Photochemically induced fluorimetric detection of tianeptine and some of its metabolites. Application to pharmaceutical preparation†

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The photochemically induced fluorescence (PIF) properties of tianeptine and some of its metabolites were investigated in acidic (pH 2.3) water–alcohol mixtures at room temperature. Two PIF methods were developed, including bulk solution and flow injection analysis (FIA). Linear calibration plots were established over a concentration range of more than one order of magnitude. Limits of detection ranged from 15 ng ml$^{-1}$ for FIA-PIF to 25 ng ml$^{-1}$ in bulk solution. The RSDs were between 3 and 5%. The PIF methods were applied to the determination of tianeptine in a pharmaceutical preparation with recoveries varying from 96 to 106% in bulk solutions and from 98 to 106% for FIA-PIF.

Tianeptine is an original dibenzothiazepine tricyclic antidepressant agent. Its novelty proceeds from its chemical structure, which distinguishes it from the classical dibenzazepine derivatives such as imipramine, but also from its pharmacological profile. In fact, tianeptine, in contrast to typical tricyclic antidepressants or selective serotonin uptake inhibitors (SSRIs), stimulates presynaptic serotonin (5-hydroxytryptamine) uptake in the brain and platelets after short and long term administration. On the other hand, tianeptine does not directly affect the uptake or release of dopamine.†

Unlike other antidepressant agents, tianeptine also has anxiolytic properties in patients with co-existing depression and anxiety. It appears to have a more favourable tolerability profile than amitriptyline in terms of anticholinergic and cardiovascular adverse effects. This last point can be useful in the elderly and patients withdrawing from the effect of alcohol.2

Pharmacokinetic studies showed that tianeptine is mainly metabolised by the extrarenal route. β-Oxidation is the major metabolic pathway and the pentanoic (MC5) and propionic (MC3) acid side-chain derivatives are the major metabolites in urine and plasma, further displaying therapeutic activity.3–5

In addition to pharmacological work, a number of analytical studies concerning tianeptine have been undertaken. Most of them involved HPLC determination techniques from classical UV detection6 or with diode array detection.7,8 We are not aware of any research on the luminescence properties of tianeptine or an assay method based on its photochemistry.

In this work we investigated the photochemically induced fluorescence (PIF) properties of tianeptine (7-(3-chloro-6-methyl-5,5-dioxo-6,11-dihydrodibenzo[f,j]-1,2-thiazepin-11-yl)amino)heptanoic acid, sodium salt) and its major metabolites MC5 (3-(3-chloro-6-methyl-5,5-dioxo-6,11-dihydrodibenzo[f,j]-1,2-thiazepin-11-yl)amino)pentanoic acid) and MC3 (3-(3-chloro-6-methyl-5,5-dioxo-6,11-dihydrodibenzo[f,j]-1,2-thiazepin-11-yl)amino)propanoic acid, chlorhydride) (Fig. 1) and their behaviour in environments of different pH and/or solvent. Taking into account the advantage of photochemical derivatization as a simple, clean and efficient analytical approach,9–11 we demonstrate the usefulness of this novel and rapid method, which is also combined with flow injection analysis (FIA), for the determination of tianeptine in pharmaceutical preparations.

**Experimental**

**Apparatus**

Varian (Palo Alto, CA, USA) Cary-210 and a Perkin-Elmer (Norwalk, CT, USA) Lambda-2 UV/VIS spectrophotometers were used for recording the electronic absorption spectra. Corrected fluorescence excitation and emission spectra were measured at 20 ± 2 °C on an Aminco-Bowman (Urbana, IL, USA) SLM Series 2 luminescence spectrometer and uncorrected spectra on a Kontron (Zurich, Switzerland) SFM-25 spectrofluorimeter using a quartz cuvette (10 mm optical pathlength).

Fluorescence lifetimes of tianeptine were measured on a laboratory-made multichannel phase fluorimeter,12,13 equipped with a 15 mW He–Cd continuous-wave laser ($\lambda_{\text{ex}}$ = 325 nm), a Pockels cell for frequency modulation (0.1–200 MHz) and a detection system by cross-correlation.

An Osram (Germany) 200 W high-pressure mercury arc lamp with an Oriel (Les Ulis, France) Model 8500 power supply was utilized for the photochemical studies in bulk solutions. The FIA-PIF system consisted of a peristaltic Ismatec (Switzerland) pump connected to a rotary injection valve fitted with an interchangeable polytetrafluoroethylene (PTFE) loop.

![Fig. 1 Structures of tianeptine and its metabolites MC3 and MC5.](Image)
an on-line Knauer (Berlin, Germany) photoreactor including PTFE tubing wound around a germicide UV irradiation lamp (8 W, 254 nm) and a spectrophotofluorimetric detection system with a compact quartz cell (volume 25 μl, optical pathlength 1.5 mm). The connections between the different parts of the system were made with PTFE tubing (0.5 mm id) and various end-fittings and connectors.

Reagents

The tianeptine sodium salt and the metabolites were a kind gift from Laboratoires ARDIX (Orleans, France). No further purification was performed. The pharmaceutical preparation Stablon (12.5 mg) was purchased commercially. Working solvents were of spectroscopic grade purchased from Aldrich (Milwaukee, WI, USA) or Merck (Darmstadt, Germany). The pH buffers were obtained from Merck or Arcos. HCl was of Normapur analytical grade purchased from Prolabo (Paris, France) and HClO₄ was obtained from Aldrich (spectroscopic grade). De-ionized water was used in all experiments.

Solutions

Stock standard solutions of tianeptine or its metabolites (10⁻⁴ m) were freshly prepared by dissolving an appropriate amount of the compound in de-ionized water.

PIF studies in bulk solutions

Several optimization experiments were carried out. For the solvent effect studies, samples of constant volume and concentration of tianeptine and of its metabolites were irradiated in different solvents or mixtures for the same period of time. A similar procedure was applied for the pH effect study, using the optimum alcohol content previously determined for tianeptine (spectroscopic grade). De-ionized water was used in all experiments.

Results and discussion

Direct PIF study

Absorption and fluorescence spectra of irradiated tianeptine solutions. Tianeptine presents a strong UV absorption band at 271 nm (εₘₚₙₐₓ = 7650 l mol⁻¹ cm⁻¹), which indicates the existence of an extended delocalized π-electron system. Based on the tianeptine structural features, including two benzene and a thiazepine ring, it was expected that this compound would exhibit native fluorescence. However, tianeptine displayed no fluorescence at room temperature in polar solvents such as water, methanol, ethanol, propanol and ethylene glycol. This can be rationalized by the existence of the lowest excited singlet state of the n,π* type occurring at lower energy than the corresponding π,π* states, owing to the thiazepine nucleus; under these conditions, the electronic transition yielding fluorescence emission is inhibited.¹⁴

In contrast, upon UV irradiation of tianeptine (1.5 × 10⁻⁵ m) for 4 min in alcohols or in acidic water–alcohol media, an intense fluorescence band appeared, with an emission maximum at 422 nm and a shoulder at 446 nm; the 266 nm main intense fluorescence band (Fig. 2). Interestingly, the 266 nm main peak of the corrected PIF excitation spectrum corresponds precisely to the 262 nm absorption band of the UV-irradiated tianeptine solutions.

This important result demonstrates that tianeptine undergoes a photochemical reaction, yielding strongly fluorescent photoproduct(s). Moreover, tianeptine fluorescence lifetime measurements, performed by the multifrequency phase method (see details of the measurements in the Experimental section), indicated the presence of two exponential decays (τ₁ = 10.54 ± 0.1 ns and τ₂ = 2.28 ± 0.02 ns). Both steady state and kinetic fluorescence measurements suggest that at least two fluorescent photoproducts are formed. Kinetic and structural studies of these photoproducts are in progress.

Similar PIF excitation and emission spectra were obtained for both metabolites MC3 and MC5 under the same experimental conditions as used for tianeptine (Fig. 2).

In pharmaceuticals. For this application, the classical standard additions procedure was selected. Four tablets of Stablon (12.5 mg of tianeptine) were weighed and pulverized. The whole amount of the resulting powder was then used in order to prepare 100 ml of aqueous stock standard solution. In contrast to tianeptine, which is soluble in water, the excipients contained in the tablets were poorly soluble. For this reason, the stock standard solution was centrifuged for 25 min. Subsequently, the supernatant was discarded and used for further dilutions.

A constant volume of the diluted pharmaceutical sample was spiked with increasing volumes of 10⁻⁵ m tianeptine aqueous standard solution. Each spiked solution was then diluted to 5 ml.
Solvent effect. We investigated the solvent effect on the photochemically induced fluorescence properties of tianeptine. In Table 1, we report the uncorrected excitation and emission wavelengths and relative PIF intensities of tianeptine in various solvents. These wavelength values were used for all subsequent analytical measurements. The PIF spectral properties and the photoreactivity of tianeptine are virtually independent of the nature of the alcohol (Table 1). Water–alcohol (methanol, ethanol, propan-2-ol) mixtures were found to be the most efficient solvents, yielding the highest PIF signals with special emphasis in the case of propan-2-ol. The percentage of propan-2-ol had a significant effect on the PIF signal; indeed, the PIF intensity increased sharply for propan-2-ol contents ranging from 0 to 30%, then reached a plateau and decreased slowly for propan-2-ol percentages > 40%. We decided to adopt 30% as the optimum propan-2-ol concentration in further studies.

Assuming a radical mechanism for the tianeptine photochemical reaction, the fact that, among the alcohols tested, propan-2-ol produces a more intense PIF signal can be related to its capacity to generate more stable radicals than methanol or ethanol.

pH effect. We studied the effect of pH on the tianeptine PIF signal. The curves of the fluorescence intensity $I_f$ versus pH established in water–propan-2-ol (70 + 30 v/v), shows an increase in $I_f$ which reached a maximum at pH 2.3, followed by a decrease for higher pH values (Fig. 3). It may be assumed that tianeptine $N$-protonation occurs at this pH value, producing the PIF signal enhancement. It is worth noting that buffer solutions inhibited the PIF signals; therefore, it was necessary to use dilute solutions of either HCl or HClO₄ of appropriate pH. For both acids identical results were obtained.

**Optimum irradiation time.** The curves of fluorescence intensity of tianeptine or tianeptine metabolite versus UV irradiation time obtained in water–propan-2-ol (70 + 30 v/v) (pH 2.3) exhibited an initial rapid increase in the fluorescence signal, which reaches a well marked plateau between about 4 and 12 min, and finally a decrease for irradiation times longer than 12 min (Fig. 4). This decrease indicates that the fluorescent photoproducts were photolyzed. For both tianeptine and its metabolites, the optimum irradiation time (see definition in the Experimental section) was 4 min. This irradiation time was adopted in subsequent studies.

**FIA-PIF study**

The effects of several parameters on the FIA-PIF intensity and width of the flow injection peaks of tianeptine were investigated. It was found that the injection volume ($V_i$), the flow rate ($V_f$) and the reactor length ($L_r$) produced important changes in the FIA-PIF signals. Changes in the flow rate between 0.2 and 2 ml min⁻¹ produced a significant variation in the FIA-PIF intensities; to avoid excessive band broadening, we adopted a compromise flow rate of 1.1 ml min⁻¹, which was satisfactory for the whole range of tianeptine concentrations (Fig. 5). As expected, the FIA-PIF intensity increased proportionally with the injection volume (in the range 200–408 μl) and an optimum $V_i$ value of 408 μl was chosen. The FIA-PIF signal increased markedly with the reactor length in the range 100–440 cm. A reactor length of 440 cm was selected for subsequent studies.

**Analytical applications and figures of merit**

**Bulk solutions and FIA-PIF.** Table 2 gives the analytical figures of merit for the bulk solution and FIA-PIF determination.
of tianeptine and its metabolites. Linear plots of the product intensity versus initial tianeptine (or metabolite) concentration were established over a concentration range of more than one order of magnitude; the correlation coefficients were very close to unity, indicating a satisfactory precision for the analytical curves. The limits of detection (LOD) were low, ranging from 15 ng ml$^{-1}$ for the FIA-PIF procedure to 25 ng ml$^{-1}$ for the bulk solutions. The small RSDs, between 3 and 5%, depending on the method, demonstrated the good reproducibility of PIF measurements. Our LOD values compare favourably with literature data. Nicot et al.$^6$ reported LODs > 10 ng ml$^{-1}$ for tianeptine and its metabolites using an HPLC method with UV detection. In other HPLC studies concerning a number of antidepressants, Turcant et al.$^7$ and Tracqui et al.$^8$ obtained LODs of about 100 and 2.5–15 ng ml$^{-1}$, respectively, with diode array detection. It must be stressed also that these HPLC techniques are much slower than our FIA methods, with diode array detection.

Both methods were applied to the determination of tianeptine in a pharmaceutical preparation (Stablon), using the standard additions procedure. Good linearity was obtained for the standard addition plots. The slopes were close to those measured for the calibration curves, which indicates the absence of significant interference from matrix effects in the pharmaceutical preparations. Satisfactory recoveries were found at various tianeptine concentrations, ranging from 96 to 106% in bulk solutions and from 98 to 106% for the FIA-PIF studies (Table 3).

### Conclusion

We have demonstrated the usefulness of PIF detection for the easy determination of tianeptine and its metabolites using both stationary and dynamic (FIA) conditions. The assay of tianeptine in a pharmaceutical preparation was performed with good sensitivity, rapidity and precision. Concerning bioanalytical applications, the fact that tianeptine and its metabolites show similar PIF characteristics underlines the interest in combining PIF detection with HPLC in order to increase both sensitivity and selectivity. Work is in progress to explore the feasibility and reliability of such an approach.

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### References