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Capillary electrophoresis enantioselective separation of vigabatrin enantiomers by precolumn derivatization with dehydroabietylisothiocyante and UV–vis detection

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Abstract

A simple and reliable capillary electrophoresis (CE) method with UV–vis detection is presented for the enantioselective separation and determination of vigabatrin enantiomers. Dehydroabietylisothiocyante (DHAIC), a novel chiral derivatizing reagent, was used for precolumn derivatization of vigabatrin enantiomers. Optimal separation was obtained with a running buffer consisting of 50 mM Na_2HPO_4 (pH 9.0), 17 mM sodium dodecyl sulfate (SDS) and 25% acetonitrile. The enantiomeric separation of vigabatrin derivatives was achieved within 25 min, and the resolution was found to be 2.1. Detection was followed by direct UV absorptiometric measurements at 202 nm. A calibration curve ranging from 0.3 to 6.0 μg/ml was shown to be linear, and the limit of detection was 0.15 μg/ml. The developed method has been applied to the determination of vigabatrin enantiomers spiked in human plasma, no interferences were found from endogenous amino acids.

Keywords: Capillary electrophoresis; Enantiomer separation; Vigabatrin; Dehydroabietylisothiocyante

1. Introduction

Vigabatrin (4-amino-hex-5-enoic acid) is an anticonvulsant drug developed by Merrell Dow Res. Inst. It is a structural analogue of γ-aminobutyric acid (GABA) and a selective catalytic inhibitory of enzyme GABA transaminase in brain. Clinically vigabatrin is used for the treatment of epilepsy [1]. Vigabatrin possesses a chiral center in molecular structure and is supplied as a racemic mixture of the enantiomers. But it is reported that only the (*S*)-(+)-enantiomer possess pharmacologically activity [2], and another study has also shown the difference in the pharmacokinetics and pharmacological activity of (*R*)-(−)- and (*S*)-(+)-vigabatrin after epileptic patients was treated with vigabatrin racemate [3]. Therefore, the chiral separation and determination of vigabatrin enantiomers are of great interest in pharmacology and pathology study.

In the past decade, numerous analytical methods, such as high-performance liquid chromatography (HPLC) method [4–6] and capillary electrophoresis (CE) method [7,8] for the determination of vigabatrin have been developed. However, only a few reports are involved in the analysis of vigabatrin enantiomers. Haegele et al. [9] reported a gas chromatography–mass spectrometry (GC–MS) method for the determination of vigabatrin enantiomers in plasma. Schramm et al. [10] developed a gas–liquid chromatography (GLC) method to detect content of vigabatrin enantiomers in plasma or serum. An HPLC method with pre-column derivatization has also been presented for the analysis of vigabatrin enantiomers in human serum [3]. But, both GC–MS and GLC imply expensive chiral capillary columns and time-consuming. While for HPLC, although have given sensitive results, but usually requires long analysis time, complicate operation and large amount solvent consuming.

CE is a microanalytical technique, which provides advantages in term of simplicity, high efficiency, low cost and short analysis time. In recent years, CE has been considered to be an important technology for the enantioseparation of many chiral compounds, but this technology has not been applied to the separation and determination of vigabatrin enantiomers yet. In the present study, a CE method with UV–vis detection was developed for the separation and determination of vigabatrin enan-

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tiomers. The procedure is based on the derivatization of vigabatrin enantiomers with a new chiral derivatizing reagent, dehydroabietylisothiocyante (DHAIC) that was synthesized recently for first in our laboratory [11], to form diastereomeric derivatives, and enantioseparation was completed in an achiral running buffer. The DHAIC as chiral derivatizing reagent has only been used in our laboratory for the resolution of six α-amino acids enantiomer; no other studies were carried out. This work demonstrates the first application of DHAIC for the analysis of an amine-containing drug in human plasma by CE method.

2. Experimental

2.1. Reagent

RS-Vigabatrin and *R*-vigabatrin were obtained from Sigma (St. Louis, MO, USA). Na_2HPO_4 and triethylamine (TEA) were obtained from Guangzhou Chemical Reagent (Guangzhou, China). Sodium dodecyl sulfate (SDS) was purchased from Shanghai Chemical Reagent (Shanghai, China). Acetonitrile was obtained from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals and organic solvents used in this work were of analytical grade. Vigabatrin stock solution was prepared in water and stored at 4 °C. DHAIC was dissolved in acetonitrile. Water was doubly distilled.

2.2. Synthesis of DHAIC

To a mixture of dehydroabietylamine (28.5 g, 0.1 mol) and Et_3N (30.3 g, 0.3 mol) in 75 ml dried diethyl ether cooled at 0 °C, CS_2 (15.2 g, 0.2 mol) was added. The reaction mixture was stirred at 0 °C for 2 h, and at room temperature for 2 h. Then a solution of 0.1 mol of $POCl_3$ in 30 ml of dried diethyl ether was added. The resulting mixture was stirred at temperature for another 4 h. After evaporation of solvent, the residue was dissolved in petroleum ether and the precipitate was filtered off. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel (petroleum ether as eluent) to give white viscous oil, and solidify at 0 °C (24.6 g, yield 76%). IR (KBr, ν, cm^{-1}): 3109, 1611, 1497 (Ph), 2097, 2186 (N=C=S); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.14 (s, 3H), 1.39–1.43 (m, 9H), 1.60–1.63 (m, 2H), 1.67 (d, J = 13.3 Hz, 1H), 1.75 (d, J = 13.3 Hz, 1H), 1.86–1.92 (m, 4H), 2.47 (d, J = 12.9 Hz, 1H), 3.00–3.03 (m, 1H), 3.09–3.11 (m, 2H), 3.43 (d, J = 14.0 Hz, 1H), 3.59 (d, J = 14.0 Hz, 1H), 7.09 (s, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 146.6, 145.8, 134.4, 130.3, 127.0, 124.5, 124.2, 56.8, 45.7, 38.5, 38.3, 37.7, 36.5, 33.6, 30.3, 25.3, 24.1, 24.1, 19.2, 18.7, 18.2. *m/z* (FAB), 43 (26.79%), 173 (75.72%), 312 (100%), 327 ($M^+ + 1$, 55.15%); $[\alpha]_D^{20} = -52^\circ$ (2 mg/ml, CH_3CN).

2.3. Capillary electrophoresis

All separations were performed on a HP^{3D} capillary electrophoresis system (Hewlett Packard, Waldbronm, Germany) with a diode array detector (190–600 nm). Detection was a fixed wavelength of 202 nm with date processed on a HP chemstation. The capillary used for separation was 50 μm i.d. × 360 μm o.d. × 55 cm total length. The effective length of capillary is 50 cm. Prior to sample injection; the capillary was rinsed sequentially with 0.1 M NaOH, doubly distilled water and running buffer solution for 2 min each. Electrolyte composition was 50 mM Na_2HPO_4 (pH 9.0) containing 17 mM SDS and 25% acetonitrile. Samples were injected into capillary by applying hydrodynamic pressure (50 mbar) for 10 s. The applied voltage was 20 kV. The capillary temperature was set to 20 °C. All solutions were filtered through a 0.45 μm pore-size filter before use.

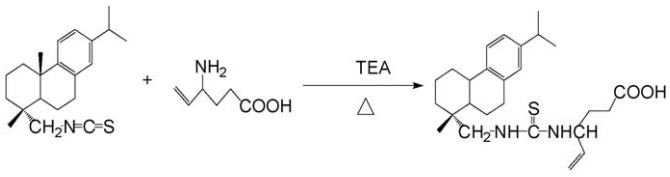

Fig. 1. General reaction scheme for the derivatization of vigabatrin with DHAIC.

2.4. Preparation of plasma sample

The plasma sample was prepared as described in ref. [8]. The blood sample was centrifuged immediately to obtain the plasma, which was stored at −20 °C until analysis. A 0.5 ml plasma sample was deproteinized by adding 0.5 ml of acetonitrile. After centrifugation at 12,000 × g for 15 min, 90 μl of the supernatant liquid was spiked with 10 μl of vigabatrin. Plasma samples of various vigabatrin contents were similarly prepared by spiking the plasma with the desired amount of vigabatrin.

2.5. Pre-column derivatization

A 10 μl volume of the vigabatrin standard solution or plasma sample spiked with vigabatrin was mixed with 100 μl of 1.5% TEA acetonitrile solution in a 0.5 ml derivatization vial. Derivatizing reagent—50 μl of DHAIC (30 mM in acetonitrile) was added. The mixture was vortexed, and then heated with a dry heating block at 65 °C for 45 min. After cooling, the derivative solution was injected for separation. The derivatization reaction scheme was shown in Fig. 1.

3. Results and discussion

3.1. Derivatization

It was found that DHAIC reacts with vigabatrin under alkaline conditions and in organic solvent (acetonitrile). To optimize the derivatization condition, the peak areas of CE analysis for vigabatrin were used to compare and examine the effects of TEA concentration, the molar ratio of DHAIC to vigabatrin, the heating temperature and time on the derivatization reaction. The result showed that when 0.94% TEA concentration, the molar ratio of 15:1 (DHAIC:vigabatrin), the heating temperature of 65 °C and heating time of 45 min were selected as the derivatization condition, the derivatization reaction was found to have gone to completion, i.e. the largest peak area was obtained. It was also found that the standard vigabatrin deriva-

tives (DHAIC–vigabatrin) solutions are stable, and the largest peak area was still obtained after the derivative stand for two weeks at room temperature.

3.2. Chiral separation of vigabatrin enantiomers

Since DHAIC and DHAIC–vigabatrin had low solubility in water, the CE separation of DHAIC–*R*/*S*-vigabatrin has to perform in a SDS micellar medium and in presence of acetonitrile. Therefore, the concentration of SDS and acetonitrile, pH of running buffer, the concentration of running buffer, applied voltage and temperature of capillary were examined to optimize the experimental conditions for the chiral separation of DHAIC–*R*/*S*-vigabatrin.

3.2.1. Effect of SDS concentration

The presence of micelles in the running buffer is essential for the chiral separation. When CE running buffer, which do not contain SDS including 50 mM Na_2HPO_4 (pH 9.0) and 25% acetonitrile was tested for the separation of DHAIC–*R*/*S*-vigabatrin, no resolution between enantiomers was obtained. After SDS was added to the running buffer, the continuous increase of the separation selectivity was observed with increasing SDS concentration, and a baseline separation was achieved for the DHAIC–*R*/*S*-vigabatrin enantiomers after SDS concentrations were higher than 15 mM. But an increased SDS concentration also led to the prolongation of the migration time of analytes (see Fig. 2). Therefore, a SDS concentration of 17 mM is considered optimal as it provides the fastest analysis time with best resolution.

3.2.2. Effect of organic modifier

Addition of organic modifiers, such as methanol and 2-propanol, could enhance the solubility of DHAIC–*R*/*S*-vigabatrin enantiomers. However, no optical resolution could be obtained. By using acetonitrile instead of methanol or 2-propanol as an organic modifier, the optical resolution of the DHAIC–*R*/*S*-vigabatrin enantiomers was obtained. The concentration of acetonitrile in the running buffer affects also the separation. The enantiomeric resolution of DHAIC–*R*/*S*-vigabatrin decreases dramatically with increasing acetonitrile concentration from 22% to 28% (see Fig. 3). Although a higher resolution could be achieved for DHAIC–*R*/*S*-vigabatrin enantiomers at lower acetonitrile concentration, which led to a reduction of solubility of DHAIC–vigabatrin, and as a consequence, to very long separation times. Thus, 25% acetonitrile is considered optimal.

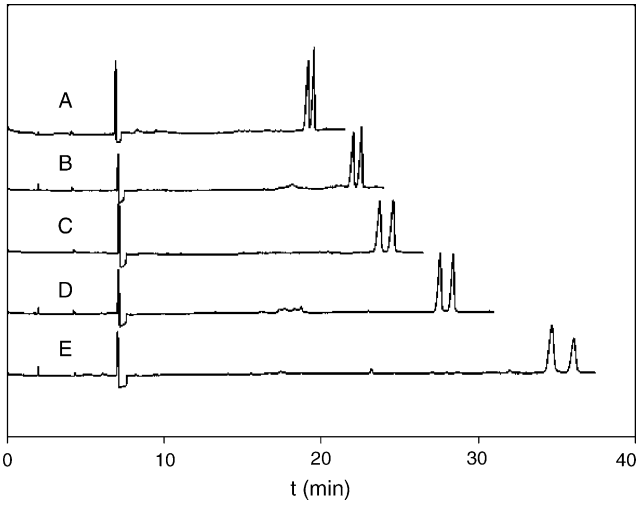

Fig. 2. Electropherogram of vigabatrin enantiomers derivatives under different SDS concentration. Electrolyte composition was 50 mM Na_2HPO_4 (pH 9.0) containing 25% acetonitrile and 13 mM SDS (A); 15 mM SDS (B); 17 mM SDS (C); 19 mM SDS (D) and 21 mM SDS (E). Capillary was 50 μm i.d., 55 cm in length. Applied voltage was 20 kV; capillary temperature was 20 °C; UV detection wavelength was 202 nm.

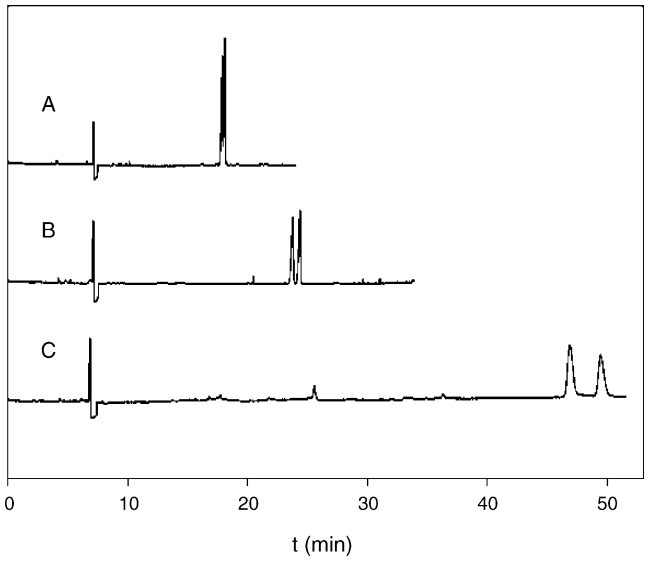

Fig. 3. Electropherogram of vigabatrin enantiomers derivatives under different acetonitrile concentration. Electrolyte composition was 50 mM Na_2HPO_4 (pH 9.0) containing 17 mM SDS and 28% acetonitrile (A); 25% acetonitrile (B) and 22% acetonitrile (C). Other conditions as in Fig. 2.

3.2.3. Effect of pH of running buffer

The effect of pH of running buffer on derivatives migration and enantiomeric separation was investigated by using a 50 mM Na_2HPO_4 buffer containing 17 mM SDS and 25% acetonitrile as running buffer. In the pH range of 8.0–10.0, an increase in pH of the running buffer was found to result in a decrease in the resolution of enantiomer. On the other hand, the migration time of DHAIC–*R*/*S*-vigabatrin enantiomers also decrease with the increase of pH value of running buffer. This is due to the fact that higher electroosmotic flow (EOF) was obtained at higher pH value, which led to a faster migration time for DHAIC–vigabatrin. The results demonstrate that the Na_2HPO_4 buffer at pH 9.0 is the pH that is most optimal for successful chiral separation within the shortest time.

3.2.4. Effect of buffer concentration

This series experiments were performed at the optimal pH of 9.0 with 17 mM SDS and 25% acetonitrile to measure the optimum buffer concentration. In the concentration range of 35–60 mM Na_2HPO_4 buffer, the resolution of DHAIC–*R*/*S*-vigabatrin enantiomers increases with the increase of Na_2HPO_4

concentration; the resolution is more than 2.0 after the concentration is more than 50 mM. Therefore, a concentration of 50 mM Na_2HPO_4 was chose as the optimization.

3.2.5. Effect of capillary temperature and applied voltage

The change of capillary temperature results in the change of viscosity of the solution and then the change of EOF, which led to the change of resolution of enantiomer. Generally, a higher resolution can be obtained at a lower capillary temperature. In this test, 20 °C was considered as the optimal capillary temperature. Low voltage can also improve the resolution because the mobility was low and the long time is required. Thus 20 kV should be the appropriate applied voltage considering the resolution and the migration time at the same time.

After a careful study on the effects of above several parameters, the separation conditions of DHAIC–*R*/*S*-vigabatrin enantiomers were selected as following: 20 kV applied voltage, and a running buffer consisted of 50 mM Na_2HPO_4 (pH 9.0), 17 mM SDS and 25% acetonitrile. Capillary temperature was at 20 °C. Under optimum conditions, the electropherogram for enantiomeric separation of DHAIC–*R*/*S*-vigabatrin was shown in Fig. 4. As can be seen, the enantiomeric separation of vigabatrin derivatives was achieved within 25 min, and the resolution was found to be 2.1.

3.3. Method validation

Before a method is used for biological and clinical analyses, it must be validated. In the present work, the CE method for the determination of vigabatrin enantiomers was validated by determining its performance characteristics regarding linearity, limit of detection and reproducibility (precision).

3.3.1. Linearity and limit of detection

A series of vigabatrin enantiomers standard solutions were tested to determine the linearity between the vigabatrin concentration and peak area. Linear regression analysis of the results yielded the following equation:

$$R: \quad y = 34.09x - 0.4291 \quad (r^2 = 0.9983)$$

$$S: \quad y = 34.65x - 1.4968 \quad (r^2 = 0.9991)$$

where y is peak area, and x is the concentration of vigabatrin (μg/ml) in the derivative solution. The calibration curves exhibited an excellent linear behavior over the concentration range of from 0.3 to 6.0 μg/ml.

The limit of detection was established considering a signal-to-noise ratio of 3. From above calibration curves, the limit of detection was calculated to be 0.15 μg/ml (1.3×10^{-6} M) for both *R*- and *S*-vigabatrin.

3.3.2. Reproducibility

The reproducibility was investigated by injecting three standard solutions of vigabatrin enantiomer derivatives at 0.5, 3.0 and 6.0 μg/ml each five times and recording the migration time and the peak areas. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.). The results obtained indicate that the R.S.D. were less than 5.0%, which makes possible the use of this method with quantitative purposes in a reproducible way.

3.4. Determination of vigabatrin enantiomers in human plasma

Owing to vigabatrin drug is not available in china, real plasma samples from epileptic patients treated with vigabatrin drug cannot be obtain. In order to evaluate the applicability of the method for biological and clinical analyses, human plasma spiked with vigabatrin enantiomers was used as the test sample. Since DHAIC can react with primary amines, the separation and determination of DHAIC–vigabatrin enantiomers may be affected by the endogenous plasma components such as amino acids, primary amine and protein. However, the laborious sample cleanup might not be necessary because of the high separating power of CE. The only pretreatment we performed was deproteinizing the plasma with the addition of acetonitrile, followed by centrifugation.

A series of human plasma samples spiked with vigabatrin enantiomers at different amounts were analyzed. The typical electropherograms obtained from separations of plasma blank and plasma spiked with 0.3 μg/ml vigabatrin are shown in Fig. 5. As can be seen, there are no peaks observed across the migration time for the DHAIC–vigabatrin derivatives in the electropherogram of plasma blank (Fig. 5A), which indicate that the endogenous components in plasma such as amino acids do not interfere the determination of vigabatrin enantiomers.

The determination results of vigabatrin enantiomers spiked in plasma samples are summarized in Table 1. The recoveries of vigabatrin enantiomers are in the range of 96.7–108%. According to the report in literature [3,6], for an adult epileptic patient, the concentrations of vigabatrin enantiomers in serum are in the range of 2–18 μg/ml within 0–6 h after intake of

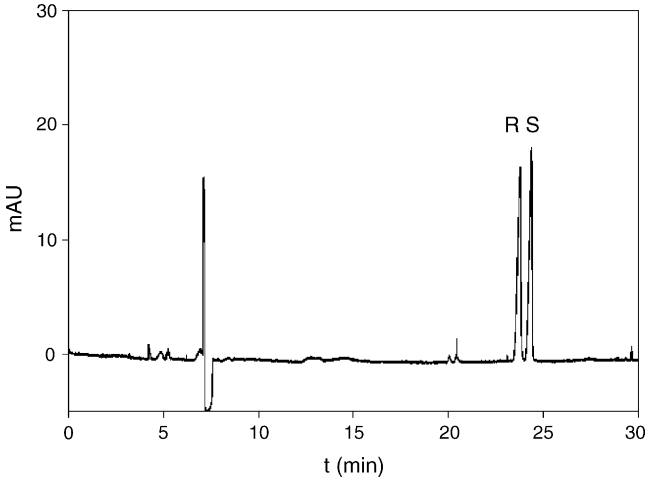

Fig. 4. Electropherogram of vigabatrin enantiomers derivatives. Electrolyte composition was 50 mM Na_2HPO_4 (pH 9.0) containing 25% acetonitrile and 17 mM SDS. Capillary was 50 μm i.d., 55 cm in length. Applied voltage was 20 kV; current flow was 35 μA; capillary temperature was 20 °C; UV detection wavelength was 202 nm.

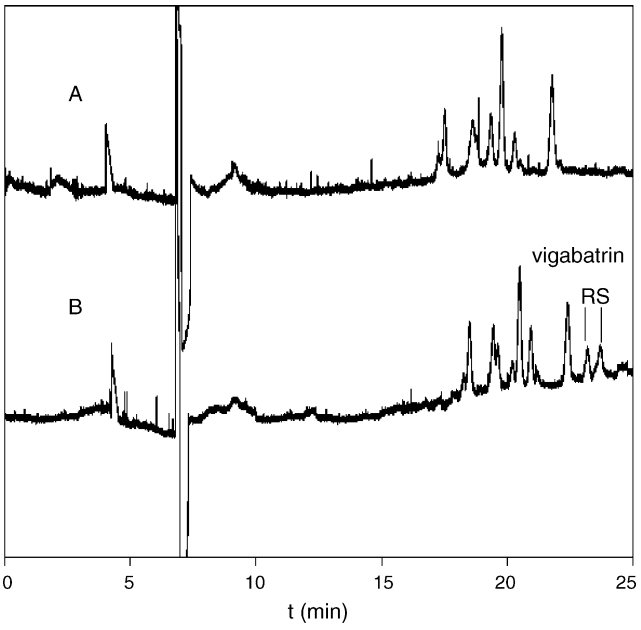

Fig. 5. Electropherogram of human plasma samples: (A) plasma blank; (B) plasma spiked with 0.3 μg/ml vigabatrin enantiomers. CE conditions as in Fig. 4.

Table 1
Precision and recovery of vigabatrin enantiomers ($n = 4$)

Concentration added (μg/ml)	Found[a] (μg/ml)		Precision (R.S.D., %)		Recovery (%)	
	R	*S*	*R*	*S*	*R*	*S*
0.3	0.31	0.32	5.3	7.0	103	107
0.6	0.60	0.65	5.1	3.2	100	108
0.9	0.95	0.91	4.7	3.3	106	101
1.5	1.51	1.55	2.9	4.6	100	103
3.0	2.96	2.90	0.6	3.1	98.7	96.7

[a] Average of four determinations.

1000 mg *RS*-vigabatrin. For a healthy volunteer, the concentrations of vigabatrin in plasma are in the range of 1–24 μg/ml within 0.5–24 h after a single 1000 mg vigabatrin oral dose. In our experiments, the concentrations of vigabatrin enantiomers spiked in plasma samples are in the range of 0.3–3.0 μg/ml. If the concentration is higher than 3.0 μg/ml, it can also be analyzed by diluting the sample solution. Therefore, the presented method fulfilled completely the requirements for the pharmacokinetic study of vigabatrin enantiomers and is suitable for routine therapeutic drug monitoring.

4. Conclusion

A simple and reliable CE method with UV–vis detection is presented for the determination vigabatrin enantiomers. DHAIC, a novel chiral derivatizing reagent, was used for precolumn derivatization of vigabatrin. The enantiomeric separation was completed in an achiral running buffer within 23 min. To the best of our knowledge, this is the first report on a CE method that was utilized for the separation and determination of vigabatrin enantiomers. And its successful application to the determination of vigabatrin enantiomers spiked in human plasma has been demonstrated.

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