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Implementation and characterization of analytical procedures for the investigation of the metabolic pathway of pharmaceutical drugs using stable-isotope labeling and high resolution mass spectrometry

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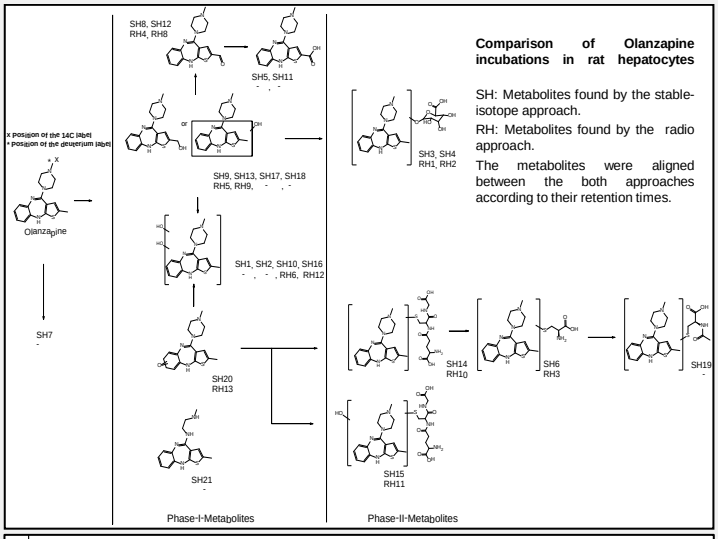
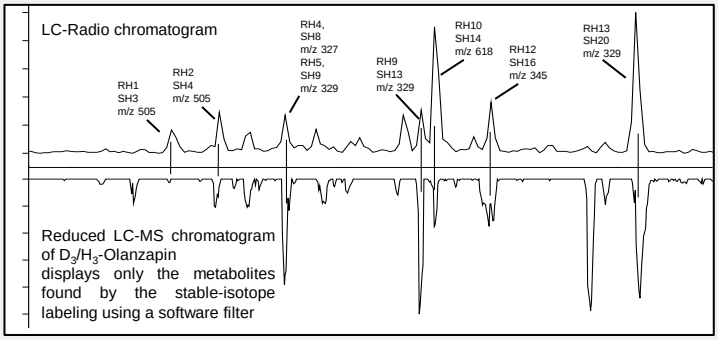
Introduction

The qualitative elucidation of the metabolic pathway of pharmaceutical drugs in *in-vitro* assays is an important part during early stages of the drug development process. Of particular interest during these studies is the selection of a suited tox species which covers all metabolites in human. Method of choice for the elucidation of the metabolic pathway is the use of radio labeled analogs of the drug in combination with LC radio detection. This approach is well established in the pharmaceutical industry. Nevertheless the use of radio labeled compounds has some inherent drawbacks. Normally this kind of compounds is available only at later stages of the development process and the synthesis is complex and cost intensive. An alternative approach is the use of stable-isotope labeled compounds, mainly deuterium analogs of the drug, in combination with LC-MS applications. Even if this technique has been frequently reported there was never a comparative study evaluating the advantages and disadvantages between the stable- and the radio-labeling approach in detail.

Concept

For a systematic comparison of the stable-isotope approach with the classical radio approach three commercially available drugs were selected. Ketoconazole, Olanzapine and Diclofenac were available as deuterium and radio (^{14}C) labeled analogs. The metabolic profile of all 'model compounds' was investigated using mixtures of the unlabeled with the deuterated and the ^{14}C labeled drug analogs, respectively. All observed metabolites were structural elucidated in incubations in rat microsomes and rat hepatocytes. The results of both approaches for all incubation experiments were aligned and compared.





Results

Overall 55 metabolites were found for the three model compounds analyzed in the two incubation types. **All metabolites found with the classical radio approach could also be found with the stable-isotope approach.** 13 metabolites were only found by the stable-isotope approach and for one metabolite the alignment between both approaches was tentative.

As example on the left hand side the results for the incubation experiments of Olanzapine in rat hepatocytes are shown in detail.

During method development it could be shown that the stable-isotope approach is more sensitive as the classical approach. This finding mainly explains the qualitative difference between both approaches. The results for all studies are summarized in the following table.

	Metabolites found with the stable isotope approach	Metabolites found with the radio approach
Olanzapine microsomes	10	7
Olanzapine hepatocytes	21	13
Diclofenac microsomes	2	2
Diclofenac hepatocytes	11	10
Ketoconazole microsomes	5	4
Ketoconazole hepatocytes	6	5

Conclusion

During the performed studies it could be shown that the applied stable-isotope approach is a reliable alternative for the classical radio-labeled metabolism-studies. The respective stable-isotope labeled drug analogues are normally available much earlier during the preclinical development of the drugs, they are much cheaper and can be handled without particular safety-precautions. The results of the metabolite-spotting are much closer to an elucidated structure since m/z values and accurate masses are directly combined with the spotting results whereas the radio-chromatograms must be linked to the MS dataset, which is a sometimes challenging.

Over all, the stable-isotope approach is highly suited for *in-vitro* investigations, e.g. tox species selection where a very reliable qualitative finding of the metabolites is required.

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