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Fluorescence determination of enantiomeric composition of pharmaceuticals via use of ionic liquid that serves as both solvent and chiral selector

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Abstract

A new method has been developed for the sensitive and accurate determination of enantiomeric compositions of a variety of drugs, including propranolol, naproxen, and warfarin. The method is based on the use of the fluorescence technique to measure diastereomeric interactions between both enantiomeric forms of a drug with an optically active room temperature ionic liquid (RTIL) followed by partial least squares analysis of the data. The chiral RTIL used in this study, *S*-[(3-chloro-2-hydroxypropyl) trimethylammonium] [bis((trifluoromethyl)sulfonyl)amide] (*S*-$[CHTA]^+$ $[Tf_2N]^-$), is a novel chiral RTIL that has been synthesized successfully recently in our laboratory in optically pure form using a simple one-step reaction with commercially available reagents. The high solubility power and strong enantiomeric recognition ability make it possible to use this chiral RTIL to solubilize a drug and to induce diastereomeric interactions for the determination of enantiomeric purity, that is, to use it as both solvent and chiral selector. Enantiomeric compositions of a variety of pharmaceutical products with different shapes, sizes, and functional groups can be determined sensitively (microgram concentration) and accurately (enantiomeric excess as low as 0.30% and enantiomeric impurity as low as 0.08%) by use of this method.

Keywords: Chiral analysis; Room temperature ionic liquid; Fluorescence; Drug; Partial least square; Enantiomeric excess; Enantiomeric purity

It is a well-known fact that enantiomeric forms of many compounds have different physiological and therapeutic effects [1–4]. In general, only one form of enantiomeric pair is pharmacologically active [1–4]. The other (or others) can reverse or otherwise limit the effect of the desired enantiomer. The importance of chiral effects prompted the Food and Drug Administration to issue a 1992 mandate requiring pharmaceutical companies to verify the enantiomeric purity of chiral drugs that are produced [1–4]. As a consequence, the pharmaceutical industry needs effective methods to determine enantiomeric purity.

Currently, there are a variety of methods (e.g., high-performance liquid chromatography [HPLC],[1] gas chromatography [GC], capillary electrophoresis [CE], circular dichroism

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[1] *Abbreviations used:* HPLC, high-performance liquid chromatography; GC, gas chromatography; CE, capillary electrophoresis; CD, circular dichroism; NMR, nuclear magnetic resonance; MS, mass spectrometry; NIR, near-infrared; PLS, partial least squares; RTIL, room temperature ionic liquid; IL, ionic liquid; *R*-propranolol, (*R*)-(+)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol; *S*-propranolol, (*S*)-(–)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol; *R*-naproxen, (*R*)-(–)-2-(6-methoxy-2-naphthyl) propionic acid; *S*-naproxen, (*S*)-(+)-2-(6-methoxy-2-naphthyl) propionic acid; *R*-warfarin, *R*-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone; *S*-warfarin, *S*-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone; $CHTA^+$ Tf_2N^-, [*S*-(3-chloro-2-hydroxypropyl) trimethylammonium] [bis(trifluoromethyl)sulfonylamide]; DSC, differential scanning calorimetry; TGA, thermal gravimetric analysis; RMSEP, root mean standard error of prediction; SEP, standard error of prediction; LOD, limit of detection; FTIR, Fourier transform infrared.

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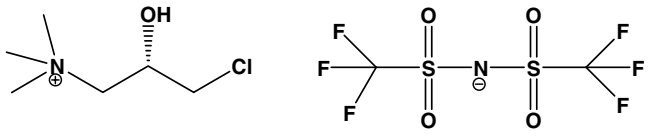
[CD], nuclear magnetic resonance [NMR], mass spectrometry [MS]) available for the determination of enantiomeric purity [5–10]. Although these methods have proven to be effective, they all have some drawbacks, including that they are time-consuming, have low sensitivity, and are destructive [5–10]. More important, none of them is truly universal; that is, they cannot be used for all types of compounds. We have, however, demonstrated recently that it is possible to develop a novel method that not only is universal but also has relatively higher sensitivity and accuracy [11,12]. The method is based on the use of the near-infrared (NIR) technique to measure diastereomeric interactions between added carbohydrate compounds (e.g., α-/β-/γ-cyclodextrin or sucrose), with both enantiomeric forms of an analyte followed by partial least squares (PLS) analysis of the data [11,12]. Compared with other existing methods, this technique not only has relatively higher sensitive and accuracy but also is universal [11,12]. Specifically, it can be used to determine enantiomeric compositions of all types of compounds, including amino acids and pharmaceutical products (e.g., propranolol, atenolol, ibuprofen) with only microgram concentration and enantiomeric excess as low as 0.8% [11,12]. It is noteworthy to add that although this method has proven to be very effective, it still has some limitations such as the need to add a carbohydrate compound (to induce the diastereomeric interactions) and the fact that the analysis must be performed in a solvent that can dissolve both analyte and the carbohydrate compound. Because of the latter requirement (and because of the different solubilities of various types of analytes), it may be necessary to perform the analysis in a variety of solvents, including water or a mixture of water and organic solvents. Therefore, a separated calibration curve must be constructed for each set of carbohydrate–analyte in each specific solvent system. This cumbersome and time-consuming task somewhat limits the application of the method. Therefore, it is desirable to modify this method by eliminating the added carbohydrate and using only one solvent system for the analysis of all types of compounds. Chiral room temperature ionic liquid (RTIL) may offer a solution to this problem.

RTILs are a group of organic salts that are liquid at room temperature. They have unique chemical and physical properties, including that they are air and moisture stable, have a high solubility power, and have virtually no vapor pressure [13,14]. Because of these properties, they can serve as a "green" recyclable alternative to the volatile organic compounds that traditionally are used as industrial solvents [13,14]. The RTILs have, in fact, been used successfully in many applications, including replacing traditional organic solvents in organic and inorganic syntheses, solvent extractions, liquid–liquid extractions, and electrochemical reactions as well as a medium to enhance the sensitivity of thermal lens measurements [13–19]. Advances in RTILs have made synthesis of chiral RTILs a subject of intense study during recent years [14,20,21]. The popularity stems from the fact that it is possible to use chiral ionic liquids (ILs) as chiral solvents for optical resolutions, for asymmetric induction in synthesis, and as chiral stationary phase in chromatography [14,20,21]. It may also be possible to use chiral ILs to replace the solvent as well as the added carbohydrate compound for the enantiomeric purity determination method. Specifically, the chiral IL, with its high solubility power, should dissolve many different types of analytes. Its chirality may produce the needed diastereomeric interactions with both enantiomeric forms of an analyte. Unfortunately, in spite of their potentials, chiral ILs are not commercially available. Only a few chiral ILs have been synthesized, and the synthesis of reported chiral ILs requires rather expensive reagents and elaborated synthetic schemes [14,20,21]. However, we demonstrated recently that it is possible to synthesize both enantiomeric forms of a novel chiral IL by using a simple one-step metathesis reaction from commercially available reagents [22]. This new chiral IL has many advantages, including its high thermal stability (up to 500 °C) and relatively lower background absorption compared with other ILs based on imidazolium [22–24]. More important, it exhibits relatively strong enantiomeric recognition [22].

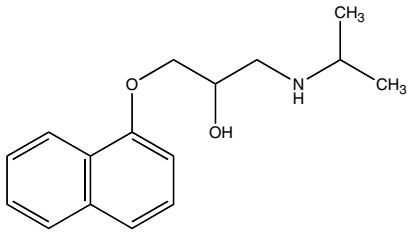
The information presented is indeed provocative and clearly demonstrates that it is possible for a chiral IL to serve as a solvent as well as an added chiral selector for the enantiomeric determination method. Such considerations prompted us to initiate this study, which aims to explore the use of the newly synthesized chiral IL as both the solvent and added chiral selector for the fluorescence determination of enantiomeric compositions of various pharmaceutical products, including propranolol, naproxen, and warfarin.

Materials and methods

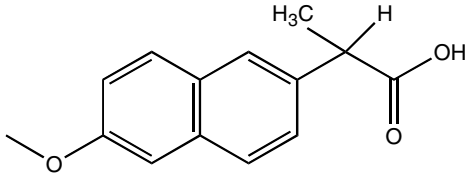
(*R*)-(+)- and (*S*)-(–)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol (*R*- and *S*-propranolol, respectively) and (*R*)-(–)- and (*S*)-(+)-2-(6-methoxy-2-naphthyl) propionic acid (*R*- and *S*-naproxen, respectively) were purchased from Aldrich Chemical (Milwaukee, WI, USA). *R*- and *S*-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone (*R*- and *S*-warfarin, respectively) were obtained from Wako Pure Chemical Industries (Osaka, Japan). [*S*-(3-chloro-2-hydroxypropyl) trimethylammonium] [bis(trifluoromethyl)sulfonylamide] ($CHTA^+$ Tf_2N^-) was synthesized from (*S*)-(–)-(3-chloro-2-hydroxypropyl) trimethylammonium chloride and *N*-lithiotrifluoromethanesulfonimide using a procedure reported in our earlier publication [22]. The product obtained was dried under vacuum overnight and characterized by ^1H NMR, CD, differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA) [22].



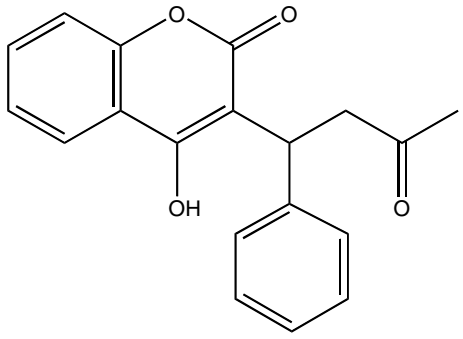
[S-(3-chloro-2-hydroxypropyl) trimethylammonium] [bis(trifluoromethyl)sulfonyl amide] or $CHTA^+$ Tf_2N^-



1-(isopropylamino)-3-(1-naphthyloxy)-2-propranol or Propranolol



2-(6-Methoxy-2-naphthyl) propionic acid or Naproxen



4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone or Warfarin

Fluorescence spectra were taken on the Jasco model FP 6500 spectrofluorometer. Normally, each spectrum of a sample was an average of 20 spectra measured at 0.1-nm intervals and 3.0-nm slit widths for both excitation and emission. Propranolol and naproxen were excited at 280 nm, and warfarin was excited at 320 nm. Multivariate analysis of data was performed using Unscrambler (version 8.0, Camo ASA, Woodbridge, NJ, USA), similar to the procedures used in our previous publications [11,12].

Results and discussion

As described in the introductory paragraphs, a variety of RTILs have been developed and used successfully in various applications, including solvent for electrochemical reactions, organic and inorganic synthesis, and solvent extraction. However, their application in spectroscopy is rather limited. A variety of reasons might account for the lack of application, but the most likely one probably is that reported RTILs, especially those based on alkyl imidazolium cation, have strong background absorption and fluorescence background in the UV and visible region [23,24]. It was reported that these imidazolium-based ILs have rather strong residue absorption in the visible region up to approximately 450 nm and that their fluorescence background is very complicated and can be attributed to the copresence of many molecular species, including protonated and deprotonated forms [23,24]. As a consequence, these RTILs cannot be used as solvent for spectroscopic measurements, particularly for trace chemical characterization. The chiral RTIL, $CHTA^+$ Tf_2N^- (which we successfully synthesized), has many advantages compared with currently available RTILs as well as chiral RTILs [22]. These include the fact that both *R*- and *S*-$CHTA^+$ Tf_2N^- can be readily synthesized in enantiomeric pure form in a one-step synthesis from commercially available reagents. More important, $CHTA^+$ Tf_2N^- has virtually no background absorption and fluorescence signal in the long-wavelength UV and visible region. This can be seen in Fig. 1, where absorption spectra of $CHTA^+$ Tf_2N^- and $BMIm^+$ Tf_2N^- are shown. As illustrated, $BMIm^+$ Tf_2N^- has relatively strong background absorption up to approximately 450 nm. $CHTA^+$ Tf_2N^- has relatively much lower absorption in the UV region and virtually no absorption in the visible region. Furthermore, no detectable fluorescence was observed when $CHTA^+$ Tf_2N^- was excited. Taken together, these results clearly demonstrate that $CHTA^+$ Tf_2N^- can be used successfully as solvent for trace characterization by the fluorescence technique.

Propranolol, a beta blocker drug, was found to be readily soluble in *S*-$CHTA^+$ Tf_2N^- IL. To be used successfully as both solvent and chiral selector for enantiomeric determination, the chiral *S*-$CHTA^+$ Tf_2N^- must have chiral recognition toward *R*- and *S*-propranolol, and this diastereomeric interaction would lead to changes in the fluorescence spectra of propranolol. This possibility was investigated by preparing 24 solutions of propranolol in *S*-$CHTA^+$ Tf_2N^- IL having the same total concentration of 10 μM with relatively different enantiomeric compositions (for compositions of these solutions, see Table 1), and their fluorescence spectra were measured. Fig. 2 shows spectra of

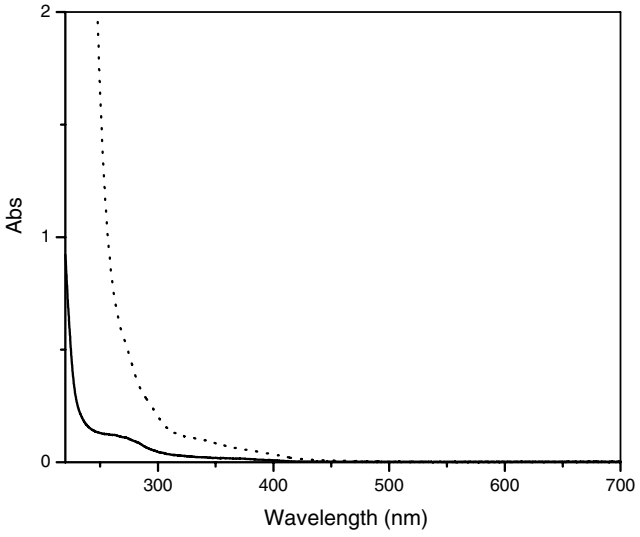


Fig. 1. Absorption spectra of *S*-$CHTA^+$ Tf_2N^- and $BMIm^+$ Tf_2N^- in a 5-mm path length cell.

Table 1
Compositions of solutions used for calibration

Sample	Mole fraction of *R*-propranolol	Mole fraction of *S*-propranolol
1	0.0000	1.0000
2	0.0250	0.9750
3	0.2250	0.7750
4	0.2750	0.7250
5	0.4000	0.6000
6	0.4450	0.5550
7	0.4650	0.5350
8	0.4750	0.5250
9	0.4900	0.5100
10	0.4970	0.5030
11	0.4990	0.5010
12	0.5000	0.5000
13	0.5010	0.4990
14	0.5020	0.4980
15	0.5040	0.4960
16	0.5050	0.4950
17	0.5150	0.4850
18	0.5450	0.4550
19	0.5750	0.4250
20	0.6250	0.3750
21	0.7000	0.3000
22	0.8250	0.1750
23	0.9250	0.0750
24	1.0000	0.0000

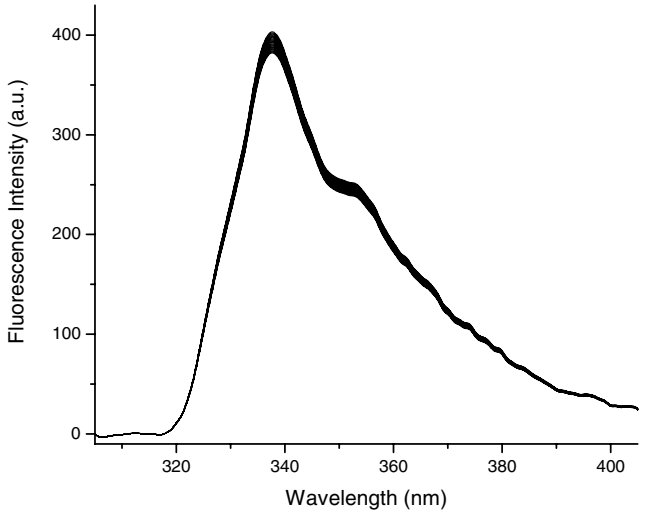


Fig. 2. Fluorescence spectra of 24 solutions of propranolol in *S*-CHTA with a total concentration of 10 μM but different enantiomeric compositions. Each spectrum was an average of 20 spectra taken with a 280-nm excitation wavelength.

the 24 solutions of propranolol in *S*-$CHTA^+$ Tf_2N^-. It is evident from the figure that adding propranolol to the *S*-$CHTA^+$ Tf_2N^- solution leads to changes in the spectra. Because these propranolol solutions have the same total concentration (10 μM) but different enantiomeric compositions, the observed differences clearly indicate that the chiral *S*-$CHTA^+$ Tf_2N^- IL can differentiate *R*-propranolol from *S*-propranolol, and as expected, the diastereomeric interactions lead to changes in the fluorescence spectra. Similar to the procedures used in our previous studies

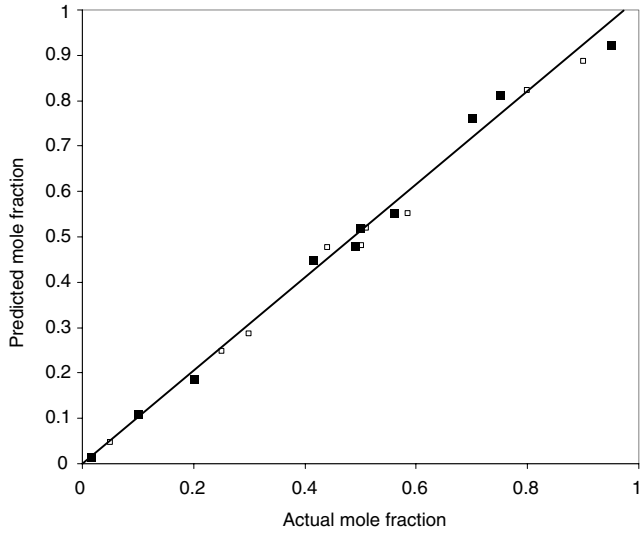


Fig. 3. Predicted enantiomeric composition versus actual composition for 10 μM propranolol in *S*-$CHTA^+$ Tf_2N^- IL. ■, *S*-propranolol; □, *R*-propranolol.

[11,12], the multivariate method of analysis (i.e., PLS) was used to develop calibration models for subsequent determination of enantiomeric purity of unknown samples. Results from the PLS cross-validation show that calibration for 24 models require a relatively small number of factors for optimal performance (10 for *R*-propranolol and 6 for *S*-propranolol). The root mean standard error of prediction (RMSEP) values are 0.097 and 0.096 for *R*- and *S*-propranolol, respectively, whereas the standard error of prediction (SEP) values are 0.094 and 0.092 for *R*- and *S*-propranolol, respectively.

To evaluate the effectiveness of this method, 10 samples of propranolol with the same total concentration of 10 μM but different enantiomeric compositions were prepared, and the concentrations of *R*- and *S*-propranolol in each sample were calculated using the calibration models. Results obtained are shown in Fig. 3, where the calculated concentrations of *R*- and *S*-propranolol in ten samples were plotted against actual concentrations. To illustrate the accuracy of the method, calculated concentrations of *R*-propranolol (in 10 samples) were plotted separately from those of *S*-propranolol (of the same sample) (Fig. 3). As expected, the calculated concentrations for both *R*- and *S*-propranolol are linearly related to actual concentrations. Furthermore, the linear relationship obtained for *R*-propranolol $[y = (1.02 \pm 0.04)x + (0.00 \pm 0.02)]$ is, within experimental error, the same as that for *S*-propranolol $[y = (1.06 \pm 0.04)x + (0.02 \pm 0.02)]$. In fact, both concentrations of *R*- and *S*-propranolol fit well into a single equation with $y = (1.04 \pm 0.03)x + (0.01 \pm 0.01)$ and a correlation coefficient of 0.99999.

The chiral recognition ability of *S*-$CHTA^+$ Tf_2N^- was found not to be specific to propranolol; rather, it is also effective with other chiral compounds. Shown in Figs. 4 and 5 are spectra of 34 solutions of naproxen with the same total concentration of 10 μM and different enantiomeric

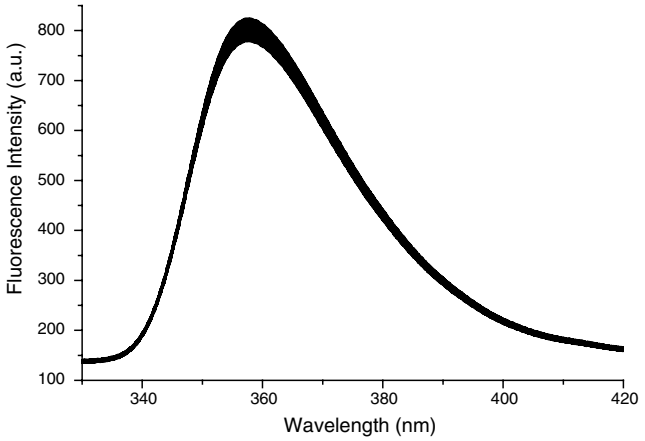


Fig. 4. Fluorescence spectra of 34 solutions of naproxen in *S*-CHTA with a total concentration of 10 μM but different enantiomeric compositions. Each spectrum was an average of 20 spectra taken with a 280-nm excitation wavelength.

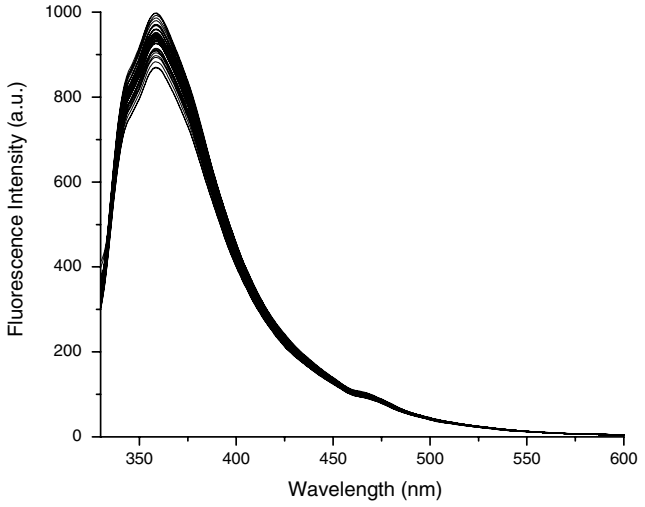


Fig. 5. Fluorescence spectra of 34 solutions of warfarin in *S*-CHTA with a total concentration of 10 μM but different enantiomeric compositions. Each spectrum was an average of 20 spectra taken with a 320-nm excitation wavelength.

compositions (Fig. 4) and spectra of 34 solutions of 10 μM warfarin with different enantiomeric compositions (Fig. 5). As in the case of propranolol, the chiral IL *S*-$CHTA^+$ Tf_2N^- exhibits chiral recognition toward *R*- and *S*-naproxen and *R*- and *S*-warfarin, and the diastereomeric interactions lead to changes in the fluorescence spectra of these solutions. Therefore, these chiral discriminations can be used to determine enantiomeric compositions of naproxen and warfarin. Plots of calculated versus actual concentrations of *R*- and *S*-naproxen and of *R*- and *S*-warfarin are shown in Figs. 6A and B, respectively. As illustrated, the calculated concentrations for all 10 solutions of naproxen and 15 solutions of warfarin agree well with actual concentrations.

It is expected that the method should have high sensitivity. Its sensitivity can be evaluated from the following values: the lowest enantiomeric excess (ee% = [(*R*-enantiomer − *S*-enantiomer)/(*R*-enantiomer + *S*-enantiomer)] × 100) and the lowest percentage enantiomeric impurity (ei% = [(minor enantiomer)/(both enantiomer)] × 100) that can be determined at the lowest concentration of a sample. It should be noted that both ee% and ei% values are dependent on the sample concentration; that is, the limits of detection (LODs) on ee% and ei% can be improved by increasing sample concentration or vice versa. In an attempt to estimate the sensitivity of the method, we performed measurements of ee% on all three drugs: propranolol, naproxen, and warfarin. For each drug, at least 10 samples (propranolol and naproxen) or 15 samples (warfarin) of 10 μM with different ee% values in *S*-$CHTA^+$ Tf_2N^- were prepared, their fluorescence spectra were taken, and ee% for each

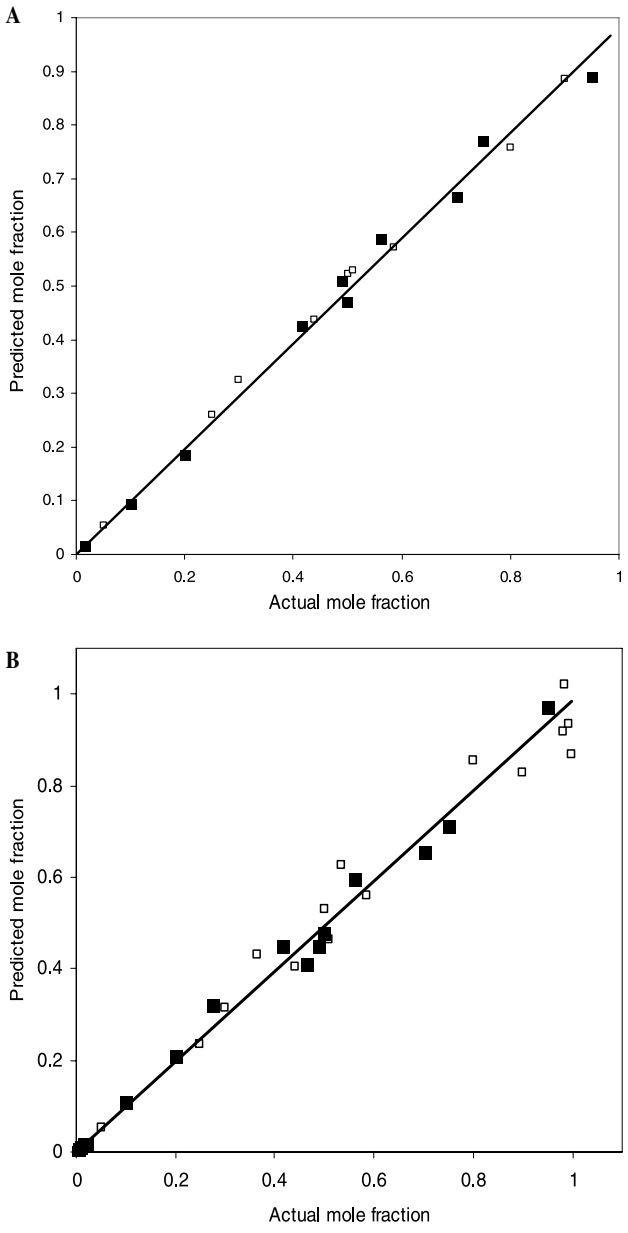


Fig. 6. Predicted enantiomeric composition versus actual composition for 10 μM of naproxen (A) and warfarin (B) in *S*-$CHTA^+$ Tf_2N^- IL. ■, *S* enantiomers; □, *R* enantiomers.

sample was calculated using PLS methods. Results for propranolol, naproxen, and warfarin are listed in Tables 2–4. It is evident from the tables that the method is very sensitive for all three drugs. It can accurately determine samples with concentrations as low as micrograms having an ee% value as high as 99.90% or as low as 0.30%. Furthermore, even at an ee% value as low as 0.30%, the relative errors were only 5.88% for propranolol, 3.90% for naproxen, and 6.79% for warfarin.

Table 5 lists ei% values for warfarin. As listed, even at a concentration as low as 10 μm, this method is capable of detecting 0.60% of *S*-warfarin impurity in the presence of 99.40% of *R*-warfarin. More important, even at this low ei% level, the relative error was only 5.98%. A much lower ei% can, in fact, be determined by this method (e.g., 0.08%), but at a relatively higher error (12.05%).

Collectively, the results presented clearly demonstrate that a novel chiral IL *S*-$[CHTA]^+$ $[Tf_2N]^-$ exhibits high sol-

Table 2
Actual and calculated ee% of solution of 10 μM propranolol in *S*-$CHTA^+$ Tf_2N^- IL

Sample	*R*-propranolol mole fraction	*S*-propranolol mole fraction	Actual ee%[a]	Calculated ee%	Relative error[b](%)
1	0.0500	0.9500	−90.00	94.19	4.66
2	0.2500	0.7500	−50.00	47.85	4.31
3	0.4400	0.5600	−12.00	12.77	6.42
4	0.5015	0.4985	0.30	0.32	5.88
5	0.5030	0.4970	0.60	0.64	7.13
6	0.5100	0.4900	2.00	2.14	7.09
7	0.5350	0.4650	7.00	6.37	8.94
8	0.7250	0.2750	45.00	47.95	6.56
9	0.9000	0.1000	80.00	75.30	5.87
10	0.9850	0.0150	97.00	93.18	3.94

[a] ee% = [(*R*-propranolol − *S*-propranolol)/(*R*-propranolol + *S*-propranolol)] × 100.
[b] Relative error = (actual value − calculated value) × 100.

Table 3
Actual and calculated ee% of solution of 10 μM naproxen in *S*-$CHTA^+$ Tf_2N^- IL

Sample	*R*-naproxen mole fraction	*S*-naproxen mole fraction	Actual ee%[a]	Calculated ee%	Relative error[b](%)
1	0.0500	0.9500	−90.00	−94.05	4.50
2	0.2500	0.7500	−50.00	−51.70	3.40
3	0.3000	0.7000	−40.00	−42.04	5.10
4	0.4400	0.5600	−12.00	−12.48	4.00
5	0.5015	0.4985	0.30	0.29	3.90
6	0.5100	0.4900	2.00	2.09	4.30
7	0.5850	0.4150	17.00	15.98	6.00
8	0.8000	0.2000	60.00	56.94	5.10
9	0.9000	0.1000	80.00	82.96	3.70
10	0.9850	0.0150	97.00	91.57	5.60

[a] ee% = [(*R*-propranolol − *S*-propranolol)/(*R*-propranolol + *S*-propranolol)] × 100.
[b] Relative error = (actual value − calculated value) × 100.

Table 4
Actual and calculated ee% of solution of 10 μM warfarin in *S*-$CHTA^+$ Tf_2N^- IL

Sample	*R*-warfarin mole fraction	*S*-warfarin mole fraction	Actual ee%[a]	Calculated ee%	Relative error[b] (%)
1	0.0500	0.9500	−90.00	−94.48	4.98
2	0.2500	0.7500	−50.00	−46.52	6.97
3	0.3000	0.7000	−40.00	−37.74	5.65
4	0.4800	0.5200	−4.00	−3.66	8.48
5	0.5015	0.4985	0.30	0.32	6.79
6	0.5100	0.4900	2.00	2.12	6.06
7	0.5850	0.4150	17.00	16.03	5.70
8	0.7250	0.2750	45.00	41.64	7.46
9	0.9000	0.1000	80.00	75.60	5.12
10	0.9800	0.0250	96.00	103.7	8.02
11	0.9850	0.0150	97.00	92.17	4.98
12	0.9900	0.0100	98.00	90.12	8.04
13	0.9920	0.0080	98.40	92.33	6.17
14	0.9940	0.0060	98.80	104.6	5.87
15	0.9990	0.0010	99.80	109.7	9.92

[a] ee% = [(*R*-propranolol − *S*-propranolol)/(*R*-propranolol + *S*-propranolol)] × 100.
[b] Relative error = (actual value − calculated value) × 100.

Table 5
Actual and calculated relative concentrations of *S*-warfarin and *R*-warfarin in five solutions of 10 μM warfarin in *S*-$CHTA^+$ Tf_2N^-

Actual mole fraction of *S*-warfarin	Calculated mole fraction of *S*-warfarin	Relative error (%)
0.0010	0.0008	12.05
0.0060	0.0064	5.98
0.0080	0.0087	8.84
0.0100	0.0093	7.14
0.0200	0.018	7.76
0.0500	0.053	5.48
0.2500	0.235	6.12
0.3000	0.315	4.97
0.4400	0.404	8.17
0.5015	0.529	5.54
0.5100	0.464	9.06
0.5850	0.561	4.09
0.8000	0.854	6.78
0.9000	0.829	7.89
0.9850	1.021	3.62
Actual mole fraction of *R*-warfarin	Calculated mole fraction of *R*-warfarin	Relative error (%)
0.9990	0.868	13.08
0.9940	1.104	11.05
0.9920	0.934	5.84
0.9900	1.069	8.07
0.9800	0.919	6.22
0.9500	0.971	2.19
0.7500	0.712	5.04
0.7000	0.655	6.48
0.5600	0.596	6.50
0.4985	0.477	4.22
0.4900	0.452	7.81
0.4150	0.451	8.64
0.200	0.208	4.14
0.1000	0.108	7.98
0.0150	0.016	6.67

ubility power and strong enantiomeric recognition. Because of these features, it is possible to use this chiral IL to solubilize an analyte and to induce diastereomeric interactions for the determination of enantiomeric purity. We have, in fact, successfully developed a new method based on the fluorescence technique where this chiral IL serves both as a solvent and as a chiral selector for the determination of enantiomeric purity. Enantiomeric compositions of a variety of drugs, including propranolol, naproxen, and warfarin, can be determined sensitively (microgram concentration) and accurately (enantiomeric excess as low as 0.30% and enantiomeric impurity as low as 0.08%) by use of this method. As stated in the previous paragraph, both ee% and ei% values are dependent on the sample concentration; that is, the ee% and ei% values (and their associated errors) can be improved by increasing sample concentration and vice versa. To our knowledge, the method reported here has the highest sensitivity (sample concentrations in the micromolar or microgram range) and highest accuracy (ee% as high as 97% and as low as 0.30% and ei% as low as 0.08%). To date, other reported methods based on a variety of techniques, including HPLC, GC, Fourier transform infrared (FTIR), MS, and NMR, have lower sensitivity and/or lower accuracy [25–30]. For example, all of these methods can detect samples only in the millimolar concentration range, which is approximately 1000 times higher than the LOD of our method [25–32]. Furthermore, even at these high LODs, the lowest ee% and ei% values that these methods can determine are in the 1 and 0.1% range, respectively, but with associated error as high as 5% [25–32]. Even at concentrations as low as the microgram level, the ee% and ei% values obtained by our method for all three drugs are much lower than those obtained by all other reported methods. More important, because with this method the chiral IL serves as both solvent and chiral selector, it is not necessary to add either a chiral selector or a chiral column to perform the analysis. Relatively fewer and simpler calibration models are needed. As a consequence, the method will have wider applications and universal utility because it can be used for the analysis of all types of compounds with relatively shorter analysis time and an easier procedure. The three drugs used in this study were selected because they are commercially available in both enantiomeric forms. This does not mean that the method reported here is limited to these three drugs. In fact, the method is rather general because we used this method recently for the ee% and ei% determinations of other compounds, including tryptophan and dansyl-amino acids that absorb and emit in relatively different spectral regions. Furthermore, these chiral IL-facilitated ee% and ei% determinations are not limited to fluorescent chiral compounds. We have, in fact, demonstrated successfully that by the combined use of the chiral IL (*S*-$[CHTA]^+$ $[Tf_2N]^-$) and the NIR spectrophotometric detection method, we can detect ee% and ei% values of all types of fluorescent as well as nonfluorescent chiral compounds [22]. Experiments are now in progress in our laboratory to extend the use of this method for the ee% determination of multicomponent samples.

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