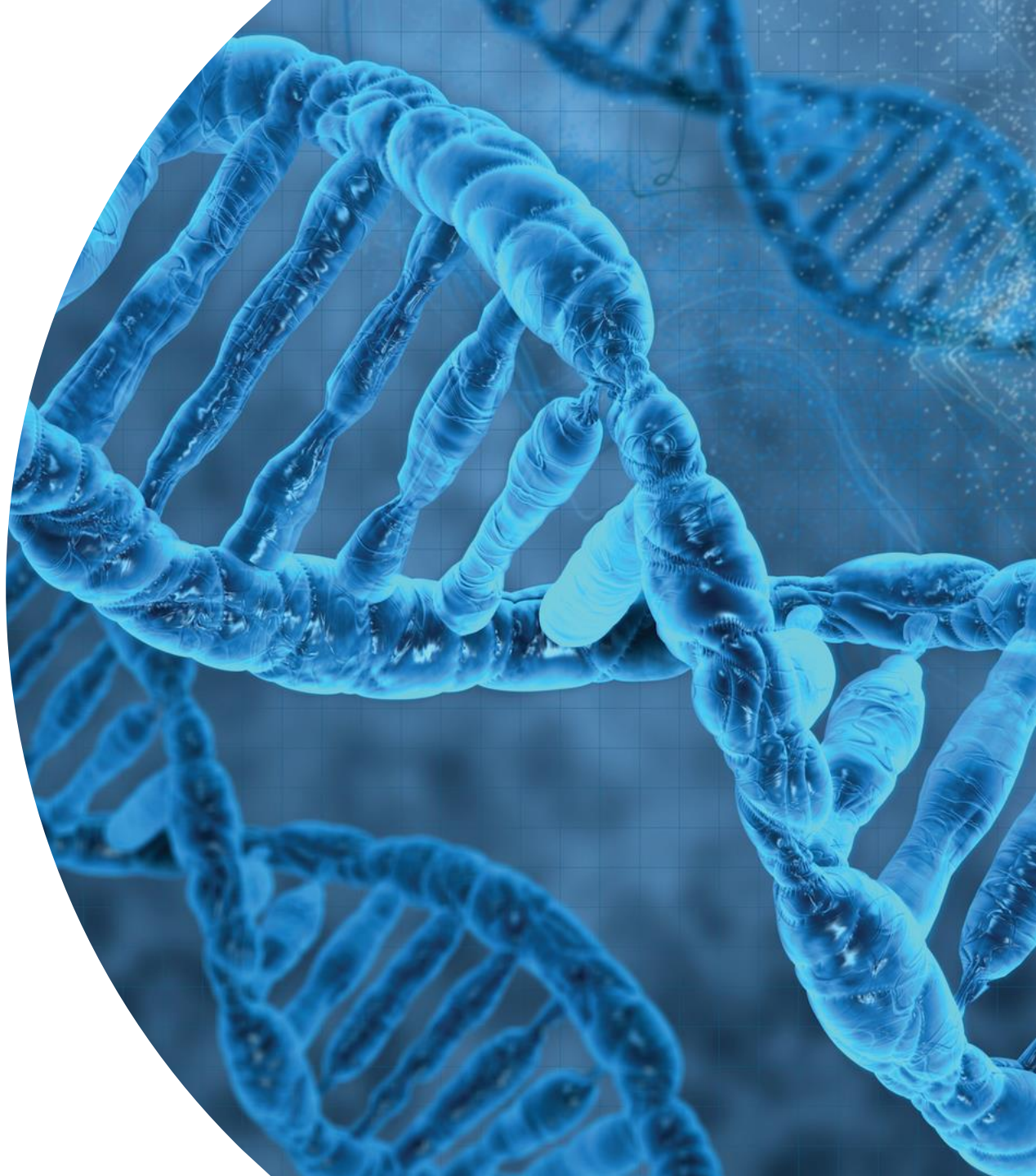
Hydrochlorothiazide – a twist in the tail

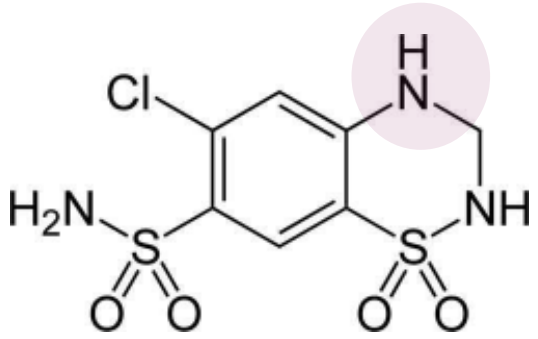
Dr Andrew Teasdale

Senior Principal Scientist, Chemical Development, Pharmaceutical Technology and Development, Operations, AstraZeneca, Macclesfield, UK

Mitigation strategies for Nitrosamine Drug Substance Related Impurities: Quality and Bioequivalence considerations for Generic Products – June 15th 2023



Hydrochlorothiazide – Nitrosamine risk

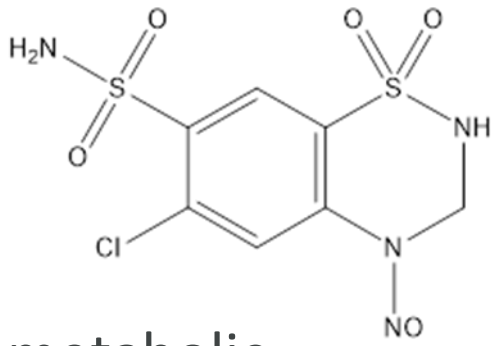


ANILINIC SECONDARY AMINE

- Hydrochlorothiazide (HCTZ) is a thiazide-type diuretic medication approved in 1959 for the treatment of hypertension and oedema (Herman LL, 2020; Reynolds, 1989; Rosendorff, 2011).
- It is the most commonly prescribed antihypertensive drug and used both as a single agent and in combination with other APIs such as beta blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blocking agents, statins, and other diuretics.
- In 2019 it was reported to be the second most commonly prescribed drug (as part of a combination product) in the US with some 40 million prescriptions and close to 10 million patients

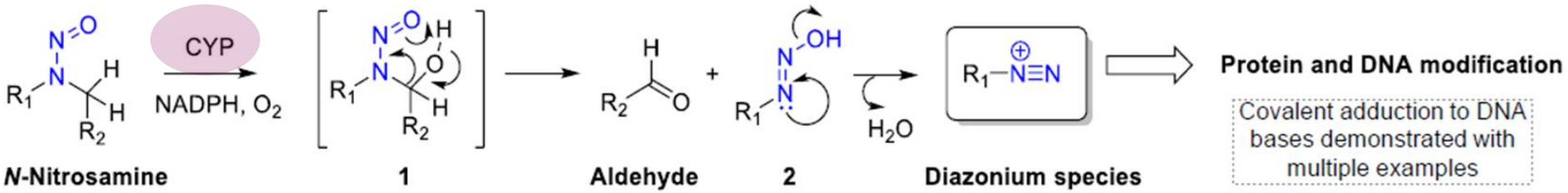
- HCTZ is susceptible to Nitrosation
- The Nitrosamine of HCTZ is positive in the Ames test but the profile is not typical of an N-Nitrosamine
  - This indicates the situation is more complex than first appears
- Investigations have shown the Nitrosamine of HCTZ to be highly unstable at physiological pH.
- *SO WHAT ?*

Nitroso- hydrochlorothiazide HCTZ - What do we know ?



Mutagenic Profile

- AZ has repeatable Ames test for HCTZ – Ames positive
- Results (repeatable – see later) show that active both with and without metabolic activation
  - Inconsistent with anticipated profile

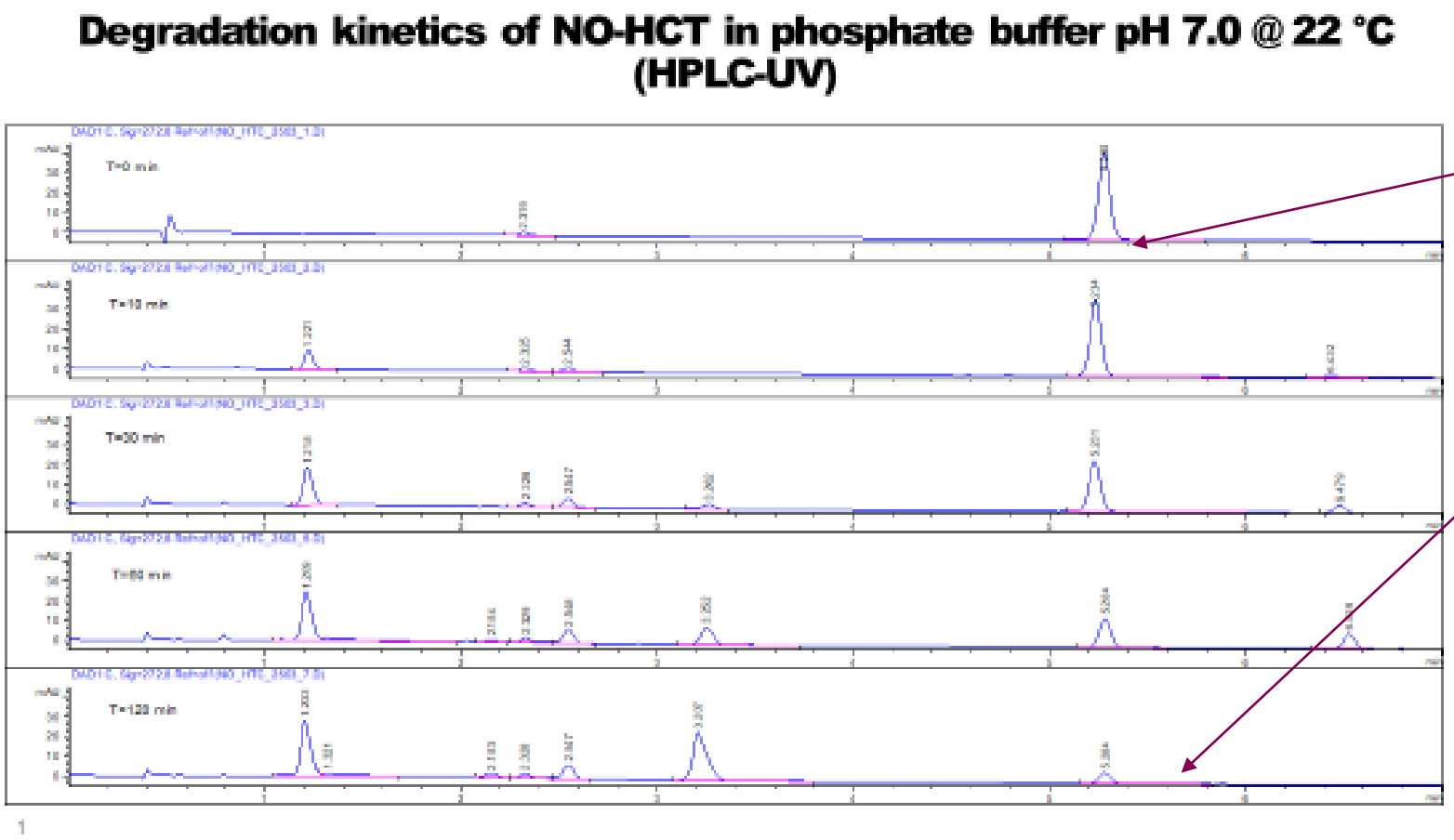


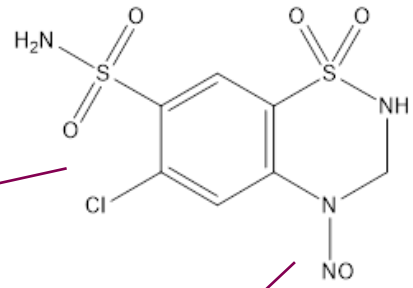
Metabolic activation (rat/hamster/none)	Strain	AZ result
Rat	1535	Neg
	1537	Neg
	98	Pos
	100	Pos
	e. coli	Pos
Hamster	1535	Neg
	1537	Neg
	98	Pos
	100	Pos
	e. coli	Pos
None	1535	Neg
	1537	Neg
	98	Neg
	100	Pos
	e. coli	Pos

  - What is this telling us? That there is a direct acting (Non Nitrosamine) alkylating agent present in the system – Next question – What is it and How ?

HCTZ - What do we know?

- Nitroso HCTZ is unstable under conditions analogous to the Ames test



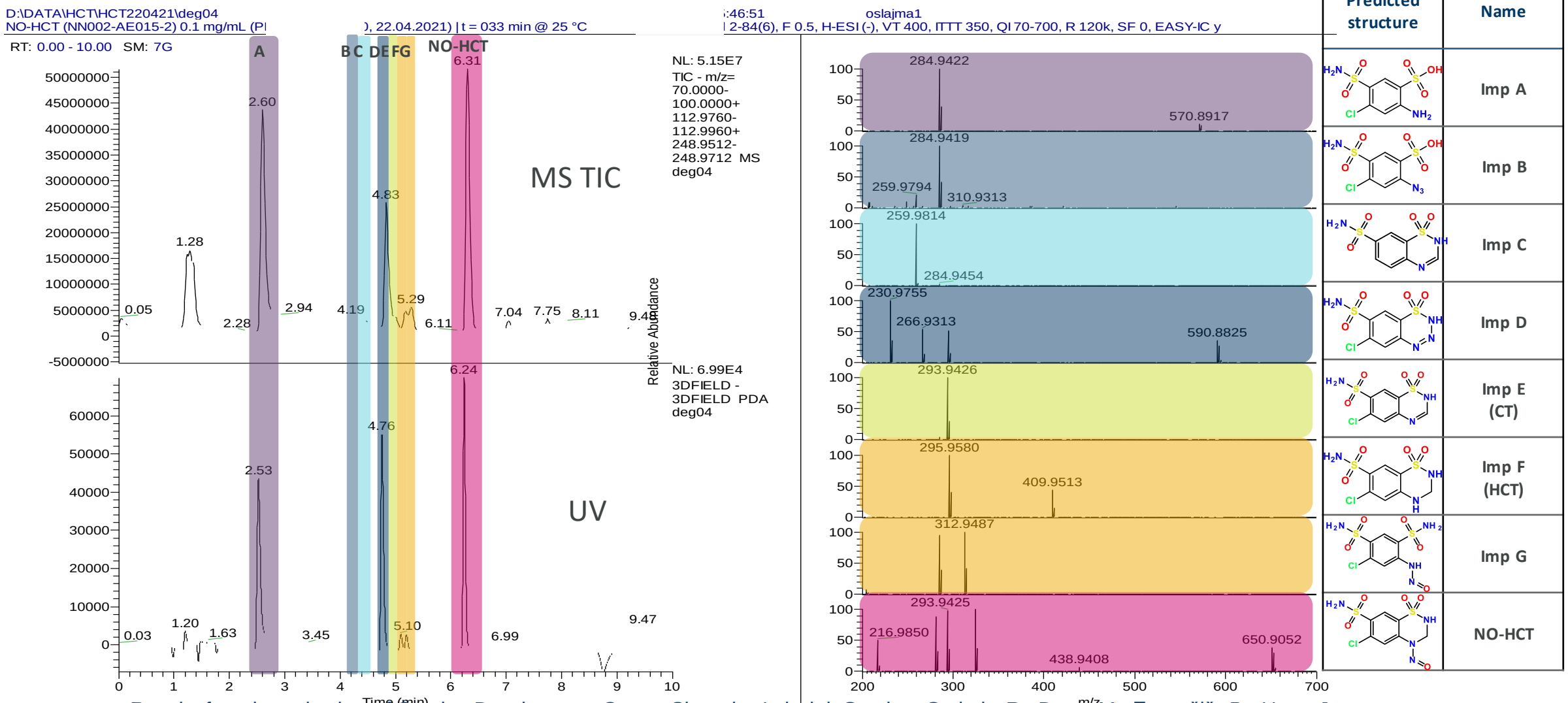


Ames test involves pre-incubation at 37° C for 2 hours

Results from investigation of Sandoz Development Center Slovenia, Lek d.d. Sandoz: Grahek, R.; Drev , M.; Zupančič, B.; Hren, J.; Ošlaj , M.; Bastarda , A.; Kocijan , A.; Časar, Z. On the Stability and Degradation Pathways of *N*-Nitroso-Hydrochlorothiazide and the Corresponding Aryl Diazonium Ion. *Org. Process Res. Dev.* **2023**, submitted.

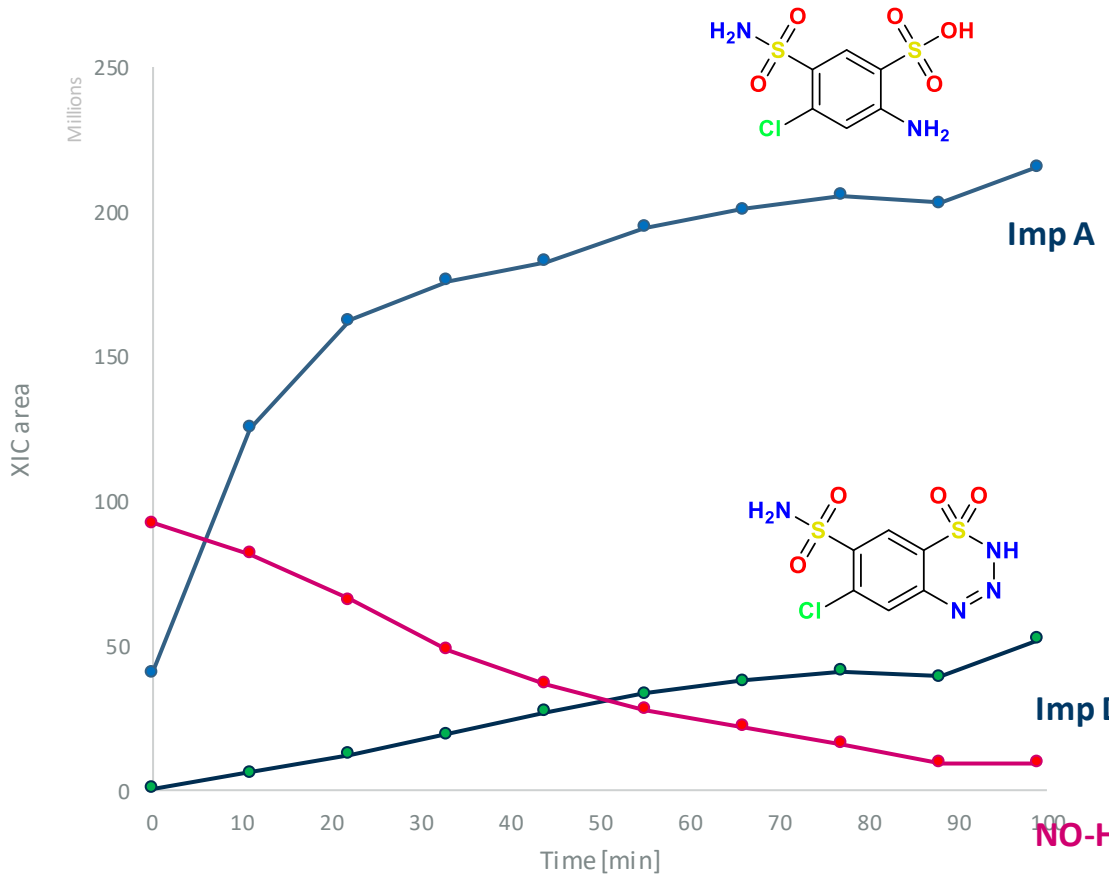
HCTZ – So what is happening ?

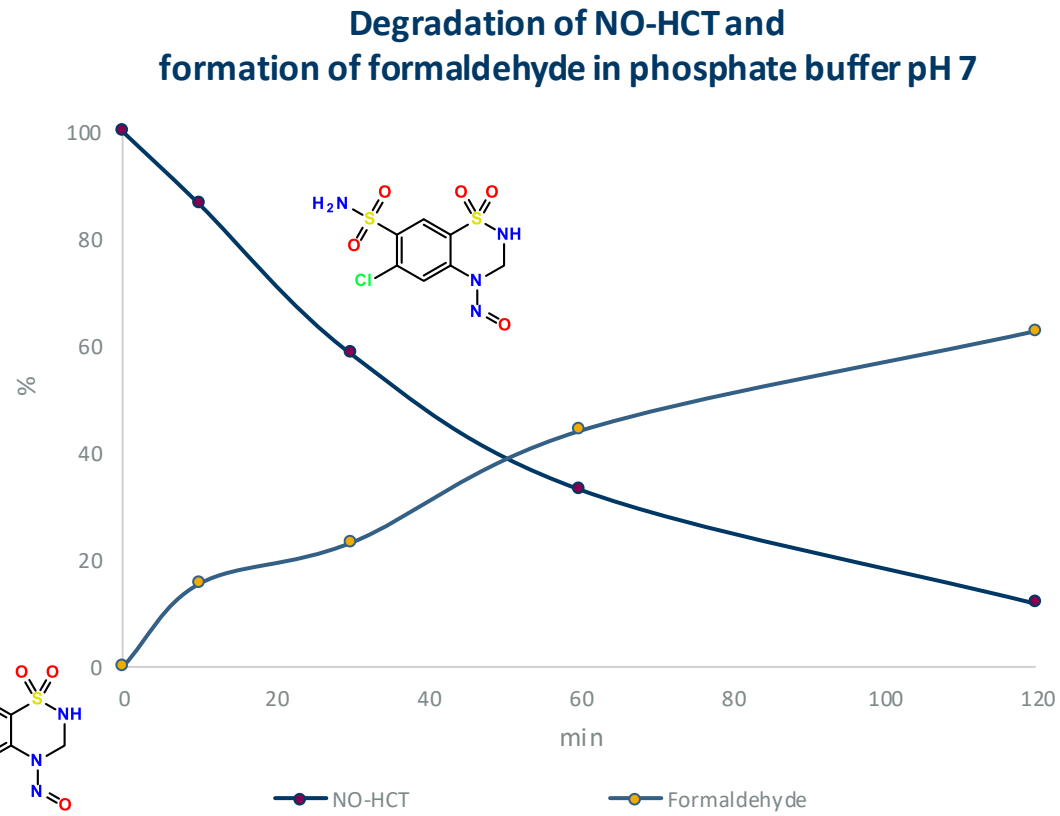
Series of new species are being generated



Results from investigation of Sandoz Development Center Slovenia, Lek d.d. Sandoz: Grahek, R.; Drev, M.; Zupančič, B.; Hren, J.; Ošlaj , M.; Bastarda , A.; Kocijan , A.; Časar, Z. On the Stability and Degradation Pathways of *N*-Nitroso-Hydrochlorothiazide and the Corresponding Aryl Diazonium Ion. *Org. Process Res. Dev.* **2023**, submitted.

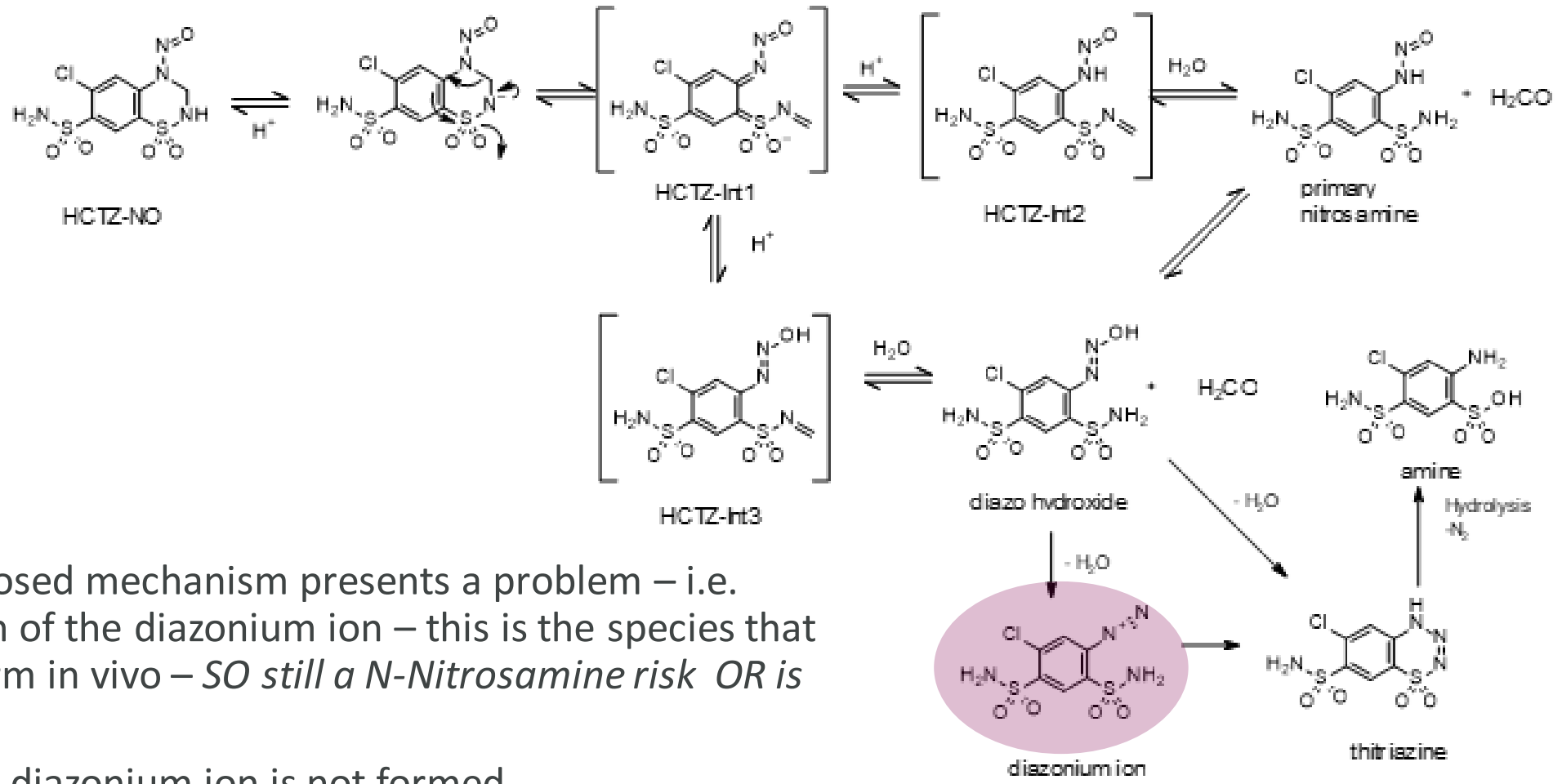
NO-HCT degradation kinetics in phosphate buffer (10 mM, pH 7.0 @ 22 °C)





➢ Degradation of NO-HCT rapidly leads to the formation of two related species – Impurity A and D AND Formaldehyde

Proposed degradation pathway of NO-HCT to triazine

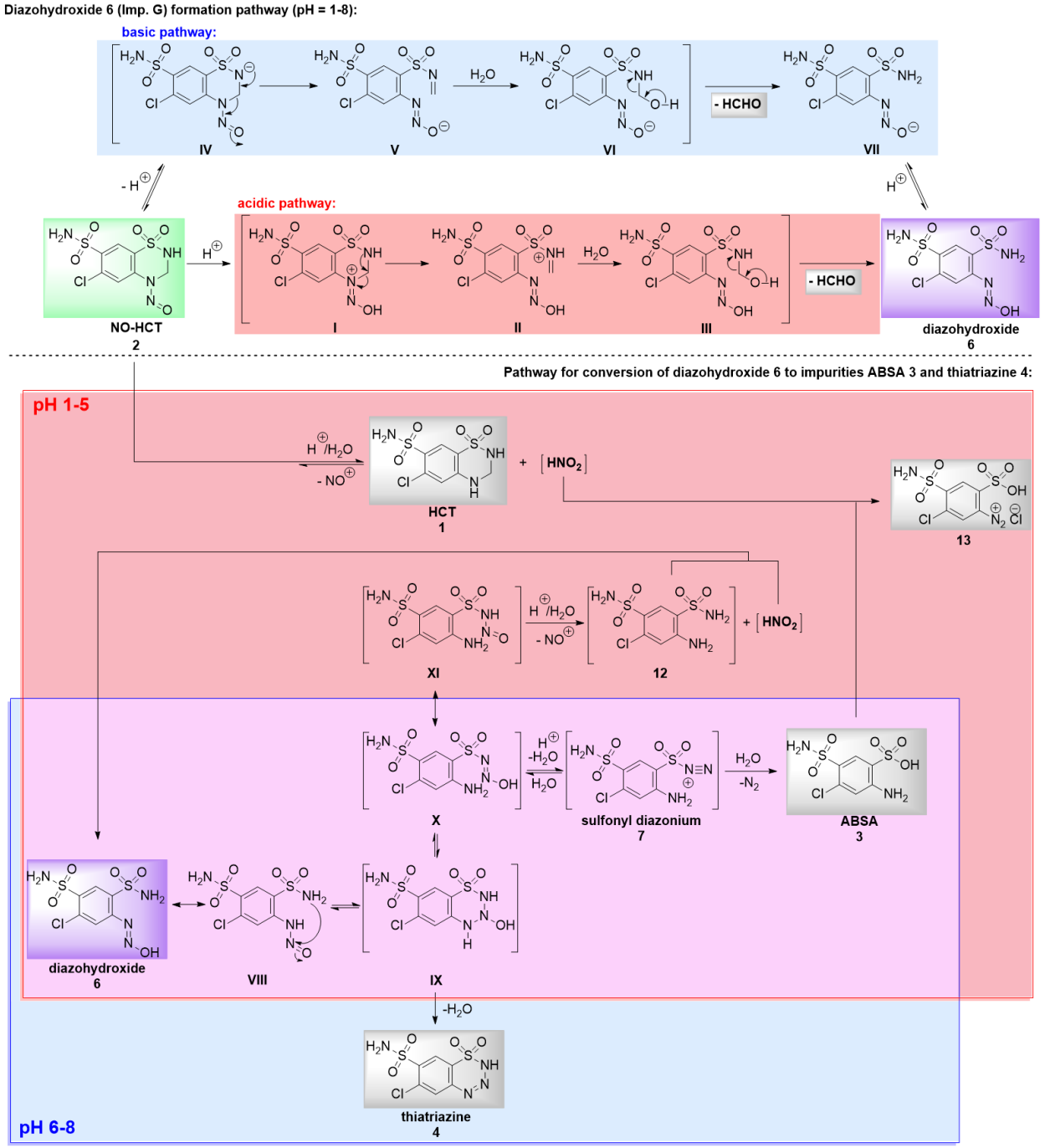


- The proposed mechanism presents a problem – i.e. formation of the diazonium ion – this is the species that would form in vivo – *SO still a N-Nitrosamine risk OR is there ?*
- Not if the diazonium ion is not formed

Results from investigation of Sandoz Development Center Slovenia, Lek d.d. Sandoz: Grahek, R.; Drev, M.; Zupančič, B.; Hren, J.; Ošlaj, M.; Bastarda, A.; Kocijan, A.; Časar, Z. On the Stability and Degradation Pathways of *N*-Nitroso-Hydrochlorothiazide and the Corresponding Aryl Diazonium Ion. *Org. Process Res. Dev.* **2023**, submitted.

Revised mechanism

- By generating authentic samples of postulated species, including the diazonium ion, the following reaction mechanism is proposed, which is consistent with all experimental observations.



Results from investigation of Sandoz Development Center Slovenia, Lek d.d. Sandoz: Grahek, R.; Drev , M.; Zupančič, B.; Hren, J.; Ošlaj , M.; Bastarda , A.; Kocijan , A.; Časar, Z. On the Stability and Degradation Pathways of *N*-Nitroso-Hydrochlorothiazide and the Corresponding Aryl Diazonium Ion. *Org. Process Res. Dev.* **2023**, submitted.

Outcome of Further investigation

- Key observation: HCTZ diazonium is not observed during decomposition of NO-HCT
- HCTZ diazonium was prepared in MeCN solution in extremely acidic conditions (12 eq. of H_2SO_4), where it is reasonably stable. Such conditions are not physiologically relevant. In the presence of water, it rapidly transforms to other derivatives.
- Sulfonyl diazonium species were postulated as a key intermediate in degradation of NO-HCTZ, which is supported by 1H and 15 N NMR spectroscopy as well as formation of imp. A
- Sulfonyl diazonium species should be considered as analogues to sulfonic acid halides (RSO_2X) and should therefore not be considered as a CoC compound
- The secondary diazonium salt (ca. 3 %) was observed during the degradation of NO-HCTZ only at pH = 1. Furthermore stability studies showed that it decomposes immediately at pH 6; which suggests that it would be degraded immediately before absorption

Results from investigation of Sandoz Development Center Slovenia, Lek d.d. Sandoz: Grahek, R.; Drev, M.; Zupančič, B.; Hren, J.; Ošlaj, M.; Bastarda, A.; Kocijan, A.; Časar, Z. On the Stability and Degradation Pathways of *N*-Nitroso-Hydrochlorothiazide and the Corresponding Aryl Diazonium Ion. *Org. Process Res. Dev.* **2023**, submitted.

Ames profile – comparison to Formaldehyde

- Ames profile of NO-HCTZ is consistent with that of formaldehyde
- Triazine profile (impurity D) – Ames +ve one strain without activation is completely different to that expected of an N-Nitrosamine

Overview of NO HCTZ Ames data

Metabolic activation (rat/hamster/none)	Strain	NO HCTZ AZ result March 21	NO HCTZ AZ result Sept 21	Triazine AZ result	Formalin AZ result
Rat	1535	Neg	Neg	Pos	Neg
	1537	Neg	Neg	Neg	Neg
	98	Pos	Pos	Neg	Neg
	100	Pos	Pos	Neg	Pos
	e. coli	Pos	Pos	Neg	Pos
Hamster	1535	Neg	Neg	Pos	Neg
	1537	Neg	Neg	Neg	Neg
	98	Pos	Pos	Neg	Pos
	100	Pos	Pos	Neg	Pos
	e. coli	Pos	Pos	Neg	Neg
None	1535	Neg	Neg	Pos	Neg
	1537	Neg	Neg	Neg	Neg
	98	Neg*	Pos*	Neg	Pos
	100	Pos	Pos	Neg	Pos
	e. coli	Pos	Pos	Neg	Pos

*1.7-fold increase in March data vs 2.0-fold increase in September data

NO-HCTZ - Conclusions

- NO-HCTZ is extremely unstable
- Under physiological pH it generates the following species
  - Equimolar yield of formaldehyde
  - An Aromatic amine (Impurity A)
  - A Thiatriazene (Impurity D)
  - Formaldehyde has a limit of 10mg /day – also formed endogenously – no concern

- NONE of these belong to the Cohort of concern and are not N-Nitrosamines
- Therefore it is proposed that levels of Impurity A / Impurity D be managed through application of the TTC – 1.5ug/day.

Transgenic Study

- Results of Transgenic Study – Negative
- This fully corroborates the results / findings of the mechanistic studies
- Nitroso hydrochlorothiazide is non mutagenic i.e., class 5 and thus can be controlled to limits defined by ICH Q3A / 3B