# CASE REPORT

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# Analysis of 2-Hydroxyimipramine in an Imipramine-Related Fatality

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**ABSTRACT:** A fatality following ingestion of the tricyclic antidepressant imipramine (Novopramine®), acetaminophen, and ethyl alcohol is described. Imipramine, desipramine, acetaminophen, and 2-hydroxyimipramine were quantitated by high performance liquid chromatography, and ethyl alcohol by gas liquid chromatography. Concentrations of imipramine, desipramine, 2-hydroxyimipramine, and acetaminophen were: in blood—9.0, 1.1, 3.9, and 11 mg/L; in urine—92, 14, and 42 mg/L (acetaminophen not quantitated in urine). Ethyl alcohol concentration in blood was $<10$ mg/dL and 105 mg/dL in the urine by headspace gas chromatography. These findings are compared to previous reports of imipramine-related fatalities. To our knowledge, this is the first fatality reported involving imipramine where analysis included quantitation of 2-hydroxyimipramine in blood and urine.

**KEYWORDS:** toxicology, imipramine, chromatographic analysis, 2-hydroxyimipramine, antidepressants

Imipramine hydrochloride (Novopramine®, Novopharm Limited, Scarborough, Ontario) is a tricyclic antidepressant drug used in the treatment of neurotic or psychotic depressive states including endogenous and reactive depression, involutional melancholia, senile depressions, and persistent nocturnal enuresis [*1*].

The metabolism and pharmacokinetics of imipramine have been extensively studied, experimentally and clinically [*2,3*]. After the introduction of sensitive methods for analysis of imipramine and desipramine, plasma studies revealed substantial interindividual differences in steady state concentration and independent variations in demethylation and hydroxylation of imipramine [*4,5*].

Experimental studies have shown that 2-hydroxyimipramine and 2-hydroxydesipramine exert cardiac effects and inhibit reuptake of norepinephrine and serotonin much like imipramine and desipramine [*6*]. It has been reported that 2-hydroxyimipramine is more cardiotoxic than imipramine [*7*].

The concentration of tricyclic antidepressant drug-hydroxylated metabolites are generally

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much lower than the parent drug. Increased 2-hydroxydesipramine [*8*] and trans-10-hydroxynortriptyline [*9*] concentrations, however, are seen in the elderly and often at concentrations higher than the parent drugs. Two-hydroxyimipramine pharmacokinetics have been followed in the therapeutic situation and after overdose [*3,10*]. Evidence suggests that the elimination of the hydroxy metabolites is faster than their formation in the liver. The increased concentrations in the elderly correlate inversely with decreased renal function in aging [*3*].

Several recent reports summarized fatal and nonfatal intoxications for the common tricyclic antidepressant drugs [*11–14*]. This report describes analytical findings of imipramine, 2-hydroxyimipramine, desipramine, acetaminophen, and ethyl alcohol in a single death case.

## Case History

This 26-year-old woman had a long history of depression and had attempted suicide by overdose 5 years ago. In the evening of 7 August, the decedent was drinking with her husband. The next morning, her husband noted that some whiskey was drunk after he went to bed. He left the house at 0800 h and returned at 1130 h and found his wife collapsed, but breathing, on the floor. Two empty bottles were found nearby (Anacin-3® and Novopramine®). She was taken to a local hospital and treated for 2 h. While being transferred to this hospital, she had a cardiac arrest in the ambulance. Cardiopulmonary resuscitation (CPR) was performed and she survived until arrival at this hospital, but died shortly thereafter, at 1550 h.

On 16 July, she received a prescription for Novopramine (150 of 50-mg tablets).

An autopsy was not performed. Toxicologic analysis on blood and urine is described below. External examination revealed no significant findings. A mixed drug overdose was considered the cause of death.

## Toxicologic Analysis

### *Standards and Reagents*

Acetonitrile and methanol were HPLC grade and glass distilled (Caledon Laboratories Ltd., Georgetown, Ontario and Fisher Scientific, Dartmouth, Nova Scotia). Doxepin hydrochloride was obtained from Pfizer Canada, Inc., Pointe Claire-Dorval, Quebec. Clomipramine hydrochloride, imipramine hydrochloride, and desipramine hydrochloride were supplied by Ciba Geigy, Mississauga, Ontario. Ciba Geigy, Basle, Switzerland provided 2-hydroxyimipramine, 2-hydroxydesipramine, and 8-hydroxyclomipramine. Acetaminophen was a gift from McNeil Consumer Products Company, Guelph, Ontario, and 8-chlorotheophylline was purchased from Aldrich Chemical Company, Montreal, Quebec.

### *Thin-Layer Chromatography*

Urine drug screening by thin-layer chromatography (TLC) was performed as reported previously [*15*].

### *Gas Liquid Chromatography*

Ethyl alcohol quantitation by gas chromatography was performed as reported previously [*16*].

*High Performance Liquid Chromatography*

Liquid chromatography was performed on a Model 740 solvent delivery system by Spectra Physics, SF 770 variable wavelength ultraviolet (UV) detector by Schoeffel Instrument, and an Omniscribe recorder by Houston Instruments (all obtained from Technical Marketing Associates, Halifax, Nova Scotia). Analysis was performed at ambient temperature using a 250- by 4.6-mm RP-8 column with 5-$\mu$m particle size (Brownlee Labs, Santa Clara, CA). Detector wavelength was set at 205 nm.

The mobile phase for imipramine and desipramine analysis was a buffer solution consisting of 0.01 mol/L of potassium dihydrogen phosphate mixed with acetonitrile and *n*-nonylamine (550/450/0.6). This mixture was adjusted to pH 3.2 with phosphoric acid. The flow rate was 1.6 mL/min.

Doxepin and clomipramine were used as internal standards for imipramine/desipramine analysis. Stock drug solutions at 100 $\mu$g/mL (as free bases) were prepared in methyl alcohol. A drug-free serum was added to give stock standards of imipramine and desipramine at 500 ng/mL. To 2.0 mL of serum standard and unknown blood (diluted with Red Cross blank blood) were added 5 mL of hexane: isoamyl alcohol (97:3) containing the two internal standards (50 ng/mL) and 0.1 mL of saturated sodium carbonate. The tubes were mixed for 20 min and centrifuged. The organic extract was transferred to an 8-mL screw cap tube with a plastic pasteur pipette. A 0.1-mL mixture of mobile phase: 0.1 *M* phosphoric acid (1:1) was added followed by vortexing and centrifugation. The organic layer was aspirated to waste and the aqueous phase injected onto the HPLC column. The range was set at 0.02 absorbance units full scale and 20 to 40 $\mu$L of aqueous phase was injected.

For 2-hydroxyimipramine analysis, a buffer solution consisting of 0.01 mol/L of potassium dihydrogen phosphate was mixed with acetonitrile and *n*-nonylamine to form the mobile phase (800/200/0.6). This mixture was adjusted to pH 3.0 with phosphoric acid. The flow rate was 1.6 mL/min.

Doxepin and 8-hydroxyclomipramine were used as internal standards for 2-hydroxyimipramine. Stock drug solutions at 100 $\mu$g/mL (as free bases) were prepared in methyl alcohol.

The specimens were extracted and analyzed as described for imipramine and desipramine.

Urine quantitation was performed in the same manner as the blood specimens after dilution (1/100).

Acetaminophen was analyzed according to Baselt [*17*] with minor modifications.

Peak height ratios were used to calculate antidepressant concentrations using the average factor obtained from the two internal standards.

## Results

Qualitative analysis of the urine gave a TLC pattern consistent with tricyclic antidepressant metabolites and a positive screen for ethyl alcohol by head space analysis.

A summary of the quantitative results appears in Table 1.

Chromatograms of the blood extract for imipramine, desipramine, 2-hydroxyimipramine, and 2-hydroxydesipramine are found in Figs. 1 and 2. Two-hydroxydesipramine was not quantitated.

## Discussion

The relationship between serum concentration and clinical response for imipramine has been more straightforward than for other antidepressant drugs. Studies by Glassman [*18*] showed a linear relationship between blood concentration and clinical outcome. That study and another by Reisby [*5*] measuring imipramine and desipramine found that, as combined

TABLE 1—*Summary of toxicologic analysis.*

| Drug | Concentration | |
|---|---|---|
| | Blood | Urine |
| Imipramine, mg/L | 9.0 | 92 |
| Desipramine, mg/L | 1.1 | 14 |
| 2-hydroxyimipramine, mg/L | 3.9 | 42 |
| Acetaminophen, mg/L | 11 | |
| Ethyl alcohol, mg/dL | <10 | 105 |

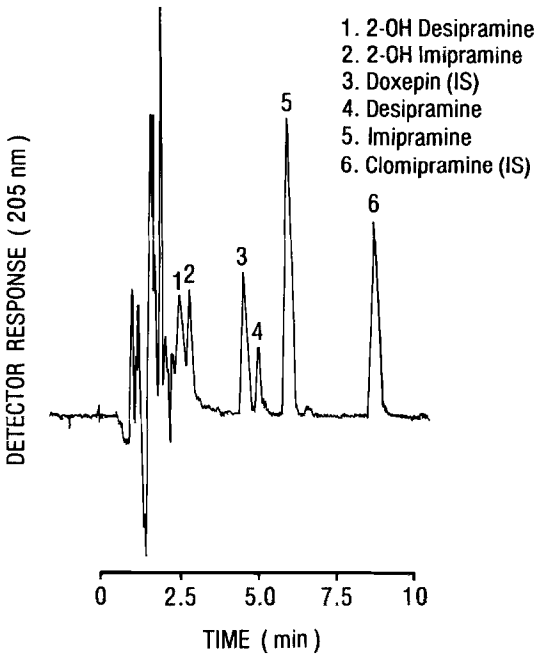


FIG. 1—*Chromatogram of blood extract used for imipramine/desipramine analysis:* (1) *2-hydroxydesipramine,* (2) *2-hydroxyimipramine,* (3) *doxepin internal standard,* (4) *desipramine (1.1 mg/L),* (5) *imipramine (9.0 mg/L), and* (6) *clomipramine internal standard. For chromatographic conditions, see text.*

concentrations exceeded 200 ng/mL, the response was better than at total concentrations below 200 ng/mL. Both studies concluded that concentrations greater than 250 ng/mL are associated with more side effects.

All early studies of the antidepressant drugs made the assumption that only the parent drug and demethylated metabolite were active compounds and that hydroxymetabolites did not penetrate the brain. It is now clear that most tricyclic antidepressant drugs have hydroxy derivatives which are pharmacologically active and penetrate the brain [*6–7,19,20*]. It has been stated that the most important hydroxy metabolites are the 10-hydroxy derivatives of amitriptyline and nortriptyline since these metabolites frequently exceed parent drug concentrations in therapeutic situations. However, it is also known that imipramine hydroxy metabolites are markedly elevated in the elderly [*8,9*], and high trans-10-hydroxynortripty-

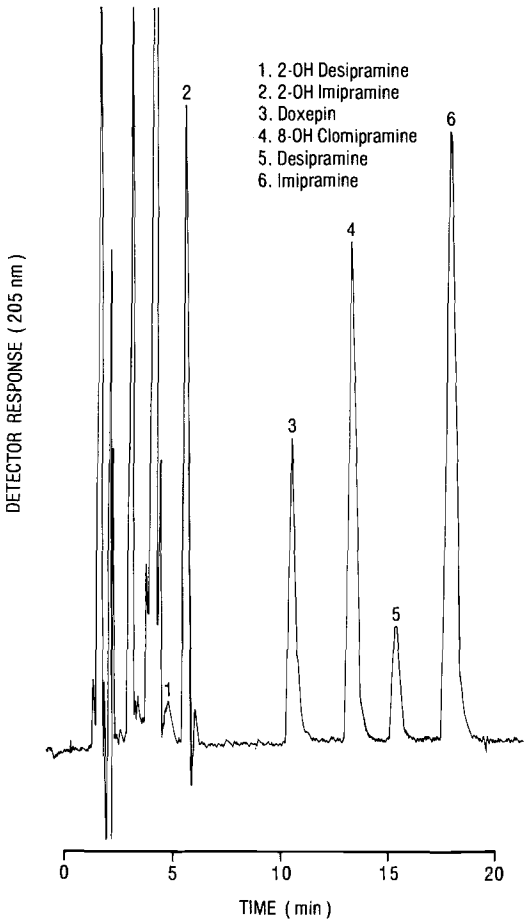


FIG. 2—*Chromatogram of blood extract used for 2-hydroxyimipramine quantitation:* (1) *2-hydroxydesipramine,* (2) *2-hydroxyimipramine (3.9 mg/L),* (3) *doxepin internal standard,* (4) *8-hydroxyclomipramine internal standard,* (5) *desipramine, and* (6) *imipramine. For chromatographic conditions, see text.*

line in blood has been associated with congestive heart failure [*21*]. In one report [*3*] of hydroxyimipramine concentrations in three overdose cases, the concentration was less than imipramine. The drug concentration versus time curve for hydroxy derivates declined in parallel with their corresponding parent compounds.

Serum concentrations of imipramine, desipramine, and their hydroxy metabolites were measured in an imipramine overdose case in conjunction with drug removal by hemoperfusion [*22*]. This study also reported quantitation of all four substances in brain and liver, at autopsy, 96 h after hospital admission. Liver concentrations of imipramine, 0.338 mg/kg, and desipramine, 0.640 mg/kg, were low for fatalities according to Baselt [*23*]. Brain concentrations of imipramine, 0.091 mg/kg, and desipramine, 0.407 mg/kg, were considered low for fatalities.

In this report, the 2-hydroxyimipramine concentration of 3.9 mg/L in blood represents 26% of the total tricyclic drug/metabolites measured.

In a large study of tricyclic antidepressant drug-related fatalities [*11*], the ratio of parent

to desmethyl metabolite had a mean value of 4.7 with combined concentrations greater than 1.0 mg/L considered clearly toxic and generally associated with fatalities. Bailey et al. [*12*] reported an average ratio of 1.43 in blood for fatal overdose cases. In our experience, high ratios are generally seen only when a short time interval separated ingestion from death. In this case, the ratio of blood imipramine/desipramine is 8.1 and imipramine/2-hydroxyimipramine is 2.3. The estimated time between ingestion and death, in this case, was 5 to 8 h.

The blood ethyl alcohol concentration was not considered significant, at death, but may have been at the time of ingestion.

Interpreting the postmortem acetaminophen blood concentration (11 mg/L) is difficult without knowing the time of ingestion. This drug was not considered a major factor, but its hepatic metabolism via the mixed function oxidase system may have competed with the metabolism of imipramine.

It is known that certain patients requiring high doses of imipramine or nortriptyline to maintain therapeutic blood concentrations are fast hydroxylators and have very high concentrations of hydroxylated metabolites in their blood [*24*].

The sum of blood imipramine, desipramine, and 2-hydroxyimipramine concentrations, in this case, was 14 mg/L. Cardiac and respiratory toxicity leading to death have been associated with combined blood concentrations greater than 1.0 mg/L [*11*]. This report describes a fatality with very high concentrations of imipramine/desipramine and the quantitation of 2-hydroxyimipramine. Earlier studies of imipramine-related fatalities using UV absorbance for quantitation probably included hydroxy metabolites in the total concentrations reported [*25*]. The contribution of 2-hydroxyimipramine to the overall toxic effects leading to death is unknown.

## Conclusion

In conclusion, postmortem hydroxy imipramine/desipramine may be important to measure in the elderly, in individuals known to require large doses therapeutically, and when the total imipramine/desipramine concentration in blood is near the toxic fatal range (approximately 1.0 mg/L).

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