

Summary

The purpose of this work, the coupling of the photochemistry in the purely watery environment with the high performance liquid chromatography, was moved successfully. The HPLC on-line method was developed by using a hyphenated technique comprising a photoreactor and HPLC separation. The enrichment and chromatography occurs with two special aqua columns.

The developed HPLC on-line method was used successfully for photostability testing with variable pH factors and with different sources of light. The described method allows the investigation of the most different drugs. Thus, polar as well as lipophilic drugs could be investigated with this method.

Especially the application of this method the screening on phototoxic or photoallergic reaction by drugs is to be emphasised.

For screening phototoxic reactions the DNA base guanosinemonophosphate was used as model substance. The drugs chlorpromazine and ofloxacin showed under exposure with UVA light interaction and reaction with the DNA base and the phototoxic effect of these drugs could be confirmed with this method. On the other side the diuretic drug hydrochlorothiazide showed no reaction with GMP, although the drug was described in the literature as phototoxic. A reaction of hydrochlorothiazide could be proved by the use of short DNA antisense single-strand instead of the base GMP under exposure with UVA light. However, in attempts with the DNA sense single-strand no interaction could be ascertained.

For the screening on photoallergic reaction of drugs the dipeptide alanyltryptophan was used as model substance. The test results to the screening on photoallergy well correlated with the information from the literature. The photoallergic potential could be confirmed for the phenothiazines, hydrochlorothiazide, ofloxacin and mefloquine by the interaction or reaction with the dipeptide.

The theory was confirmed that often halogenated drugs are involved in photochemical reactions with biomolecules. The halogenated drugs chlorpromazine, hydrochlorothiazide, ofloxacin and mefloquine turned out especially reactive in presence of biomolecules. The not halogenated drugs etodolac and nifedipine showed to the comparison weak reaction with biomolecules. All together distinctive pH dependence has appeared in the carried out investigations.

A damage dependent on sequence of the DNA single-strand can be booked with the investigation results. The chromatograms of the DNA antisense strand showed in presence of

hydrochlorothiazide, eosine and protoporphyrine a damage or structure change of the DNA antisense strand, while the comparative investigation results showed higher stability to the DNA sense strand.

The main application of the described HPLC on-line method is the screening on photoallergic and phototoxic potential of drugs. Drugs in development could be tested with this method in early stage. This method is a low cost alternative to conventional methods. In comparison animal experiments are very time-consuming and are also cost-intensive with it.

Outgoing from the introduced results numerous other application possibilities arise. With the developed method new knowledge could be won to understand mechanisms by photosensitizers in photodynamic therapy.