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Separation of the $S(+)$ and $R(-)$-enantiomers of tiagabine·HCl and its two chiral precursors by chiral chromatography: application to chiral inversion studies

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Abstract

Chiral HPLC methods were developed and validated for tiagabine·HCl and its two chiral precursors to determine the chiral purity of the three compounds to ensure the quality of the final product which is used as a new antiepileptic drug. Tiagabine·HCl was derivatized with 1-napthalenemethylamine and was chromatographed on a Pirkle type phenyl glycine column with a mobile phase of 69:31, 0.1 M ammonium acetateacetonitrile (v/v). The two chiral precursors were chromatographed on a Chiralcel-OG column with a mobile phase of hexane, isopropanol etc. Each of the three HPLC methods have a selectivity factor (α) of 1–2 or higher. The validation of the methods was done by conducting standard addition and recovery studies of the $S(+)$-enantiomers in the samples. The %RSD of all three methods were < 5 with a limit of quantification of 0.05% (peak area) or lower. By using these methods, a study was conducted to investigate the effect of pH, temperature, and trace levels of transition metals such as Fe^{3+}, Co^{2+}, and Ni^{2+} on the conversion of $R(-)$-enantiomer to the $S(+)$-enantiomer of tiagabine·HCl and its two chiral precursors. The results of this study demonstrated that the two chiral precursors of tiagabine·HCl under reflux conditions are more sensitive to chiral inversion than tiagabine·HCl. Under reflux conditions, in the presence of trace metal ions and different pH, approximately 10, 11, and 1% of the $R(-)$-enantiomer was converted to the $S(+)$-enantiomer for ethyl nipecotate, ethylester of tiagabine, and tiagabine·HCl, respectively. However, at room temperature, tiagabine·HCl appears to be less chirally stable than its two chiral precursors. Approximately 0.4% $R(-)$-enantiomer of tiagabine·HCl was converted to the $S(+)$-enantiomer at room temperature and acidic conditions. Under similar conditions, the $S(+)$-enantiomer of ethyl nipecotate and ethylester of tiagabine·HCl was $< 0.05\%$. The initial $S(+)$-enantiomer content for all three compounds was $< 0.1\%$.

1. Introduction

Stereoselective biological activity of chiral molecules was recognized by scientists previously [1]. In recent years, research has been intensified

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to understand the aspects of the molecular mechanism for stereoselective biological activities of the chiral molecules [2–4]. The technology in the chiral stationary phases of chromatography has improved exponentially in the last decade. Various types of chiral molecules can now be analyzed using GC or HPLC for the enantiomeric purity of a compound [5–19].

Tiagabine·HCl is synthesized as a single enantiomer [20], because $R(-)$-enantiomer is pharmacologically more potent than the $S(+)$-enantiomer. The chiral center of tiagabine·HCl is in the nipecotic acid moeity of the molecule. Nipecotic acid is a β-cyclic amino acid in which the carboxylic acid group is at the chiral carbon of the nipecotic acid ring. The $R(-)$-enantiomer of ethyl nipecotate, which is used in the synthesis of tiagabine·HCl, typically contains $<0.1\%$ of the $S(+)$-enantiomer. However, the ethyl ester of tiagabine·HCl (the only chiral intermediate) and also tiagabine·HCl, always contained higher-percentages of the $S(+)$-enantiomers than was originally present in the starting chiral material, the $R(-)$-ethyl nipecotate. These results indicated that chiral conversion of the $R(-)$-enantiomer to the $S(+)$-enantiomer (chiral inversion) had been occurring during the synthetic process.

In the literature, there is no reference on the effect of metal ions in the chiral inversion of amino acid type molecules. However, from theory, there is always a potential for chiral inversion if the chiral center of a molecule can interact physico-chemically with an external chemical entity. Metal ions such as Fe^{3+}, Ni^{2+}, and Co^{2+} were used in this experiment because stainless steel vessels are frequently used in organic syntheses and trace amounts of different metals can leach from the steel into the reaction mixture at elevated temperature and low pH. All three chiral molecules used in this experiment have a carboxyl group attached at the chiral center. Therefore, any type of interaction of the carbonyl group with the metal ions could potentially interrupt the spatial configuration of the adjacent chiral carbon and hence facilitate chiral inversion. Chiral inversion can also occur in the presence of acid or base. Triethylamine and HCl were used in these experiments to determine the effect of the pH in racemization of the chiral molecules tested. The solvents used in these experiments to prepare the samples of each of the three chiral molecules, were chosen to mimic the solvents used in the synthesis.

This report describes the results of the experiments which were designed to mimic the conditions of the actual synthetic process in order to obtain information on the factor(s) causing the chiral inversion of ethyl nipecotate, ethyl ester of tiagabine, and tiagabine·HCl. The chemical structures of tiagabine·HCl, ethylester of tiagabine·HCl, and ethyl nipecotate are shown in Fig. 1.

2. Experimental

2.1. Equipment

A high performance liquid chromatography solvent delivery system (SP 8800) equipped with an injector/autosampler (SP 8780), an integrator (SP4270), and a variable wavelength UV-visible detector (SP 8450) were used in the experiments (Spectra Physics, San Jose, CA). A 25 cm × 4.6 mm, Chiralcel-OG (Daicel, USA) column was

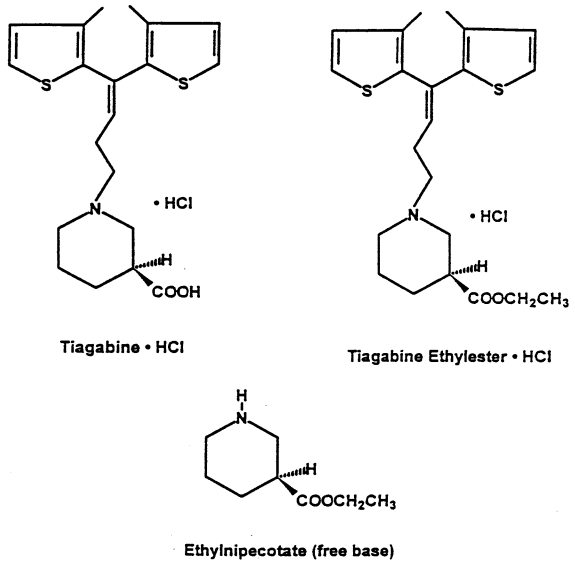

Fig. 1. Chemical structure of tiagabine·HCl, ethylester of tiagabine·HCl, and ethylnipecotate.

used to analyze the ethyl ester and ethyl nipecotate samples. The chiral stationary phase column used to analyze tiagabine·HCl samples was a 25 cm × 4.6 mm, 5 μm, L-phenylglycine (Regis, Morton Grove, IL).

2.2. Materials

HPLC grade hexane, 1-octanol, acetonitrile, ammonium acetate, acetic acid, and triethylamine were purchased from Fisher Scientific (Fairlawn, NJ). Diethylamine (reagent grade) and 2-methyl-2-propanol were purchased from Aldrich (Milwaukee, WI). The racemic mixture, $R(-)$- and $S(+)$-enantiomers of tiagabine·HCl, ethylester of tiagabine·HCl, and ethyl nipecotate were from Abbott Laboratories (North Chicago, IL).

3. HPLC conditions for chiral separation of the $S(+)$ and $R(-)$-enantiomers of tiagabine·HCl and its two precursors

3.1. HPLC conditions for ethyl nipecotate

The chromatographic conditions of the chiral HPLC method developed and validated for ethyl nipecotate are summarized below.

Column:	25 cm × 4.6 mm, Chiralcel-OG
Mobile phase:	941412, hexane/isopropanol/2-methyl-2-propanol (v/v), 0.05%, diethylamine was also added into the mobile phase
Flow rate:	0.8 ml min^{-1}
Detector:	230 nm, 0.10 AUFS
Injection volume:	10 μl (for a 1 mg ml^{-1} sample)

A typical chromatogram of a racemic ethyl nipecotate sample is shown in Fig. 2.

3.2. HPLC conditions for ethyl-ester of tiagabine·HCl

The conditions of the analytical method which was developed and validated to determine the content of $S(+)$-enantiomer are summarized below.

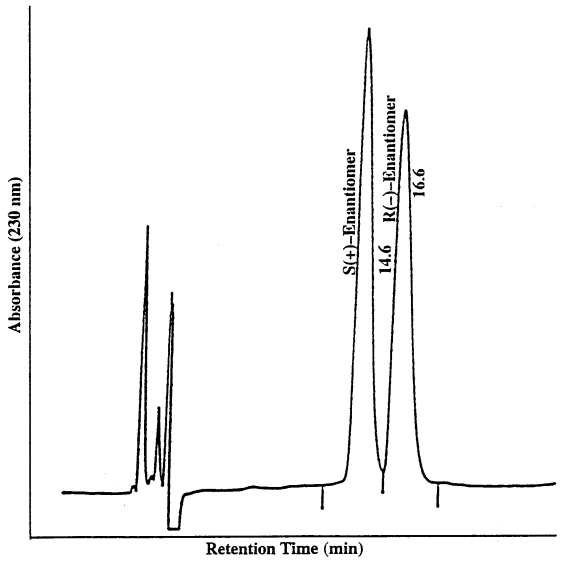

Fig. 2. Typical chromatogram of the racemic (±) mixture of ethyl nipecotate.

Column:	25 cm × 4.6 mm, Chiralcel-OG from Daicel
Mobile phase:	98.95/0.80/0.20/0.05, hexane/2-methyl-2-propanol/1-octanol/ diethylamine (v/v)
Flow rate:	0.8 ml min^{-1}
Detector:	280 nm, 0.10–0.20 AUFS
Injection volume:	5–10 μl for a 1.0 mg ml^{-1} sample

A typical chromatogram of racemic ethyl-ester of tiagabine·HCl sample is shown in Fig. 3.

3.3. HPLC Conditions of tiagabine·HCl

The chromatographic conditions of the chiral HPLC method which was developed and validated for tiagabine·HCl are listed below.

Column:	25 cm × 4.6 mm, 5 μm, L-phenylglycine
Mobile phase:	69/31, 0.1 M ammonium acetate (pH ~3.7)/CH_3CN (v/v)
Flow rate:	1.5 ml min^{-1}
Detector:	260 nm, 0.20 AUFS
Injection volume:	25 μl for 1 mg ml^{-1} sample

The pH of the mobile phase was adjusted with glacial acetic acid. In this procedure, tiagabine·HCl was derivatized with 1-naphthalene-methylamine (to form an amide) prior to chromatography. A typical chromatogram of racemic tiagabine·HCl is shown in Fig. 4.

4. Analysis and work-up of the samples for chiral HPLC

Samples of ethyl nipecotate and the ethyl ester of tiagabine·HCl were neutralized. The solvents were removed by rotary evaporation. Ethyl nipecotate and ethyl ester of tiagabine·HCl gave oily products. These samples were dissolved in the appropriate mobile phase prior to analysis.

The pH of the solutions of tiagabine·HCl were adjusted to ~6 and the free base was extracted into methylene chloride. The hydrochloride salt was precipitated by bubbling dry HCl gas to the mixture. Methylene chloride was subsequently removed by rotary evaporation. The solid product of tiagabine·HCl was then triturated with isopropyl ether to obtain the final product. The tiagabine·HCl was derivatized with 1-naphthalenemethylamine (NMA) to its amide derivative by following the procedure described below.

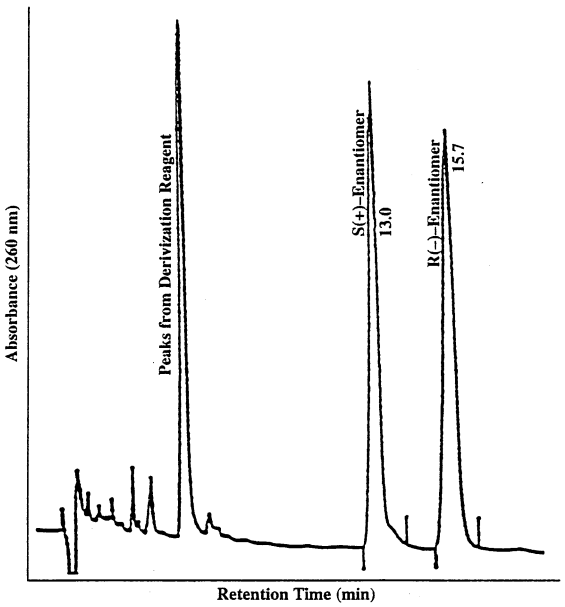

Fig. 4. Typical chromatogram of the racemic mixture (±) of tiagabine·HCl.

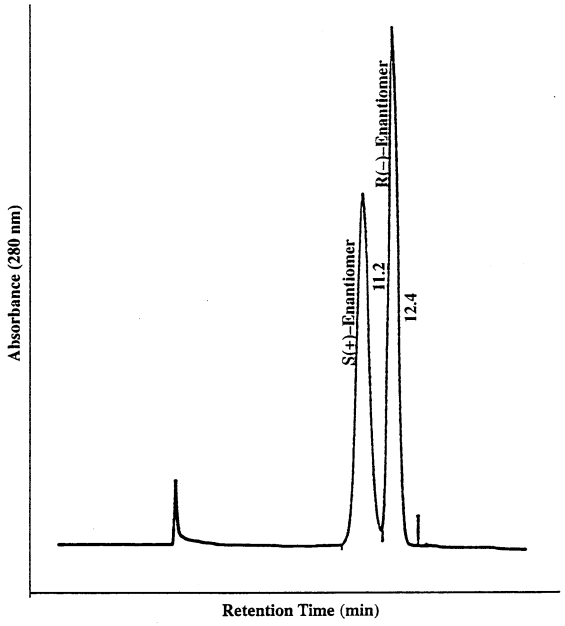

Fig. 3. Typical chromatogram of the racemic mixture (±) of ethyl ester of tiagabine·HCl.

Approximately 10 mg of 1-hydroxybenzotriazole hydrate (HOBT), 20 mg of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide-HCl (EDC) and 8 ml of methylene chloride was added to a glass scintillation vial. Approximately 25 mg of the tiagabine·HCl sample prepared as described above was transferred into the scintillation vial and sonicated for ~15 min. Approximately 15 μl of NMA was added to the mixture and sonicated for another 10 min. The reaction mixture was filtered, and the filtrate was evaporated to dryness with nitrogen. The residue was dissolved in ~10 ml of the mobile phase used to determine the *S*(+)-enantiomer of tiagabine·HCl in the samples. This solution was directly injected into the HPLC system.

5. Results and discussion

5.1. Chiral HPLC methods for tiagabine · HCl and its two chiral precursors

Tiagabine · HCl is a very difficult molecule for chiral separation. Various types of chiral stationary phases (Chiralcel-OG, Chiralcel-OJ, Chiralcel-AD, cyclobond-B, protein columns, AGP and BSA types, etc.) were investigated for direct separation (without derivatization) of the two enantiomers. Except for the protein (AGP and BSA) columns, no separation was obtained between the $S(+)$ and $R(-)$-enantiomers of tiagabine · HCl. The protein columns were able to separate the two enantiomers. However, the reproducibility (day–day, column–column) and chromatographic efficiency was unacceptable for routine analysis. The phenylglycine column gave good separation of the amide derivative of the two enantiomers when derivatized with 1-naphthalenemethylamine.

Separation of the two enantiomers of tiagabine · HCl ethylester was successfully achieved on a Chiralcel-OG column. All the stationary phases which were investigated for tiagabine · HCl was also investigated for the ethylester of the drug. Again, separation of the two enantiomers of ethylester was also obtained on protein stationary phases. However, the chromatographic efficiency and reproducibility was much poorer than the Chiralcel-OG column.

Separation of the two enantiomers of ethyl-nipecotate was also obtained on a Chiralcel-OG column. All other stationary phases either showed no separation of the two enantiomers or poor chromatographic efficiency and reproducibility making them impossible to use for routine analysis.

The reproducibility of each of the three methods were $< 5\%$ RSD for 0.1% level of the $S(+)$-enantiomer (unwanted) in the samples. Standard addition and recovery studies (from ~ 0.03 to 1.0%) showed $98 \pm 4\%$ or better for each of the three methods. The detector signal for all three molecules (at their detection wavelengths) was linear over a three order of magnitude with correlation coefficient of 0.998 or better. The regression line for all three compounds essentially passes through the origin with negligible y-intercept.

5.2. Chiral inversion of ethyl nipecotate

The $R(-)$-enantiomer of ethyl nipecotate did not racemize to the $S(+)$-enantiomer in ethyl acetate either in the presence of HCl or triethylamine at room temperature for approximately 18 h. Chiral inversion of ethyl nipecotate also did not occur when a sample was refluxed (in ethyl acetate) for ~ 20 h. The results of these experiments are summarized in Table 1.

However, the inversion of the $R(-)$-enantiomer to the $S(+)$-enantiomer did occur when ethyl nipecotate was refluxed in the presence of HCl or triethylamine. The apparent pH of the HCl and triethylamine solutions of ethyl nipecotate were 2.0 and 9.8, respectively. The rate of chiral inversion was enhanced (in most cases) when the HCl and triethylamine solutions of ethyl nipecotate also contained small amounts ($\sim 2\%$ w/w) of Co^{2+}, Ni^{2+}, or Fe^{3+}. The data presented in Table 1 demonstrates that the rate of chiral inversion is most rapid when ethyl nipecotate is refluxed in the presence of HCl and cobalt ion. The rate of chiral inversion was also enhanced in the presence of Fe^{3+} in basic or acidic conditions. The presence of Ni^{2+} enhanced the rate of chiral inversion only under acidic conditions.

5.3. Chiral inversion of the ethyl ester of tiagabine · HCl

No chiral inversion occurred when the ethylester of tiagabine · HCl was dissolved in 55/45, ethyl acetate/toluene (v/v), in the presence of HCl or triethylamine (TEA) and refluxed or stored at room temperature for ~ 21 h. The apparent pH of the HCl and TEA solutions of tiagabine · HCl ethyl ester used in this experiment were 3 and 8.8, respectively. However, chiral inversion did occur when similar solutions of ethyl ester of tiagabine · HCl containing small amount of Co^{2+}, Ni^{2+}, or Fe^{3+} ($\sim 2\%$ by weight) were refluxed. The data for this experiment has been tabulated in Table 2.

Inspection of the data in Table 2 reveals that the presence of the metal ions (Co^{2+}, Ni^{2+}, and Fe^{3+}) promotes the inversion of the $R(-)$-enan-

Table 1
Results of the chiral inversion study of ethyl nipecotate under various experimental conditions

Metal ion added	Acid/base added	Condition used	Time (h)	% of $S(+)$-enantiomer[a]
—	—	RF	20	ND
—	HCl	RT	1	ND
—	HCl	RT	18	ND
—	HCl	RF	1	4.4
—	HCl	RF	18	5.1
—	TEA	RT	1	ND
—	TEA	RT	18	ND
—	TEA	RF	1	1.3
—	TEA	RF	18	4.6
Co^{2+}	HCl	RF	1	4.4
Co^{2+}	HCl	RF	18	9.6
Co^{2+}	TEA	RF	1	3.6
Co^{2+}	TEA	RF	18	4.7
Fe^{3+}	HCl	RF	1	4.3
Fe^{3+}	HCl	RF	18	6.2
Fe^{3+}	TEA	RF	1	3.6
Fe^{3+}	TEA	RF	18	6.3
Ni^{2+}	HCl	RF	1	3.7
Ni^{2+}	HCl	RF	18	6.5
Ni^{2+}	TEA	RF	1	3.6
Ni^{2+}	TEA	RF	18	3.9

[a] $S(+)$-enantiomer was not detected in the starting material (<0.1%).
TEA, triethylamine; RT, room temperature; RF, reflux; ND, not detected (<0.05%).

tiomer to the $S(+)$-enantiomer. The initial rate of the chiral inversion of ethyl ester of tiagabine·HCl in the presence of Fe^{3+} and Ni^{2+} under acidic and basic conditions (for ~1 h) is very slow. However, the rate is enhanced when the samples are refluxed for a longer time period. It is also seen that the rate of chiral inversion is higher in the presence of HCl and metal ions than that in the presence of triethylamine (TEA) and metal ions, except for Co^{2+}. Cobalt ion showed the same effect in the presence of HCl or TEA. Furthermore, the initial rate of chiral inversion (in acid or base) was much higher in the presence of Co^{2+} than that seen in the presence of Ni^{2+} or Fe^{3+}. The amount of chiral inversion of the $R(-)$-enantiomer appears to the highest degree with a combination of HCl and either Co^{2+} or Fe^{3+}.

5.4. Chiral inversion of tiagabine·HCl

A slow rate of chiral inversion of tiagabine·HCl was observed after 19 h reflux in water (pH ~3.0). Chiral inversion of tiagabine·HCl was also not observed in water at an apparent pH of 0.6 (HCl) or pH of 10.8 (NaOH) either at room temperature or reflux.. The results of these experiments are tabulated in Table 3.

The rate of chiral inversion of tiagabine·HCl in HCl was enhanced significantly in the presence of Co^{2+}, Ni^{2+}, or Fe^{3+} at reflux for extended periods. Inspection of the data in Table 3 indicates that ~1% of the $R(-)$-enantiomer is converted to $S(+)$-enantiomer when refluxed for ~23 h in the presence of HCl and Co^{2+}, Ni^{2+}, or Fe^{3+} ions. The net chiral inversion of these

Table 2
Results of the chiral inversion study of ethyl ester of tiagabine·HCl under various experimental conditions

Metal ion added	Acid/base added	Condition used	Time (h)	% of $S(+)$-enantiomer[a]
—	TEA	RT	1	ND
—	TEA	RT	21	ND
—	TEA	RF	1	ND
—	TEA	RF	21	ND
—	HCl	RT	1	ND
—	HCl	RT	21	ND
—	HCl	RF	1	ND
—	HCl	RF	21	ND
Ni^{2+}	HCl	RF	1	0.07
Ni^{2+}	HC1	RF	22	9.8
Ni^{2+}	TEA	RF	1	0.2
Ni^{2+}	TEA	RF	2	6.3
Fe^{3+}	HCl	RF	1	0.1
Fe^{3+}	HCl	RF	23	10.7
Fe^{3+}	TEA	RF	1	0.2
Fe^{3+}	TEA	RF	22	3.6
Co^{2+}	HCl	RF	1	3.6
Co^{2+}	HCl	RF	22	11.4
Co^{2+}	TEA	RF	1	3.4
Co^{2+}	TEA	RF	13	10.2

[a] $S(+)$-enantiomer was not detected in the starting material ($<0.1\%$).
TEA, triethylamine; RT, room temperature; RF, reflux; ND, not detected ($<0.05\%$).

samples is $\sim 0.7\%$ from the initial level which is much less than its two chiral precursors for similar stress conditions.

The data shown in Table 3 also demonstrates that under reflux conditions, the rate of chiral inversion of tiagabine·HCl in the presence of Ni^{2+}, Co^{2+}, or Fe^{3+} ($\sim 2\%$ by weight) in acid (pH ~ 0.6) is faster than in base (pH ~ 10.8), except with Ni^{2+}. In a solution of NaOH, the chiral inversion in the presence of Ni^{2+} was approximately half of that observed for Co^{2+}, or Fe^{3+}.

Inversion of the chiral center of ethyl nipecotate occurs at elevated temperature in the presence of HCl or TEA, but not at room temperature. The results of the experiment described in this report show that the $R(-)$-enantiomer of ethyl nipecotate is converted to the $S(+)$-enantiomer in the presence of acid or base in the absence of metal ions. The ethyl ester of tiagabine·HCl was chirally stable in acid or base in the absence of metal ions.

In the presence of metal ions such as Co^{2+}, Ni^{2+}, or Fe^{3+}, chiral inversion of ethyl nipecotate, ethyl ester of tiagabine·HCl, and tiagabine·HCl was observed both at acidic and basic pH. The presence of metal ions generally accelerated the rate of chiral inversion at either pH. Approximately 4–9% of the $R(-)$-enantiomer of ethyl nipecotate was converted to $S(+)$-enantiomer when refluxed for ~ 20 h in the presence of acid or base with metal ions. Under similar conditions, the ethylester of tiagabine·HCl showed a net inversion of 3–11%, and tiagabine·HCl showed a net inversion of $< 1.5\%$. This observation is a significant outcome of this study as one would have expected opposite to these findings if predicted from the chemical structure of the three compounds which were studied in this investigation.

From the chemical structures of ethylnipecotate, ethylester of tiagabine·HCl, and tiagabine·HCl, it will be expected that tiagabine·HCl will interact most strongly with the metal ions because of the presence of free carboxylic acid. Therefore, the perturbation of the chiral center is expected to be the strongest for

Table 3
Results of the chiral inversion study of tiagabine·HCl under various experimental conditions

Metal ion added	Acid/base added	Condition used	Time (h)	% of $S(+)$-enantiomer[a]
—	HCl	RT	1	0.33
—	HCl	RT	19	0.43
—	HCl	RF	1	0.34
—	HCl	RF	19	0.56
—	NaOH	RT	1	0.38
—	NaOH	RT	19	0.42
—	NaOH	RF	1	0.31
—	NaOH	RF	19	0.43
—	H_2O	RF	1	0.44
—	H_2O	RF	19	0.47
Co^{2+}	HCl	RF	1	0.52
Co^{2+}	HCl	RF	23	1.04
Co^{2+}	NaOH	RF	1	0.51
Co^{2+}	NaOH	RF	23	0.69
Fe^{3+}	HCl	RF	1	0.29
Fe^{3+}	HCl	RF	23	1.01
Fe^{3+}	NaOH	RF	1	0.31
Fe^{3+}	NaOH	RF	23	0.50
Ni^{2+}	HCl	RF	1	0.48
Ni^{2+}	HCl	RF	23	0.93
Ni^{2+}	NaOH	RF	1	0.38
Ni^{2+}	NaOH	RF	23	1.24

[a] $S(+)$-enantiomer was not detected in the starting material ($<0.1\%$).
TEA, triethylamine; RT, room temperature; RF, reflux; ND, not detected ($<0.05\%$).

tiagabine·HCl and hence the maximum racemization. However, the data obtained in this study shows the opposite except for the samples studied at room temperature. The mechanism by which the two precursors of tiagabine·HCl racemizes faster than tiagabine·HCl at elevated temperature may be due to the formation of an enol-metal complex, at much higher percentages than tiagabine·HCl. The formation of an enol-metal complex will effect the chiral center of the molecules much more severely that the formation of a metal complex with the carboxylic acid group of tiagabine·HCl. Also, the free carboxylic acid of tiagabine·HCl can form an internal salt with the protonated nitrogen of the nipecotic acid moeity of the molecule. Therefore, the carboxylic acid will not be readily available for metal complexation which will result in less racemization than its two precursors which have a higher potential to form an enol-metal complex.

Because metals are leachable from stainless steel vessels, these results predict a potential impact on chiral inversion resulting from processing in such vessels, especially at elevated temperatures and extreme pH and incubation for long periods of time.

The data presented in Tables 1–3 clearly demonstrates that tiagabine·HCl is the molecule less susceptible to chiral inversion when compared with its two chiral precursors, ethyl nipecotate and ethylester of tiagabine·HCl. Therefore, extra precautions in handling and storage of the two chiral precursors of tiagabine·HCl is necessary to avoid chiral inversion.

6. Conclusions

The analytical methods developed for tiagabine·HCl and its two chiral precursors are

simple, sensitive, and reproducible for day–day analysis. These methods have helped to determine the causes of chiral inversion of the parent and the two precursors during synthesis and manufacturing of the bulk drug.

Ethyl nipecotate did not undergo any chiral inversion when dissolved in ethyl acetate and mixed with HCl or triethylamine and allowed to stand for 18 h at room temperature. However, the $R(-)$-enantiomer of ethyl nipecotate slowly converts to its $S(+)$-enantiomer when refluxed in neat ethyl acetate and in the presence of HCl or triethylamine. The rate of chiral inversion was increased when Abbott-68937 samples dissolved in ethyl acetate/triethylamine or ethyl acetate/HCl were refluxed in the presence of trace metal ions such as Fe^{3+}, Co^{2+} or Ni^{2+}.

The ethyl ester of tiagabine·HCl did not show any chiral inversion when dissolved in ethyl acetate-toluene (55/45, v/v) in the presence of HCl gas or triethylamine, either at reflux or room temperature for 18 h. However, chiral inversion did occur when the same samples were refluxed in the presence of Fe^{3+}, Co^{2+}, or Ni^{2+} ions.

Tiagabine·HCl, dissolved in water/HC1 or water NaOH, did not show any significant chiral inversion when refluxed or stored at room temperature for 19 h. However, when the above samples were refluxed for approximately 23 h in the presence of Fe^{3+}, Co^{2+}, or Ni^{2+}, slow chiral inversion took place.

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