Journal of Chromatography B, 867 (2008) 32–36

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simultaneous analysis of 1,2,3,4-tetrahydroisoquinolines by high-performance liquid chromatography using 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride as a fluorescent labeling reagent

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ARTICLE INFO

Article history:
Received 5 November 2007
Accepted 3 March 2008
Available online 8 March 2008

Keywords:
HPLC
Fluorometric detection
4-(5,6-Dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride
1,2,3,4-Tetrahydroisoquinolines
Brain

ABSTRACT

A highly sensitive high-performance liquid chromatographic method for the simultaneous analysis of 1,2,3,4-tetrahydroisoquinolines (TIQs) in the rat brain was developed. 1,2,3,4-Tetrahydroisoquinoline (TIQ), 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BeTIQ) were derivatized with 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride to produce fluorescent sulfonamides. The labeling reaction was carried out at 50 °C for 15 min at pH 8.5. The fluorescent derivatives were separated on a reversed-phase column by gradient elution using (A) water–(B) acetonitrile/methanol (55:45) at 55 °C and detected by fluorescence measurement at 318 nm (excitation) and 398 nm (emission). The detection limits (signal-to-noise ratio = 3) were 8–9 fmol per injection. The relative standard deviations ($n = 6$) of TIQs were 2.6–10.5% and the recoveries were 87.6, 101.8 and 75.2%, respectively. The concentrations of TIQ, 1-MeTIQ and 1-BeTIQ in normal rat brains ($n = 6$) were 0.7 ± 0.3 (0.10 ± 0.04), 3.4 ± 1.5 (0.50 ± 0.22) and 1.3 ± 1.8 pmol/g (0.30 ± 0.41 ng/g), respectively.

1. Introduction

Parkinson's disease is characterized by a degeneration of neurons in the zona compacta of the substantia nigra and a decrease in the dopamine content in the caudate nucleus and putamen, although the cause of nigral degeneration is still unknown. Since 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to induce Parkinsonism in humans, monkeys and mice [1–3], various structurally MPTP-like endogenous amines have been screened for neurotoxicity, and 1,2,3,4-tetrahydroisoquinoline (TIQ) was assumed to be one of the endogenous and environmental substances which induce Parkinsonism [4]. The 1,2,3,4-tetrahydroisoquinolines (TIQs) such as TIQ, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BeTIQ) were found in the brains of several mammalian species and their concentrations in the brain were related to Parkinson's disease [5–14]. The concentration of 1-MeTIQ decreases significantly in the brains of patients with Parkinson's disease [10] and the concentration of 1-BeTIQ tends to increase in the cerebrospinal fluid of patients with Parkinson's disease [11], while there is no significant difference in the concentration of TIQ in the brains of normal subjects and patients with Parkinson's disease [9]. Furthermore, although TIQ and 1-BeTIQ, as well as MPTP, induced Parkinsonism in rats [12,13], pretreatment with 1-MeTIQ prevented MPTP- and TIQ-inducing Parkinsonism [14]. Therefore, to understand the relationship among TIQs and Parkinson's disease, it is required that TIQs in the brain are simultaneously determined with highly sensitive detection.

At present, some methods for the determination of TIQ, 1-MeTIQ and 1-BeTIQ in the brain including gas chromatography–mass spectrometry (GC–MS) [5,7,11,15], high-performance liquid chromatography (HPLC) [16] and radioimmunoassay [17] have been reported. Although the radioimmunoassay method is highly sensitive in comparison with the GC–MS method, it is non-specific because of cross-reactivity. In the GC–MS and HPLC methods, although TIQs extracted from the homogenized brain with an organic solvent were reacted with a derivatization reagent and then analyzed, they were not determined simultaneously.

We previously developed an extremely sensitive fluorescent derivatization reagent, 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride (DMS-Cl), which reacts quantitatively with amino acids and amines to form corresponding stable and highly fluorescent sulfonamides [18–20]. In this paper, a highly sensitive HPLC method for the simultaneous determination of TIQ, 1-MeTIQ and 1-BeTIQ in the rat brain with fluorescence detection after pre-column derivatization with DMS-Cl is described.

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doi:10.1016/j.jchromb.2008.03.002

2. Experimental

2.1. Chemicals and solvents

All chemicals were of analytical-reagent grade, unless stated otherwise. The labeling reagent, DMS-Cl, was prepared as described in a previous paper [18]. 1,2,3,4-Tetrahydroisoquinoline hydrochloride was purchased from Aldrich (Milwaukee, WI 53233, USA). 1-Methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride and 1-benzyl-1,2,3,4-tetrahydroisoquinoline hydrochloride were synthesized by the method of Kotake et al. [11] and the structures of the isolated 1-MeTIQ and 1-BeTIQ were confirmed by MS and ^{1}H NMR spectra. *N*-ethylbenzylamine as an internal standard (IS) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetonitrile and methanol (HPLC grade) and *o*-phthalaldehyde (OPA) were obtained from Wako (Osaka, Japan). Deionized-distilled water was purified with the Milli-QII system (Yamato, Tokyo, Japan) prior to use.

2.2. Washing of glass vials

The glass vials were reused after the treatment described below. The glass vials used were soaked overnight in nitric acid (40%) after washing with a cleaner, and then rinsed well with water. The dried glass vials were silanized with dimethyldichlorosilane, followed by successive rinsing with absolute toluene and methanol prior to each use.

2.3. Preparation of the derivative of TIQ with DMS-Cl (DMS-TIQ)

A mixture of TIQ hydrochloride (51.2 mg, in borate buffer (0.2 M, pH 9.0) 10 ml) and DMS-Cl (100 mg, in acetonitrile 40 ml) was allowed to stand for 15 min at 50 °C. The reaction mixture was evaporated to remove acetonitrile under reduced pressure, and the precipitates of DMS-TIQ were filtered off. DMS-TIQ was recrystallized from methanol (white needle, yield 79.5 mg).

2.4. Instrumental conditions

The fluorescence intensities were monitored at excitation and emission wavelengths of 318 nm and 398 nm, respectively. Uncorrected fluorescence spectra of DMS-TIQ were measured with a Shimadzu RF-530 spectrofluorometer (Shimadzu, Kyoto, Japan) using a quartz cell (optical path length, 10 mm). An Agilent 1100 Series HPLC system (Agilent, California, USA) consisted of a binary pump, a micro vacuum degasser, a micro autosampler, a thermostatted column compartment, a programmable 3D fluorescence detector, a control module and a chemistation were used. A TSK gel ODS-80Ts (150 mm × 4.6 mm, i.d., 5 μm, Tosoh, Tokyo, Japan) connected to a TSK guard-gel ODS-80Ts (15 mm × 3.2 mm, i.d., 5 μm, Tosoh) as a guard column was employed at 55 °C with an elution program using (A) water–(B) acetonitrile/methanol = (55:45). The elution program consisted of an isocratic elution of 55% B for 15 min, followed by a linear gradient elution from 55 to 70% of B for 30 min, then a stepwise increase to 100% of B to wash the column for 10 min, and finally a stepwise decrease to 55% of B to re-equilibrate the column for 10 min. The flow rate was 1 ml/min.

2.5. Derivatization procedure

To a test solution (50 μl, in acetonitrile) of TIQ, 1-MeTIQ, 1-BeTIQ and IS placed in a screw-capped glass vial, borate buffer (0.1 M, pH 8.5, 50 μl) and DMS-Cl (7.5 mM, in acetonitrile, 50 μl) were successively added and mixed. The mixture was allowed to stand for 15 min at 50 °C. After cooling, the reaction mixture was mixed with L-proline (0.1 M, 50 μl). An aliquot (20 μl) of the mixture was subjected to HPLC.

2.6. Analytical procedure for the rat brain

A rat brain sample was prepared according to the method of Kohno et al. [5] with some modifications. Each brain (1.36–1.93 g) was homogenized in 10 volumes (v/w) of perchloric acid (0.4 M) solution containing disodium ethylenediaminetetraacetic acid (0.1%) and ascorbic acid (0.1%). After centrifugation (14,000 × *g*, 15 min), the supernatant was collected. The residue was treated again as mentioned above. The portion of the combined supernatant (20 ml) spiked with IS (50 pmol, 50 μl) was washed with ether (10 ml). The aqueous layer was adjusted to pH 11 with sodium hydroxide (5 M) and extracted with dichloromethane (2 ml, twice). The combined organic layer was dried over anhydrous sodium sulfate and then evaporated to dryness after addition of HCl (0.5 M, in methanol, 50 μl). To the residue, OPA (4%, w/v, in acetonitrile, 50 μl) and borate buffer (0.1 M, pH 8.5, 50 μl) were added. After standing for more than 3 min at room temperature, a derivatization reaction with DMS-Cl was carried out. The reaction mixture was centrifuged (4000 × *g*, 3 min) and an aliquot of the supernatant (20 μl) was subjected to HPLC.

2.7. Preparation of the rat brains

After anesthetization with ether, each male Wistar rat (10 weeks or older) was decapitated. The brain was removed immediately under ice cooling and then stored at −20 °C until assay. The brain from a rat treated with standard of TIQ, 1-MeTIQ and 1-BeTIQ was also obtained. The standard TIQs (1 μmol each) in physiological saline (1 ml) were administered intraperitoneally to a male Wistar rat. After 24 h, the rat was killed and the brain was removed immediately.

3. Results and discussion

3.1. Structure and fluorescence spectra of DMS-TIQ

Primary and secondary amino compounds reacted with DMS-Cl in a basic medium to give the corresponding fluorescent sulfonamides. The structure of DMS-TIQ confirmed by MS and ^{1}H NMR spectral data are shown in Fig. 1. The analytical data of DMS-TIQ is as follows: m.p. 238.0–239.5 °C. MS (*m*/*z*): 494 ([M]$^+$). ^{1}H NMR (*δ*, ppm, in $CDCl_3$): 2.77 and 3.61 (2H each, t each, H of CH_2–CH_2, *J* = 5.9 Hz each), 3.75, 3.96 and 3.99 (3H each, s each, –OCH_3), 4.53 (2H, s, H of CH_2 of tetrehydroisoquinoline), 4.80 (2H, s, CH_2 of phthalimidine), 6.98 (1H, d–d, H of benzene ring, *J* = 2.2 and 8.8), 6.98 and 7.32 (1H each, s each, aromatic-H of phthalimidine), 7.03–7.20 (4H, m, aromatic-H of tetrehydroisoquinoline), 7.99 (1H, d, H of benzene ring, *J* = 8.8 Hz), 8.26 (1H, d, H of benzene ring, *J* = 2.20 Hz).

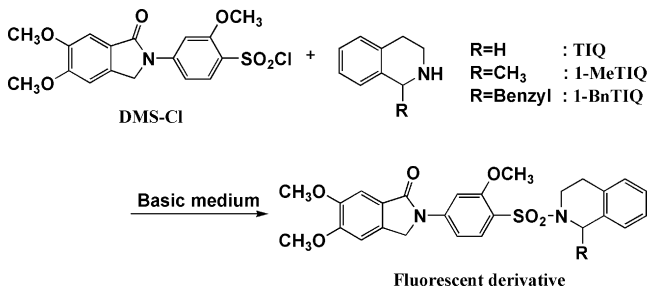

Fig. 1. Labeling reaction of 1,2,3,4-tetrahydroisoquinolines with DMS-Cl.

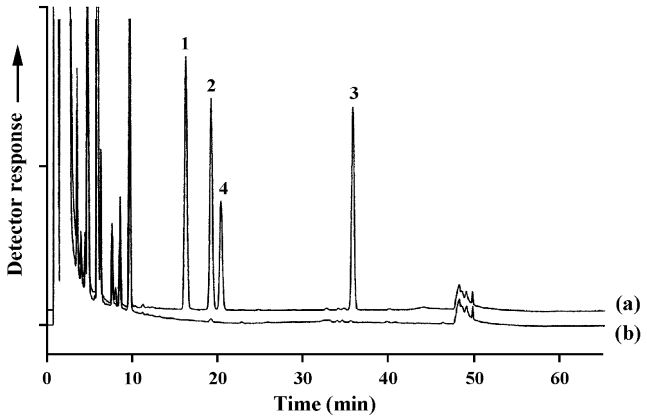

Fig. 2. Chromatograms obtained from (a) standard solution of TIQs and IS (50 pmol each) labeled with DMS-Cl and (b) reagent blank according to the derivatization procedure described in Section 2.5. HPLC conditions as in Section 2.4. Peaks: 1, TIQ; 2, 1-MeTIQ; 3, 1-BeTIQ; 4, IS.

The fluorescence spectra of DMS-TIQ in water, aqueous acetonitrile (25, 50 and 75%) and acetonitrile were measured. Sample solutions were prepared by the dilution of dimethylsulfoxide solutions of the derivatives (5 mM) with acetonitrile to 100 μM and then successively with the measuring solvents to 0.05 μM. The excitation and emission maximum wavelengths of DMS-TIQ were 318 nm and 398 nm, respectively, in aqueous acetonitrile and acetonitrile, and were changed little in water (Ex 322 nm and Em 395 nm). The fluorescence intensities in 25–50% aqueous acetonitrile showed a maximum and were about 11.6 and 2.6 times more intense than those in water and acetonitrile, respectively. The effect of pH on the fluorescence spectra of the DMS-TIQ was also examined by measurement in a 50% (v/v) acetonitrile solution of phosphoric acid (10 mM, pH 2.3), phosphate buffer (10 mM, pH 5.0–8.0) and trisodium phosphate (10 mM, pH 11.8). The fluorescence wavelengths and intensity of DMS-TIQ were not influenced by the pH.

3.2. HPLC chromatogram

The derivatives of TIQs and IS labeled with DMS-Cl were successfully separated on a reversed-phase column. Typical chromatograms obtained from a standard solution (50 pmol each) and a reagent blank according to the derivatization procedure described in Section 2.5 are shown in Fig. 2. The peaks due to TIQ, 1-MeTIQ, 1-BeTIQ and IS were successfully separated from the peaks due to the reagent blank and eluted at 16.1, 19.1, 35.6 and 20.2 min, respectively. The fluorescence was monitored at the excitation and emission maximum wavelengths of the prepared DMS-TIQ (318 nm and 398 nm, respectively).

3.3. Reaction conditions

A standard solution of TIQ, 1-MeTIQ and 1-BeTIQ (250 pmol each, in acetonitrile, 50 μl) was used to determine the optimum labeling conditions. The labeling reaction of TIQs with DMS-Cl proceeded in a basic medium. When the effect of pH was examined in the range of pH 7–10 using a borate buffer (0.1 M), the pH did not practically affect the labeling reactions. Therefore, borate buffer (pH 8.5) was adopted for the labeling reaction. The reaction mixture was stable for at least 24 h at room temperature.

The effect of reaction time on the labeling reaction was tested at 25, 50 and 70 °C. The labeling reaction of TIQs with DMS-Cl proceeded more quickly at high temperature. The maximum peak area was obtained from the reaction after 15 min at 50 °C and 5 min at 70 °C. Therefore, the labeling reaction carried out at 50 °C for 15 min.

The optimum concentration of DMS-Cl in acetonitrile was determined. The most intense and constant peak areas were obtained when the concentration of the reagent solution was more than 5 mM.

After the labeling reaction, proline was added to convert the excess DMS-Cl to the proline-derivative because excess DMS-Cl caused the guard column to degrade, the peaks to be broadened and the retention times to be delayed after several injections. Incidentally, the reaction took place at room temperature within 2 min and the derivative of proline was eluted after about 2 min.

The efficiency of the conversion of TIQ into the fluorescent derivative was examined by comparing the peak area under the reaction conditions with that of the prepared DMS-TIQ. The extent of the conversion was about 97.0 ± 3.9% (n = 3).

3.4. Washing of glass vials

Glass vials were soaked in nitric acid before treatment with dimethyldichlorosilane. When a glass vial was reused without soaking in nitric acid, the peaks corresponding to TIQ, 1-MeTIQ and 1-BeTIQ were observed in the blank. However, by using glass vials soaked over night in nitric acid and silanized with dimethyldichlorosilane, the peaks corresponding to TIQs in the blank chromatogram became negligibly small.

3.5. Linearity and detection limit

Linearity was studied over wide ranges of concentration (between 0.5 and 500 pmol) of TIQs. The peak-area ratios of analytes to IS were linear in the concentration ranges investigated (r > 0.998 each). When the precision was tested using standard solutions (5 μM each), the relative standard deviations (R.S.D., n = 10) were less than 2%. The detection limits (signal-to-noise ratio = 3) for TIQ, 1-MeTIQ and 1-BeTIQ were 8, 9 and 9 fmol/injection, respectively.

3.6. HPLC chromatograms obtained from rat brains

Typical chromatograms obtained from standards TIQs (50 pmol/g each), blank and normal rat brains unspiked and spiked with standards TIQs (50 pmol/g each) according to the analytical procedure for the rat brain described in Section 2.6 are shown in Fig. 3. The peaks due to TIQs in the rat brain were identified by comparing the retention times with those of the standard solution and by co-chromatography of the standard TIQs and the brain sample.

3.7. Selection of IS

Some secondary amines (50 pmol each) were examined to select an IS. The retention times and detection response of secondary amines labeled with DMS-Cl are shown in Table 1.

The reaction mixtures of 1-phenylpiperazine and 1-(2-chlorophenyl)piperazine were not subjected to HPLC because a precipitation appeared in those reaction mixtures. No peak due to *N*-isopropylcyclohexcylamine was detected, and the peaks due to 1,2,3,4-tetrahydro-9-acridanone, *N*-isopropylbenzyl amine, *N*-methylcyclohexcylamine and *N*-ethylcyclohexcylamine were small (about one sixth in comparison with *N*-ethylbenzylamine). The peaks due to *N*-methylbenzylamine, 4-benzylpiperizine, 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine, dibenzylamine, *N*-*n*-buthylbenzylamine, *cis*-decahydroisoquinoline, *trans*-decahydroisoquinoline, *N*-benzyl-2-phenylethylamine and *N*-propylbutylamine partially overlapped with those of TIQs, the reagent blank or other biogenic components. The peak due to 1-benzylpiperazine eluted at about 21 min, but its retention time was shortened by repeated runs. The peak due to *N*-ethylbenzylamine

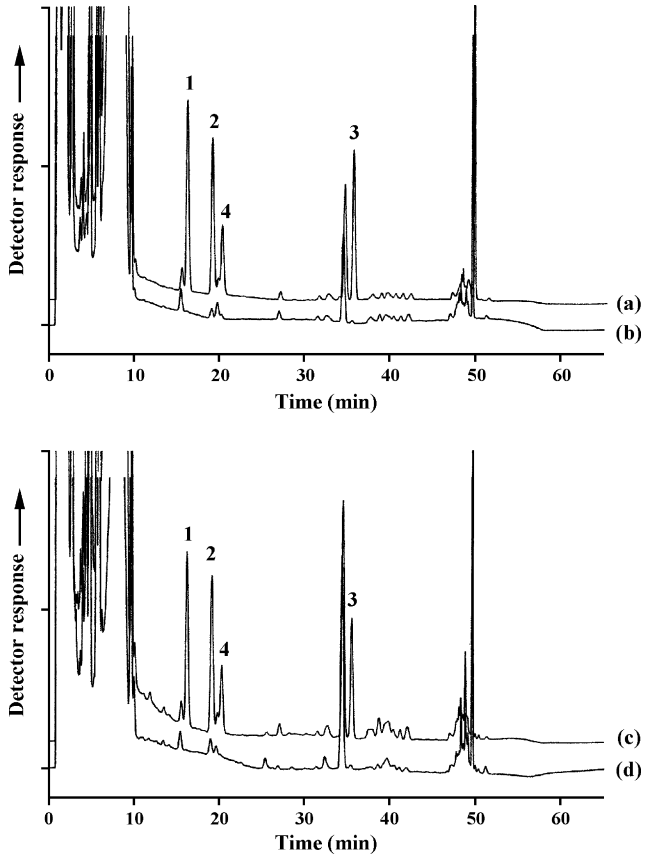

Fig. 3. Chromatograms obtained from (a) perchloric acid solution spiked with standard TIQs (50 pmol/g each) without rat brain, (b) perchloric acid solution without rat brain (blank), (c) supernatant of homogenate spiked with standard TIQs (50 pmol/g each) and (d) supernatant of homogenate according to the analytical procedure for the rat brain described in Section 2.6. HPLC conditions as in Section 2.4. Peaks: 1, TIQ; 2, 1-MeTIQ; 3, 1-BeTIQ; 4, IS.

was successfully separated from those of TIQs and other biogenic components. Although the peak due to *N*-ethylbenzylamine overlapped with a part of the small peak due to the blank (Fig. 3), *N*-ethylbenzylamine was used as IS in this study.

3.8. Extraction with and evaporation of dichloromethane

Extraction of TIQs was examined by use of chloroform and dichloromethane. The recoveries of TIQ, 1-MeTIQ, 1-BeTIQ and IS by extraction with dichloromethane were 93.1, 86.1, 77.1 and 71.1%, respectively. Similar results were obtained with chloroform, except for the low recovery of TIQ (24%). Therefore, dichloromethane was used in this study. When an aliquot (4 ml) of the dichloromethane layer without the addition of HCl (0.5 M, in methanol, 50 μl) was evaporated with a stream of nitrogen, the peak areas of TIQs decreased to about 60%. The presence of HCl prevented TIQs from being lost during evaporation.

3.9. OPA treatment

It is known that primary amino compounds such as amino acids in samples are effectively eliminated from the derivatization reaction with the labeling reagents by treatment with OPA [18,19]. Actually, when the labeling reaction was carried out without OPA treatment, a complicated chromatogram was obtained, as shown in Fig. 4, and the measurement of TIQs was disturbed. As shown in Fig. 3(c), the peaks due to TIQs and IS are able to be measured by employing the OPA treatment, so the OPA treatment of brain samples was adopted prior to the labeling reaction of TIQs with DMS-Cl. Incidentally, OPA treatment did not affect the labeling reaction of TIQs.

Table 1
Retention time and detector response of secondary amino compounds as IS

Compound	Retention time (min)	Detector response[a]
1-Benzylpiperazine	19.8–21.3[b]	49.2
4-Benzylpiperizine	32.4	120.5
1-Phenylpiperazine	–[c]	–[c]
1,2,3,4-Tetrahydro-9-acridanone	15.4	7.3
1-(2-Chlorophenyl)piperazine	–[c]	–[c]
4-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridine	33.2	144.5
(*S*)-(−)-1,2,3,4-Tetrahydro-3-isoquinoline	15.4	7.5
Dibenzylamine	34.1	99.4
N-methylbenzylamine	15.4	149.2
N-ethylbenzylamine	20.2	100.0
N-isopropylbenzyl amine	25.0	13.1
N-*n*-buthylbenzylamine	33.8	121.5
cis-Decahydroisoquinoline	31.5	100.6
trans-Decahydroisoquinoline	33.0	126.6
N-methylcyclohexcylamine	18.3	8.5
N-ethylcyclohexcylamine	26.3	14.6
N-isopropylcyclhexcylamine	n.d.[d]	n.d.[d]
N-propylbutylamine	26.9	82.6
N-benzyl-2-phenylethylamine	37.8	138.0

[a] The peak area of *N*-ethylbenzylamine labeled with DMS-Cl was taken as 100.
[b] The retention time was varied.
[c] Not subjected to HPLC because of generation of precipitates.
[d] n.d. = not detected.

3.10. Recovery and precision

The recovery test was examined using the supernatant of homogenate (20 ml) spiked with various amounts of standard TIQs (10, 25 and 50 pmol each). The relationships between the peak-area ratios of the analytes to IS and the concentrations of the analytes

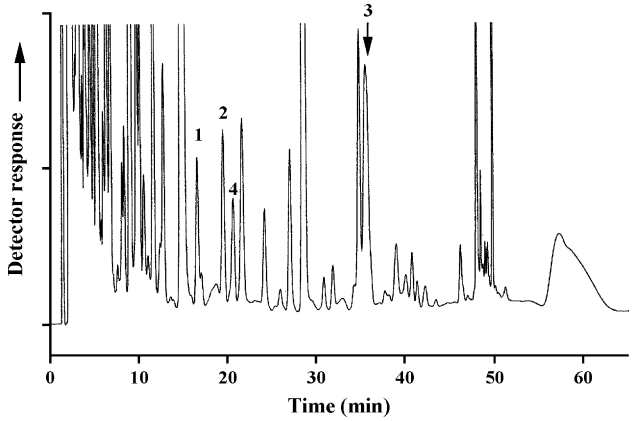

Fig. 4. Chromatogram obtained from the supernatant of a homogenate spiked with standard TIQs (50 pmo/g each) according to the procedure without OPA treatment described in Section 2.6. HPLC conditions as in Section 2.4. Peaks: 1, TIQ; 2, 1-MeTIQ; 3, 1-BeTIQ; 4, IS.

Table 2
Linear regression analysis and recoveries of TIQs

	Regression equations of standard[a]		Recovery (%)[b]
	With rat brain	Without rat brain	
TIQ	$y = 0.0367x + 0.0004$ ($r = 0.998$)	$y = 0.0419x + 0.0006$ ($r = 0.999$)	87.6
1-MeTIQ	$y = 0.0400x + 0.1338$ ($r = 0.995$)	$y = 0.0393x + 0.0539$ ($r = 0.999$)	101.8
1-BeTIQ	$y = 0.0228x + 0.1094$ ($r = 0.985$)	$y = 0.0303x + 0.0377$ ($r = 0.999$)	75.2

[a] x, amounts of TIQs (pmol/g, range: 10–50); y, peak-area ratios of TIQs to IS.
[b] The recovery was obtained from the slope ratio of a regression equation of the analyte with the brain to that without the brain.

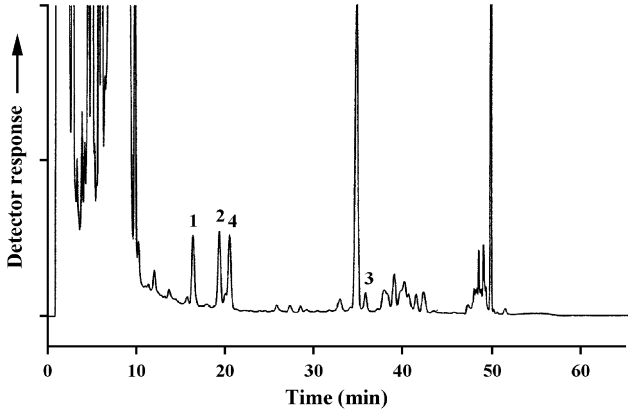

Fig. 5. Chromatogram obtained from the brain of a rat treated with intraperitoneal administration of standard TIQs (1 nmol) according to the procedure described in Section 2.6. HPLC conditions as in Section 2.4. Peaks: 1, TIQ; 2, 1-MeTIQ; 3, 1-BeTIQ; 4, IS. Concentration: TIQ, 29.4 pmol/g; 1-MeTIQ, 28.0 pmol/g; 1-BeTIQ, 7.7 pmol/g.

were linear. The recoveries were obtained from the slope ratio of regression equations of TIQ, 1-MeTIQ and 1-BeTIQ with/without rat brains. As shown in Table 2, those were 87.6, 101.8 and 75.2%, respectively. As the recoveries of TIQ and 1-BeTIQ were low, the estimated values of TIQ and 1-BeTIQ were corrected using these recoveries.

The precisions were tested using perchloric acid solution and the supernatant of homogenate (20 ml) spiked with standard TIQs (50 pmol each) with six replicate assays in 1 day. The relative standard deviations (R.S.D.) of TIQ, 1-MeTIQ and 1-BeTIQ in perchloric acid were 2.6, 2.4 and 2.3%, respectively, and those in the supernatant of the homogenate were 3.0, 4.2 and 10.5%, respectively.

3.11. Determination of TIQ, 1-MeTIQ and 1-BeTIQ in rat brains

The concentrations of TIQs in brains from normal rats ($n = 6$) were determined by the present method. The mean values (mean ± S.D.) of TIQ, 1-MeTIQ and 1-BeTIQ were 0.7 ± 0.3, 3.4 ± 1.5 and 1.3 ± 1.8 pmol/g, respectively. These mean values of TIQ and 1-MeTIQ were lower than the values (TIQ for 5.4 ng/g (40.5 pmol/g) and 1-MeTIQ for 2.0 ng/g (14.1 pmol/g)) reported previously [5], while the concentration of 1-BeTIQ in the rat brain has not been reported.

We also tried to detect TIQ, 1-MeTIQ and 1-BeTIQ in the brain of a rat after treatment with intraperitoneal administration of standard TIQs (1 μmol each), since it was reported that TIQs pass through blood–brain barrier [21,22]. The chromatogram obtained from a rat treated with TIQs is shown in Fig. 5. The concentrations of TIQ, 1-MeTIQ and 1-BeTIQ in the brain were 29.4, 28.0 and 9.7 pmol/g, respectively. These results suggests that determination of TIQ, 1-MeTIQ and 1-BeTIQ in the brain can be achieved by the present method if TIQs are present in the brain at least at the pmol/g level.

4. Conclusion

We established an HPLC method for the simultaneous analysis of TIQ, 1-MeTIQ and 1-BeTIQ in the rat brain using a pre-column fluorescent labeling reagent. As the highly sensitive, reliable and simultaneous analysis of TIQs can provide useful information for clinical and biochemical investigations, the proposed method may be useful for understanding the relationships between TIQs and Parkinson's disease.

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