Enantiomeric discrimination and quantification of Zolmitriptan by ^{1}H NMR spectroscopy using (R)-(-)-α-Methoxy phenyl acetic acid as chiral solvating agent

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ABSTRACT

A simple, accurate, precise, convenient and economical ^{1}H-NMR method for enantiomeric resolution and quantitative determination of the two enantiomeric forms of Zolmitriptan utilizing (R)-(-)-α-methoxy phenyl acetic acid ((R)-MPA) as a chiral solvating agent is developed. Optimal experimental conditions were evaluated by studying the interaction of substrate with different concentrations of chiral solvating agents viz., (R)-(-)-α-methoxy phenyl acetic acid ((R)-MPA), (R)-(+)-α-Methoxy-α-trifluoromethyl phenyl acetic acid ((R)-MTPA), (S)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol ((S)-TFAE), and (S)-(-)-1,1'-(2-naphthol) ((S)-BINOL) for ^{1}H NMR spectroscopic resolution and determination of Zolmitriptan enantiomers in bulk drugs. Effects of the nature and mole ratio of CSA to analyte on enantiomeric discrimination were investigated. Among different chiral selectors (R)-MPA showed the highest resolution of ^{1}H-NMR signals of Zolmitriptan enantiomers. Hydrogen bonding interaction between the analyte and CSA was the driving force for desired resolution. A mechanism was proposed to explain the interactions between ZMT enantiomers and (R)-MPA. The mole ratio of (R)-MPA with each of the enantiomers of analyte was determined by Job plots which were used to study the complex formation between (R)-MPA and analytes. The method was validated in terms of LOD, LOQ, linearity and recovery. The method was applied successfully to determine the enantiomeric purity of ZMT in bulk drugs.

Key words: Enantiomeric purity, Zolmitriptan, Chiral solvating agent, NMR titration, Hydrogen bonding
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INTRODUCTION

Zolmitriptan, (4*S*)-4-((3-(2-(dimethylamino) ethyl)-1*H*-indol-5-yl) methyl)-oxazolidin-2-one (ZMT) (Fig.1), a novel serotonin 5-hydroxytryptamine receptor agonist of the 1B and 1D subtypes used in the acute oral treatment of migraine with or without aura [1, 2]. ZMT mimics the action of serotonin directly by stimulating the serotonin receptors in the brain and relieves the pain of migraines. ZMT is marketed as (*S*)-enantiomer, since it is pharmacologically more potent than (*R*)-enantiomer. Moreover (*R*)-ZMT is toxic in nature and the allowed limit in bulk drug is 0.15% (w/w) [1, 3].

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(a)

(*S*)-4-((3-(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)methyl)oxazolidin-2-one ((S)-ZMT)

(b)

(*R*)-4-((3-(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)methyl)oxazolidin-2-one ((R)-ZMT)
Fig.1 Chemical structures of (a) (*S*)-ZMT and (b) (*R*)-ZMT
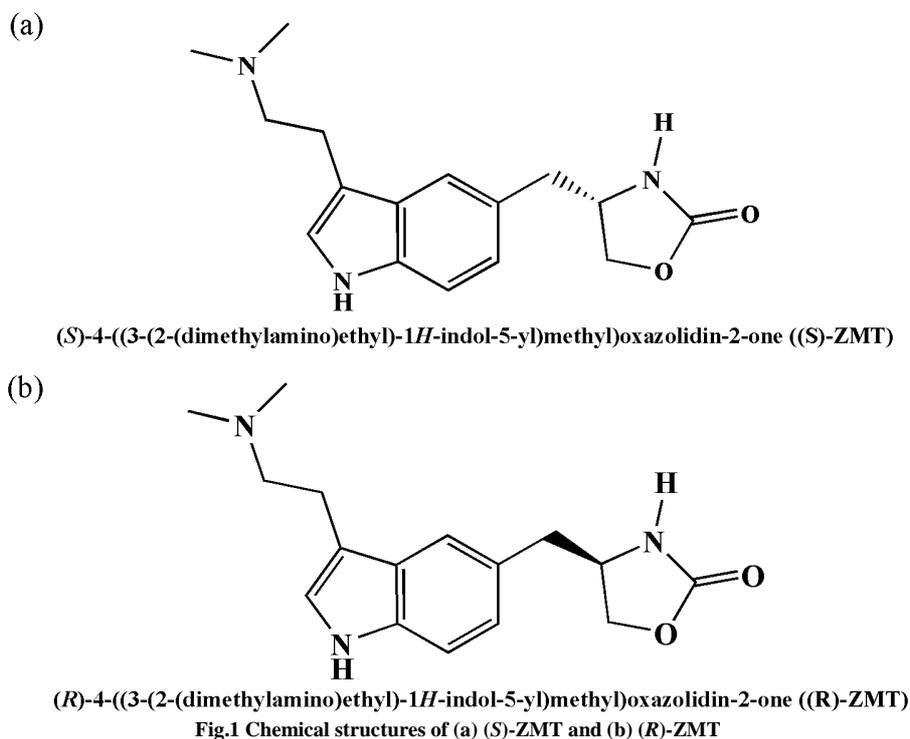
A literature survey reveals that a few techniques have been reported for quantification of ZMT in plasma [4-6]. Srinivasu *et al.* have reported the quantification of ZMT enantiomers in bulk drugs and pharmaceutical formulations by chiral liquid chromatography [7]. Zhang *et al.* determined the enantioseparation of ZMT on vancomycin-bonded chiral stationary phase [8]. Pang *et al.* carried out the enantioresolution of ZMT using hydroxylpropyl-beta-cyclodextrin by capillary electrophoresis [9]. Yu *et al.* determined the ZMT enantiomers in rat liver microsomes [10]. Reported methods in literature for determination of ZMT enantiomers were based on chromatography using expensive chiral stationary phases. ^{1}H-NMR was also used for discrimination of ZMT enantiomers without carrying out any quantitative measurements [11,12]. Hence, development of simple and rapid methods for quantification of enantiomers of ZMT is highly essential, because quantitative NMR method not only reduces the analysis time but also cost effective. Enantiomeric purity determination gained an importance in both the pharmaceutical industry and regulatory authorities [13]. NMR spectroscopy is one of the most common techniques used to determine the enantiomeric purity of the chiral drug substances [14-18]. The current manuscript describes a systematic study on enantiomeric discrimination of ZMT by ^{1}H-NMR spectroscopy using a variety of chiral solvating agents (CSAs). Effects of kinds of CSAs and mole ratios to analyte on enantiomeric discrimination were investigated. A mechanism was also proposed based on interaction between ZMT and CSA. The method was validated in terms of linearity, recovery, limit of detection (LOD) and limit of quantification (LOQ).

EXPERIMENTAL SECTION

Instrumentation
^{1}H NMR spectra were recorded in CDCl$_3$ on Unity INOVA 500 spectrometer (Varian) at an operating frequency of 499.13 MHz in the deuterium lock mode. Chemical shifts were reported in parts per million (ppm) with respect to tetra methyl silane (TMS) as an internal standard. NMR tubes of 5 mm i.d. containing 0.6 mL of solution were used. Typical operating conditions: probe temperature 25°C; tip angle of 30°; acquisition time 2.5 sec; pulse width 13.25 μsec; pulse delay 5 sec; number of scans 64; sweep width 6442.18 Hz; data point resolution 0.25 Hz/point.

Reagents and Chemicals
(*R*)-(-)-α-methoxy phenyl acetic acid ((*R*)-MPA), (*R*)-(+)-α-Methoxy-α-trifluoromethyl phenyl acetic acid ((*R*)-MTPA), (*S*)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol ((*S*)-TFAE), and (*S*)-(-)-1,1´-(2-naphthol) ((*S*)-BINOL) (Fig.2), deuterated chloroform (CDCl$_3$, 98.8 atom% D,), and hexadeuteriodimethyl sulfoxide (DMSO-d6, 99.9 atom% D)

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were purchased from Sigma Aldrich (Saarbrücken, Germany). (*S*)-ZMT and (*R*)-ZMT enantiomers (Fig.1) were obtained from a local manufacturing unit (Hyderabad, India) as a gift samples.

Sample preparation
The samples were prepared by adding 2mg of ZMT enantiomers (1:1) to 2-20mg of CSAs in 0.6 mL CDCl$_3$ and allowed to stand for 10 min. The solutions were then transferred to NMR tubes to record spectra.

(R)-MPA   (R)-MPAA   (S)-TFAE   (S)-BINOL

ZMT
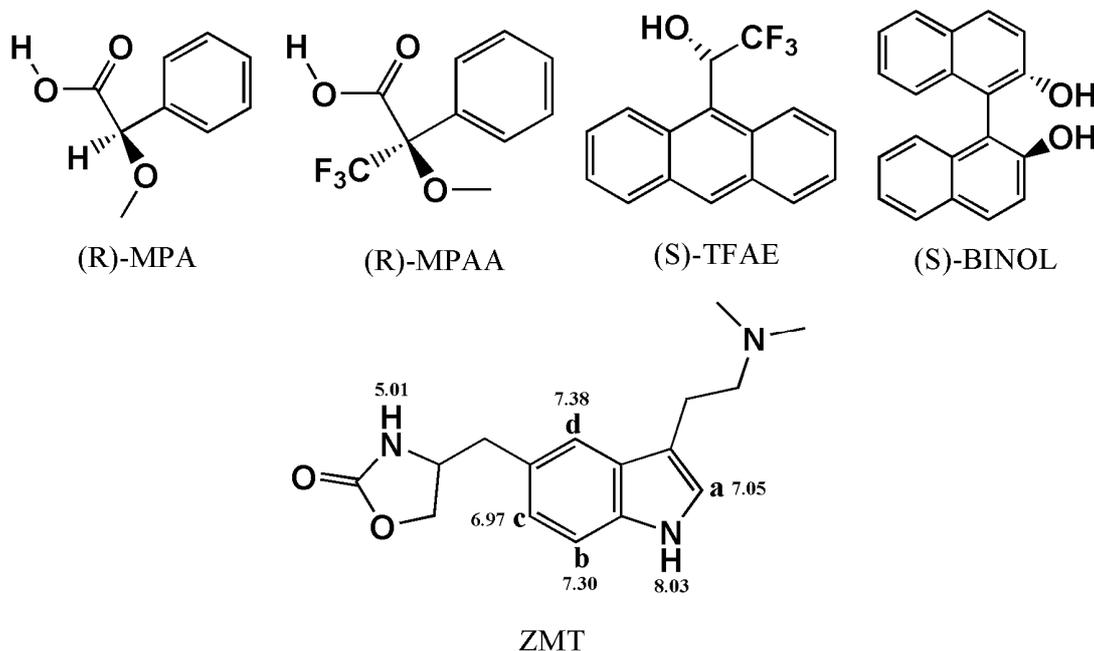
Fig.2 Chemical structures of CSAs and proton assignment of ZMT in ppm

Determination of enantiomeric purity of ZMT
Samples containing ZMT with 0, 33, 50, 66, 83 and 100% ee were accurately placed into NMR tubes. All the samples were prepared by adding 1:1 mole ratio of ZMT and (*R*)-MPA) in 0.6 mL CDCl$_3$. All samples were shaken well to ensure complete dissolution. For linearity, 1.0 to 5.0 mg of (*R*)-ZMT content was added to (*S*)-ZMT to give total concentration of 6.0 mg/0.6 mL in CDCl$_3$.

RESULTS AND DISCUSSION

Selection of solvent
ZMT and all CSAs are well soluble in CDCl$_3$ and DMSO-d$_6$. Organic soluble CSAs are often more effective in nonpolar solvents. Because, these non polar solvents cannot effectively solvate the polar groups of the CSAs and analyte which involves in the non-covalent interactions (viz. hydrogen bonding). As nonpolar in nature, CDCl$_3$ has been opted as a suitable dissolving solvent instead of DMSO-d$_6$.

Nature of CSAs on resolution
Enantiomers have identical physical and chemical properties in an achiral environment, whereas in chiral environment they behave as different. ^{1}H NMR spectroscopy is most commonly used technique to investigate the chiral recognition ability of CSAs (optically active) and enantiomeric discrimination of chiral compounds. The association of enantiomers with CSAs results in the formation of transient diastereomers due to formation of non-covalent intermolecular interactions like hydrogen bonding with analyte, π-π interaction with aromatic ring of analyte.

The chiral discrimination ability of CSAs was investigated from 1:1 to 1:10 mole ratio of analyte and CSA in CDCl$_3$. It was observed that N-methyl, aromatic (a-d in Fig.2), amide and amine protons did not overlap with other

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proton signals in ^{1}H NMR spectrum. Fig.3 shows the ^{1}H NMR spectra of ZMT enantiomers with CSAs at equimole ratio. In case of (*R*)-MPAA, non equivalence was observed for the methylene protons around 30Hz. However, resolved methylene protons of ZMT enantiomers were overlapped with the other proton signals of ZMT and its enantiomer. These differences in chemical shifts between enantiomers were not significant for quantification. In case of (*S*)-TFAE, non-equivalence was observed in methyl proton at 6.2Hz even at 10 mole ratio of CSA. While using (*S*)-BINOL as a CSA, non equivalence in chemical shifts of ZMT enantiomers was observed even increasing the concentration to 10 mole ratio. From the above discussion, (*R*)-MPA is found to be the best CSA for discrimination of ZMT enantiomers. Besides all CSAs, (*R*)-MPA resolved H-a (14 Hz) and H-b (20 Hz) protons of ZMT enantiomers with upfield chemical shift, respectively. However, observed non equivalence in chemical shifts of ZMT enantiomers the methyl, methylene and methine proton signals were overlapped with each other hence they were not considered for quantification. While, the amine protons of ZMT enantiomers were shifted to downfield with 28Hz. This could be the probes for the quantitation of ZMT enantiomers.
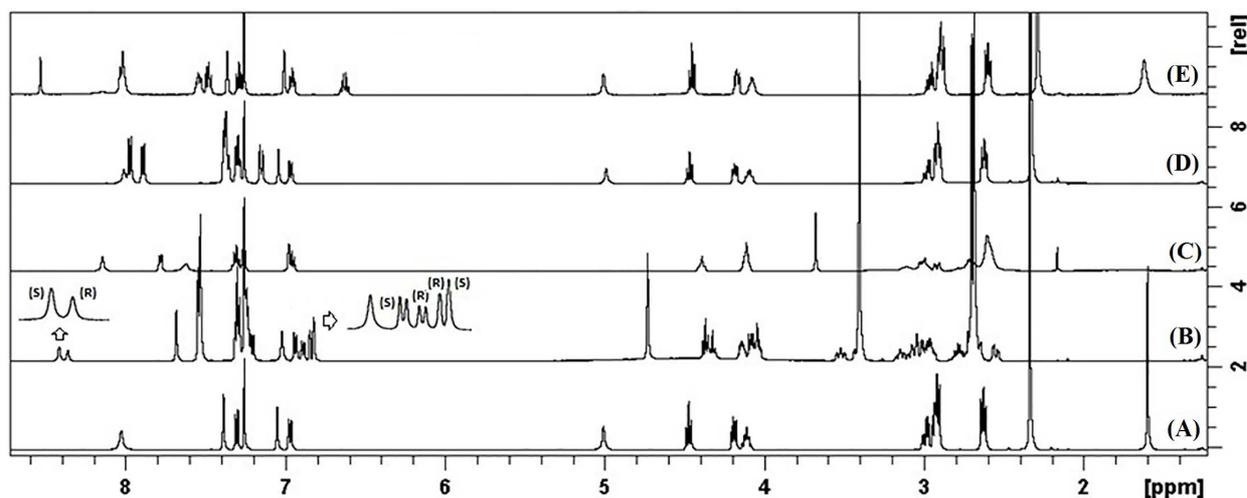
Fig.3 ^{1}H NMR spectra of ZMT A) with no CSA, B) (*R*)-MPA, C) (*R*)-MPAA, D) (*S*)-TAFE and E) (*S*)-BINOL at 1:1 mole ratio

Effect of Substrate to CSA mole ratios

The effect of varying substrate to CSA mole ratio on the discrimination of enantiomeric signals was studied. The downfield chemical shift of amide proton is characteristic of hydrogen bonding interactions. While, due to the π-π interactions upfiled chemical shifts in aromatic protons were observed. Upon increasing the concentration of (*R*)-MPA, H-a, H-b protons of ZMT are overlapped due to π-π interactions between the aromatic rings of ZMT and (*R*)-MPA, whereas amide proton shifted to different extents in down field. However, even increasing in concentration of CSA to 10 mole ratios, the amine proton was not much shifted and was found to be relatively static (Fig.4a). The effect of (*R*)-MPA concentration on ZMT enantiomers is shown in Fig.4b. The optimised performance for the determination of enantiomeric purity was accomplished at 1:1 mole ratio of ZMT with (*R*)-MPA in CDCl$_3$.

(a)

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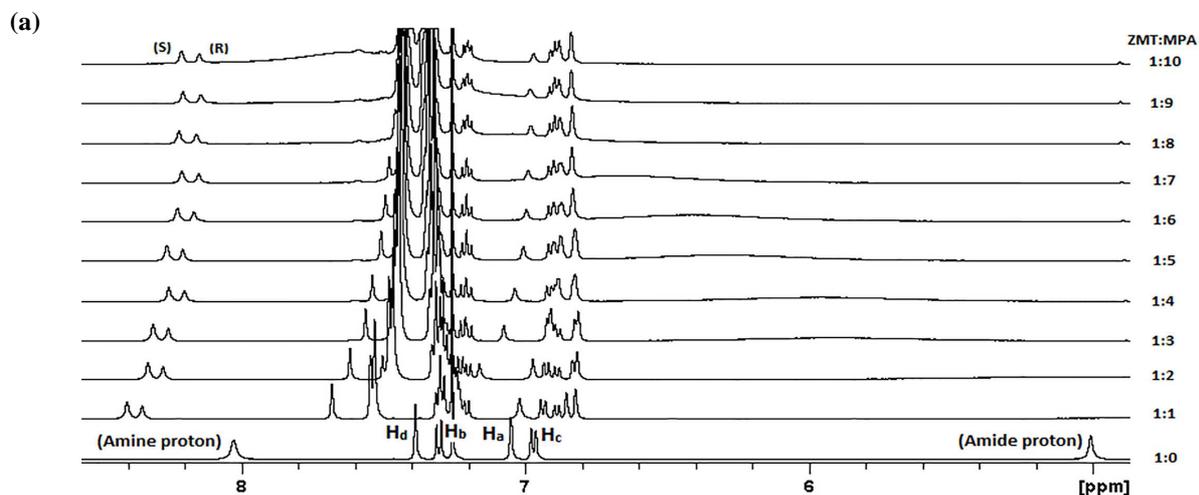
(b)
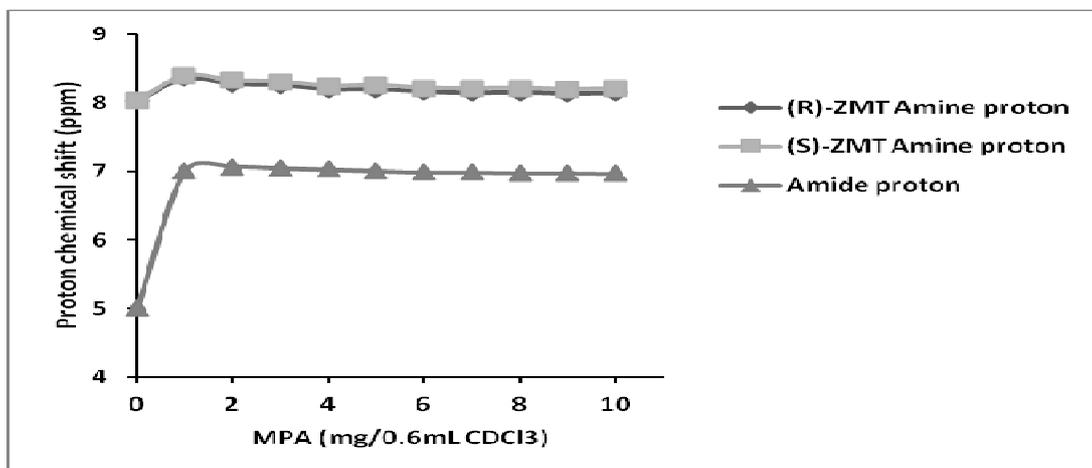
Fig.4 Effect of (*R*)-MPA concentration on ZMT enantiomers discrimination in 0.6 mL of CDCl₃ at 298 K. (a) ¹H NMR spectra of racemic-ZMT with (*R*)-MPA in 1:0 to 1:10 mole ratio and (b) chemical shift verses (*R*)-MPA concentration

Stoichiometry of (R)-MPA-ZMT complex

An attempt was performed to gain better understanding of the stoichiometry of diastereomeric complex formed between (*R*)-MPA and ZMT enantiomers. The stoichiometry of the (*R*)-MPA and ZMT complex was determined by using the Job plot method [19]. The total concentration of the (*R*)-MPA and analyte was kept constant at 10 mM in CDCl₃, whereas the molar fraction of the analyte $\{[G]/([H]+[G])\}$ was varied continuously. The Job plots for the complexation of (*R*)-MPA with the (*R*)-ZMT and (*S*)-ZMT in CDCl₃ are shown in Fig.5. Maxima was observed when the molar ratio was 1:1 (X = 0.5), which indicates that (*R*)-MPA and ZMT enantiomers forms instantaneous 1:1 complex.
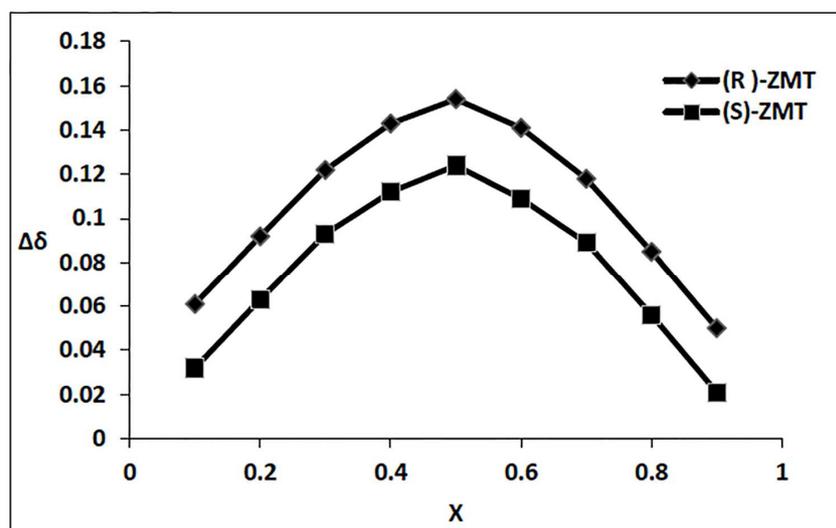
Fig.5 Job's plots of (*R*)-ZMT and (*S*)-ZMT with (*R*)-MPA [X=molar fraction of ZMT, Δδ=Chemical shift change in amine proton of ZMT] (♦) with pure (*R*)-ZMT, (■) with pure (*S*)-ZMT

Conformational studies

To confirm the interactions between the ZMT enantiomers and (*R*)-MPA (i) solvent titrations and (ii) effect of MPA concentration on H-bonding were investigated.

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(a)

(b)
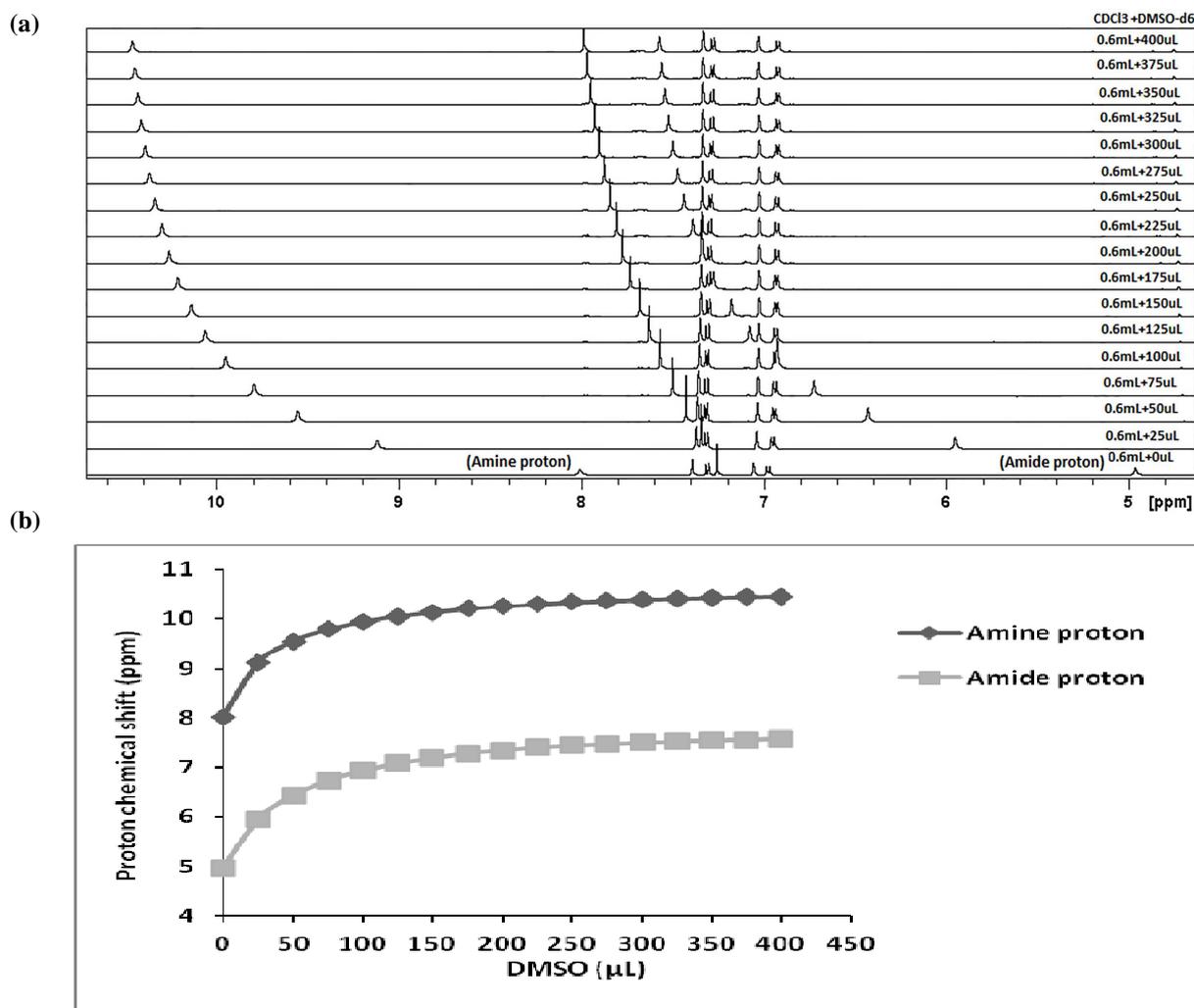
Fig.6 Solvent dependence of amine and amide protons a t 298 K. (a) ^{1}H NMR spectra of ZMT at varying concentrations of DMSO-d_6 in 0.6mL of CDCl$_3$ and (b) chemical shift verses DMSO-d$_6$ concentration

NMR solvent titrations

Solvent titrations were performed to know the effect of solvent on the chemical shift variations and also investigated the nature of hydrogen bonding between substrate and CSA. The H-bonding usually results in deshielding of the signal. The extent of signal shifting towards down field indicates the strength of hydrogen bonding [20, 21]. Initially 2 mg of ZMT was dissolved in the 0.6 mL of CDCl$_3$, followed by gradual increase of its polarity by addition of DMSO-d$_6$ at increment of 25 μL each up to 450 μL (Fig.6a). As the concentration of DMSO-d$_6$ increases, the chemical shift value of amide and amine protons were shifted to downfield with change in chemical shift (Δ ppm) 2.61 and 2.45 ppm respectively. In fact, chemical shifts of all other protons remain intact. Fig.6b shows the variation in chemical shift of amide and amine proton against the DMSO-d$_6$. Thus, it indicated that amide and amine protons might be involved in the inter-molecular H-bonding.

(R)-MPA concentrations on H-bonding

The effect of (*R*)-MPA concentration on chemical shift variation of amide and amine protons was investigated. From Fig 4b, as the concentration of (*R*)-MPA increased from 1 mole to 10 mole ratios, the amide proton shifted towards the down field from 5.01ppm to 6.97ppm, whereas the amine proton shifted to down field from 8.03ppm to 8.21ppm. The overall change in chemical shift in amine proton was 0.18ppm, whereas in amide proton, it was 1.96ppm (Fig.4b). This revealed that the amide protons involved in the inter-molecular hydrogen bonding, which might be with the carboxylic group of (*R*)-MPA. The mechanism was proposed based on the interactions between

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ZMT enantiomers and (*R*)-MPA (Fig.7). Hence amide groups present in ZMT play an important role for chiral recognition with CSA. In addition, (*S*)-enantiomer of ZMT appeared at lower field than corresponding (*R*)-enantiomer in presence of (*R*)-MPA indicates the strong binding of (*R*)-enantiomer with CSA than (*S*)-enantiomer (Fig.4a).
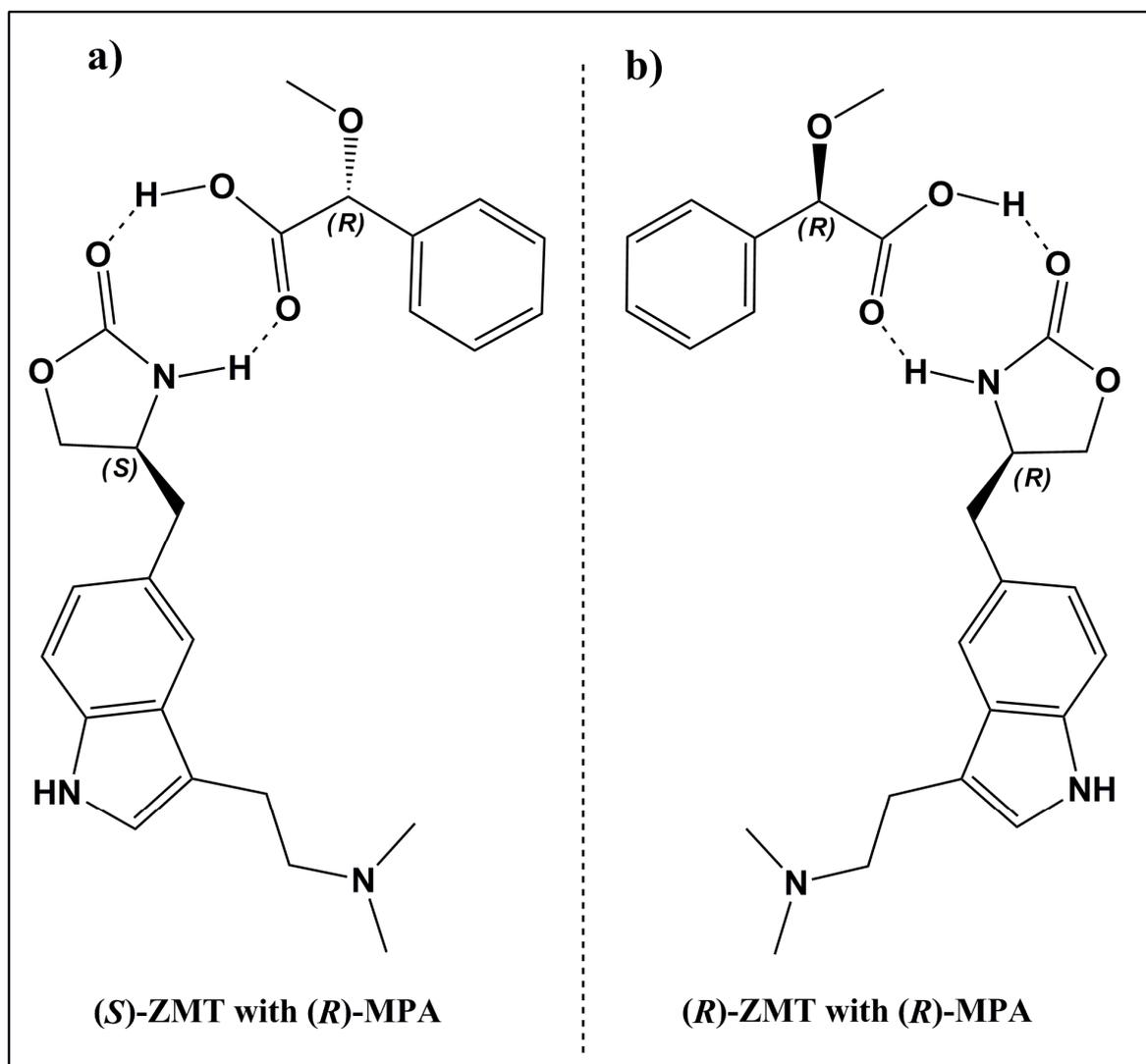
Fig.7 Proposed intermolecular hydrogen bonding between enantiomers of (*S*)-ZMT and (*R*)-ZMT with (*R*)-MPA

Validation
The developed method was validated with respective specificity, linearity, recovery, limit of quantification (LOQ) and limit of detection (LOD). There was no interference of amine proton signal over the (*R*)-MPA. The results revealed that the developed method was specific with respective enantiomeric signals. The method was validated from 0% to 100% enantiomeric excess (ee) in the presence of (*R*)-ZMT (Fig.8).

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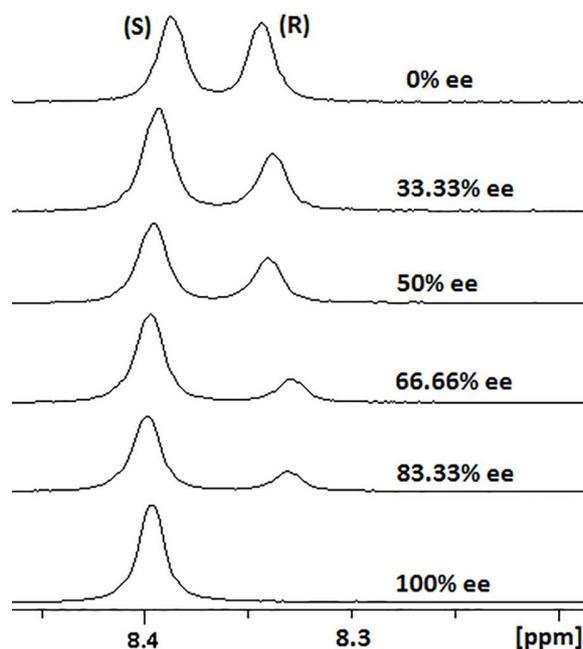
Fig. 8 ^{1}H NMR spectra for various enantiomeric purities of ZMT in the presence of (*R*)-MPA

A linear graph was obtained when recovery values were correlated to the concentration of (*S*)-ZMT and (*R*)-ZMT. The relationship between the found values and theoretical values were found to be linear from 1.00 to 6.00 mg in CDCl$_3$. The linear equation and r^2 values for (*S*)-ZMT and (*R*)-ZMT were y = 0.9897x + 0.036, y = 0.975x + 0.1074, 0.9993 and 0.9991, respectively (Fig. 9).
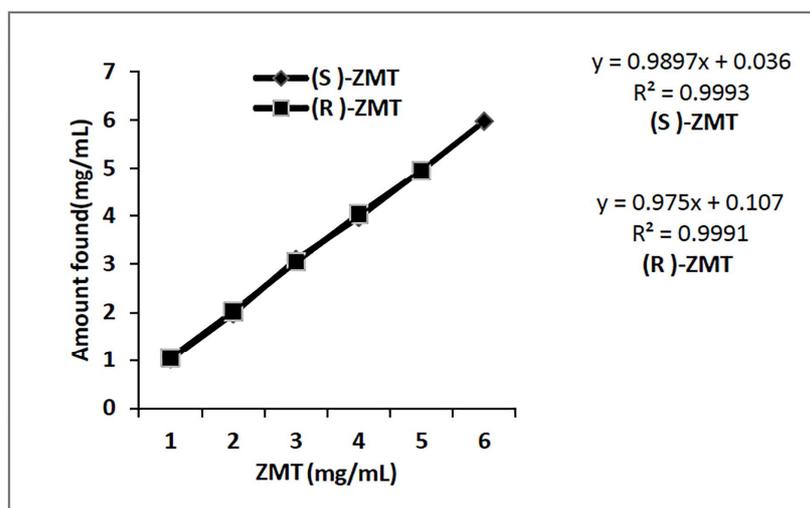
Fig.9 Linearity of (*S*)-ZMT and (*R*)-ZMT enantiomers

LOD and LOQ were determined as the concentration with a signal-to-noise (S/N) ratio of 3 and 10, respectively (Table 1). The amount of (*R*)-ZMT in presence of (*S*)-ZMT of bulk drug-I and II at different concentrations were evaluated and its RSDs were also calculated. The mean ± SD recovery values for (*S*)-ZMT enantiomer was 97-103% of added antipode (Table 2).

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Table 1 Linearity, LOD and LOQ of (S)-ZMT and (R)-ZMT enantiomers

Analyte	Linear range (mg/0.6mL)	Correlation coefficient (n=6)	LOD[a] (mg/0.6mL)	LOQ[b] (mg/0.6mL)
(S)-ZMT	1.0-6.0	0.9993	0.06	0.15
(R)-ZMT	1.0-6.0	0.9991	0.05	0.14

[a]*LOD is limit of detection at 3:1 signal-to-noise ratio.*
[b]*LOQ is limit of quantification at 10:1 signal-to-noise ratio.*

Table 2 Recovery of (S)-ZMT and (R)-ZMT enantiomers in synthetic mixture-I and II by ^{1}H NMR.

(S)-ZMT (mg)	Amount of (S)-ZMT found (mg)[a]	% Recovery[c] of (S)-ZMT	% RSD (n=5)	(R)-ZMT (mg)	Amount of (R)-ZMT found (mg)[b]	% Recovery[c] of (R)-ZMT	% RSD (n=5)
Synthetic mixture-I							
5.00	4.96	99.2	0.22	1.00	1.03	103	0.24
4.00	3.97	99.3	0.26	2.00	2.02	101	0.31
3.00	3.06	102	0.32	3.00	3.05	101.7	0.41
2.00	1.97	98.5	0.34	4.00	4.04	101	0.28
1.00	1.02	102	0.25	5.00	4.95	99	0.24
Synthetic mixture-II							
5.00	4.94	98.8	0.24	1.00	1.03	103	0.21
4.00	4.02	100.5	0.20	2.00	1.96	98	0.35
3.00	3.08	102.7	0.36	3.00	3.06	102	0.44
2.00	1.95	97.5	0.28	4.00	3.98	99.5	0.32
1.00	1.03	103	0.32	5.00	5.05	101	0.36

[a]*The amount of (S)-ZMT found(mg) was calculated from: $A_{(S)} \times$ mg taken / $[A_{(S)} + A_{(R)}]$*
[b]*The amount of (R)-ZMT found(mg) was calculated from: $A_{(R)} \times$ mg taken / $[A_{(S)} + A_{(R)}]$*
[c]*The recovery of ZMT was calculated from: (amount found x 100)/amount added*
n=no. of replicates.

CONCLUSION

A simple and rapid quantitative ^{1}H-NMR spectroscopic method was developed. The diasteriomeric complexation between Zolmitriptan and (R)-MPA and enantiomeric discrimination of Zolmitriptan were investigated. Out of four chiral solvating agents, (R)-MPA has effectively resolved the signals at equimolar ratio. The enantiomeric excess of Zolmitriptan was also determined at different concentrations. The proposed method could be an alternative technique to chiral HPLC for quantification of enantiomeric purity of Zolmitriptan. The suggested method proved to be advantageous with regard to i) easy performance of quality testing within a short time, ii) easy to obtain CSAs from commercial sources and finds applications in quality control of Zolmitriptan and its formulations.

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