Research Paper

Studies on the analysis of 1,2,3,4-tetrahydroisoquinoline (TIQ) and 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) in biological samples using liquid chromatography-tandem mass spectrometry

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Abstract A specific and sensitive method for the analysis of 1,2,3,4-tetrahydroisoquinoline (TIQ) and 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) as endogenous amines obtained from biological samples is described. These compounds, processed by a combination of solvent and solid-phase extraction (SPE), have been analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The levels of TIQ and 1-MeTIQ in biological samples were determined with the aid of the deuterated internal standard (IS) 1-MeTIQ-d_4 using multiple reaction monitoring (MRM, product ions *m/z* 90.9 of *m/z* 133.8 for TIQ, *m/z* 130.8 of *m/z* 147.8 for 1-MeTIQ and *m/z* 133.8 of *m/z* 151.8 for 1-MeTIQ-d_4). The chromatographic separation was conducted on a reversed phase 5CN-MS column (150×2.0 mm, i.d.) using a mobile phase comprised of methanol and 5 mM ammonium formate (90 : 10, v/v) at a flow rate of 0.2 mL/min. The calibration curves for TIQ and 1-MeTIQ using 1-MeTIQ-d_4 were linear (r^2>0.99) in the selected concentration range for each compound. The lower limits of detection of each compound were 0.10 ng/mL for TIQ and 0.01 ng/mL for 1-MeTIQ. The good recoveries for TIQ (>93.2%) and 1-MeTIQ (>94.1%) in this assay precluded the need to concentrate samples prior to analysis. TIQ and 1-MeTIQ contents in mouse brains following intraperitoneal administration of haloperidol (HP) were measured, and TIQ content did not differ significantly from those in control group, but 1-MeTIQ content decreased significantly. This result agrees well previous findings in human parkinsonism and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse brain.

Key words: TIQ and 1-MeTIQ in biological samples, Haloperidol (HP), Deuterated internal standard, 1-MeTIQ-d_4, Solid-phase Extraction (SPE), LC-MS/MS

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Introduction

The endogenous amines 1,2,3,4-tetrahydroisoquinoline [TIQ (**1**)], 1-methyl-1,2,3,4-tetrahydroisoquinoline [1-MeTIQ (**2**)] and 1-benzyl-1,2,3,4-tetrahydroisoquinoline [1-BnTIQ (**3**)] are structurally related to the Parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**4**)]. These compounds have been detected in the brains of rodents and humans[1−5] and could be related to the pathogenesis of Parkinson's disease (PD). TIQ and 1-BnTIQ have been reported to be neurotoxic[6−14] in rodent and primate

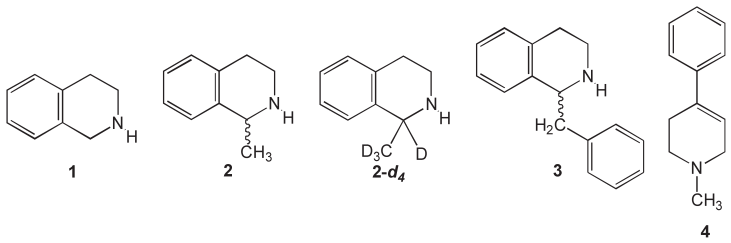


Fig. 1. Chemical structures discussed in the text.
1=TIQ, 2=1-MeTIQ, 2-d_4=1-MeTIQ-d_4 (I.S.), 3=1-BnTIQ, 4=MPTP

models of PD. Although the TIQ levels in the brains of individuals with PD were not significantly different from those in control brains, the levels of 1-MeTIQ were significantly lower in PD brains than in control brains[3]. Furthermore, the 1-MeTIQ level tended to decrease with age[15]. In contrast, the 1-BnTIQ levels tend to increase in the cerebrospinal fluid of PD patients[5]. Moreover, the 1-MeTIQ level was significantly lower in the brains of MPTP-[3,4] or haloperidol (HP, a neuroleptic agent with structural similarities to MPTP) -treated mice[16]. Interestingly, MPTP- or TIQ-induced bradykinesia was completely prevented by pretreatment with 1-MeTIQ[3]. Therefore, measurements of the levels of these endogenous amines in the brain could prove of value in understanding the pathogenesis of PD.

A highly specific and sensitive assay is required for the estimation of these compounds that are present in only trace levels in the brain. A gas chromatography-mass spectrometry (GC-MS) method has been reported for the detection of TIQ, 1-MeTIQ and 1-BnTIQ[1–5,16–18]. However, GC-MS analysis is complicated by the need to form derivatives with the required GC properties. In this paper, we report a method for the detection of TIQ and 1-MeTIQ using liquid chromatography-electrospray ionization/tandem mass spectrometry (LC-MS/MS) and a combination of solvent and solid-phase extraction (SPE). The solid-phase step has allowed us to avoid evaporation of the solvent that we know from earlier work[16] leads to significant losses of the analytes. This assay has been applied successfully to the determination of TIQ and 1-MeTIQ levels in the biological samples (brain and liver) obtained from rat and mice. In addition, we examined the change of TIQ and 1-MeTIQ levels in brain tissue of HP-treated mice with this assay.

Materials and Methods

Chemicals and reagents

TIQ hydrochloride was purchased from Wako Pure Chemical Industries (Osaka, Japan). 1-MeTIQ hydrochloride was offered by Dr. S Ohta (Graduate School of Biomedical Sciences, Hiroshima University, Japan). The hydrochloride salt of the IS 1-MeTIQ-d_4 (**2-d_4**) was synthesized in our laboratory by the procedures described previously[19,20]. The cartridge, OASIS® HLB (60 mg) for solid-phase extraction was purchased from Waters Co. (Milford, MA, USA). All other chemicals and reagents were of analytical purity from commercial sources.

Preparation of standard solutions

Calibration curves for TIQ and 1-MeTIQ were prepared as follows: Stock solutions (1 μg/mL) of each compound were prepared in methanol. Prior to analysis, the stock solution of 1-MeTIQ was diluted with 0.4 M perchloric acid (containing 0.1% w/v EDTA and 0.1% w/v ascorbic acid) to give final concentrations of 0.02, 0.1, 0.5, 1.0, 5.0 and 10.0 ng/mL in the spiked samples. EDTA and ascorbic acid were added to the 0.4 M perchloric acid solution in order to protect against auto oxidation of the test compounds during the extraction procedure as reported previously[2]. For TIQ the final concentrations were 0.2, 1.0, 2.0, 3.0, 6.0 and 10.0 ng/mL. The final volume in all cases was 3 mL. Each standard was subjected to the same 'work-up' procedure described below for the sample analyses.

Sample collection

Biological samples

All animal experiments were conducted in accordance with the Guideline for Animal Experimentation of Department of research development, Association of medicinal analysis. Male ddY mice weighing 28−30 g (6 weeks old) and male Wistar rats weighing 230−250 g (9 weeks old) (SLC, Shizuoka, Japan) were euthanized by cervical dislocation. The brain and liver were quickly removed. The brain and liver were weighed and added to the equivalent of two volumes of 0.4 M perchloric acid solution (containing 0.1% w/v EDTA and 0.1% w/v ascorbic acid). The tissues were homogenized,

and the homogenates were centrifuged at 12,000 g for 30 min at 4°C and then the supernatant was separated. All of these samples subsequently were processed as described below.

Moreover, male ddY mice weighing 28−30 g (6 weeks old) were injected with 4 mg/kg of haloperidol (HP, 20% v/v Tween 20 in saline solution) intraperitoneally for four days. The mice were euthanized by cervical dislocation at 24 h after the last injection and the brain was quickly removed. The brain was weighed and added to the equivalent of two volumes of 0.4 M perchloric acid solution (containing 0.1% w/v EDTA and 0.1% w/v ascorbic acid). The tissues were homogenized, and the homogenates were centrifuged at 12,000 g for 30 min at 4°C and then the supernatant was separated. The following operations were performed as described below.

Sample preparation for LC-MS/MS analyses

Solvent extraction of TIQ and 1-MeTIQ

To a sample (3 mL) of each preparation obtained as described above was added 1 mL of 28% v/v ammonium hydroxide solution and 30 μL of the IS solution (1-MeTIQ-d_4, 1 μg/mL in 0.1 M HCl). The resulting mixtures were extracted twice with 5 mL of dichloromethane. After the centrifugation, 8 mL of the organic phase was added to 8 mL of 0.4 M perchloric acid solution (containing 0.1% w/v EDTA and 0.1% w/v ascorbic acid). The mixture was shaken for five min and, after separation of the supernatant; the aqueous phase was processed by solid-phase extraction.

Solid-phase extraction (SPE)

SPE was conducted using Waters OASIS® HLB extraction cartridge (60 mg) that had been preconditioned with methanol (5 mL) followed by water (5 mL). A 6 mL aliquot of each sample to be analyzed was loaded onto an HLB extraction cartridge that then was washed with 6 mL of water. The analyte was subsequently eluted with 0.01% formic acid in methanol (2 mL). This solution then was transferred to an autosampler vial and 20 μL was injected into the LC-MS/MS system.

Instrumentation and chromatographic conditions

LC-MS/MS was performed using a triple quadrupole tandem mass spectrometer (LCMS-8030, Shimadzu Corp., Kyoto, Japan) equipped with electrospray ionization (ESI). The HPLC system consisted of a Shimadzu LC-30AD pump equipped with a Shimadzu Sil-30AC auto sampler. The reversed phase column used for chromatographic separation was COSMOSIL® 5CN-MS column (2.0 mm I.D., 150 mm length, Nacalai Tesque, Inc., Kyoto, Japan). The mobile phase [methanol and 5 mM ammonium formate (90 : 10, v/v)] was delivered in isocratic mode at a flow rate of 0.2 mL/min. Ionization conditions for LC-MS/MS were as follows: capillary voltage, 3.0 kV; source temperature, 150°C; desolvation temperature, 400°C; cone voltage, 15 V; collision energy, 30 eV for TIQ, 16 eV for 1-MeTIQ and IS. Argon was used as the collision gas.

Results and Discussion

ESI mass spectra

The ESI mass spectra of TIQ, 1-MeTIQ and deuterated 1-MeTIQ-d_4 (IS) were shown in Fig. 2. TIQ, 1-MeTIQ and IS showed the calculated MH^+ values at *m/z* 133.8, 147.8 and 151.8, respectively. These ions were the only dominant species present.

At the first, selected ion monitoring (SIM) using these ions was examined for quantitative analysis of TIQ and 1-MeTIQ. However, SIM of *m/z* 147.8 for 1-MeTIQ displayed some interfering background peaks. Consequently, we examined the technique of multiple reaction monitoring (MRM) using the characteristic product ions from the parent ions. Fig. 3 showed the product ion spectra (PIS) of TIQ (*m/z* 133.8), 1-MeTIQ (*m/z* 147.8) and 1-MeTIQ-d_4 (*m/z* 151.8). The PIS of 1-MeTIQ and 1-MeTIQ-d_4 at 16 V of collision energy gave the base peaks at *m/z* 130.8 and 133.8, respectively. The corresponding spectrum of TIQ, however, was more complicated with a number of relatively intense ions appearing in the spectrum including an ion at *m/z* 90.9, corresponding to a benzyl carbocation or tropylium ion.

Scheme 1 presents our interpretation of the fragmentation pattern observed in the PIS of 1-MeTIQ (**2**). The base peak in the spectrum appears at *m/z* 131. The proposed pathway leading to this ion proceeds via the *N*-protonated species $\mathbf{2H}^+$ (*m/z* 148) that fragments to the benzylic carbocation $\mathbf{i}^+$. Proton migration to the amino group leads to the *N*-protonated aminylethylstyryl species $\mathbf{ii}^+$ that undergoes a further transformation to the tetralinyl cation $\mathbf{iii}^+$ (*m/z* 131) with ammonia as the neutral loss species.

The corresponding PIS for 1-MeTIQ-d_4 (**2**-$\boldsymbol{d_4}$) are shown in Scheme 2. Consistent with the pathway proposed for 1-MeTIQ (**2**), the base peak in this spectrum appears at *m/z* 134. The sequences **2**-$\boldsymbol{d_4}$ → $\mathbf{2H}^+$-$\boldsymbol{d_4}$ (*m/z* 152) → **i**-$\boldsymbol{d_4}^+$ →

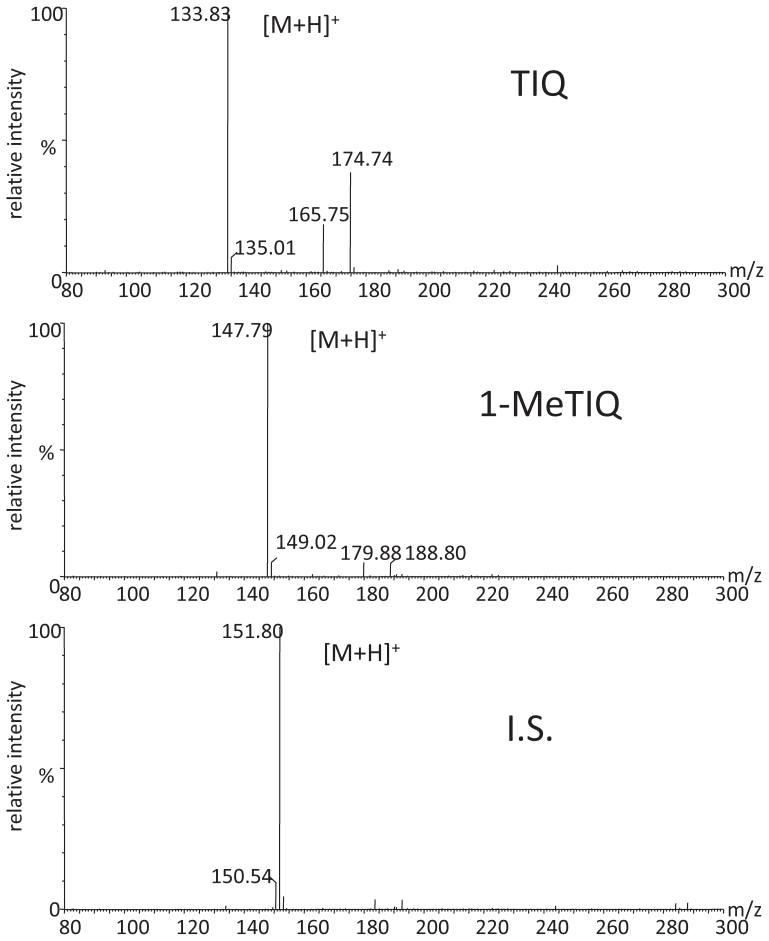


Fig. 2. LC-ESI mass spectra of TIQ, 1-MeTIQ and 1-MeTIQ-d_4 (I.S.).

ii$^+$-d_4 → **iii**$^+$-d_3 (*m/z* 134) are completely analogous to the corresponding sequences [**2** → **2H**$^+$ (*m/z 148*) → **i**$^+$ → **ii**$^+$ → **iii**$^+$ (*m/z* 131)] for 1-MeTIQ (**2**) shown in Scheme 1.

The PIS of TIQ (**1**) (Fig. 3, top panel) at collision dissociation energy of 30 V is more complicated than the PIS of 1-MeTIQ (**2**) and 1-Me-TIQ-d_4 (**2**-d_4) that were obtained at collision dissociation energy of 16 V. This probably is a consequence of the higher collision dissociation energy required to fragment TIQ (**1**). It may be reasonable to suspect that the absence of stabilization of the initially formed carbocation fragments (**ii**$^+$ and **ii**$^+$-d_4) by the 1-methyl group may account for the differences in energy requirement. Scheme 3 attempts to rationalize the fragmentation pathway of TIQ (**1**). As with the 1-methyl analogs, initial protonation occurs on nitrogen to give **1H**$^+$ (*m/z* 134). Fragmentation of **1H**$^+$ gives the unstable primary benzylic carbocation **iv**$^+$ that is converted to the more stable iminium ion **v**$^+$ via a hydride migration. Fragment ion **v**$^+$ rearranges to the cycloheptatrienyl species **vi**$^+$ that will lose the neutral vinylamine leading to the tropylium fragment **vii**$^+$ (*m/z* 91).

The ions that were selected for the MRM studies were the base peaks *m/z* 130.8 for 1-MeTIQ and *m/z* 133.8 for the I.S. In the case of TIQ, we elected to monitor *m/z* 90.9 since this intense ion appeared in a region of the spectrum that was relatively free of other fragment ions.

MRM chromatograms

Fig. 4 shows the MRM chromatograms of TIQ (0.20 ng/mL), 1-MeTIQ (0.02 ng/mL) and IS (5.0 ng/mL) of standard solutions and of extracts obtained from rat brain using these characteristic product ions. Similar chromatograms were obtained with the other matrices. The chromatograms did not display any interfering contaminants although the peaks observed in the MRM chromatograms did not achieve baseline resolution. This was not a problem since each analyte has a unique MH$^+$ value. It should be noted that the ion intensity at *m/z* 133.8 for the IS was below the limits of detection in all tissue extracts that had not been spiked with the IS.

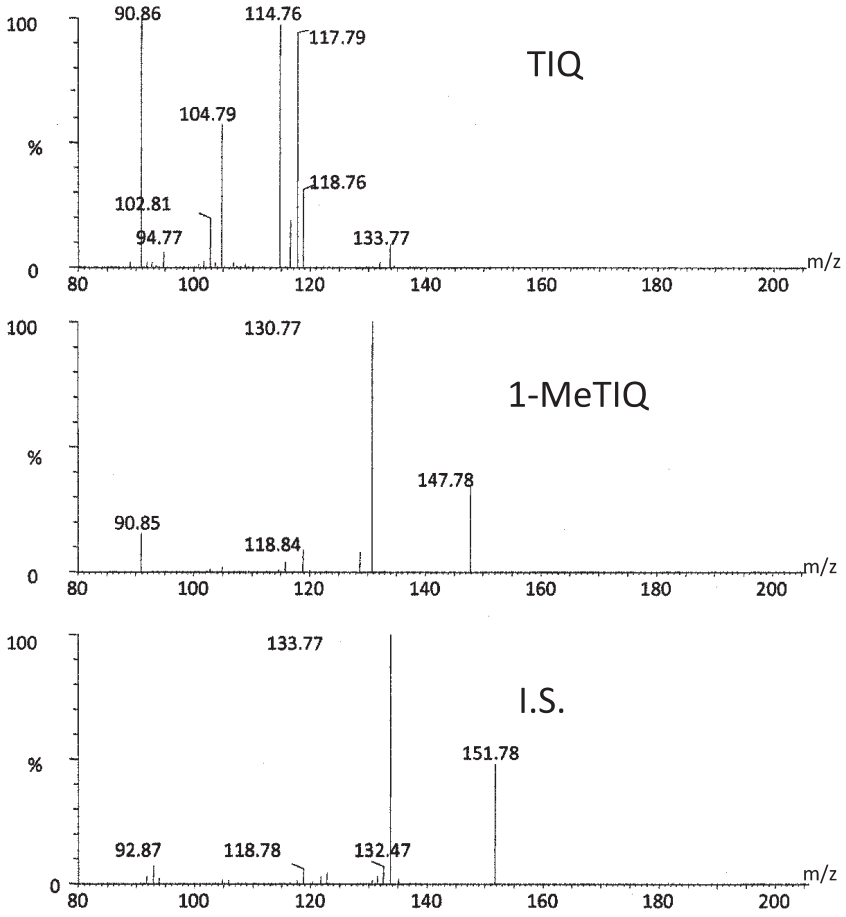
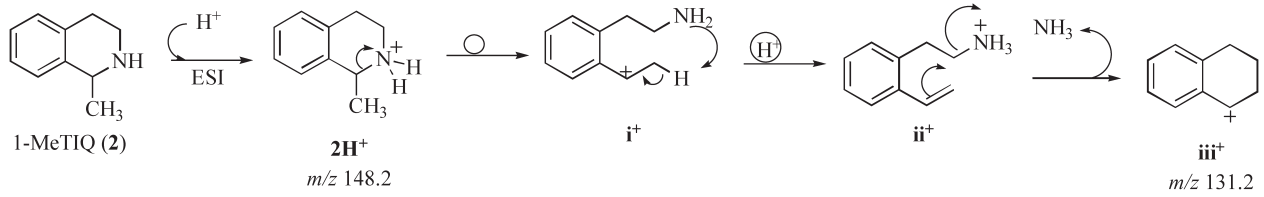
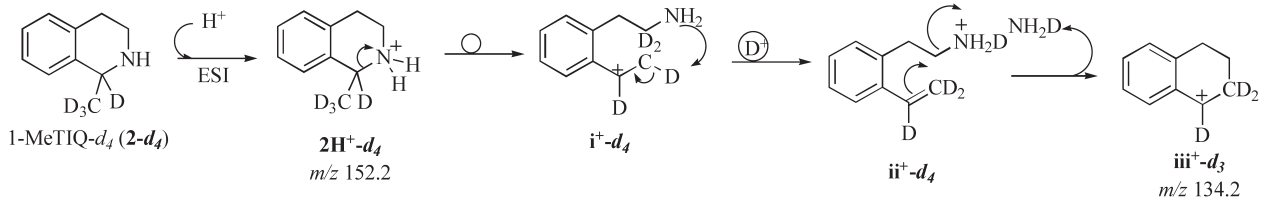


Fig. 3. PIS (LC-MS/MS) of TIQ, 1-MeTIQ and 1-MeTIQ-d_4 (I.S.).



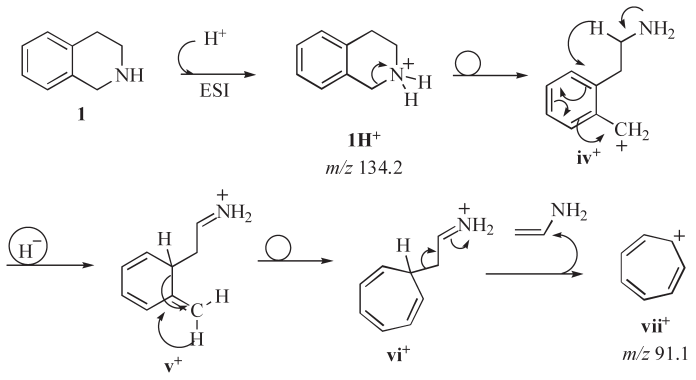
Scheme 1. Proposed fragmentation pathway for the PIS of 1-MeTIQ (2) observed at a collision energy of 16 V.



Scheme 2. Proposed PIS fragmentation pathway of 1-MeTIQ-d_4 (2-d_4) observed at a collision energy of 16 V.

Method validation

Calibration curves for TIQ (product ion *m/z* 90.9) and 1-MeTIQ (product ion *m/z* 130.8) were generated with standard solutions containing varying concentrations of both analytes. The data were obtained by plotting peak area ratios (analyte/IS) vs. the added amounts of TIQ and 1-MeTIQ to the samples. As a result, good linearity was observed over the concentration ranges examined (0.2−10.0 ng/mL, y=0.1598x+0.0733, r^2=0.9912 for TIQ and 0.02−10.0 ng/mL, y=0.1656x+0.0024, r^2=0.9998 for 1-MeTIQ). The calibration curves for each compound showed little intra-day and inter-day variability in slopes



Scheme 3. Proposed PIS fragmentation pathway of TIQ (1) observed at a collision energy of 30 V.

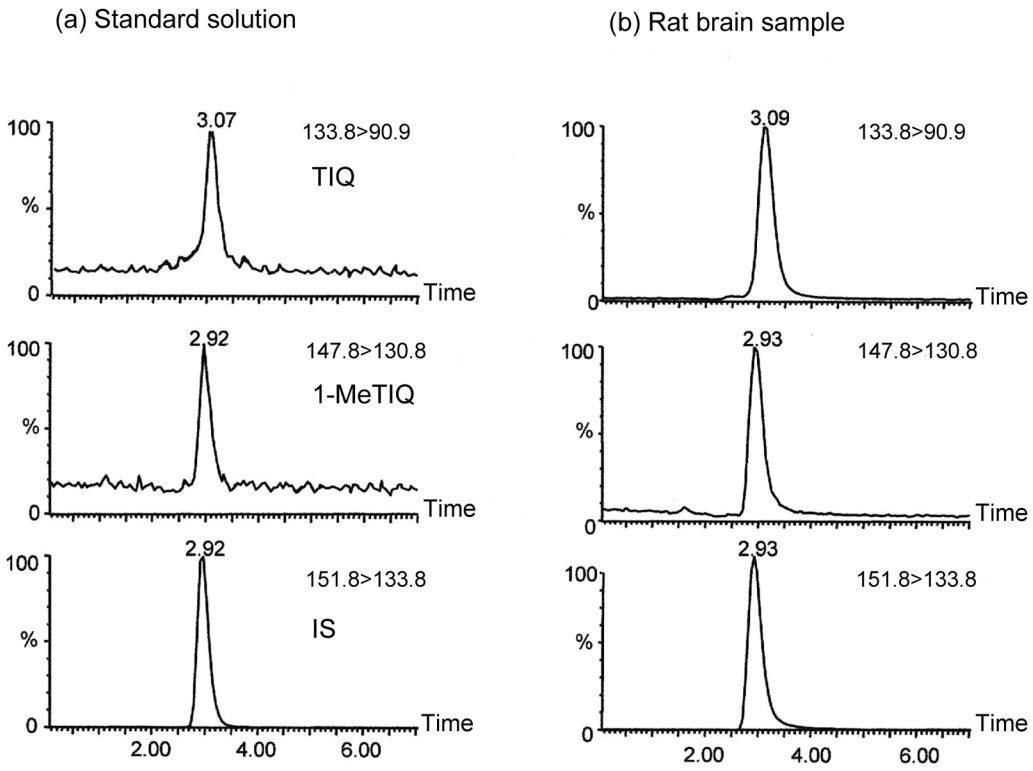


Fig. 4. Typical MRM chromatograms (LC-MS/MS) of (a) solutions of standards (0.2 ng/mL for TIQ, 0.02 ng/mL for 1-MeTIQ and 5.0 ng/mL for I.S.) and (b) rat brain sample (7.22 ng/g of tissue for TIQ and 2.15 ng/g of tissue for 1-MeTIQ).

and intercepts [coefficient of variation (C.V.), ＜6%, n=5]. The lower limits of detection were approximately 0.10 ng/mL for TIQ and 0.01 ng/mL for 1-MeTIQ (S/N=3).

The recovery experiments of TIQ and 1-MeTIQ were undertaken using the rat brain and liver homogenate samples. A constant amount of TIQ and 1-MeTIQ standard solutions were added to the centrifugation supernatants obtained from each tissue homogenate (the spiked concentrations of TIQ and 1-MeTIQ were 1.0 and 0.5 ng/mL, respectively), and the TIQ and 1-MeTIQ concentrations were determined in the same manner as described above. As shown in Table 1, the recoveries of TIQ at 1.0 ng/mL (n=5) in rat brain and liver homogenate samples were 92.1±5.6%, 93.2±6.0%, respectively. The recoveries of 1-MeTIQ at 0.5 ng/mL (n=5) in rat brain and liver homogenate samples were 96.3±8.1% and 94.1±8.8%, respectively.

From these results, it was suggested that the good recoveries for TIQ and 1-MeTIQ in this assay precluded the need to concentrate samples prior to analysis. This advantage avoided the sample loss (up to 40% for TIQ and 55% for 1-MeTIQ) that accompanied evaporation of the organic required in the GC-MS assay for these compounds[16].

Table 1. Recoveries (%) of TIQ and 1-MeTIQ from spiked samples of rat tissue homogenates

	Spiked (ng/mL)	Concentration (ng/mL)		Recovery (%)
		Intact*	Measured**	
Brain				
TIQ	1.00	1.29	2.21±0.16	92.1±5.6
1-MeTIQ	0.50	0.12	0.60±0.11	96.3±8.1
Liver				
TIQ	1.00	1.02	1.95±0.13	93.2±6.0
1-MeTIQ	0.50	0.09	0.56±0.12	94.1±8.8

*Mean measured concentration (n=5). **Mean values±S.D., n=5 (the number of animals per group). The homogenate supernatants were used as described in methods.

Table 2. Intra- and inter-day precisions of TIQ and 1-MeTIQ contents in rat tissues by LC-MS/MS assay

Compound		Conc. (ng/g of tissue)	Intra-day CV (%)	Inter-day CV (%)
TIQ	Brain	6.33	4.6	7.3
	Liver	6.91	3.8	7.8
1-MeTIQ	Brain	3.12	5.8	8.6
	Liver	2.64	6.1	9.1

The data showed the intra-day and inter-day variability of five measurements.

Moreover, we confirmed the intra-day and inter-day variability in TIQ and 1-MeTIQ levels in rat brain and rat liver samples (Table 2). The C.V. value in intra-day (n=5) for TIQ was about 4.6% in rat brain, 3.8% in rat liver, respectively. For 1-MeTIQ, the C.V. value (n=5) was about 5.8% in rat brain, 6.1% in rat liver, respectively. The C.V. value in inter-day (n=5) for TIQ was about 7.3% in rat brain, 7.8% in rat liver, respectively. For 1-MeTIQ, the C.V. value (n=5) was about 8.6% in rat brain, 9.1% in rat liver, respectively.

The levels of TIQ and 1-MeTIQ in biological samples obtained from rats and mice were shown in Table 3. TIQ and 1-MeTIQ levels in brain and liver obtained from rats ranged from 6.74 to 7.31 and 2.72 to 3.24 ng/g of tissue on average, respectively. TIQ and 1-MeTIQ levels in brain and liver obtained from mice ranged from 4.83 to 5.22 and 1.61 to 2.08 ng/g of tissue on average, respectively.

In addition, this method was applied to the determination of the endogenous amines, TIQ and 1-MeTIQ contents in the brain after drug administration. The levels of TIQ and 1-MeTIQ in haloperidol (HP)-treated mouse brain were shown in Fig. 5. Whereas the levels of TIQ and 1-MeTIQ in brain were 4.83±0.88 and 2.08±0.32 ng/g of tissue in control mice, respectively, those in HP-treated mice were 4.67±0.85 and 0.44±0.09 ng/g of brain tissue, respectively.

Table 3. The levels of TIQ and 1-MeTIQ in biological samples obtained from rats and mice

Sample	TIQ (ng/g of tissue)	1-MeTIQ (ng/g of tissue)
Rat Brain	6.74±0.91	3.24±0.63
Mouse Brain	4.83±0.85	2.08±0.32
Rat Liver	7.31±1.52	2.72±0.15
Mouse Liver	5.22±1.19	1.61±0.18

The data are mean values±S.D., n=10 (the number of animals per group).

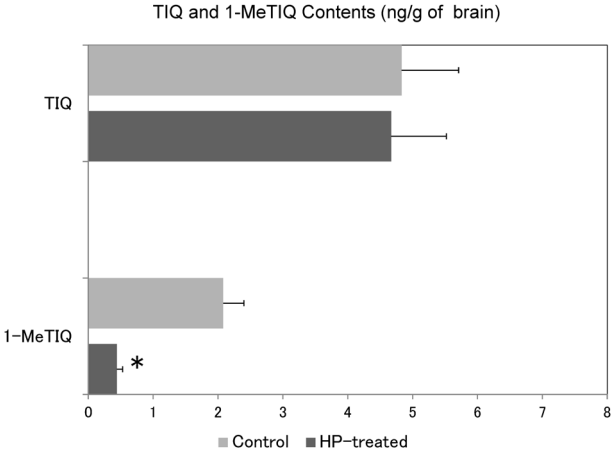


Fig. 5. TIQ and 1-MeTIQ contents in control and HP-treated mouse brains.
HP was dosed intraperitoneally at 4 mg/kg for 4 days.
*Significantly different from the corresponding control (P<0.01, Student's t-test).

A significant decrease in the endogenous amine, 1-MeTIQ level was observed in the HP-treated mouse brain in comparison with that in control brain. However, there was no difference in the endogenous TIQ level between control and HP-treated mice. This result agrees well with the previous finding of MPTP-treated mice[4]. We also have been very interested in these results because 1-MeTIQ content was reduced in parkinsonian brains[3]. As far as the structures of TIQ and 1-MeTIQ are concerned, the difference is whether or not they have a methyl group. TIQ has been reported to be neurotoxic[21,22], while 1-MeTIQ has the opposite effect and is reported to be neuroprotective[23–27]. It is possible that HP and MPTP may inhibit the enzymatic formation of 1-MeTIQ in brain from 2-phenylethylamine

and acetaldehyde. If this endogenous amine 1-MeTIQ plays an important role in the development of exogenous compound-induced parkinsonism, 1-MeTIQ may be a lead compound for anti-parkinsonism drugs.

Next, it will be interesting to know whether these endogenous amines, TIQ and 1-MeTIQ, can be used as biomarkers for examination and diagnosis of Parkinson's disease by knowing changes in the content of these endogenous amines in biological samples. In particular, TIQ and 1-MeTIQ have been detected not only in brain, liver, and kidney tissues but also in blood and urine samples[28]. Moreover, TIQ and 1-MeTIQ are known to be present naturally in plants and in a variety of food products[18,29–31]. We also measured the TIQ (209.5±8.4 ng/g) and 1-MeTIQ (58.4±0.9 ng/g) contents in Japanese tea, white wine and cocoa for reference. TIQ (209.5±8.4 ng/g) and 1-MeTIQ (58.4±0.9 ng/g) contents in Japanese tea were much higher than white wine (2.0±0.2 ng/g for TIQ, 0.2±0.03 ng/g for 1-MeTIQ) and cocoa (88.3±2.5 ng/g for TIQ, 5.75±0.50 ng/g for 1-MeTIQ) beverages. Considering that we drink Japanese tea regularly, it is thought that large amounts of TIQ and 1-MeTIQ are detected in body fluids. Therefore, it may be difficult to use the changes in TIQ or 1-MeTIQ contents in body fluid samples as diagnostic markers for Parkinson's disease. This marker seems to require further investigation.

Conclusions

The levels of the endogenous amines TIQ and 1-MeTIQ were determined by LC-ESI/MS/MS in biological samples by a combination of solvent and solid-phase extractions. TIQ and 1-MeTIQ in biological samples were quantified with good reproducibility by this method. Moreover, TIQ and 1-MeTIQ contents in the brain tissues of HP-treated mice were measured, and TIQ contents did not differ significantly from those in the control group, but 1-MeTIQ contents decreased significantly.

Acknowledgments

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Conflict of Interest

All authors declare that they have no conflict of interest.

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