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## Some analytical-chemical aspects of butoprozine metabolism in the rat

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### Summary

The subject of the first part of this thesis is the screening for unknown compounds of metabolic origin. Several aspects of detection (UV spectroscopic, liquid scintillation counting) and HPLC separation (also in comparison with GLC and TLC) are discussed, while advantages and limitations are dealt with. Examples are given, taken from the area of chemical and radiochemical purity control. An approach towards screening for unknown metabolites in biological samples is described. The biological sample is introduced directly into the HPLC separation system (without pretreatment like e.g. extraction). A reversed phase separation system is used.

The second part of this thesis deals with preparative applications of HPLC. Different situations are discussed: isolation of the main component in a mixture, containing closely related compounds; isolation of a number of compounds, present in low concentration, from a sample in which one component is present in large excess; isolation of the main component from a mixture, where the solubility is a limiting factor.

Finally, in this part of the isolation of butoprozine metabolites from rat bile is described, as an example of the use of pre-columns for trace enrichment in a preparative application.

In the third part the identification of isolated compounds using spectroscopic techniques is treated. For several reference substances NMR and mass spectrometric data are given ( $^1\text{H}$  and  $^{13}\text{C}$ -NMR, EI and CI-MS, the latter with both positive and negative ions). Using these data, a number of unknown compounds could be identified. Finally, some preliminary results of current investigations are shown. Some examples of the use of packed microbore HPLC columns are given together with the results of the on-line coupling of these columns to a mass spectrometer. In addition, some results of on-line

radioactivity detection for HPLC are given, in which the storage and processing of the data are of special interest.