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# Postmortem Stability and Interpretation of $\beta_2$-Agonist Concentrations

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**ABSTRACT:** This paper describes a series of stability and redistribution studies aimed at understanding the presence and significance of $\beta_2$-agonists in asthma deaths. Salbutamol and terbutaline were shown to be stable in postmortem blood at 23°C for 1 week, 4°C for 6 months and −20°C for 1 to 2 years. However, fenoterol was shown to degrade at 23°C (83% loss), 4°C (93% loss) and −20°C (66% loss) over the same time. Salbutamol concentrations detected in blood taken at the time of body admission to the mortuary were not significantly different from the concentrations detected in blood taken from the same cases at the time of autopsy (45 h later). This suggests that significant postmortem redistribution of salbutamol is unlikely to occur during this period. Postmortem blood concentrations of at least salbutamol are likely to reflect the concentration of these drugs in the body at the time of death.

**KEYWORDS:** forensic science, salbutamol, terbutaline, postmortem stability, redistribution, GC/MS

$\beta_2$-Agonists are the most commonly used anti-asthmatic medications, with salbutamol and terbutaline being the most frequently prescribed in Australia. Due to their relative safety, the $\beta_2$-agonists are rarely measured in asthma deaths.

However, previous work has shown that extremely high concentrations of salbutamol are detected in some asthma deaths (1), with 50% of the cases analyzed having salbutamol concentrations above the reported therapeutic concentration (20 ng/mL). Salbutamol concentrations above 50 ng/mL have been known to produce such side effects as sinus tachycardia, increased respiratory rate, and skeletal muscle tremors (2). Importantly, $\beta_2$-agonists are also known to partly induce hypokalaemia and this may predispose patients to potentially fatal cardiac arrhythmias (3,4). Consequently, measurements of $\beta_2$-agonists may provide useful information to medical investigations of asthma deaths. Unfortunately, little is known concerning the stability of these drugs in tissues after death and whether $\beta_2$-agonists undergo redistribution. A series of stability and redistribution studies were conducted in an attempt to address these issues.

## Methods

A solid phase extraction method was used to extract salbutamol, terbutaline and fenoterol from antemortem and postmortem blood specimens (1). In brief, after applying the blood to SepPak $C_{18}$ cartridges (Waters, Australia), the analytes of interest were eluted using methanol:acetonitrile. Following derivatization of the analytes to their trimethylsilyl derivatives, the drugs were analyzed using gas chromatography/electron impact mass spectrometry. Orciprenaline was used as an internal standard. The limit of quantitation in blood was 1 ng/mL for all analytes, and the co-efficients of variation for both intra-assay precision and inter-assay reproducibility ranged between 2.2 and 13.0%.

### *Short-term and Long-term Stability Studies*

Drug-free postmortem blood was collected from the abdominal cavity of numerous cadavers at autopsy, and pooled. Abdominal cavity blood was chosen due to the large volume required. To 30 mL of postmortem blood, terbutaline, salbutamol, and fenoterol solutions were added to give final concentrations of 15, 25 and 15 ng/mL, respectively. After thorough mixing, 5-mL aliquots were dispensed into 6 separate plastic tubes, each containing 1% [w/v] sodium fluoride/potassium oxalate preservative. These tubes were then stored at room temperature (23°C). This process was repeated for postmortem blood at 4°C and −20°C. A blank blood sample representing a drug-free control was also stored under the same conditions.

An aliquot of 1 mL, from each of the 6 spiked blood tubes and the 1 drug-free control, was taken on the day of preparation and analyzed for $\beta_2$-agonist content. Another 1-mL aliquot was taken from each of the incubations at various time periods thereafter.

### *Re-analysis of Blood Specimens*

Postmortem blood specimens from 8 asthma cases positive for salbutamol were reanalyzed 12 months after the original drug analyses. Eight separate cases were also reanalyzed after 24 months of the original analyses.

### *Redistribution Studies*

Femoral blood was taken from 20 deceased persons at the time of body admission to the mortuary, and analyzed for salbutamol. The concentrations detected were then compared to salbutamol concentrations in femoral blood taken from the same deceased persons at autopsy. The average length of time between body admission and autopsy was 45 h (range 13 to 117 h). Salbutamol concentrations detected in femoral blood from 11 deceased persons were compared to concentrations detected in cubital and heart blood taken from the same cases.

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*Statistical Analysis*

Statistical analysis of the data was performed using the In Stat V2.01 program, run on an Apple Macintosh personal computer. Student's paired t-test was used for statistical evaluation of the short-term stability study, the re-analysis of blood specimens, and the comparison of admission and autopsy blood specimens. Analysis of variance (repeated measures, with Dunnett's post hoc multiple comparisons) was used for the statistical evaluation of the long-term stability studies, and the alternate Welch t-test was used to compare femoral, cubital and heart blood salbutamol concentrations. A value of $p < 0.05$ was considered significant.

## Results

In spiked postmortem blood specimens, terbutaline and salbutamol appeared to be stable at room temperature over 7 days. The mean salbutamol concentration after 7 days was 24.9 ± 2.6 ng/mL, compared to 25.1 ± 2.0 ng/mL at $t$ = zero. The mean terbutaline concentration after 7 days was 13.8 ± 0.9 ng/mL, compared to an original concentration of 15.0 ± 2.8 ng/mL at $t$ = zero. Both of these were not significant.

In contrast, fenoterol was unstable in postmortem blood at room temperature, with the concentration after 7 days being only 17% of the initial spiked concentration. The mean fenoterol concentration after 7 days was 2.2 ± 0.3 ng/mL, compared to 12.7 ± 1.1 ng/mL at $t$ = zero [$p < 0.001$].

Similarly, salbutamol and terbutaline, but not fenoterol, were stable in postmortem blood stored at 4°C for 3 and 6 months, Fig. 1. The mean salbutamol concentrations after 3 and 6 months storage were 27.3 ± 2.7 and 24.1 ± 2.5 ng/mL, respectively, compared to 25.1 ± 2.0 ng/mL at $t$ = zero. The mean terbutaline concentrations after 3 and 6 months storage were 12.2 ± 1.6 and 12.9 ± 1.4 ng/mL, compared to an original value of 15.0 ± 2.8 ng/mL.

There was almost a complete loss of fenoterol in blood after 3 months storage at 4°C. A mean fenoterol concentration of only 0.85 ± 0.80 ng/mL was observed after the 3 month period, compared to an initial concentration of 12.7 ± 1.1 ng/mL at $t$ = zero [$p < 0.0001$]. Furthermore, no fenoterol was detected after 6 months incubation.

Salbutamol and terbutaline, but not fenoterol, were also stable in postmortem blood kept at −20°C over 6 months, Fig. 2. Mean salbutamol concentrations were 25.1 ± 2.0, 26.4 ± 3.2, 24.2 ± 2.5 and 24.1 ± 1.9 ng/mL at zero, 2, 4 and 6 months, respectively. For terbutaline, the mean concentrations were 15.0 ± 2.8, 16.4 ± 1.4, 17.0 ± 1.4 and 15.6 ± 1.3 ng/mL at zero, 2, 4 and 6 months, respectively.

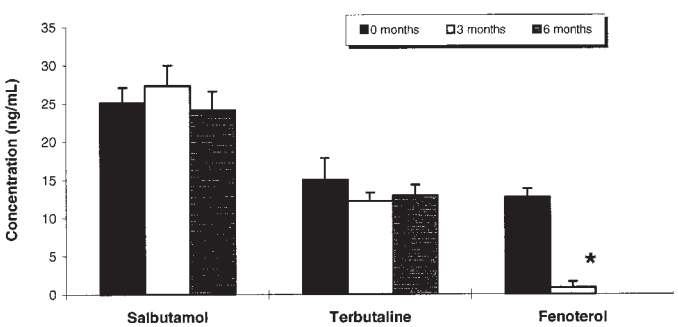


FIG. 1—*Stability profile of salbutamol, terbutaline and fenoterol in postmortem blood stored at 4°C over 6 months. Values are expressed as the mean ± SD of 5 to 6 blood specimens. [* p < 0.0001].*

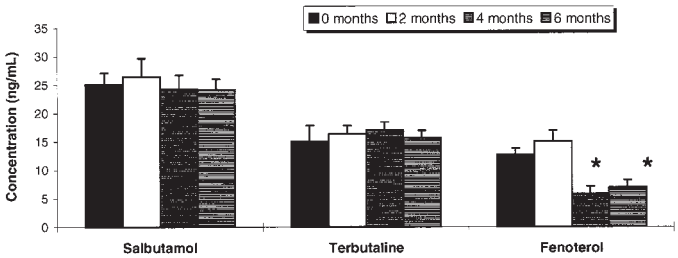


FIG. 2—*Stability profile of salbutamol, terbutaline and fenoterol in postmortem blood stored at −20°C over 6 months. Values are expressed as the mean ± SD of 6 blood specimens. [* p < 0.01].*

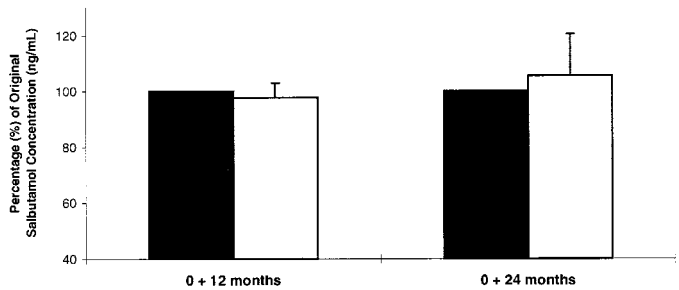


FIG. 3—*Long-term stability profile of salbutamol in postmortem blood stored at −20°C for 12 and 24 months* (n = *8 each). Values are expressed as mean percentages ± SD of the original blood result measured at time zero (taken as representing 100%).*

Fenoterol appeared to be stable at −20°C for 2 months, then showed a marked decrease in concentration thereafter. The mean concentrations at zero, 2, 4 and 6 months, respectively, were 12.7 ± 1.1, 15.0 ± 1.9, 5.8 ± 1.3 and 6.9 ± 1.3 ng/mL [$p < 0.05$].

Blood salbutamol concentrations in 8 asthmatic cases were measured within a week of autopsy and the subsequent result was taken as representing 100%. The blood specimens were then stored at −20°C, and 12 months later reanalyzed for salbutamol. The values obtained were expressed as a percentage of the original concentration, Fig. 3. The mean percentage for the 8 cases following this 12 month interval was 97.7 ± 5.3% which was not significantly different from the original measurements.

Salbutamol concentrations were also reanalyzed in the blood of another 8 asthmatic cases, stored at −20°C for 24 months. The mean percentage for the 8 cases following the 24 month interval was 105.5 ± 15.0%, which was not significantly different from the concentrations measured originally.

The mean concentrations for admission and autopsy blood specimens were 33.2 ± 42.8 ng/mL (range 1.1 to 136 ng/mL) and 32.7 ± 41.4 ng/mL (range 1.2 to 151 ng/mL), respectively. There was no significant difference between the blood salbutamol concentrations measured in these 2 periods of sampling, and there was a good correlation between the admission and autopsy blood concentrations, Fig. 4.

The concentrations of salbutamol in 8 matched femoral and cubital blood specimens, 10 matched femoral and heart blood specimens, and 7 matched cubital and heart blood specimens were also compared. Table 1 displays the raw data of salbutamol concentrations in the 3 different sites of sampling. Corresponding details of each of the deceased's last known usage of salbutamol and other asthmatic medications are also shown.

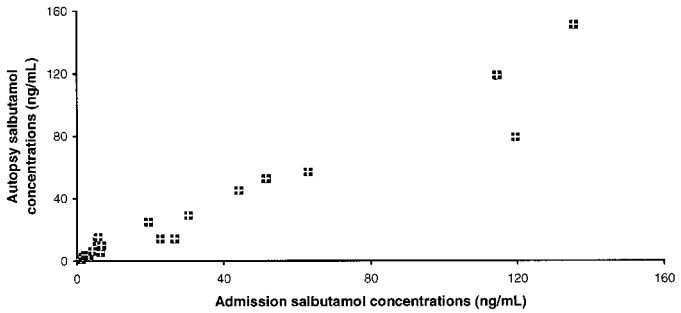


FIG. 4—*Scattergram showing correlation between admission and autopsy blood concentrations of salbutamol [*n *= 20; nonparametric linear correlation:* $r^2 = 0.927$*, 95% confidence interval 0.90037 − 0.9858].*

The majority of the cubital and heart blood salbutamol concentrations, in cases 1 to 6, were between 84 and 111% of the matched femoral blood salbutamol concentration. These patients had been prescribed regular medication for their asthmatic condition. On the other hand, cases 7 to 11 had cubital and heart blood salbutamol concentrations ranging from 100% to almost 900% of the corresponding femoral blood salbutamol concentrations. Interestingly, 4 out of 5 of these individuals were known to have administered salbutamol, either via a nebulizer or a metered dose inhaler, shortly before death.

## Discussion

Since days or weeks may pass between death, specimen collection and drug analysis, it is important to establish the stability, or otherwise, of $\beta_2$-agonists. Spontaneous chemical hydrolysis of drugs such as heroin and cocaine (5,6), or bacterial or enzymatic degradation of drugs such as the nitrobenzodiazepines and dothiepin (7,8), are known to occur during this interval. If any of these factors were to occur for the $\beta_2$-agonists, the concentrations detected in postmortem specimens would not necessarily reflect the actual concentration of the drug at the time of death, and thereby affect the interpretation of postmortem data.

Salbutamol and terbutaline were found to be stable in spiked postmortem blood at room temperature for 7 days, at 4°C for 6 months and at −20°C for 6 months. These conditions represent environments that blood specimens may possibly be subjected to in actual cases.

In contrast, fenoterol was shown to be unstable under the same conditions. The greatest loss of fenoterol was observed at room temperature and at 4°C. A loss of 83% was observed for fenoterol in spiked postmortem blood at room temperature over 7 days, 93% loss at 4°C over 6 months, and a loss of 66% at −20°C over 6 months. The instability of fenoterol is most likely a result of the presence of the phenolic group attached to the side chain N-atom, which is susceptible to oxidization. In contrast, salbutamol and terbutaline which have a t-butyl group attached to this N-atom were relatively stable under all storage conditions.

The loss of fenoterol at these temperatures has important implications in the interpretation of blood specimens from asthmatic patients suspected of taking this drug. As a recommendation, blood specimens should be stored immediately at −20°C or colder, and analyzed for fenoterol as soon as possible.

Another important consideration when measuring $\beta_2$-agonists is the stability of their metabolites. The major metabolite of salbutamol is the 4′-O-sulfate ester. In plasma, the salbutamol:sulfate metabolite ratio is approximately 1:4 following inhalation and oral therapy (9,10). In theory, their corresponding sulfate conjugates may be converted back to the parent $\beta_2$-agonist as a result of chemical, enzymic, or bacterial degradation. This would consequently result in falsely elevated concentrations of the parent drug.

When blood specimens were re-analyzed up to 24 months after the original analyses, the salbutamol concentrations detected were not significantly different. This would suggest that the salbutamol sulfate conjugate does not break down and convert back into the parent drug when stored at −20°C long-term.

The concentration of salbutamol in femoral blood taken at body admission was not significantly different from that measured in femoral blood taken at autopsy, on average 45 h later. This would suggest that the process of redistribution does not influence the postmortem concentration of salbutamol at this site. As a consequence, the concentrations of salbutamol detected in femoral blood

TABLE 1—*Salbutamol concentrations in postmortem femoral, cubital and heart blood specimens, and corresponding salbutamol use.*

| Case # | Blood Concentration (ng/mL) | | | Details of Regular Drug Use and Drug Use Prior to Death |
|---|---|---|---|---|
| | Femoral | Cubital* | Heart* | |
| 1 | 0.6 | 0.5 (83%) | — | Prescribed salbutamol and beclomethasone inhaler, and prednisolone |
| 2 | 0.7 | 0.8 (114%) | 0.82 (114%) | Prescribed salbutamol and beclomethasone inhalers, theophylline and prednisolone |
| 3 | 28 | — | 26 (93%) | Ipratropium bromide nebulizer mask in hand but machine not on; also prescribed salbutamol, terbutaline, sodium cromoglycate and theophylline |
| 4 | 35 | 34 (97%) | 34 (97%) | Ipratropium bromide 4 hourly; also prescribed salbutamol and cortisone |
| 5 | 45 | 38 (84%) | 29 (64%) | Prescribed salbutamol 1 week prior to death |
| 6 | 76 | — | 73 (96%) | Nebuliser 4 times a day; salbutamol, ipratropium bromide, theophylline and prednisolone also prescribed |
| 7 | 30 | 37 (123%) | 47 (157%) | Took salbutamol, then collapsed |
| 8 | 221 | 222 (100%) | 326 (148%) | Found clutching a salbutamol inhaler |
| 9 | 6 | — | 21 (350%) | Prescribed salbutamol, ipratropium bromide and theophylline |
| 10 | 43 | 74 (172%) | 144 (335%) | Found with salbutamol nebulizer mask in hand |
| 11 | 119 | 324 (272%) | 1063 (893%) | Salbutamol nebulizer continuously during attack, then treated by ambulance officers |

* Percentages of salbutamol in cubital and heart blood compared to femoral blood are shown in parentheses. Cases 1, 4, 5, 7, and 9 died as a result of natural heart disease (e.g., ischaemic heart disease); Cases 3, 6, 8, 10, and 11 died as a result of natural respiratory disease (e.g., asthma, chronic obstructive airways disease); Case 2 died as a result of combined drug toxicity to amitriptyline and benzodiazepines.

obtained at autopsy are likely to reflect drug concentrations at the time of death. However, it cannot be entirely excluded that postmortem changes have already occurred before body admission, namely in the first hour or so after death.

Blood concentrations of drugs can vary in specimens taken from various anatomical sites. After death, drugs may leak out of organs in which they had accumulated in during life, thereby increasing the blood concentration of the drug during the postmortem interval. Drugs such as some of the antidepressants have higher concentrations in blood from central vessels than corresponding peripheral vessel concentrations (11).

In this study it was observed that salbutamol concentrations were evenly distributed between femoral, cubital, and heart blood in cases in which the asthmatic patient had not used salbutamol shortly prior to death. These data would seem to suggest a relatively even distribution of salbutamol throughout the body, and postmortem changes do not appear to have taken place. Conversely, when salbutamol concentrations were lower in femoral blood, compared to levels detected in cubital and heart blood, the circumstances indicated that the drug was taken immediately prior to death, and quite possibly in large and continuing amounts. This would imply that there may not have been enough time to allow pulmonary circulation to reach equilibrium within the body before death, especially as the absorption half life of inhaled salbutamol is less than 1 h.

Tissue equilibrium of salbutamol, therefore, plays an important role in determining the salbutamol concentrations in different blood sites throughout the body at the time of death.

It was essential to ascertain whether the high concentrations of salbutamol detected in heart blood occurs antemortem or postmortem. Since salbutamol was shown not to undergo significant redistribution, concentrations measured in postmortem blood specimens are probably a true reflection of the concentration at the time of death. Since $\beta_2$-agonists are known to partly induce hypokalaemia and predispose patients to cardiac arrhythmias, high antemortem levels in heart blood could be potentially dangerous. In particular, Case 11, Table 1 had a heart blood salbutamol concentration of 1063 ng/mL, with a corresponding femoral blood concentration of 119 ng/mL.

*Acknowledgments*

Details of salbutamol usage were obtained from reports and statements made available at the Victorian State Coroner's Office.

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