Analyte instability issues in blood

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janssen
PHARMACEUTICAL COMPANIES
OF Johnson&Johnson

Outline

- Why and when stability in plasma but not in blood
  - Literature overview
- Janssen in-house examples
  - Instability
  - Red Blood Cell (RBC) / plasma distribution
- Conclusions and recommendations

Literature

- Reduction of hydroxamic acids to the corresponding amides catalysed by rabbit blood.
  K. Sugihara et al., Xenobiotica 30 (2000) 457-467

- Nonenzymatic Reduction of N-hydroxy-2-acetylaminofluorene to 2-Acetylaminofluorene by Heme in the Presence of Hydroquinones. ***K. Shigeyuki et al., Journal of Health Science, 46 (2000) 66-69***

- A unique tertiary amine N-oxide reduction system composed of quinone reductase and heme in rat liver preparations.
  K. Shigeyuki et al., Drug Metabolism and Disposition 27 (1999) 92

- Reductive dechlorination of p,p'-DDT mediated by Hemoproteins in the hepatopancreas and blood of goldfish.
  K. Shigeyuki et al., Journal of Health Science 45 (1999) 217 – 221

- Quinone-dependent tertiary amine N-oxide reduction in rat blood.
  K. Shigeyuki et al., Biol. Pharm. Bull. 21 (1998) 1344-1347

- Debromination of (a-bromoiso-valeryl) urea catalysed by rat blood.
  S. Kitamura et al., Journal of PARMACY AND Pharmacology 51 (1999) 73-78

- N-oxide reduction by hemoglobin, cytochrome C and ferrous ions.
  G. Powis et al., Res Commun Chem Pathol Pharmacol 1 (1980) 143-150

- Reduction of the prodrug loperamide oxide to its active drug loperamide in the gut of rats, dogs and humans.
  K. Lavrijsen et al., Drug Metabolism and Disposition 23 (1995) 354-362

Literature

- Two-step reduction in blood, catalyzed by haemoglobin
- Published compound classes:
  - *N*-oxides (reduction to amine)
  - Hydroxamic acids (reduction to amide)
  - Halogenated compounds
    (reductive dehalogenation; I > Br > Cl > F)

Literature – *N*-oxides

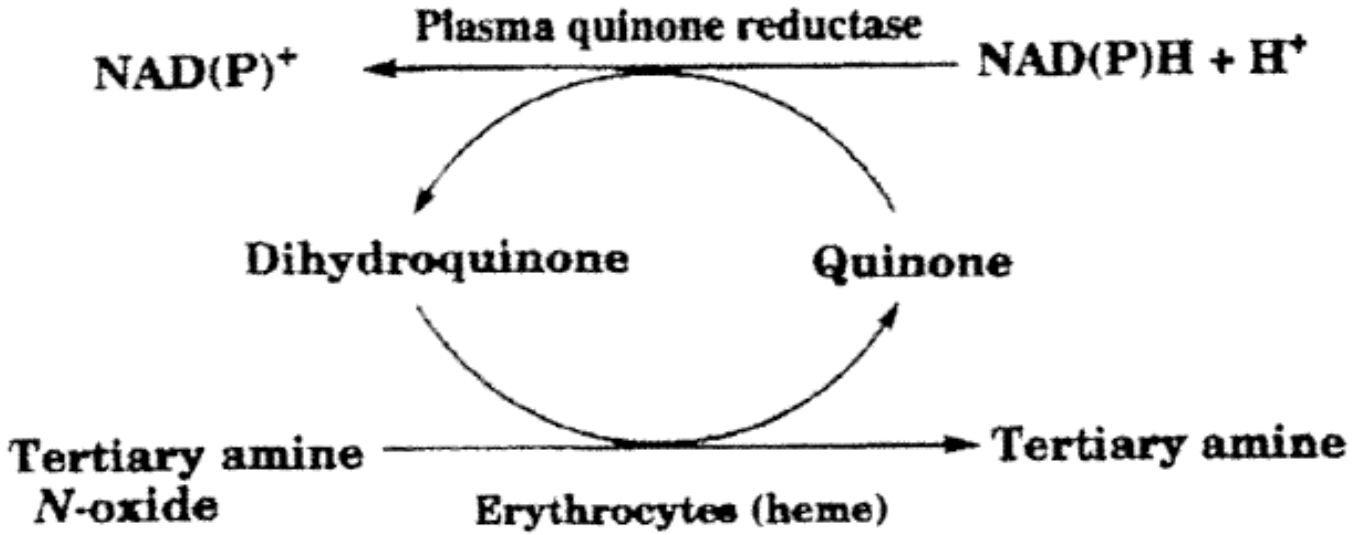
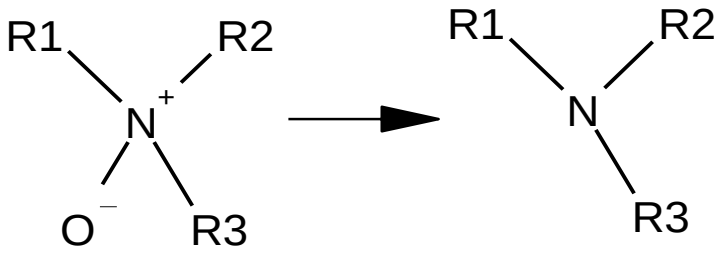


Fig. 3. Proposed Mechanism for Quinone-Dependent *N*-Oxide Reduction in Rat Blood

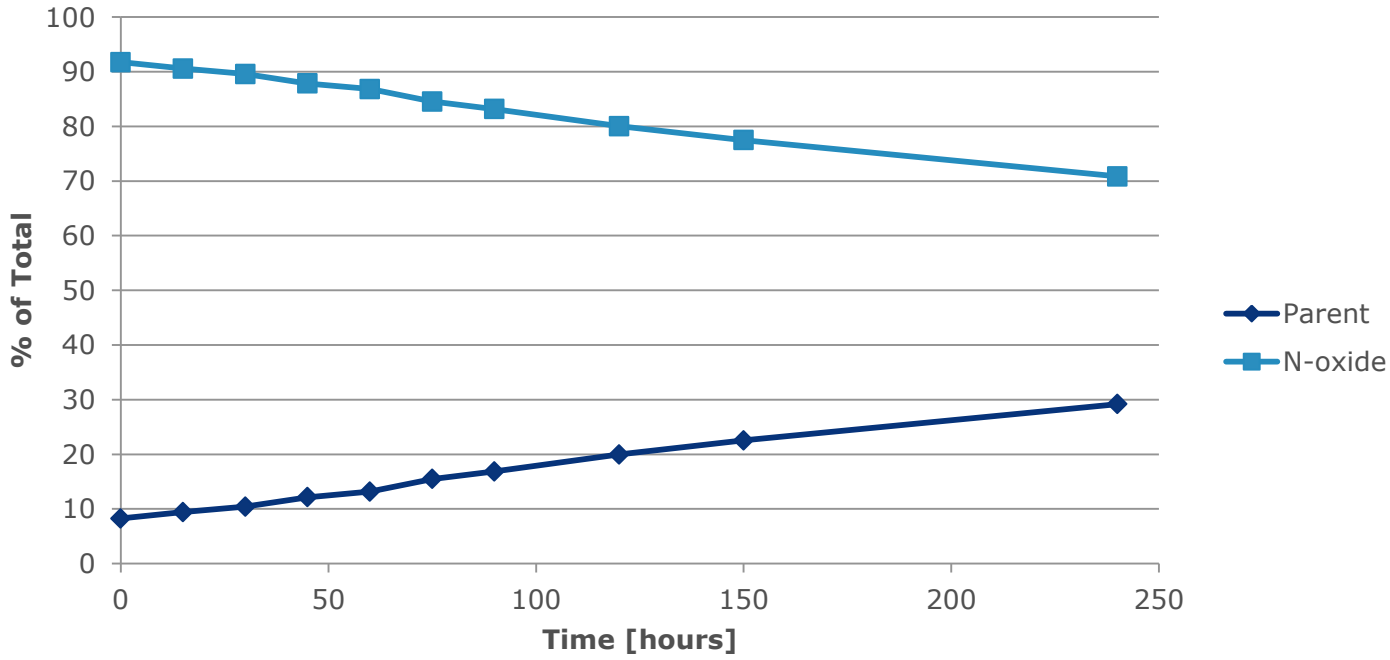
Quinone-dependent tertiary amine N-oxide reduction in rat blood.
K. Shigeyuki et al., Biol. Pharm. Bull. 21 (1998) 1344-1347

Example 1 – *N*-oxide

Incubated in human blood at 37 ° C – whole blood analyzed

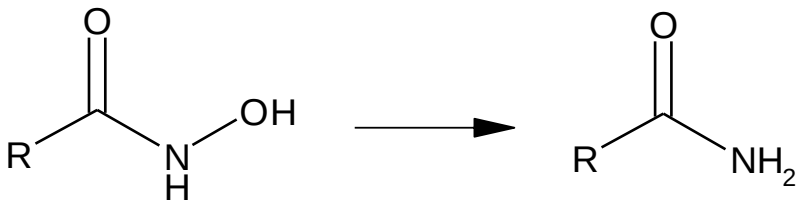


N-oxide Parent

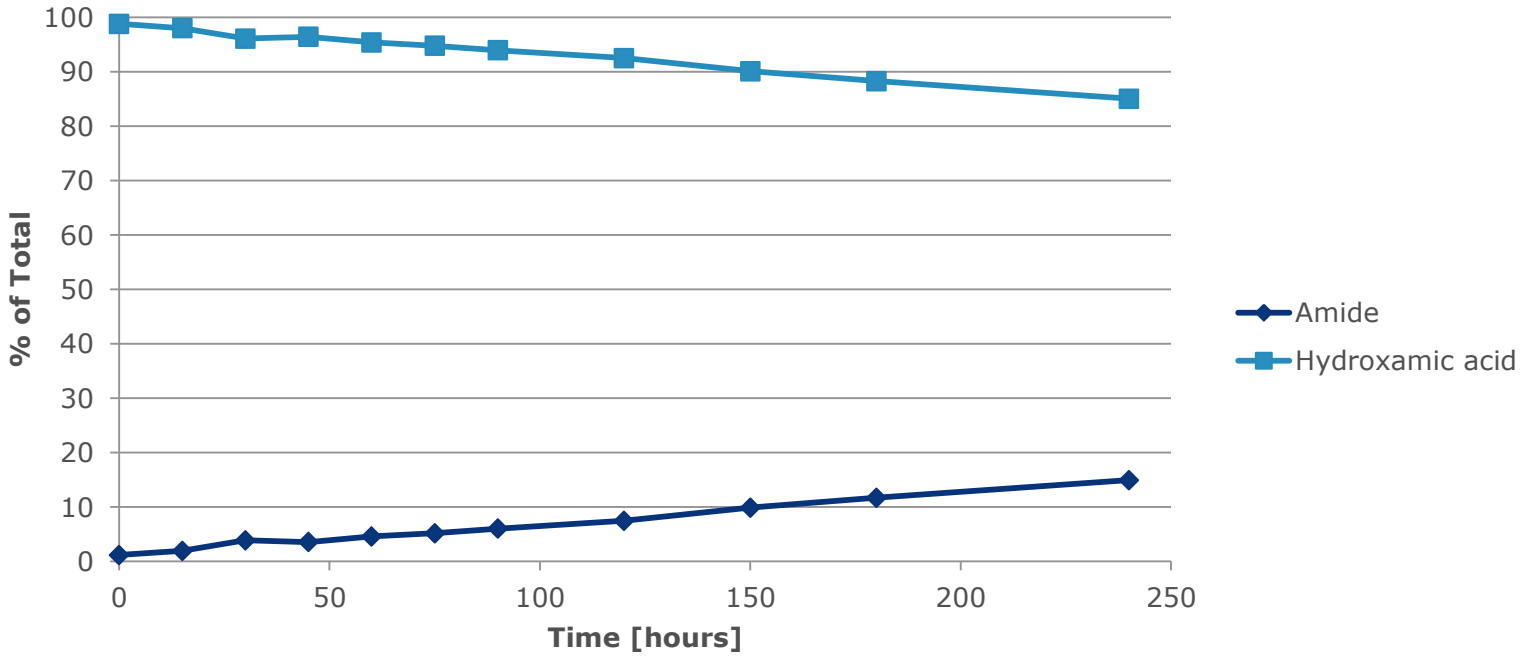


Example 2 – Hydroxamic acid

Incubated in human blood at 37 ° C – whole blood analyzed



Hydroxamic acid Amide



RBC/plasma distribution

Whole blood OK, plasma fraction NOT OK

Incubation conditions	Spiked (ng/ml blood)	% of Ref Whole blood	% of Ref Plasma fraction
2 hours 4 °C	5.00	89	91
2 hours RT	5.00	101	81
2 hours 37 °C	5.00	93	72
2 hours 4 °C	900	95	93
2 hours RT	900	91	84
2 hours 37 °C	900	92	82

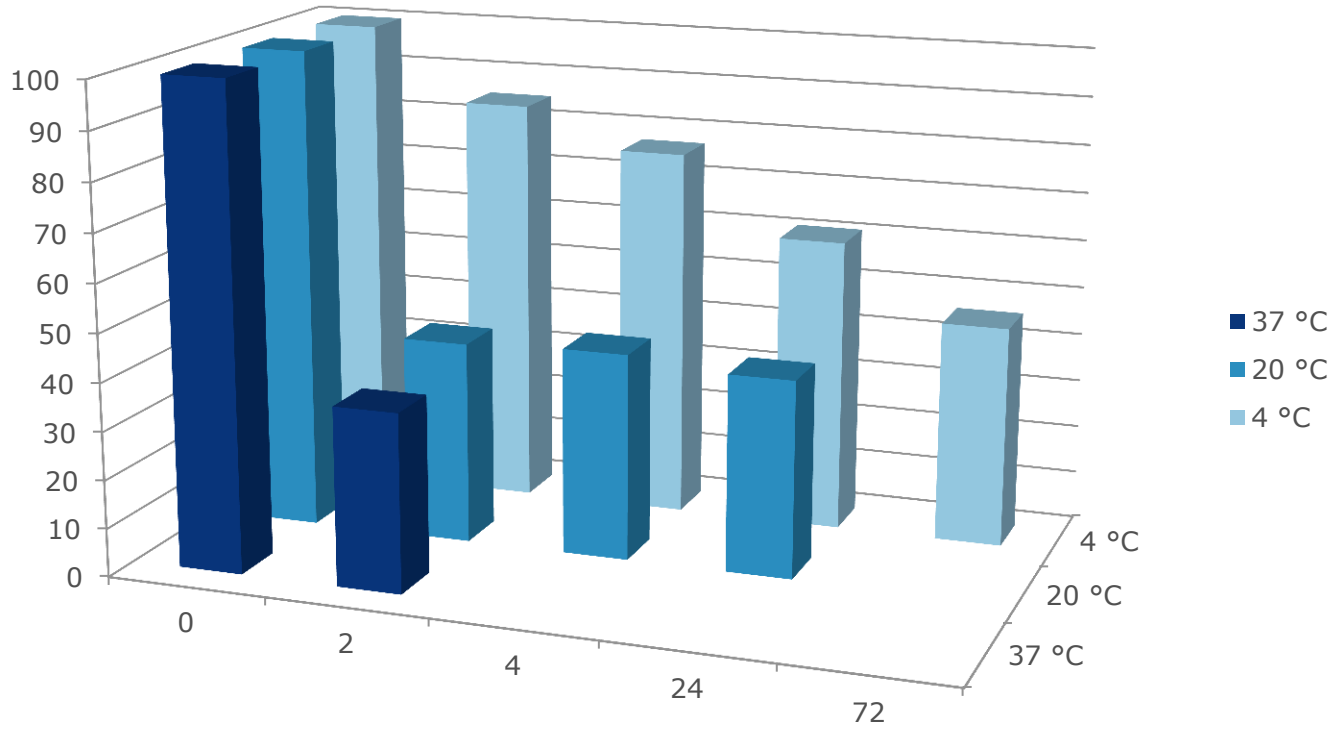
RBC/plasma distribution

High concentration OK, low concentration NOT OK (whole blood was not analyzed, only the plasma fraction was analyzed)

Incubation conditions	Spiked (ng/ml blood)	% of blood conc.	% of Ref Plasma fraction
Reference	0.52	83	
2 hours at 4 °C	0.52	59	71
2 hours at RT	0.52	73	88
24 hours at RT	0.52	63	76
2 hours at 37 °C	0.52	84	102
Reference	120	150	
2 hours at 4 °C	120	149	100
2 hours at RT	120	151	101
24 hours at RT	120	150	100
2 hours at 37 °C	120	151	101

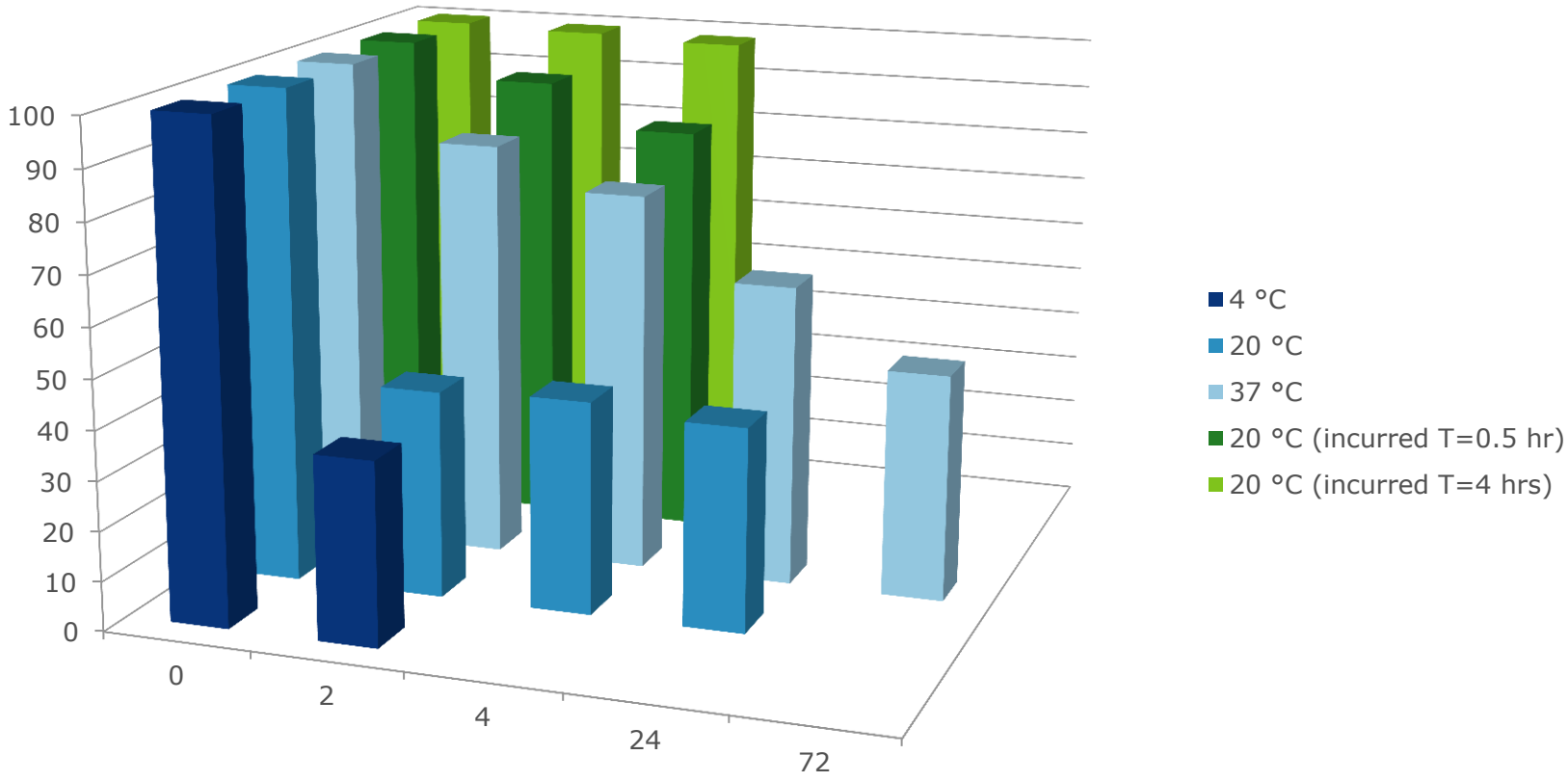
RBC/plasma distribution

Apparent degradation at all tested temperatures, but actually redistribution



RBC/plasma distribution

Apparent degradation at all tested temperatures, but actually redistribution



Conclusions and recommendations

- Compounds can be stable in plasma but not in blood
  - Plasma stability is not always predictive for blood stability
  - Conduct of blood stability experiments cannot be ignored
- Compound classes with concerns can be identified
- Special caution with *N*-oxides as major metabolites
  - Incurred plasma samples can be spiked to blood to conduct experiment in absence of available reference compound
- RBC / plasma distribution makes interpretation difficult
  - Recommendation to analyze whole blood instead of plasma

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