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Enantiomeric determination of tramadol and its main metabolite *O*-desmethyltramadol in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

Pharmacokinetic studies require sensitive analytical methods to allow the determination of low concentrations of drugs and metabolites. When drugs present an asymmetric center, the enantiomeric determination of the compounds of interest should be performed. The method developed is based on on-line LC–MS–MS using atmospheric pressure chemical ionization as an interface determination of enantiomers of tramadol (T) and its active metabolite *O*-desmethyltramadol (ODT) in human plasma. This determination is preceded by an off-line solid-phase extraction (SPE) on disposable extraction cartridges (DECs), performed automatically by means of a sample processor equipped with a robotic arm (ASPEC system). The DEC filled with ethyl silica (50 mg) was first conditioned with methanol and water. The washing step was performed with water and the analytes were finally eluted by dispensing methanol. The collected eluate was then evaporated to dryness before being dissolved in the LC mobile phase and injected into the LC system. The enantiomeric separation of tramadol and ODT was achieved on a Chiralpak AD column containing amylose tris-(3,5-dimethylphenylcarbamate) as chiral selector. The mobile phase was isohexane–ethanol–diethylamine (97:3:0.1, v/v). The LC system was then coupled to a tandem mass spectrometry system with an APCI interface in the positive ion mode. The chromatographed analytes were detected in the selected reaction monitoring mode. The MS–MS ion transitions monitored were 264→58 for tramadol, 250→58 for ODT, and 278→58 for ethyltramadol, used as internal standard. The method was validated. The recoveries were around 90% for both T and ODT. The method was found to be linear for each enantiomer of both compounds (r^2>0.999). The mean RSD values for repeatability and intermediate precision were 3.5 and 6.4% for T enantiomers and 5.0 and 5.6% for ODT enantiomers, respectively. Moreover, the method was found to be selective towards other metabolites, *N*-desmethyltramadol and *N*,*O*-desmethyltramadol (NODT). The method developed was successfully used to investigate plasma concentration of enantiomers of T and ODT in a pharmacokinetic study. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Tramadol; *O*-Desmethyltramadol

1. Introduction

Tramadol hydrochloride is a centrally acting analgesic drug presenting an analgesic efficacy and potency that ranges between weak opioids and morphine [1,2]. Tramadol is used in therapy as the racemate of the *trans*-isomer, the latter being more active than the *cis*-isomer [3]. On the other hand, it has also been demonstrated that the (+)-form {1*R*,2*R*-2[(dimethyl-amino)-methyl]-1-(3-methoxy-

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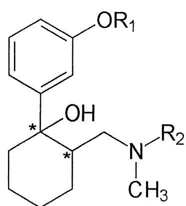
phenyl)-cyclohexanol} of the *trans*-isomer is 10-fold more active than the (−)-form {1*S*,2*S*-2[(dimethyl - amino) - methyl] - 1 - (3 - methoxyphenyl)-cyclohexanol} [3].

Tramadol (T) is metabolized in the liver mainly to *O*-desmethyltramadol (ODT), *N*-desmethyltramadol (NDT) and *N*,*O*-desmethyltramadol (NODT) (Fig. 1). Of these three metabolites, only ODT is pharmacologically active. The methods previously reported for the determination of tramadol in biological samples involve gas chromatography with nitrogen-selective detection [4], gas chromatography–mass spectrometry [5–7] or liquid chromatography (LC) coupled to UV [8–10], fluorometric [11–15] or electrochemical detection [16]. Some methods involving capillary electrophoresis [17,18] or LC coupled to mass spectrometry (MS) were also reported [19,20]. The enantiomeric separation of tramadol using different techniques such as chiral LC [9,13,15,21] or capillary electrophoresis [16–18,22,23] were previously reported but only some of them were applied to biological samples such as urine [9,17,18] or plasma [13,15,16].

Cellulose and amylose derivatives are chiral selectors that are widely used as chiral stationary phases (CSPs) in LC. These CSPs are now well-known to provide some nice enantioseparations in LC and were previously successfully used in the field of



	R_1	R_2
Tramadol (T)	CH_3	CH_3
O-desmethyltramadol (ODT)	H	CH_3
N-desmethyltramadol (NDT)	CH_3	H
N,O-desmethyltramadol (NODT)	H	H
Ethyltramadol (IS)	C_2H_5	CH_3

Fig. 1. Structures of tramadol (T), *O*-desmethyltramadol (ODT), *N*-desmethyltramadol (NDT), *N*,*O*-desmethyltramadol (NODT) and ethyltramadol (I.S.). The stereogenic centers are marked with an asterisk.

determination of enantiomers in biofluids, either in the normal [27–29] or reversed mode [24–26].

A sample handling step is usually necessary prior to an LC analysis of drugs in biological fluids in order to remove proteins and to increase the selectivity and sensitivity of the method. The sample preparation step is particularly important when an enantioselective LC analysis is performed using CSPs [24]. Moreover, the sample clean-up, either by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) is still recommended before LC–MS analysis of biological complex matrices [30]. In most methods reported for the determination of tramadol in biofluids, the sample clean-up of the biological sample was based on LLE [4,5,7,9–12,14–18]. An alternative to this often tedious and time-consuming extraction technique consists in the isolation of drugs by SPE using disposable extraction cartridges (DECs) [6,13,18,19]. Indeed, the SPE procedure can be easily automated by using sample processors equipped with a robotic arm such as the ASPEC system (Gilson) and the total number of samples analyzed within 24 h can therefore be increased.

Among the different possible detection techniques that can be coupled to LC, mass spectrometric detection has been increasingly used in the last few years to perform bioanalytical determinations with maximum selectivity and sensitivity [30–36]. The development of atmospheric pressure ionization (API) interfaces such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) contributed to the increasing utilization of MS detection in LC. APCI is particularly suited to the analysis of low or moderately polar compounds introduced at typical flow-rates ranging from 0.2 to 1.0 ml/min [31,37,38] and frequently used in the field of bioanalytical determination of drugs [19,30,31,33,35,36]. In the APCI source, the liquid is sprayed into a heated quartz tube causing the finely dispersed sample drops to vaporize. The vaporized sample molecules are carried through an ion molecule reaction region by means of a flow of heated gas. The primary ionization, initialized by a corona discharge, creates a reagent ion from the solvent vapor. Sample molecules are therefore ionized by proton or electron transfer and attracted inside the mass spectrometer.

This study reports a validated method combining

automated SPE, normal-phase chiral LC and MS detection to perform the enantiomeric determination of tramadol and its main metabolite ODT in presence of NDT and NODT. Tandem mass spectrometry coupled to normal-phase liquid chromatography was selected in order to obtain a selective and sensitive determination of T and ODT. The chiral LC conditions and the MS optimization were investigated in order to select the most appropriate operating conditions. The limits of quantitation (LOQs) for the enantiomers of each compound were determined to be less than 0.5 ng/ml. The method was successfully used for a pharmacokinetic study.

2. Experimental

2.1. Chemical and reagents

Tramadol hydrochloride was obtained from Chemagis (Tel-Aviv, Israel). The metabolites of tramadol, ODT, NDT and NODT were synthesized in our laboratories (SMB Laboratoires, Brussels, Belgium). The (+)- and (−)-enantiomers of tramadol and ODT were kindly supplied by the Department of Analytical Pharmaceutical Chemistry (University of Liège, Liège, Belgium). Ethyltramadol was synthesized in our laboratories and was used as an internal standard (I.S.). As this compound is eluted in two peaks, the first one was used for calculations. Tramadol, *O*-desmethyltramadol, *N*-desmethyltramadol and *N*,*O*-desmethyltramadol and ethyltramadol were used as the racemic form of the *trans*-isomer.

Isohexane, *n*-hexane, isopropanol, methanol and water were of HPLC-grade while ethanol and diethylamine were of analytical-reagent grade quality, all from Merck (Darmstadt, Germany). Nitrogen was produced by an on-site nitrogen generator from Air Liquide (Milmort, Belgium).

Isolute DECs (1 ml capacity) filled with 50 mg end-capped ethyl silica (C_2) were purchased from International Sorbent Technology (IST) (Mid-Glamorgan, UK).

The CSP used for the determination of tramadol enantiomers was a Chiralpak AD column (250×4.6 mm I.D.) packed with amylose tris-(3,5-dimethylphenylcarbamate) coated on silica (10 μm) from Daicel Chemical Industries (Tokyo, Japan). The analytical column was preceded by a LiChroCart guard column (4×4 mm I.D.) packed with LiChrospher 100 Diol (5 μm) from Merck. The other CSP tested in this study was a Chiralcel OD-H column (250×4.6 mm I.D.) containing cellulose tris-(3,5-dimethylphenylcarbamate) coated on silica (5 μm) from Daicel. This CSP was also preceded by a guard column from Merck, packed with a diol bonded phase.

2.2. Apparatus

The LC system consisted of a Model 1100 Series liquid chromatograph equipped with a binary pump, a vacuum degasser, a thermostatted column compartment and an autosampler, all from Hewlett-Packard (Palo Alto, CA, USA). Mass spectrometric detection was carried out using a PE Sciex API 365 Triple Quadrupole instrument (Thornhill, Canada) equipped with an APCI interface. A Power Mac 8600 (200 MHz) computer from Apple MacIntosh (Austin, TX, USA) equipped with a sample Control version 1.3 and a MacQuan version 1.3 software from PE Sciex was used to control the LC–MS–MS system and to collect and treat the data.

The ASPEC XL4 system (automated sample preparation with extraction cartridges) from Gilson (Villiers-le-Bel, France) consisted of an automatic sampling injector module equipped with four needles, four Model 401 dilutor pipettors and a set of racks and accessories for handling DECs, plasma samples and solvents.

2.3. Chromatographic technique

All chromatographic experiments were carried out in the isocratic mode. For the determination of suitable conditions for the enantioseparation of tramadol and its metabolites, different mobile phases consisting of hexane or isohexane, ethanol or isopropanol and diethylamine were tested on both Chiralpak AD and Chiralcel OD-H CSPs.

The mobile phase finally used for the complete separation of the enantiomers of tramadol, ODT, NDT and NODT consisted of isohexane–ethanol–diethylamine (97:3:0.1, v/v). The flow-rate was 1.0

ml/min and the mobile phase was thermostatted at 25±0.1°C.

2.4. Mass spectrometric detection

Mass spectrometric detection was carried out using a PE Sciex API 365 apparatus equipped with an APCI source in the positive ion mode. The following conditions were applied: the auxiliary gas (N_2) flow-rate was 2 l/min, the curtain gas flow-rate was 1.2 l/min at 60 p.s.i., the heated nebuliser temperature was 250°C and the corona discharge was 1 μA (1 p.s.i.=6894.76 Pa). The mass spectrometer was set to generate and select the pseudomolecular ion ([M+H$^+$]) at m/z 264 for tramadol, m/z 250 for O- and NDT, m/z 236 for NODT and m/z 278 for I.S. via the first quadrupole mass filter (Q1). The MS–MS fragmentation was achieved by introducing the pseudomolecular ions into the collision cell (Q2) with a collision energy of 15 eV (collision gas N_2) for all compounds investigated. Signal for product ions at m/z 58 for T, ODT and I.S., and m/z 44 for NDT and NODT, were monitored via the third quadrupole mass filter (Q3).

2.5. Standard solutions

2.5.1. Solutions used for method development

A stock solution of tramadol hydrochloride racemate was prepared by dissolving the appropriate amount of the compound in ethanol to give a final concentration of 500 ng/ml. A second stock solution containing 500 ng/ml of ODT, NDT and NODT, all racemates, was also prepared in ethanol.

2.5.2. Solutions used for method validation

Four 1 μg/ml solutions containing, respectively, the single enantiomers of tramadol and ODT were prepared in water and used for the determination of the elution order of each enantiomer. Seven mixed solutions of tramadol hydrochloride and ODT (racemate) were prepared by diluting stock solutions of racemic tramadol and ODT with water to achieve concentrations ranging from 20 to 10 000 ng/ml for T and 20 to 2000 ng/ml for ODT. These solutions were then used to spike plasma samples for calibration curves (from 0.5 to 250 ng/ml for each enantiomer of T and 0.5 to 50 ng/ml for each enantiomer of ODT).

2.6. Sample preparation

The plasma sample was centrifuged at 3000 g for 10 min and a 1.0-ml volume of plasma was transferred manually to a vial on the appropriate rack of the ASPEC system. A 200-μl volume of internal standard solution (500 ng/ml) was then added automatically. The DEC sorbent was first conditioned with 1.0 ml of methanol and then with 1.0 ml of water. A 1.0-ml volume of plasma sample was aspirated by the autosampler needle from the corresponding vial and dispensed onto the DEC. The washing step was then performed by dispensing 1.0 ml of water twice. The elution was performed by applying a 0.5-ml volume of methanol twice, both eluates being collected in the same vial and homogenized. The sample preparation procedure was performed automatically by the ASPEC system in the batch mode. The resulting extract was then evaporated for 120 min in a rotational-vacuum concentrator at 45°C, and reconstituted in 500 μl of mobile phase and transferred manually to the LC autosampler for analysis. The injection volume was 50 μl.

2.7. Pharmacokinetic study

The LC–MS–MS procedure developed was used to investigate the plasma profiles of T and ODT enantiomers after a single oral dose of an immediate release formulation of tramadol (Topalgic, Houde, France). A clinical study on 30 healthy male volunteers was conducted. The subjects received a single dose of 200 mg (four capsules of Topalgic 50 mg). Sixteen blood samples were withdrawn at different times until 36 h after the administration of the medication.

3. Results and discussion

3.1. LC conditions

As reported in previous studies, the complete enantiomeric separation of tramadol was obtained on a cellulose tris-(3,5-dimethylphenylcarbamate) CSP used in the reversed-phase mode with mixtures of

organic modifiers and aqueous buffers [12,14], or in normal-phase [9]. The simultaneous separation of tramadol and ODT enantiomers was also observed on a Chiralcel OD-R CSP [12,14] but the selectivity of the separation towards the enantiomers of other metabolites such as NDT or NODT was not mentioned since it was not tested [12] or not extracted from plasma [14]. Elsing and Blaschke performed the determination of T, NDT and ODT but using two different CSPs and by extracting selectively T and NDT at pH>10 and ODT at pH<9 [9].

The aim of the present study was to develop a method allowing the simultaneous determination of T and ODT enantiomers in presence of other metabolites such as NDT or NODT using SPE as a sample preparation technique. MS–MS detection was then selected. However, mobile phases used with the Chiralcel OD-R CSP are not suitable for a determination with a mass spectrometric detector due to the high content of sodium perchlorate generally used in the LC mobile phase. Some experiments were therefore performed under the normal-phase conditions with the Chiralcel OD-H and Chiralpak AD CSPs.

The Chiralcel OD-H column was first tested due to the previous enantioseparation reported in the literature [9,12,14]. Different parameters such as water or diethylamine concentration in the mobile phase, nature of organic modifiers (ethanol and isopropanol were tested) or temperature were investigated. By varying these parameters, a complete enantioseparation was obtained for T, ODT and NODT. However, only a partial separation of the NDT enantiomers was observed (Fig. 2).

The same investigations as mentioned above were performed on the Chiralpak AD CSP. A complete enantioseparation of NDT was obtained under the same conditions as described by Elsing and Blaschke [9]. However, the enantiomers of ODT were coeluted while T and NDT were enantioseparated. NODT was particularly strongly retained in these conditions (retention time, t_R>70 min). No enantioseparation of the active metabolite was observed by varying the diethylamine or the isopropanol concentrations in the mobile phase.

Finally, the complete enantioseparation of T, NDT, ODT and NODT (cf. Fig. 3) was obtained on the Chiralpak AD CSP by modifying the nature of the mobile phase and by using a mixture of isohexane, ethanol and diethylamine as mobile phase. It must be noted that normal-phase chromatography can be combined safely with an APCI interface. Indeed, the ionization source used in this study is protected against overpressure by a pressure relief valve. Moreover, when a small amount of polar modifier is used in combination with the apolar majority of the mobile phase, the ignition of an explosion is highly unlikely.

3.2. Optimization of MS conditions

The LC–MS–MS method for the determination of tramadol and ODT enantiomers in human plasma was investigated. The APCI interface was selected since ESI is not recommended when LC mobile phases containing hexane are used [39]. For the optimization of MS conditions, each compound was directly introduced into the MS detector using APCI ionization and parameters such as corona discharge, orifice voltage, ring voltage, flow of nebulizer and auxiliary gas (N_2) and temperature of auxiliary gas (N_2) were optimized in order to obtain the protonated pseudomolecular ions of T, ODT, NDT, NODT and I.S.

The most suitable collision energy was determined by observing the response obtained for the fragment ion peak m/z. The product ion mass spectra presented in Fig. 4 illustrate a predominant fragment ion peak m/z 58 for T, ODT and I.S. and 44 for NDT and NODT, observed when the collision energy was 15 eV.

Selective reaction monitoring (SRM) mode ion chromatograms were used to determine T and ODT. SRM spectra selectively filter out ions that are not related to the target compounds and a very clean ion chromatogram can thus be obtained, due to the great selectivity and sensitivity of this operational mode.

3.3. Validation

Even if the method developed allows the determination of three metabolites enantiomers simultaneously with the enantiomers of tramadol, the validation of the method was reduced to the quantitation of the enantiomers of T and its active metabolite ODT.

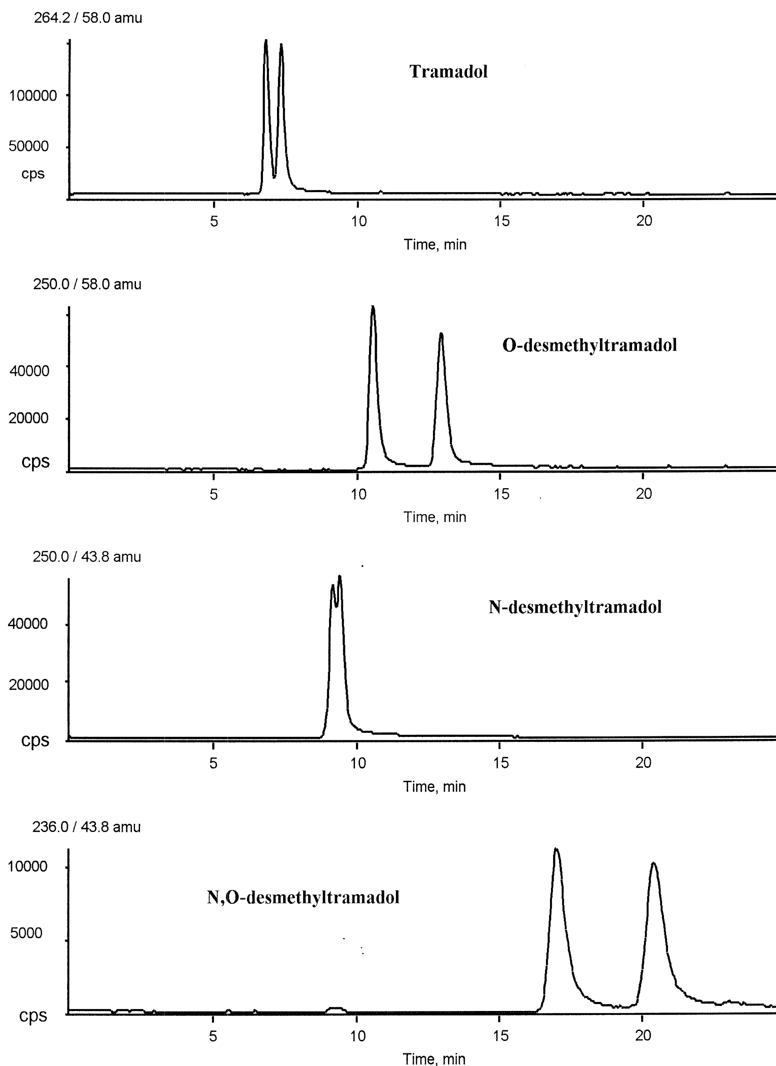


Fig. 2. SRM ion chromatograms illustrating the enantiomeric separation of tramadol and its three metabolites on a Chiralcel OD-H CSP. Stationary phase: Chiralcel OD-H, mobile phase: hexane–ethanol–water–diethylamine (96:4:0.1:0.1 v/v), flow-rate: 1.0 ml/min, temperature: 20°C, analyte concentrations: 500 ng/ml for all racemates.

3.3.1. Stability

Table 1 shows the stability of the whole procedure (stock solution, eluate, plasma sample, freeze–thaw cycle, plasma sample storage). No significant degradation of T and ODT enantiomers was observed after stability tests.

3.3.2. Selectivity

Fig. 5 shows a typical chromatographic trace of a plasma extract containing racemic tramadol and racemic ODT. The absence of interfering endogenous components at the retention times of the enantiomers of T and ODT is also demonstrated (chromatograms A). The order of elution of T and ODT enantiomers was determined by separately injecting solutions of each enantiomer.

3.3.3. Recovery

The recoveries of tramadol and ODT over the entire concentration range were determined by com-

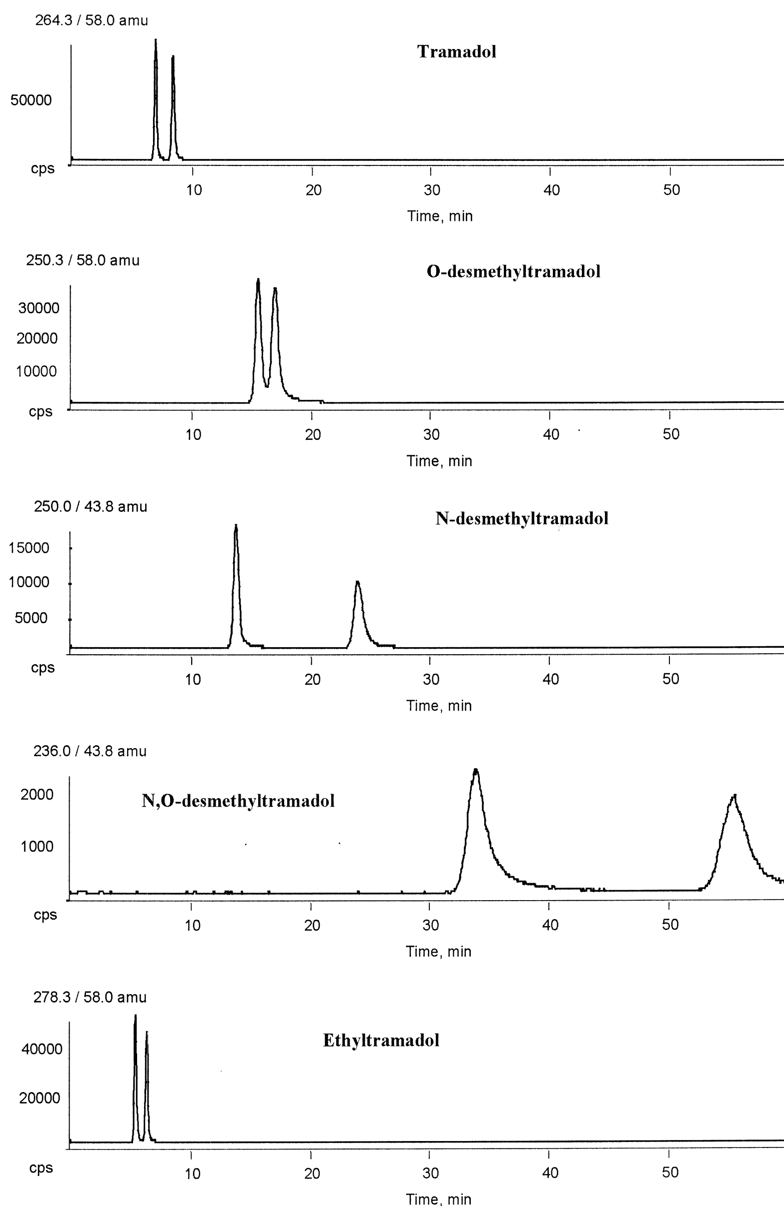


Fig. 3. SRM ion chromatograms illustrating the enantiomeric separation of tramadol and its three metabolites on a Chiralpak AD CSP. Stationary phase: Chiralpak AD, mobile phase: isohexane–ethanol–diethylamine (97:3:0.1 v/v), flow-rate: 1.0 ml/min, temperature: 25°C, analyte concentrations: 500 ng/ml for all racemates.

paring peak areas obtained from plasma sample and those found by direct injection of an aqueous standard solution at the same concentration, using the same autosampler equipped with the same loop [40]. The mean recoveries of T and ODT were 90±9% and 90±3%, respectively (cf. Table 2).

3.3.4. Linearity

The linear regression analysis of T and ODT enantiomers was made by plotting the peak area ratio (y) versus the inverse of analyte concentration ($1/x$) in ng/ml. The following equations were obtained (concentration range: 0.5–250 ng/ml for tramadol

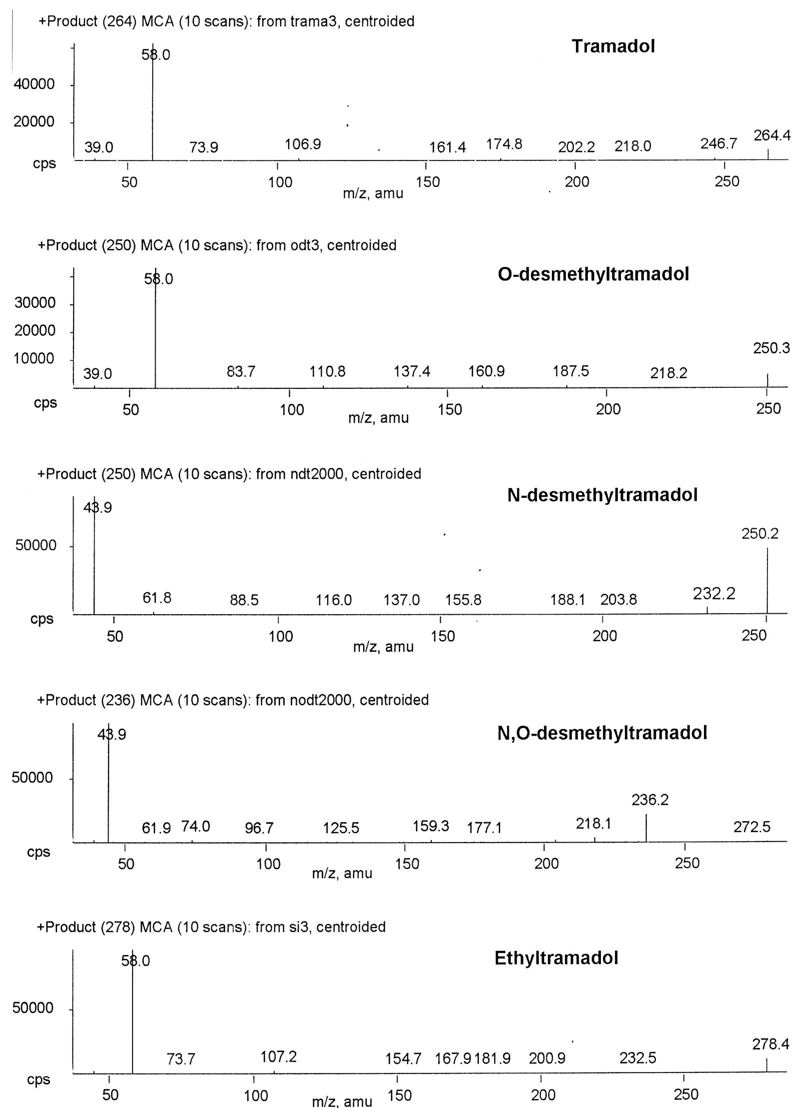


Fig. 4. Product ion mass spectra of tramadol, its three metabolites and internal standard. See text for operating conditions.

enantiomers and 0.5–50 ng/ml for ODT enantiomers):

(+)-Tramadol: $y = 0.0186x + 0.0019$, $r^2 = 0.9988$

(−)-Tramadol: $y = 0.0201x + 0.0029$, $r^2 = 0.9988$

(+)-ODT: $y = 0.01x + 0.0011$, $r^2 = 0.9996$

(−)-ODT: $y = 0.0113x + 0.0020$, $r^2 = 0.9991$

The linearity of the relationship between peak area ratios and concentration is demonstrated by the determination coefficients (r^2) obtained for the regression lines in the case of the four enantiomers studied.

3.3.5. Detectability

The limits of detection (LODs) and quantitation (LOQs) were determined as analyte concentrations giving rise to signal-to-noise ratios of 3 and 10,

Table 1
Stability of tramadol and *O*-desmethyltramadol enantiomers in plasma control samples

	(+)-Tramadol		(−)-Tramadol		(+)-ODT		(−)-ODT	
	0.5 ng/ml	250 ng/ml	0.5 ng/ml	250 ng/ml	0.5 ng/ml	50 ng/ml	0.5 ng/ml	50 ng/ml
Stock solution (24 h, 18±2°C, $n=2$)								
ng/ml	0.57	242.5	0.51	258.6	0.50	45.5	0.49	46.6
% of initial	103.7	96.5	95.4	102.3	100.2	95.0	104.7	102.2
Eluate (24 h, 18±2°C, $n=2$)								
ng/ml	0.50	253.1	0.53	252.2	0.44	54.4	0.45	51.2
% of initial	97.0	98.3	92.4	98.1	98.9	119.8	97.9	112.0
Plasma sample (24 h, 18±2°C, $n=2$)								
ng/ml	0.44	233.9	0.51	225.3	0.48	53.0	0.49	53.5
% of initial	87.8	97.0	102.7	95.9	101.7	93.0	98.3	106.2
Freeze–thaw ($n=2$)								
ng/ml	0.53	241.1	0.56	241.0	0.48	50.9	0.492	56.7
3rd cycle (% of initial)	106.2	106.8	106.3	108.6	92.1	105.1	97.7	102.9
Plasma sample storage (30 days, −80±5°C, $n=3$)								
ng/ml	0.52	246.5	0.50	249.8	0.50	51.4	0.48	51.2
% of initial	103.2	102.2	100.9	106.4	104.6	90.2	97.0	101.6

respectively. The LODs and LOQs for the enantiomers of T were found to be 0.05 and 0.15 for (+)-T, 0.05 and 0.17 for (−)-T, respectively. Using this approach, the LODs and LOQs for the enantiomers of ODT were found to be 0.09 and 0.3 for (+)-ODT and 0.09 and 0.33 for (−)-ODT, respectively (cf. Table 2). However, it is probably better to consider that LOQ is around 0.5 ng/ml for all compounds since the validation has demonstrated that the method is precise and accurate at this concentration.

3.3.6. Precision

The precision of the bioanalytical method was determined by measuring repeatability and intermediate precision for the four compounds at three different concentration levels ranging from 0.5 to 250 ng/ml for T enantiomers and 0.5 to 50 for ODT enantiomers. The mean values for repeatability and intermediate precision were 3.5 and 6.1 for (+)-T, 3.5 and 6.6 for (−)-T, 4.2 and 5.8 for (+)-ODT and 3.8 and 5.4 for (−)-ODT, respectively (cf. Table 2).

3.3.7. Accuracy

The accuracy of the procedure was assessed by calculating the ratio between the analyte amount found versus the amount spiked in the plasma, ranging from 0.5 to 250 ng/ml for T enantiomers and 0.5 to 50 ng/ml for ODT enantiomers. The accuracy, defined as mean% ±interval of confidence ($P>0.05$), shows that the LC–MS–MS procedure developed for the enantiomeric determination of T and ODT enantiomers can be considered as accurate and linear within the concentration range investigated (Table 2).

3.4. Pharmacokinetics

The LC–MS–MS procedure developed was used to investigate the enantiomeric plasma profile of tramadol and its active metabolite ODT, after a single oral dose of an immediate release formulation of tramadol (Topalgic, 50 mg). Pharmacokinetic parameters [area under the curve ($AUC_{0\rightarrow36}$), C_{max}, T_{max}] calculated for each enantiomer are presented in Table 3.

4. Conclusions

A sensitive, accurate and precise method based on LC–MS–MS has been developed for the simultaneous and enantioselective determination of tramadol

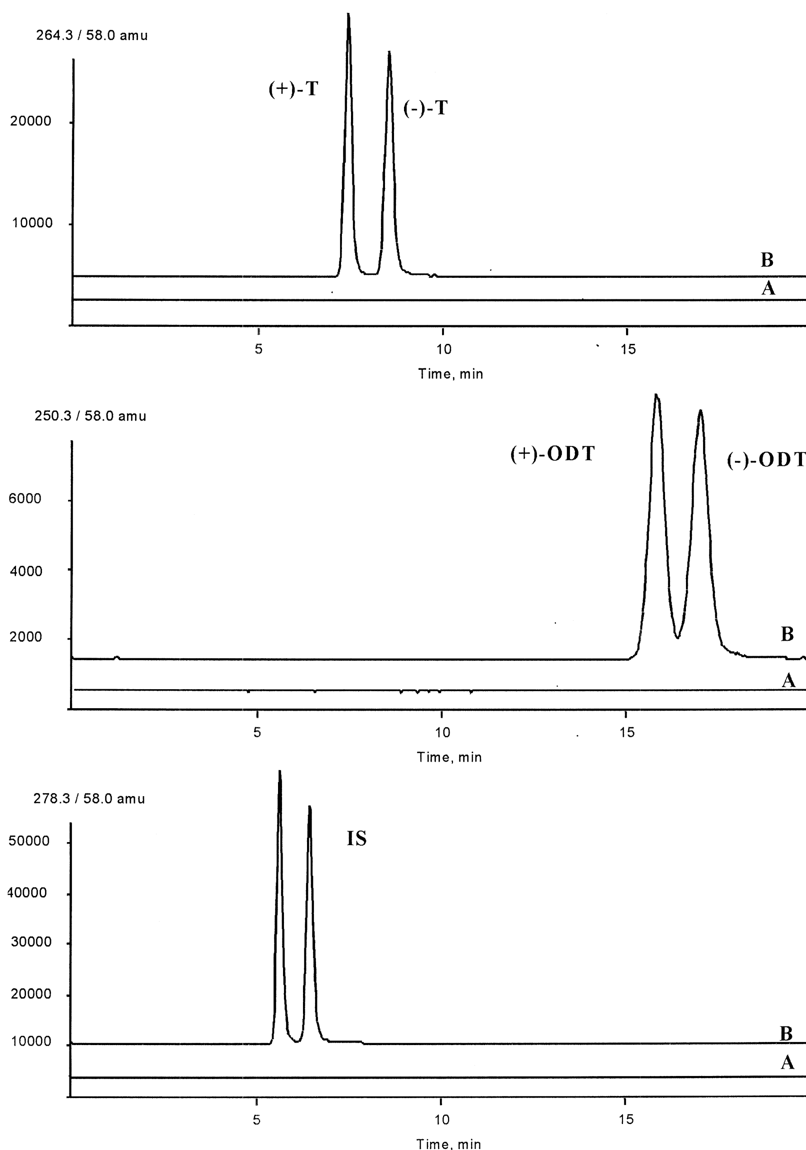


Fig. 5. SRM ion chromatogram of blank plasma (A) and spiked plasma (B) samples with 25 ng/ml for T and 10 ng/ml for ODT racemates. See text for conditions.

and its active metabolite *O*-desmethyltramadol in human plasma. The method was validated to meet the requirements of the pharmacokinetic investigation of these two chiral compounds. The procedure developed was successfully applied to the determination of (+)- and (−)-T and (+)- and (−)-ODT plasma levels for investigating a pharmacokinetic study.

Acknowledgements

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Table 2
Validation of the LC–MS–MS method for the enantioselective determination of T and ODT

Validation criterion		(+)-Tramadol	(−)-Tramadol	(+)-ODT	(−)-ODT
Recovery (mean±SD, $n=9$, %)		90±8	89±9	89±6	91±8
Linearity ($n=7$, $k=1$)	Range (ng/ml)	0.5–250	0.5–250	0.5–50	0.5–50
	Slope	0.0186	0.0201	0.0100	0.0113
	Intercept	0.0019	0.0029	0.0011	0.0020
	r^2	0.9988	0.9988	0.9996	0.9991
LOD (ng/ml)		0.05	0.05	0.09	0.09
LOQ (ng/ml)		0.15	0.17	0.29	0.33
Repeatability ($n=6$, %)					
Tramadol	*ODT*				
0.5 ng/ml	0.5 ng/ml	5.7	4.8	5.6	5.6
12.5 ng/ml	5 ng/ml	1.9	2.0	3.4	3.7
250 ng/ml	50 ng/ml	3.0	3.8	3.5	2.2
	Mean	3.5	3.5	4.2	3.8
Intermediate precision ($n=6$; 3 days, %)					
Tramadol	*ODT*				
0.5 ng/ml	0.5 ng/ml	6.7	8.2	6.1	6.6
12.5 ng/ml	5 ng/ml	5.4	5.3	3.4	3.8
250 ng/ml	50 ng/ml	6.3	6.2	7.8	5.8
	Mean	6.1	6.6	5.8	5.4
Accuracy (recovery±IC, %; $n=6$)					
Tramadol	*ODT*				
0.5 ng/ml	0.5 ng/ml	102.6±4.7	100.1±1.3	98.2±3.7	96.6±5.0
12.5 ng/ml	5 ng/ml	100.2±0.8	98.7±1.5	98.9±2.5	104.7±6.1
250 ng/ml	50 ng/ml	100.9±2.6	100.3±2.3	99.3±1.8	99.6±1.1

Table 3
Pharmacokinetic parameters

Parameter	(+)-Tramadol	(−)-Tramadol	(+)-ODT	(−)-ODT
T_{max} (h)	1.8	1.8	2.1	2.1
C_{max} (ng/ml)	323.2	289.5	61.2	81.1
$AUC_{0\rightarrow36}$ (ng/ml per h)	2784	2227	697	761

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