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Short Communication

Liquid chromatographic determination of mianserin in plasma by fluorescence detection after on-line photochemical reaction*

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Introduction

The tetracyclic antidepressant mianserin (Tolvin®) belongs to a new generation of antidepressant drugs with low anticholinergic and cardiotoxic side effects. The use of liquid chromatography (LC) has been described [1] when this drug has to be measured in plasma to check the efficiency of the pharmacotherapy and the compliance of patients, but low plasma levels (therapeutic range: 15–70 ng ml^{-1}) [2] and the poor absorbance coefficients in the UV above 220 nm put high demands onto sample preparation and chromatography. This paper describes the LC determination of mianserin with fluorescence detection after "on-line" photochemical reaction and its consequences on detection limits and specificity of analysis.

Experimental

Chromatographic system

The isocratic HPLC system consisted of a Gilson solvent delivery pump Model 302 (Villiers le Bel, France), a WISP autosampler from Waters (Millipore, Eschborn, FRG). As detectors a Shimadzu SPD-6A variable wavelength UV detector (Shimadzu Europe, Duisburg, FRG) or a 820-FP spectrofluorometer from Jasco (Barzano, Italy) equipped with a XBO 150 W 1 ofr xenon light source (Osram, Berlin, FRG) connected to a LDC 10B integration system (LDC-Milton Roy, Riviera Beach, FL, USA) were used. On-line absorbance spectra were recorded with a 1000S diode array detector from Applied Biosystems (Weiterstadt, FRG), fluorescence spectra on the 820-FP spectrofluorometer equipped with a cuvette holder instead of the flow cell.

Mianserin was separated using a 150 × 4.6 mm Asahipak ODP-50 column (obtained from Amchro, Sulzbach, FRG) run with a mobile phase consisting of methanol–acetonitrile–water (50:25:25, v/v/v) at a flow of 1 ml min^{-1}. All solvents used were obtained in HPLC-grade from Scharlau (Barcelona, Spain). Mianserin HCl was a gift from Organon (Oss, The Netherlands).

Photochemical reaction

For post-column irradiation a "Beam-Boost" photochemical reactor was used (ICT, Frankfurt, FRG), connected between the column outlet and the detector. The eluate was irradiated "on-line" in a capillary PTFE tubing in crocheted geometry by a tubular 8W-low pressure mercury lamp with prominent spectral emission at 254 nm. When using 25 m of illuminated reaction coil the resulting irradiation time at a flow rate of 1 ml min^{-1} was 150 s. By exchanging reaction coils of different lengths (up to 25 m), various irradiation times could be obtained. In off-line irradiation experiments a "Beam-Box" photoreactor

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(ICT) was used to irradiate cuvettes with four low-pressure mercury lamps. Mianserin solutions (3 ml) were irradiated in 10 × 10 mm quartz cuvettes for 90 s, which corresponds approximately to the on-line photoreaction condition in the "Beam-Boost" photochemical reactor.

Analysis of plasma

Blood plasma was deproteinized by the addition of an equal volume of acetonitrile. After centrifugation and dilution of the supernatant with water (1:1), 200 µl were injected into the chromatographic system.

Results

Photochemical reaction of mianserin

In contrary to other tricyclic drugs, mianserin does not exhibit significant UV-absorbance above 230 nm. But following irradiation with UV-light in aqueous solution, a distinct change in the UV-spectrum of the drug was observed, showing a spectral band in the range 220–250 nm, with a long tailing edge extending up to 280 nm.

Similarly, following photochemical reaction, mianserin, which does not show any significant native fluorescence activity, became strongly fluorescent with an emission maximum at 430 nm after excitation at 270 nm (Fig. 1). Although this photochemical effect was already observed after irradiation with UV-light for a few seconds, the fluorescence of mianserin was further enhanced at longer reaction times, without reaching a maximum after 150 s (Fig. 2). This observed photochemical process was found to be linear for mianserin concentrations from at least 0.5–1000 ng injected. In off-line experiments the conditions for this photochemical process were further examined. The formation of the fluorescent product of mianserin seemed to be dependent on the kind of organic modifier in the mobile phase: the yield in fluorescent product formation was four to five times greater when methanol instead of acetonitrile was used. While the presence of higher concentrations of buffer salts (100 mM and above) attenuated the photoreaction product, the fluorescence intensity of the formed mianserin photoproduct itself was slightly enhanced under acidic conditions.

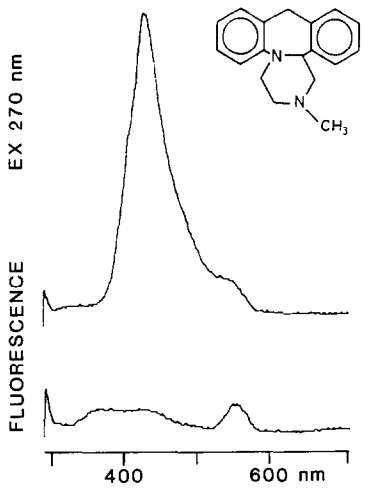

Figure 1
Fluorescence spectra of mianserin in 50% methanol before (lower trace) and after irradiation with 254 nm UV-light for 90 s (upper trace).

Chromatographic separation of mianserin

The above results suggested that mianserin should be separated with a mobile phase system low in buffer concentration and high in percentage of the organic modifier methanol. In previous experiments mianserin exhibited pronounced tailing on reversed-phase silica indicating strong polar (silanol) interactions parallel to the reversed-phase retention. This could only be suppressed by high concentrations of an amine salt added to the mobile phase.

Consequently, a separation system without buffer salts in the mobile phase was used, choosing a column packed with a vinyl alcohol polymer modified with C-18 chains. The resulting chromatograms showed very good peak symmetry and acceptable column performance, which otherwise could not be found on a reversed-phase silica system. Using a methanol–acetonitrile–water mobile phase (50:25:25) mianserin eluted from a 15 cm column with a retention time of approximately 9 min.

Analysis of mianserin in plasma

The formation of a fluorescent product by on-line photochemical reaction provided the basis for a specific and sensitive chromatographic analysis of mianserin in plasma. Using UV-detection at 220 nm mianserin cannot be determined in plasma without extensive sample clean-up, but when on-line post-column photoreaction in combination with fluorescence detection is chosen, the same sample could be easily quantified using the

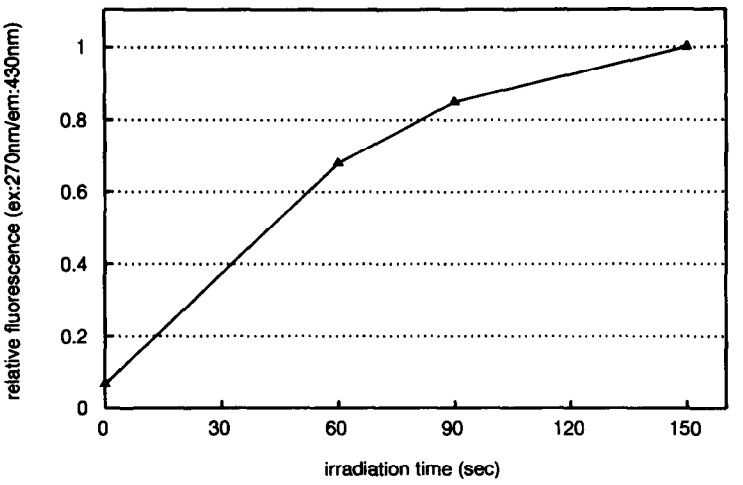

Figure 2
Dependence of fluorescence intensity (excitation at 270 nm, emission at 430 nm) on irradiation time of mianserin.

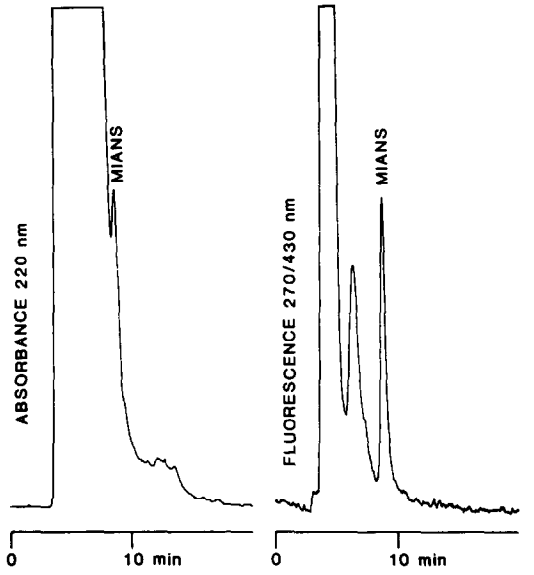

Figure 3
Chromatogram of 50 µl plasma sample spiked with 100 ng ml^{-1} mianserin after protein precipitation as sample preparation step. Left: detection at 220 nm. Right: fluorescence detection (ex:270 nm/em:430 nm) after on-line irradiation for 150 s (25 m reaction coil).

same chromatographic conditions, with increased sensitivity (Fig. 3).

The low detection limit (0.2 ng mianserin) of this detection method allows analysis of plasma samples with minimal sample preparation down to the 5 ng ml^{-1} level.

Discussion

On-line irradiation in HPLC analysis has been proven to be a very efficient and easy-to-perform post-column reaction procedure for a number of drug classes [3, 4], because it neither requires complex equipment nor the addition of reagents to modify analytes, as is normally necessary in other post-column reaction systems. Under UV-light mianserin undergoes a molecular modification (possibly ring opening or radical formation in the azepine ring) leading to a conformational change, that allows the molecule to fluoresce.

Changing from absorbance detection below 220 nm to the more specific fluorescence detection in analysis of blood plasma samples not only lowers the detection limits, but furthermore, reduces drastically the need for sample preparation. Reduced sample preparation not only has the consequence in higher analysis speed but also in increased accuracy and reproducibility of analysis without sacrificing the specificity of the analysis, an important prerequisite for the acceptance of LC in clinical routine measurements.

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