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Short communication

Improved HPLC method for the simultaneous determination of tramadol and *O*-desmethyltramadol in human plasma

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Abstract

This paper describes an HPLC method for the determination of tramadol and its major active metabolite, *O*-desmethyltramadol (ODT), in human plasma. Sample preparation involved liquid–liquid extraction with diethyl ether–dichloromethane–butanol (5:3:2, v/v/v) and back extraction with sulphuric acid. Tramadol, ODT and the internal standard, sotalol, were separated by reversed phase HPLC using 35% acetonitrile and an aqueous solution containing 20 mM sodium phosphate buffer, 30 mM sodium dodecyl sulphate and 15 mM tetraethylammonium bromide pH 3.9. Detection was by fluorescence with excitation and emission wavelengths of 275 and 300 nm, respectively. The method was linear for tramadol (3–768 ng/ml) and ODT (1.5–384 ng/ml) with mean recoveries of 87.2% and 89.8%, respectively. Intra- and inter-day precisions were 10.34% and 8.43% for tramadol and 9.43% and 8.75% for ODT at the respective limits of quantitation (3 and 1.5 ng/ml). Accuracy for tramadol ranged from 96.2% to 105.3%. The method was applied to a pharmacokinetic study of tramadol in human volunteers.

Keywords: Tramadol; *O*-Desmethyltramadol; Sotalol

1. Introduction

Tramadol hydrochloride, (±)-*trans*-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol (Fig. 1A), is a centrally-acting analgesic used in the treatment of mild to moderate pain [1]. Its therapeutic plasma concentration is in the range 100–300 ng/ml [2]. Tramadol is rapidly and almost completely absorbed after oral administration but its absolute bioavailability is only 65–70% due to first-pass metabolism [3]. The metabolism of tramadol in human is mediated by cytochrome P4502D6 (CYP2D6) to *O*-desmethyltramadol (ODT, Fig. 1B) and *N*-desmethyltramadol (NDT). ODT is pharmacologically active and contributes to the analgesic efficacy of tramadol [4].

Although the tramadol molecule contains a benzene ring, UV detection is unsuitable for its analysis in plasma due to lack of sensitivity and selectivity [5]. Early analytical methods for tramadol and its metabolites in biological samples involved gas chromatography (GC) with nitrogen selective detection [6] and GC–mass spectrophotometry (GC–MS) [7]. More recent methods include the use of electrochemistry [8], mass spectrometry [9], capillary zone electrophoresis [10] and HPLC with fluorescence detection [5,11]. The latter provides good selectivity and sensitivity and was used to determine tramadol and ODT but it required an extensive washout period and different excitation and emission wavelengths for tramadol, ODT and the internal standard [11].

This paper describes an improved method for the simultaneous determination of tramadol and ODT in human plasma involving simple sample preparation followed by HPLC with fluorescence detection. The method is sensitive, rapid and suitable for routine application to pharmacokinetic, bioavailability and bioequivalence studies.

2. Experimental

2.1. Reagents and chemicals

Tramadol hydrochloride and sotalol hydrochloride were obtained from Sigma–Aldrich (St Louis, MO, USA). *O*-Des-

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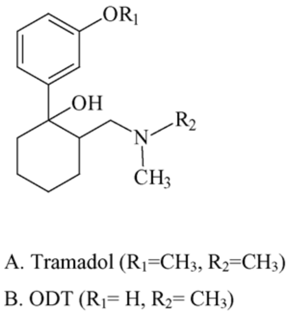

Fig. 1. Chemical structures of tramadol and *O*-desmethyltramadol (ODT).

methyltramadol hydrochloride, *N*-desmethyltramadol and *O*,*N*-didesmethyltramadol (ONDT) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada). Acetonitrile, methanol, diethyl ether, dichloromethane and 1-butanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), sodium dihydrogenphosphate and tetraethylammonium bromide (TEA) were analytical grade from BDH (Poole, UK). Deionized water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA).

2.2. Chromatography

The HPLC system was an LC Workstation Class LC10 (Shimadzu, Kyoto, Japan) consisting of a SIL-10ADvp autosampler maintained at 4 °C, LC-10ADvp pump, CTO-10Avp column oven, SCL-10Avp system controller and RF-10A spectrofluorometric detector. Data was processed by an LC solution Workstation (Shimadzu Corporation). Chromatographic separation was performed on a Hypersil C18 analytical column (100 mm × 2.1 mm i.d., 5 μm particle size) maintained at 30 °C. The mobile phase consisted of acetonitrile:buffer (20 mM sodium dihydrogenphosphate, 30 mM sodium dodecyl sulphate and 15 mM tetraethylammonium bromide adjusted to pH 3.9 with phosphoric acid) (35:65, v/v) at a flow rate of 0.5 ml/min. Fluorescence detection employed excitation and emission wavelengths of 275 and 300 nm, respectively.

To achieve stable ion pair chromatography, the mobile phase was optimised for the content of SDS (10–80 mM) at various acetonitrile concentrations (20–60%). Capacity factors were obtained using sodium benzenesulfonate as unretained substance. A typical plot of capacity factor (k') versus SDS concentration is shown in Fig. 2. At the optimum SDS concentration, the addition of TEA (5–30 mM) and variation in buffer pH (2.5–7.0) were evaluated to optimise peak shape and retention on the column. Sotalol was chosen as the internal standard (IS) after comparing with verapamil, metoprolol and citalopram.

2.3. Stock solutions

Stock solutions of tramadol and ODT (both 1.0 mg/ml as free bases) were prepared by dissolving 11.38 and 11.46 mg of the respective hydrochlorides in 10 ml methanol. Stock solutions containing 16 μg/ml tramadol and 8 μg/ml ODT were prepared by dilution. An IS stock solution (1.0 mg/ml) was prepared by dissolving 10.0 mg sotalol hydrochloride in 10 ml methanol. The working IS solution (8 μg/ml) was prepared by successive 1:10 and 0.8:10 dilutions with water. All stock solutions (tramadol, ODT and IS) were stored at −16 °C and other solutions were prepared daily.

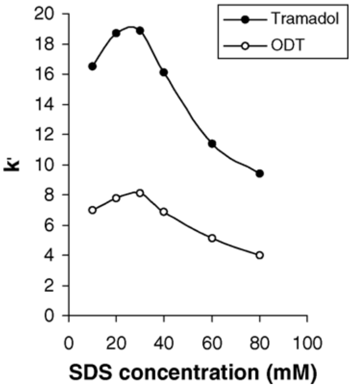

Fig. 2. Plots showing the variation in capacity factor, k', with sodium dodecyl sulfate concentration (SDS) for tramadol and ODT using a mobile phase containing 35% acetonitrile.

2.4. Sample preparation

Into a 10 ml glass tube was added 1.0 ml plasma followed by 100 μl IS solution and 0.5 ml 0.2 M borate buffer (pH 9.3). After vortex-mixing, 7.0 ml of extraction solvent (diethyl ether:dichloromethane:1-butanol 5:3:2) was added and the tube shaken for 20 min and then centrifuged for 10 min at 2600 × *g*. The organic layer was transferred to a clean 10 ml glass conical tube, shaken with 200 μl back-extraction solvent (0.05 M H_2SO_4:acetonitrile 9:1) for 20 min and centrifuged for 10 min at 2600 × *g*. The aqueous phase (100 μl) was injected into the HPLC system.

2.5. Validation procedures

Outdated Blood Bank plasma (Dunedin Hospital) was screened to ensure the absence of interfering peaks at the retention times of tramadol, ODT and the IS The assay was also tested for potential interference from the following basic drugs; propranolol, metoprolol, gallopamil, carvedilol, naftopidil and sertraline.

Calibration standards were prepared by spiking plasma with tramadol (3, 6, 12, 24, 48, 96, 192, 384, 691.2 and 768 ng/ml) and ODT (1.5, 3, 6, 12, 24, 48, 96, 192, 345.6 and 384 ng/ml). QC samples containing LOQ, low, medium and high concentrations of tramadol (3, 9, 74 and 614 ng/ml) and ODT (1.5, 4.5, 37 and 307 ng/ml) were similarly prepared.

Linearity of calibration curves based on peak height ratios (tramadol/IS) was assessed by weighted least squares regression analysis ($1/y^2$). Limit of detection (LOD) and quantita-