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Simultaneous determination of tianeptine and its active metabolite tianeptine MC5 in rat plasma and brain tissue using high performance liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS)

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A selective and sensitive method was developed to simultaneously quantify tianeptine and its main active metabolite tianeptine MC5 in rat plasma and brain tissue. A one-step liquid–liquid extraction was developed for sample preparation. The linear range for plasma was from 1.0–500.0 ng mL^{-1} and for brain tissue it was from 1.0–500.0 ng g^{-1}. The method was validated in accordance with US FDA guidelines (precision within 15% relative standard deviation (RSD) and accuracy within 15% relative error (RE) with a consistent recovery). Stable isotope-labeled tianeptine and tianeptine MC5 (tianeptine-D4 and tianeptine MC5-D4) were used as the internal standards achieving good reproducibility while reducing the matrix effects. This method was successfully applied to a preclinical study for the simultaneous determination of tianeptine and tianeptine MC5 in rat plasma and brain tissue.

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1. Introduction

Tianeptine is an atypical tricyclic antidepressant drug possessing novel neurochemical properties. It acts by enhancing the reuptake of serotonin, in contrast to typical tricyclic antidepressants or selective serotonin reuptake inhibitors (SSRIs).[1] Tianeptine is as effective as other antidepressant agents, and yet, it exhibits less side effects and complications.[2] It can be used in the treatment of panic disorders due to its anti-anxiety properties.[3] Moreover, it possesses neuroprotective properties and has been shown to improve mental stability in patients with depression. Also it may prevent atrophy in the human hippocampus and dysfunction related to aging and Alzheimer's type dementia.[4,5]

Tianeptine is rapidly and completely absorbed when given orally and is extensively metabolized through β-oxidation of its heptanoic acid side chain to form tianeptine MC5, a pentanoic acid analogue (main active metabolite) (Fig. 1).[6] Several methods have been published for the determination of tianeptine and its active MC5-metabolite in biological fluids. High performance liquid chromatography with UV detection and ion-pair liquid–liquid extraction have been developed for the determination of tianeptine and its metabolites with a lower limit of quantification (LLOQ) of 5 ng mL^{-1} in plasma,[7] 10 ng mL^{-1} in plasma and urine and 10 ng g^{-1} in brain tissues.[8] An HPLC method with fluorescence detection has been reported for the determination of tianeptine in human plasma but it required compound derivatization and solid-phase extraction and the method sensitivity was LLOQ = 5 ng mL^{-1}.[9] Moreover HPLC-mass spectrometry has been applied for the determination of tianeptine in post-mortem samples (blood, urine, liver, and stomach contents) with an LLOQ of 10 ng mL^{-1} using liquid–liquid extraction, where the MC5 metabolite was only detected in the urine and the liver, but not quantified,[10] and for the simultaneous determination of tianeptine and other fourth-generation antidepressants in human plasma using solid-phase extraction with an LLOQ of 0.01 ng mL^{-1}.[11] Ultra-high-performance liquid chromatography-mass spectrometry has been described for studying the ion suppression and enhancement effects of co-eluting analytes, including tianeptine, in plasma using liquid–liquid extraction.[12] The different chromatographic methods used for the determination of tianeptine in biological samples have been summarized in Table 1. For these methods, only partial validations were performed. To date, no LC-MS method has been reported for the simultaneous quantification of tianeptine and its MC5 metabolite in plasma and its main target organ, the brain.

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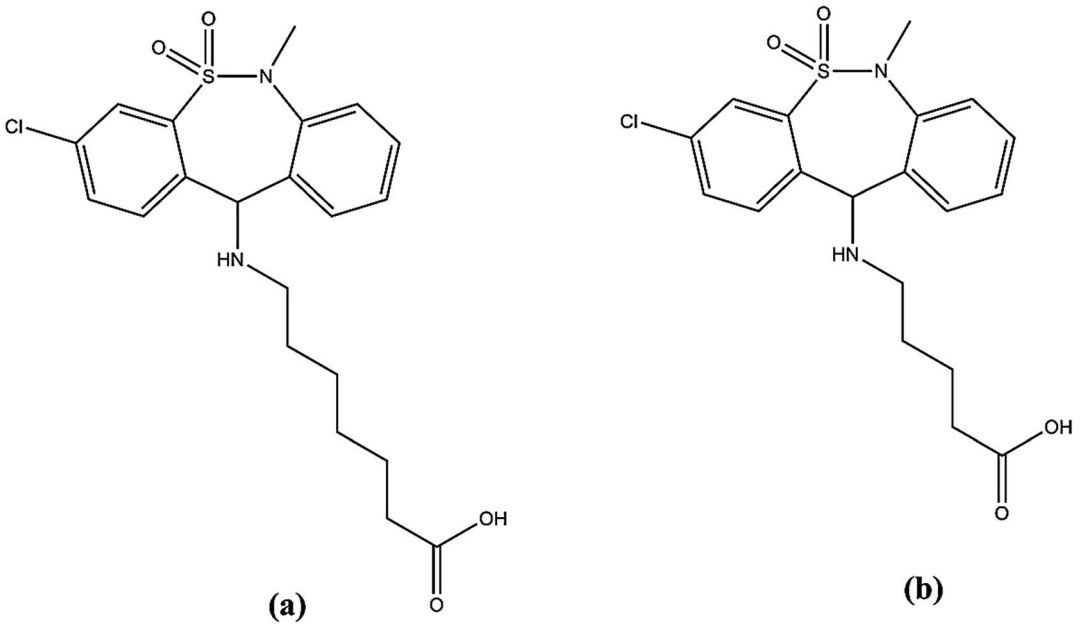

Fig. 1 Chemical structures of tianeptine (a) (MW = 436.95 g mol^{-1}) and tianeptine MC5 (b) (MW = 408.90 g mol^{-1}).

The purpose of this investigation is to develop and validate a simple, selective and sensitive LC-MS/MS method for the quantification of tianeptine and its active metabolite tianeptine MC5 in biological fluids (rat plasma and brain tissue) by means of a one-step liquid–liquid extraction sample preparation approach using LC-MS/MS detection. The bioanalytical method validation has been adopted according to the current FDA guidelines which include the accuracy and precision of inter- and intra-batch variation, specificity, linearity, limit of detection, limit of quantitation, recovery, and stability.

2. Experimental

2.1. Chemicals and reagents

Tianeptine, tianeptine MC5, tianeptine-D4 and tianeptine MC5-D4 were purchased from Toronto Research Chemicals (Toronto, Canada). Ammonium formate, formic acid, chloroform, ethyl acetate, hexane, methyl tert-butyl ether and diisopropyl ether were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol and water were supplied as LC-MS grade from Sigma-Aldrich (St. Louis, MO). Rat plasma with K EDTA and brain homogenate (tissue normal saline ratio: 1/2) collected from male Sprague Dawley were obtained from Bioreclamation IVT (Westbury, NY). Spin-X® centrifuge tube filters were supplied by Sigma-Aldrich (St. Louis, MO). Lobind microcentrifuge tubes were purchased from Eppendorf (Hauppauge, NY).

2.2. Instrumentation

LC-MS/MS analysis was conducted *via* an Agilent 1100 binary pump HPLC system (Santa Clara, CA) interfaced to a Waters Micromass Quattro Micro triple quadrupole mass spectrometer with a positive electrospray ionization source (Milford, MA). Instrument control and data processing were performed using Masslynx 4.1 software by Waters Corporation (Beverly, MA). Sample evaporation was done using a Labconco CentriVap Complete Vacuum Concentrator (Kansas City, MO).

2.3. LC-MS/MS conditions

The analytes were separated on a Waters Atlantis dC-18 (2.1 × 30 mm, 3 μm) column coupled with a Phenomenex Security Guard C-18 guard column (4.0 mm × 2.0 mm). The column temperature was controlled at 25 °C. The mobile phase A was 10 mM ammonium formate/0.1% formic acid (aqueous buffer), mobile phase B was acetonitrile (ACN) and the volume of injection was 15 μL. Analyte separations were done using a flow rate of 0.3 mL min^{-1} under gradient conditions (time/minute, % mobile phase B): (0, 20), (5, 75), (6, 90), (7, 90), (8, 20) and (12, 20). ACN was used for washing the autosampler injection needle after each injection. The mass spectrometer was operated in positive ion ESI mode using nitrogen as the desolvation gas at a flow rate of 800 L h^{-1} and a temperature of 450 °C. The capillary and the cone voltages were 3.50 kV and 10 V, respectively, and the source temperature was set to 130 °C. Argon was used as the collision gas with a collision cell pressure of 5.5 × 10−4 mbar and a collision energy of 15 eV. A multiple reaction monitoring (MRM) function was used for the analyte quantification; the ion transitions were monitored as 437 → 292 for tianeptine, 409 → 292 for tianeptine MC5, 441 → 292 for tianeptine-D4 and 413 → 292 for tianeptine MC5-D4.

2.4. Solutions and standards

The primary stock solutions of 1.0 mg mL^{-1} for tianeptine, tianeptine MC5, tianeptine-D4 and tianeptine MC5-D4 were prepared in 100% methanol. All dilutions were made with 100% methanol. Analyte secondary stock solutions were prepared

Table 1 Summary of the different chromatographic methods used for the determination of tianeptine

Stationary phase	Mobile Phase	Detection	Sample preparation	Concentration range	Applications
Nucleosil C18 (150 × 4.6 mm, 5 μm)	(A) acetonitrile : (B) sodium heptane sulfonate buffer, pH 3.0	UV detection at 220 nm	Ion pair liquid–liquid extraction using a heptane–octanol–tetraheptylammonium bromide mixture with phosphate buffer, pH 7.0	5.00–500.00 ng mL^{-1}	Determination of tianeptine and its main metabolite MC5 in human plasma[7]
Hypersil C18 (150 × 4.6 mm, 5 μm)	(A) acetonitrile : (B) sodium pentane sulfonate buffer, pH 3.0	UV detection at 220 nm	Ion pair liquid–liquid extraction using a heptane–octanol–tetraheptylammonium bromide mixture with phosphate buffer, pH 7.0	10.00–1000.00 ng mL^{-1} in plasma and urine 10.00–1000.00 ng g^{-1} in brain tissue	Determination of tianeptine and its metabolites MC5 and MC3 in human plasma, urine and tissues[8]
Phenomenex C18 (250 × 4.6 mm, 5 μm)	(A) acetonitrile : (B) 10 mM orthophosphoric acid, pH 2.5	Fluorescence detection at λ_{ex} 458.0 nm, λ_{em} 520.0 nm	Derivatization with 4-chloro-7-nitrobenzofurazan–solid phase extraction using silica cartridge	5.00–300.00 ng mL^{-1}	Determination of tianeptine in human plasma[9]
Poroshell 120 EC-C18 (100 × 2.1 mm, 2.7 μm)	(A) 0.1% formic acid aqueous solution : (B) 0.1% formic acid in acetonitrile	MS detection	Solid phase extraction using Oasis HLB cartridge	0.01–100.00 ng mL^{-1}	Simultaneous analysis of fourth-generation antidepressants including tianeptine in human plasma[10]
XTerra MS™ C18 (150 × 2.1 mm, 5 μm)	(A) acetonitrile : (B) 10 mM ammonium acetate buffer with 0.1% formic acid, pH 3.0	MS detection	Liquid–liquid extraction using *n*-hexane : ethyl acetate, *n*-hexane : 2-propanol	10.00–10 000.00 ng mL^{-1}	Determination of tianeptine in post-mortem samples (blood, urine, liver, and stomach contents)[11]
TF Hypersil GOLD phenyl column (100 × 2.1 mm, 1.9 μm)	(A) 10 mM aqueous ammonium formate, 0.1% formic acid, pH 3.4 : (B) 0.1% formic acid in acetonitrile	MS detection	Liquid–liquid extraction using butyl acetate : ethyl acetate mixture	250 ng mL^{-1}	Studying the ion suppression and enhancement effects of co-eluting analytes (including tianeptine) in plasma[12]

independently to prepare calibration standards and QC samples through the dilution of the primary stock solutions to 10 μg mL^{-1}. Tianeptine and tianeptine MC5 standard working solutions were 10.0, 20.0, 40.0, 60.0, 100.0, 200.0, 600.0, 1000.0, 2000.0 and 5000.0 ng mL^{-1} while quality control (QC) working solutions were 10.0, 30.0, 400.0 and 4000.0 ng mL^{-1}. The IS working solution containing a mixture of tianeptine-D4 and tianeptine MC5-D4 was prepared at a single concentration of 500.0 ng mL^{-1}. All solutions were kept in a freezer at −20 °C when not in use.

2.5. Spiked samples and real samples

10 μL of standard or QC working solution was spiked into 100 μL of blank plasma or blank brain homogenate to generate the corresponding standard or QC samples. Calibration standard samples with final concentrations of 1.0, 2.0, 4.0, 6.0, 10.0, 20.0, 60.0, 100.0, 200.0 and 500.0 ng mL^{-1}, and QC samples with concentrations of 1.0, 3.0, 40.0 and 400.0 ng mL^{-1} were obtained.

Dosed animal samples were taken from rats following the administration of tianeptine (10 mg kg^{-1}, ip) 60 min prior to sacrifice. Plasma was drawn from the left ventricle and transferred to EDTA-containing Vacutainers. Brain samples were homogenized similarly to the blank brain. Biological samples were kept at −80 °C until use. Calibration standard samples and QC samples were freshly prepared every day.

2.6. Sample preparation

Sample preparation was performed using a one-step liquid–liquid extraction. To each 100 μL rat plasma or brain homogenate sample, 10 μL IS working solution (500.0 ng mL^{-1}) and 1.2 mL of an ethyl acetate : diisopropyl ether mixture 50 : 50 v/v were added. The mixture was vortexed for 5 min and then centrifuged at 15 000 × *g* at 5 °C for 10 min. The organic supernatant from the plasma and brain homogenate samples (1.0 mL) was transferred and evaporated in a vacuum concentrator at 50 °C for 20 min until complete dryness. The samples

were reconstituted using 100 μL ACN/mobile phase A (20 : 80, v/v), sonicated, vortexed, and centrifuged at 15 000 × *g* at 5 °C for 10 min. Following this process, 90 μL of the supernatant from plasma samples was transferred for injection. However, the supernatants from brain homogenate samples were transferred to Spin-X® centrifuge tube filters (pore size: 0.22 μm) and centrifuged at 10 000 × *g* at 5 °C for 10 min. The filtrate was transferred for injection into the mass spectrometer.

2.7. Method validation

The bioanalytical method validation was done according to the current FDA guidelines[13] which include selectivity, linearity, intra/inter-day precision, accuracy, recovery, stability, dilution tests and robustness.

The selectivity ($n = 6$) was assessed by comparing analyte chromatograms in blank plasma or blank brain homogenate, with analyte chromatograms in the same matrix at the LLOQ (1.0 ng mL^{-1}).

The linearity was determined using calibration standard samples within the range of 1.0–500.0 ng mL^{-1} for plasma and 1.0–500.0 ng g^{-1} for brain tissue. The calibration curves were constructed from the ratios of peak areas between analytes and IS using $1/x$ weighted linear regression.

The intra-day ($n = 5$) and inter-day ($n = 15$) precision (% RSD) and accuracy (% RE) were measured at the QC levels 1.0, 3.0, 40.0 and 400.0 ng mL^{-1}.

The recovery ($n = 3$), including absolute recovery (AR) and relative recovery (RR), and matrix effect (ME) in plasma and brain homogenate were calculated from the peak areas of spiked samples, post preparation spiked samples and neat standard solutions at the QC levels 3.0, 40.0 and 400.0 ng mL^{-1}.

The autosampler stability (25 °C, 18 h), bench-top stability (25 °C, 18 h) and freeze–thaw stability (3 freeze–thaw cycles, −80 °C, 72 h) of analytes in plasma and brain homogenate were assessed at low (3.0 ng mL^{-1}) and high (400.0 ng mL^{-1}) concentrations ($n = 3$) by comparing freshly spiked samples and samples subjected to the stability conditions.

The dilution test ($n = 5$) was performed through the dilution of spiked plasma and brain homogenate samples at 2000.0 ng mL^{-1} to the upper limit of quantitation (ULOQ = 500.0 ng mL^{-1}) with the corresponding blank matrix accommodating real samples with analyte concentrations over the ULOQ. The precision (% RSD) and accuracy (% RE) were calculated.

The robustness of the analytes in plasma and brain homogenate was assessed at 3.0 ng mL^{-1} concentration ($n = 3$) by changing the initial ratio of the system, mobile phase A : acetonitrile from (80 : 20) to (85 : 15). To evaluate this change the % RSD was calculated.

2.8. Animal study

Male Sprague–Dawley rats (3–4 months old; $N = 4$) were injected with tianeptine (10 mg kg^{-1}, ip) 60 min prior to sacrifice. Subjects were then anesthetized with isofluorane and blood was drawn directly from the left ventricle, collected in EDTA 4 mL Vacutainers (BD, Franklin Lakes, NJ, USA) and centrifuged for 15 min in a clinical centrifuge. The plasma was removed, placed in aliquots, frozen and stored at −70 °C. After the blood was drawn, the animals were quickly decapitated, brains were removed within 3 minutes, rinsed three times with saline, weighed, and stored at −70 °C. The tianeptine dose was calculated as the weight of the salt, dissolved in saline and injected in a volume of 1 mL kg^{-1}. All procedures applied in this work were revised and officially accepted by the Augusta University Institutional Animal Care and Use Committee and are in agreement with AAALAC guidelines. Actions were taken to reduce pain and discomfort according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23) reviewed in 1996.

3. Results and discussion

3.1. Sample preparation method development

Sample preparation has been optimized to attain acceptable recovery and matrix effects for all analytes. Considering the polarities of the analytes and IS, protein precipitation and liquid–liquid extraction (LLE) were tested for plasma and brain homogenate extraction in order to develop a sensitive and accurate method. Using protein precipitation, the evaporation and dryness steps took more time and the samples still contained impurities after reconstitution. So, further sample clean-up would be necessary after protein precipitation.

LLE was first tested with different solvents, ethyl acetate, diisopropyl ether, chloroform, hexane and methyl tertiary butyl ether. The LLOQ (1 ng mL^{-1}) was detected using a mixture of ethyl acetate and diisopropyl ether while the other solvents failed to extract tianeptine and tianeptine MC5. Ethyl acetate provided higher recovery than diisopropyl ether. However a mixture of ethyl acetate : diisopropyl ether 50 : 50 v/v provided the highest recovery (1-fold and 30% higher than diisopropyl ether and ethyl acetate, respectively) and analyte signal-to-noise ratio (S/N) and the lowest time for the evaporation step. It also provided a clean extract for plasma while for the brain an extra filtration step was needed. Yet, LLE extraction for brain homogenate was still better than the protein precipitation extraction.

Buffers with different pH ranges were tested for increasing the extraction efficiency by increasing the neutralization of analytes in the aqueous phase to be more distributed in the organic phase. Consistent with the fact that tianeptine and its active metabolite contain a carboxylic group and amino group, a buffer at pH 7 was expected to give the highest recovery. However no statistical difference was observed for the recovery with or without the addition of the buffer pH 7. Therefore, there is no need for the use of a buffer or pH adjustment in this study.

Stable isotope-labeled ISs were used in this study to improve precision and accuracy and to achieve good reproducibility while reducing the matrix effects. Consequently, the matrix effects became less prominent and the recovery was more directly related to the sensitivity of the method.[14]

3.2. LC-MS/MS method development

To achieve higher sensitivity and selectivity for the simultaneous quantification of tianeptine and tianeptine MC5, different parameters were optimized in tandem mass spectrometry and

liquid chromatography. MS tuning parameters for all analytes have been optimized by direct infusion of 10 μg mL^{-1} standard solutions of tianeptine and tianeptine MC5 and the internal standards tianeptine-D4 and tianeptine MC5-D4 at 50 μL min^{-1} into the instrument to define the precursor and the product ion transition pairs. The detection of analytes and ISs was conducted using the MRM function, producing both high sensitivity and selectivity. The product ion mass spectra for tianeptine and tianeptine MC5 and the ISs tianeptine-D4 and tianeptine MC5-D4 were acquired using collision activated dissociation (CAD) with argon as the collision gas. The most abundant fragment ion for each analyte was chosen to monitor ion-transitions in MRM mode. The ion transitions monitored were 437 → 292 for tianeptine, 409 → 292 for tianeptine MC5, 441 → 292 for tianeptine-D4 and 413 → 292 for tianeptine MC5-D4. This fragmentation resulted from the loss of the side chain from the ring system. No other ion transitions were detected for tianeptine and its major metabolite. This prevented the addition of qualifying ion transitions that could be used to verify the identity of the analytes (Fig. 2).

3.3. Selectivity

For plasma and brain samples, the method selectivity ($n = 6$) was evaluated through representative comparison between the chromatograms of blank matrix samples, blank matrix samples with IS (50.0 ng mL^{-1}) and spiked matrix standard samples at the LLOQ (1.0 ng mL^{-1}) without IS together with spiked matrix standard samples at the LLOQ (1.0 ng mL^{-1}) and IS (50.0 ng mL^{-1}).

No significant interference was observed among the MRM channels in both blank plasma and blank brain homogenate (see Fig. 3), indicating that the LC-MS/MS method provided acceptable selectivity for the simultaneous quantification of the analytes at the LLOQ for both plasma and brain homogenate.

3.4. Linearity and sensitivity

The method linearity was validated over the concentration range from 1.0–500.0 ng mL^{-1} and 1.0–500.0 ng g^{-1} for plasma and brain tissue, respectively. Calibration curves were constructed from peak area ratios between analytes and ISs using $1/x$ weighted linear regression. Non-weighted, $1/x$ weighted and $1/x^2$ weighted linear regression have been tested where the relative error for the calibration standards and the QC samples was the lowest using $1/x$ weighted linear regression.

From these calibration curves, slopes, intercepts and R^2 values were calculated and are shown in Table 2. Good linearity ($R^2 > 0.995$) was observed for all of the analytes in either matrix within the tested range.

The sensitivity of the method was expressed by the lower limit of quantitation (LLOQ), which was the lowest concentration within 20% precision and accuracy. LLOQs for all the analytes were 1.0 ng mL^{-1} in plasma or 1.0 ng g^{-1} in brain tissue. The analyte signal-to-noise ratio (S/N) was also used to evaluate the sensitivity.

3.5. Precision and accuracy

Intra-day ($n = 5$) and inter-day ($n = 15$) precision and accuracy were calculated using LLOQ and QC samples at 1.0, 3.0, 40.0 and 400.0 ng mL^{-1} in either matrix. Precision presenting the closeness of a series of measurements from multiple samples with the same concentration was assessed by relative standard deviation (RSD), while accuracy presenting the closeness of a measured value to the true one was assessed by % relative error (RE) between measured concentrations and nominal concentrations calculated as ((nominal concentration − measured concentration/nominal concentration) × 100). Table 3 shows the % RSD and % RE values for each analyte in QC plasma samples and brain homogenate samples. The values

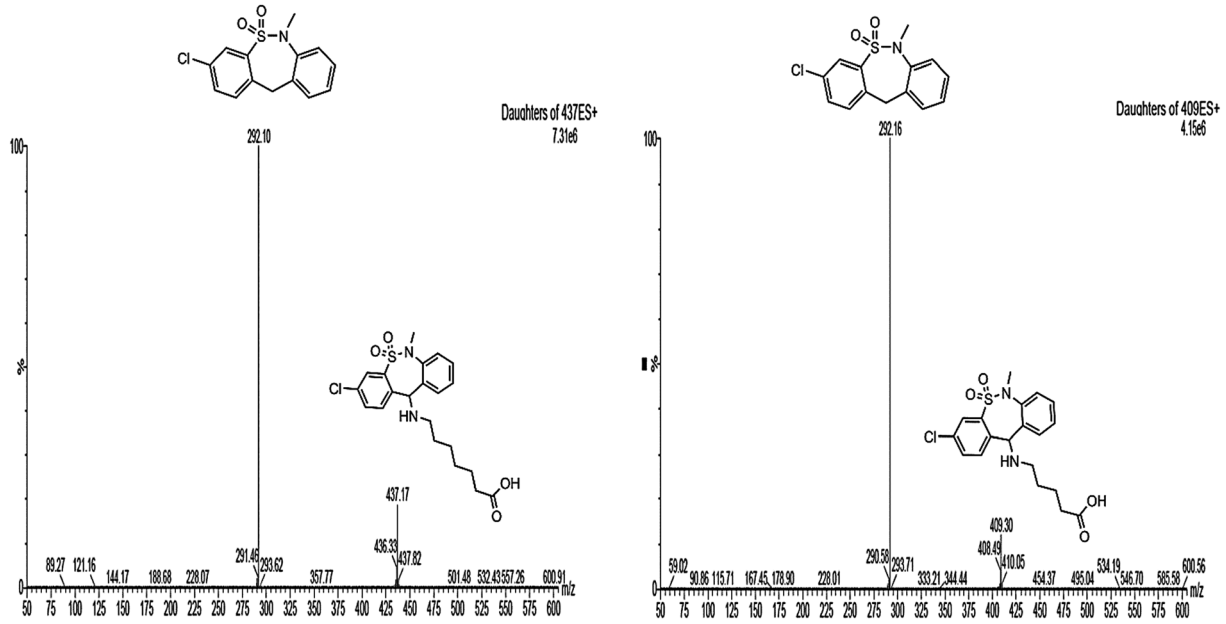

Fig. 2 Product ion mass spectra for tianeptine (left) and tianeptine MC5 (right).

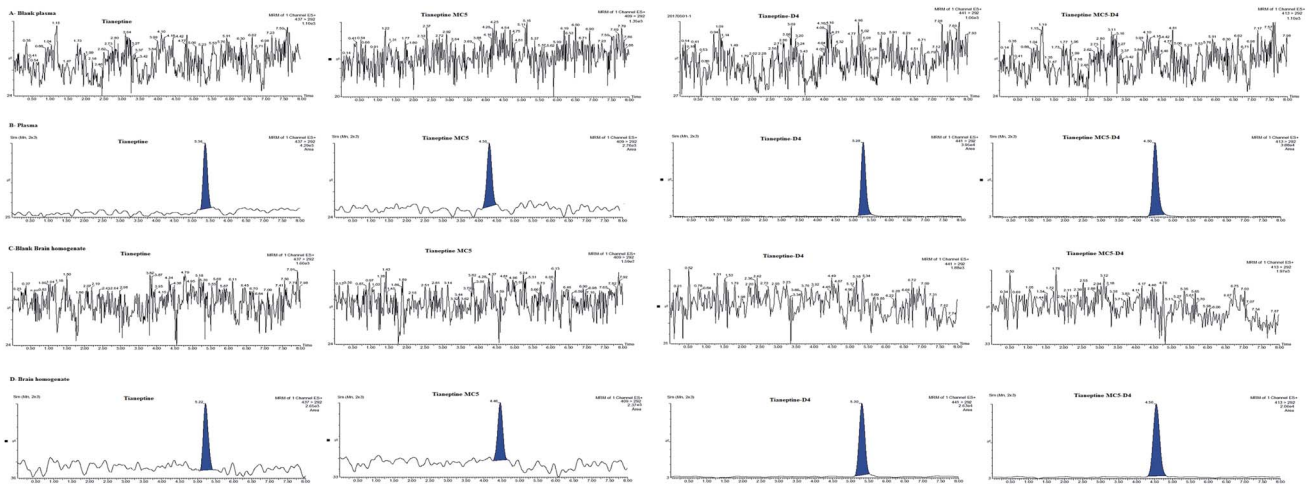

Fig. 3 Chromatograms of tianeptine, tianeptine MC5, tianeptine-D4 and tianeptine MC5-D4 in blank plasma (A), in plasma at the LLOQ (1.0 ng mL^{-1}) for tianeptine and tianeptine MC5 and at 50 ng mL^{-1} for tianeptine-D4 and tianeptine MC5-D4, (C) in blank brain homogenate and (D) in brain homogenate at the LLOQ (1.0 ng mL^{-1}) for tianeptine and tianeptine MC5 and at 50 ng mL^{-1} for tianeptine-D4 and tianeptine MC5-D4.

Table 2 Calibration curves for tianeptine and tianeptine MC5 extracted from rat plasma and brain homogenate

	Plasma ($n = 3$)			Brain homogenate ($n = 3$)		
Analyte	Slope	Intercept	R^2	Slope	Intercept	R^2
Tianeptine	1.5199 ± 0.0217	0.8064 ± 0.1680	0.9981 ± 0.0011	1.0079 ± 0.0539	0.6440 ± 0.24878	0.9966 ± 0.0014
Tianeptine MC5	1.1035 ± 0.0152	0.5448 ± 0.2046	0.9979 ± 0.0006	1.3019 ± 0.1993	0.9609 ± 0.33430	0.9960 ± 0.0017

are less than 15% for the QC samples and less than 20% for the LLOQ for either analyte in either matrix. These data are in accordance with the requirements from Guidance for Industry (bioanalytical method validation) by FDA.[13]

3.6. Recovery

AR, RR and ME ($n = 3$) were assessed in plasma and brain homogenate, using spiked samples, post-preparation spiked samples and neat standard solutions at three QC concentrations 3.0, 40.0 and 400.0 ng mL^{-1}. Post-preparation spiked samples were prepared by spiking standard working solutions into blank matrices treated with the same sample preparation. The AR was evaluated by using peak area ratios between spiked samples and the average of neat standard solutions at the same concentration. The RR, presented as the loss due to sample preparation, was evaluated by peak area ratios between spiked samples and the average of the corresponding post-preparation spiked samples. The ME, presented as the influence of the matrix on the signal response, was evaluated using the peak area ratios between post-preparation spiked samples and the average of the corresponding neat standard solutions. The types of matrix effects (enhancement demonstrated as a ratio over 100%, or suppression demonstrated as a ratio below 100%) and the enhancement or suppression percentages calculated as the difference from 100% could be determined. Table 4 shows AR, RR, matrix effects and the type in each matrix. The AR and RR vary for different analytes in different matrices but were consistent, accurate, specific and reproducible at the three QC concentrations for the same analyte in the same matrix. Ion enhancement was detected for the analytes, at all concentrations in either matrix.

3.7. Stability

Three sets ($n = 3$) of plasma and brain homogenate samples spiked at two concentrations 3.0 ng mL^{-1} and 400.0 ng mL^{-1} were prepared for the validation of autosampler stability (25 °C, 18 h), bench-top stability (25 °C, 18 h) and freeze–thaw stability (3 freeze–thaw cycles, −80 °C, 72 h). The first set was analyzed instantly and considered to be a time zero control, and then used for testing the autosampler stability. The subsequent set was placed on the bench at room temperature (25 °C) for 18 h and then analyzed. The latest set was kept at −80 °C for 24 h and then fully thawed at 25 °C. This process was repeated for another 2 freeze–thaw cycles. The ratio between concentrations in tested samples and the time-zero control was calculated for all stability tests, as shown in Table 5. The results revealed that a difference of less than 10% from the time-zero control was observed indicating that the chemical stability of these analytes was maintained in either matrices under common sample treatment and storage conditions.

3.8. Dilution

Tianeptine and tianeptine MC5 might possess different concentrations in the different biological samples depending on the dose, route of administration, intervariability of

Table 3 Intra-day and inter-day precision (% RSD) and accuracy (% RE) of the LC-MS/MS method used for the quantification of tianeptine and tianeptine MC5 extracted from rat plasma and brain homogenate

			Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Matrix	Analyte	Nominal conc. (ng mL^{-1})	Measured conc. (ng mL^{-1})	% RSD	% RE	Measured conc. (ng mL^{-1})	% RSD	% RE
Plasma	Tianeptine	1.0	1.03 ± 0.09	8.61	−2.67	1.07 ± 0.11	9.90	−7.50
		3.0	2.86 ± 0.20	7.09	4.67	2.81 ± 0.19	6.60	6.25
		40.0	39.63 ± 1.77	4.47	0.92	39.99 ± 3.18	7.94	0.02
		400.0	387.91 ± 9.72	2.50	3.02	402.91 ± 11.49	2.85	−0.73
	Tianeptine MC5	1.0	1.07 ± 0.01	11.92	−7.33	1.03 ± 0.14	14.00	−3.33
		3.0	2.90 ± 0.21	7.37	3.33	2.87 ± 0.18	6.09	4.22
		40.0	40.62 ± 1.73	4.26	−1.53	40.77 ± 3.45	8.46	−1.93
		400.0	392.21 ± 7.69	1.96	1.95	396.01 ± 11.17	2.82	0.99
Brain homogenate	Tianeptine	1.0	1.01 ± 0.16	15.77	−1.33	1.05 ± 0.15	13.84	−5.33
		3.0	3.01 ± 0.32	10.70	−0.22	3.10 ± 0.29	9.44	−3.33
		40.0	41.99 ± 1.57	3.73	3.39	39.37 ± 2.39	6.07	1.58
		400.0	378.88 ± 12.85	5.07	5.28	392.62 ± 11.11	2.83	1.85
	Tianeptine MC5	1.0	0.95 ± 0.14	14.76	4.67	1.05 ± 0.16	15.16	−5.33
		3.0	3.07 ± 0.28	9.11	−2.22	3.11 ± 0.32	10.15	−3.78
		40.0	41.77 ± 1.80	4.31	−4.41	38.61 ± 2.19	5.68	3.47
		400.0	368.97 ± 10.01	2.71	7.76	371.93 ± 14.08	3.78	7.02

Table 4 Absolute recovery (% AR), relative recovery (% RR) and matrix effect (% ME) of the LC-MS/MS method

Matrix	Analyte	Conc. (ng mL^{-1})	AR% ($n = 3$)	RR% ($n = 3$)	ME% ($n = 3$)	Type of ME	
Plasma	Tianeptine	3.0	82.51 ± 1.87	79.79 ± 2.51	108.13	8.13	Enhancement
		40.0	91.16 ± 1.86	77.54 ± 1.87	122.29	22.29	Enhancement
		400.0	93.92 ± 2.62	84.33 ± 2.66	119.22	19.22	Enhancement
	Tianeptine MC5	3.0	77.14 ± 3.28	78.26 ± 3.37	104.99	4.99	Enhancement
		40.0	85.53 ± 0.98	79.68 ± 0.86	113.80	13.80	Enhancement
		400.0	87.72 ± 2.65	85.89 ± 1.53	114.25	14.25	Enhancement
Brain homogenate	Tianeptine	3.0	83.62 ± 1.71	74.8 ± 1.75	115.82	15.82	Enhancement
		40.0	78.93 ± 1.52	72.20 ± 2.50	107.49	7.49	Enhancement
		400.0	86.21 ± 1.96	77.58 ± 1.66	116.96	16.96	Enhancement
	Tianeptine MC5	3.0	86.61 ± 1.80	80.83 ± 1.50	107.14	7.14	Enhancement
		40.0	79.74 ± 2.13	77.45 ± 1.05	104.23	4.23	Enhancement
		400.0	88.30 ± 1.30	83.59 ± 1.71	112.41	12.41	Enhancement

experimental subjects and several other factors. Therefore, a dilution test ($n = 5$) was done in order to adjust the method for analytes in real samples at concentrations above the upper limit of quantitation (ULOQ = 500.0 ng mL^{-1}). Dilution of spiked plasma and brain homogenate samples at 2000.0 ng mL^{-1} to the ULOQ was made using the corresponding blank matrix. Table 6 shows the calculated concentrations, precision and accuracy ($n = 5$). All % RSD and % RE values for these analytes

Table 5 Stability testing (autosampler stability, bench-top stability and freeze–thaw stability) of tianeptine and tianeptine MC5 at 3.0 and 400.0 ng mL^{-1} in rat plasma and brain homogenate. Stabilities are displayed as the percentage of relative concentration to time zero control (mean ± SD)

Matrix	Analyte	Conc. (ng mL^{-1})	Autosampler stability (25 °C, 18 h) ($n = 3$)	Benchtop stability (25 °C, 18 h) ($n = 3$)	Freeze–thaw stability (3 cycles, −80 °C, 72 h) ($n = 3$)
Plasma	Tianeptine	3.0	97.01 ± 1.56	95.76 ± 2.49	96.96 ± 3.75
		400.0	99.87 ± 2.48	96.90 ± 3.32	94.38 ± 2.86
	Tianeptine MC5	3.0	98.57 ± 0.89	96.56 ± 3.36	95.59 ± 1.87
		400.0	101.59 ± 2.00	95.11 ± 2.89	97.46 ± 3.33
Brain homogenate	Tianeptine	3.0	103.77 ± 1.79	103.51 ± 2.72	97.62 ± 3.30
		400.0	101.33 ± 2.59	104.73 ± 1.79	96.65 ± 3.44
	Tianeptine MC5	3.0	103.45 ± 1.81	101.72 ± 2.54	99.72 ± 4.86
		400.0	99.02 ± 2.45	104.39 ± 2.17	97.12 ± 4.55

Table 6 Precision (% RSD) and accuracy (% RE) of spiked samples at 2000.0 ng mL^{-1} in rat plasma and brain homogenate after 4-fold dilution to the ULOQ concentration (500.0 ng mL^{-1})

		Plasma ($n = 5$)			Brain ($n = 5$)		
Analyte	Nominal conc. (ng mL^{-1})	Measured conc. (ng mL^{-1})	% RSD	% RE	Measured conc. (ng mL^{-1})	% RSD	% RE
Tianeptine	2000.0	1827.88 ± 46.04	2.52	8.61	1802.50 ± 58.46	3.24	9.88
Tianeptine MC5	2000.0	1747.98 ± 51.75	2.96	12.60	1763.60 ± 41.26	2.34	11.82

Table 7 Concentrations of tianeptine and tianeptine MC5 in plasma and brain tissue collected from four rats 60 min after treatment with tianeptine (10 mg kg^{-1}, ip)

Analyte	Plasma conc. (ng mL^{-1})	Brain conc. (ng g^{-1})	Brain-to-plasma ratio
Tianeptine	823.25 ± 82.39	68.15 ± 6.17	0.08
Tianeptine MC5	674.38 ± 69.78	34.71 ± 4.44	0.05

in either matrix are less than 15%, suggesting that a 4-fold matrix matched dilution can be applicable to preclinical samples over the ULOQ.

3.9. Robustness

The robustness of the method is a measure of its capacity to remain unaffected by small but deliberate changes that might occur during routine analysis to the method parameters. This provides an indication of the reliability and consistency of the method. Robustness was assessed by the analysis of tianeptine and tianeptine MC5 (3.00 ng mL^{-1}) with a change in the initial mobile phase ratio of the system, mobile phase A : acetonitrile from (80 : 20) to (85 : 15). The % RSDs were found to be 1.39 and 1.61 for tianeptine in plasma and brain samples, respectively. The % RSDs were found to be 1.44 and 1.79 for tianeptine MC5 in plasma and brain samples, respectively. The low values of % RSDs for both analytes in either matrix indicate the robustness of the method.

3.10. Application

This validated bioanalytical method was successfully applied to analyze plasma and brain homogenate samples collected from rats ($n = 4$) dosed with tianeptine (10 mg kg^{-1}, ip) 60 min prior to sacrifice. Parallel experiments either with or without a 4-fold dilution were achieved. Plasma and brain homogenate samples taken from every rat were analyzed in duplicate, and the average concentration was recorded as shown in Table 7.

Preparation of the brain homogenate was done with 1 weight unit of brain tissue and 2 weight units of water, so the concentrations of analytes in brain homogenate represent one third of the concentrations in brain tissue. Tianeptine and tianeptine MC5 could be detected in the brain. However, their concentrations in plasma were higher than the ULOQ, but could still be measured using a 4-fold dilution.

Assuming 1 g brain tissue is equivalent to 1 mL of plasma, the ratio of brain-to-plasma for each analyte was calculated. The ratio of brain-to-plasma for tianeptine is 0.08 and for tianeptine MC5 it is 0.05. The ratios are far less than the ratios for the antipsychotics, quetiapine,[15] and aripiprazole[16] and the active metabolite of nicotine (cotinine) which has been considered as a potential therapy for Alzheimer's disease.[17] These findings are in agreement with earlier pharmacokinetic studies for tianeptine.[8,18] Tianeptine and tianeptine MC5 have been shown to quickly diffuse into the brain. However, their low overall concentrations are likely related to their polarity and log *P* values. Tianeptine possesses a quite low log *P* (1.05 at pH 7.4) which makes it different from other tricyclic antidepressants.[18,19] Tianeptine is effective at low concentrations. So even with its relatively low penetration into the brain, its pharmacological and biochemical performance is not affected.[1,20]

4. Conclusions

A sensitive, selective and robust liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method was developed and validated for the simultaneous quantitation of tianeptine and tianeptine MC5 in rat plasma and brain homogenate. The adopted method exhibited good precision and accuracy. The method linearity was within the range of 1.0–500.0 ng mL^{-1} and 1.0–500.0 ng g^{-1} for plasma and brain tissue, respectively, and the LLOQ was 1.0 ng mL^{-1} and 1.0 ng g^{-1} for plasma and brain tissue, respectively. For the sample preparation, a small sample volume (100 μL) of plasma or brain homogenate, and a one-step liquid–liquid extraction for sample preparation were required. The method has been successfully applied to a preclinical study of tianeptine and tianeptine MC5 in rats.

Conflicts of interest

There are no conflicts to declare.

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