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Improved separation and detection of picolinic acid and quinolinic acid by capillary electrophoresis-mass spectrometry: Application to analysis of human cerebrospinal fluid

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"Quinolinic acid (QA)", a metabolite of the kynurenine pathway (KP), is implicated as a major neurological biomarker, which causes inflammatory disorders, whereas there is an increase evidence of the role of picolinic acid (PA) in neuroinflammation. Therefore, there is an urgent need to develop new clinical test for early diagnosis of neuroinflammatory disorders. A comparison is made between three different platforms such as high performance liquid chromatography–electrospray mass spectrometry (HPLC–ESI-MS/MS), nano LC–Chip/ESI-MS/MS, as well as the use of cationic (quaternary ammonium) and anionic (sulfonated) coated capillaries in capillary electrophoresis (CE)-ESI-MS/MS. The comparison revealed that CE-ESI-MS/MS method using a quaternary ammonium coated capillary is the best method for analysis of PA and QA. A simple stacking procedure by the inclusion of acetonitrile in the artificial cerebrospinal fluid (CSF) sample was employed to improve the peak shape and sensitivity of KP metabolites in CE-ESI-MS/MS. The developed CE-ESI-MS/MS assay provided high resolution, high specificity and high sensitivity with a total analysis time including sample preparation of nearly 12 min. In addition, excellent intra-day and inter-day repeatability of migration times and peak areas of the metabolites were observed with respective relative standard deviation (RSD) of less than 2.0% and 2.5%. Somewhat broader variations in repeatability for a 3 independently prepared coated capillary (total 35 runs each) with % RSD up to 3.8% and 5.8% was observed for migration time and peak areas, respectively. Artificial CSF was used as a surrogate matrix to simultaneously generate calibration curves over a concentration range of 0.02–10 μM for PA and 0.4–40 μM for QA. The method was then successfully applied to analyze PA and QA in human CSF, demonstrating the potential of this CE-ESI-MS/MS method to accurately quantitate with high specificity and sensitivity.

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1. Introduction

The bulk of tryptophan (TRYP) not used for protein synthesis is catabolized via kynurenine pathway (KP). Picolinic acid (PA) and quinolinic acid (QA) are two spontaneous metabolites form in KP, which cyclize nonenzymatically through unstable aldehyde intermediates. Alterations KP metabolism have been implicated in the pathophysiology of a variety of neuro-degenerative diseases including Alzheimer's disease, multiple sclerosis, Parkinson's, cerebral malaria, amyotropia lateral sclerosis and HIV infection [1–4]. One endogenous metabolite of L-TRYP catabolism in the KP is PA, which has been reported to possess a wide range of neuro-protective, immunological and antiproliferative affects within the body [5,6]. Another TRYP metabolite, QA is identified as a neurotoxin when its concentrations in the cerebrospinal fluid (CSF) and blood are sufficiently elevated [1,2,4,7]. Hence a reliable and sensitive analytical method need to be developed to quantitate PA and QA concentrations for understanding of their endogenous function in the brain and their potential use as early diagnostic markers for the neurodegenerative diseases. Furthermore, the evaluation of early disease diagnostic markers in CSF seems to be promising because CSF is in direct contact with the CNS, from which normal and pathological substances may diffuse without any significant hindrance [8].

Analysis of CSF sample by capillary electrophoresis-mass spectrometry (CE-MS) and high performance liquid chromatography (HPLC)–MS is troublesome because they often show great diversity and complexity. In addition, to the presence of salts, this biological sample usually includes proteins, cells, and carbohydrates etc., all making the analysis more complex. Numerous HPLC–MS [9–13] and gas chromatography (GC)–MS [14,15] methods for separation and detection of tryptophan (TRYP) metabolites in

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biological samples have been reported. However, there are few reports on the analysis of TRYP metabolites in CSF [14]. Most of the aforementioned HPLC–MS techniques are time consuming, require gradient elution or specialized chromatographic column for separation of PA and QA and other KP metabolites. On the other hand, the use of GC–MS requires sample derivatization to yield volatile and thermally stable analytes.

CE has been extensively developed as a complementary analytical technique to conventional HPLC in the past decade [16–19]. In contrast to HPLC–MS and GC–MS, CE-MS is particularly suitable for analyzing a wide range of small polar and charged metabolites [20]. To-date, only two CE-MS methods have been reported to analyze TRYP metabolites [21]. In our recent studies [22], a CE-MS method was developed for the simultaneous analysis of the TRYP metabolites in the multi-enzyme system. However, the run time, peak shape and detection sensitivity of TRYP metabolites by the CE-MS method still need to be improved for its routine and efficient analysis in clinical samples.

The use of covalently coated capillaries is a promising approach to minimize the variability of migration time and peak area on bare fused-silica (BFS) capillary in CE and CE-MS [23,24]. In this article, we focused on the development of CE-electrospray ionization (ESI)-MS/MS method for simultaneous separation and quantitation of the PA and QA in human CSF. First, R_s and S/N of PA and QA using three hyphenation techniques such as HPLC–ESI-MS/MS, nano-HPLC–ESI-MS/MS and CE-ESI-MS/MS with cationic (quaternary ammonium) or anionic (sulfonated) coated capillaries were compared. Next, the feasibility of employing acetonitrile (ACN) in the artificial CSF sample was tested to improve the peak shapes and on-column preconcentration of PA and QA in CSF sample containing inorganic salts [25–27]. After screening for appropriate internal standard, simultaneous quantitation was performed in high throughput fashion resulting in a highly sensitive and repeatable CE-ESI-MS/MS methodology for the determination of PA and QA in human CSF.

2. Materials and methods

2.1. Chemicals and reagents

2,2′-Azobis (2-methylpropionitrile) (AIBN), 3-(trimethoxysilyl) propyl methacrylate (γ-MAPS), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), (vinylbenzyl) trimethylammonium chloride (VBTA), 3-hydroxypicolinic acid, 2-hydroxynicolinic acid, tyramine, tryptamine, tyrosine and baclofen were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), anhydrous methanol (MeOH), ammonium carbonate ($(NH_4)_2CO_3$) and analytical-grade ammonium acetate (as 7.5 M NH_4OAc solution) were also purchased from Sigma–Aldrich. Ammonium hydroxide (NH_4OH), formic acid (FA) and acetic acid (HOAc) were obtained from Fisher Scientific (Springfield, NJ, USA). Quinolinic acid (QA), >98%, and picolinic acid (PA), >99.9% were purchased from VWR International (Atlanta, GA, USA). Water used in all experiments was triply deionized and obtained from Barnstead Nanopure II water system (Barnstead International, Dubuque, IA, USA). The bare fused-silica capillary (BFS) capillary (375 μm O.D., 50 μm I.D.) was obtained from Polymicro Technologies (Phoenix, AZ, USA). Human cerebrospinal fluid (CSF) was provided by Dr. Andrew Miller of Emory University.

2.2. Preparation of standard solutions and artificial CSF

The stock solutions of QA, PA, 3-hydroxy-PA, 2-hydroxynicolinic acid, tyramine, tryptamine, tyrosine and baclofen were prepared at 10 mM in triply deionized water. Artificial CSF was prepared according to a procedure from DURECT Corporation (Cupertino, CA) [28]. Briefly, 8.66 g sodium chloride, 0.224 g potassium chloride, 0.206 g calcium chloride, and 0.163 g magnesium chloride were dissolved in triply deionized water and prepared in 0.5 L of volumetric flask (solution A). The solution B was prepared by dissolving 0.214 g sodium phosphate dibasic heptahydrate and 0.027 g sodium phosphate monobasic monohydrate in 0.5 L of triply deionized water in another volumetric flask. Solutions A and B were then mixed in equal proportions before spiking with PA and QA.

2.3. CE-ESI-MS/MS analysis

2.3.1. Preparation of coated capillaries

The schematic diagram on the preparation of the negatively charged (AMPS) and positively charged (VBTA) coated capillaries is shown in Fig. 1. First, the capillary was flushed under vacuum with water, 1.0 M sodium hydroxide, water, 0.1 M hydrochloric acid, water, and MeOH for 0.5, 3, 0.5, 0.5, 0.5, and 0.5 h, respectively. Next, the silanization of the capillary inner wall with γ-MAPS was performed as described elsewhere [29]. Briefly, The γ-MAPS was dissolved in MeOH at 1:1 (v/v), then filled through the above pretreated capillary under vacuum. The filled capillary was sealed with rubber septa and kept at 60 °C for 20 h in a GC oven. The capillary was rinsed with MeOH to flush out the residual reagents. Next, it was dried by nitrogen gas at 60 °C for 3 h. Following silanization of the capillaries, the reaction mixtures consisting of charged monomers (20.0 mg AMPS or 40.0 mg VBTA), 10.0 mg AIBN, and 2.5 mL anhydrous MeOH was sonicated for 20 min to obtain a homogeneous solution and then filled into the pretreated capillary. After the pretreated capillary was completely filled with the reaction mixture, it was sealed at both ends with rubber septa. The sealed capillary was reacted at 60 °C for 24 h and then flushed with MeOH to remove the unreacted materials.

2.3.2. Buffer and sheath liquid preparation

The background electrolyte (BGE) was prepared by dissolution of the appropriate salt [NH_4OAc, or $(NH_4)_2CO_3$] in triply deionized water followed by pH adjustment with 14.8 M NH_4OH to the desired pH values using an Orion 420A pH meter (Beverly, MA, USA). The sheath liquid was prepared by mixing aqueous solutions with appropriate volume ratio of MeOH (e.g., 80/20 (v/v) of MeOH/H_2O containing 40 mM HOAc. The running buffers and sheath liquid were filtered with 0.45 μm PTFE membranes and degassed for 20 min before use.

2.3.3. CE-ESI-MS/MS

The CE-ESI-MS/MS experiments were performed on an Agilent CE system interfaced to an Agilent 6410 series triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA) equipped with an Agilent CE-MS adapter kit (G1603A), and an Agilent CE-ESI-MS sprayer kit (G1607). The sheath liquid was delivered using an Agilent 1100 series isocratic HPLC pump with a 1:100 splitter. Nitrogen was used as a nebulizing gas, drying gas and collision gas. The Agilent ChemStation was employed for instrument control. The Agilent MassHunter Work-station (version B.02.05) was used for data acquisition, qualitative and quantitative analysis.

The CE-ESI-MS/MS experiments were carried out with an AMPS coated capillary (60 cm total length) or a VBTA capillary (60 cm total length). Both capillaries were rinsed with the running buffer for 30 min before its first use. In addition, the capillaries were flushed with the running buffer for 4 min after each CE-MS run. Cathodic electroosmotic flow (EOF) was induced on the AMPS capillary by applying a voltage of +15 kV, whereas anodic EOF was generated on VBTA capillary at −15 kV. In both cases, the capillaries were subjected to a voltage ramp of 3 kV/s. A 100 mbar of internal pressure was applied to the coated capillaries during electrophoresis

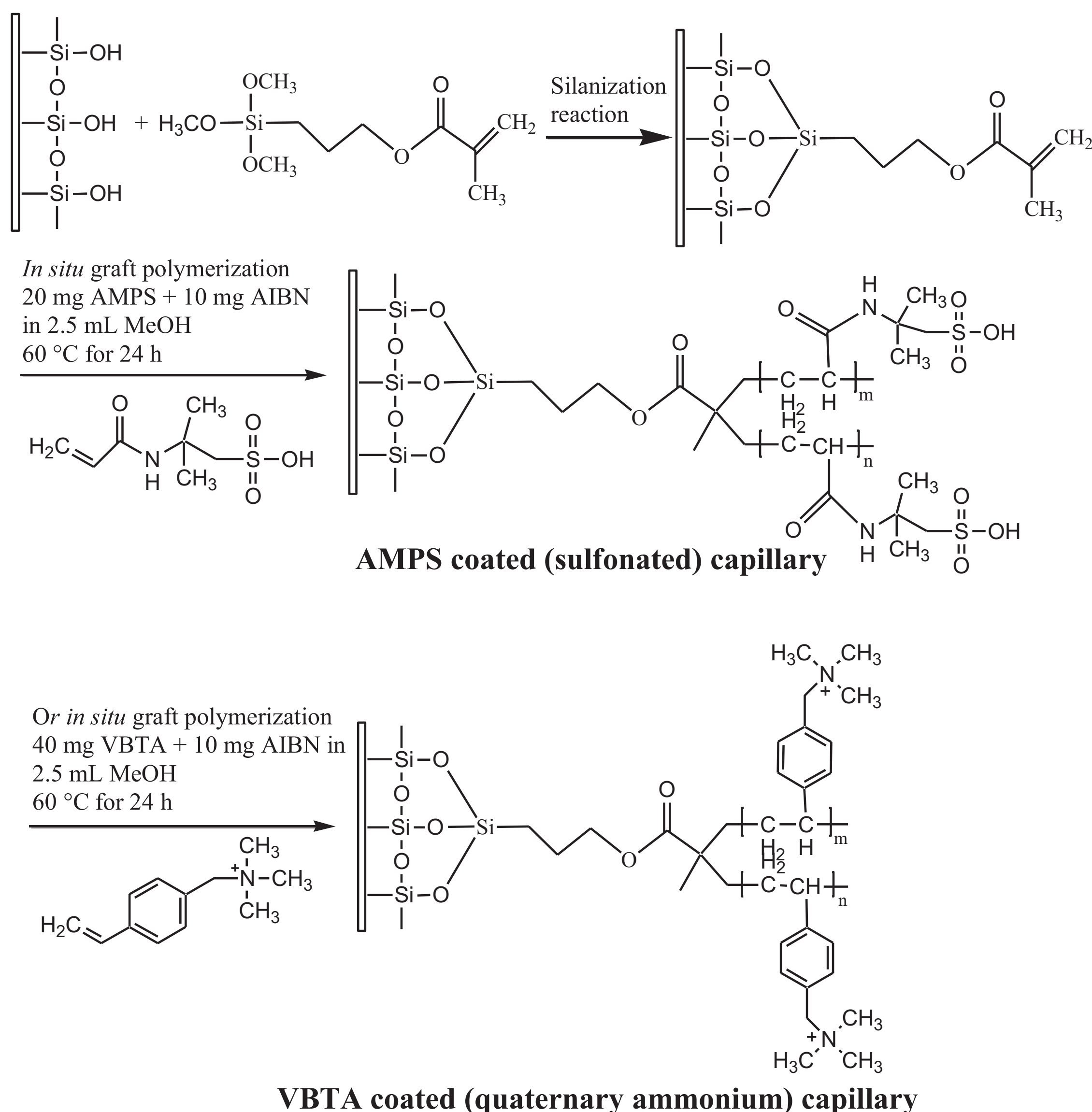

Fig. 1. Schematic for the preparation of sulfonated (AMPS) and quaternary ammonium (VBTA) coated capillaries.

runs. To ensure a good run-to-run repeatability, a fresh buffer vial was always used for each run. Analytes were kept at 10 °C in the autosampler and injected hydrodynamically at the pressure of 5 mbar for 100 s. Unless otherwise stated, the following ESI-MS/MS conditions were used: sheath liquid, MeOH/H_2O (80/20, v/v) containing 40 mM HOAc; sheath liquid flow rate, 5 μL/min; capillary voltage, +3500 V; drying gas flow rate, 5.0 L/min; drying gas temperature, 200 °C; nebulizer pressure, 7 psi; collision cell gas (N_2) pressure was ~30 psi. The multiple reaction monitoring (MRM) in the positive ion mode was used. The Agilent MassHunter Optimizer software (V. B.02.00) was used for auto optimization of analyte-dependent multiple reaction monitoring (MRM) transitions and other mass spectrometric parameters (summarized in Table 1). The noise level and S/N ratio were calculated by the Agilent Mass Hunter Workstation (version B.02.05). The S/N ratio was calculated in the extracted ion chromatogram by taking the ratio of the peak height and noise, where the noise was measured as six times the standard deviation of the linear regression. The value for noise used in this work was determined in the time range closest to the peak.

Table 1
MRM transitions, fragmentor voltage (FV) and collision energy used for the studied analytes.

Compound	Precursor ion (m/z)	Product ion (m/z)	FV (V)	Collision energy (eV)
QA	168.0	78.4	58	25
PA	124.0	78.1	64	21
3-Hydroxypicolinic acid	140.0	94.2	78	21
2-Hydroxynicotinic acid	140.0	94.1	74	21
Tyramine	138.1	121.2	64	5
Tryptamine	161.1	144.2	49	5
Tyrosine	182.1	91.1	64	29
Baclofen	214.1	151.1	93	13

2.3.4. Calibration curves and sample preparation

Calibration solutions were prepared by spiking a 100 μL of blank artificial CSF with 2 μL of 20, 37.5, 50, 100, 200, 500, 1000 and 2000 μM of QA stock solutions, 2 μL of 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 μM PA stock solutions, and 2 μL of 100 μM IS solution. The calibration curves was prepared by diluting the stock solution at the desired concentrations of 0.4, 0.75, 1, 2, 4, 10, 20 and 40 μM for QA 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 μM for PA and a constant concentration of 2 μM for IS. Prior to analysis, 50% (v/v) ACN was added to each working calibration solutions to achieve sample stacking. The calibration curves for QA and PA were obtained by plotting the peak area ratio of each analyte to the IS versus concentrations of the PA and QA in artificial CSF. The CSF patient samples were stored at −80 °C. Before use, the samples were thawed at room temperature. An aliquot of 100 μL of CSF patient sample was spiked with 2 μL of 100 μM IS and 102 μL of ACN was added to obtain a sample containing 50% (v/v) ACN.

3. Results and discussion

3.1. Comparison of HPLC, nano LC–Chip and CE coupled to ESI-MS/MS for the simultaneous separation of PA and QA

In recent years, effort has been made to improve both separation and detection techniques for the analysis of TRYP metabolites using HPLC–MS [9–13], GC–MS [14,15] and CE-MS [21,22]. Moreover, recently, chip-based nanoLC–MS has generated increased interest because of the minimal sample consumption and high sensitivity [30–32]. To develop a selective and sensitive analytical technique for the analysis of PA and QA in CSF sample, three liquid-based hyphenation techniques such as HPLC–ESI-MS/MS, nano LC–Chip/ESI-MS and CE-ESI-MS/MS, were compared for such analysis (Fig. 2). Several differences are worth mentioning. First, partial resolution in HPLC–MS/MS and co-elution of PA and QA in nano LC–Chip but fast run times were observed in both HPLC–MS/MS and nano LC–Chip/ESI-MS/MS (Fig. 2A and B). Second, both PA and QA are polar charged compounds, hard to retain and separate by the common C-18 reversed-phase LC columns, in which the retention and separation mechanism rely upon the analytes hydrophobicity. Interestingly, the S/N values of PA and QA obtained in HPLC–MS/MS and nano LC–Chip/MS/MS were lower compared to CE-MS/MS. This may be attributed the presence of aqueous mobile phases, which are often required to achieve adequate retention and low separation of polar analytes, but results in decreased sensitivity in ESI-MS [33,34]. Third, as shown in Fig. 2C and D, a reversal of elution order of analytes on AMPS and VBTA coated capillaries was observed with two CE-MS/MS approaches. Therefore, selectivity changes based on mobility differences can be implemented simply by switching between capillary that contain different functional group on its inner surface.

On the VBTA coated capillary, anodic electroosmotic flow (EOF) was generated by applying a voltage of −15 kV. Because QA contains one more ionizable carboxyl group than PA (see Fig. 2 inset), it is not too surprising that QA acquires more effective negative charge than PA. Hence, its electrophoretic mobility toward the cathodic (detector) end will be larger than PA, thus leading to faster migration time. As for the AMPS coated capillary, in which the cathodic EOF was generated at +15 kV, the directions of EOF and electrophoretic mobility of analytes are reversed. Thus, longer migration time, reversal migration order and asymmetric peak shape were observed. Comparing the separation on the two coated capillaries, clearly shows that good peak shapes, shorter run time and high *S/N* of analytes were obtained on the VBTA coated capillary at the mobile phase of 15 mmol/L $(NH_4)_2CO_3$ pH 11.0 (Fig. 2D). Because our ultimate aim is to increase the detection sensitivity of

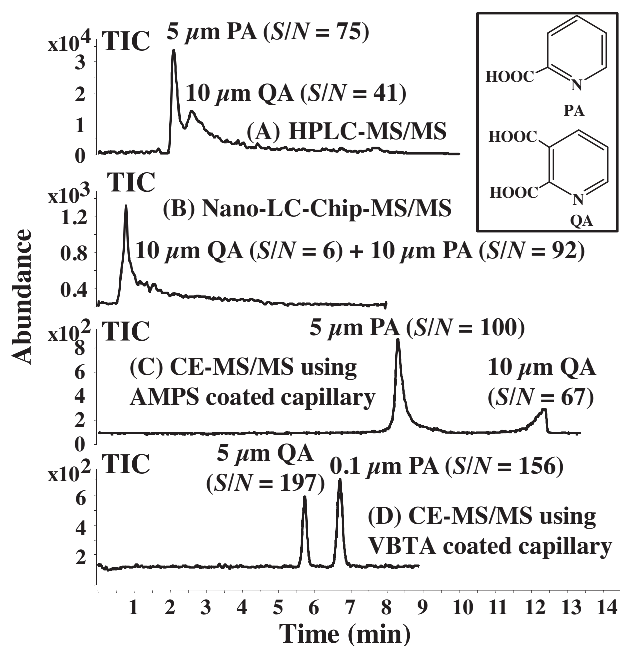

Fig. 2. Comparison of HPLC–ESI-MS/MS (A), nano-LC–Chip/ESI-MS/MS (B) and CE-ESI-MS/MS (C and D) for the simultaneous separation of quinolinic acid (QA) and picolinic acid (PA) dissolved in triply deionized water. Conditions for HPLC–ESI-MS/MS and nano-LC–Chip/ESI-MS/MS are described in the supplemental. CE-ESI-MS/MS using AMPS coated capillary (C) and VBTA coated capillary (D) were performed on 60 cm long (50 μm I.D.). Running buffer, 15 mmol/L NH_4OAc, pH 7.0, +15 kV and 15 mmol/L $(NH_4)_2CO_3$ pH 11.0, −15 kV was used on AMPS and VBTA coated capillary, respectively. Capillary temperature, 20 °C. Injection, 5 mbar, 100 s. A 100 mbar of internal pressure was applied to the coated capillaries during electrophoresis runs. Spray chamber parameters for C and D are described in Section 2.3.3.

PA and QA in biological samples, CE-ESI-MS/MS with a VBTA coating was considered better than AMPS coating.

3.2. Optimization of CE-ESI-MS/MS assay

3.2.1. Effect of % (v/v) ACN in artificial CSF samples

Most of the biological samples, such as CSF and urine, contain high levels of salt, which may cause band broadening with decrease resolution and sensitivity in CE. Thus, it is important to optimize the analyte separation in artificial CSF sample, which has a composition similar to one would expect in human CSF. Stacking by the inclusion of ACN in the sample offers an interesting approach for the on-column preconcentration of analytes in CE-MS. First, the addition of ACN to the sample would eliminate the effect of protein adsorptions and enable tolerance of high salt concentration [35]. Second, because ACN has low conductivity, it can produce stacking due to the high field strength, especially for the analysis of CSF samples with high salt concentrations [25–27]. In the previous reports [25,36], a concentration of ACN above 50% (v/v) was considered essential for CE stacking. In this study, we explore simple methods based on ACN mediated stacking to improve the peak shape and sensitivity of PA and QA in the presence of high levels of salts. As shown in Fig. 3, upon increasing the concentration of ACN from 0% (v/v) to 50% (v/v) in the artificial CSF samples improve peak sharpness, consequently S/N of the analytes improved. We hypothesized from the electropherogram that the unknown peaks in low % (v/v) ACN may be resulted due to unusual split of PA peaks and not due to the impurity in the PA or QA standard solution. To test this hypothesis, the blank artificial CSF sample with 50% (v/v) ACN was analyzed by CE-MS/MS. The baseline in the extracted ion chromatograms for PA and QA was clear, and no impurity peaks were observed in the

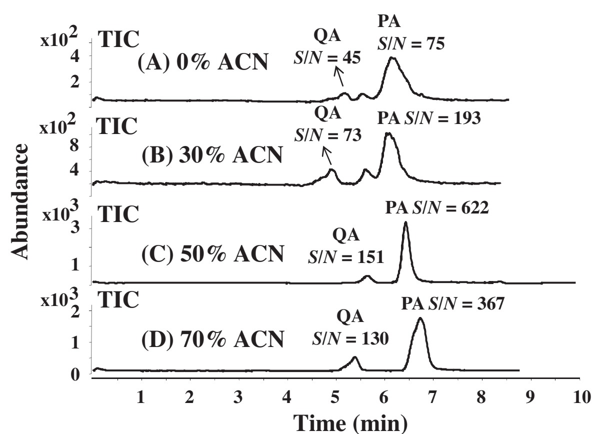

Fig. 3. Effect of % (v/v) ACN in artificial CSF samples by CE-ESI-MS/MS using VBTA coated capillary. Analytes: 5 μM QA and 1 μM PA spiked in artificial CSF with different % (v/v) ACN: (A) 0% (v/v) ACN; (B) 30% (v/v) ACN; (C) 50% (v/v) ACN; (D) 70% (v/v) ACN. Other conditions are the same as described in Fig. 2D.

chromatogram (Fig. S1). However, the peak broadening at 70% (v/v) ACN resulted in a slight decrease in S/N. Nevertheless, the results showed that the PA and QA are well separated and the S/N of PA and QA are significantly improved by 8.3 and 3.4 times, respectively when 50% (v/v) is added to the artificial CSF sample.

3.2.2. Optimized separation of PA, QA and internal standard

The use of an internal standard (IS) in CE-MS eliminates injection volume-related sources of error and leads to dramatically improved precision in bioanalytical assay of metabolites. The main requirement is that the IS must not be the same as one of the endogenous component in the sample. In addition, IS must be structurally similar, readily available, and provide a good peak shape. The use of IS is commonly practiced in ESI-MS quantitation due to the day-to-day variation in abundance. Fig. 4 showed the simultaneous separation of PA and QA with six possible IS spiked in the artificial CSF sample. Clearly, only tyramine showed good peak shape and was well separated from PA and QA. Thus, it was chosen as the optimum IS.

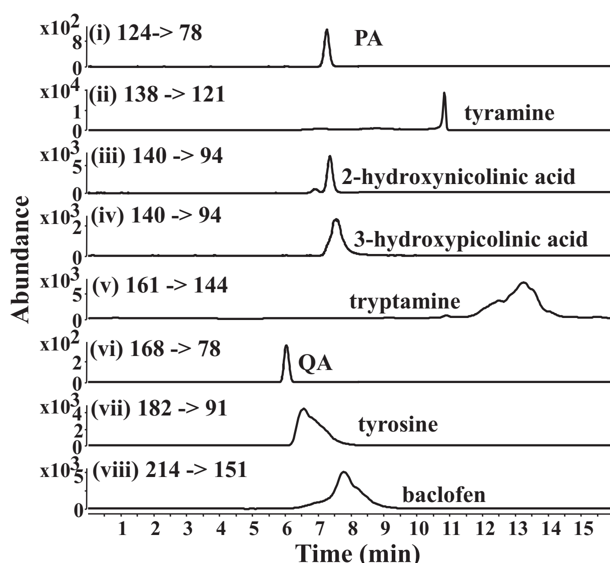

Fig. 4. Separation of PA and QA with various choices of internal standards. Analytes: 5 μM concentration for QA and tyrosine, 1 μM concentration for PA, 3-hydroxypicolinic acid, 2-hydroxynicolinic acid, tyramine, tryptamine and baclofen spiked in artificial CSF with 50% (v/v) ACN. Other conditions are the same as described in Fig. 2D.

Using this IS the artificial CSF could be analyzed within 11 min by CE-ESI-MS/MS (Fig. S2A).

3.2.3. Limits of detection and quantitation

As shown in the inset electropherogram (Fig. S2B), the limit of quantitation (LOQ) of PA and QA in the artificial CSF were 20 nM and 400 nM, respectively (based on S/N of 10:1). The limit of detection (LOD) of PA and QA were 10 nM and 200 nM, respectively (based on S/N of 3:1). In this study, the VBTA coated capillary generates anodic EOF, which migrates in the same direction with the mobility of the negatively charged PA and QA, thus shorting the migration time and improving the peak shape, consequently taller peaks with higher S/N were observed. Furthermore, the use of a triple quadrupole mass spectrometer improves the sensitivity of the TRYP metabolites compare to single quadrupole. This is because triple quadrupole with two mass filters and a collision cell between them drastically reduces noise resulting in higher detection sensitivity. To the best of our knowledge, there is only one published work on the analysis of PA and QA using CE-TOF [21]. The authors of this work [21] reported LOD of several TRYP metabolites to be as low as 20 nM but did not mention the LOD of PA and QA. Hence, we were unable to compare the LOD of PA and QA with others. However, very recently, we develop an enzymatic CE-MS assay for PA and QA production [22]. Compared to the previous CE-ESI-MS method developed by our group [22], the LOD in this study is ~3800 folds lower for PA and 50 fold lower for QA.

3.2.4. Repeatability of migration times and peak areas

To improve the variability of migration times and peak areas as well as to prevent capillary tip erosion in CE-MS with a BFS capillary, the use of chemically modified covalently coated capillary is a promising approach [23,24]. The repeatability of the CE-ESI-MS/MS method with the VBTA coated capillary (Fig. 1) illustrated by analyzing the artificial CSF sample spiked with PA, QA and IS. The represented electropherograms for run numbers 1, 15, 30 and 45 under the optimized CE-ESI-MS/MS conditions are shown in Fig. S3. The % RSD values of the migration times and peak area ratios of the analytes using the same coated capillary are shown in Table 2. Excellent intra-day and inter-day repeatability of migration times and peak areas of the metabolites were observed with respective % RSD of less than 2.0% and 2.5%. Somewhat broader variations in the capillary-to-capillary repeatability for a total of 3 independently prepared capillaries (35 runs each) with % RSD up to 3.8% and 5.8% was observed for migration times and peak areas, respectively. Therefore, all intra capillary and inter capillary data suggests that the CE-ESI-MS/MS method using VBTA coated capillary was feasible for the determination of PA and QA in artificial CSF samples.

3.3. Analysis of CSF sample

Human CSF typically contains endogenous TRYP metabolites, which would interfere with its use as a blank matrix in a bioanalytical assay. It is also comparatively difficult to obtain CSF from humans in the volumes necessary for validation and conducting assays on a routine basis. Based on these limitations, the artificial CSF was used as the blank matrix in this study. First, the calibration curves were set up to simultaneously quantitate PA and QA in the artificial CSF. The peak area ratios of each analyte PA/tyramine (IS) and QA/tyramine (IS) were plotted versus the concentrations of PA and QA (Fig. S4). The calibration curves (in artificial surrogate matrix) provided good linearity for at least three orders of magnitude. For PA and QA, the linearity range from 0.02 to 10 μM (R^2 = 0.9958) and 0.4 to 40 μM (R^2 = 0.9970), respectively.

The developed CE-ESI-MS/MS method using the VBTA coated capillary was finally applied to the simultaneous determination of concentrations of PA and QA in the patient CSF sample. A

Table 2
Repeatability of the migration times and peak area ratios on the VBTA capillaries.[a]

	QA (% RSD)		PA (% RSD)		IS (% RSD)
	Time	Area ratio$_{QA/IS}$	Time	Area ratio$_{PA/IS}$	Time
Intra-day and inter-day repeatability					
Day 1 (n = 15)	1.5	1.7	1.7	2.2	1.9
Day 2 (n = 15)	1.3	1.6	1.6	2.3	1.8
Day 3 (n = 15)	1.4	1.8	1.8	1.8	1.8
n = 45	1.4	2.4	1.7	2.4	1.8
Capillary-to-capillary repeatability					
Capillary 1 (n = 35)	2.2	5.0	2.6	5.6	3.8
Capillary 2 (n = 35)	2.0	4.1	3.1	4.7	3.6
Capillary 3 (n = 35)	1.9	4.1	2.8	5.1	3.7
Capillary to capillary (n = 105)	2.1	5.4	3.0	5.8	3.8

[a] All the conditions are the same as in Fig. 5A.

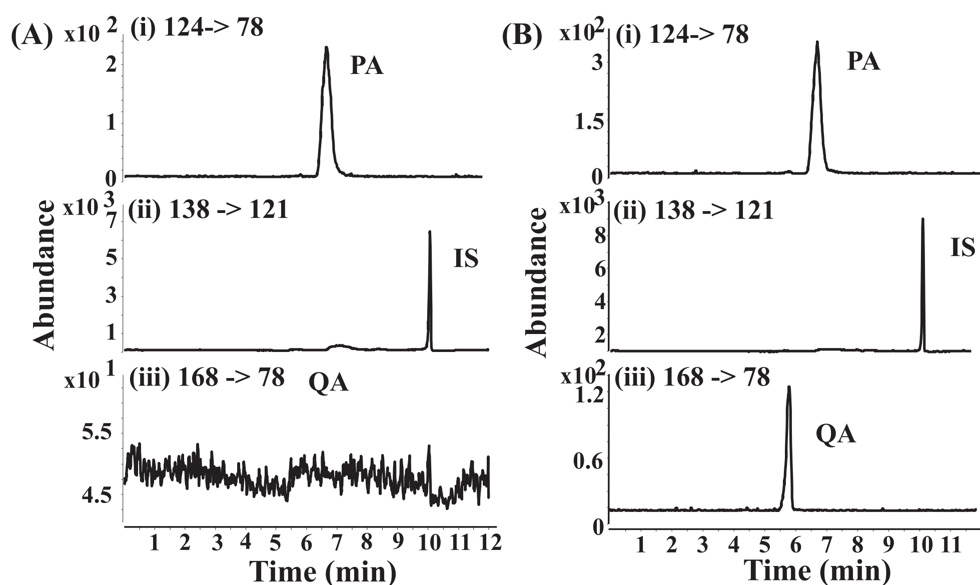

Fig. 5. Electropherograms of (A) CSF patient sample and (B) 1 μM QA spiked in CSF patient sample. Analytes: (A) 1 μM IS spiked in CSF patient sample dissolved in 50% (v/v) ACN and (B) 1 μM QA and IS spiked in CSF patient sample dissolved in 50% (v/v) ACN. Other conditions are the same as in Fig. 2D.

representative electropherogram of the patient CSF sample showed good selective separation of PA from IS and clear background (Fig. 5A). The measured PA concentration (obtained from the slope of the calibration curve of artificial CSF sample) suggested 1002 ± 25 nM (n = 3) was in the patient CSF sample, but concentration of QA was below the LOQ. To evaluate the developed CE-ESI-MS/MS method using the VBTA coated capillary for the determination of QA, 1 μM QA was spiked into the same CSF samples and analyzed in the same manner as Fig. 5A. The obtained electropherograms (Fig. 5B) is highly comparable to Fig. 5A with good peak shape and repeatable migration time. Therefore, our results demonstrate that this CE-ESI-MS/MS method using the VBTA coated capillary is very suitable for the sensitive analysis of PA and QA in human CSF, which unlike artificial CSF contains multiple potentially interfering factors including proteins, carbohydrates, and cells.

4. Conclusions

Compared to HPLC–ESI-MS/MS and nano LC–Chip/ESI-MS/MS, the CE-ESI-MS/MS method using the VBTA coated capillary was the best method because it provided better separation selectivity, symmetrical peak shapes and the highest S/N essential for analysis of both PA and QA. Moreover, the combined use of a VBTA coated capillary and a triple quadrupole MS provided a simple hyphenation method, which produces high repeatability of migration times and peak areas for selective and sensitive MS/MS detection. To improve the peak shapes in artificial sample, simple ACN stacking method was used, which provided higher efficiency both PA and QA. The CSF samples preparation in this study is also significantly less time consuming than HPLC and GC methods. The results suggest that the developed CE-ESI-MS/MS method with the VBTA coated capillary has the potential to provide an efficient diagnostic tool to measure the tryptophan metabolites of KP at trace levels in human CSF samples. The opportunity to determine the relationship between central CSF and peripheral KP metabolites will ultimately allow the substantiation of specific peripheral KP pathway metabolites as surrogate markers of KP activation in the brain, thereby providing early clinical indicators for potential therapeutic intervention. Future studies will concentrate on the development of clinical correlation, which may lead to the development of a simple blood test for the analysis of KP metabolites as possible diagnostic and therapeutic biomarkers for neuroinflammatory disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.09.085.

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