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A Simple HPLC–MS/MS Method for Determination of Tryptophan, Kynurenine and Kynurenic Acid in Human Serum and its Potential for Monitoring Antidepressant Therapy

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Abstract

The kynurenine pathway, in which tryptophan is metabolized to kynurenine and kynurenic acid, has been linked to depression. A rapid and highly reproducible liquid-chromatography-tandem mass spectrometry (LC-MS/MS) method were established for determining tryptophan, kynurenine and kynurenic acid in human serum. Biological samples were precipitated with methanol before separation on an Agilent Eclipse XDB-C18. The stable-isotope-labeled internal standards (kynurenine-$^{13}C_4{}^{15}N$ and kynurenic acid-d_5) were used for quantification. Detection was performed using multiple reaction monitoring in electrospray ionization mode at m/z 205.1→188.1 for tryptophan, m/z 209.1→146.1 for kynurenine, m/z 190.1→144.1 for kynurenic acid. Good linearity of analyte to internal standard peak area ratios was seen in the concentration range 1,000–50,000 ng/mL for tryptophan, 100–5,000 ng/mL for kynurenine and 1–60 ng/mL for kynurenic acid. Pooled drug-free human serum was purified using activated charcoal and the method was shown to be linear, with validation parameters within acceptable limits. The newly developed method was successfully used to determine concentrations of tryptophan, kynurenine and kynurenic acid in serum from 26 healthy volunteers and 54 patients with depression. Concentrations of tryptophan and kynurenine were lower in serum from depressed individuals than from healthy individuals.

Introduction

Depression is one of the most prevalent and debilitating mental illnesses, affecting millions of people and their families worldwide. The most commonly used treatments for patients with depression are tricyclic antidepressants and selective serotonin reuptake inhibitors, such as clomipramine and paroxetine. Although these drugs undoubtedly provide some benefit, precise evaluation of their therapeutic effects is difficult and the identification of suitable biomarkers in patients with depression would allow more objection evaluation of drug efficacy and disease prognosis.

The kynurenine pathway is essential for several fundamental physiological processes and also mediates interactions between immunological and neuronal functions (1). These interactions have been implicated in the pathophysiology of many diseases, including human immunodeficiency virus (HIV)-infection, major schizophrenia and depression (2).

We have now developed a simple and reliable HPLC–MS/MS method for the precise determination of L-tryptophan (Trp), L-kynurenine (Kyn) and kynurenic acid (Kyna) in the serum of patients suffering from depression. We used a simple protein precipitation method to prepare human serum samples and simultaneously examined Trp, Kyn and Kyna in serum from 26 normal subjects and 54 patients with depression. To minimize matrix effects, we used stable-isotope-labeled internal standards (SIL–ISs), Kyn-$^{13}C_4{}^{15}N$ and Kyna-d_5, which are structurally similar to the analytes and behave in a similar way under the analytical conditions (3).

The newly developed method was validated using principles of good clinical practice, in accordance with the guidelines issued by the China Food and Drug Administration (FDA) and European Medicines Agency (EMEA).

Materials and methods

Chemicals and reagents

L-Trp (purity 99.0–101.0%, Lot # SLBD9323V), L-Kyn (purity ≥98%, Lot # BCBH77844V), Kyna (purity ≥98%, Lot # SLBC5374V) and activated charcoal (DARCO®, 100 mesh particle size) were all obtained from Sigma-Aldrich. L-Kyn-$^{13}C_4{}^{15}N$ (purity: 98%, lot. 1971-070A6) and Kyna-d_5 (purity: 98%, lot. 10-ABY-121-1) were supplied by Toronto Research Chemicals Inc. (Toronto, Canada). The structures of L-Trp, L-Kyn and Kyna are shown in Figure 1. HPLC grade methanol was purchased from Merck KGaA, (Darmstadt, Germany) and mass spectrometry grade ammonium formate was purchased from Sigma-Aldrich Corporation (Bangalore, India). Purified water (conductivity 18 MΩ) was obtained using a Milli-Q water purification system (Millipore Corporation, Billerica, MA, USA).

Liquid chromatography and mass spectrometry

HPLC was carried out using an Agilent 1,200 series HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA), equipped with a quaternary pump, a column oven degasser, an autosampler and a temperature-controlled column. Chromatographic separations were achieved using an Agilent Eclipse XDB-C18 (4.6 mm × 150 mm, 5 μm) reversed phase analytical column maintained at 35°C. The mobile phase for analysis of Trp and Kyn was methanol/water (45:55, v/v) containing ammonium formate (5 mM) and the mobile for analysis of Kyna was methanol/water (35:65, v/v) containing ammonium formate (5 mM). The flow rate for all analyses was 0.5 mL/min.

Mass spectrometry was carried out using an Agilent 6,410 triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Experiments were conducted in positive ionization mode and quantification was carried out in multiple reaction monitoring (MRM) mode. Conditions, including high voltage capillary, impact energy, split voltage, nebulizing gas, drying gas flow rate, drying gas temperature and dwell time, were optimized manually. The precursor/product transitions (*m/z*) were at *m/z* 205.1 → 188.1 for Trp, *m/z* 209.1 → 146.1 for Kyn, *m/z* 190.1 → 144.1 for Kyna, *m/z* 214.1 → 149.1 for Kyn-$^{13}C_4{}^{15}N$ and *m/z* 195.1 → 149.1 for Kyna-d_5. The ion spray temperature and ion spray voltage were maintained at 350°C and 3,500 V, respectively. The drying gas flow rate was 10 L/min and the nebulizing gas pressure was 45 psi. A summary of the mass spectrometric conditions for each analyte is provided in Table I.

Preparation of stock solutions and blank serum samples

Stock solutions of Trp (10 mg/mL), Kyn (1 mg/mL), Kyna (0.1 mg/mL), Kyna-d_5 (100 μg/mL) and Kyn-$^{13}C_4{}^{15}N$ (250 μg/mL) were prepared in 96% methanol. A working solution containing a mixture of both SIL and ISs was prepared in methanol by dilution of the stock solutions. Mixed calibration standards containing the three analytes at seven different concentration levels were prepared from the stock solutions by serial dilutions with methanol (it is called working solutions). Quality control (QC) standards were prepared from the same as dilution process of calibration standards. The working solutions contained three different concentration levels, low (LQC), medium (MQC) and high (HQC), including two points in common with the calibration standard. The working solutions of calibrators and QCs were made from different stock solution. All solutions were stored at 4°C.

Fresh blank human serum samples were obtained from the Clinical Division of Therapeutic Drug Monitoring (TDM) of Guangzhou Brain Hospital and treated with activated charcoal to remove endogenous tryptophan metabolites from the serum, as previously described (4). Briefly, serum (1 mL) was treated with activated charcoal (50 mg) and the mixture was stirred moderately on a magnetic stirrer for 8 h at room temperature. It was usually take 30 mL serum treated with 1.5 g activated charcoal to prepared calibrators and QCs. After centrifugation at 20,293 × g for 10 min (Centrifuge 5,424, Eppendorf AG, Hamburg, Germany) at 4°C, the supernatant was filtered through a 0.45 μm membrane filter. The filtrate was centrifuged at 20,293 × g for 30 min at 4°C and the purified serum supernatant was transferred to clean tubes and used immediately.

Sample extraction procedures

Sample preparation was performed using generic protein precipitation with methanol. Briefly, serum (100 μL) was transferred by pipette into a 2 mL plastic tube and IS working solution (40 μL; Kyn-$^{13}C_4{}^{15}N$ (10 μg/mL) and Kyna-d_5 (150 ng/mL)) was added. The mixture was vortexed (XW-80A Vortex Mixer, Shanghai Medical University Instrument Factory, Shanghai, China) for 5 s, and then deproteinized by vortexing with methanol (500 μL) for 30 s. The mixture was then centrifuged at 20,156 × g for 5 min and a sample of the supernatant (100 μL) was transferred to an autosampler vial. An aliquot (3 μL) was injected into the HPLC–MS/MS system for analysis of Trp and Kyn. Analysis of Kyna required an additional step. After centrifugation, a sample of the supernatant (about 600 mL) was collected in a clean 1.5 mL tube and evaporated to dryness under vacuum at 45°C. The residue was redissolved in methanol/water (35:65, v/v, 100 μL), vortexed for 30 s and centrifuged at 20,156 × g for 5 min. An aliquot (5 μL) of the supernatant was then injected into the HPLC–MS/MS system for analysis of Kyna.

Data analysis

Agilent Mass Hunter Chemstation software (B.01.03) was used to evaluate chromatographic data. Microsoft Office Excel 2010 was used to calculate intra- and inter-assay means, standard deviations (SD), relative standard deviations (RSD) or coefficients of variation (CV).

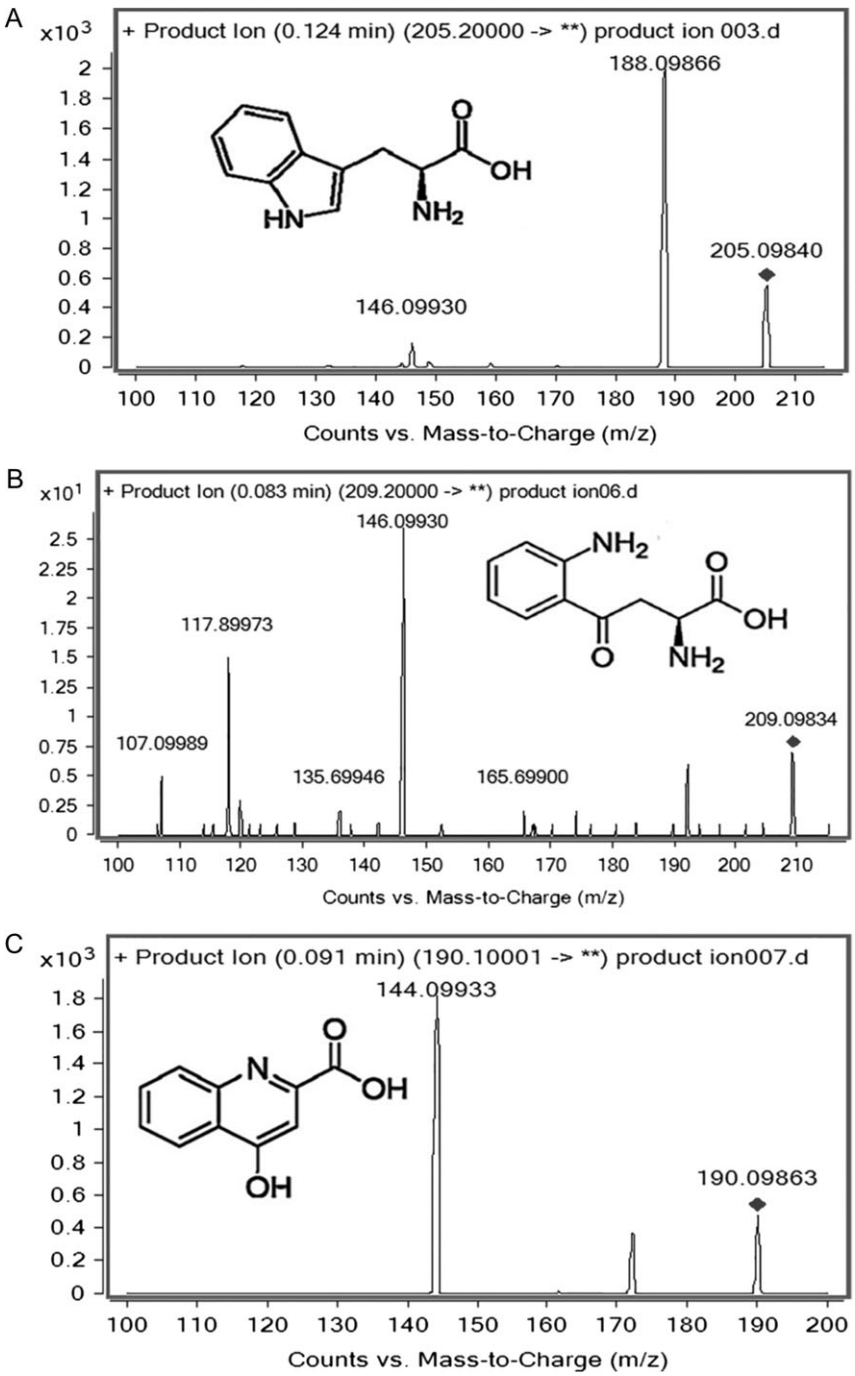

Figure 1. Chemical structures and mass spectra of tryptophan $[M + H]^+$ (A), kynurenine $[M + H]^+$ (B) and kynurenic acid $[M + H]^+$ (C).

Table I. Mass spectrometric conditions: Dwell, collision energy (CE) , fragmentor voltage (FV), retention time (RT)

Analytes	Precursor ion (*m/z*)	Production (*m/z*)	Dwell (ms)	CE (V)	FV (V)	RT (min)
Tryptophan	205.1	188.1	100	5	100	3.67
L-Kynurenine	209.1	146.1	100	15	90	3.16
L-Kynurenine-$^{13}C_4{}^{15}N$	214.1	149.1	100	15	100	3.16
Kynurenic acid	190.1	144.1	200	15	100	3.65
Kynurenic acid-d_5	195.1	149.1	100	15	105	3.62

Results were analyzed using Statistical Package for Social Sciences (SPSS) software, version 16.0. Independent samples *t*-tests were used to compare normal and control data. A $P < 0.05$ was considered to be statistically significant.

Method validation

Selectivity relative to endogenous matrix (particularly phospholipids and inorganic salts) of the SIL–ISs was tested using non-pooled, drug-free human serum. Six individual serum samples were treated with activated charcoal and chromatograms of these samples were compared with chromatograms of blank serum samples spiked with IS to evaluate specificity.

Sensitivity was initially assessed for the lower limit of quantification (LLOQ), with a signal to noise (S/N) ratio at least 5-fold greater than average background noise. The LLOQ was evaluated by injecting five independent pretreated serum samples spiked with Trp, Kyn and Kyna at the LLOQ together with the ISs.

Carryover was determined by immediately and repeatedly injecting a blank sample after an injection at the upper limit of quantification (ULOQ) of the calibration curve, and then injecting the LLOQ standard once (5). Carryover was calculated by dividing the analyte peak area of the blank specimen by the analyte peak area of the LLOQ standard and multiplying by 100. A carryover effect not exceed 20% at the LLOQ concentration generally indicates a negligible residue in the apparatus.

As described above, calibration curves were obtained using the three analytes at seven concentration levels. Calibration standards were prepared by serial dilution of stock solution with methanol (final concentrations: 1, 2, 5, 10, 20, 40 and 50 μg/mL of Trp; 0.1, 0.2, 0.5, 1, 2, 4 and 5 μg/mL of Kyn; and 1, 2, 5, 10, 30, 50 and 60 ng/mL of Kyna). QC final concentration in serum was: 2, 8, 40 μg/mL Trp; 0.2, 0.8, 4 μg/mL Kyn; 2, 8 50 ng/mL Kyna. Each calibration level of the concentration curve and QC was duplicated daily before sample analysis. The calibration curves were calculated using $1/x^2$ weighted least squares regression equations. A coefficient of determination (R^2) value >0.99 indicated a good correlation. The peak area ratio, intercept and linear coefficient generated by a representative calibration curve were used to determine the concentrations of the QC and test samples within the calibration range. The accuracy was within ±20% at the LLOQ concentration and within ±15% for other calibration levels.

Intra-day and inter-day precisions, expressed as CV or RSD, were evaluated by analysis of QC for Trp, Kyn and Kyna at the LLOQ, LQC, MQC and HQC, respectively, each as five replicates in three independent analytical runs. As recommendation in FDA and EMEA guideline: Bioanalytical Method Validation required that the CV of the IS-normalized matrix factor (MF) calculated from 6 lots of matrix should not be greater than 15% (6, 7). Acceptable criteria for intra-day and inter-day accuracy are the same as for intra-day and inter-day precision.

The concept of matrix effects was introduced by Matuszewski *et al.* (8), and is defined as the effect of different matrixes on assay accuracy. In the present study, the matrix effect was evaluated by estimating the recovery of analytes spiked into six different healthy human serum samples. To analyze the matrix effect, three types of sample are needed. These are a post-spiked sample set (A), in which Trp, Kyn and Kyna at three QC levels and IS were added to post-extracted drug-free biological specimens, a pre-extracted set (B), which was prepared and processed as described in *Sample extraction procedures*, and an un-extracted sample set (C), in which individual un-extracted samples were diluted directly using mobile phase, as described by Shang *et al.* (9). The matrix effect was calculated by dividing the mean peak areas of (A) by those of (C) and multiplying by 100. The measure of extraction efficiency used in this study is relative recovery, which is the ratio of the average peak areas of (B) to those of (A), multiplied by 100 (10).

The stability of the three analytes was investigated at two QC levels, LQC and HQC. The stability of the stock solutions was analyzed after storing for 6 h at room temperature and after storing for 1, 15, 24 and 36 days at 4°C. The concentrations of Trp, Kyn and Kyna stock solutions after storage were compared with freshly prepared stock solutions. The short-term stability of Trp, Kyn and Kyna was examined after samples had been exposed to laboratory conditions for 6 h at 4°C and after samples had been in the autosampler for 24 h. The long-term stability was evaluated by comparing freshly analyzed samples (control samples) with spiked serum samples stored for 1, 7, 16 or 30 days at −20°C. Freeze-thaw stability of the samples was evaluated over three freeze-thaw cycles, in which the samples were frozen at −20°C for at least 24 h, thawed, and then stored at ambient temperature for 2 h. The concentrations of the QC and stability samples of Trp, Kyn and Kyna were calculated based on the daily calibration curves. Current EMEA guidance stipulates that the RSD for sample stability should be within ±15% of the nominal concentration.

In cooperation with clinicians at Guangzhou Brain Hospital, we collected serum from 26 normal subjects and 54 patients with depression and simultaneously examined Trp, Kyn and Kyna in the samples. All the subjects underwent a complete physical examination and gave written informed consent before the study, following approval from the independent Ethical Committees of Guangzhou Huiai Hospital.

Results and discussion

In the present study, we were used both rapid and suitable protein precipitation method. Undoubtedly, we recognize that solid-phase extraction (SPE) should offer greater precision, however, high cost and low reproducibility are the main drawbacks for SPE method, and liquid–liquid extraction (LLE) is also performed but usually requires a big sample size for better sensitivity. Trp is metabolized sequentially to Kyn and Kyna and the concentrations of Trp and Kyn in human serum are higher than those of Kyna. In an attempt to eliminate interferences between similar drugs, we developed a new method to concentrate Kyna and detection methods for all three substances. Interference, however, remained a problem and, although we have created detection methods for all three substances, we were unable to obtain good precision for Kyna at the LLOQ. Kyn-$^{13}C_4{}^{15}N$ and Kyna-d_5 were selected as IL-ISs, since they were unlikely to give large differences in ionization because of their structural and chromatographic similarity to Kyn and Kyna, respectively.

Comparing the solvents methanol and acetonitrile, we chose a mixture of methanol and water as the mobile phase because of the superior proton donating ability of methanol (11). Ammonium formate (5 mM) is often added to the mobile phase to provide a stable pH for the analytes and to enhance the mass-to-charge, $[M + H]^+$, precursor ion. By optimizing the mobile phase composition, we were able to achieve elution of all analytes within 3–4 min. We evaluated Agilent Eclipse Plus C18 (4.6 × 100 mm^2, 3.5 μm) and Agilent Eclipse XDB-C18 (4.6 × 150 mm^2, 5 μm) columns and found that

the latter column resulted in better peak shapes and better separation. The chromatographic run time was 4.2 min per sample.

Möller *et al.* (12) have validated a method for the analysis of Trp, Kyn and Kyna in plasma but the new method described here was validated by us.

The specificity of the method was demonstrated by obtaining chromatograms of six batches of non-pooled, drug-free human serum (blank human serum treated with activated charcoal) (Figures 2 and 3). Although Trp is present in blank serum, levels are below 20% of LLOQ and the influence of blank serum can be ignored. The retention times for Trp, Kyn, Kyna and ISs were < 4min and no other significant endogenous interference responses were seen in any of the samples. Our assay provided higher sensitivity while use a smaller injection volume, and the LLOQ concentration of Trp, Kyn and Kyna ($n = 5$) were 1 μg/mL, 100 ng/mL and 1 ng/mL, respectively (Table II).

Good linearity of the ratios of analyte peak area to IS peak area was observe in the concentration range 1,000–50,000, 100–5,000 and 1–60 ng/mL for Trp, Kyn and Kyna, respectively. A $1/x^2$ weighting factor was applied since this achieved optimal precision and accuracy. The regression coefficients (R^2) were >0.99 for the serum standard curves. Trp can't be completely removed by treatment with activated charcoal, and there was a peak in blank serum (panel A-2 in Figure 2), but its peak area was below 20% of LLOQ (panel A-3 in Figure 2) and the influence of blank serum can be ignored. It is important that freshly prepared blank serum is used to establish standard curves.

The accuracy of the determination for Trp, Kyn and Kyna, measured by repeated injection of a blank sample after an injection at the ULOQ of the calibration curve or of the LLOQ standard, is 100–115% and no ghost peaks appeared in the blank plasma samples after injection at the ULOQ, suggesting that there is no cumulative carryover.

Accuracy and precision of serum samples at LQC, MQC, HQC and LLOQ concentrations are shown in Table II. The intra- and inter-assay accuracy for four concentration levels of Trp, Kyn and Kyna were in the range 88.20–108.31%. Similar results were obtained for intra- and inter-assay precision of all three analytes; intra-day CVs were 0.55–2.67% and inter-day CVs were 2.80–10.73%. Compared with Trp and Kyn, inter-assay precision for Kyna is higher since the concentration in serum is very low.

Matrix effects, which are also called total or overall matrix effects, arise because of variations in extraction and mass ionization (13) and are one of the biggest challenges for mass detection. In the present method, the matrix effect increases apparent Kyna levels in serum about 260%. The CV of IS-normalized matrix effects of three levels concentration are all less than 6.86% (Table II). Addition of ammonium formate increases the hydrophobicity of the analytes by forming ion pairs with their charged groups and increases their separation from endogenous interferences when using a hydrophobic stationary phase (14).

Extraction recoveries of 98.02 ± 2.61%, 100.28 ± 1.50% and 100.69 ± 2.33% (mean ± SD, $n = 5$) were achieved for Trp at the LQC, MQC and HQC levels (2,000, 8,000 and 40,000 ng/mL),

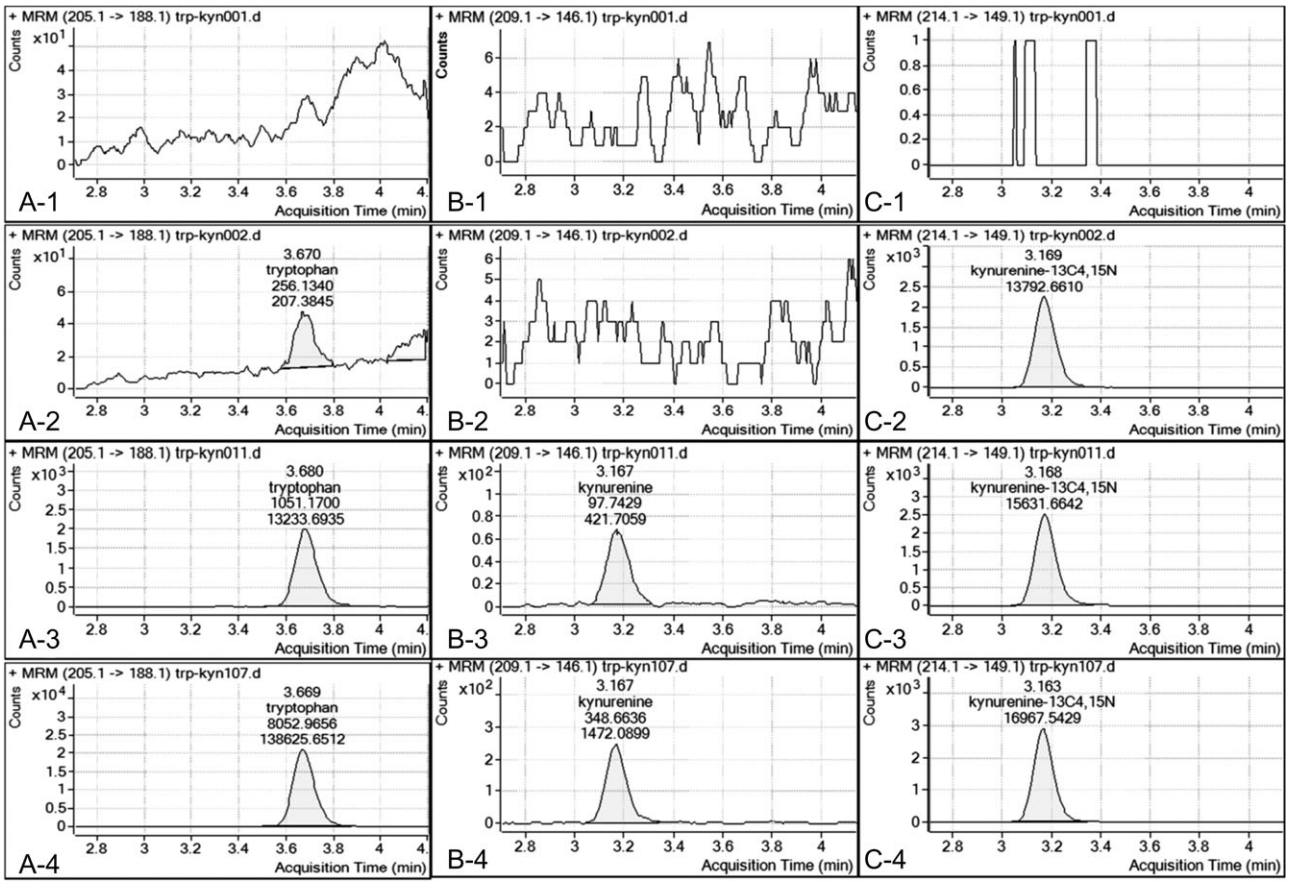

Figure 2. MRM chromatograms of (A) tryptophan, (B) kynurenine and (C) Kynurenine-$^{13}C_4{}^{15}N$. Drug-free serum with activated charcoal (1), drug-free serum spiked with IS (10 μg/mL) (2), LLOQ spiked with IS (3) and a healthy volunteer with IS (4).

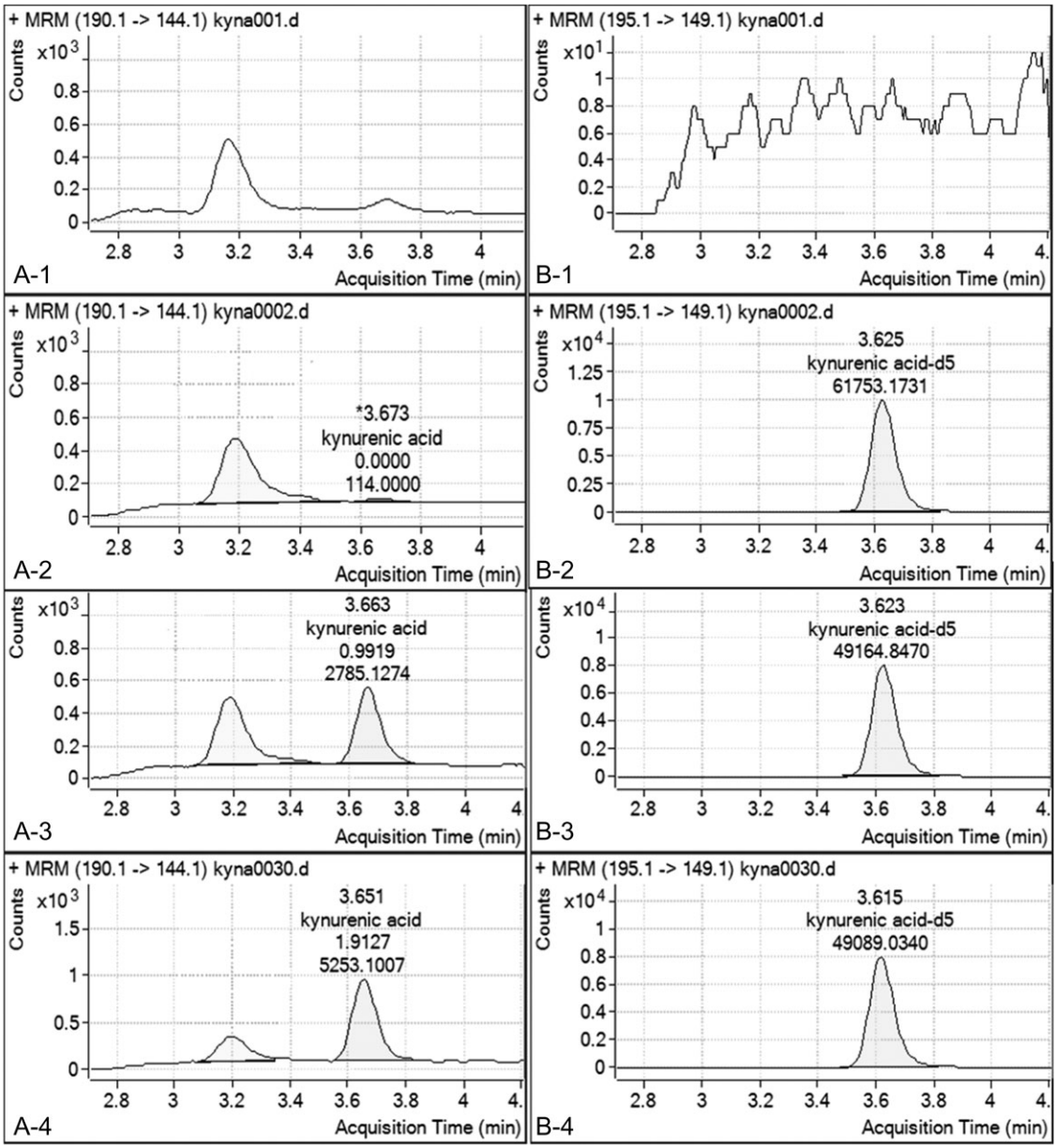

Figure 3. MRM chromatogram of (A) kynurenic acid and (B) Kynurenic Acid-d_5 (150 ng/mL). (1) Drug-free serum, (2) drug-free serum spiked with IS, (3) drug-free serum spiked with kynurenic acid (1 ng/mL) and (4) a healthy volunteer.

Table II. Precision, accuracy, recovery and matrix effect of tryptophan, kynurenine and kynurenic acid

Analytes	Conc. (ng/mL)	Intra-day ($n = 5$)		Inter-day ($n = 15$)		Recovery (mean ± SD, $n = 5$)	Matrix effects (%CV, $n = 6$)
		Accuracy (%)	Precision (%CV)	Accuracy (%)	Precision (%CV)		
Tryptophan	1,000	108.31	1.48	106.79	7.06		
	2,000	97.59	0.55	96.52	4.26	98.02 ± 2.61	3.53
	8,000	88.40	1.03	88.20	2.80	100.28 ± 1.50	
	40,000	103.87	1.88	106.00	4.10	100.69 ± 2.33	4.30
Kynurenine	100	97.77	4.33	98.33	7.60		
	200	106.45	3.28	103.61	5.37	96.20 ± 4.19	5.65
	800	100.63	1.55	98.37	5.70	99.39 ± 2.29	
	4,000	102.62	0.92	100.68	9.85	99.61 ± 1.67	1.40
Kynurenic acid	1	103.28	2.67	103.72	5.80		
	2	100.18	4.08	102.76	10.73	92.78 ± 2.75	6.86
	8	92.68	1.55	95.06	9.32	95.27 ± 1.48	
	50	97.91	1.37	100.48	5.34	100.05 ± 1.09	1.51

Table III. Stability of tryptophan, kynurenine and kynurenic acid in stock solution and human serum under tested conditions (mean ± SD, $n = 3$)

Analytes	Conc. (ng/mL)	Stock solution	Serum sample			
		Short term (6 h, 25°C)	Short term (6 h, 25°C)	Autosampler (24 h, 4°C)	Freeze-thaw (three cycles, −70°C)	Long term (30 d, 4°C)
Tryptophan	2,000	1832.36 ± 7.24	1867.31 ± 47.43	2121.23 ± 20.71	1767.06 ± 68.77	2014.71 ± 94.75
	40,000	42330.65 ± 1094.73	42330.65 ± 1094.73	43506.50 ± 714.09	43068.94 ± 1498.32	45335.92 ± 216.95
Kynurenine	200	200.80 ± 7.35	207.72 ± 1.35	208.77 ± 0.80	217.17 ± 6.63	229.88 ± 3.28
	4,000	4076.90 ± 111.14	4087.42 ± 12.53	3912.31 ± 23.30	4337.97 ± 41.78	4441.98 ± 102.85
Kynurenic acid	2	2.11 ± 0.04	1.94 ± 0.09	1.81 ± 0.04	2.13 ± 0.05	2.16 ± 0.16
	50	45.04 ± 2.68	49.15 ± 1.23	43.16 ± 0.50	51.98 ± 0.54	54.72 ± 0.93

respectively. Kyn and Kyna also had good extraction recoveries. These were 96.20 ± 4.19%, 99.39 ± 2.29% and 99.61 ± 1.67% (mean ± SD, $n = 5$) for Kyn and 92.78 ± 2.75%, 95.27 ± 1.48% and 100.05 ± 1.09% for Kyna at the LQC, MQC and HQC levels, respectively. Relative extraction efficiency achieved using protein precipitation was higher than that achieved using other methods.

Observed stabilities are summarized in Table III. The stock solutions and human serum samples were stable for at least 6 h when stored at room temperature and the concentrations of Trp, Kyn and Kyna in processed samples did not change after 24 h in the autosampler. Over time, the concentration of all three stock solutions gradually decreased, even at low temperatures. However, human serum samples frozen to −20°C for 30 days and subjected to three freeze-thaw cycles were unchanged. There is a little endogenous released of blank serum, but levels are within 20% of LLOQ. According to the required of method in this study and reduce time consume, we were stable in low temperature for one month and at least six month or more rigorous.

We simultaneously detected Trp, Kyn and Kyna in serum samples from 26 normal subjects and 54 patients with depression, using fresh calibration curves. The mean concentrations of Trp, Kyn and Kyna in volunteers were 10,349.24 ± 1,755.05, 375.20 ± 72.82, 5.21 ± 1.89 ng/mL, respectively and, in patients with depression, the mean concentrations were 8,193.45 ± 2,239.36, 305.84 ± 114.74, 4.55 ± 2.31 ng/mL, respectively. The data were analyzed using SPSS and analysis of independent samples *t*-tests showed significantly lower levels ($P < 0.006$) of Trp and Kyn in patients with depression than in normal individuals. There were, however, no significant differences ($P > 0.05$) in Kyna levels between healthy and depressed individuals.

Conclusion

A simple and rapid HPLC–MS/MS method has been developed to quantify Trp, Kyn and Kyna in human serum in a single chromatographic run. A combination of protein precipitation, extraction and chromatographic separation achieved undifferentiated and quantitative recovery of Trp, Kyn and Kyna. Generic protein precipitation can be used for the detection of Trp and Kyn but the sample must be further concentrated for determination of Kyna. The method has been validated in terms of selectivity, sensitivity, carryover, linearity, accuracy, precision, matrix effects, recovery and short and long-term stability. For accurate results, calibration curves should be prepared with fresh blank serum for each analysis. Using the newly developed method, Trp, Kyn and Kyna concentrations were measured in serum samples from 26 healthy human volunteers and 54 patients with depression. The concentrations of Trp and Kyn were lower in serum from depressed individuals than from healthy individuals, but there was no difference in levels of Kyna between the two groups. Concentrations of Trp and Kyn in human serum could thus be used to monitor the effects of antidepressant drugs and predict likely treatment outcomes.

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