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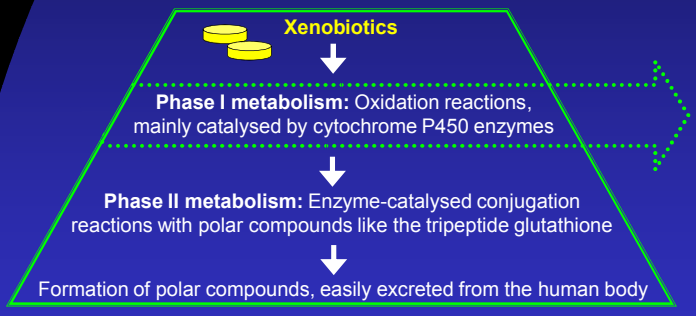
Simulation of the oxidative phase I metabolism of tetrazepam using online electrochemistry/HPLC/MS

Degradation pathway of xenobiotics



Conventional methods for metabolism studies

- *In vivo* and *in vitro* methods, using laboratory animals, isolated liver cells or microsomes
- ⇨ Metabolites may form adducts with the cell matrix, hence isolation and identification of reactive metabolites is hampered
- ⇨ Complex and time consuming
- ⇨ Variations in the expression of cytochrome P450 isoforms in each organism have to be taken into account


Laboratory animals


Liver cell microsomes

Instrumental approach: Electrochemical simulation

- Electrochemical cell for the simulation of the oxidative phase I metabolism
- ⇨ Fast method for direct identification of oxidative labile sites in a drug molecule
- ⇨ High potential for high-throughput screening of metabolites in drug development

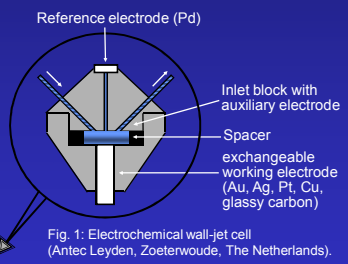


Fig. 1: Electrochemical wall-jet cell (Antec Leyden, Zoeterwoude, The Netherlands).

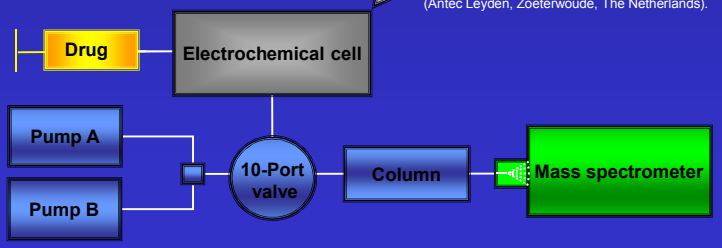
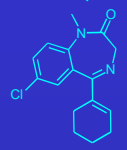


Fig. 2: ALEXYS OxMet™ HPLC system (Antec Leyden, The Netherlands) for electrochemical simulation of oxidative phase I metabolism. The oxidation products, eluting from the electrochemical cell, are collected in a sample loop, which is implemented in the 10-port valve. After loop filling, the oxidation products are flushed onto the column and further detected by mass spectrometry.

Metabolic studies of tetrazepam

- Pharmaceutical specialty: Myolastan®, used for example in the treatment of muscular rheumatism or slipped spinal disks
- Main metabolites according to the manufacture: Demethylation to nortetrazepam and hydroxylation to 3-hydroxy-tetrazepam followed by a glucuronidation
- Recently published : Metabolic transformation of tetrazepam to diazepam, known as the active compound in Valium® [1,2]

Tetrazepam



Tetrazepam metabolites in urine samples

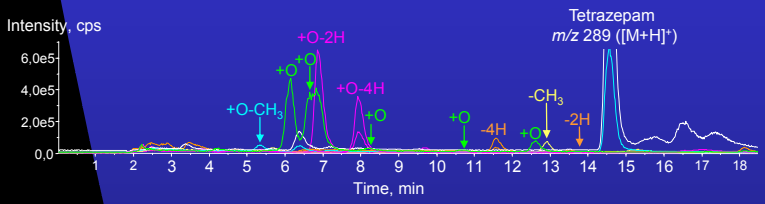


Fig. 3: Mass traces of the metabolites detected in a urine sample from a patient 6 h after intake of one tablet Myolastan®. HPLC separation was performed on a phenyl column. Exact masses have been determined by time-of-flight mass spectrometry.

Proposed structures

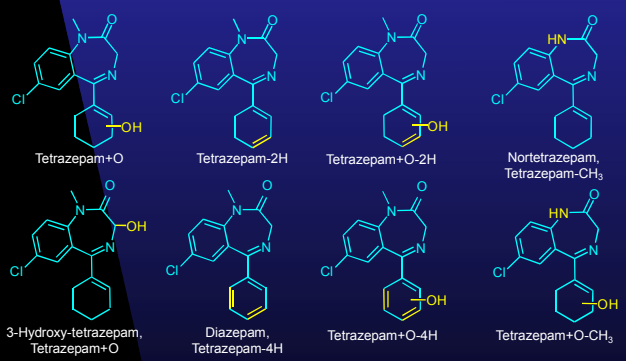


Fig. 4: Proposed structures of the metabolites detected in the urine sample. The structures are in accordance with the postulated degradation of tetrazepam to diazepam[1,2].

[1] Pavilic M, Libiseller K, Grubwieser P, Schubert H, Rabl W (2007), Int J Legal Med 121:169
[2] Laloup M, del Mar Ramirez Fernandez M, Wood M, Maes V, De Boeck G, Vanbeckevoort Y, Samyn N (2007) Anal Bioanal Chem 388:1545

Comparison: Electrochemical simulation - microsomal approach

Electrochemical simulation

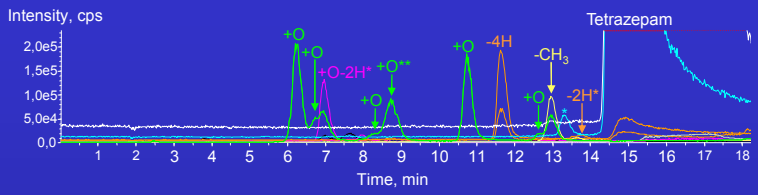


Fig. 5: Mass traces of metabolites formed in the electrochemical wall-jet cell, equipped with a Pt-electrode at a potential of 2 V. *Compounds also present in Myolastan tablets without oxidation. **Metabolites, not found in urine samples.

Microsomal approach

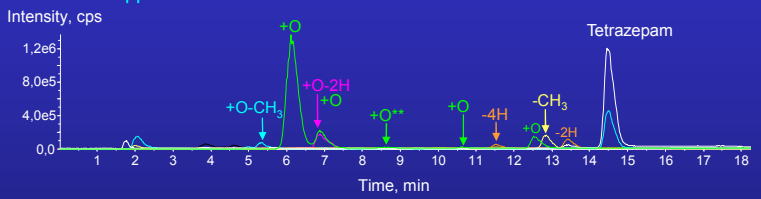


Fig. 6: Mass traces of metabolites formed in the microsomal approach. Experiments were performed using pooled rat liver microsomes from male Sprague Dawley rats. **Metabolites, not found in urine samples.

Conclusion

- Main metabolites of tetrazepam were detected in the conventional microsomal approach as well as in the electrochemical simulation (HPLC-EC)
- One metabolite of the urine sample (+O-4H) was neither found in the electro-chemical simulation nor in the microsomal approach
- Both approaches lead to an additional hydroxylation product, not found in urine. In the human body, this metabolite might takes part in subsequent phase II conjugation reactions.

The results clearly indicate that the purely instrumental approach, based on electrochemical conversion, is as well eligible for metabolism studies with significant time savings compared to the conventional microsomal approach.

Acknowledgement

Urine samples from patients after Myolastan® intake were kindly provided by B. Schubert, H. Oberacher, Institute of Legal Medicine, Innsbruck Medical University. For financial support the Fonds der Chemischen Industrie (Frankfurt/Main, Germany) is gratefully acknowledged.