

First-in-class HPLC-fluorescence method for quantification of roscovitine in plasma: Greenness assessment and application to pharmacokinetic studies in rats

IBRAHIM A. DARWISH* 
NOURAH Z. ALZOMAN 

Department of Pharmaceutical
Chemistry, College of
Pharmacy, King Saud
University, P.O. Box 2457
Riyadh 11451, Saudi Arabia

ABSTRACT

This research introduces a first-in-class high-performance liquid chromatography with fluorescence detection (HPLC-FD) method for the reliable quantification of ROS in plasma. The method relies on the native fluorescence of ROS, with excitation and emission set at 280 nm and 340 nm, resp. Sample preparation involved a simple one-step protein precipitation with methanol. Chromatographic resolution of ROS and pemigatinib (PEM), which served as the internal standard (IS), was achieved using Zorbax Eclipse Plus C18 HPLC column. The mobile phase consisted of methanol and formate buffer solution. The method was validated in compliance with ICH M10 guidelines. It showed linearity across concentration range of 50–1,500 ng mL⁻¹ ($R^2 = 0.9991$), with a lower limit of quantitation of 50 ng mL⁻¹. Accuracy ranged from 96.8 to 103.4 %, and intra- and inter-day precision (RSD) were between 3.2 and 6.1 %. The method was successfully applied to quantify ROS in plasma samples from rats following administration of a single oral dose (25 mg kg⁻¹), enabling the determination of key pharmacokinetic parameters, including $C_{\max}$ (361.31 ng mL⁻¹), $t_{\max}$ (1.75 h, $t_{1/2}$ (2.867 h), V_d (0.077 L kg⁻¹), CL (0.018 L h⁻¹ kg⁻¹), AUC (ng h mL⁻¹) and MRT (h). The greenness and practical applicability of the method were confirmed using four comprehensive independent metric tools. In comparison to previously reported bioanalytical approaches, the proposed method offers several practical advantages, such as reduced complexity and cost, a shorter run time, full validation, established greenness, and suitability for the clinical laboratory setting.

Keywords: roscovitine, HPLC-FD, pharmacokinetic study, therapeutic drug monitoring, green analytical chemistry

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INTRODUCTION

Roscovitine (ROS) is a synthetic purine analog that acts as a potent inhibitor of cyclin-dependent kinases (CDKs). Its chemical structure is given in Fig. 1a, and its chemical name is: (2*R*)-2-[[6-(benzylamino)-9-(propan-2-yl)-9*H*-purin-2-yl]amino]butan-1-ol. It has a molecular formula of C₁₉H₂₆N₆O and a molar mass of 354.46 g mol⁻¹ (1).

* Correspondence; e-mail: idarwish@ksu.edu.sa

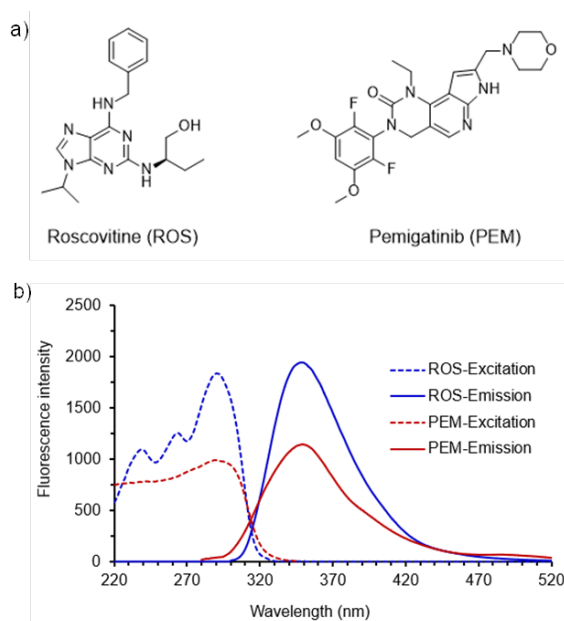


Fig. 1. The chemical structures of: a) roscovitine (ROS) and pemigatinib (PEM); b) fluorescence spectra (excitation and emission) of their methanolic solutions. The concentrations were 2500 and 500 ng mL⁻¹ for ROS and PEM, resp.

However, advancing the pharmacological understanding of ROS for clinical development has faced challenges, particularly concerning efficacy and toxicity. These difficulties largely stem from the limited number of bioanalytical methods for reliably quantifying ROS in blood plasma, which is essential for studying its pharmacokinetics and conducting therapeutic drug monitoring. Although many methods have been reported in the literature for identifying/characterizing and quantifying ROS in bulk drug substance and/or synthetic formulations (5–8), few bioanalytical methods have been reported for the determination of ROS in biological matrices for assessing tissue distribution, kinetics, and metabolite identification (9–12). These encompass high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) (9), liquid chromatography-tandem mass spectrometry (LC-MS/MS) complemented with nuclear magnetic resonance (NMR) spectroscopy (10, 11), and HPLC coupled with electrochemical electrospray ionization mass spectrometry (HPLC-EC/ESI-MS) (12). Despite their valuable contribution, these methods present significant drawbacks for routine bioanalysis. The HPLC-UV method suffers from relatively low sensitivity, poor recovery, and lacks an internal standard, compromising accuracy and precision. LC-MS/MS and HPLC-EC/ESI-MS techniques, while highly sensitive and specific, require expensive instrumentation, specialized operational expertise, complex sample preparation procedures, and extended run times, making them impractical for routine clinical laboratories. The use of NMR, although powerful for structural elucidation, is entirely unsuitable for routine quantification due to its high cost, low sensitivity, and complexity. Therefore, a new analytical method for quantifying ROS levels in human plasma that offers straightforward sample preparation, sufficient sensitivity and

excellent accuracy must be urgently developed and validated. To date, no HPLC-FD method has been reported for the quantification of ROS in plasma, despite the fact that ROS possesses native fluorescence properties.

To the best of our knowledge, this is the first report describing HPLC-FD for the quantification of ROS in plasma, offering a sensitive, cost-effective, and widely accessible alternative to existing UV and mass spectrometric methods.

EXPERIMENTAL

Instruments

The HPLC instrument, manufactured by Shimadzu Corporation (Japan) was used. The system was equipped with an LC-10AD VP pump with an FCV-10AL VP low-pressure flow-control valve, an SCL-10A VP system controller, a Rheodyne 7725 injection valve with a 20- μ L loop, SPD-10A VP, and an RF-10A XL fluorescence detector (set at λ_{ex} 280 nm and λ_{em} at 340 nm). Shimadzu's LCSolution software (version 1.25) was used for instrument control and data acquisition. A Hanna pH 211 meter (Romania) was used to adjust the buffer solution pH, a Heidolph D-91126 vortex mixer (Germany) to blend standards with plasma samples, and a Sigma 1-15PK centrifuge (Germany) to separate the precipitated proteins after treatment of the plasma samples with methanol.

Materials

Roscovitine (ROS) and pemigatinib (PEM) (purity > 99 %) were obtained from LC Laboratories (USA). A Zorbax Eclipse Plus C18 HPLC column (150 mm $\times$ 4.6 mm, 5 μ m particle size) manufactured by Agilent (USA) was used. A guard column from Macherey-Nagel GmbH & Co. (Germany) was placed upstream of the analytical column. HPLC-grade solvents were acquired from Merck (Germany), while all other reagents were of analytical grade.

Human plasma, obtained from the blood bank of King Khaled University Hospital in Riyadh, Saudi Arabia, was stored at -70°C until analysis. The obtaining of samples and study have been approved by the Research Ethics Committee (REC) at King Saud University (Riyadh, Saudi Arabia), under reference No. KSU-SE-20-51.

Preparation of standard solutions and quality control samples

Stock solutions of ROS and the internal standard (PEM) of 1.0 mg mL⁻¹, were prepared by separately transferring accurately weighed 10-mg portions of each compound into individual 10-mL volumetric flasks. Each substance was first dissolved in approximately 8 mL of acetonitrile, and then the final volume was adjusted to 10 mL using the same solvent. Serial dilutions of these stocks with acetonitrile yielded working standard solutions of 3,000 ng mL⁻¹ for ROS and 1,200 ng mL⁻¹ for PEM.

Calibration standards were generated by spiking ROS and PEM into drug-free human plasma. The final ROS concentrations ranged from 10 to 1,500 ng mL⁻¹, while the IS level remained constant at 600 ng mL⁻¹ across all preparations. Each calibration sample was then combined with an equal volume of methanol, vortex-mixed for 30 seconds, and centrifuged at 13,000 rpm for 10 minutes. The clear supernatant was passed through syringes

equipped with 0.2- μm Millipore filters before injection. A 10- μL portion of each filtrate was introduced into the HPLC system for chromatographic analysis.

Quality control (QC) samples were prepared at three distinct ROS concentration levels: low (100 ng mL⁻¹), medium (400 ng mL⁻¹), and high (800 ng mL⁻¹). The same protocol used for calibration standards was followed for QC sample preparation. All prepared samples were kept refrigerated until analysis, which was carried out on separate days, with daily evaluation of system suitability parameters.

HPLC analysis and statistical data treatment

Chromatographic separation by HPLC was performed under isocratic conditions using a mobile phase composed of methanol and acetate buffer (pH 5) in a 40:30 (V/V) ratio, delivered at 1.5 mL min⁻¹. Aliquots of various preparations (10 μL) including ROS standards, blank plasma, blank plasma fortified with PEM (IS), and blank plasma fortified with both ROS and PEM, were introduced into the system using the autosampler. A calibration curve was constructed by plotting the peak area ratio (ROS/PEM) against the known ROS concentrations.

Results are presented as mean $\pm$ RSD. Regression analysis of the HPLC-FD calibration curves was performed, applying a significance threshold of $p < 0.05$.

Validation of HPLC-FD method

Validation protocol was carried out for the proposed HPLC-FD method, following the International Council on Harmonisation guideline for bioanalytical method validation and study sample analysis (14). This validation procedure was carried out to assess the method's reliability in quantifying ROS in plasma samples, with respect to required key parameters.

Linearity and sensitivity. – To evaluate linearity, three independent calibration curves were generated to obtain regression equations and their corresponding coefficients of determination (R^2). The sensitivity of the method was assessed by determining the lower limit of quantification (LLOQ), defined as the lowest calibration standard concentration (at a signal-to-noise ratio = 10).

Precision and accuracy. – Intra-assay precision and accuracy were determined by analyzing six replicate samples of each quality control (QC) level (low, medium and high) plus LLOQ, within the same run. For inter-assay evaluation, three replicates of each QC concentration were analyzed on three separate days. Precision was expressed as percent relative standard deviation (RSD, %) derived from the replicate measurements. Accuracy, reported as percent recovery, was calculated by comparing the experimentally determined concentrations with the nominal (theoretical) values.

Intermediate precision. – Method intermediate precision was evaluated by having two different analysts to conduct ROS analysis under standard operating conditions over three consecutive days. The resulting data variability (analyst-to-analyst and day-to-day) was expressed as RSD.

Stability studies of ROS in samples. – The stability of ROS was examined under a range of conditions, including autosampler handling, bench-top exposure, freeze-thaw cycling,

and both short- and long-term storage. For autosampler stability, QC samples were kept under autosampler conditions for approximately 48 hours before analysis. Bench-top stability was tested by allowing plasma samples to sit at room temperature for roughly 6 hours. Freeze-thaw stability was assessed by subjecting samples to three cycles of freezing at -70°C followed by thawing at ambient temperature.

Short-term storage stability was evaluated after keeping the spiked plasma samples at 8°C for 30 days, while long-term stability was assessed following storage at -70°C for 60 days, with analysis conducted using the proposed HPLC-FD method. Stock and working solutions of ROS were tested for stability by storing them at room temperature for 24 hours and under refrigeration (below 10°C) for 30 days prior to analysis. All stability experiments used freshly prepared calibration standards. ROS was considered stable in plasma if the deviation between the measured concentration and the mean concentration of the stability QC was within $\pm 15\%$.

Pharmacokinetic study in rats

Five healthy male Wistar rats, with an average body mass of 250 ± 30 g, were housed under controlled conditions (25°C and 50% RH). Each rat received an oral dose of 25 mg kg^{-1} of ROS, formulated in 1% DMSO in saline. Blood samples of approximately $300\text{ }\mu\text{L}$ were drawn into heparinized tubes prior to dosing and at various time points between 0.25 and 12 hours after administration. Plasma was separated by centrifugation at $4,500\text{ rpm}$ and 4°C for 15 minutes, then stored at -20°C until further analysis.

All animal procedures were conducted in strict accordance with the guidelines established by the Research Ethics Committee (REC) for live animal studies at King Saud University (Riyadh, Saudi Arabia), under approval reference No. KSU-SE-20-51.

The calculated pharmacokinetic parameters included maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), volume of distribution (V_d), elimination rate constant (K_{el}), elimination half-life ($t_{1/2}$), clearance (CL), area under the curve from 0 to 24 hours (AUC_{0-24}) and from 0 to infinity ($AUC_{0-\infty}$), and mean residence time (MRT).

Assessment of greenness of HPLC-FD procedure

The greenness of HPLC-FD procedure was evaluated using three assessment tools: the Analytical Eco-Scale (AES) (15), the Green Analytical Procedure Index (GAPI) (16) and Analytical Greenness (AGREE) (17).

In the AES too, the environmental impact of the analytical process is assessed based on four intrinsic parameters, each assigned penalty points (PPs) that reflect its ecological repercussions. The total PPs are subtracted from an ideal score of 100, and the resulting score classify the procedure's eco-friendliness into three categories; green (total score > 75 points), moderately green ($50\text{--}75$ points), or inadequately green (< 50 points).

The GAPI tool evaluated the entire analytical workflow across 15 parameters (1–15), covering activities from sample extraction to post-analysis waste management. The criteria are presented in a pictogram consisting of 15 segments, each color-coded (green, yellow or red) to indicate its environmental impact. Green represents sustainability, while red signals a lack of ecological merit, with detailed guidelines for colour assignment.

The AGREE tool is a user-friendly software that assesses the environmental performance of analytical methods based on the 12 principles of green analytical chemistry (GAC). Results are presented in a circular pictogram, with segments shaded from deep green (score = 1, fully sustainable) to bright red (score = 0, non-sustainable). A total score between 0 and 1 is displayed at the centre, summarizing the method's overall analytical sustainability.

Assessment of HPLC-FD method applicability

The overall practicality, efficiency, and environmental impact of the HPLC-FD method were evaluated using the recently described BAGI (Blue applicability grade index) metric tool (18). The evaluation was performed following the guidelines provided with the open-source BAGI application. Ten predefined criteria, encompassing the parameters of type of analysis, multi-/single-element analysis, type of analytical technique/instrumentation, number of samples simultaneously analysed, sample preparation involved, number of samples analysed per hour, type of reagents/materials used, requirement for preconcentration, degree of automation, and amount of sample, were each scored based on the method's performance characteristics. Scores for each criterion were recorded, and the tool automatically generated a 10-segment asteroid-shaped pictogram, where each segment corresponds to one criterion. Segment colours range from light to dark blue, with darker shades reflecting higher compliance with the ideal conditions for that attribute. The central numerical score (out of 100) represents the overall applicability of the method. The tool scores the method from 25 to 100 (the higher the score, the more practical the method). A higher BAGI score indicates a method that is not only environmentally sustainable but also practical, safe, rapid, and cost-effective for routine laboratory or industrial use.

RESULTS AND DISCUSSION

Strategy for method development

Due to ROS's therapeutic efficacy against various cancers, researchers are actively refining treatment strategies to improve clinical outcomes, minimize side effects, enhance target specificity, overcome resistance, and advance personalized medicine. Achieving these goals requires a reliable method for quantifying ROS in plasma, which is essential for four key reasons: (i) conducting pharmacokinetic studies on absorption, distribution, metabolism, and excretion; (ii) enabling therapeutic drug monitoring to optimize dosing; (iii) correlating plasma concentrations with safety and efficacy outcomes; and (iv) meeting regulatory requirements for drug approval. Given that existing bioanalytical methods for ROS quantification in plasma are limited, this study aimed to develop a new analytical method to fill that gap.

Building on evidence that ROS exhibits intrinsic fluorescence (5) and our laboratory's prior success with simple non-extractive HPLC methods for plasma analysis (13), this study aimed to develop a sensitive HPLC-FD method for quantifying ROS in plasma samples.

Green Analytical Chemistry (GAC) plays a pivotal role in pharmaceutical and biomedical analysis by promoting sustainable practices that minimize environmental impact while enhancing the efficiency and safety of analytical processes. Through the develop-

ment of green methodologies, such as reducing waste generation, minimizing energy consumption, and using greener solvents, GAC contributes to the overall greenness of pharmaceutical and biomedical research (19). By prioritizing environmentally conscious approaches, researchers can achieve accurate and reliable results while reducing the ecological footprint of their analytical procedures, thus fostering a healthier and more sustainable future for drug development and biomedical investigations. In line with these principles, this study was also designed to develop a green HPLC-FD method for ROS.

Optimization of the HPLC-FD conditions

Selection of detection wavelength and internal standard. – The fluorescence spectra (excitation and emission) of ROS revealed maximum fluorescence intensity at an emission wavelength of 340 nm with excitation at 280 nm (Fig. 1b). Consequently, these wavelengths were adopted for ROS detection during the development of the HPLC-FD method. A previous study conducted in our laboratory (20) demonstrated that PEM is also a native fluorescent molecule, exhibiting its fluorescence emission at the same emission wavelength as ROS (Fig. 1b). For this reason, PEM was selected as the internal standard for the HPLC-FD method described herein.

Mobile phase composition and flow rate. – Experiments were conducted to determine the optimal mobile phase composition and flow rate. The results are summarized and presented in Fig. 2 and Table I and discussed below.

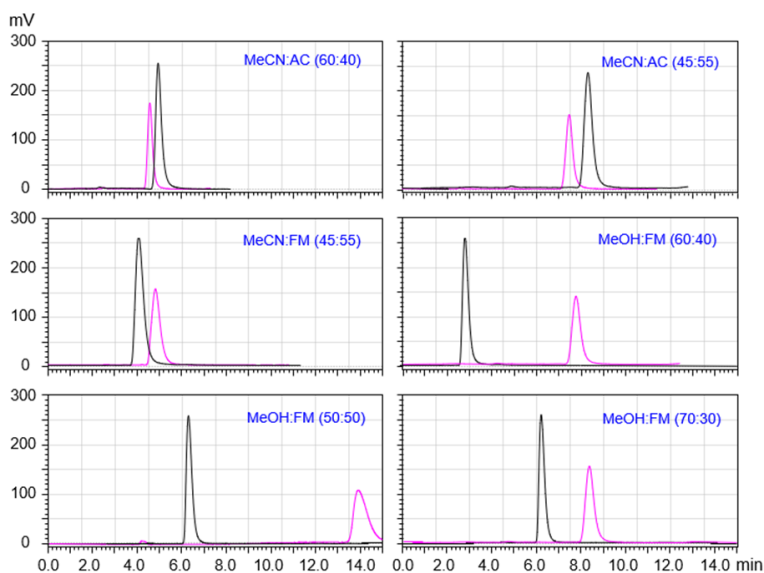


Fig. 2. HPLC chromatograms obtained when using mobile phases of different compositions for separation of ROS (pink) and the internal standard, PEM (black). The concentrations of ROS and IS injected were 800 and 600 ng mL⁻¹, resp. (column: Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 µm particle size) at 25 ± 2 °C; mobile phase: pH 5.0, isocratic conditions; flow rate 1.5 mL min⁻¹). MeCN – acetonitrile, AC – acetate buffer, MeOH – methanol, FM – formate buffer.

Table I. Optimization of mobile phase composition for separation of ROS and PEM (IS) of HPLC-FD method

Organic solvent/ buffer solution	Organic solvent:buffer ratio (%, <i>V/V</i>)	Retention time (min)	
		ROS	IS
Acetonitrile			
Acetate buffer, pH 5	60:40	4.32	4.91
Acetate buffer, pH 5	45:55	7.60	8.52
Formate buffer, pH 5	45:55	4.92	3.68
Methanol			
Formate buffer, pH 5	60:40	7.79	2.81
Formate buffer, pH 5	50:50	13.82	6.10
Formate buffer, pH 5	70:30	8.60	6.21
Formate buffer, pH 5 ^a	70:30	5.73	4.10

^a Flow rate in this experiment was 1.5 mL min⁻¹, however, it was 1.0 mL min⁻¹ in all other experiments.

Initial separation trials were conducted on an analytical Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 µm particle size) kept at 25 ± 2 °C under isocratic conditions. The initial mobile phase (acetonitrile:acetate buffer, 60:40, pH 5, 1.0 mL min⁻¹) gave a rapid ROS peak (4.32 min) but co-elution with the IS (4.91 min). Reducing acetonitrile to 45 % extended ROS and PEM retention to 7.60 and 8.52 min, yet resolution remained insufficient. Replacing acetate with formate buffer (pH 5, same 45:55 ratio) reversed the elution order (ROS 4.92 min, IS 3.68 min) without resolving the pair. Switching to methanol:formate (60:40) separated ROS (7.79 min) and IS (2.81 min) within 10 min, but the early IS peak risked interference from plasma components. A 50:50 ratio delayed ROS to 13.82 min, whereas increasing methanol to 70 % achieved baseline separation with both peaks under 10 min (ROS 8.60 min, IS 6.21 min). To improve the separation further, the flow rate was raised from 1.0 to 1.5 mL min⁻¹. This adjustment shortened the total run time to 7 minutes while preserving resolution, with ROS and IS now eluting at 5.73 and 4.10 min, resp.

Based on these results, all subsequent experiments employed a mobile phase consisting of methanol and formate buffer (pH 5) in a 70:30 (V/V) ratio, delivered at a flow rate of 1.5 mL min⁻¹.

Column packing and dimensions. – Several reversed-phase columns were tested to identify the optimal packing and dimensions, including a phenyl column (250 mm × 4.6 mm, 5 µm; Phenomenex, USA) and a Nucleosil CN column (250 mm × 4.6 mm, 5 µm; Macherey-Nagel, Germany), with performance compared against a Zorbax Eclipse Plus C18 column (250 mm × 3.9 mm, 5 µm). The C18 column yielded superior results and was chosen for all subsequent analyses, providing baseline resolution of ROS and IS within a 10-minute run time (ROS at 5.73 min, IS at 4.10 min; Fig. 3). System suitability parameters, namely chromatographic parameters like capacity factor, separation factor, resolution, peak symmetry, and theoretical plates, are summarized in Table II.

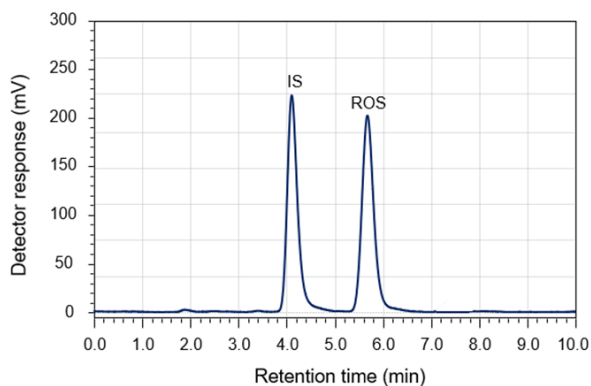


Fig. 3. A representative HPLC chromatogram of standard solution containing ROS and IS (PEM). The concentrations of ROS and IS were 800 and 600 ng mL⁻¹, resp. (column: Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 μm particle size) at 25 ± 2 °C; mobile phase: methanol and formate buffer (pH 5), 70:30 (V/V) ratio, isocratic conditions, flow rate 1.5 mL min⁻¹). Retention times were 4.10 and 5.73 min for IS and ROS, resp.

Table II. Chromatographic parameters and peak purity profile of the HPLC-FD method

Parameter	Value	
	ROS	PEM (IS)
System suitability		
Retention time of ROS (min)	5.728	4.100
Capacity factor (<i>k'</i>)	1.148	1.949
Resolution factor (<i>R_s</i>)	3.856	6.434
Peak asymmetry factor	1.040	1.488
Number of theoretical plates (<i>N</i>)	16,909	11,839
Peak purity		
Impurity detection	Not detected	Not detected
Purity index	0.99998	0.99999
Single point threshold	0.92574	0.98504
Minimum purity index	74,237	14,955

Validation of HPLC-FD method

The proposed HPLC-FD method was partly validated according to ICH bioanalytical guidelines (14), assessing linearity, sensitivity (*LLOQ*), accuracy, precision, stability and selectivity.

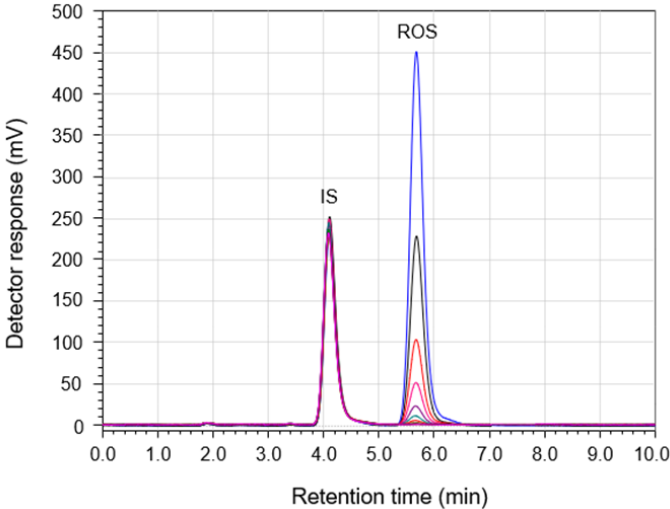


Fig. 4. Overlaid HPLC chromatograms of standard solutions containing varying concentrations (10–1,500 ng mL⁻¹) of ROS and a fixed concentration of IS (600 ng mL⁻¹) (column: Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 µm particle size) at 25 ± 2 °C; mobile phase: methanol and formate buffer (pH 5), 70:30 (V/V) ratio, isocratic conditions, flow rate 1.5 mL min⁻¹).

Linearity and sensitivity. – A series of ROS concentrations were analysed under the specified conditions (overlaid chromatograms shown in Fig. 4). Linear regression demonstrated high coefficient of determination and a near-zero intercept. The resulting regression equation was $y = 0.0341 + 0.0015x$ ($R^2 = 0.9991$), where y is the peak area ratio and x is the ROS concentration. Linearity and statistical parameters are summarized in Table III.

The method's *LLOQ* was determined, as 5 times of the response noise and found to be 50 ng mL⁻¹ (Table III). This *LLOQ* confirms adequate sensitivity for measuring therapeutic ROS concentrations in patients, which range from 307 to 3,783 ng mL⁻¹ after an 800 mg oral

Table III. Calibration parameters of the HPLC-FD method for the determination of ROS

Parameter	Value
Linearity range (ng mL ⁻¹)	50–1,500
Intercept (a)	0.0341
Standard deviation of intercept (SD_a)	0.0075
Slope (b)	0.0015
Standard deviation of slope (SD_b)	0.0008
Determination coefficient (R^2)	0.9991
Standard deviation of residuals ($S_{y/x}$)	0.0055
Lower limit of quantitation (<i>LLOQ</i> , ng mL ⁻¹)	50

Table IV. Precision and accuracy of HPLC-FD method in spiked human plasma

Spiked ROS (ng mL ⁻¹)	Intra-day ^a		Inter-day ^a	
	Accuracy (%)	Precision (RSD, %)	Accuracy (%)	Precision (RSD, %)
LLOQ (50)	98.8	3.9	99.4	5.8
100	103.4	3.2	96.2	4.6
600	96.8	4.1	103.5	3.2
1200	98.6	3.8	97.4	6.1

^a Values are mean of 6 replicates.

Table V. Intermediate precision of HPLC-FD method

Parameter	Recovery (mean ± RSD) (%) ^a
Analyst to-analyst	
Analyst-1	96.8 ± 2.8
Analyst-2	103.1 ± 3.6
Day-to-day	
Day-1	96.4 ± 2.8
Day-2	101.8 ± 4.5
Day-3	96.6 ± 5.1

^a Values are the mean of three determinations.

dose (21). Accordingly, measurements can be performed from a small plasma volume (*e.g.*, 100–200 µL instead of 1 mL or more).

Accuracy and precision. – Accuracy (recovery, %) and precision (RSD, %) were assessed at three QC levels (100, 600 and 1,200 ng mL⁻¹), as shown in Table IV. Intra-day recovery ranged from 98.3 to 103.2₅ %, while inter-day recovery ranged from 96.8 to 103.4 %. Intra-day RSD values were between 3.2 and 4.1 %, and inter-day RSD ranged from 3.2 to 6.1 %. The intermediate precision in terms of analyst-to-analyst and day-to-day variation was assessed. As summarized in Table V, recovery ranged from 96.4 to 103.1 %, with RSD values below 5.1 %, confirming the method's reproducibility.

Selectivity. – Fig. 5 compares chromatograms of blank plasma (no ROS, no IS) and plasma spiked with ROS (800 ng mL⁻¹) and IS (600 ng mL⁻¹). No interfering peaks appeared at the retention times of ROS or IS. Peak purity plots (Fig. 6, numerical data in Table II) showed purity curves below threshold lines, indicating that spectral variations stem only from background noise. Peak purity indices and single-point thresholds were near 1 for all peaks, confirming no co-eluting compounds with ROS or IS.

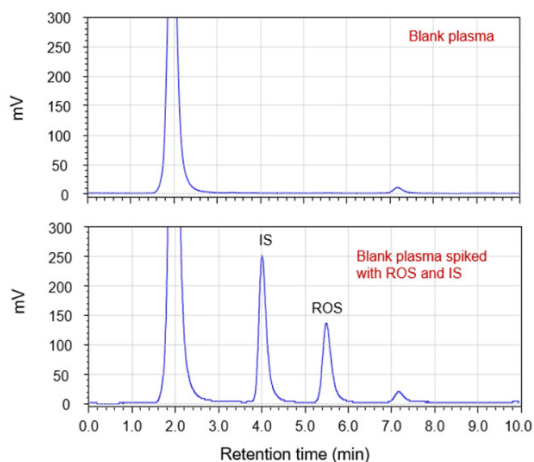


Fig. 5. Representative HPLC chromatograms of blank (drug-free) human plasma (upper panel) and plasma spiked with both ROS and IS at concentration of 800 and 600 ng mL⁻¹, resp. (lower panel) (column: Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 µm particle size) at 25 ± 2 °C; mobile phase: methanol and formate buffer (pH 5), 70:30 (V/V) ratio, isocratic conditions, flow rate 1.5 mL min⁻¹).

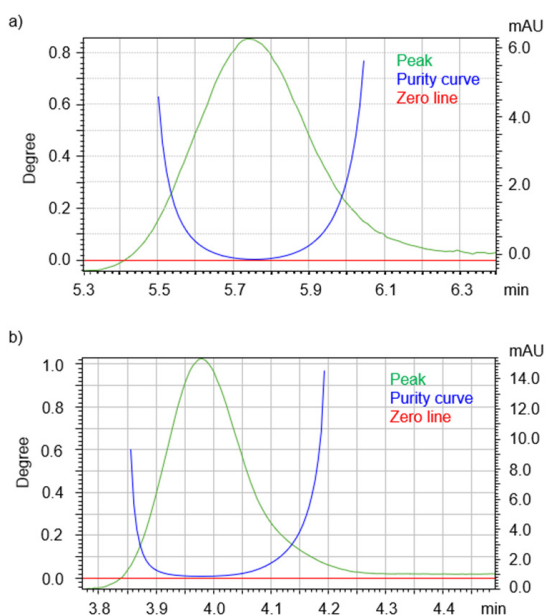


Fig. 6. Peak purity plots of: a) ROS and b) IS obtained from HPLC chromatograms of plasma samples spiked with both ROS and IS at concentration of 800 and 600 ng mL⁻¹, resp. (column: Zorbax Eclipse Plus C18 HPLC column (250 mm × 3.9 mm i.d., 5 µm particle size) at 25 ± 2 °C; mobile phase: methanol and formate buffer (pH 5), 70:30 (V/V) ratio, isocratic conditions, flow rate 1.5 mL min⁻¹).

Plasma ROS stability studies. – Stability results for ROS are summarized in Table VI. ROS remained stable in spiked human plasma for at least 6 hours at room temperature and 48 hours under autosampler conditions. It also withstood three freeze-thaw cycles, 30 days at 8 °C, and 60 days at –70 °C, with recovery values between 97.8 and 102.5 % and RSD below 5.4 %.

Table VI. Stability data for ROS in human plasma by HPLC-FD method

Stability	Recovery (%) ^a	Precision (RSD, %) ^a
Bench top (6 h)	102.5	3.8
Autosampler (48 h)	98.2	5.2
Freeze-thaw (3 cycle)	99.4	4.1
30 days at 8 °C	101.2	2.8
30 days at –70 °C	97.8	5.4

^a Values are mean of 3 determinations.

Dilution integrity. – Plasma samples were prepared to contain ROS at 6 concentration levels (100, 200, 400, 600, 800, and 1200 ng mL^{–1}). The results (Table VII) showed that the measured concentrations after 10-fold dilution lied within the acceptable limits for accuracy and precision, as evident from the good recovery (97.4–103.5 %) and RSD values within the range of 1.5–4.2 %. These results confirm that the method is free from significant dilution effects and that samples can be reliably diluted without compromising accuracy or precision.

Table VII. Dilution and integrity studies of the proposed HPLC-FD for determination of ROS in spiked human plasma samples

Nominal concentration (ng mL ^{–1})	Measured concentration (ng mL ^{–1})	Recovery (mean ± RSD) (%) ^a
100	97.4	97.4 ± 2.3
200	197.2	98.6 ± 3.1
400	411.2	102.8 ± 1.5
600	595.2	99.2 ± 2.6
800	827.2	103.5 ± 4.2
1200	1174.8	97.9 ± 3.8

^a Values are mean of three determinations.

Comparison with reported method. – Table VIII demonstrates the results obtained by the application of the proposed HPLC-FD method for the determination of ROS in spiked plasma samples at three concentration levels (200, 400 and 800 ng mL^{–1}), with results com-

pared to those obtained for the same samples by the reported HPLC-UV method (9). The proposed HPLC-FD method yielded a mean recovery of 99.7 ± 1.4 %, while the reported HPLC-UV method produced 99.6 ± 0.8 %. Statistical analysis using Student's *t*-test and *F*-test confirmed no significant differences between the proposed and reported methods: for HPLC-FD *vs.* HPLC-UV $t_{\text{calc}} = 0.1074$ ($t_{\text{tab}} = 2.306$) and $F_{\text{calc}} = 0.4923$ ($F_{\text{tab}} = 6.39$) at 95 % confidence, showing no statistically significant difference in either accuracy or precision between the proposed HPLC-FD and the reported HPLC-UV method (9).

Table VIII. Analysis of plasma samples spiked with ROS by the proposed HPLC-FD and reported HPLC-UV methods

Nominal ROS conc. (ng mL ⁻¹)	Recovery (mean $\pm$ SD, %) (RSD, %)	
	Proposed HPLC-FD ^a	Reported HPLC-UV method (9) ^a
200	101.2 $\pm$ 1.4 (1.4)	98.8 $\pm$ 1.6 (1.6)
400	98.5 $\pm$ 1.8 (1.8)	99.5 $\pm$ 1.5 (1.5)
800	99.5 $\pm$ 1.5 (1.5)	100.4 $\pm$ 1.2 (1.2)
Mean	99.7 $\pm$ 1.4 (1.4)	99.6 $\pm$ 0.8 (0.8)
<i>t</i> -value	0.1074 (2.306) ^b	
<i>F</i> -value	0.4923 (6.39) ^b	

^a Values are mean of five determinations $\pm$ SD (RSD).

^b Values between brackets are the tabulated values at 95 % confidence interval.

Pharmacokinetic study in rats

The developed HPLC-FD method was applied to measure ROS levels in rat plasma following a single oral dose of 25 mg kg⁻¹. Rats were chosen as the animal model owing to its adequacy (22). Fig. 7 presents the mean plasma concentration-time curve of ROS, and Table IX lists the key pharmacokinetic parameters.

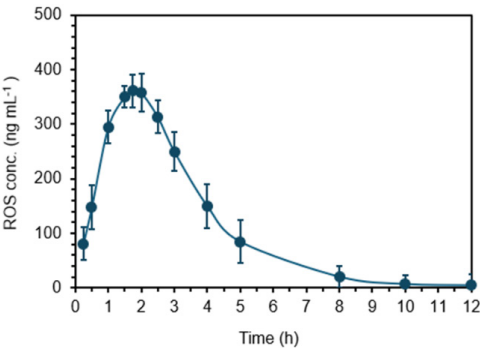


Fig. 7. Concentration-time profile of ROS in rats after single oral gavage administration at a dose of 25 mg kg⁻¹. The measured ROS concentrations are expressed as means obtained from 5 rats $\pm$ SD.

Table IX. The pharmacokinetic parameters of ROS in rat plasma after oral administration^a

Parameter	Unit	Value ^b
Maximum plasma concentration ($C_{\max}$)	ng mL ⁻¹	361.31
Time required for maximum plasma concentration ($t_{\max}$)	h	1.75
Volume of distribution (V_d)	L kg ⁻¹	0.077
Elimination rate constant (K_{el})	h ⁻¹	0.242
Elimination half-life time ($t_{1/2}$)	h	2.867
Clearance (CL)	L h ⁻¹ kg ⁻¹	0.018
Area under curve from time 0 to last concentration (AUC_{0-t})	ng h mL ⁻¹	1339.56
Area under curve at infinite time ($AUC_{0-\infty}$)	ng h mL ⁻¹	1342.01
Area under curve ratio ($AUC_{0-t}/AUC_{0-\infty}$)	%	99.82
Mean residence time (MRT)	h	3.25

^a Dose of 25 mg kg⁻¹.

^b Values are mean of five determinations.

After oral administration, ROS was rapidly absorbed, with a peak plasma concentration ($C_{\max}$) of 361.31 ng mL⁻¹ reached at 1.75 h ($t_{\max}$). The elimination half-life ($t_{1/2}$) was 2.867 h. A low volume of distribution ($V_d = 0.077$ L kg⁻¹) suggests that ROS remains largely within the bloodstream, showing limited tissue distribution. Clearance (CL) was also low (0.018 L h⁻¹ kg⁻¹), indicating relatively slow elimination, primarily *via* the kidneys. The AUC ratio ($AUC_{0-t}/AUC_{0-\infty}$) was 99.8 %, confirming that the method adequately captures the drug's elimination phase. These parameters are essential for determining the appropriate dosage and dosing schedule to attain the desired safe therapeutic outcomes in patient treatment.

Greenness of the HPLC-FD procedures

In developing this HPLC-FD method, the organic solvent content and run time were deliberately reduced to cut solvent consumption and waste while preserving optimal resolution between ROS and IS. Three assessment tools: AES (15), GAPI (16) and AGREE (17) were used to evaluate the method's greenness.

Analytical eco-scale. – Table X presents the AES results. Penalty points (PPs) were assigned for acetonitrile (2 PP) and ammonium acetate (4 PP) based on quantities. Hazard impact added 1 PP for acetonitrile and 2 PP for ammonium acetate. Energy use by the HPLC-FD system contributed 1 PP, while occupational hazards added none. Waste production and treatment accumulated 4 and 3 PPs, resp., as the method generates ≤ 10 mL of untreated waste per sample. Total PPs were 17, yielding an eco-scale score of 83 (100–17), indicating excellent environmental sustainability.

Green analytical procedure index. – The GAPI pictogram (15 parameters) is shown in Fig. 8a. Five parameters (1, 7, 12, 14, 15) are red (non-compliant), parameter 5 is yellow (direct quantitative method), parameters 6 and 10 are yellow (microscale extraction and moderate

Table X. Analytical eco-scale tool in assessing greenness of the HPLC-FD method

Eco-scale score parameters	Penalty points (PPs)
Solvent/reagent	
Amount: methanol (£ 10 mL per sample)	2
Ammonium acetate buffer (10 mL per sample)	4
Hazards: methanol	1
Ammonium acetate buffer	2
Instrument: Energy used (kWh per sample)	
HPLC-FD	1
Occupational hazardous	
Analytical process hermetic	0
Emission of vapors and gases to the air	0
Waste	
Production (£ 10 mL per sample)	4
Treatment (No treatment involved)	3
Total PPs	17
Eco-scale score	83

health risk solvents), the remaining eight parameters are green (fully compliant). Overall, 53.3 % green, 13.3 % yellow, and 33.3 % red, reflecting satisfactory greenness.

Analytical greenness. – The AGREE pictogram (Fig. 8b) shows parameter 1 (sample treatment) in yellow (manual handling), parameter 3 (off-line analysis) is red, parameter 7 is reddish-yellow (10 mL waste mobile phase), parameter 8 is dark yellow (10 min run time), parameter 9 is yellow (energy consumption), and all other parameters are green. The overall score of 0.69 out of 1 demonstrates high greenness for the proposed method.

Blue applicability grade index (BAGI)

The applicability of the proposed HPLC-FD method was evaluated using the BAGI metric tool, which assesses the overall practicality, efficiency, and environmental impact of an analytical procedure (18). As described in the tool's protocol, a 10-segment asteroid-shaped pictogram was generated, where each segment corresponds to a specific method attribute. Segments are coloured in varying shades of blue, with darker hues indicating better compliance with the respective criterion, and the centre displays the overall numerical score. The resulting asteroid pictogram for the proposed method is shown in Fig. 8c. The method achieved a BAGI score of 77.5 out of 100, confirming that it is not only environmentally friendly but also practical for routine laboratory or industrial applications.

Comparison of the proposed HPLC-FD method with the reported methods

A comparative evaluation of the proposed HPLC-FD method *vs.* reported ROS bio-analytical methods (9–12) is summarized in Table XI and discussed below. The proposed

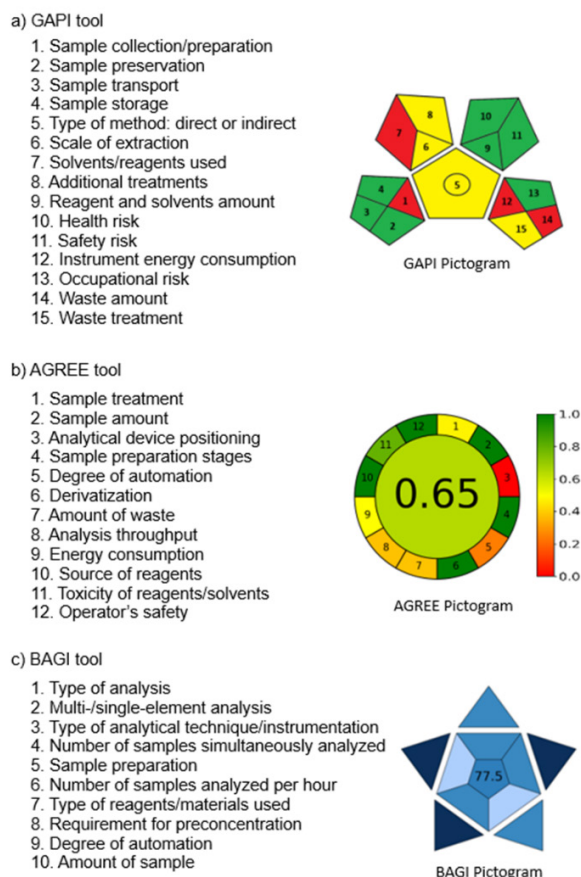


Fig. 8. Pictograms of greenness and applicability of the proposed HPLC-FD method for the determination of ROS by: a) GAPI; b) AGREE and c) BAGI tools. The evaluation parameters are given on the left-hand side of the corresponding pictograms.

HPLC-FD method exploits ROS native fluorescence, offering superior selectivity over the HPLC-UV method (9), which suffers from endogenous interference. While LC-MS/MS (10, 11) and HPLC-EC/ESI-MS (12) provide high selectivity or oxidation characterization, they require expensive, complex instrumentation unsuitable for routine use. For sample preparation, the proposed method uses rapid, cost-effective methanol protein precipitation *vs.* acetonitrile (9), SPE (11) or microextraction by packed sorbent (MEPS) (10), which are more labor-intensive or hazardous. The proposed method operates at 1.5 mL min⁻¹ isocratically, achieving a 7-min run time comparable to the HPLC-UV method (6 min) (9), but faster than gradient methods (16–45 min) (10–12), improving throughput.

The proposed method shows a linear range of 50–1,500 ng mL⁻¹, with LLOQ 50 ng mL⁻¹ and is twice as sensitive as HPLC-UV (LOQ 100 ng mL⁻¹) (9), though inferior to LC-MS/MS with MEPS (LOQ 1.0 ng mL⁻¹, range 0.5–2000 ng mL⁻¹) (10), which requires greater expertise. The

Table XI. Comparative evaluation of the proposed HPLC-FD method with the reported methods

Parameter	Proposed HPLC-FD method (this paper)	Reported method (ref.)			
		HPLC-UV (9)	LC-MS/MS-NMR (10)	LC-MS/MS (11)	HPLC-EC/ESI/MS (12)
Detection principle	Fluorescence	UV	MS/MS and NMR	MS/MS	EC/ESI-MS
Sample preparation	PP	PP	PP and SPE	MEPS	None
Flow rate (mL min ⁻¹)	1.5 (isocratic)	1.0 (isocratic)	0.2-0.5 (gradient)	0.15 (gradient)	0.25 (gradient)
Run time (min)	7	6	~16-45	~16	~30
Linearity range (ng mL ⁻¹)	10-1500	100-5000	100-5000	0.5-2000	NR
LOQ (ng mL ⁻¹)	50	100	NR	1.0	NR
Accuracy (recovery, %)	96.8-103.4	84	NR	96-115 %	NR
Precision (RSD, %)	3.2-6.1	≤ 13.9	NR	1.7-11.4	NR
Application	PK/TDM	PK	TD/metabolite ID	PK/PD	OxPs ID
Greenness level	Excellent	NR	NR	NR	NR
Through (samples per h)	High (~8-9)	High (~10)	Very low	Low (~4)	Very low
Clinical lab feasibility	High	Moderate	Very low	Low	Very low
Instrument cost	Moderate	Moderate	Extremely high	Very high	High
Operational expertise	Routine	Specialized	Highly specialized	Highly specialized	Specialized

EC/ESI/MS – electrochemical-electrospray ionization-mass spectrometry, ID – identification, MEPS – microextraction in packed syringe, MS/MS – tandem mass spectrometry, NMR – nuclear magnetic resonance, NR – not reported, OxPs – oxidation products, PD – pharmacodynamics, PK – pharmacokinetics, PP – protein precipitation, TDM – therapeutic drug monitoring, SPE – solid phase extraction, TD – tissue distribution

LC-MS/MS-NMR (11) and HPLC-EC/ESI-MS (12) methods did not report their quantitative parameters. Accuracy and precision for the proposed method (recovery 96.8–103.4 %; RSD 3.2–6.1 %) comply with ICH M10 guidelines, outperforming the HPLC-UV method (recovery 84 %; RSD up to 13.9 %) (9) and matching LC-MS/MS with microextraction in packed syringe (96–115 %; RSD 1.7–11.4 %) (10); the other reports (11, 12) did not disclose these data.

CONCLUSIONS

This study presents the first HPLC-FD method for ROS quantification in rat plasma, using simple methanol protein precipitation and isocratic elution (methanol:formate buffer – pH 5, 70:30 V/V, 1.5 mL min⁻¹) with a 7-min run time suitable for high-throughput analysis. The calibration curve was linear from 50 to 1,500 ng mL⁻¹ using 1/x² weighted regression ($R^2 = 0.9991$), with back-calculated accuracy and precision for all QC levels within $\pm 15\%$ ($\pm 20\%$ at LLOQ), and the LLOQ (50 ng mL⁻¹) confirmed at a signal-to-noise ratio = 10. These results reveal that the proposed HPLC-FD method is accurate and precise for the determination of ROS in plasma samples, matching the performance of the reported HPLC-UV method.

Applied to rat plasma following oral administration, it enabled determination of key PK parameters ($C_{\max}$, $t_{\max}$, $t_{1/2}$, V_d , CL , MRT , AUC) to guide dosing. The results indicate applicability of HPLC-FD method for pharmacokinetic studies of ROS.

Greenness was confirmed by ESA (83/100), GAPI (8/15), AGREE (0.69/1), and BAGI (77.5), supporting routine lab practicality.

In conclusion, this first-in-class HPLC-FD method provides a sensitive, green, and eminently practical tool for routine quantification of ROS in plasma, making it highly suitable for supporting pharmacokinetic studies and therapeutic monitoring in both pre-clinical and future clinical settings.

Acronyms, abbreviations, symbols. – AES – analytical eco-scale, AGREE – analytical GREENness, AUC – area under the curve, $AUC_{0-\infty}$ – area under the curve from time zero to infinity, AUC_{0-t} – area under the curve from time zero to the last measurable concentration, BAGI – blue applicability grade index, CDKs – cyclin-dependent kinases, CL – clearance, $C_{\max}$ – maximum plasma concentration, EC/ESI-MS – electrochemical-electrospray ionization mass spectrometry, FD – fluorescence detection, GAC – green analytical chemistry, GAPI – Green analytical procedure index, K_{el} – elimination rate constant, LC-MS/MS – liquid chromatography-tandem mass spectrometry, LLOQ – lower limit of quantification, MEPS – microextraction in packed syringe, MRT – mean residence time, PEM – pemigatinib, PK – pharmacokinetics, PP – protein precipitation, PPs – Penalty points, QC – quality control, ROS – roscovitine, SPE – solid-phase extraction, $t_{1/2}$ – elimination half-life, TDM – therapeutic drug monitoring, $t_{\max}$ – time to reach maximum plasma concentration, V_d – volume of distribution

Data availability. – The authors confirm that the data of this study are available within the article.

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Conflict of interest. – 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.

Author's contribution. – Conceptualization, I.A.D.; methodology, I.A.D. and N.Z.A.; analysis, I.A.D.; investigation, I.A.D.; writing, original draft preparation, N.Z.A.; writing, review and editing, I.A.D. and N.Z.A. Both authors have read and agreed to the published version of the manuscript.

REFERENCES

1. H. K. Alhashimi, M. A. A. Najm and H. N. K. AL-Salman, Targeting cyclin-dependent kinases (CDKs) for cancer therapy: An in-depth review of molecular candidates, their synthesis, evaluation, and future directions, *Farm. Polska* **81** (2025) 489–499; <https://doi.org/10.32383/farmopol/218224>
2. N. Raje, S. Kumar, T. Hideshima, A. Roccaro, K. Ishitsuka, H. Yasui, N. Shiraishi, D. Chauhan, N. C. Munshi, S. R. Green and K. C. Anderson, Seliciclib (CYC202 or R-roscovitine), a small-molecule cyclin-dependent kinase inhibitor, mediates activity via down-regulation of Mcl-1 in multiple myeloma, *Blood* **106** (2005) 1042–1047; <https://doi.org/10.1182/blood-2005-01-0320>
3. K. Bettayeb, D. Baunbæk, C. Delehouze, N. Loaëc, A. J. Hole, S. Baumli, J. A. Endicott, S. Douc-Rasy, J. Bénard, N. Oumata, H. Galons and L. Meijer, CDK inhibitors roscovitine and CR8 trigger Mcl-1 down-regulation and apoptotic cell death in neuroblastoma cells, *Genes Cancer* **1** (2010) 369–380; <https://doi.org/10.1177/1947601910369817>
4. J. Cienas, K. Kalyan, A. Sorokinas, E. Stankunas, J. Levy, I. Meskinyte, V. Stankevicius, A. Kaupinis and M. Valius, Roscovitine in cancer and other diseases, *Ann. Transl. Med.* **3**(10) (2015) Article ID 135 (12 pages); <https://doi.org/10.3978/j.issn.2305-5839.2015.03.61>
5. M. S. Alsalhi, M. Al-Outaibi and I. A. Darwish, Development of three innovative green microwell spectrometric methods with high throughput for determination of seliciclib in its bulk form and capsules, *Sust. Chem. Pharm.* **37** (2024) Article ID 101443; <https://doi.org/10.1016/j.scp.2024.101443>
6. I. A. Darwish and N. Z. Alzoman, Development of green and high throughput microplate reader-assisted universal microwell spectrophotometric assay for direct determination of tyrosine kinase inhibitors in their pharmaceutical formulations irrespective the diversity of their chemical structures, *Molecules* **28** (2023) Article ID 4049 (19 pages); <https://doi.org/10.3390/molecules28104049>
7. G. Xie, M. Liao, B. Wang and C. Zhang, Determination of seliciclib enantiomeric purity by HPLC on chiral stationary phase, *Chromatographia* **79** (2016) 641–646; <https://doi.org/10.1007/s10337-016-3067-3>
8. P. Orság, L. H. I. Fojt, J. Coufal, V. Brázda and M. Fojta, Voltammetric behavior of a candidate anti-cancer drug seliciclib at carbon electrodes in aqueous buffers and a cell culture medium, *Monatsh. Chem.* **150** (2019) 461–467; <https://doi.org/10.1007/s00706-018-2333-5>
9. M. Vita, L. Meurling, T. Pettersson, M. Cruz-Sidén, Å. Sidén and M. Hassan, Analysis of roscovitine using novel high performance liquid chromatography and UV-detection method: Pharmacokinetics of roscovitine in rat, *J. Pharm. Biomed. Anal.* **34**(2) (2004) 425–431; [https://doi.org/10.1016/S0731-7085\(03\)00534-X](https://doi.org/10.1016/S0731-7085(03)00534-X)
10. M. Vita, M. Abdel-Rehim, S. Olofsson, Z. Hassan, L. Meurling, A. Sidén, M. Sidén, T. Pettersson and M. Hassan, Tissue distribution, pharmacokinetics and identification of roscovitine metabolites in rat, *Pharm. Sci.* **25**(1) (2005) 91–103; <https://doi.org/10.1016/j.ejps.2005.02.001>
11. M. Vita, P. Skansen, M. Hassan and M. Abdel-Rehim, Development and validation of a liquid chromatography and tandem mass spectrometry method for determination of roscovitine in plasma and urine samples utilizing on-line sample preparation, *J. Chromatogr. B* **817** (2005) 303–307; <https://doi.org/10.1016/j.jchromb.2004.12.018>
12. M. Karady, O. Novák, A. Horna, M. Strnad and K. Doležal, High performance liquid chromatography-electrochemistry-electrospray ionization mass spectrometry (HPLC/EC/ESI-MS) for detection and characterization of roscovitine oxidation products, *Electroanalysis* **23** (2011) 2898–2905; <https://doi.org/10.1002/elan.201100383>
13. A. Y. Sayed, N. Y. Khalil, A. Almomen, A. A. Almhizia and I. A. Darwish, A highly sensitive nonextraction-assisted HPLC method with fluorescence detection for quantification of duvelisib in plasma samples and its application to pharmacokinetic study in rats, *Drug Des. Dev. Ther.* **15** (2021) 2667–2677; <https://doi.org/10.2147/dddt.s318714>

14. ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, *ICH Harmonised Guideline, Bioanalytical Method Validation and Study Sample Analysis: M10*, ICH, Geneva 2022.
15. A. Gałuszka, P. Konieczka, Z. M. Migaszewski and J. Namieśnik, Analytical eco-scale for assessing the greenness of analytical procedures, *Trends Anal. Chem.* **37** (2012) 61–72; <https://doi.org/10.1016/j.trac.2012.03.013>
16. J. Płotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green analytical procedure index, *Talanta* **181** (2018) 204–209; <https://doi.org/10.1016/j.talanta.2018.01.013>
17. F. Pena-Pereira, W. Wojnowski and M. Tobiszewski, AGREE-analytical greenness metric approach and software, *Anal. Chem.* **92** (2020) 10076–10082; <https://doi.org/10.1021/acs.analchem.0c01887>
18. N. Manousi, W. Wojnowski, J. Płotka-Wasyłka and V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality, *Green Chem.* **25** (2023) 7598–7604; <https://doi.org/10.1039/D3GC02347H>
19. J. Płotka-Wasyłka and J. Namieśnik (Eds.), *Green Analytical Chemistry: Past, Present and Perspectives*, Springer Nature Singapore Pte Ltd., Singapore 2019; <https://doi.org/10.1007/978-981-13-9105-7>
20. I. A. Darwish, N. Z. Alzoman and M. S. Alsali, A green and highly sensitive microwell spectrofluorimetric method with high throughput for the determination of pemigatinib based on dual fluorescence enhancement by photoinduced electron transfer blocking and micellization: Application to the analysis of tablets, content uniformity testing, and human plasma, *Luminescence* **39**(6) (2024) e4813; <https://doi.org/10.1002/bio.4813>
21. L. Meijer, G. Hery-Arnaud, C. Leven, E. Nowak, S. Hillion, Y. Renaudineau, I. Durieu, R. Chiron, A. Prevotat, I. Fajac, D. Hubert, M. Murris-Espin, S. Huge, I. Danner-Boucher, B. Ravoninjatovo, S. Leroy, J. Macey, T. Urban, G. Rault, D. Mottier and R. Le Berre, Safety and pharmacokinetics of roscovitine (seliciclib) in cystic fibrosis patients chronically infected with *Pseudomonas aeruginosa*, a randomized, placebo-controlled study, *J. Cyst. Fibrosis* **21** (2022) 529–536; <https://doi.org/10.1016/j.jcf.2021.10.013>
22. E. M. Blais, K. D. Rawls, B. V. Dougherty, Z. I. Li, G. L. Kolling, P. Ye, A. Wallqvist and J. A. Papin, Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions, *Nat. Commun.* **8** (2017) Article ID 14250 (15 pages); <https://doi.org/10.1038/ncomms14250>