Philippine Journal of Science
154 No. 1: 131-134, February 2025
ISSN 0031 - 7683
Date Received: 20 Sep 2024

RESEARCH NOTE

Validated SPE-UHPLC Method for the Quantification of Rotenone at Trace Levels in Rat Plasma

Harriet Jane R. Caleja-Ballesteros*, Angel Mhae T. Lingaya, Jeanne Lorraine P. Amponin, Conrado S. Pabustan IV, Liezel Mari M. Abaya, Lisette Kjell Z. Lorenzo, and Irene M. Villaseñor

Institute of Chemistry, University of the Philippines Diliman,
Diliman, Quezon City, Metro Manila 1101 the Philippines

Pharmacokinetics studies rely on different quantitative methods to determine the effect of the drugs on organisms and to assess their safety and efficiency. Although rotenone is known to be toxic against non-target cells, it possesses anti-cancer properties that can make it a potential drug candidate upon structure modification. An ultra-high-performance liquid chromatography method for the quantification of rotenone was developed and validated. The rotenone in rat plasma samples was extracted by a solid phase extraction method and rotenone was identified in ppb levels. The developed method was found to be simple, rapid, accurate, and precise and can be used for routine therapeutic drug monitoring. The method presented in this study can be used as the reference chromatographic conditions for further studies on the routine drug monitoring of structurally modified rotenone derivatives.

Keywords: bioanalytical method validation, drug monitoring, rat plasma, rotenone, solid phase extraction, UHPLC

While much of the literature has focused on the extent and harmful effects of the toxicity of rotenone, emerging studies have also begun applying the mechanism of this toxicity for clinical purposes (Ling 2003). Rotenone's ability to induce cell apoptosis is explored for potential anti-cancer and anti-tumor activities. Some studies attempt to lessen its negative effects on non-target cells by using it in combination with other drugs or introducing modifications in its structure (Bak *et al.* 2021; Lawana and Cannon 2020). With the increasing interest in these studies, there is a need for a method to quantify rotenone and its derivatives on biological samples.

Liquid chromatography–tandem mass spectrometry (LC-MS-MS) was previously used for the quantification of rotenone in biological fluids wherein rotenone was eluted at 12 min (Caboni *et al.* 2008; Rhee *et al.* 2016). Our goal is to improve the analysis time for rotenone in biological fluids which will be more suitable for a routine analysis. As such, the objective of this work is to develop and optimize a rapid method of quantification for rotenone traces in rat plasma using ultra-high-pressure liquid chromatography (UHPLC) coupled with a photodiode array (PDA) detection. The results using UHPLC provide higher chromatographic resolution with enhanced sensitivity, and shorter analytical run times (Behnoush *et al.* 2015; Yu *et al.* 2006).

The HPLC-grade solvents acetonitrile (ACN), and water (H_2O) were used as the mobile phase. Standard solutions of concentrations from 500–1000 ppb were prepared by weighing rotenone (purity ≥ 95%) using Sartorius Cubis II microbalance, with caffeine as internal standard, and diluted using LC-MS grade methanol (MeOH). The solutions were then filtered using nylon 0.22-µm syringe

*Corresponding author: hrcaleja@up.edu.ph

filters and collected in amber HPLC vials. The rat plasma samples for the rotenone analysis were obtained from Pharmalytics Corporation, a partner institution of this study. Rotenone from the rat plasma samples was extracted by solid phase extraction (SPE) procedure adopted from Waters Corporation (Zhang *et al.* 2014).

The analysis of the standard and sample solutions was performed using a Shimadzu Nexera X3 UHPLC System, coupled with a PDA detector. The optimized method used in the analysis was an isocratic flow of 45 ACN: 55 H_2O at a constant flow rate of 0.3 mL/min. The oven temperature was set at 40 °C, and an injection volume of 1 μL was used. All the chromatograms were acquired at 295 nm, and the total run time was 5 min.

A 10-µL amount of the as-received rat plasma samples was added with 90-µL blank rat plasma to make the 100-µL plasma sample and subjected to the SPE protocol. The initial analysis of these samples, however, did not detect rotenone, as the concentrations were below the detection limit, and the actual concentrations were immeasurable. This agrees with the low bioavailability of rotenone (Niederberger *et al.* 2022). To quantify the rotenone in the sample, a spike analysis was conducted after the SPE. The collected eluate after extraction was evaporated to dryness using CentriVap Cold Trap and spiked with rotenone and IS in final concentrations of 500-ppb rotenone and 10-ppm caffeine.

The optimal condition was selected based on the different chromatographic factors (Table 1). Among the tested elution programs, the method with the mobile phase composition of 45 ACN: 55 H_2O gave the best set of chromatographic results and was used as the analytical method for rotenone. Further identification and verification of rotenone peak was done by varying the concentration of rotenone while maintaining the same concentration for caffeine, as shown in Figure 1. The peak area of rotenone at 3.3 min and the caffeine peak at 0.6 min were used as the quantitative parameter.

The developed method was validated according to the International Conference on Harmonisation (1994) guidelines. A positive linear response for rotenone in the concentration range of 500–1000 ppb was observed with

Table 1. The chromatographic factors of rotenone and caffeine at different elution conditions.

Mobile phase	Resolution (Rs > 2)	Tailing factor (0.9 < T < 2)	Plates/m (N > 2000)	Capacity factor (k' > 4)
65 ACN: 35 H_2O	2.020	1.399	2857	0.691
55 ACN: 45 H_2O	4.486	1.429	6182	1.550
45 ACN: 55 H_2O	11.667	1.311	20314	4.349

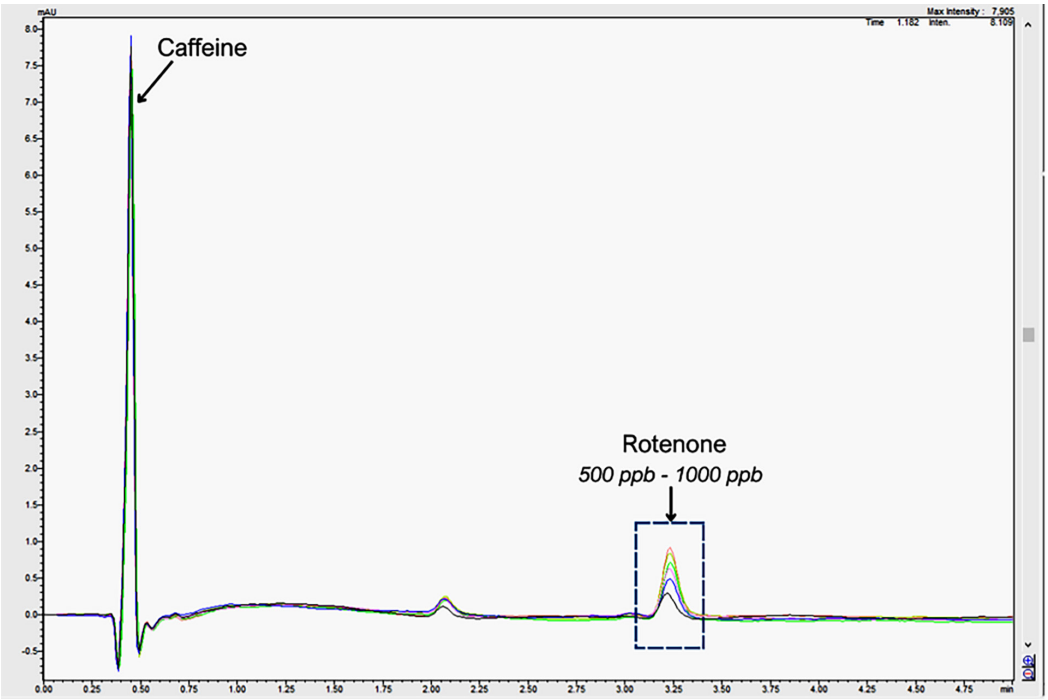

Figure 1. UHPLC chromatogram of increasing concentration of rotenone with 10 ppm of caffeine IS under isocratic flow of 45 ACN: 55 H_2O.

an average R^2 value of 0.99. Residual analysis and F-test statistical analysis were conducted to evaluate the linear relationship between rotenone concentration and the ratio of peak areas of rotenone to caffeine. Table 2 summarizes the figures of merit for the developed analytical method for rotenone.

Using the SPE protocol, the rotenone from the rat plasma samples was extracted and the absolute extraction recovery was calculated to be 69.98%. A study quantifying rotenone in yam bean seeds displayed a higher analyte recovery after undergoing SPE (Lautié *et al.* 2012). The lower recovery in this study may be due to the complexity of blood plasma, as opposed to interferences found from plant samples. Nevertheless, the recovery obtained from the presented SPE technique is considered high-value and adequate (US EPA 2007).

A blind analysis was conducted to assess the accuracy and precision of the extraction method. The blank rat plasma, also obtained from Pharmalytics Corporation, was spiked with rotenone and the concentrations were reported in Table 3. The difference between the expected and calculated values was not significantly different based on the paired samples t-test ($p > 0.05$). These suggest that the developed UHPLC method can quantify rotenone accurately and precisely in rat plasma samples.

Four sets with nine rat plasma samples collected at different time intervals per set were provided by Pharmalytics Corporation for rotenone quantification. Using the SPE and spiking methods, the rotenone in the rat plasma samples was detected and was found in ppb levels. The concentrations of rotenone obtained from the received rat plasma samples using this method were further used in the pharmacokinetic studies of rotenone.

Table 2. Validation parameters for the UHPLC method of analysis for rotenone.

Figures of merit	Values
Linear range	500 to 1000 ppb
LOD	74.2 ppb
LOQ	224.9 ppb
Recovery	96.5% to 105.5%
Precision, RSD	
Intra-day	4.49%
Inter-day	0.76%

Table 3. Blind analysis of blank rat plasma samples spiked with rotenone (n = 3).

Rotenone concentration, ppb		Accuracy	Precision
Expected	Calculated	Recovery	RSD
500	520	4.02%	1.73%
800	843	5.35%	6.88%
1000	898	10.23%	6.68%

A straightforward SPE-UHPLC method was developed and validated for the quantification of rotenone in rat plasma. The developed method was found to be simple, precise, and accurate, suitable for the analysis of rotenone. Moreover, the UHPLC elution was completed in less than 5 min, a significant advantage for routine analysis. The results obtained from this method have been shared with the research group of Dr. Rita Alvero for pharmacokinetics interpretation. The developed method can be used as a starting reference for the drug monitoring of rotenone and rotenone derivatives that are currently studied as potential treatments for cancer (Hernandez *et al.* 2024).

ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Science and Technology–Philippine Council for Health Research and Development for funding this research titled "Project 3: HPLC and Mass Spectral Studies to Support Various ADMET assays" under the program "Bridging Efficacy and Safety: IND-enabling Suite of ADME-Tox Assays." We also acknowledge the laboratory of Dr. Rita Alvero (Pharmalytics Corporation) of "Project 5: Determination of Pharmacokinetic Properties" under the same program for providing us with the rat plasma samples that are essential in this study.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflicts regarding this study.

REFERENCES

BAK DH, KANG SH, PARK CH, CHUNG BY, BAI HW. 2021. A novel radiolytic rotenone derivative, rotenoisin A, displays potent anticarcinogenic activity in breast cancer cells. J Radiat Res 62(2): 249–258. https://doi.org/10.1093/jrr/rrab005

BEHNOUSH B, SHEIKHAZADI A, BAZMI E, FATTAHI A, SHEIKHAZADI E, SABERI ANARY SH. 2015. Comparison of UHPLC and HPLC in benzodiazepines analysis of postmortem samples. Medicine (United States) 94(14). https://doi.org/10.1097/MD.0000000000000640

CABONI P, SARAIS G, VARGIU S, DE LUCA MA, GARAU VL, IBBA A, CABRAS P. 2008. LC-MS-MS determination of rotenone, deguelin, and rotenolone in human serum. Chromatographia 68(9–10): 739–745. https://doi.org/10.1365/s10337-008-0830-0

HERNANDEZ RD, GENIO FAF, CASANOVA JR, CONATO MT, PADERES MC. 2024. Antiproliferative activities and SwissADME predictions of physicochemical properties of carbonyl group-modified rotenone analogues. ChemistryOpen 13(1). https://doi.org/10.1002/open.202300087

INTERNATIONAL CONFERENCE ON HARMONISATION. 1994. Validation of Analytical Procedures: Text and Methodology Q2(R1).

LAUTIÉ E, ROZET E, HUBERT P, QUETIN LECLERCQ J. 2012. Quantification of rotenone in seeds of different species of yam bean (*Pachyrhizus* sp.) by a SPE HPLC-UV method. Food Chem 131(4): 1531–1538. https://doi.org/10.1016/j.foodchem.2011.09.125

LAWANA V, CANNON JR. 2020. Rotenone neurotoxicity: relevance to Parkinson's disease. In Adv Neurotoxicol 4: 209–254. https://doi.org/10.1016/bs.ant.2019.11.004

LING N. 2003. Rotenone : a review of its toxicity and use for fisheries management. Dept. of Conservation, New Zealand. Retrieved on 27 Jul 2024 from https://www.doc.govt.nz/documents/science-and-technical/sfc211.pdf

[US EPA] United States Environmental Protection Agency. 2007. Method 3535A: solid-phase extraction (SPE); part of test methods for evaluating solid waste, physical chemical methods. Retrieved on 27 Jul 2024 from https://www.epa.gov/esam/epa-method-3535a-sw-846-solid-phase-extraction-spe

NIEDERBERGER E, WILKEN-SCHMITZ A, MANDERSCHEID C, SCHREIBER Y, GURKE R, TEGEDER I. 2022. Non-reproducibility of oral rotenone as a model for Parkinson's disease in mice. Int J Mol Sci 23(20). https://doi.org/10.3390/ijms232012658

RHEE J, YUM H, MOON S, IN S, LEE S, SEO J. 2016. Rotenone analysis by liquid chromatography–tandem mass spectrometry with information-dependent acquisition in a fatal case of rotenone poisoning with a commercial organic insecticide being sold in Korea. J Anal Toxicol 40(6): 460–465. https://doi.org/10.1093/jat/bkw032

YU K, LITTLE D, PLUMB R, SMITH B. 2006. High-throughput quantification for a drug mixture in rat plasma – a comparison of Ultra Performance^{TM} liquid chromatography/tandem mass spectrometry with high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 20(4): 544–552. https://doi.org/10.1002/rcm.2336

ZHANG X, IRANETA PC, MARSZALKOWSKI FJ, FOUNTAIN KJ. 2014. A simplified solid phase extraction (SPE) protocol for bioanalysis using Oasis HLB. Waters Corporation.