Method Article

Simultaneous determination of tryptophan, 5-hydroxytryptophan, tryptamine, serotonin, and 5-HIAA in small volumes of mouse serum using UHPLC-ED

Anthony Gallegos, Roslyn Rivkah Isseroff

University of California, Davis

Abstract

In this paper we report a simple and efficient method for the concurrent analysis of tryptophan, 5-HTP, tryptamine, serotonin, and 5-HIAA in mouse serum using UHPLC-ED after protein precipitation and dilution. These compounds are neuroactive and are of interest in studies of mood and behavior; They are also biomarkers for the presence of neuroendocrine tumors and are used in the diagnosis of these cancers. After a brief series of validation experiments, this method was applied to serum from mouse behaviour experiments.

• A convenient UHPLC method with electrochemical detection for concomitant analysis of the serotonin pathway in serum, including, for the first time, tryptamine.
• The method met all performance criteria established for use in our lab and was applied in rodent experiments.

© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Article Info

Method name: Chromatographic analysis of tryptophan, 5-hydroxytryptophan, tryptamine, serotonin, and 5-HIAA in small volumes of mouse serum

Keywords: Serotonin, Biogenic amines, Indoleamines, Chromatographic analysis, Electrochemical detection, Serum measurement, Protein precipitation, Neuroendocrine tumors, Mood, Behavior

Article history: Received 18 August 2021; Accepted 18 January 2022; Available online 20 January 2022

* Corresponding author.
E-mail address: rriseroff@ucdavis.edu (R.R. Isseroff).

https://doi.org/10.1016/j.mex.2022.101624
2215-0161 © 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Specification table

| Subject Area | Biochemistry, Genetics and Molecular Biology |
|--------------|---------------------------------------------|
| Analytical Chemistry | Chromatographic analysis of tryptophan, 5-hydroxytryptophan, tryptamine, serotonin, and 5-HIAA in small volumes of mouse serum |

| Name and reference of original method | N/A |
| Resource availability | Reagents: |
| | • Acetonitrile, gradient grade (Sigma Aldrich, St. Louis, Missouri) |
| | • Methanol, gradient grade (Sigma Aldrich) |
| | • Citric acid monohydrate, for analysis (Acros Organics, Belgium) |
| | • EDTA disodium salt dihydrate, for analysis (Acros Organics) |
| | • Sodium hydroxide 50%, for HPLC (Honeywell (Fluka), Charlotte, North Carolina) |
| | • Perchloric acid 70%, for analysis (Acros Organics) |

Chromatography:

| | ALEXYS Neurotransmitters Analyzer (Antec Scientific, Netherlands) |
| | Acquity UPLC BEH C18 column, 1.0 mm x 100 mm, 1.7 um particle diameter (Waters, Ireland) |
| | Clarity CDS (DataApex, Prague, Czech Republic) |

Background

The serotonin metabolic pathway consists of the amino acid precursor L-tryptophan, the intermediates 5-hydroxytryptophan (5-HTP) and tryptamine, serotonin (5-HT), and a carboxylated metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) (Fig. 1). Serotonin is present at high concentrations in blood and is structurally related to melatonin and kynurenine, although these compounds play disparate roles in the body. The serotonin pathway has been most widely studied in the contexts of mood regulation and oncology.

The physiological roles and diagnostic potential for these pathway metabolites have been examined in a number of clinical scenarios. In the brain, serotonin is essential to signaling in serotonergic neurons and is related to mood and behavior. On this basis, one widely used approach to treat major depressive disorder (MDD) is through selective serotonin reuptake inhibitors (SSRIs) which increase extracellular concentrations of serotonin in the brain [1,2] but have also been shown to reduce blood serotonin levels in depressed patients [3,4]. Measurement of plasma levels of serotonin has been used to predict response to SSRI treatment in patients with MDD [3,4]. Although the mechanism of action of SSRIs has not yet been fully elucidated, recent theories have focused on modulation of 5-HT receptor expression or sensitivity rather than a direct change in localized 5-HT concentrations [3,5]. 5-HTP, a precursor of serotonin and intermediate in its conversion from tryptophan, has shown therapeutic potential for some depressed individuals through an apparently different mechanism. After one week of loading with oral 5-HTP, depressed patients showed elevated serum serotonin levels along with improvement of depressive symptoms [6]. Blood 5-HIAA concentrations have been correlated to the severity of depression in individuals with COPD [7]. Tryptamine also possesses demonstrated neuroactive properties [8] although a correlation between serum tryptamine and mood has not been established.

Besides their roles in mood regulation, 5-HTP, serotonin, and 5-HIAA levels in the blood are key biomarkers for some types of neuroendocrine tumors and are used during diagnosis [9–12]. Carcinoid tumors are slow growing malignant or non-malignant masses in the digestive tract that secrete hormones such as 5-hydroxytryptophan and serotonin, as well as catecholamines [13–15]. The overproduction of hormones results in carcinoid syndrome, with symptoms including skin flushing,
Fig. 1. The metabolic pathway for serotonin in mammals.

facial lesions, diarrhea, difficult breathing, and elevated heart rate [14,16]. The elevation of tryptophan metabolites in carcinoid tumor patients results from diversion of dietary tryptophan which normally is used in large part for the production of niacin [17]; Thus, carcinoid syndrome is sometimes accompanied by low blood tryptophan (associated with elevated cortisol and irritability) [18] and by pellagra (low blood niacin) [16,17]. Tryptamine is not routinely measured in blood and to our knowledge no chromatographic method for blood analysis has been reported. However, concentrations of tryptamine between 31.2 – 124.8 nM have been detected in the blood of healthy individuals [19] and elevated levels reported in a patient with carcinoma [20].

Several methods have been reported for measurement of serotonin in blood, either alone [21–30] or in combination with 5-HIAA [31]; 5-HTP and 5-HIAA [32]; tryptophan [33,34]; or tryptophan and 5-HIAA [35,36]. Methods also exist to independently measure blood 5-HIAA [37] and tryptophan [38]. Only two methods are reported for measurement of the entire serotonin pathway in blood, excluding tryptamine: by gradient reverse-phase LC with fluorescence detection after online solid-phase extraction (SPE) [39], and by isocratic reverse-phase LC with amperometric detection after acidification with 0.2 N HClO₄ and washing with chloroform [40]. Thus, the currently reported method is the first reported that includes tryptamine in the measurement of the serotonin pathway in serum.

Besides the inclusion of both possible tryptophan-serotonin intermediates, this method has several key features which, taken together, result in important advantages over existing methods: First, sample preparation requires only a protein precipitation and dilution procedure which is quick and easy to perform; second, this method utilizes electrochemical detection which is sensitive and selective for redox-active compounds such as indoleamines; in addition, electrochemical detectors are is simple to operate and maintain; and third, this method requires only 10 μL of serum, a feature which is useful when sample volumes are limited or when samples must be divided for several different analyses.
Method details

Instrumentation and chromatographic conditions

The method uses an ALEXYS Neurotransmitters Analyzer UHPLC system (Antec Scientific, Netherlands) consisting of an LC 110 S pump, OR 110 degasser, AS 110 autosampler, and Decade Elite detector/column oven. The autosampler was equipped with a refrigerated sample compartment maintained at 4°C, and the detector was equipped with a SenCell flow cell with a 2 mm glassy carbon working electrode. The analytes were separated on an Acquity UPLC BEH C18 column (1.0 mm x 100 mm, 1.7 um particle diameter, Waters, Ireland) using a mobile phase of 4:96 acetonitrile: citrate buffer pH = 4.3. Mobile phase was delivered at a flow rate of 100 uL/minute and the separation was conducted at 37°C. The working electrode was held at 1.0 v against the Ag/AgCl reference electrode with a noise filter of 0.5 Hz and a full-scale range of 500 nA. A 2 uL injection was performed in partial-loop mode.

Standard preparation

Separate 1 mM freezer-stocks were prepared from each standard compound, aliquoted into microcentrifuge tubes, and stored at -80°C until use. The freezer-stocks were prepared in water with the exceptions of tryptophan, which was prepared in 20:80 methanol: water, and 5-HIAA, which was prepared in methanol, due to solubility concerns.

New aliquots of the freezer-stocks were thawed for each day of analysis. Standards were prepared by appropriate dilution into a series of 10 mL volumetric flasks and completed with 2 mL methanol and the remainder of 0.1 N HClO₄ containing 2 mM EDTA. All stocks and working standards were prepared using class A volumetric glassware.

Calibration

Internal standardization was chosen for two reasons: Firstly, because sample preparation involved only protein precipitation, transfers, and dilutions, and the presence of an internal standard would help to account for volume errors arising during these steps; and secondly, because injections were performed in partial-loop mode, which is generally less precise than full-loop mode, and the presence of an internal standard would help to correct for variability in the injected volumes.

N-methylserotonin and 5-hydroxyindolecarboxylic acid (5-HICA), two synthetic analogs, were evaluated for use as internal standards. Under this method’s final operating conditions, N-methylserotonin was sufficiently resolved from all other compounds while 5-HICA was not adequately resolved from tryptophan. Thus, N-methylserotonin was chosen as the internal standard.

Calibrators were prepared at the instrument LLOQ of 0.050 uM and at 3 levels evenly spaced to the instrument upper limit of 8,000 uM. N-methylserotonin was added at each calibration level at a concentration of 8,000 uM, and calibration plots were generated using peak area ratios. The LLOQ calibrator was injected in duplicate during each day of analysis.

Blood collection

C57BL/6 mice that were being tested for behavior [41] were anesthetized using isofluorane, and blood was collected via retro-orbital draw using a glass capillary. Only the first 150 uL of blood was collected in order to limit stress-related elevation of catecholamines, and potentially, compounds within the serotonin pathway [42]. Blood was collected into 1.5 mL microcentrifuge tubes and allowed to coagulate on ice for 30 minutes to an hour, after which the samples were centrifuged at 1,500 x g for 10 minutes, and the sera transferred to a new microcentrifuge tube. Serum samples were immediately frozen and stored at -80°C until analysis.
Table 1
Performance characteristics for the proposed method.

|                              | mean ± SD; %RSD |
|------------------------------|-----------------|
| **Calibration**              |                 |
| Range (μM)                   | 0.050 – 8.000   |
| **Equation (n=3 days, average terms)** |               |
| 5-HTP                        |                 |
| serotonin                    |                 |
| tryptophan                   |                 |
| 5-HIAA                       |                 |
| tryptamine                   |                 |
| **Correlation Coefficient (R², n=3 days)** |               |
| 5-HTP                        | 0.9999909±0.0000067; 0.000667 |
| serotonin                    | 0.9999853±0.0000194; 0.001940 |
| tryptophan                   | 0.9999990±0.0000012; 0.000116 |
| 5-HIAA                       | 0.9999952±0.0000024; 0.000240 |
| tryptamine                   | 0.9999966±0.0000030; 0.000298 |
| **Percent Accuracy (n=3 days)** |               |
| Spiked plasma, 0.500 μM      |                 |
| 5-HTP                        | 101.6±16.7; 16.4 |
| serotonin                    | 94.9±4.2; 4.4   |
| tryptophan                   | 100.4±1.4; 1.4  |
| 5-HIAA                       | 80.1±3.7; 4.7   |
| tryptamine                   | 90.8±6.4; 7.1   |
| Spiked plasma, 80.000 μM     |                 |
| 5-HTP                        | 97.9±0.9; 0.9   |
| serotonin                    | 99.9±1.4; 1.4   |
| tryptophan                   | 101.4±1.8; 1.8  |
| 5-HIAA                       | 94.4±0.9; 1.0   |
| tryptamine                   | 100.4±0.8; 0.7  |
| **Daily Precision (%RSD, n=3 days)** |       |
| Spiked plasma, 0.500 μM      |                 |
| 5-HTP                        | 4.0             |
| serotonin                    | 2.8             |
| tryptophan                   | 1.7             |
| 5-HIAA                       | 5.1             |
| tryptamine                   | 6.6             |
| Spiked plasma, 80.000 μM     |                 |
| 5-HTP                        | 1.1             |
| serotonin                    | 1.1             |
| tryptophan                   | 1.6             |
| 5-HIAA                       | 1.0             |
| tryptamine                   | 1.2             |
| **Inter-Day Precision (%RSD, n=15 determinations)** | |
| Spiked plasma, 0.500 μM      |                 |
| 5-HTP                        | 14.5            |
| serotonin                    | 4.6             |
| tryptophan                   | 2.4             |
| 5-HIAA                       | 6.1             |
| tryptamine                   | 8.6             |
| Spiked plasma, 80.000 μM     |                 |
| 5-HTP                        | 1.3             |
| serotonin                    | 1.6             |
| tryptophan                   | 2.2             |
| 5-HIAA                       | 1.3             |
| tryptamine                   | 1.4             |
| **Sensitivity**              |                 |
| Instrument LLOQ (μM)         | 0.050           |
| **S/N Ratio at LLOQ**        |                 |
| 5-HTP                        | 39.5±2.4; 6.1   |
| serotonin                    | 17.3±7.4; 42.7  |
| tryptophan                   | 24.9±1.2; 4.9   |
| 5-HIAA                       | 19.1±1.7; 8.7   |
| tryptamine                   | 10.4±0.2; 2.3   |

(continued on next page)
Table 1 (continued)

| Instrument LLOD (uM, S/N=3.0) | mean ± SD; %RSD |
|-------------------------------|-----------------|
| 5-HTP                         | 0.004           |
| serotonin                    | 0.009           |
| tryptophan                   | 0.006           |
| 5-HIAA                        | 0.008           |
| tryptamine                    | 0.014           |

Internal standard/protein precipitation solution (IS/PP Solution)

To reduce the number of small-volume transfers and sample preparation steps, the internal standard was added to the protein precipitation solution. 400uL of 1 mM N-methylserotonin freezer-stock and 10 uL of formic acid were added to a 10 mL volumetric flask and completed with methanol. The final formulation of the IS/PP solution was 40 uM N-methylserotonin and 0.1% (v/v) formic acid in methanol.

Sample preparation

10 uL human plasma or mouse serum was carefully transferred to a microcentrifuge tube. Reverse-pipetting was used to improve accuracy since serum is viscous and will not fully evacuate from pipette tips when forward-pipetting. 20 uL IS/PP solution was added to each sample using a Hamilton gastight syringe, and the sample tubes were briefly centrifuged to bring the two drops of liquid together in the bottoms of the tubes. The samples were allowed to sit for 1 minute, and were then vortexed for 30 seconds and centrifuged for 10 minutes at 21,000 x g. The supernatants were transferred to new microcentrifuge tubes and made up to 100 uL by addition of 70 uL of 0.1 N HClO₄ containing 2 mM EDTA. The finished samples were loaded into a 96-well plate which was then placed in the autosampler compartment for analysis. The final dilution factor for plasma and serum samples was 10.

Method validation

A brief series of spike-recovery experiments was performed to assess the accuracy and precision of the method before its application to experimental samples. For spiking experiments, human citrate-dextrose plasma for infusion (Vitalant, Scottsdale, Arizona) was chosen as a surrogate sample matrix. Aliquots of human plasma were analyzed un-spiked and spiked to two concentration levels: at the method LLOQ and at the method upper limit. Each spike level was analyzed in 5 replicates, and assay results from spiked samples were compared to their un-spiked counterparts to determine the amount of the spike which was recovered. Acceptance criteria for % accuracy were 80-120% at the LLOQ and 90-110% for the high concentration spike. Acceptance criteria for both daily precision and inter-day precision were an imprecision of ±20% RSD (relative standard deviation) at the LLOQ and ±10% RSD for the high concentration spike. Results of the method validation experiments are given in Table 1, and representative chromatograms are shown in Fig. 2.

Calibration curves were assessed over the course of several days of validation experiments to determine the ideal parameters for curve fitting. First, linear regressions were applied and analysis of residuals was performed. It was determined that the response for all compounds was reliably non-linear and that calibration data was heteroscedastic; Accordingly, a quadratic fit and a weighting factor of 1/concentration were applied to improve the quality of data. Calibrations were considered acceptable if $R^2 \geq 0.999$ for each compound and if all calibrators back-calculated to within 5% of their nominal concentrations.

Our ongoing experience in the analysis of these compounds has demonstrated that they are stable for several months or more when stored in water or dilute aqueous acids at -80°C. To test temporal stability at 4°C, an extracted serum sample was left in the refrigerated autosampler compartment and
Fig. 2. Chromatograms of (panel A) a calibrator solution containing 8 uM of each compound; (panel B) unspiked (lower) and spiked (upper) human plasma from method validation experiments; (panel C) serum from a wounded mouse after behavioral experiments - 5-HTP and 5-HIAA were present at low but quantifiable concentrations, while tryptamine was not detected; and (panel D) a close view of the 5-HTP and 5-HIAA peaks in mouse serum (top) and a calibrator solution containing 0.050 uM each (bottom).
re-analyzed throughout the day. 5-HIAA in the extracted samples degraded noticeably over the course of several hours, and addition of 2 mM EDTA to the sample diluent stabilized 5-HIAA.

Calculations

Percent accuracy was calculated for each compound in each QC sample according to the formula:

$$\text{percent accuracy} = \frac{S}{S_0} \times 100$$

where $S$ represents the amount of spike recovered and $S_0$ represents the nominal amount of the spike. 5 replicates were analyzed on each day of testing, and the average of these was taken as the result for that day.

Daily precision corresponds to a percent relative standard deviation ($\%\text{RSD}$) and was calculated for each day of testing according to:

$$\frac{\sigma_{\text{daily}}}{\text{mean}_{\text{daily}}} \times 100 = \%\text{RSD}_{\text{daily}}$$

where $\text{mean}_{\text{daily}}$ and $\sigma_{\text{daily}}$ represent the mean and standard deviation of analysis results on that day. Inter-day precision corresponds to a $\%\text{RSD}$ and was calculated at the end of testing according to:

$$\frac{\sigma}{\text{mean}} \times 100 = \%\text{RSD}$$

where $\text{mean}$ and $\sigma$ represent the mean and standard deviation of analysis results for all replicates at that concentration level over the course of testing.

Baseline noise was determined in software using the ASTM noise function with 30 second intervals. Then, the limits of sensitivity were estimated according to:

$$\text{limit} = \frac{S/N \times \text{noise}}{F}$$

where $S/N$ is the desired signal-to-noise ratio and $F$ is the response factor which is determined for each compound according to:

$$F = \frac{\text{peak height}}{\text{concentration}}$$

from a low concentration calibrator. Signal-to-noise ratios of 3 and 10 were chosen for the limit of detection (LOD) and lower limit of quantitation (LLOQ), respectively.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] D. Barton, et al., Elevated brain serotonin turnover in patients with depression: effect of genotype therapy, Arch. Gen. Psychiatry 65 (1) (2008) 38–46.
[2] Selective serotonin reuptake inhibitors (SSRIs), Mayo Clinic. Accessed March 03, 2021.
[3] A. Holck, et al., Plasma serotonin levels are associated with antidepressant response to SSRIs, J. Affect. Disord. 250 (2019) 65–70.
[4] J-C. Alvarez, et al., Plasma serotonin level after 1 day of fluoxetine treatment: a biological predictor for antiderpressant response? Psychopharmacology 143 (1999) 97–101.
[5] T. Smith, et al., In vivo microdialysis assessment of extracellular serotonin and dopamine levels in awake monkeys during sustained fluoxetine administration, Synapse 38 (4) (2000) 460–470.
[6] M. Kaneko, et al., L-5-HTP treatment and serum 5-HT level after L-5-HTP loading on depressed patients, Neuropsychobiology 5 (4) (1979) 232–240.
[7] T. Sekiduka-Kumano, et al., Positive association between the plasma levels of 5-hydroxyindoleacetic acid and the severity of depression in patients with chronic obstructive pulmonary disease, BMC Psychiatry 13 (2013) 159.
[8] R. Jones, Tryptamine: a neuromodulator or neurotransmitter in mammalian brain? Prog. Neurobiol. 19 (1982) 117–139.
[9] U. Guller, et al., Detecting pheochromocytoma: defining the most sensitive test, Ann. Surg. 243 (1) (2006) 102–107.
[10] W. Meijer, et al., Discriminating capacity of indole markers than in the diagnosis of carcinoid tumors, Clin. Chem. 46 (10) (2000) 1588–1596.
[11] J. Adaway, et al., Serum 5-HIAA: a better biomarker than urine for detecting and monitoring neuroendocrine tumors? Endocrine Abstracts 34 (2014) 45.
[12] M. Wewin, et al., The role of serum 5-HIAA as a predictor of progression and an alternative to 24-h urine 5-HIAA in well-differentiated neuroendocrine neoplasms, Biology (Basel) 10 (2) (2021) 76.
[13] E. Williams, M. Sandler, The classification of carcinoid tumors, Lancet 1 (7275) (1963) 238–239.
[14] W. Peart, et al., Carcinoid syndrome due to pancreatic duct neoplasm secreting 5-hydroxytryptophan and 5-hydroxytryptamine, Lancet 1 (7275) (1963) 239–243.
[15] W. Meijer, et al., Catecholamine-synthesizing enzymes in carcinoid tumors and pheochromocytomas, Clin. Chem. 49 (4) (2003) 586–593.
[16] R. Fleischmajer, A. Hyman, Clinical significance of derangements of tryptophan metabolism - a review of pellagra, carcinoid, and H disease, Arch. Dermatol. 84 (563) (1961).
[17] J. Mota, L. Sousa, R. Riechelmann, Complications from carcinoid syndrome: review of the current evidence, ECanermedicals cience 10 (2016) 662–678.
[18] M. Tanke, et al., Low plasma tryptophan in carcinoid patients is associated with increased urinary cortisol excretion, Psychoneuroendocrinology 33 (9) (2008) 1297–1301.
[19] Franzén, F. and Gross, H., Tryptamine, N,N-dimethyltryptamine, N,N-dimethyl-5-hydroxytryptamine and 5-methoxytryptamine in human blood and urine. 1965, 1965, 206(988): p. 1052.
[20] D. Eccleston, T. Crawford, G. Ashcroft, Tryptamine in the blood and urine of a patient with a carcinoid tumour, Nature 197 (1963) 502–503.
[21] D. Koch, P. Kissinger, Determination of serotonin in serum and plasma by liquid chromatography with precolumn sample enrichment and electrochemical detection, Anal. Chem. 52 (1) (1980) 27–29.
[22] E. Kwart, J. Kwart, H. Rutgers, A simple paired-ion liquid chromatography assay for serotonin in cerebrospinal fluid, platelet-rich plasma, serum and urine, Ann. Clin. Biochem. 21 (5) (1984) 425–429.
[23] T. Audhya, J. Adams, L. Johansen, Correlation of serotonin levels in CSF, platelets, plasma, and urine, Biochim. Biophys. Acta 150 (10) (2012) 1496–1501.
[24] Y. Hirohata, et al., High-performance liquid chromatographic method with column-switching and post-column reaction for determination of serotonin levels in platelet-poor plasma, Clin. Biochem. 37 (3) (2004) 191–197.
[25] E. Pussard, et al., Validation of an HPLC-amperometric detection to measure serotonin in plasma, platelets, whole blood, and urine, Clin. Chem. 42 (7) (2016) 1086–1091.
[26] P. Tagari, D. Boullin, C. Davies, Simplified determination of serotonin in plasma by liquid chromatography with electrochemical detection, Clin. Chem. 30 (1) (1984) 131–135.
[27] C. Korse, et al., A serum and platelet-rich plasma serotonin assay using liquid chromatography tandem mass spectrometry for monitoring of neuroendocrine tumor patients, Clin. Chim. Acta 469 (2017) 130–135.
[28] M. Lindstrom, et al., Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: a pilot study, Clin. Chim. Acta 482 (2018) 78–83.
[29] N. Narasimhachari, Quantitation of serotonin in human plasma, serum and cerebrospinal fluid samples by HPLC-EC using 6-hydroxytryptamine as an internal standard, J. Liq. Chromatogr. Relat. Technol. 7 (13) (1984) 2679–2689.
[30] B. Marasini, et al., High-performance liquid chromatographic assay of serotonin in human plasma, Ric. Clin. Lab. 15 (1) (1985) 63–69.
[31] Taylor, C. and Woodmansey, K., Clinical research method for the quantitative determination of free plasma serotonin and 5-hydroxyindolacetic acid (5-HIAA) in human plasma by UHPLC-MS/MS. Thermo Scientific Technical Note 21966.
[32] L. Cui, et al., Serum metabolomics reveals serotonin as a predicator of severe dengue in the early phase of dengue fever, PloS Negl. Trop. Dis. 10 (4) (2016).
[33] G. Anderson, et al., Liquid-chromatographic determination of serotonin and tryptophan in whole blood and plasma, Clin. Chem. 27 (5) (1981) 775–776.
[34] E. Johnsen, et al., Liquid chromatography-mass spectrometry platform for both small neurotransmitters and neuropeptides in blood, with automatic and robust solid phase extraction, Sci. Rep. 5 (2015) 9308.
[35] X. Zheng, et al., Quantitative analysis of neurochemical panel in rat brain and plasma by liquid chromatography-tandem mass spectrometry, Anal. Chem. 84 (2012) 10044–10051.
[36] J. Danaceau, et al., A liquid chromatographic-tandem mass spectrometric method for the analysis of related and altered indoles in human whole blood, J. Anal. Toxicol. 27 (7) (2003) 440–444.
[37] P. Hofmann, et al., 5-Hydroxy-indolacetic-acid (5-HIAA) serum level in depressive patients and ECT, J. Psychiatr. Res. 30 (3) (1996) 209–216.
[38] R. Romainhoni, et al., Quantification of tryptophan in plasma by high performance liquid chromatography, Quim. Nova 35 (3) (2012) 623–626.
[39] I. Kema, et al., Profiling of tryptophan-related plasma indoles in patients with carcinoid tumors by automated on-line solid-phase extraction and HPLC with fluorescence detection, Clin. Chem. 47 (10) (2001) 1811–1820.
[40] Y. Sagara, et al., Determination of plasma 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, tryptophan and melatonin by high-performance liquid chromatography with electrochemical detection, J. Chromatogr. Biomed. Appl. 431 (1988) 170–176.
[41] D. Fregoso, et al., Skin-brain axis signaling mediates behavioral changes after skin wounding, Brain Behav. Immun. (2021) 55.
[42] E. Grouzmann, et al., Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice, Pflugers Arch.: Eur. J. Physiol. 447 (2003) 254–258.