlap in the relative frequency distributions of AM and MA concentrations in blood are evident from Figure 1.

Because both amines are stimulants of the central nervous system, the total concentration (AM + MA) might be more relevant when questions about impairment and toxicity arise in forensic casework. In this present case series (\(N=8,496\)), the mean (median) and highest concentration (AM + MA) were 0.96 mg/L (0.78 mg/L) and 10.5 mg/L, respectively.

### Concentration ratios of MA to AM in blood

Table II presents information about median concentrations of AM and MA in blood arranged as a function of the MA concentration ratio. The correlation between AM and MA was poor when all drug-positive cases were analyzed \((r=−0.13)\). However, when MA/AM was \(>1\) (\(N=3,561\)), the Pearson correlation coefficient was statistically significant at \(r=0.41\) \((P<0.001)\), and the median concentration of MA (0.58 mg/L) was significantly higher than that of AM (0.10 mg/L) \((P<0.001)\). When the MA/AM ratio was \(>10\), the median concentrations of MA and AM were 0.87 and 0.05 mg/L, respectively \((P<0.001)\), and the correlation coefficient was \(r=0.86\) \((P<0.001)\).

Table II also gives information about changes in the median concentrations of AM and MA in blood for selected ranges of the MA/AM concentration ratio. When MA/AM ratios were evaluated within selected intervals, for example, \(>3.0\) to \(<4.0\) and \(>9.0\) to \(<10.0\), the Pearson correlation coefficients were \(r=0.99\), indicating very strong positive associations (Table II). Furthermore, the median concentrations of AM varied from 11 to 27\% of the MA concentration. Taking all cases with MA/AM ratios \(>3.0\) and \(<10.0\) to indicate abuse of MA alone \((N=1,342)\), the Pearson correlation coefficient was \(r=0.87\), the median concentration of AM was 0.1 mg/L and that of MA was 0.56 mg/L \((P<0.001)\).

Figure 2 shows the relative frequency distribution of MA concentrations in blood along with the AM metabolite for people abusing the secondary amine alone, as judged by MA/AM ratios \(<3.0\) and \(<10.0\).

![Figure 1](https://academic.oup.com/jat/article-abstract/36/9/634/784762/635.png)
Table II
Median Concentrations of AM and MA in Blood and Correlation between MA and AM Shown as a Function of MA/AM Ratio

<table>
<thead>
<tr>
<th>Concentration ratio, MA/AM</th>
<th>N</th>
<th>Conc. of AM median (mg/L)</th>
<th>Conc. of MA median (mg/L)</th>
<th>Pearson’s r, AM versus MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>8,496</td>
<td>0.35*</td>
<td>0.22</td>
<td>−0.13†</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>3,561</td>
<td>0.10</td>
<td>0.58†</td>
<td>0.41‡</td>
</tr>
<tr>
<td>&gt;1.0 and &lt;2.0</td>
<td>753</td>
<td>0.30</td>
<td>0.41</td>
<td>0.96</td>
</tr>
<tr>
<td>&gt;2.0 and &lt;3.0</td>
<td>400</td>
<td>0.21</td>
<td>0.50</td>
<td>0.98</td>
</tr>
<tr>
<td>&gt;3.0 and &lt;4.0</td>
<td>275</td>
<td>0.15</td>
<td>0.55</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;4.0 and &lt;5.0</td>
<td>197</td>
<td>0.13</td>
<td>0.61</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;5.0 and &lt;6.0</td>
<td>134</td>
<td>0.09</td>
<td>0.52</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;6.0 and &lt;7.0</td>
<td>148</td>
<td>0.09</td>
<td>0.57</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;7.0 and &lt;8.0</td>
<td>133</td>
<td>0.07</td>
<td>0.52</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;8.0 and &lt;9.0</td>
<td>98</td>
<td>0.08</td>
<td>0.67</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;9.0 and &lt;10.0</td>
<td>94</td>
<td>0.08</td>
<td>0.75</td>
<td>0.99</td>
</tr>
<tr>
<td>10.0</td>
<td>843</td>
<td>0.05</td>
<td>0.87</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Significantly higher concentration of AM than MA in blood (P < 0.001).
†Weak negative correlation between MA and AM.
‡Significantly higher concentration of MA than AM in blood for all MA/AM ratios >1.0 (P < 0.001).
§All correlation coefficients with MA/AM >1.0 highly significant (P < 0.001).

Figure 2. Relative frequency distributions of the concentrations of AM and MA in blood samples from impaired drivers when the MA/AM concentration ratio was >3.0 and <10.0. N = 12 cases with MA >3.0 mg/L (0.7%) were not included for clarity.

Discussion
AM has been a major drug of abuse in Sweden for many decades, and intravenous injection is the common mode of administration among people attending outpatient treatment centres and those arrested by the police for impaired driving (17). The seizures of AM have decreased slightly over the years, whereas the seizures of MA have increased. There is no evidence that amphetamines are manufactured illicitly in Sweden; instead, they seem to be smuggled from the Baltic countries, Russia and Netherlands. The increasing availability of MA on the illicit drug market has meant a higher prevalence of this central stimulant amine, Logan (3) reported a median concentration of 0.55 mg/L, but this was in only 28 cases. The concentration of AM was always lower, at 5–38% of the MA concentration, although no correlation analysis was presented. In the present study, cases with MA/AM concentration ratios >1.0 (Table I) are more likely instances of abuse of MA, rather than intake of a mixture of the two amines. The purity of street drugs is never known with certainty and some batches of MA might have contained AM as an impurity or vice versa. Interpretations also become complicated if one of the amines is taken at a time when there is a residual amount of the other in the blood from prior episodes of abuse.

Other research studies show that the concentration of parent drug and primary metabolite in blood are correlated, as exemplified by 6-acetylmorphine and morphine, r = 0.61 (21), codeine and morphine r = 0.89 (22) and diazepam and nordiazepam (r = 0.58) (Jones, unpublished). After patients were chronically dosed with diazepam, the correlation between di­azepam and its N-desmethyl metabolite was r = 0.76 (23). The Pearson correlation coefficient between AM and MA in all cases of DUID was low and insignificant (r = −0.13), which suggests that many traffic offenders have taken a mixture of AM and MA or AM with MA as an impurity.

When the ratio of MA/AM in blood was >1.0 (N = 3,561), the correlation between AM and MA was statistically highly significant (r = 0.41, P < 0.001). In these cases, the median concentration of AM (0.10 mg/L) was almost six times lower than the median concentration of MA (0.58 mg/L), which is more in line with abuse of MA and not a mixture of stimulants (3). Much higher correlations between MA and AM were observed when MA/AM ratios were selected at increasing intervals (>3.0 and <4.0), as shown in Table II. The Pearson correlation coefficients between MA and AM were highly significant at r = 0.99 (P < 0.001).

In conclusion, when ratios of MA/AM are >3.0 and <10.0, this is strongly suggestive of the abuse of MA alone (N = 1,342). Using this criterion, the mean (median) and highest concentrations of this secondary amine in blood were 0.72 mg/L (0.56 mg/L) and 4.2 mg/L, respectively (Figure 2). This article seems to be the first to consider the correlation between parent drug and primary metabolite as a way to distinguish people who had taken AM containing MA as an impurity (18). The intention with this article is to report correct descriptive statistics for the concentrations of MA in blood samples from impaired drivers arrested in Sweden.

The median concentration of AM in blood was 0.133 mg/L in a Danish study, whereas no mention was made of the concentration of MA (19). Drivers apprehended in Finland (N = 128) had a median concentration of AM in blood of 0.455 mg/L (1). In 878 drivers apprehended in Norway, there were 691 cases with AM the only drug, 31 cases with MA only, and 156 cases with both AM and MA. The median sum concentration (AM + MA) of stimulants was 0.52 mg/L (2), which compares with a median of 0.78 mg/L in the present study. An investigation of traffic offenders in the Netherlands found a median concentration of AM of 0.22 mg/L in 208 cases (20). The concentrations of blood AM in impaired drivers apprehended in Sweden, where intravenous abuse is very common, have always been higher than in other nations (16, 18).
users of pure MA from people taking a mixture of the two central stimulants.

The authors declare that they have no conflicts of interest in publishing this article.

References