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Capillary electrophoresis methods for the determination of tramadol: a review

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Abstract:

Tramadol is a widely used opioid analgesic frequently prescribed for the treatment of moderate to severe, acute and chronic pain. It has a complex mechanism of action, acting both as a central opiate agonist and as a of norepinephrine and serotonin reuptake inhibitor. It is a chiral substance, having two chiral centers in its structure and it is used in therapy as a racemic mixture of two of its enantiomers, (*S*,*S*)-tramadol and (*R*,*R*)-tramadol. In the last 25 years several analytical procedures have been published in the literature for the achiral and chiral determination of tramadol from pharmaceutical formulations and biological matrices. Among these capillary electrophoresis methods has proved to be an efficient, reliable and cost-effective solution. The purpose of the present review is to provide a systematic survey in which to present and discuss the electrodriven methods available in the literature for the achiral and chiral analysis of tramadol.

Keywords: tramadol, capillary electrophoresis, chiral separation, pharmaceutical analysis

1. Introduction

Tramadol (*trans*-(+/-)-2-(dimethylaminomethyl)-1-(m-methoxyphenyl)cyclohexanol) is a centrally acting synthetic opioid analgesic used in the treatment of acute and chronic pain of moderate to severe intensity. It is a pure, non-selective agonist on μ, δ and k opioid receptors, with considerable higher affinity for the μ receptor. Other synergistic mechanisms contributing to its analgesic effect are the inhibition of neuronal norepinephrine reuptake and increased serotonin release.[1]

Unlike morphine, the analgesic doses of tramadol do not cause respiratory depression, does not affect gastrointestinal motility and cardiovascular effects are mild. The tramadol analgesic potency is 1/10 - 1/6 of that of morphine and is equally potent when compared to codeine. It has a relatively safe pharmacological profile and its tolerance and abuse potential is low.[2]

From the stereochemistry point of view, tramadol presents two chiral centres in its structure, which leads to the existence of four enantiomers; however, it is used in therapy as a racemic mixture of two of its enantiomers, (*1R,2R*)-tramadol and (*1S,2S*)-tramadol. The chemical characteristics of tramadol enantiomers are presented in **Figure 1**.

The differences between the pharmacological profiles of the two enantiomers are well known and documented; as (+) (*1R,2R*)-tramadol presents higher affinity for the μ opioid receptors and preferentially inhibits serotonin reuptake while (-)(*1S,2S*)-tramadol preferentially inhibits norepinephrine reuptake by stimulating α2-adrenergic receptors.[3] Clinical studies suggest that there is a synergistic and complementary interaction between the two enantiomers after the administration of the racemic.[4]

Administered orally, tramadol is rapidly and almost completely absorbed; the maximum plasma concentration is reached after 1.5-2 hours. Its bioavailability is approximately 70% after a single administration due to the first-pass effect; bioavailability can increase to 90% - 100% after repeated administrations. Tramadol plasma proteins binding is 20%; and it has distribution volume of 3-4 l kg^{-1}.[5]

Tramadol is metabolized (approximately 85%) by N- and O-demethylation phase I reactions, using cytochrome P450 isoenzymes, mainly CYP3A4 and CYP2D6; then by phase II reaction through conjugation with glucuronic acid of the O-demethylated. All phase I metabolites possess chiral centres, and O- and N-demethylation of tramadol proved to be highly stereoselective. The main metabolites were identified as O-demethyltramadol (M1), N-demethyltramadol (M2) and O-demethyl-N-demethyltramadol (M5); as the renal elimination of other two metabolites N-bis-demethyl tramadol (M3), O-demethyl-N-bis-demethyltramadol (M4) is lower than 1% of the administered dose. A scheme representing the main metabolic routes of tramadol is presented in **Figure 2**.[6]

So far, eleven different metabolites were identified in urine, and considerable inter-individual quantitative differences between metabolites was observed. Among the main metabolites, only M1 is pharmacologically active. Animal studies showed that M1 is 2-4 times more potent than tramadol.[7]

The half-life elimination of tramadol is around 6 hours. More than 90% of tramadol is excreted in urine (70% of this amount is excreted as metabolites), the rest being excreted in faeces.[5]

Non-clinical studies revealed no special risks for humans based on pharmacological conventional study assessment of safety, genotoxicity, repeated dose toxicity or carcinogenic potential.[5]

Tramadol is available in several pharmaceutical preparations like capsules, drops and sustained-release formulations for oral use, suppositories for rectal use and injectable solution for intramuscular and intravenous injection.[5]

Taking into consideration the aspects mentioned above the development of efficient and reliable analytical methods for the achiral and chiral determination of tramadol from different matrices are needed, and capillary electrophoresis (CE) techniques are very efficient and reliable candidates for the task, being considered nowadays an alternative for the more frequently used chromatographic techniques.[8]

CE has developed over the years into a highly efficient separation technique and is currently applied in several different areas of drug analysis such as chiral separations, with advantages related with the small expenditure of reagents, analytes and chiral selectors, rapid method development, fast migration times and high diversity in choosing and changing chiral selectors.[9,10] Basically, in CE usually a direct method of enantioseparation is applied, by merely dissolving the chiral selector into the background electrolyte (BGE) solution; method which has the advantage of promoting highly efficient separations at a reasonable cost.[11]

Tramadol is a basic analyte, a tertiary amine, with a pKa value of around 9.5, consequently can be ionized in an acidic environment and hence its determination by CE becomes feasible.

In the current review applications of CE methods for the achiral and chiral determination of tramadol are presented; the methods used for this purpose are described and briefly discussed.

2. Achiral determination of tramadol by capillary electrophoresis

Several CE methods have been published for the determination of tramadol from pharmaceutical products, fixed-dose combinations or urine and plasma samples.

Cationic capillary isotachophoresis (ITP) with conductometric detection has been applied for the determination of tramadol from different pharmaceutical formulations. The optimised ITP electrolyte

system consisted of 5 mM potassium picolinate and 5 mM picolinic acid at pH 5.25 as leading electrolyte and 10 mM formic acid at pH 2.58 as the terminating electrolyte; the driving and detection currents were 50 µA and 10 µA, respectively. pKa value of tramadol (9.44) was determined by UV spectrophotometry as a preliminary step in the CE method development. The effective mobility of tramadol using the conditions mentioned above was 24.26 10^{-9} m^2 V^{-1} s^{-1} (tetraethylammonium ion was used as standard for mobility). The method was used to determine 50 mg tramadol in capsules, 100 mg tramadol in suppositories and 50 and 100 mg mL^{-1} tramadol in injections and drops. The linearity of the method was verified over a concentration range between 15–180 mg L^{-1}; the limit of detection (LOD) and limit of quantification (LOQ) was 2 and 5 mg mL^{-1}. ITP proved to be a reliable solution for the determination of tramadol from pharmaceutical formulations.[12]

CE with UV laser-induced native fluorescence detection (CE-UV-LINF) was applied for the direct determination of tramadol in urine without previous extraction or preconcentration preparation steps. The use of LIF detection system enhanced the LOD of the method about 1000-fold by comparison to UV absorption detection; the high sensitivity of the method allowed the direct quantification of tramadol from urine samples. 150 mM sodium tetraborate buffer at pH 6.0 was used as BGE, and naphazoline nitrate was used as internal standard (IS). The linearity of the method was examined over the range of 20 ng mL^{-1} – 5 mg mL^{-1} in human urine; the LOD of the method was 1 ng mL^{-1}. Tramadol and metabolites M2 and M5 were quantified while metabolites M1 and M3 were only identified but not quantified from the urine samples.[13]

CE with UV detection was used for the separation of tramadol and its five phase I (M1, M2, M3, M4, M5) and three phase II metabolites (glucuronides of M1, M4 and M5). The baseline separation of all analytes was achieved when using a 65 mM sodium tetraborate BGE at pH 10.65. The method was applied on a urine sample collected after the administration of a 50 mg single oral dose of tramadol. The samples were purified and a five-fold concentration of the sample was applied through solid-phase extraction (SPE) before CE analysis. Diastereomeric separation of the glucuronide metabolites was obtained using 10 mM ammonium acetate–100 mM formic BGE at pH 2.75 and with a micellar 25 mM sodium tetraborate–70 mM SDS BGE at pH 10.45. The lowest concentrations that could be detected by CZE were 1.5–2 µM for the phase I metabolites and 6–7 µM for the phase II metabolites. Both methods showed that in vitro glucuronidation processes produces glucuronide diastereomers in different amounts.[14]

CE with electrochemiluminescence (CE-ECL) detection by end column mode, was used to determine simultaneously tramadol and lidocaine from urine. A 10 mM phosphate buffer at pH 9.0 was used as BGE; 50 mM phosphate buffer and 5 mM tris(2,2-bipyridyl) ruthenium(II) ($Ru(bpy)^{2+}_3$) was used as ECL solution.[15] The same technique was used for the simultaneous determination of etahmsylate, lidocaine and tramadol in urine, combination used as local haemostatic, local anaesthetic and analgesic

in surgery. A 20 mM phosphate buffer at pH 9.0 was used as BGE, 50 mM phosphate at pH 9.0 containing 5.0 mM $Ru(bpy)^{2+}_3$ was added to the cell for CE-ECL detection.[16] In another study applicability of the CE-ECL for the analysis of tramadol spiked in human serum was also verified.[17] Tramadol and lidocaine were chosen as model compounds to evaluate microchip a CE-ECL detection system in both pre-column and post-column detection modes.[18] The application of CE-ECL in monitoring ethamsylate, lidocaine and tramadol in urine shows that this technique can be a viable solution in clinical laboratories for both pharmaceutical analysis and pharmacokinetic studies. CE-ECL offers the advantages of improved selectivity, enhanced sensitivity and reduced cost.

CE-ECL was used for the determination of diltiazem and tramadol in water. The detection cell was filled with 50 mM $Ru(bpy)_3^{2+}$ in a phosphate buffer at pH 8.0; the phosphate buffer was used as BGE. The calibration plot was linear over the range of 6 10-6 – 3 10^{-4} mol L^{-1} while the LOD was 1.038 10^{-8} mol L^{-1}.[19]

Capillary zone electrophoresis (CZE) was used for the simultaneous determination of paracetamol and tramadol from fixed-dose combinations. Fourteen formulations containing tramadol hydrochloride 37.5mg and paracetamol 325mg with different excipients in different ratio were prepared and CZE was used to determine both the drug content of the formulations and for the characterization of dissolution profiles.[20]

CE with capacitively coupled contactless conductivity detection (CE-C^4D) was used for the simultaneous determination of codeine, orphenadrine, paracetamol, promethazine, tramadol, and scopolamine from different fixed-dose combinations. A complex BGE containing 20 mM β-alanine, 4 mM sodium chloride and 4 µM sodium hydroxide at pH = 9.6 was used throughout the determinations. Each sample was analysed in a single run and a LOD of 0.62 µmol L^{-1} was obtained for tramadol.[21]

CE coupled with a carrier assisted electromembrane extraction was used for the determination of pseudoephedrine and tramadol in urine samples. Nonionic lipophilic surfactants were added in the supported liquid membrane and acted as carriers thus promotinng the migration of ionic analytes toward the acceptor phase. Optimal extraction conditions were achieved when using 2-nitrophenyl octyl ether, containing 0.25 mM Span 80 as supported liquid membrane, with 200 V voltage and with pH 5.0 in donor and pH 1.0 in acceptor solution.[22]

The analytical characteristics of the methods presented above are synthetized in **Table 1**.

3. Chiral determination of tramadol enantiomers by capillary electrophoresis

Taking into consideration the stereoselective aspects of tramadol pharmacokinetics and pharmacological profile most of CE methods from literature deal with the enantioseparation of tramadol enantiomers.

The chiral separation of tramadol and two of its major metabolites, M1 and M2 was studied using CZE and methyl-β-CD (M-β-CD) as chiral selector. The enantiomers of the three substances were separated using a 50 mM phosphate BGE at pH 2.5 containing 75 mM M-β-CD as chiral selector, 220 mM urea and 0.05% (w/v) hydroxypropylmethyl cellulose (HPMC).[23]

CZE was successfully used in another method for the enantioseparation of tramadol; both uncoated and polyacrylamide-coated capillaries and charged and neutral CDs were tested in the preliminary optimization process. During the CD screening process, two anionic CDs, carboxymethylated-β-CD (CM-β-CD) and sulfobuthylether-β-CD (SBE-β-CD) showed steric interaction with tramadol. The best results were achieved when using a 50 mM phosphate BGE at pH 2.5 and 5 mM CM- β-CD as chiral selector. Linearity was verified over the range of 1.0 – 12.5 μg mL^{-1} for each enantiomer, and the LOD and LOQ for both enantiomers were 30 ng mL-1 and 100 ng mL^{-1} respectively. The method was applied for the determination of tramadol enantiomers in pharmaceutical preparations.[24]

The previously mentioned CE method using CM- β-CD as chiral selector was adapted for the enantiomeric determination of tramadol and its phase I metabolites in urine. Chiral determinations of tramadol and its three main metabolites, M1, M2 and M5 in urine was performed after a liquid–liquid extraction (LLE) used to pre-concentrate and clean the sample before the CE procedure. The O-methylated analytes (tramadol, M2, M3) exhibited stronger CD complexation than the demethylated (M1, M4, M5) ones, suggesting that O-methylation plays an important role in the complexation with CM-β-CD, likely due to the more hydrophobic character of the analytes. Also, it seems that N-demethylation lead to secondary interactions of the analytes with CM-β-CD, thereby producing more stable diastereoisomeric complexes. It is assumed that O-methylation influence analyte migration times while N-methylation influence chiral resolution. The enantiomeric ratios of all the excreted metabolites, M1, M2 and M5, differed from 1.0, demonstrating the stereoselective metabolism profile of tramadol.[25]

CZE using a basic BGE was applied in an achiral and a chiral separation of tramadol and its main metabolite M1 in urine samples. The best results for the achiral separation were obtained when using a 50 mM sodium tetraborate BGE at pH 10.6 while the enantiomeric separation of tramadol and M1 enantiomers was achieved by adding 20 mM CM-β-CD as chiral selector to the BGE. A preconcentration by SPE was used for the achiral determination while an LLE was used for the chiral determination. The optimised method was used for the determination of tramadol and M1 in human urine after an LLE.[26]

Partial filling CE coupled with electrospray ionization mass spectrometry (CE-ESI-MS) was used for the simultaneous determination of tramadol and phase I metabolites after a preliminary LLE. Partial

filling technique was used in order to prevent MS contamination with the chiral selector. The best enantioseparation of the analytes was obtained when using a coated polyvinyl alcohol (PVA) capillary, 40 mM ammonium acetate BGE at pH 4.0, and 2.5 mM SBE-β-CD as the chiral selector. The order of migration of the analytes in the presence of SBE- β -CD was the following: M1, tramadol, M5, M4, M2, M3; all compounds were separated at baseline in less than 20 minutes. The use of the anionic CD allowed the enantioseparation of the analytes and improved the selectivity of metabolites with the same molecular mass (M1 and M2, M3 and M5). Migration behaviour and the stereoselective interactions of the analytes proved to be strongly influenced by their structural characteristics. The use of anionic CDs is extremely efficient in the partial-filling technique because, in the absence of electro osmotic flow (EOF), the applied voltage will lead to a counter current process in which the enantiomers and the chiral selector will migrate in opposite directions. The optimized method was used for the determination of tramadol and its main metabolites enantiomers in plasma after a LLE.[27]

CE with laser-induced fluorescence detection (CE-LIF) was used for the chiral separation of tramadol and its phase II metabolite O-demethyl tramadol glucuronide from urine. The best results were achieved when using 50 mM sodium tetraborate BGE at pH 10.0, and a dual CD system consisting of an anionic CD, 40 mM CM- β-CD and a neutral CD, 22.5 mM M-β-CD. The method was used for the direct quantification of tramadol enantiomers and O-demethyl tramadol glucuronide in urine due to the high sensitivity of the LIF detector. 150 mg tramadol was administered to a healthy volunteer and urinary excretion was monitored during 24 hours and the ratio of the diasteromers was determined; 11.4% of the dose was excreted as *S,S*-tramadol, 16.4% as *R,R*-tramadol and 23.7% as O-demethyl tramadol glucuronide. The amount of *S,S*-O-demethyl tramadol glucuronide was more than three times higher than the *R,R*-O-demethyl tramadol glucuronide; which demonstrates once again the stereoselective metabolism of tramadol.[28]

Using the carrier ability of the multicharged highly sulphated CDs (HS-CDs) infinite chiral resolution of tramadol and its metabolites was obtained; this situation occurs when the two enantiomers migrate in opposite directions. Among the tested CD derivatives, HS-γ-CD proved to be the most efficient chiral selector. Infinite resolution was obtained using ultrashort separation zones by partial filling technique. A classic CE method was used to estimate complex mobilities and complexation constants; the results point out the fundamental role of the differences between the complex mobility of the enantiomers in the enantiodiscrimination mechanism.[29]

In another study HS-CDs were used for the enantioseparation of tramadol, all of the three available HS-CDs (α,β and-γ) proved to be useful for the chiral determination of tramadol enantiomers at pH above 7.0, but HS-γ-CD showed again better resolution and a baseline separation was achieved with this selector at concentration as low as 0.5% w/v. The interaction of positively charged tramadol with the negatively charged HS-CDs buffers does not allow detection of the enantiomers in an acidic BGE.[30]

Tramadol enantiomers migration order was established using a computational design method and the mechanisms responsible for the enantiodiscrimination of the enantiomers was elucidated. A sequential methodology was applied, by using a semiempirical Parametric Model 3 (PM3) and by calculations based on density functional theory. The best results were achieved using a 100 mM phosphate BGE at pH 10.0 and sulphated-β CD (S-β-CD) or CM-β-CD as chiral selectors. It seems that enantiorecognition depends on the formation of association complexes between the anionic β-CD and certain functional groups of tramadol.[31]

Tramadol along with other four basic analytes (amlodipine, hydroxyzine, fluoxetine, tolterodine) was used as model compound for the assessment of sulphated maltodextrin potential as chiral selector in CE. Using a 50 mM phosphate BGE at pH 3.0 and 2% (w/v) sulphated maltodextrin as chiral selector baseline enantioseparation was achieved for all studied analytes. Sulphated maltodextrin proved to be a more efficient chiral selector than neutral maltodextrin due to the electrostatic interaction between sulphated groups and the ionized chiral analytes and to the counter current mobility of negatively charged chiral selector which permit more stereoselective interactions with the positively charged tramadol.[32]

Maltodextrin was used as chiral selector in a chiral stability indicating CE determination in a stability evaluation study of tramadol enantiomers in pharmaceuticals. Tramadol tablets were subject to heat, photolysis, hydrolysis and oxidation to evaluate stress testing. The best results were achieved when using a 50 mM sodium tetraborate BGE at pH 10.2 and 10 % (m/v) maltodextrin as chiral selectors. The linearity range for both enantiomers was verified over a range of 5–100 mg mL^{-1}; and LOD and LOQ were 1.5 mg mL^{-1} and 5 mg mL^{-1}, respectively. Tramadol proved to be more stable in solid state rather than in solution under thermolytic and photolytic stress conditions. Degradation products resulting from the stability testing were the same for both enantiomers and did not interfere with their detection.[33]

Maltodextrin was also used as chiral selector in another CE method for the simultaneous chiral separation of tramadol and methadone, opioid drugs with similar chemical characteristics and as a result similar electrophoretic behaviour. The best results for the enantioseparation of both substances were obtained when using a 100 mM phosphate BGE at pH 8.0 containing 20% (w/v) maltodextrin; under optimized condition the baseline separation of the four enantiomers was obtained in approximately 12 minutes. The LOD of the method were 2 μg mL^{-1} for tramadol and 1.5 μg mL^{-1} for methadone respectively. The method was used for the determination of the analytes in pharmaceutical preparations, urine, and plasma samples.[34]

Tramadol along with other three (chlorpheniramine, hydroxyzine, propranolol) basic analytes was used to study the influence of acidic amino acids (aspartic acid, glutamic acid) as chiral additives on their enantioseparation. No enantioseparation was achieved when using only the amino acids, but the use of

a dual selector system containing an amino acid and a CD improved chiral resolution by comparison with the situations when only CD was used. The best results were obtained when using a 100 mM phosphate BGE at pH 3.0 containing 5.0 mM HP-α-CD/HP-β-CD and 20 mM aspartic acid as chiral selectors. The LOD and LOQ of the enantiomers were over the range of 0.6-1.5 and 2.0-5.0 μg mL^{-1}, respectively. The results show that at a certain pH, the electrostatic interactions between the anionic amino acids and the positively charged basic drugs like tramadol could lead to an improvement of the enantioresolution. A ^{1}H NMR study was subsequently performed in order to investigate the effects of the anionic amino acid in a mixture of an analyte and CD on the proton chemical shifts of analytes, the results were in concordance with those obtained previously by CE which support the synergistic effect of the amino acids on the enantioseparation.[35]

A CE method using partial filling technique with two chiral plugs was used for the enantioseparation of 10 model molecules, including tramadol. This innovative method uses two adjacent chiral plugs containing the same BGE, but with different chiral selectors; using this technique the chiral selector in each plug can interact independently with the analyte and does not disturb the enantioseparation with the other chiral selector. The best results were obtained when a 100 mM phosphate BGE at pH 3.0 containing 10 mM HP-α/β-CD and 10% (w/v) maltodextrin as chiral selectors. The proposed method was compared with a mixed chiral selector CE method using combination of HP-α-CD/HP-β-CD and maltodextrin in the BGE.[36]

Recently our research group elaborated a new CE method for the enantioseparation of tramadol enantiomers in pharmaceutical preparation. The best results were obtained when using a basic BGE containing 25 mM sodium tetraborate at pH 11.0 and an anionic CD, 5 mM CM-β-CD as chiral selector. The optimization of the method was carried out using an experimental design strategy, a faced-centred central composite design being employed in the optimization process.[37]

The analytical characteristics of the methods presented above are synthetized in **Table 2**.

4. Conclusions

In the last twenty years CE has become an interesting option in drug analysis, being considered a viable alternative to the more frequently used HPLC mechanism, especially in the field of chiral analysis. Chiral HPLC is the most frequently used technique in the analysis of chiral drugs; however, it has certain drawbacks, related with the high costs of chiral stationary phases, laborious precolumn or post-column derivatization processes and consumption of large quantities of organic mobile phase. CE experiment require low amounts of sample, reagents and chiral selectors, lead to high efficiencies and chiral resolutions and a wide choice of chiral selectors are available. Another advantage of chiral CE

analysis is related to the fact that is carried out usually using a direct method of separation, by simply dissolving the chiral selector in the BGE.

In some cases, CE enantioseparation can be advantageous by comparison with HPLC in terms of feasibility, efficiency and price as well as from an environmental point of view. However, CE still presents the drawback of lower reproducibility and higher detectability values in comparison with HPLC techniques.

CE have been applied successfully for the achiral and chiral analysis of tramadol from pharmaceutical preparations and in different biological matrices (plasma, serum, urine). In most methods UV detection measurement is used but also more structure-selective and sensitive detection methods, like LIF or MS have been successfully employed. Several methods have been developed for pharmacokinetic studies and applied successfully for the simultaneous determination of tramadol and its phase I and phase II metabolites from biological matrices.

CDs are the most frequently used chiral selectors in CE; stereoselective analysis of tramadol enantiomers by CE using derivatized neutral (M-β-CD) and derivatized anionic CDs (CM-β-CD, SBE-β-CD, HS-γ-CD) as chiral selectors has been reported. The successful use of anionic CDs is due to the basic characteristics of tramadol, which is consequently easily positively chargeable under acidic pH conditions. Also, maltodextrin and its derivatives have been successfully used as chiral selectors for the enantioresolution of tramadol.

The data presented in the current review allow the claim that electrodriven methods are suitable for the achiral and especially chiral analysis of tramadol, being sometimes equally efficient and less expensive than more established techniques such as HPLC.

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Table 1 Achiral analysis of tramadol and its metabolites by electromigration techniques

Analytes	Method	Detection	Sample	Analytical conditions	Reference
Tramadol	ITP	Conductometric	Pharmaceutical preparations (capsules, drops, suppositories, injections)	5 mM potassium picolinate, 5 mM picolinic acid (pH 5.25) – leading BGE; 10 mM formic acid (pH 2.58) – terminating BGE; 50 µA driving current, 10 µA detection current	12
Tramadol	CE	LIF (excitation wavelength 257 nm)	Urine	75 cm length (55 cm to detector) x 50 mm i.d. capillary; 150 mM sodium tetraborate (pH 10.6) – BGE; 25 kV, 19.5 ^{0}C	13
Tramadol, M1, M2, M3, M4, M5, glucuronides of M1, M4, M5	CE	UV (200 nm)	Urine	47 cm length (40cm to detector) x 50 mm i.d. capillary; 65 mM sodium tetraborate (pH 10.65) – BGE; 9 kV; 25 ^{0}C	14
	NACE			10 mM ammonium acetate, 100 mM formic acid (pH 2.75) – BGE; 17 kV; 25 ^{0}C	
	MEKC			25mM sodium tetraborate –70mM SDS (pH 10.45) - BGE; 13 kV; 25 ^{0}C	
Tramadol, Lidocaine	CE	ECL	Urine	50 cm length x 25 mm i.d. capillary; 10 mM phosphate (pH 9.0), 15 kV ECL solution 50 mM phosphate buffer (pH 9.0), 5 mM tris(2,2'-bipyridyl) ruthenium(II)	15
Tramadol, Lidocaine, Ethamsylate	CE	ECL	Urine	50 cm length x 25 mm i.d. capillary; 20 mM phosphate (pH 9.0), 15 kV ECL solution 50 mM phosphate buffer (pH 9.0), 5 mM tris(2,2'-bipyridyl) ruthenium(II)	16
Tramadol	CE	ECL	Serum	40 cm length x 25 mm i.d. capillary; 20 mM phosphate (pH 8.0), 20 kV	17

Tramadol, Paracetamol	CZE	UV (210, 240 nm)	Pharmaceutical preparations (fixed dose combination)	28 cm length (20cm to detector) x 50 mm i.d. capillary; 25 mM sodium tetraborate (pH 9.30) – BGE, 20 kv, 20 ^{0}C	20

Table 2 Chiral analysis of tramadol and its metabolites by electromigration techniques

Analytes	Method	Detection	Sample	Analytical conditions	Reference
Tramadol, M1, M2	CZE	UV (200, 272 nm)	Bulk substances	50 cm length (42 cm to detector) x 50 µm i.d. capillary; 50 mM phosphate (pH 2.5), 75 mM M-β-CD, 220 mM urea, 0.05% (w/v) HPMC – BGE; 25 kV, 15 ^{0}C	23
Tramadol	CZE	UV (195 nm)	Pharmaceutical formulation	50 cm length (45.5 cm to detector) x 50 µm i.d. capillary; 50 mM phosphate (pH 2.5), 5 mM CM-β-CD – BGE; 20 kV, 25 ^{0}C	24
Tramadol, M1, M2, M3, M4, M5	CZE	UV (195 nm)	Urine	40.5 cm length (36 cm to detector) x 50 µm i.d. capillary; 50 mM phosphate (pH 2.5), 5 mM CM-β-CD – BGE; 20 kV, 25 ^{0}C	25
Tramadol, M1, M2, M3, M4, M5	CZE	UV (214 nm)		57 cm length (50 cm to detector) x 50 µm i.d. capillary; 50 mM sodium tetraborate (pH 10.2), 20 mM CM-β-CD – BGE; 20 kV, 20 ^{0}C	26
Tramadol, M1, M2, M3, M4, M5	CE-MS	ESI-MS	Serum	70 cm length x 50 µm i.d. PVA coated capillary; 40 mM ammonium acetate (pH 4.0), 2.5 mM SBE-β-CD-BGE, 25 kV, 20 ^{0}C MS: SIM positive ion mode (4 ions); capillary voltage, 3 kV; fragmentor, 70 V; drying gas N flow and temperature, 6 l/min and 1508C; nebulizer pressure 4 p.s.i.; sheath liquid, 0.5% formic acid in water–isopropanol (50:50, v/v); sheath flow, 3 ml/min,	27
Tramadol, O-demethyl tramadol glucuronide	CZE	LIF (257 nm laser)	Urine	70 cm length (50 cm to detector) x 50 µm i.d. capillary; 50 mM sodium tetraborate (pH 10.0), 40 mM CM- β-CD, 22.5 mM M-β-CD – BGE; 25 kV, 19 ^{0}C	28
Tramadol, M1, M2, M3, M4, M5	CZE	UV (200 nm)	Urine	64.5 cm length (56 cm to detector) x 50 µm i.d. capillary; 50% capillary filled; 20 mM ammonium formate (pH 2.5), 0.2% HS-γ-CD – BGE; 25 kV, 20 ^{0}C	29

Tramadol	CZE	UV (200 nm)	Bulk substance	40 cm length (32 cm to detector) x 75 µm i.d. capillary; 25 mM phosphate (pH 7.0), 2% HS-γ-CD – BGE; 10 kV, 20 ^{0}C	30
Tramadol, Amlodipine, Hydroxyzine, Fluoxetine, Tolterodine	CZE	UV (214 nm)	Bulk substance	60 cm length (50 cm to detector) x 50 µm i.d. capillary; 50 mM phosphate (pH 3.0), 2% sulfated maltodextrin – BGE; 18 kV, 20 ^{0}C	32
Tramadol	CZE	UV (214 nm)	Pharmaceutical formulation	60 cm length (50 cm to detector) x 75 µm i.d. capillary; 50 mM sodium tetraborate (pH 10.2), 10 % maltodextrin – BGE; 20 kV, 20 ^{0}C	33
Tramadol, Methadone	CZE	UV (214 nm)	Pharmaceutical formulation	60 cm length (47 cm to detector) x 50 µm i.d. capillary; 100 mM sodium tetraborate (pH 8.0), 20% maltodextrin – BGE; 22 kV, 20 ^{0}C	34
Tramadol, Chlorpheniramine, Hydroxyzine, Propranolol	CZE	UV (214 nm)	Pharmaceutical formulation	60 cm length (50 cm to detector) x 50 µm i.d. capillary; 100 mM phosphate (pH 3.0), 5.0 mM HP-α-CD/HP-β-CD 20 mM aspartic acid – BGE; 18 kV, 25 ^{0}C	35
Tramadol, Baclofen, Carvedilol, Cetirizine, Chlorpheniramine, Citalopram, Fluoxetine, Hydroxyzine, Propranolol, Trihexyphenidyl	CZE	UV (214 nm)	Bulk substances	60 cm length (50 cm to detector) x 50 µm i.d. capillary; 100 mM phosphate (pH 3.0), 5.0 mM HP-α-CD/HP-β-CD 10% (w/v) maltodextrin – BGE; 18 kV, 25 ^{0}C	36
Tramadol	CZE	UV (210 nm)	Pharmaceutical formulation	38 cm length (30 cm to detector) x 50 µm i.d. capillary; 25 mM sodium tetraborate (pH 11.0), 5 mM CM-β-CD – BGE; 17,5 kV, 15 ^{0}C	37

Figure 1 Tramadol enantiomers chemical structures

Figure 2 Phase I metabolism of tramadol (O -demethyl tramadol (M1), N-demethyl tramadol (M2), N-bis-demethyl tramadol (M3), O-demethyl-N-bis-demethyl tramadol (M4), O-demethyl-N-demethyl tramadol (M5))

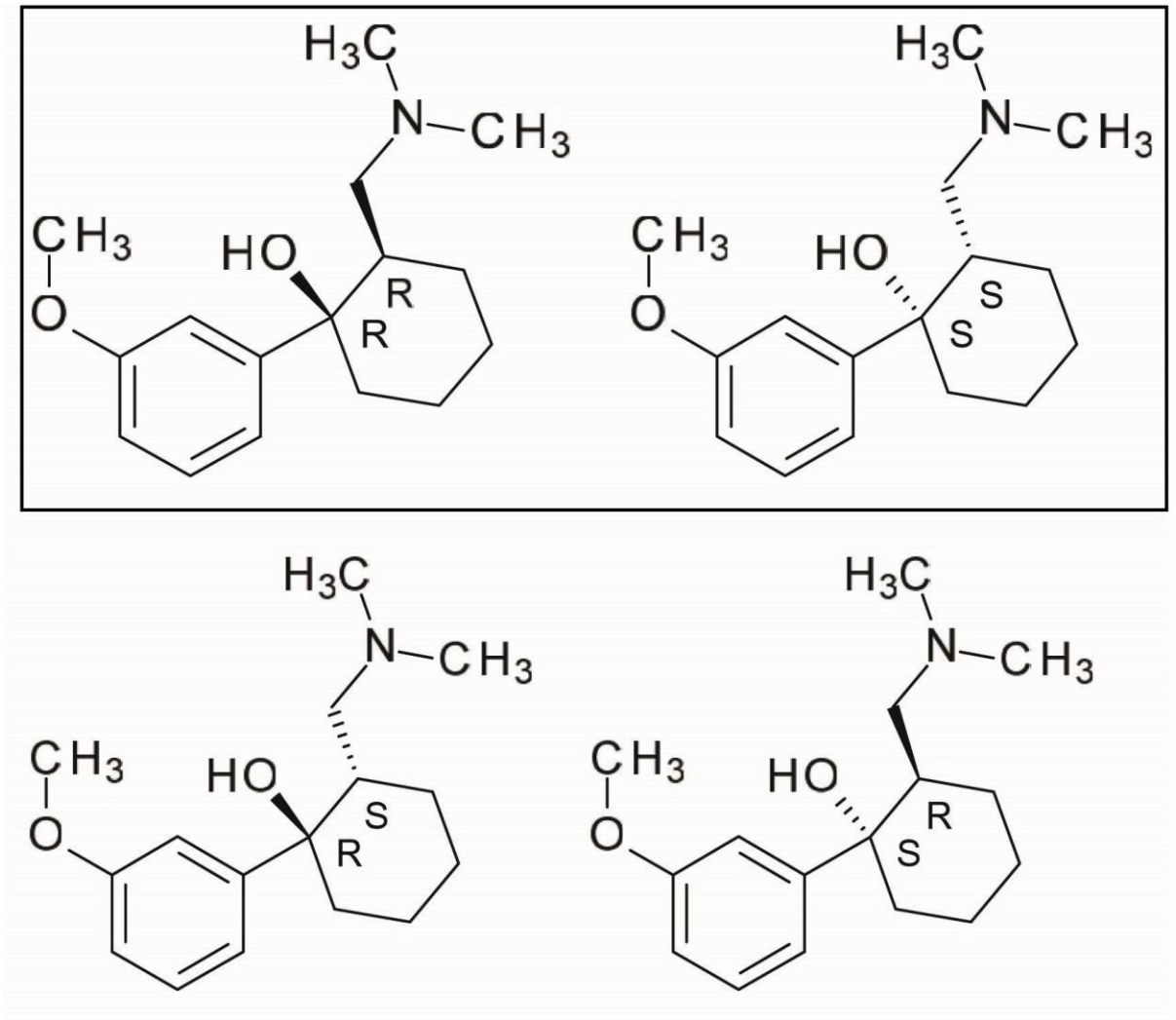

Figure 1

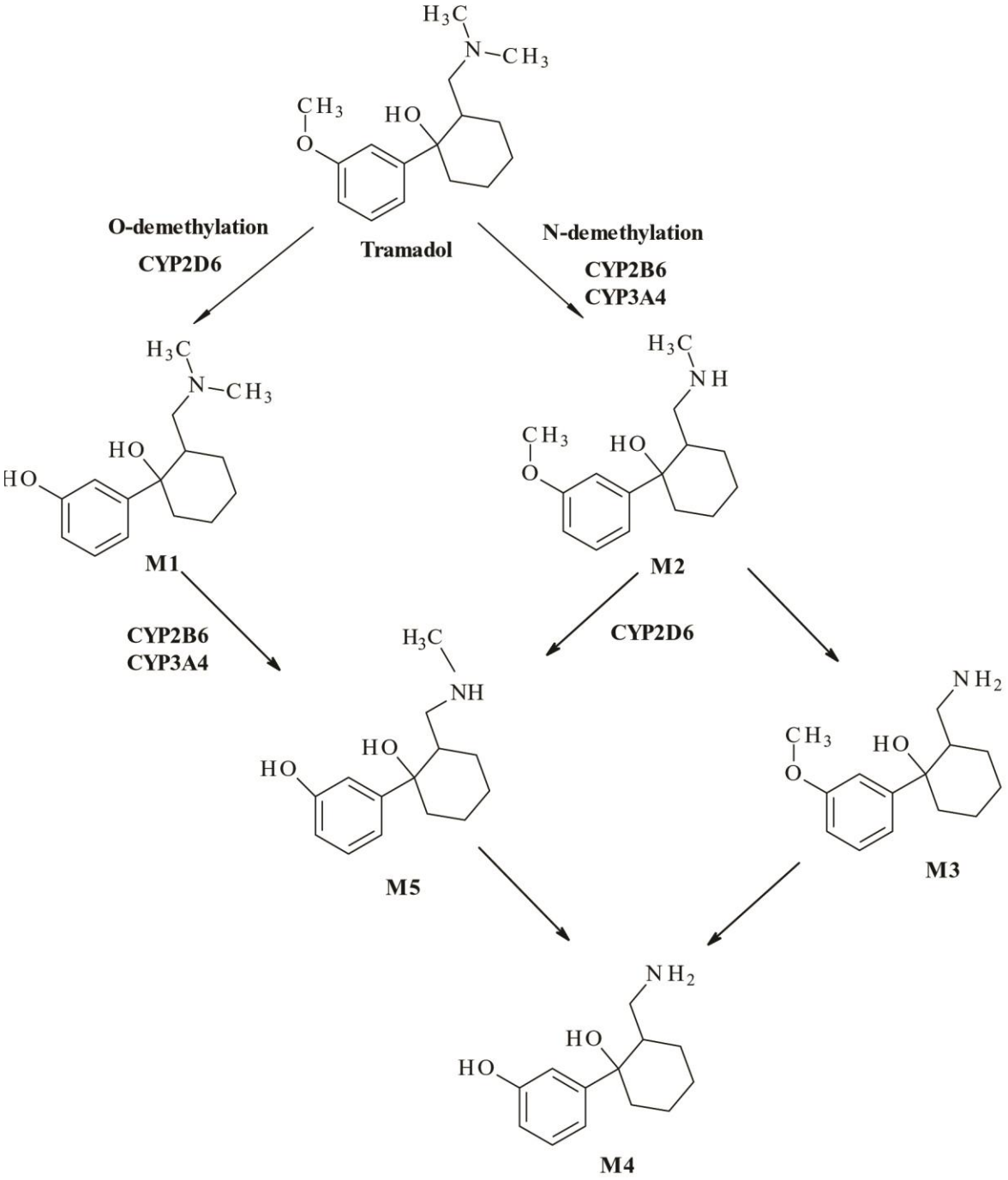
Tramadol
O-demethylation
CYP2D6
N-demethylation
CYP2B6
CYP3A4
M1
M2
CYP2B6
CYP3A4
CYP2D6
M5
M3
M4

Figure 2