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Reverse-phase chromatographic determination and intrinsic stability behavior of 5-[(4-chlorophenoxy)methyl]--1,3,4-oxadiazole-2-thiol

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The study describes the development and preliminary validation of a simple reverse-phase chromatographic method for determination of a novel drug candidate, 5-[(4-chlorophenoxy) methyl]-1,3,4-oxadiazole-2-thiol (OXCPM), in bulk and stressed solution, in order to find out the intrinsic stability behavior of the compound. Isocratic elution was carried out at a flow rate of 1.0 mL min^{-1} through a Promosil C_{18} column maintained at 25 °C, using the mobile phase comprising acetonitrile and aqueous *o*-H_3PO_4 (pH 2.67) (1:1, *V*/*V*). Detection was performed at 258 nm. The response of the detector was linear in a concentration range of 1.25–50.00 μg mL^{-1} with the correlation coefficient of 0.9996 ± 0.0001. Cumulative intra-day, inter-day and inter-instrument accuracy (99.5 ± 1.0, 100.2 ± 1.0 and 100.3 ± 0.4 %, resp.) with RSD less than 5 % indicated that the method was accurate and precise. The resolution and selectivity factor (>2 and >1, resp.), particularly in copper metal- and dry-heat-stress solutions, depicted the selectivity of the method. OXCPM remained stable under hydrolytic (acidic and neutral pH, ≤ 37 °C), photolytic and moist heat stress conditions. Under alkaline conditions (hydrolytic and photolytic), polar products were formed that eluted very fast through the column (t_R < 3.75 min). At room temperature, the compound was susceptible to oxidation by hydrogen peroxide and transition metals. The ionogram of most of the stress solutions indicated the presence of a product having *m*/*z* 256, which might be a result of N- or S-methylation or -SH oxidation. The results of the study indicate that the method is selective, sensitive and suitable to be used for determination of OXCPM in bulk and under stress conditions.

Keywords: 5-[(4-chlorophenoxy)methyl]-1,3,4-oxadiazole--2-thiol, forced degradation, stability, RP-HPLC/DAD

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5-[(4-Chlorophenoxy)methyl]-1,3,4-oxadiazole-2-thiol (OXCPM) (Fig. 1) was synthesis to be used as a precursor for a number of less cytotoxic antimicrobial and antienzymatic derivatives (1). The compound is a 2,5-disubstituted oxadiazole, which is an important heterocyclic active moiety – pharmacophore – due to the presence of cyclic nitrogen (2 atoms) and oxygen (one atom) and exocyclic sulfur (2). Several agents possessing such pharmacophore have been found effective against a number of diseases including cancer, hypertension, arrhythmia, CNS depression and retroviral and bacterial infections (3–6). Keeping this in mind, we have investigated this parent compound for drug likeliness and found it a potential candidate for drug development (7).

For quantification of OXCPM in bulk and matrices, a simple and specific chromatographic method is needed. Therefore, the present study describes for the first time the development and validation of a reverse-phase chromatographic method for determination of OXCPM.

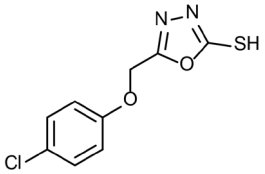

Fig. 1. Structural formula of OXCPM.

EXPERIMENTAL

Chemicals, reagents and other supplies

Analytical grade sodium hydroxide pellets, copper(II) sulphate pentahydrate, iron(II) sulphate heptahydrate, magnesium sulphate heptahydrate, hydrogen peroxide solution (35 %), fuming hydrochloric acid (37 %) and sodium dihydrogen phosphate were purchased from Merck (Germany), trifluoroacetic acid from Scharlab (Spain), *ortho*-phosphoric acid (85 %) from BDH Chemicals (UK), HPLC grade methanol from Duksan Pure Chemicals (S. Korea) and acetonitrile from Tedia Company (USA). Polytetrafluoroethylene membrane syringe filters (0.2 μm, Minisart SRP 15, Sartorius Stedim Biotech, Germany) and nylon-H filters (0.45 μm, Micropore, USA) were used. OXCPM was received as a gift from the Department of Chemistry, GCU, Lahore, Pakistan, the synthesis and characterization of witch is already reported (1). The in-house prepared ultrapure water was used throughout the study.

Instrumentation

Two HPLC systems were used to develop and validate the method: HPLC system 1200 series (Agilent Technologies, Germany), equipped with an isocratic pump (G1310 A), autosampler (G1329 A), column thermostat (G1316 A), diode array detector (G 1315 B) and operating software (Agilent ChemStation LC/LCMS for Windows, Rev. B.01.03 [204]), and a binary HPLC system (Series 200-LC Pump, PerkinElmer, USA), equipped with a gradient pump (291N6112805A), manual injection port, UV/Vis-detector (292N6110902A) and operating software (PerkinElmer TotalChrom Workstation, version 6.3.2).

Columns used during method optimization included Promosil C_{18} (5 μm, 4.60 × 250 mm), Eclipse X DB-C_{18} and Zorbax SB-C_{18} (5 μm, 4.60 × 150 mm). In addition, a gas chromatograph equipped with a mass spectrophotometer (GC-MS-qp 2010, Shimadzu, Japan) and capillary column (J&W DB 5MS, 30 m × 0.25 mm ID, Agilent, USA) was used. A UV lamp (UVGL-58, USA), sonicator (DSA50-SK1-1.8L, Germany), thermostatic oven (U10, Memmert, Germany), pH meter (Hanna Instruments, Romania), stability chamber (Curio, SC-0709) and ultra-low chiller (Curio, MOF U32V) of Sanyo Electric Corporation, Japan, were used.

Method development and optimization

Preparation of mobile phases. – Mobile phases used during the development and optimization of the method included methanol, water, acetonitrile, acetonitrile/water (1:1, *V/V*), acetonitrile/sodium dihydrogen phosphate buffer (pH 4.47) (1:4 and 1:1, *V/V*), acetonitrile/water/phosphoric acid (50:48:2, *V/V*), acetonitrile/water/trifluoroacetic acid (50:48:2, *V/V*) and acetonitrile/aqueous *o*-H_3PO_4 (pH 2.67) (1:1, *V/V*). Mobile phases were filtered through a nylon membrane and degassed using an ultrasonicator.

Preparation of standard solutions. – A standard stock solution of the compound was prepared in methanol (1.00 mg mL^{-1}). Then, a range of working standard solutions (1.25–50.00 μg mL^{-1}) were prepared by diluting the stock solution with the mobile phase, acetonitrile/aqueous *o*-H_3PO_4 (pH 2.67) (1:1, *V/V*).

Chromatographic conditions. – A sample volume (20 μL) was eluted through a Promosil C_{18} column (5 μm, 4.60 × 250 mm) isocratically using the mobile phase composed of acetonitrile and *o*-H_3PO_4 (pH 2.67) (1:1, *V/V*) at a flow rate of 1.0 mL min^{-1}. The temperature of the column was maintained at 25 °C and detection was carried out at 258 nm using DAD.

System suitability. – Chromatographic parameters, *i.e.*, the number of theoretical plates (*N*), height equivalent to the theoretical plate (*HETP*), reduced plate height, capacity factor (k'), tailing factor and peak asymmetry (As), were evaluated to ensure that the system was working accurately during the analysis (8).

Method validation

The developed method was validated according to the available guidelines and protocols (9, 10). Validation parameters are summarized as follows.

Linearity and Beer's range. – Twelve working standard solutions (1.25–50.00 μg mL^{-1}) were analyzed in triplicate, using the chromatographic conditions described above. Linearity was assessed from the plot of peak area (mAU s) *versus* concentration (μg mL^{-1}) by the linear regression analysis. Beer's range was established from the linearity studies.

Limit of detection (LOD) *and limit of quantification* (LOQ). – Working standard solutions of 1.25–50.00 μg mL^{-1} concentration were analyzed in quintuplicate and five standard curves were constructed from the obtained data. Sensitivity parameters – limit of detection (*LOD*) and limit of quantification (*LOQ*) – were determined statistically from the standard deviation of the intercept and mean slope of the calibration line (10).

Accuracy and precision. – Three concentration levels (1.25, 25.00 and 50.00 μg mL^{-1}) of working standard solutions were used to determine the accuracy and precision. Intra- and inter-day accuracy and precision were assessed by analyzing these concentrations 6 times in a single day and once daily for six consecutive days, and determining their amounts from calibration curves constructed on each day. Intermediate accuracy/precision of the developed method was assessed by performing the experiments on two different instruments and calculating the amounts of the compound from the calibration curve.

Specificity and selectivity. – Specificity of the assay towards OXCPM was determined by resolution (*Rs*) and the selectivity factor (α) of the OXCPM peak and the nearest peak in the stressed solution (prepared as mentioned below), if any.

Robustness. – The effect of small deliberate changes in column temperature, detection wavelength, mobile phase composition, sample preparation and pH on OXCPM accuracy determination was investigated to establish the robustness of the method.

Sample solution stability. – Stability of the working standard solution (50.00 μg mL^{-1}) was evaluated by storing the samples in screw-capped test tubes, protected from light at room temperature, in a refrigerator and freezer for 48 h. The content was compared with the freshly prepared solution.

Intrinsic stability behavior under stress conditions

Intrinsic stability behavior of the compound was assessed through forced degradation studies. Experimental conditions used in the study complied with the standard guidelines and previously published reports (11–14). Methanolic solution of OXCPM (200.00 μg mL^{-1}) was subjected to hydrolytic, oxidative, photolytic, transition metal and thermal stresses. For each stress condition, four samples were generated, *i.e.*, sample solution (real time and stressed) and blank (real time and stressed). Samples were withdrawn after a suitable period of time, neutralized (if needed), filtered, diluted in the mobile phase to a suitable concentration and subjected to HPLC analysis. A brief account of the procedure is given below.

For hydrolytic stress at acidic, alkaline and neutral pH, the sample solution was separately treated with equal volumes of 0.10, 1.00 and 5.00 mol L^{-1} HCl, 0.10, 1.00 and 5.00 mol L^{-1} NaOH and water, resp. Two such sets were prepared and heated at 37 ± 5 and 121 ± 5 °C for 24 and 1 h, resp. For oxidative stress, equal volumes of the sample and hydrogen peroxide solution (1, 3, 10 and 20 %) were stored at room temperature for a period of 28 h. For photolytic stress, sample solutions in 0.10 mol L^{-1} HCl, 0.10 mol L^{-1} NaOH and water were separately placed for 48 h in UV (254 nm) and visible light (sunlight, 60,000 to 70,000 lux and tungsten lamp, 100 Watt for 12 h alternatively), whereas suitable controls were kept in dark for the same time. For transition metal stress, OXCPM solutions were mixed with equal volumes of 1.50 mmol L^{-1} of copper, iron and magnesium solutions and stored for 28 h at room temperature. The effect of dry and moist heat on the aqueous solution of OXCPM was observed by placing it in a hot-air oven at 150 ± 10 °C and in a stability chamber, maintained at 60 ± 5 °C and 90 % relative humidity (RH), for 48 h, resp. As a control, OXCPM solution was placed in a refrigerator (2–8 °C), a chiller (–52 ± 5 °C) and at room temperature for the same period of time.

Identification of degradation products

The injection volume of 1 μL (split mode with 90 % split ratio) was eluted through a silica capillary column (0.25 μm, coating 5 % phenyl polysiloxane) with helium at a flow rate of 1.27 mL min^{-1}. Column temperature was initially kept at 40 °C for 5 min, raised to 180 °C (at a heating rate of 5 °C min^{-1}) and finally to 240 °C (at a heating rate of 3 °C min^{-1}) and kept there for 5 min. The temperature of the injection port was maintained at 200 °C. The mass detector was operated in the electron-impact ionization mode (ionization energy, 70 eV). Molecular ion peaks were identified and possible structures were drawn on the basis of already reported data on decomposition pattern/chemical reactions of functional groups in OXCPM (14–19).

RESULTS AND DISCUSSION

HPLC-DAD method development and optimization

Based on the UV profile of OXCPM, detection was carried out at two wavelengths, 223 and 258 nm, using DAD. However, method validation was performed at the latter since the solvents/mobile phases did not absorb in this range and also because the response of the detector showed a wider linearity range at this wavelength. OXCPM was analyzed using three types of C_{18} columns (5 μm, 4.60 × 250 mm) and elution was carried out by varying the column temperature (25 ± 5 °C). However, elution through Promosil C_{18} at 25 °C provided the best results.

Several types of mobile phases were investigated in order to achieve suitable peaks. Moreover, changes of pH of the mobile phase (2.67 ± 2) and the flow rate (1.0 ± 0.5 mL min^{-1}) were made to improve retention time (t_R) and peak symmetry. The optimum elution

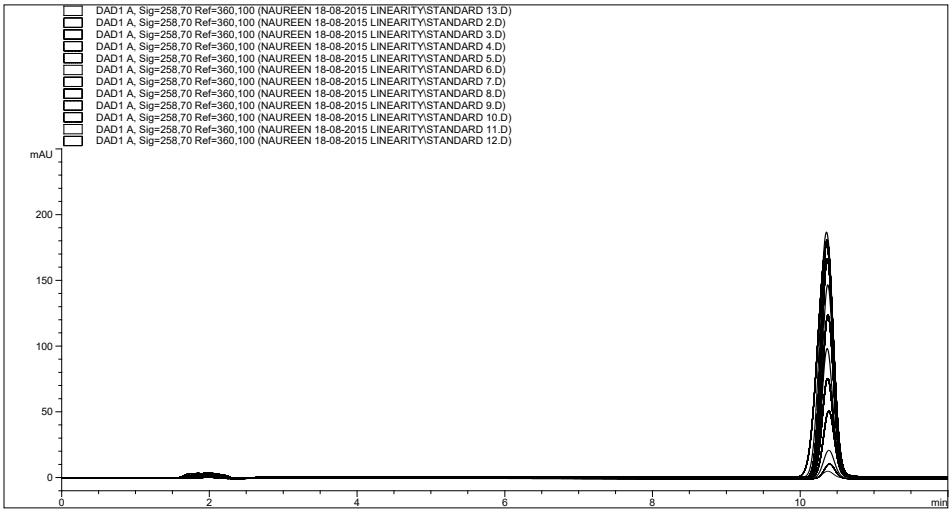

Fig. 2. Overlay of the chromatograms of OXCPM in the concentration range of 1.25–50.00 μg mL^{-1}.

Table I. Chromatographic parameters

Parameter	Value
Retention time (t_R, min)[a]	10.37 ± 0.01
Capacity factor (*k′*)	4.0
Peak repeatability (RSD, %)	0.5
Tailing factor	1.0
Number of theoretical plates (*N*)	29024.6
Height equivalent to the theoretical plate (*HETP*) (μm)	8.6
Reduced plate height (μm)	1.7
Peak symmetry	1.0

[a] Mean ± SD (n = 6)

was achieved by the mobile phase composed of acetonitrile and aqueous *o*-H_3PO_4 (pH 2.67) (1:1, *V*/*V*) at a flow rate of 1.0 mL min^{-1} at 10.37 ± 0.01 min. Chromatograms of the compound obtained using the optimized chromatographic conditions are shown in Fig. 2, which show that the peaks are Gaussian.

Chromatographic parameters

The system suitability parameters were calculated based on the eluted peak of the OXCPM solution of 50.00 μg mL^{-1}. Numerical data, shown in Table I, complied with the established guidelines (8), indicating the suitability of the method.

Method validation

Linearity and Beer's range. – The coefficient of determination of 0.9996 ± 0.0001 indicated that the method was linear over the studied concentration range (1.25–50.00 μg mL^{-1}). In the same context, the slope, y-intercept and residual sum of squares were found to be 55.15 ± 0.08 mAU s mL μg^{-1}, 10.68 ± 0.26 mAU s and 3920.90, resp.

*Limit of detection (*LOD*) and limit of quantification (*LOQ*).* – The calibration range and linear regression equations used to calculate limiting values of the compound are shown in Table II. Limiting values of 0.016 μg mL^{-1} for *LOD* and 0.047 μg mL^{-1} for *LOQ* indicated that the method was sensitive and, hence, could be used for determination of OXCPM at the sub-microgram level.

Intra-day, inter-day and inter-instrument accuracy and precision. – Results of the accuracy and precision of the method are given in Table III. The cumulative intra-day, inter-day and inter-instrument accuracy (99.5 ± 1.0, 100.2 ± 1.0 and 100.3 ± 0.4 %, resp.) with RSD less than 5 % indicated that the method was accurate and precise.

Specificity/selectivity. – The *Rs* and α of the OXCPM peak from the nearest peak, particularly in the copper metal- and dry-heat stress solutions (Figs. 7 and 8), were found to be >2 and >1, resp. These results indicated that the method was selective.

Table II. Calibration range, linear regression parameters and limiting values of OXCPM

Calibration range ($\mu g\ mL^{-1}$)	Coefficient of determination (R^2)	Slope	Intercept
1.25–50.00	0.9996 ± 0.0001[a]	55.15 ± 0.08[a]	10.68 ± 0.26[a]
	LOD = 0.016μg mL^{-1}		
	LOQ = 0.047 μg mL^{-1}		

S – slope of calibration line, σ – standard deviation of line intercept, LOD (limit of detection) = 3.3 (σ/S), LOQ (limit of quantification) = 10 (σ/S), ref. 10

[a] Mean ± SD (n = 5).

Table III. Intra-day, inter-day and inter-instrument accuracy and precision of OXCPM

Concentration ($\mu g\ mL^{-1}$)	Intra-day accuracy (%)[a]	Inter-day accuracy (%)[a]	Inter-instrument accuracy (%)[a]
1.25	99.3 ± 0.5	99.9 ± 0.6	100.0 ± 0.1
25.00	100.4 ± 0.6	100.5 ± 1.3	100.8 ± 0.2
50.00	98.7 ± 0.9	100.1 ± 1.2	100.1 ± 0.1

[a] Mean ± SD, n = 6.

Robustness. – The change in the volume of acetonitrile (± 2 mL) in the optimized mobile phase, pH of water (± 0.20), column temperature (± 2 °C), detection wavelength (± 5 nm) and sample solvent (methanol and mobile phase) did not affect accuracy, and hence the method was robust (Table IV).

Sample solution stability. – Recovery of OXCPM from the solutions stored at room temperature, in the refrigerator and in the freezer was 100.4 ± 0.6, 97.1 ± 1.2 and 101.1 ± 0.1 %, resp. These results indicated that the sample can be analyzed accurately for 48 h after preparation.

Intrinsic stability behavior under stress conditions

Hydrolytic stress. – Hydrolytic decomposition of OXCPM in acidic, alkaline and neutral solutions is shown in Fig. 3. The compound was extensively hydrolyzed in solutions of various pH at 121 ± 5 °C. The degradation pattern indicated by the appearance of additional peaks was different under three acid stress conditions. With an increase in acidity, the percentage of degradation and the number of degradation products increased. The maximum number of degradation products was observed in 5 mol L^{-1} HCl, which eluted at 4.45 ± 0.00, 4.77 ± 0.02, 5.38 ± 0.06, 7.03 ± 0.20, 7.65 ± 0.01 and 8.97 ± 0.00 min. Major degradation peaks in all acidic stress solutions were observed at 7.26 ± 0.37 and 8.97 ± 0.00 min (Fig. 3b). On the other hand, under all three alkaline conditions, the degradation pattern was similar. Highly

Table IV. Robustness of OXCPM determination under slightly changed chromatographic conditions

Chromatographic conditions	Concentration[a] (%)
Mobile phase	
Acetonitrile/aqueous *o*-H_3PO_4 (pH 2.67) (52:48)	100.0 ± 0.1
Acetonitrile/aqueous *o*-H_3PO_4 (pH 2.67) (48:52)	99.2 ± 1.0
Column temperature (°C)	
27	96.0 ± 1.9
23	100.0 ± 1.2
pH of water	
2.87	101.2 ± 0.1
2.47	100.2 ± 0.7
Detection wavelength (nm)	
253	100.1 ± 0.9
263	99.8 ± 0.7
Sample solvent	
Methanol	101.2 ± 0.8
Mobile phase	$100.0 \pm 0.0_2$

[a] Mean ± SD, $n = 3$.

polar products were retained in the column for only a few minutes and no OXCPM peak was observed (Fig. 3d). In neutral solution, degradation gave rise to three degradation products that eluted at 4.64 ± 0.01, 5.28 ± 0.01 and 6.31 ± 0.10 min. A major peak was observed at 6.31 ± 0.10 min, which demonstrated the fronting phenomenon (Fig. 3f). It is worth mentioning here that upon storage of the solutions at ambient temperature (37 ± 5 °C) for 24 h, OXCPM did not show any decomposition at acidic or neutral pH (Figs. 3a, e). However, in 0.10 mol L^{-1} NaOH, OXCPM degradation was 44.59 ± 0.10 %, and upon increasing alkalinity, a similar degradation pattern to that indicated at high temperature was observed (Fig. 3d). These results clearly indicate that OXCPM was susceptible to degradation at alkaline pH regardless of the temperature. However, at acidic and neutral pH, temperature played the primary role.

Oxidative stress. – OXCPM was extensively oxidized by hydrogen peroxide (Fig. 4); however, the extent of decomposition and degradation products did not increase with the increase in oxidant concentration (1–20 %). Degradation peaks eluted at 4.57 ± 0.03 and 7.15 ± 0.11 min. These results indicated that OXCPM was liable to oxidation at room temperature with strong oxidants, which might be due to the presence of a thiol group in the structure.

Photolytic stress. – In neutral solutions, OXCPM was found to be quite stable under both UV and visible light whilst in acidic solution, it showed slight degradation (6.68 ± 0.01 %) without any additional peak formation. In contrast, under alkaline conditions, the com-

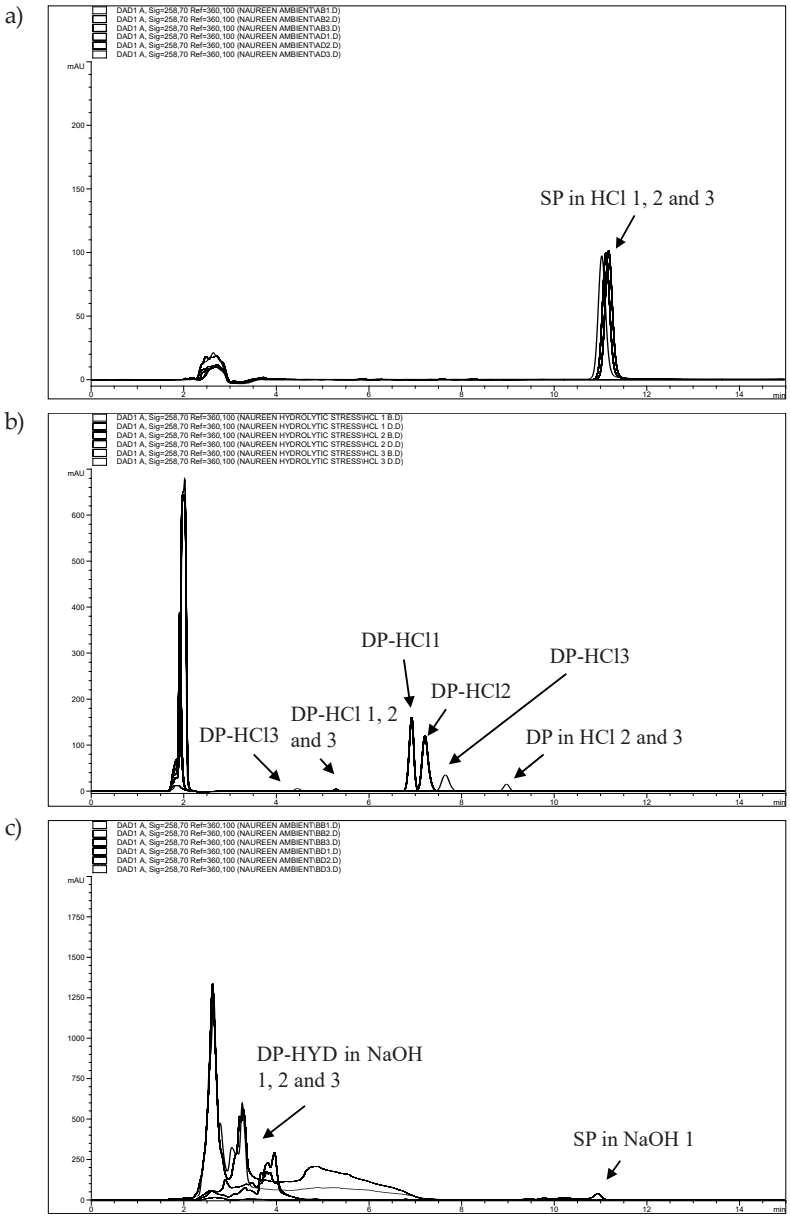

Fig. 3. Overlay of chromatograms of OXCPM after stress under different pH/temperature hydrolytic conditions: a) and b) acid hydrolysis at 37 ± 5 and 121 ± 5 °C, resp., in 0.10, 1.00 and 5.00 mol L^{-1} HCl (labeled as HCl 1, 2 and 3); c) and d) alkaline hydrolysis at 37 ± 5 and 121 ± 5 °C, resp., in 0.10, 1.00 and 5.00 mol L^{-1} NaOH (labeled as NaOH 1, 2 and 3); e) and f) neutral hydrolysis (water) at 37 ± 5 and 121 ± 5°C, resp. (labeled W).

SP – standard peak, DP-HYD – hydrolysis degradation product

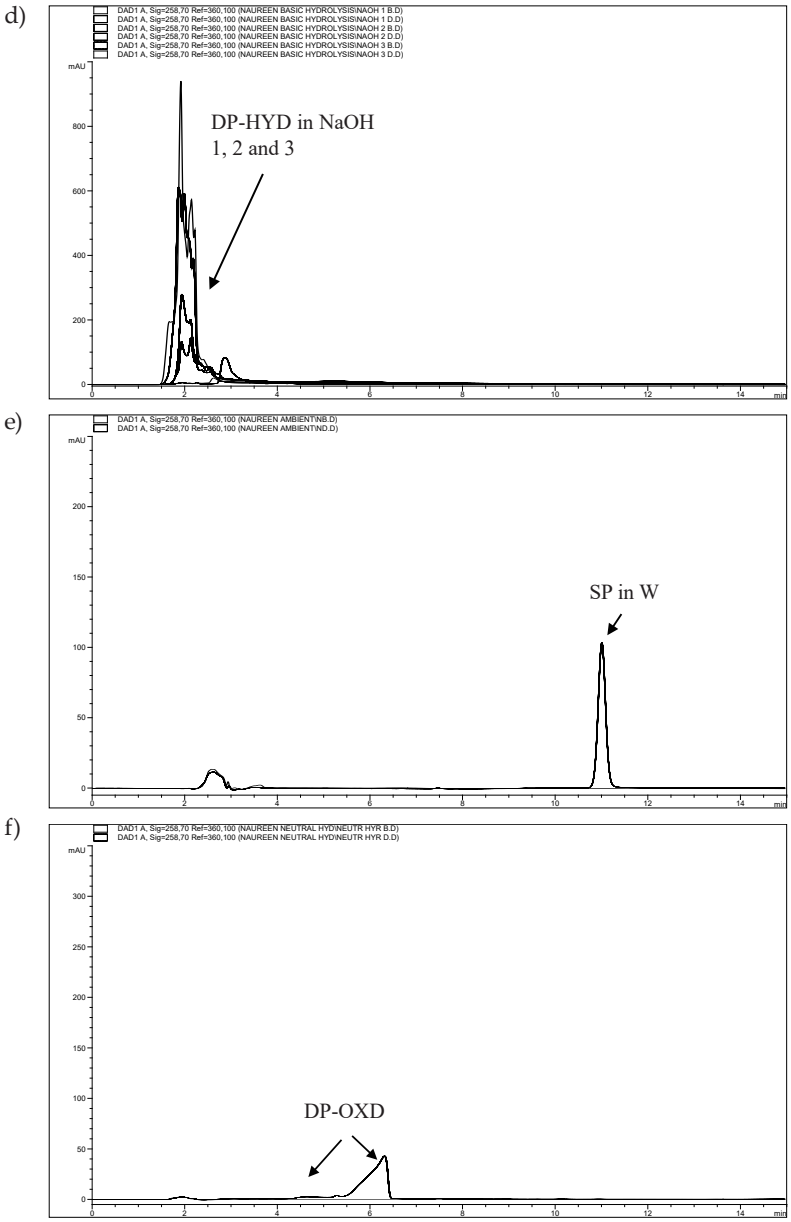

Fig. 3. Continued

pound was extensively degraded under both UV and visible light and produced polar products (t_R = 6.73 min) that rather quickly eluted from the non-polar column. Interestingly, this behavior was quite analogous to that observed under the pH/temperature hy-

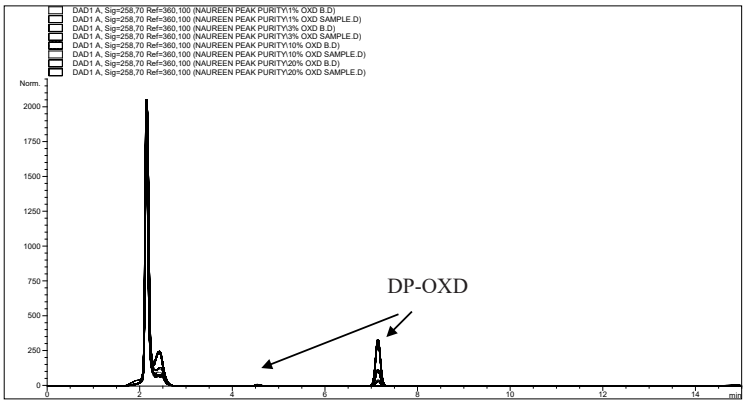

Fig. 4. Overlay of chromatograms of OXCPM in oxidative stress solutions (1, 3, 10 and 20 % hydrogen peroxide).

SP – standard peak, DP-OXD – oxidative degradation product

drolytic conditions. The neutral solution of OXCPM stored in the dark showed no decomposition (Fig. 5). These results indicated that the compound was photo-stable in acidic and neutral solutions only. It is worth mentioning that the behavior of OXCPM under visible-light-stress was quite similar to that under UV-light, which meant that the type/intensity of electromagnetic radiation did not influence the stability of OXCPM markedly.

Stress of transition metals. – In 1.50 mmol L^{-1} transition metal solutions, maximum decomposition was brought about by iron and magnesium, followed by copper. A major degradation peak in metal solutions was observed at 8.38 ± 0.04 min. In addition, another degradation product was observed in copper solution, which eluted at 6.52 ± 0.11 min (Fig. 6).

Thermal stress. – Decomposition of the compound was observed in dry heat at 150 ± 10 °C, whereby only 27.60 ± 0.04 % of OXCPM was recovered and three distinct peaks appeared at 4.10 ± 0.09, 5.02 ± 0.08 and 5.54 ± 0.00 min. Upon storage under moist heat at room temperature, no degradation took place. Similarly, solutions stored in the refrigerator and chiller showed no instability. These results indicated that the aqueous solution of the compound decomposed only in dry heat at high temperature and was stable in moist heat and at room temperature (Fig. 7).

Characterization of degradation products

Degradation products formed under different stress conditions were characterized using mass spectrometry and the findings were compared to the decomposition of the related compound/functional groups. Ionograms of almost all stress solutions indicated the presence of compounds having *m*/*z* 256 (Fig. 8).

In addition to the above, molecular ion (M^+) peaks of *m*/*z* 213 and 169 were observed under acid hydrolysis, 482 in neutral hydrolysis, and 270 and 284 under alkaline hydrolysis

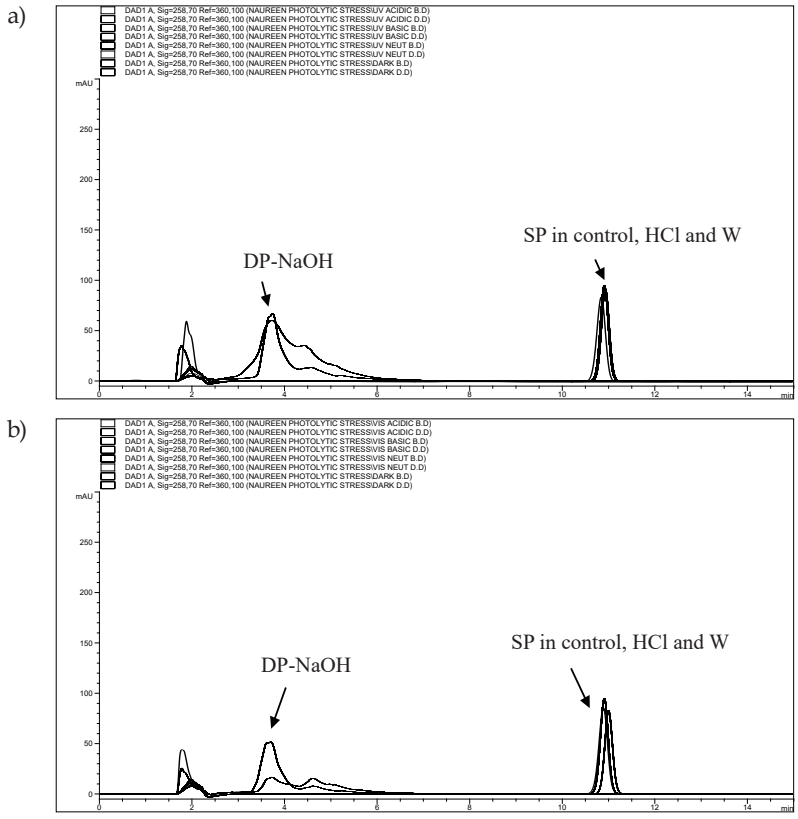

Fig. 5. Overlay of chromatograms of OXCPM in photolytic stress solutions: a) UV-light; b) visible light. SP – standard peak, DP-NaOH – photolytic degradation product in alkaline solution, HCl – hydrochloric acid, W – water.

conditions. The possible structures corresponding to these molecular ion peaks are shown in Fig. 9.

Thiols are easily oxidized compared to alcohols and such reactions result in changing the oxidation state (OS) of sulfur atom. The thiol group reacts with strong oxidants to yield sulfenic acid (OS = 0), sulfinic acid (OS = +2), sulfoxide (OS = 0), sulfones (OS = +2) or sulfonic acid (OS = +4); however, with mild oxidants, disulfides (OS = –1) are produced. In the case of hydrogen peroxide, the reaction at room temperature produces sulfoxides, which upon exposure to high temperatures (100 °C) are oxidized to sulfones. Mass spectrum of OXCPM stressed with hydrogen peroxide showed the presence of a compound having *m*/*z* 256. The structures corresponding to the product might be sulfenic acid or sulfoxide since the degradation was investigated at room temperature (A and B in Fig. 9) (14–18).

In the case of acidic, alkaline and neutral hydrolytic conditions, the compound having *m*/*z* 256 may represent the *N*- or *S*-methylated product (C and D). Compounds having *m*/*z* 169 and 213 represent the nitrile-degradation product of OXCPM formed due to proton-

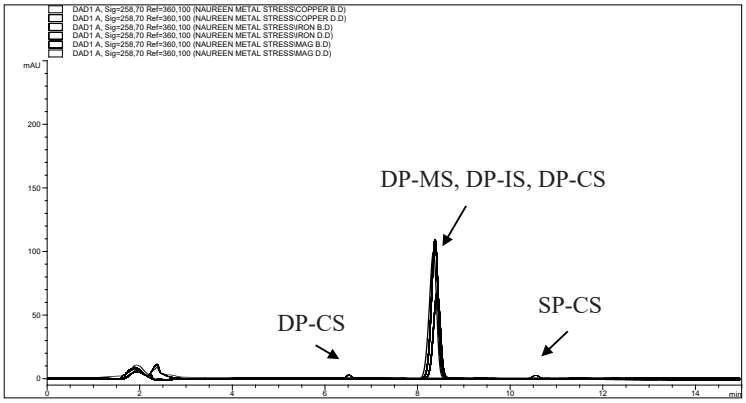

Fig. 6. Overlay of the chromatogram of OXCPM in 1.50 mmol L^{-1} stress solution of heavy metals (iron, copper, magnesium). SP – standard peak, DP-MS – degradation product in magnesium solution, DP-IS – degradation product in iron solution, DP-CS – degradation product in copper solution, SP-CS – standard peak in copper solution.

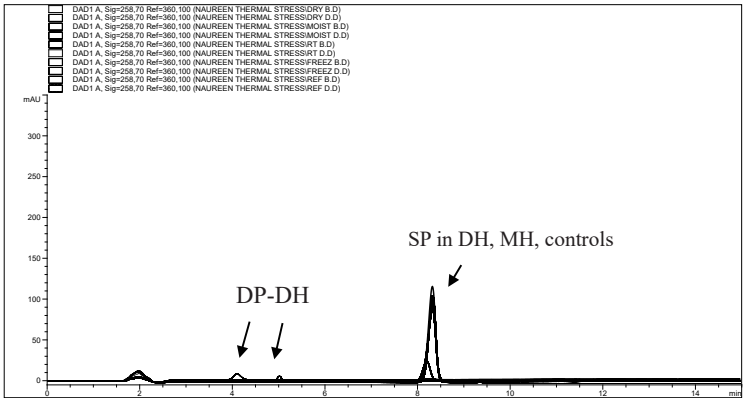

Fig. 7. Degradation behavior of OXCPM solution in dry heat. SP – standard peak, DP-DH – degradation product in dry heat at 150 ± 10 °C, MH – moist heat.

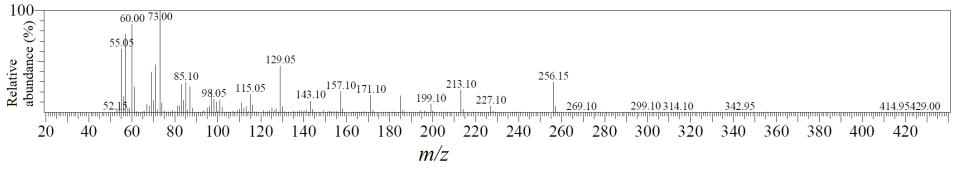

Fig. 8. The representative mass spectrum of the major degradation product of OXCPM having *m/z* 256.

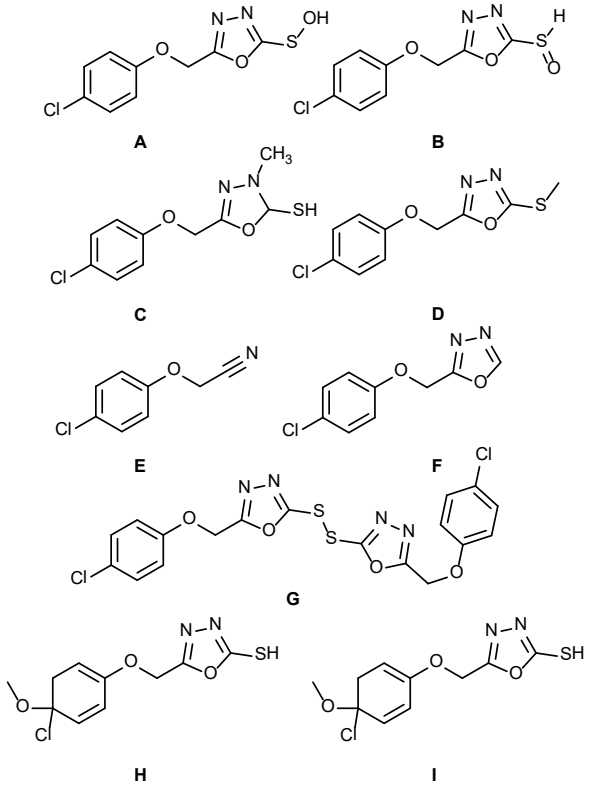

Fig. 9. Chemical structures of possible products formed as a result of forced degradation of OXCPM.

ation at *N*-3 in oxadiazole followed by ring cleavage, and des-sulfur derivative, resp. (E and F). At pH values of 7–9, two types of reactions, *i.e.*, thiol oxidation and thiol-disulfide exchange, take place in aqueous solutions of thiol containing compounds (19). These reactions take place in the presence of oxygen from air and do not require any other reagent. At neutral pH, the mass spectrum revealed the presence of the compound having *m*/*z* 480 which may be the disulfide product (G). In alkaline solutions, peaks with *m*/*z* 270 and 284 represent a product which may be formed as a result of base-catalyzed attachment of a methoxy group on the benzene ring and methylation at *S*-terminal (H and I) (Fig. 9) (20). It is worth mentioning that the photolytic degradation products in alkaline solutions were identical to those observed after basic hydrolysis. Moreover, in aqueous solutions, heat-induced degradation products were similar to the ones observed at neutral and acidic pH.

CONCLUSIONS

The results of the present study reveal that the method developed for the analysis of OXCPM is accurate, sensitive and precise. Moreover, degradation products formed under

ICH prescribed stress conditions do not interfere in determination of OXCPM at 258 nm. Hence, the developed method is stability-indicating and may be applied for routine analysis of the compound in bulk and stability studies. The intrinsic stability behavior of the compound suggests that the acidic and neutral solutions are resistant to hydrolysis at ambient temperature and photolysis under UV and visible light. However, alkaline solutions were susceptible to both hydrolysis at mild-high temperatures and photolysis. Due to the thiol group, OXCPM showed extensive oxidative degradation in hydrogen peroxide and solutions of transition metals, which indicated that the compound might be having metal chelating and free radical scavenging potential.

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