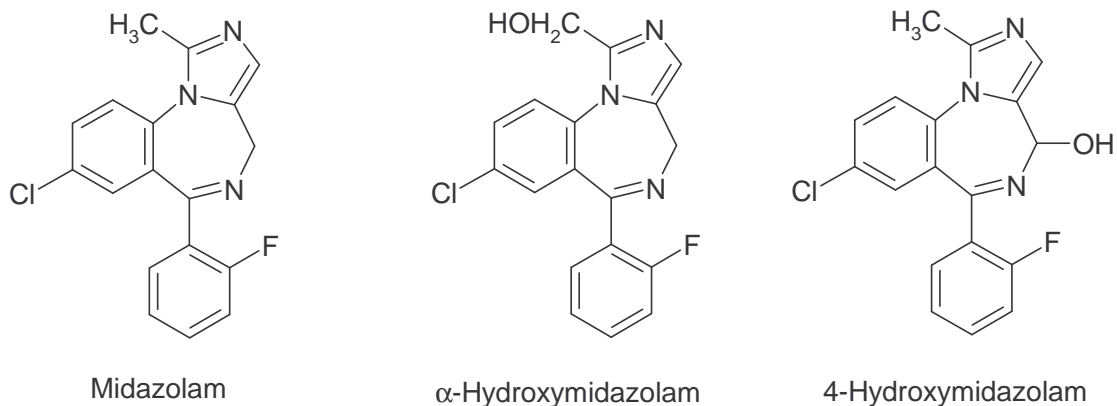


## Clinical Relevance of the Quantification of $\alpha$ -Hydroxymidazolam and/or its Glucuronide

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Midazolam is mainly metabolized to  $\alpha$ -hydroxymidazolam by the cytochrome P450 3A4. A smaller amount is hydroxylated to 4-hydroxymidazolam. Both metabolites need glucuronidation before they can be excreted renally; 60 - 80% of an applied dose can be found in urine as  $\alpha$ -hydroxymidazolamglucuronide (HMG) (1). In 1995 it has been shown that renal failure may lead to a huge increase in the HMG concentration in serum of ICU patients (2). Using in-vitro binding studies, the authors have shown that the pharmacological activity of HMG is about 10% of midazolam and  $\alpha$ -hydroxymidazolam. Whereas midazolam and  $\alpha$ -hydroxymidazolam only can be eliminated marginally by venovenous hemodialysis (3), HMG was determined to be easily removed by this procedure.



Whereas the quantification of  $\alpha$ -hydroxymidazolam straightforwardly can be integrated in any existing HPLC method for the determination of midazolam, the quantification of its glucuronide needs either an additional deglucuronidation step during sample preparation or the HPLC method has to be modified in order to allow the detection of this very polar compound. Van Rij et al. (4) have published an HPLC method for the quantification of HMG using reversed-phase ion-pair chromatography. In our laboratory we decided to perform deglucuronidation of HMG to hydroxymidazolam by adding 20  $\mu$ l of acetic acid and 20  $\mu$ l of  $\beta$ -glucuronidase/arylsulfatase to 1 ml of serum or plasma. After vortexing, the samples are incubated for 4 hours at 37°C. Following alkalization to pH 7.4 the sample is extracted as usual.

In order to evaluate the relevance of the quantification of 1-hydroxymidazolam and its glucuronide, we analyzed these 2 compounds in a randomly selected cohort of 69 patients who had a request for the quantification of midazolam. In addition creatinine was quantified in every sample. All samples were sent from intensive care units and the main indication for the analysis was prolonged sedation after application of midazolam (often in combination with other CNS depressing drugs). Some of these patients had hemofiltration. Therefore, the creatinine concentration can not be taken as real measure for kidney function. But due to the fact, that HMG as well as creatinine can easily be removed by hemofiltration the correlation of these 2 compounds can be used for the estimation of the relation between kidney function

and HMG concentration in our collective. As can be seen in Figure 1, this correlation was only very weak in our randomly selected cohort.

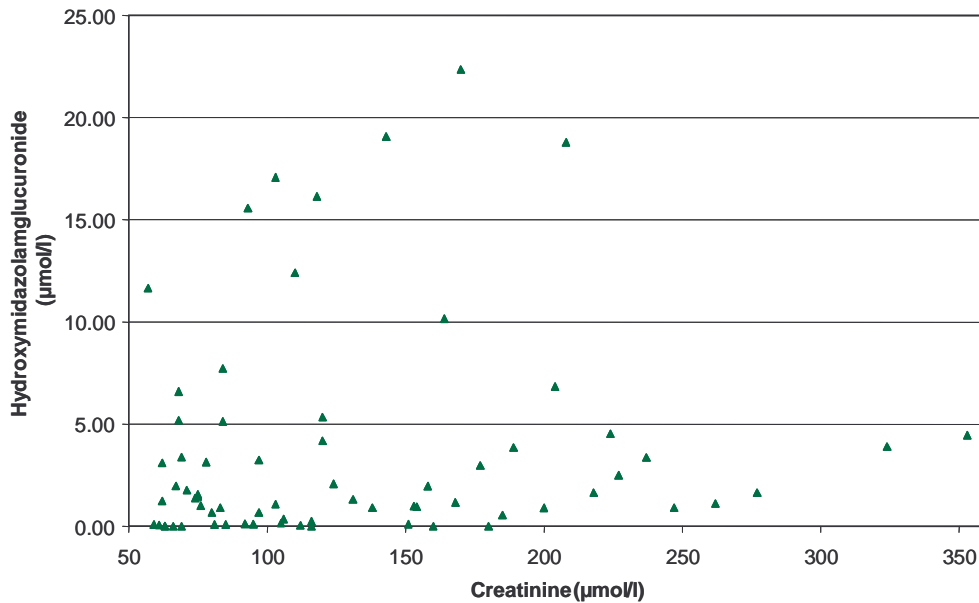


Figure 1: Correlation between creatinine and HMG concentration

In Figure 2 an activity-corrected diagram of the concentrations of midazolam and its metabolites is presented. The concentration of HMG has been divided by 10 in order to adjust for its pharmacological activity. In most of the samples midazolam is the compound with the highest concentration considering the pharmacological activity.

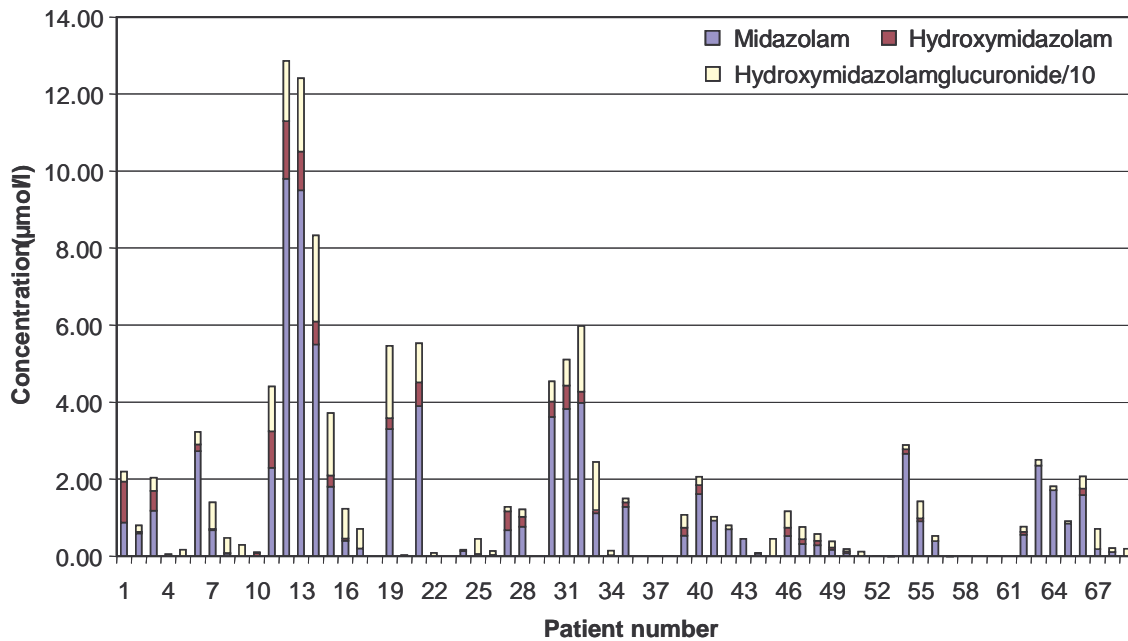


Figure 2: Concentration of midazolam and its metabolites in different patients after correction for the respective pharmacological activity

The concentrations of midazolam and of HMG of all 69 cases are plotted against each other in Figure 3 together with their therapeutic ranges. If the lower therapeutic range of midazolam (0.31 – 1.00  $\mu\text{mol/l}$ ),  $\alpha$ -hydroxymidazolam (0.20 – 0.73  $\mu\text{mol/l}$ ) or HMG (2.0 – 7.3  $\mu\text{mol/l}$ ) is used as cut-off concentration to determine if there is still a pharmacological activity of midazolam and/or its metabolites leading to prolonged sedation, 6 of 69 patients (8.7%) would have been misjudged if only the midazolam concentration would have been taken into consideration. In these patients HMG concentrations have been determined which are within the therapeutic range and may be responsible for the prolonged sedation. There was no impact of the  $\alpha$ -hydroxymidazolam concentration using this approach.

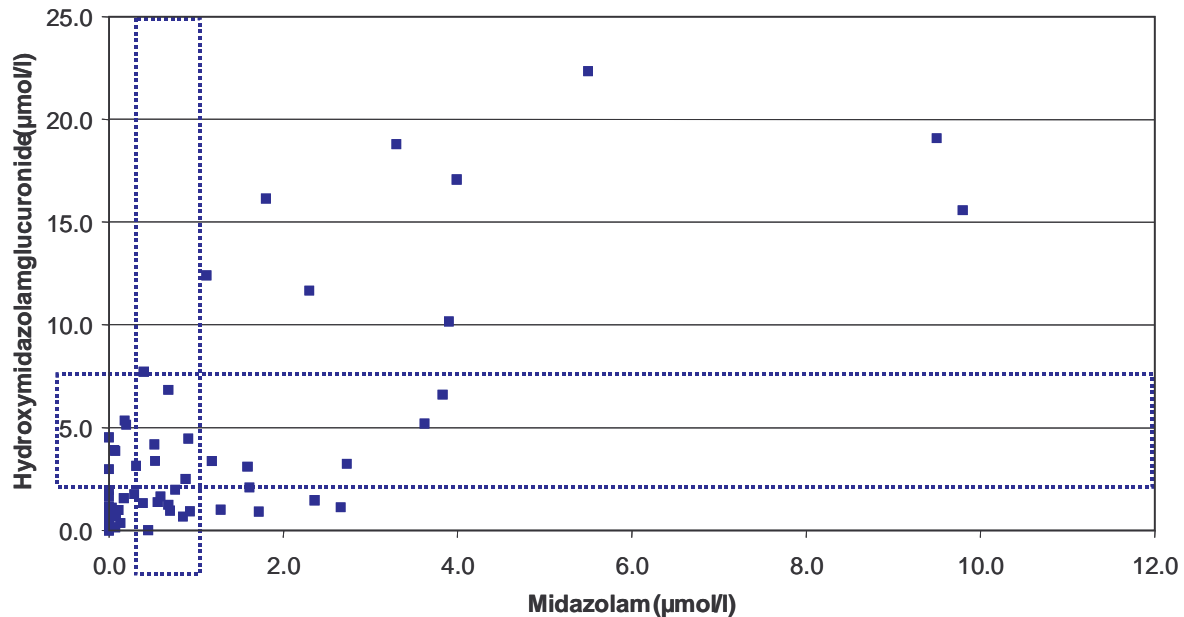


Figure 3: Concentration of midazolam and HMG with their respective therapeutic ranges

## Conclusion

In order to avoid misjudgment of patients with prolonged sedation after midazolam therapy, HMG should be determined when midazolam and  $\alpha$ -hydroxymidazolam are not present in concentrations within the therapeutic range.

## References

- (1) Arzneimittelkompendium Schweiz, 2006, Dormicum®
- (2) Bauer TM, Ritz R, Haberthür C, Ha HR, Hunkeler W, Sleight AJ, Scollo-Lavizzari, Haefeli W. Prolonged sedation due to accumulation of conjugated metabolites of midazolam. *Lancet* 1995;346:145-147.
- (3) Bolon M, Bastien O, Flamens C, Paulus S, Bouliou R. Midazolam disposition in patients undergoing continuous venovenous hemodialysis. *J Clin Pharmacol* 2001;41:959-962.
- (4) Van Rij KM, Compas D, Swart EL, de Goede PNFC, Touw DJ. Reversed-phase ion pair HPLC method for the direct analysis of 1-OH-midazolam glucuronide in human serum. *Ther Drug Monit* 1999;21:416-420.