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Determination of duloxetine in human plasma by capillary electrophoresis with laser-induced fluorescence detection

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ABSTRACT

A method based on capillary electrophoresis has been developed for the analysis of the novel antidepressant drug duloxetine in human plasma. The method makes use of laser-induced fluorescence detection after derivatisation of the analyte with 5-(4,6-dichlorotriazinyl)aminofluorescein at pH 11. A single step liquid/liquid extraction procedure with a mixture of hexane/2-propanol allows the sample clean-up with extraction yields always ≥84% and interference removal. The electrophoretic separation is achieved using uncoated fused silica capillaries (60.0 cm effective length, 75.0 cm total length, 50 μm internal diameter) and a background electrolyte composed of borate buffer (40 mM, pH 10.3), tetrabutylammonium bromide (10 mM), and acetone (10%, v/v). The applied voltage is 20 kV; the samples are injected by pressure (50 mbar × 8 s). The method has been fully validated in terms of linearity range (2.5–150 ng mL^{-1}), LOD and LOQ (1.0 and 2.5 ng mL^{-1}, respectively), precision (R.S.D. < 6.7%) and accuracy (recovery >78%). Application to samples obtained from patients under treatment with duloxetine gave good results. The method represents the first application of capillary electrophoresis to the analysis of duloxetine in human plasma.

1. Introduction

Duloxetine ((γ*S*)-*N*-methyl-γ-(1-naphthalenyloxy)-2-thiophenepropanamine, DLX, Fig. 1a) is a novel antidepressant, marketed in 2004 and approved for the acute and maintenance treatment of major depressive disorder, the acute treatment of generalized anxiety disorder and the management of diabetic peripheral neuropathic pain and fibromyalgia [1]. Recently, the drug has been approved in some countries also for the treatment of stress urinary incontinence in women [2].

DLX acts by inhibiting the uptake of serotonin and norepinephrine at neuronal synapses, while the inhibition of dopamine uptake is markedly lower [3]; on the contrary, the in-vitro affinity for other neuronal receptors including adrenergic, dopaminergic, serotonergic and muscarinic receptor subtypes is about 80-fold lower [4]. For this reason, it is classified as a serotonin and norepinephrine re-uptake inhibitor (SNRI) similar to venlafaxine, but with a more balanced activity at the two neurotransmitter reuptake sites [5]. Thanks to this receptor affinity profile, DLX shows better safety and tolerability compared to tricyclic antidepressants [6].

Administered in the form of capsules containing 20, 30 or 60 mg of DLX, the usual dose for all the approved indications is 60 mg day^{-1}, although a 40 mg day^{-1} initial treatment is recommended for the therapy of major depressive disorder. After oral administration, the drug is well absorbed, protein binding is relevant (96%) and the mean elimination half-life is 12 h [5]. DLX is metabolised in the liver by the cytocrome P450 system (in particular the CYP1A2 and CYP2D6 isoforms) to inactive metabolites (4-hydroxy duloxetine glucuronide and 5-hydroxy-6-methoxy duloxetine sulphate are the most abundant), which are excreted in the urine [7,8].

Therapeutic plasma levels of DLX have not been clearly established yet, although a recent Therapeutic Drug Monitoring (TDM) study on 37 patients (30 females and 7 males) treated with DLX (30–120 mg day^{-1}) reported steady-state serum concentrations corresponding to 5–137 ng mL^{-1} for female and 17–92 ng mL^{-1} for male patients [9].

DLX is generally safe, but it is not devoid of side effects, some of them serious (e.g. cardiovascular), although rare. The most frequent side effects include nausea, headache, insomnia, fatigue, somnolence, dry mouth, dizziness and constipation. Less common are: tachycardia, ecchymosis (rare), skin rashes and photosensitivity reactions [1]. It is therefore clear that the accurate determination of

Abbreviations: DLX, duloxetine; DTAF, dichlorotriazinylamino fluorescein; TBAB, tetrabutyl ammonium bromide; LIF, laser-induced fluorescence.

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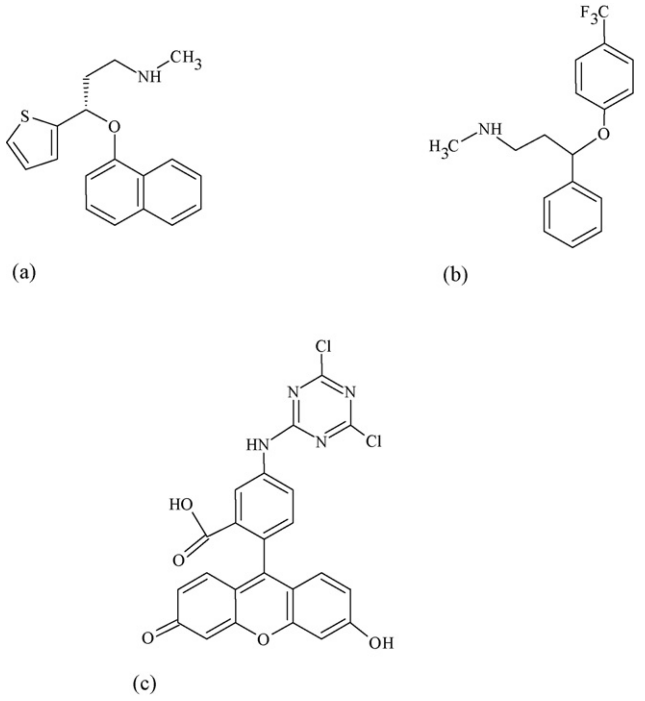

Fig. 1. Chemical structures of: (a) duloxetine (DLX); (b) fluoxetine (IS); (c) 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF).

DLX plasma levels is useful, not only for pharmacokinetic studies, but also for TDM purposes. In fact, plasma levels of psychotropic drugs may differ from one subject to another due to interindividual variability [10]. Other factors, such as polypharmacy [7] or smoking, have been demonstrated to alter DLX pharmacokinetics; a study reported that smokers tend to show remarkably lower serum drug levels, and this has been correlated to induction of CYP1A2 by polycyclic hydrocarbons contained in tobacco smoke [11].

However, only a few analytical methods have been published in the literature for the determination of DLX in biological fluids. A GC–MS method was used for DLX analysis in post-mortem specimens after a liquid–liquid extraction procedure [12]. Other methods are based on HPLC, coupled to different detectors [9,13–16]. In particular, one is based on HPLC with fluorescence detection after derivatisation with dansyl chloride, and analyses are carried out for DLX and its main metabolite in human plasma [13]. Two papers describe LC–MS methods for DLX determination and the application to pharmacokinetic studies [14,15]. HPLC with UV detection has also been used for the analysis of the drug in human plasma, adopting a column-switching procedure for sample pre-treatment [9] or a novel solid-phase extraction procedure [16].

Capillary electrophoresis (CE) represents an alternative to LC or GC, allowing fast separation and requiring very limited amounts of expensive and polluting organic solvents. Moreover, the principle that enables the separation in CE is based on migration of the analytes under the influence of an electric field and not on the partition between two phases as in LC or GC. Two methods are reported in the literature for the enantioseparation of DLX in pharmaceutical dosage forms by CE [17,18], but not for the determination of DLX in biological fluids. Most probably, this is due to the low sensitivity of this technique that limits its applicability to drug analysis in biological samples, if not enhanced by a suitable detection equipment.

The method presented here is based on CE with laser-induced fluorescence (LIF) detection; this particular detection means grants high sensitivity and allows for the first time the application of CE to the determination of DLX in plasma samples.

2. Experimental

2.1. Chemicals and solutions

DLX hydrochloride and fluoxetine hydrochloride (used as the internal standard, IS, Fig. 1b), pure standard, were kindly provided by Eli Lilly Italia (Sesto Fiorentino, Italy). Tetrabutylammonium bromide (TBAB), methanol, 2-propanol, hexane, acetone (all HPLC-grade) were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium bicarbonate and boric acid were from Carlo Erba (Milan, Italy), sodium hydroxide was from Agilent (Waldbronn, Germany). 5-(4,6-Dichlorotriazinyl)aminofluorescein (DTAF, Fig. 1c) was purchased from Invitrogen (Eugene, Oregon, USA).

Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus (Millipore, Milford, Mass., USA).

Stock solution of DLX and IS were both 100 μg mL^{-1} in methanol and resulted to be stable for at least 1 month when stored at −20 °C as assessed by electrophoretic assays.

Stock solutions were diluted daily in a mixture of water, acetone and MeOH (60/20/20, v/v/v) to obtain working solutions. DTAF solutions (10 mM in acetone) were prepared fresh every day.

Carbonate buffer (200 mM, pH 11.0) was prepared by dissolving 840.0 mg sodium bicarbonate in about 45 mL of water and adjusting the pH value of this solution to 11.0 with 2 M sodium hydroxide solution. The solution obtained was then transferred into a 50 mL volumetric flask and water was added to the mark.

The background electrolyte (BGE) was composed of acetone (10%, v/v) and an aqueous solution consisting of borate buffer (40 mM, pH 10.3), containing 10 mM TBAB; it was prepared as follows. 123.7 mg of boric acid was weighed and transferred into a beaker containing about 40 mL of water. The pH of this solution was adjusted to 10.3 using 2 M sodium hydroxide. The content of the beaker was then transferred into a 50 mL volumetric flask and water was added to the mark. The borate buffer thus obtained was used to dissolve the suitable amount of TBAB in order to obtain a final concentration of 10 mM. Finally, 10% (v/v) acetone was added to this solution.

2.2. Instrumentation

The Agilent ^{3D}CE apparatus (Agilent Technologies, Palo Alto, CA, USA) was coupled to a Picometrics (Toulouse, France) Zetalif LIF detector equipped with a 488 nm Ar-ion laser; fluorescence emission was recorded through a 488 nm filter.

Analyses were performed using uncoated fused silica capillaries (Composite Metal, Ilkley, UK) with 50 μm inner diameter, 375 μm outer diameter, 75.0 cm total length and 60.0 cm effective length. The capillary cassette was thermostatted at 25 °C. Samples were injected hydrodynamically (50 mbar × 8 s) at the anodic end of the capillary and separation was carried out by applying a 20 kV voltage.

New capillaries were conditioned with 1 M NaOH and water (10 min each). At the beginning of each working day the capillary was rinsed with water (5 min), 1 M NaOH (5 min), water (5 min) and then with the BGE for 10 min. Between the runs, the capillary was flushed with water (1 min), 1 M NaOH (1 min), water (2 min) and then with the BGE for 5 min. At the end of each working day, the capillary was washed with 1 M NaOH (5 min), water (5 min) and then air-dried.

2.3. Sample pre-treatment

2.3.1. Plasma samples

Blood samples obtained both from healthy volunteers and patients undergoing treatment with DLX were immediately centrifuged (1780 RCF, 5 °C, 15 min) to obtain the corresponding plasma aliquots, which were stored at −20 °C until analysis.