Asian Journal of Chemistry; Vol. 38, No. 9 (2026), 2492-2500
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2026.36569

Forced Degradation studies of Naringenin: Isolation, Identification and Structural Elucidation of Stress Degradation Products using NMR, FTIR, Mass Spectroscopy and Prep-HPLC

TARAK PAWAR[1,2], RAJRATNA P. TAYADE[1,*] and ANIL S. GAWADE[2]

[1]Department of Chemistry, Amity School of Applied Sciences, Amity University Maharashtra, Mumbai-410206, India
[2]Department of Research & Development, Cleanchem Laboratories LLP, A-181, Dhirubhai Ambani Knowledge City, MIDC Industrial Area, Kopar Khairane, Navi Mumbai-400710, India

*Corresponding author: E-mail: rptayade@mum.amity.edu, rajtayade158@gmail.com

Received: 3 June 2026 *Accepted*: 20 August 2026 *Published online*: 5 September 2026 AJC-22478

This study presents the forced degradation study of the antioxidant molecule naringenin (NNG) under various stress conditions following ICH guidelines Q1A(R2). Significant degradation under basic and oxidative stress were observed. It is quite stable in acidic, photolytic and thermal conditions. Under basic stress conditions, two major degradation products, NNG-DP1 and NNG-DP2, were obtained, which are isomers of each other. Under oxidative stress, one major degradation product, NNG-DP1, was observed, structurally similar to the E-isomer obtained under basic stress conditions. The degradation compounds were isolated using chromatographic separation, purified and characterized utilizing advanced analytical methods, including (^{1}H, ^{13}C, D_2O) NMR, FT-IR, COSY, NOESY and mass spectrometry techniques. Repeatability of the self-developed HPLC method was determined at 20-120 µg/mL, obtaining an RSD (%) < 2.0 and accuracy > 98%. The mean recovery, LOD and LOQ values were found to be 98.25-102.88%, 0.1 µg/mL and 1.0 µg/mL, respectively. A probable mechanism for the formation of the major degradation products was also proposed.

Keywords: Naringenin, Stress degradation, HPLC, Isomers formations, Flavonoid-related compounds, Degradation pathways.

INTRODUCTION

Naringenin (NNG), (2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-4-one ($C_{15}H_{12}O_5$) found in a variety of plants comes from a class of compounds called flavonoids exhibiting antioxidant properties [1]. It is commonly consumed as part of the diet and offers several health benefits, with reported antioxidant, anti-inflammatory, anticarcinogenic, antibacterial and vasodilatory activities [2-5]. The fruits of the citrus family, cherries, tomatoes, grapefruits and cocoa all contain the flavonoid naringenin (NNG).

Development of a new drug requires considerable time and financial resources [6]. A suitable analytical framework is therefore required for the evaluation of therapeutic compounds before regulatory approval [7,8]. Various analytical techniques such as titration, spectrophotometry, spectroscopy, mass spectrometry and chromatography, are used to assess drug quality [9,10]. Forced degradation studies provide information on degradation products and pathways, formulation stability and the development of suitable analytical methods. Such studies are useful for assessing the intrinsic stability of a molecule, identifying degradation products, understanding their formation pathways and determining the rate and extent of degradation [11,12]. Stability and forced degradation studies can further help in the selection of suitable packaging materials and storage conditions and in the characterization of degradation products [13]. HPLC and UHPLC are widely used analytical techniques for monitoring drug degradation under stress conditions [14,15].

The degradation of NNG has mainly been investigated in the context of developing analytical methods for its routine analysis. Most reported studies have used HPLC or LC-MS for the detection of degradation products [16]. Cordenonsi *et al.* [17] investigated the forced degradation of NNG under thermal stress at 60 ºC for 1-90 days and under photolytic conditions by exposing NNG solutions in ethanol to UV-A radiation (Blacklight Blue lamp-Orion, 352 nm/30 W) and UV-C radiation (Light Express lamp LE UV, 254 nm/30 W). The extent of degradation was determined using HPLC. In another study, Cordenonsi *et al*. [18] investigated the photodegradation of

NNG-loaded nanoparticles using a self-developed HPLC method and evaluated the photodegradation kinetics.

A review of the available literature revealed limited information on the forced degradation of NNG under ICH recommended acidic, alkaline, oxidative, photolytic and thermal stress conditions [16-18]. Detailed studies describing the major degradation products, their structural elucidation and the possible pathways involved in their formation are scarce. Characterization of degradation products of an active pharmaceutical ingredient (API) is important for impurity profiling, formulation stability and the selection of suitable packaging and storage conditions [19]. Based on this research gap, the present study was undertaken to investigate the stress degradation behaviour of NNG under ICH-recommended conditions. This work follows our earlier studies on the degradation of ascorbic acid [20].

To the best of our knowledge, the E-isomer has been reported through synthetic routes, whereas the formation of both E- and Z-isomers through forced degradation has not been reported. The reported compounds have previously been obtained or synthesized through different synthetic routes [21,22]. The present study provides information relevant to the isolation and purification of degradation products and to the selection of suitable storage conditions under stress-prone environments. The major degradation products were isolated, purified, and characterized using ^{1}H NMR, ^{13}C NMR, D_2O exchange, COSY, NOESY, mass spectrometry and FT-IR techniques.

Under basic stress conditions, two major degradation products were obtained. These products were identified as isomers of each other based on their 2D NMR data, particularly COSY and NOESY spectra. Under oxidative stress, the formation of other oxidation products was anticipated; in contrast, the E-isomer was obtained as the major degradation product under enhanced oxidative conditions. The study provides information on the degradation behaviour of NNG that may be useful in formulation development, storage and stability assessment. The work focuses on the identification of suitable stress conditions for enrichment of NNG degradation impurities, development of an HPLC method for their separation and isolation and structural elucidation using spectroscopic techniques. The degradation products identified in this study through forced degradation may assist manufacturers and researchers in recognizing impurities that can arise during the synthesis, processing and storage of NNG.

EXPERIMENTAL

The analytical grade chemicals and reagents were procured from Sigma Aldrich, TCI and BLD Pharma. Naringenin (NNG) was obtained from Cleanchem Laboratory, Navi Mumbai, India. The reactions were monitored using pre-coated aluminium plates procured from Merck silica gel $60F_{254}$ using the UV visualization technique, Ninhydrin reagent and iodine vapours. Column chromatographic purifications were performed on Merck silica gel (200-400 mesh). Aluminium plates pre-coated with silica gel $60F_{254}$ plates (10 cm × 10 cm with 6 mm thickness; Merck, Germany) were used for TLC.

Characterization: The reactions were monitored using pre-coated aluminium plates procured from Merck silica gel $60F_{254}$ using the UV visualization technique, Ninhydrin reagent and iodine vapours. Column chromatographic purifications were performed on Merck silica gel (60-120 mesh). The ^{1}H NMR and ^{13}C NMR spectra were recorded using JEOL (JNM-ECZ-400 s/L1) 500 MHz using $(Me)_4Si$ as the internal standard in DMSO-d_6 solvent. The exchangeable protons were further confirmed with D_2O exchange experiment. Carbon and hydrogen connectivity were proved with 2D NMR experiments like COSY and NOESY. The electrospray mass spectrum was recorded on a MD SCIEX API3200LC/MS/MS system equipped with an electrospray ionization technique. The FT-IR spectra were recorded using FT-IR 4100 (JASCO). Chromatographic analysis was performed on Thermo Ultimate 3000 reversed-phase C_{18} column with an internal diameter of 250 × 4.6 mm and a particle size of 5 μm; manufactured by WATERS (XTERRA RP18). The mobile phase consists of a mixture of methanol:water in a ratio of 70:30. The mobile phase was filtered and degassed through a 5 μ membrane filter before use and then pumped at a flow rate of 1.0 mL/min, with 20 μL volume of injection. Methanol was used as a diluent. The run time was 20 min. The HPLC spectra were recorded at a wavelength of 288 nm. The purification of degraded compounds was carried out by the column chromatography technique and preparative chromatography. The preparative isolation of degraded compounds was done by Boitage Selekt. Columns of 12 g and 40 g size were used. A mixture of methanol and dichloromethane was used as a mobile phase to obtain the desired compound, where desired compound eluted with 5% MeOH:CH_2Cl_2 solvent. A 1 bar pressure and 40 mL/min flow rate were used to run the column.

Stress degradation study: Stress degradation studies under acidic, basic, oxidative, thermal and photolytic conditions were carried out in accordance with ICH guidelines [23]. Acidic and basic conditions can induce degradation of antioxidant molecules and drugs, leading to the formation of products with structural changes. To assess the stability of NNG, degradation studies were carried out under both acidic and basic conditions.

Acidic degradation: For the acid degradation study, 100 mg of NNG was initially dissolved in 1 mL of 0.1 N HCl solution at ambient temperature for 60 h. No degradation was obtained subsequently, the concentration of HCl solution was increased to 5 N gradually and stirred for 60 h at 60 ºC. NNG does not react with 1 mL of 5 N HCl for 24 h at 60 ºC, the resultant solution was neutralized and the obtained solid was filtered. The HPLC gave no degradation in the chromatogram, hence, NNG is quite stable in acidic environment.

Basic degradation: Basic degradation was performed using 0.1 N NaOH solution. NNG (100 mg) was dissolved in 1 mL of 0.1 N NaOH and stirred continuously for 60 h at ambient temperature. Degradation was monitored by TLC and HPLC analysis, which showed degradation products at retention times (RTs) of 2.51 min (0.88%), 3.51 min (0.68%) and 3.91 min (15.47%). To accelerate the degradation process, the NaOH concentration was increased to 0.5 N and the reaction was continued for 60 h at 55-60 ºC. Under these conditions, degradation products were observed at RTs of 2.51 min (6.90%), 3.51 min (28.53%), and 3.91 min (9.24%).

To enrich the degradation products, NNG was finally treated with 3 N NaOH (10 mL) and stirred at 60 ºC for 24 h.

TLC analysis showed three prominent spots, indicating the formation of three degradation products. The reaction mixture was neutralized to pH 7 using aqueous HCl and extracted with dichloromethane. HPLC analysis showed three major degradation products at RTs of 2.51, 3.51 and 3.92 min, with degradation levels of 37.49%, 49.79% and 10.96%, respectively. The HPLC chromatogram of NNG under basic stress conditions is shown in Fig. 1.

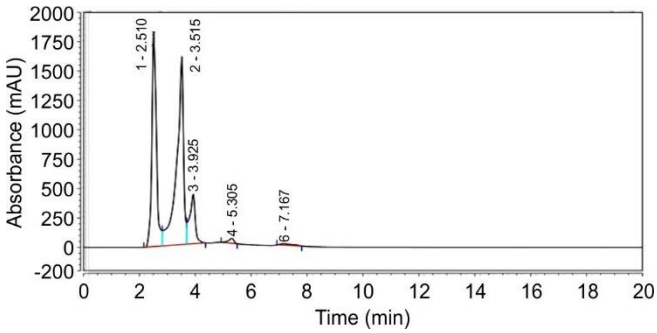


Fig. 1. HPLC chromatogram of NNG in basic degradation

For isolation, 1 g of NNG (assay >99%) was subjected to alkaline stress using 3 N NaOH. After completion of the reaction, 988 mg of crude solid was obtained. Flash chromatography afforded 395 mg of the E-isomer and 88 mg of the Z-isomer as pure compounds. The remaining fractions contained a mixture of NNG and other degradation products, including the compound observed at RT 2.51 min. Attempts to isolate this degradation product were unsuccessful due to its poor stability and volatility during solvent removal.

The E- and Z-isomers were further purified by preparative chromatography and designated as NNG-DP1 and NNG-DP2, respectively. These two degradation products are reported for the first time as degradation products of NNG under stress conditions.

Photolytic degradation: For the photodegradation study, NNG (200 mg) was exposed to UV radiation at 254 and 365 nm. Two solid samples (200 mg each) were directly exposed to UV radiation at 254 and 365 nm for 10 days. Two additional samples (200 mg each) were dissolved in alcohol and exposed to UV radiation at the same wavelengths for 10 days. After 8 days, all four samples were analyzed by HPLC, and no degradation was detected. The experiment was continued for 10 days under the same conditions, but no significant degradation was observed. These results indicate that NNG remains stable under the tested UV irradiation conditions.

Oxidative degradation: For the oxidative stress study, NNG (200 mg) was dissolved in 20 mL of 3% H_2O_2 solution and stirred for 48 h at ambient temperature. HPLC analysis of the resulting solution showed degradation products at retention times (RTs) of 2.50 and 3.54 min. The experiment was repeated by treating 200 mg of NNG with 20 mL of 3% H_2O_2 at 50 ºC. Under these conditions, degradation at RT 3.54 min increased to 5.44%, whereas the degradation at RT 2.50 min decreased.

For isolation of the major degradation product, NNG (500 mg, assay >99%) was subjected to oxidative stress using 30% H_2O_2. HPLC analysis showed a major degradation product at RT 3.54 min, corresponding to 28.53% degradation, as shown in Fig. 2. After completion of the reaction, 470 mg of crude product was obtained. Flash chromatography afforded 120 mg of the pure E-isomer, along with 335 mg of unreacted NNG and a mixture of other compounds. Based on its spectral and analytical data, the isolated E-isomer was identified as NNG-DP1.

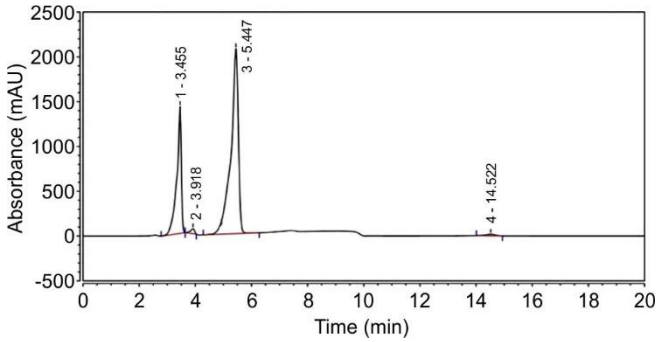


Fig. 2. HPLC chromatogram of NNG oxidative degradation

Under oxidative stress at lower H_2O_2 concentration, two degradation products were observed. Under the more stringent oxidative conditions, only NNG-DP1 was detected as the major degradation product. NNG-DP1 was also obtained under basic hydrolytic conditions.

Thermal degradation: For thermal degradation, 200 mg of NNG solid was placed on a petri dish and kept in an oven for 6 days at 100 ºC and also 200 mg NNG was dissolved in 1 mL of DMF solvent and this clear solution was kept in an oven for 100 ºC and both samples were analyzed in HPLC. No degradation occurred, hence NNG was quite stable in high thermal condition.

RESULTS AND DISCUSSION

In this study, naringenin (NNG) is subjected to forced degradation under acidic, basic, photolytic, oxidative and thermal conditions. Degradation was observed in basic as well as in oxidative conditions. NNG was found to be stable in acidic, photolytic and thermal conditions. The degraded compounds were isolated & identified by using HPLC, NMR (^1H, ^{13}C, D_2O), COSY, NOESY, FT-IR and mass spectroscopy techniques. In HPLC analysis, it was observed that three degradation products at 2.50 RT, 3.58 RT and 4.04 RT under basic stress and two degradation products at 2.50 RT and 3.58 RT in oxidative stress. The HPLC chromatogram of NNG displays a peak at 5.57 RT for the retention period as shown in Fig. 3.

The HPLC chromatograms of NNG under different stress conditions, along with the chromatogram of the parent compound, are shown in Fig. 4. The chemical structures of NNG and its degradation products were elucidated using HPLC, NMR (^1H, ^{13}C, D_2O), COSY, NOESY, FT-IR and mass spectroscopy techniques. The degradation conditions and corresponding extent of degradation are shown in Table-1.

From the characteristic data, it was interpreted that the compounds obtained at 3.58 RT (E-isomer) separately in oxidative and basic stress are structurally similar. The NNG degradation products NNG-DP1 and NNG-DP2 are characterized by their spectral and analytical data. The HPLC chro-

TABLE-1
SUMMARY OF FORCE DEGRADATION STUDY

Degradation parameter	Reaction condition	Results
Acidic	Stirred in 0.1 N HCl for 60 h at room temperature Stirred in 0.5 N HCl for 60 h at 60 ºC temperature Stirred in 5 N HCl for 24 h at 60 ºC temperature	API remained stable without any degradation
Alkaline	Stirred in 0.1 N NaOH for 60 h at room temperature	NNG-DP1 0.68%, NNG-DP2 15.47% and at 2.51 RT 0.88% degradation formed
	Stirred in 0.5 N NaOH for 60 h at 55-60 ºC temperature	NNG-DP1 28.53%, NNG-DP2 19.24% and at 2.51 RT 6.9% degradation formed
	Stirred in 3 N NaOH for 24 h at 55-60 ºC temperature	NNG-DP1 49.79%, NNG-DP2 10.96% and at 2.51 RT 37.49% degradation formed
Photolytic	Dry exposure at 254 & 365 nm for 10 days Alcoholic solution of API exposed to 254 & 365 nm for 10 days	Stable when exposed in ultraviolet radiation
Oxidative	Stirred in 3% H_2O_2 Sol^n for 48 h at room temperature	2.5 RT & NNG-DP1 0.27% degradation formed
	Stirred in 3% H_2O_2 sol^n for 48 h at 50 ºC temperature	2.5 RT & NNG-DP1 5.44% degradation formed
	Stirred in 30% H_2O_2 sol^n for 44 h at 50 ºC temperature	Only NNG-DP1 28.53% degradation formed
Thermal	Heated up to 100 ºC for 6 days	API is thermally stable

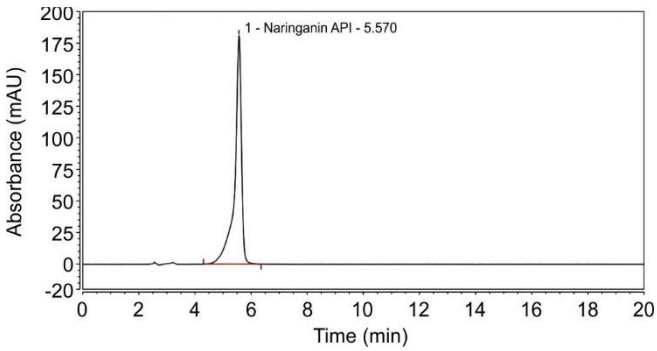


Fig. 3. HPLC chromatogram of naringenin (NNG)

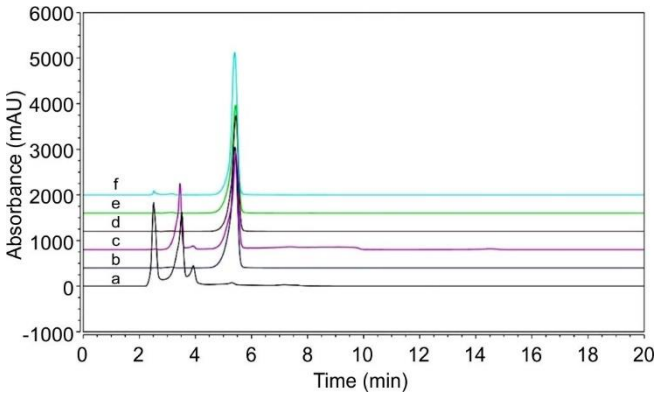


Fig. 4. Overlaid chromatogram of NNG in various stress conditions (a) basic degradation, (b) NNG API, (c) oxidative degradation, (d) photolytic degradation, (e) thermal degradation and (f) acidic degradation

matograms of the isolated degraded products NNG-DP1 and NNG-DP2 are individually overlaid with the antioxidant molecule of the NNG molecule, as shown in Fig. 5.

Characterization of isolated compounds (NNG DP1 and NNG DP2): To confirm the structure of the isolated compounds (NNG DP1 and NNG DP2), NMR, mass, FTIR and HPLC techniques were used for characterization.

Structural elucidation of NNG DP1: HPLC purity: 99.43%, m.p.: 190 ºC. ^{1}H NMR (500 Hz, DMSO-d_6, δ ppm): 6.28 (d, 1H, J = 2.1 Hz, H_a), 6.41 (d, 1H, J = 2.4 Hz, H_b),

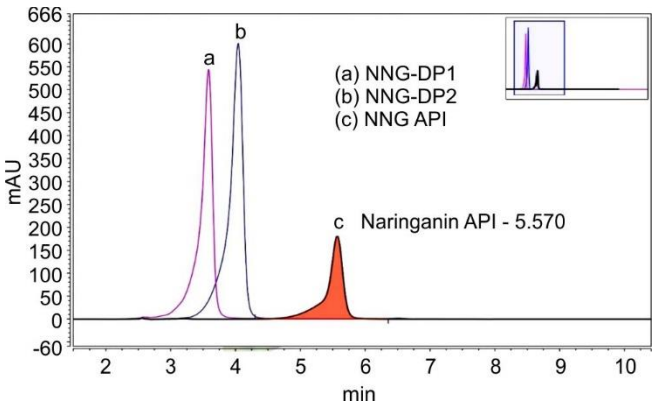


Fig. 5. HPLC chromatogram of both the degradation products obtained in oxidative and basic stress condition along with the antioxidant molecule (NNG)

6.83-6.85 (m, 2H, H_c), 7.72-7.78 (m, 4H, H_d, J = 15-16 Hz, *trans* coupling), 8.16 (d, 1H, J = 9.0 Hz, H_e), 10.26-10.53 (m, 2H, OH_f), 13.61 (s, 1H, OH_g).

The ^{1}H NMR spectrum showed signals for the 4 aromatic protons of H_d as a multiplet in the range of 7.784-7.749 ppm. The two olefinic protons exhibited a coupling constant of J = 16.5 Hz, supporting the formation of the E-isomer. The ^{1}H and ^{13}C NMR assignments are shown in Table-2. The D_2O exchange experiment showed no additional signal in the down-field region, supporting the presence of phenolic OH protons. The FT-IR spectrum showed characteristic absorption bands at 3481 cm^{-1} (OH), 1685 cm^{-1} (C=O), 1633 cm^{-1} (C=C), 1494 cm^{-1} (aromatic C–H) and 1143 cm^{-1} (aromatic C–H). The absorption band at 1633 cm^{-1} supports the presence of a C=C bond.

The COSY spectrum (Fig. 6a) showed coupling between the signals at 7.7 ppm ($C_{11}H$ and $C_{15}H$) and 6.8 ppm ($C_{12}H$ and $C_{14}H$), confirming the expected coupling between the aromatic protons. The signals at 8.16 ppm (C_3H) and 6.41 ppm (C_2H) were mutually coupled with a coupling constant of J = 8.6 Hz. The signals at 7.784 ppm (C_9H) and 7.749 ppm (C_8H) showed a coupling constant of J = 16.5 Hz, consistent with a *trans*- or E-configuration of the double bond. The

TABLE-2
ANALYTICAL DATA OF NNG-DP1 AND NNG-DP2

NNG-DP1				NNG-DP2			
^{13}C (100 MHz, DMSO-d_6) δ	Assignment	^{1}H (400 Hz, DMSO-d_6)		^{13}C (100 MHz, DMSO-d_6) δ	Assignment	^{1}H (400 Hz, DMSO-d_6)	
		δ value	Assignment			δ value	Assignment
102.59	C_9	6.28	d, *J* = 2.1 Hz, 1H, C_6H	102.55	C_9	6.28	m, 1H, C_6H
108.10	C_8	6.41	d, *J* = 8.6 Hz, 1H, C_2H	108.07	C_8	6.40	d, *J* = 3.2 Hz, 1H, C_2H
113.00	C_2	6.83-6.85	m, 2H, $C_{12}H$, $C_{14}H$	112.96	C_2	6.84	m, 2H, $C_{12}H$, $C_{14}H$
115.84	C_3	7.72-7.78	m, 4H, $C_{11}H$, $C_{15}H$, C_9H, C_8H (*J* = 16.5 Hz) *trans*	115.81	C_3	7.75-7.77	m, 4H, $C_{11}H$, $C_{15}H$, C_9H, C_8H (*J* = 8.5 Hz) *cis*
117.40	C_4, C_6	8.16	d, *J* = 9.0 Hz, 1H, C_3H	117.39	C_4, C_6	8.16	d, *J* = 3.2 Hz, 1H, C_3H
125.75	C_{15}	10.26-10.53	m, 2H, C_1-OH, C_{13}-OH	125.73	C_{15}	10.12	s, 1H, C_1-OH
131.22	C_{10}	13.61	s, 1H, C_5-OH	131.21	C_{10}	10.66	s, 1H, C_{13}-OH
132.84	C_{11}, C_{14}			132.83	C_{11}, C_{14}	13.61	s, 1H, C_5-OH
144.28	C_{12}			144.24	C_{12}		
160.27	C_1			160.24	C_1		
164.96	C_5			164.94	C_5		
165.80	C_{13}			165.76	C_{13}		
191.53	C=O (C_7)			191.50	C=O (C_7)		

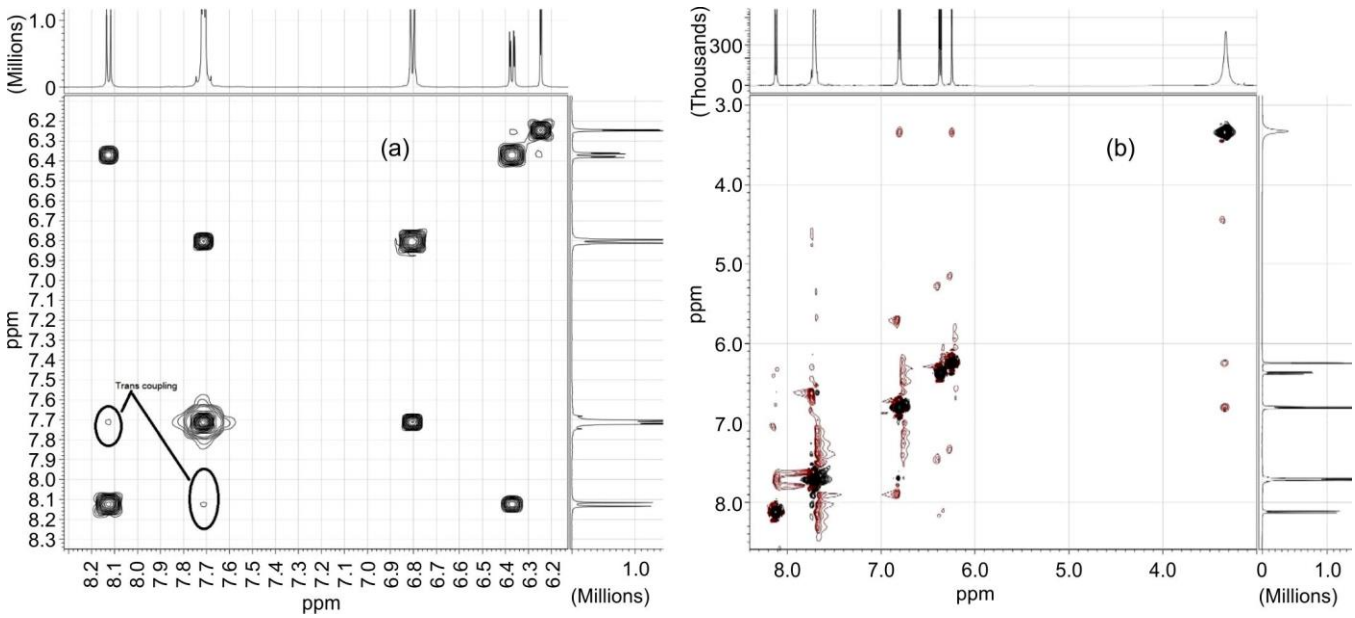
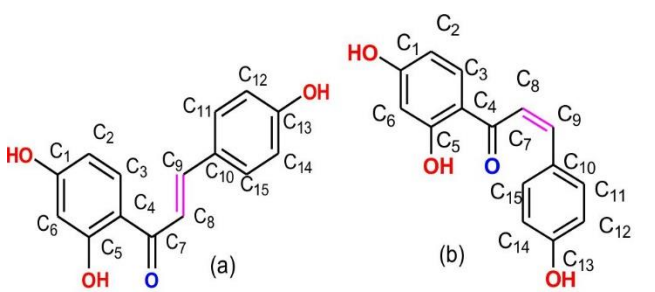


Fig. 6. (a) COSY and (b) NOESY spectra of NNG-DP1

NOESY spectrum (Fig. 6b) provided further support for the assigned E-configuration. The mass spectrum showed a major ion at *m/z* 257, corresponding to $[M+H]^+$.

Based on the ^{1}H NMR, ^{13}C NMR, COSY, NOESY, FT-IR and mass spectrometric data, NNG-DP1 was identified as (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (Fig. 7a) [24]. The proposed structure is consistent with the obtained spectroscopic and analytical data.

NNG-DP2 structural elucidation: HPLC purity: 99.32%, m.p.: 174 ºC. ^{1}H NMR, (400 Hz, DMSO-d_6, δ ppm): 6.28-6.29 (d, 1H, *J* = 2.4 Hz, H_a), 6.40-6.42 (dd, 1H, *J* = 11.2 Hz, H_b), 6.84-6.86 (d, 2H, *J* = 8.8 Hz, H_c), 7.75-7.77 (m, 4H, *J* = 8.5 Hz H_d), 8.16-8.18 (d, 1H, H_e); 10.12 (s, 1H, OH_f), 10.66 (s, 1H, OH_f), 13.61 (s, 1H, OH_g).



(*E*)-1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (m.w.: 256.25) E-isomer (NNG-DP1)

(*Z*)-1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one (m.w.: 256.25) Z-isomer (NNG-DP2)

Fig. 7. Structure of (a) NNG-DP1 and (b)NNG-DP2

For NNG-DP2, the ^{1}H NMR spectrum showed a multiplet in the range of 7.769-7.748 ppm, tentatively assigned to C_9H and C_8H, along with $C_{11}H$ and $C_{15}H$ (Table-2). The signal range was narrower than that observed for the E-isomer, consistent with the lower coupling expected for the Z-isomer. The ^{1}H NMR spectrum showed coupling constants of 2.0 Hz for both C_8H and C_9H, supporting the proposed structure of the degradation product. The FT-IR spectrum showed an absorption band at 1603 cm^{-1}, supporting the presence of a C=C bond. In the COSY spectrum (Fig. 8), the aromatic protons C_3H (8.17 ppm) and C_2H (6.41 ppm) showed mutual coupling with a coupling constant of J = 3.2 Hz. The aromatic protons $C_{12}H$ and $C_{14}H$ (6.82 ppm) showed coupling with $C_{11}H$ and $C_{15}H$ (7.74 ppm). No coupling was observed between the signals at 7.72 ppm (C_9H) and 8.16 ppm (C_3H), supporting the assignment of a *cis*- or Z-configuration. The COSY data, together with the NMR spectral features, which confirmed that NNG-DP1 and NNG-DP2 are geometric isomers. The mass spectrum showed a major ion at *m/z* 255.03 in the negative ESI mode, corresponding to $[M–H]^-$. Thus, based on these results, it is confirmed that the degraded compound is (*Z*)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (Fig. 7b).

HPLC method development and validation of NNG: The developed HPLC method was evaluated for specificity, selectivity, linearity, accuracy, precision, and robustness. The method was validated according to ICH Q2 (R1) guidelines [25,26] and USP requirements. The degradation behaviour of NNG was assessed using the developed stability-indicating HPLC method.

Method specificity and selectivity were assessed by evaluating the peak purity of NNG and its degradation products, NNG-DP1 and NNG-DP2, using a PDA detector. The peak purity results confirmed the absence of interfering peaks, supporting the specificity of the method. Linearity was evaluated over the concentration range of 20-120 µg/mL. The calibration curve showed good linearity, with a correlation coefficient (r^2) of 0.999.

Precision was evaluated at three concentration levels (80, 100 and 120 µg/mL), with each concentration analyzed in triplicate. Intra-day precision was assessed on the same day, while inter-day precision was determined on consecutive days. The %RSD values obtained for both intra-day and inter-day precision were below 2% (Table-3), confirming the precision of the method. Precision was assessed through repeatability (intra-day precision) and intermediate precision (inter-day precision) and expressed as relative standard deviation (%RSD) for a series of measurements (Table-4).

TABLE-3
SYSTEM SUITABILITY RESULTS OF NNG

SST RUN	Area (mAU × min)
1st INJ	55.4839
2nd INJ	56.1653
3rd INJ	56.932
4th INJ	57.7447
5th INJ	57.8803
6th INJ	58.4345
Average	57.10678333
SD	1.026221845
RSD	1.80

TABLE-4
LOQ, INTRA-DAY AND INTER-DAY
METHOD PRECISION DATA OF NNG

Sample	Area (mAU × min)		
	LOQ level precision	Intra-day method precision	Inter-day method precision
1st INJ	6.4634	59.8442	45.3845
2nd INJ	6.7417	59.1959	44.9264
3rd INJ	6.6525	58.2446	45.6898
4th INJ	6.6522	58.3978	45.4253
5th INJ	6.6368	58.5308	44.8312
6th INJ	7.0814	59.9376	45.6474
Average	6.704666667	59.02515	45.31743333
% SD	0.187789365	0.68065798	0.329898473
% RSD	2.80	1.15	0.73

Accuracy was assessed at concentrations of 80, 100 and 120 µg/mL. The percentage recovery ranged from 98.25 to 102.88% (Table-5), confirming the accuracy of the method.

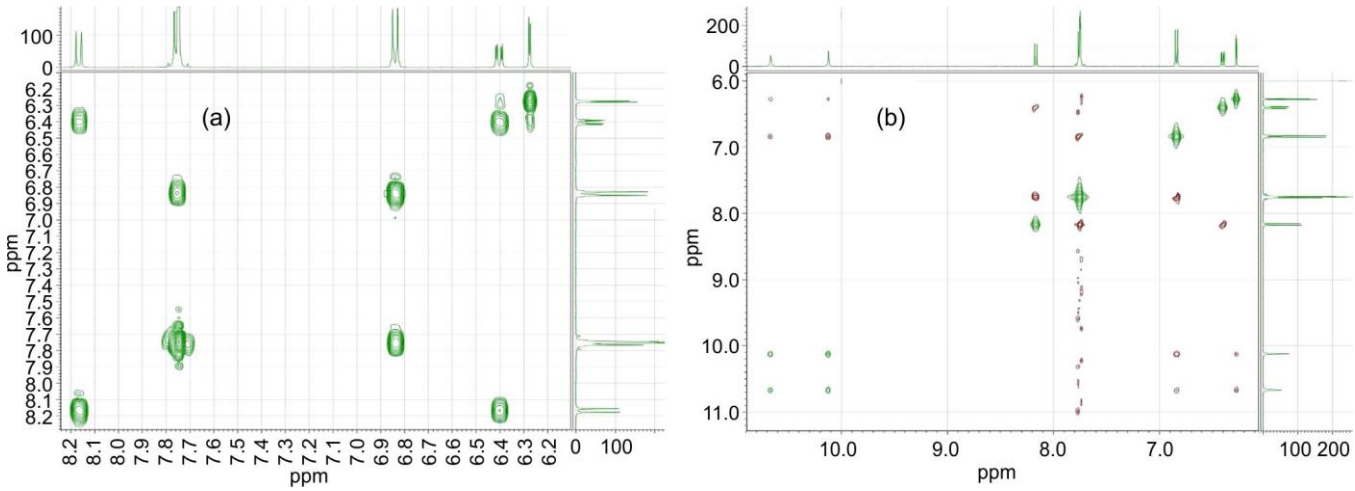


Fig 8. (a) COSY and (b) NOESY spectra of NNG-DP2

TABLE-5
INTER-DAY RECOVERY AND ACCURACY DATA STUDY OF NNG

Amount added (ppm)	Area (mAU × min)	Amount found (ppm)	Recovery (%)	± SD (%)	RSD (%)	Average area (mAU)	Mean recovery (%)	Total average recovery (%)
80	45.757	81.15	101.44	0.514	1.109	46.406	102.88	100.36
	46.446	82.37	102.97					
	47.016	83.38	104.23					
100	56.561	100.31	100.31	0.571	0.010	56.367	99.97	
	56.950	101.00	101.00					
	55.591	98.59	98.59					
120	65.040	115.35	96.12	1.189	0.028	66.475	98.25	
	65.231	115.69	96.41					
	69.155	122.65	102.21					

Linearity establishes the relationship between analyte concentration and analytical response over a specified concentration range. System suitability testing (SST) was performed to verify the performance of the HPLC system during analysis. The slope and correlation coefficient of the calibration curve were found to be 0.563853714 and 0.999, respectively.

Limit of detection (LOD) and limit of quantification (LOQ): The calculated LOD and LOQ values for NNG were 0.1 and 1.0 µg/mL, respectively. According to ICH Q2A, Q2B and Q2(R2) guidelines, an acceptable analytical method should provide an accuracy of at least 95%. Accuracy was evaluated at 80, 100 and 120 µg/mL, with each concentration prepared and analyzed in triplicate. The percentage recovery ranged from 98.25 to 102.88%, confirming the accuracy of the method. The recovery and accuracy results for NNG are shown in Table-5, while the validation parameters and their acceptance criteria are summarized in Table-6.

TABLE-6
SUMMARY OF VALIDATION PARAMETER RESULTS

Parameter	Acceptance criteria	HPLC
Recovery (%)	98-102	100.36
Linearity range (µg/mL)	–	20-120
Correlation coefficient	NLT 0.999	0.999
No. of theoretical plates	NLT 2500	3457
Precision	% RSD NMT 2%	1.15
Intermediate precision	% RSD NMT 2%	0.73
LOD (µg/mL)	–	0.1
LOQ (µg/mL)	–	1.0

The peaks corresponding to NNG and its degradation products were well resolved from each other, which further confirmed the suitability of the developed stability-indicating HPLC method. A linear relationship was observed between peak area and NNG concentration over the range of 20-120 µg/mL (Fig. 9).

Plausible mechanism: A plausible mechanism for the formation of the degradation products (**NNG-DP1** and **NNG-DP2**) is proposed in **Scheme-I**. Under basic conditions, NNG undergoes ring opening of the heterocyclic C-ring through proton-transfer and C–O bond cleavage, leading to the corresponding open-chain chalcone intermediate. Subsequent dehydration results in formation of the conjugated C=C bond, which can adopt either E- or Z-configuration. The two geometric isomers obtained under basic stress were identified as

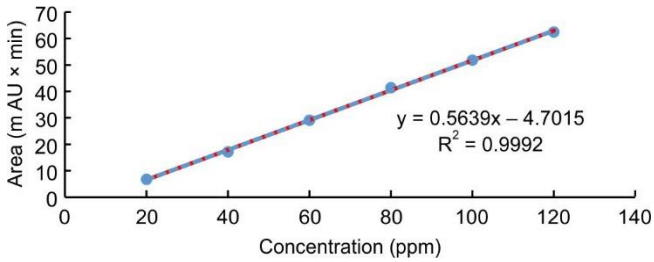


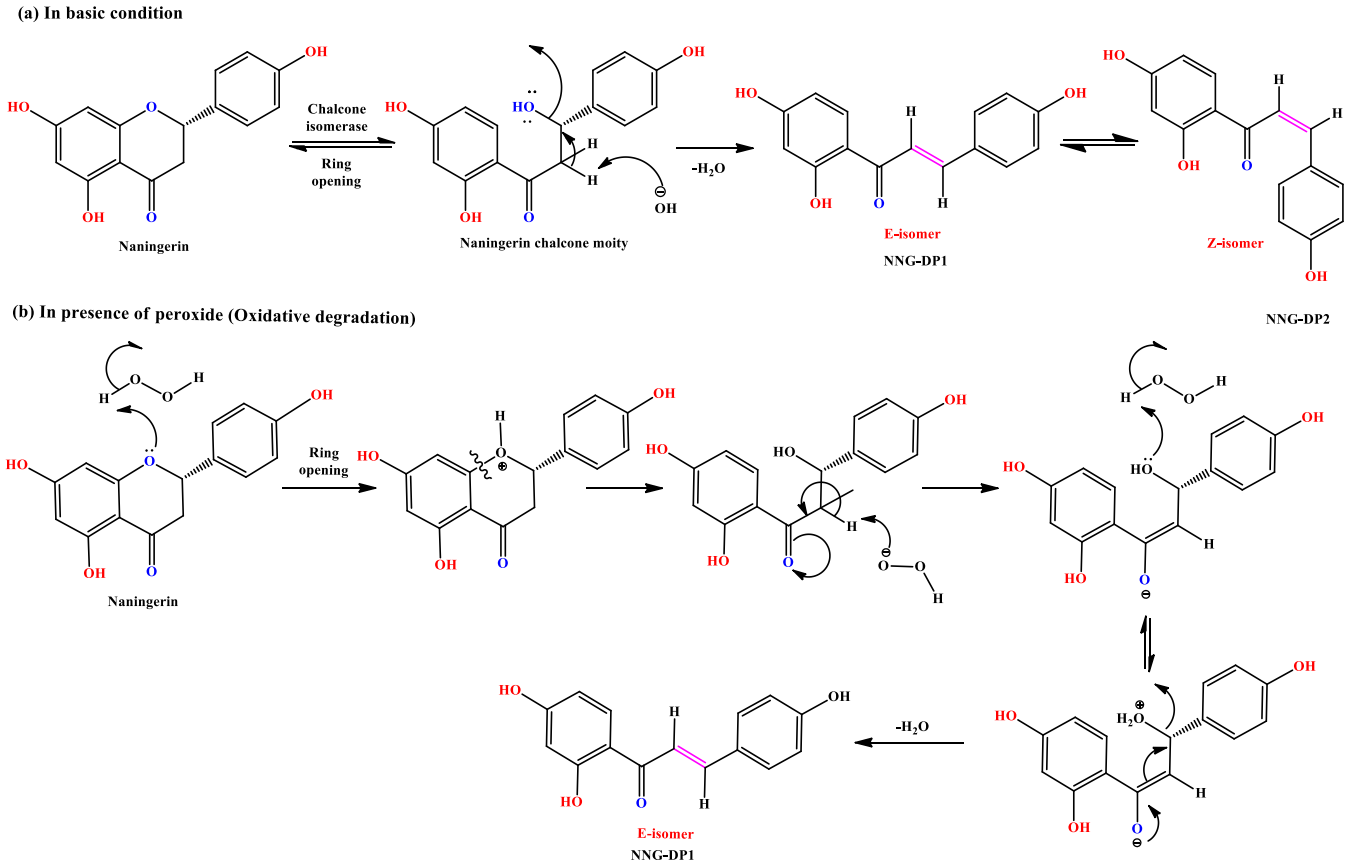
Fig. 9. Linearity plot of NNG

NNG-DP1 (E-isomer) and **NNG-DP2** (Z-isomer). The assignment was supported by their NMR data, particularly the olefinic coupling constants and NOESY spectra.

Under oxidative conditions, naringenin appears to undergo a similar ring-opening process in the presence of hydrogen peroxide, followed by formation of the chalcone system. Under the stronger oxidative conditions, **NNG-DP1** was obtained as the major degradation product, while **NNG-DP2** was not detected in significant amounts. The preferential formation of the E-isomer may be related to its higher stability under the oxidative conditions. The pro-posed pathway is based on the isolated degradation products and spectroscopic data and represents a plausible mechanism for their formation.

Conclusion

The present study addressed the limited information available on the forced degradation behaviour of naringenin under ICH-recommended stress conditions. Naringenin (NNG) showed degradation under alkaline and oxidative conditions, whereas no significant degradation was observed under the tested photolytic conditions. Two major degradation products, NNG-DP1 and NNG-DP2, were formed under alkaline stress and were isolated, purified and characterized using spectroscopic and analytical techniques. Structural elucidation established that the two products are geometric isomers. Oxidative stress resulted predominantly in the formation of NNG-DP1, which was isolated and characterized separately. NNG-DP1 was identified as (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one, with a molecular weight of 256.25 g/mol, while NNG-DP2 was identified as (Z)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one, with a molecular weight of 256.25 g/mol. Furthermore, a stability indicating HPLC method was also developed and validated for NNG and its degradation products, with satisfactory specificity, selectivity, accuracy, linearity, precision,



Scheme-I: Proposed mechanism for the formation of the degradation products

LOD, and LOQ. The identified degradation products provide useful information for impurity profiling, manufacturing, storage and packaging of naringenin. The method can be used to monitor these impurities and assess NNG stability during processing and storage.

ACKNOWLEDGEMENTS

The authors thank Amity University Maharashtra, Mumbai, for all the support. The authors are also thankful to Clean-chem Laboratories LLP, for providing state-of-the-art facilities to carry out research work and analytical studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

REFERENCES

1. A. Rauf, M.A. Shariati, M. Imran, K. Bashir, S.A. Khan, S. Mitra, T.B. Emran, K. Badalova, M.S. Uddin, M.S. Mubarak, A.S. Aljohani, F.A. Alhumaydhi, M. Derkho, S. Korpayev and G. Zengin, *Environ. Sci. Pollut. Res. Int.*, **29**, 31025 (2022); https://doi.org/10.1007/s11356-022-18754-6
2. A.V. Atoki, P.M. Aja, T.S. Shinkafi, E.N. Ondari and C.G. Awuchi, *Nutr. Neurosci.*, **27**, 637 (2024); https://doi.org/10.1080/1028415X.2023.2243089
3. M. Pun, O. Galsurker, N. Khazanov, A. Charkowski, S. Yelin, Z. Kerem, M. Weitman, H. Senderowitz and I. Yedidia, *J. Agric. Food Chem.*, **73**, 20792 (2025); https://doi.org/10.1021/acs.jafc.5c04312
4. X. Zeng, Y. Zheng, Y. He, J. Zhang, W. Peng and W. Su, *Nutrients*, **14**, 3765 (2022); https://doi.org/10.3390/nu14183765
5. L.M. Cardoso, A.B.G. de Carvalho, C. Anselmi, A.H. Mahmoud, R. Dal-Fabbro, F.G. Basso and M.C. Bottino, *Dent. Mater.*, **40**, 1353 (2024); https://doi.org/10.1016/j.dental.2024.06.019
6. B. Ramachandra, *Crit. Rev. Anal. Chem.*, **47**, 24 (2017); https://doi.org/10.1080/10408347.2016.1169913
7. J.K. Sonawane, S.M. Chavan, I.P. Narkar, S.C. Jale, N.V. Tendulkar, V. Jadhav and A. Jain, *Int. J. Res. Publ. Revs.*, **4**, 4703 (2023); https://doi.org/10.55248/gengpi.4.523.42372
8. M. Wesolowski and E. Leyk, *Pharmaceutics*, **15**, 1596 (2023); https://doi.org/10.3390/pharmaceutics15061596
9. R. Kanagaddi, V. Chintala, S.S. Nannapaneni, S. Salakolusu, N.K. Katari, J.N.S.R.C. Murty, M. Ranga and M. Kaliyaperumal, *Sep. Sci. Plus*, **7**, 2300237 (2024); https://doi.org/10.1002/sscp.202300237
10. S.Y. Gabhe, S. Anbazhegan, S. Tekawade and S.V. Mulgund, *Future J. Pharm. Sci.*, **11**, 73 (2025); https://doi.org/10.1186/s43094-025-00822-6

11. M. Dendeni, N. Cimetiere, A. Amrane and N.B. Hamida, *Int. J. Pharm.*, **438**, 61 (2012); https://doi.org/10.1016/j.ijpharm.2012.08.048
12. R. Maheshwari, P. Todke, N. Soni, N. Raval, P. K. Deb, B. Amarji, N. V.A.K. Ravipati and R.K. Tekade, in eds.: R.K. Tekade, Stability and degradation studies for drug and drug product, In: Dosage Form Design Considerations, Academic Press, Chap. 7, pp. 225-257 (2018); https://doi.org/10.1016/B978-0-12-814423-7.00007-1
13. H.M. Nassef, H.A. Ahmed, M.A. El-Atawy, T.Y. Alanazi and M.A. Mohamed, *Green Chem. Lett. Rev.*, **17**, 2301315 (2024); https://doi.org/10.1080/17518253.2023.2301315
14. S. Zokande, K. Inamdar, A. Gosar and V. Mane, *Results Chem.*, **16**, 102366 (2025); https://doi.org/10.1016/j.rechem.2025.102366
15. S. Mishra, A. Chauhan and R. Ramajayam, *Results Chem.*, **7**, 101465 (2024); https://doi.org/10.1016/j.rechem.2024.101465
16. S.S. Panda, O. Afzal, A.S.A. Altamimi and S. Beg, *Microchem. J.*, **191**, 108848 (2023); https://doi.org/10.1016/j.microc.2023.108848
17. L.M. Cordenonsi, N.G. Bromberger, R.P. Raffin and E.E. Scherman, *Biomed. Chromatogr.*, **30**, 155 (2016); https://doi.org/10.1002/bmc.3531
18. L.M. Cordenonsi, R.M. Sponchiado, J.R. Bandeira, R.C.V. Santos, R.P. Raffin and E.E.S. Schapoval, *Drug Anal. Res.*, **4**, 64 (2026); https://doi.org/10.22456/2527-2616.108783
19. K. Pilaniya, H. K. Chandrawanshi, U. Pilaniya, P. Manchandani, P. Jain and N. Singh, *J. Adv. Pharm. Technol. Res.*, **1**, 302 (2010); https://doi.org/10.4103/0110-5558.72422
20. T.S. Pawar, A. Gawade, B. Gawade and R.P. Tayade, *Int. J. Pharm. Sci. Drug Res.*, **16**, 110 (2024); https://doi.org/10.25004/IJPSDR.2024.160115
21. A. Sau, D. Mahapatra, S. Dey, D. Panja, S. Saha and S. Kundu, *Org. Chem. Front.*, **10**, 2274 (2023); https://doi.org/10.1039/D3QO00308F
22. P. Mizar, R. Arya, T. Kim, S. Cha, K.-S. Ryu, W. Yeo, T. Bae, D.W. Kim, K.H. Park, K.K. Kim and S.S. Lee, *J. Med. Chem.*, **61**, 10473 (2018); https://doi.org/10.1021/acs.jmedchem.8b01012
23. T. Zelesky, S.W. Baertschi, C. Foti, L.R. Allain, S. Hostyn, J.R. Franca, Y. Li, S. Marden, S. Mohan, M. Ultramari, Z. Huang, N. Adams, J.M. Campbell, P.J. Jansen, D. Kotoni and C. Laue, *J. Pharm. Sci.*, **112**, 2948 (2023); https://doi.org/10.1016/j.xphs.2023.09.003
24. H. Zhong, J. Zhou, X. An, Y.-R. Hua, Y.-F. Lai, R. Zhang, O. Ahmad, Y. Zhang and J. Shang, *Bioorg. Chem.*, **87**, 523 (2019); https://doi.org/10.1016/j.bioorg.2019.03.054
25. M. Blessy, R.D. Patel, P.N. Prajapati and Y.K. Agrawal, *J. Pharm. Anal.*, **4**, 159 (2014); https://doi.org/10.1016/j.jpha.2013.09.003
26. R. Gaggeri, D. Rossi, S. Collina, B. Mannucci, M. Baierl and M. Juza, *J. Chromatogr. A*, **1218**, 5414 (2011); https://doi.org/10.1016/j.chroma.2011.02.038