Manuscript Details

Manuscript number	MOLLIQ_2019_3487_R2
Title	Heterogeneous Fenton's-Like Catalysis for Degradation of Colchicine Coupled with Extraction of Its Biologically Active Metabolite
Article type	Full length article

Abstract

Nowadays, drug pollution; a form of water pollution caused by some pharmaceuticals and their metabolites resulting from consumers, industry and hospitals was reported. Colchicine (CLN) is considered one of the pharmaceutical wastewater contaminants which are not eliminated completely in municipal sewage treatment plants and are discharged into receiving water. Due to the higher toxicity of CLN, a novel heterogeneous Fenton's-like catalysis was established for complete degradation of CLN. So, a highly sensitive and specific liquid chromatographic method with quadrupole mass spectrometry (LC/Q-MS) was developed and validated for estimation of CLN in its pure form and in the presence of its degradation product. Herein, GraceSmart RP C18 column was utilized for separation of the cited drug (Retention time tR= 5.578 min) using methanol: water (55: 45, v/v) at 1.0 mL/min. Detection was performed by Agilent 6120 Quadrupole MS detector in a positive ionization mode. Thereafter and for the first time, degradation of CLN by heterogeneous Fenton's-like catalysis using modified polyacrylonitrile (PAN) as a catalyst with H_2O_2 in aqueous acidic medium was performed. This process was firstly optimized by HPLC/UV detection at 248 nm using the aforementioned chromatographic conditions. As a result, CLN degraded completely within 30 min. The only observed degradation product was the biologically active, potent and less toxic antitumor metabolite of CLN (3- demethyl CLN) which was collected, extracted, and analyzed by Fourier Transfer- Infrared Spectroscopy (FTIR) and 13Carbon-Nuclear Magnetic Resonance (13C-NMR). Finally, this method is eco-friendly and complies with the requirements of the green chemistry. It is suitable for complete removal of CLN and/or its metabolite contaminants from wastewater samples and estimation of the target drug without any interference from its degradation products. However, further study is required to expand the method applicability to the pharmaceutical wastewater treatment as well the production of 3- demethyl CLN on a large scale.

Keywords	Colchicine; 3- demethyl colchicine; Liquid chromatography-mass spectrometry; Fenton's-like catalysis; Drug pollution.
Manuscript category	Water, aqueous solutions and other hydrogen-bonded liquids

Corresponding Author	Noha M. Hosny
Corresponding Author's Institution	faculty of pharmacy, Assiut University
Order of Authors	Noha M. Hosny, Katherine Huddersman, Noha N. Atia, Samia M. El-Gizawy

Suggested reviewers	Mahmoud Omar, Yoshihiro Ohmiya, sameh ahmed

Submission Files Included in this PDF

File Name [File Type]

cover letter.doc.doc [Cover Letter]

comments_2.pdf [Response to Reviewers]

Highlights.pdf [Highlights]

Graphical abstract 1.tif [Graphical Abstract]

Noha Hosny- Revised script.docx [Manuscript File]

Figure 1.tiff.tif [Figure]

Figure 2-neww.tif [Figure]

Figure 3 new.tif [Figure]

Figure 4-new.tif [Figure]

Figure 5-new.tiff.tif [Figure]

Figure 6-new.tif [Figure]

Figure 7-neww.jpg [Figure]

Figure 8-new.tiff.tif [Figure]

Figure S1.jpg [Figure]

Figure S2.jpg [Figure]

figure S3.tiff.tif [Figure]

Figure S4 new.tif [Figure]

Conflict of intrest.docx [Conflict of Interest]

supplementary file1.pdf [Supplementary Material]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Noha M. Hosny, Honorary research assistant at DMU, Leicester, UK & Lecturer at Assiut University, Assiut, Egypt.

Editor in chief
Journal of Molecular Liquids
September, 28, 2019

Dear Prof. Yamaguchi,

Thank you very much for your effort on handling our paper.

Please kindly reconsider the attached **revised** manuscript entitled ***"Heterogeneous Fenton's-Like Catalysis for Degradation of Colchicine Coupled with Extraction of Its Biologically Active Metabolite"*** for publication as a research paper in ***Journal of Molecular Liquids***.

All comments from the reviewer were taken in consideration.

All the required corrections were done and highlighted as recommended.

Thank you in advance.
Sincerely yours,

Noha M. Hosny
*Honorary research assistant at Faculty of Health and Life Sciences, De Montfort University, Leicester, UK &
Lecturer at Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.
Tel.: +20-1144438525
E-mail address: phar_nmh2011@yahoo.com , Noha.hosni@pharm.au.edu.eg

Response to Reviewer's Comments

-Reviewer

The revised manuscript has been improved but further revision is suggested.

1. "Novel" should be removed in title. The reasons are below: 1) This heterogeneous Fenton's-Like Catalysis method has been developed (page 4, line 23-25); 2) This work is just focused on the application of heterogeneous Fenton's-like catalysis for fully degradation of CLN using the modified PAN catalyst with H2O2 in aqueous acidic medium (page 6, line 13-15).

*** Done. "Novel" was removed.**

2. All the tables should be present as three line table.

*** Done.**

3. Pay attention to some mistakes, such as, "100°c" should be "100°C" (page 9, line 11). "Robustness of the of the developed" should be changed to "Robustness of the developed". Please check similar problem again.

*** Corrected and highlighted (page 9, lines 10& 11 and page 29, line 1).**

4. Space should be existed between numbers and units except for °C, such as page 9, line 5 and line 10. Please check similar problem.

*** Done.**

5. There are too many figures and tables. Some of them can be transferred into the supporting information.

*** There are 3 tables and 8 figures inside the paper while, the others (2 tables and 4 figures) were transferred to the supplementary file.**

6. The quality of all figures needed to be greatly improved. For example, figure 7.

*** We tried to increase the resolution of all figures to more than 2000 dpi. Regarding figure 7, its resolution was increased to 10000 dpi.**

Thanks for your effort on handling of our manuscript.

Kind regards,

Noha M. Hosny

Lecturer of Pharmaceutical Analytical Chemistry- Faculty of Pharmacy- Assiut University- Assiut- Egypt

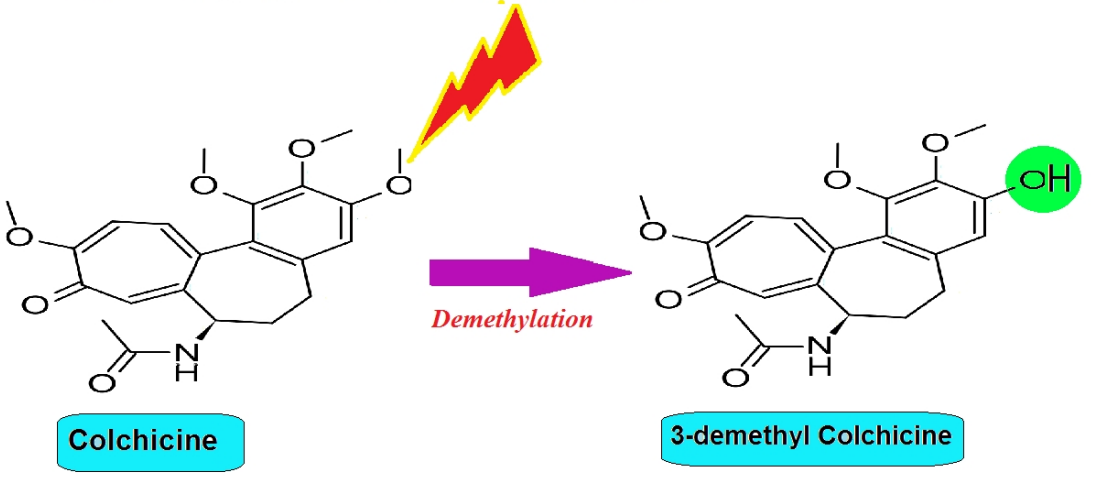
Highlights

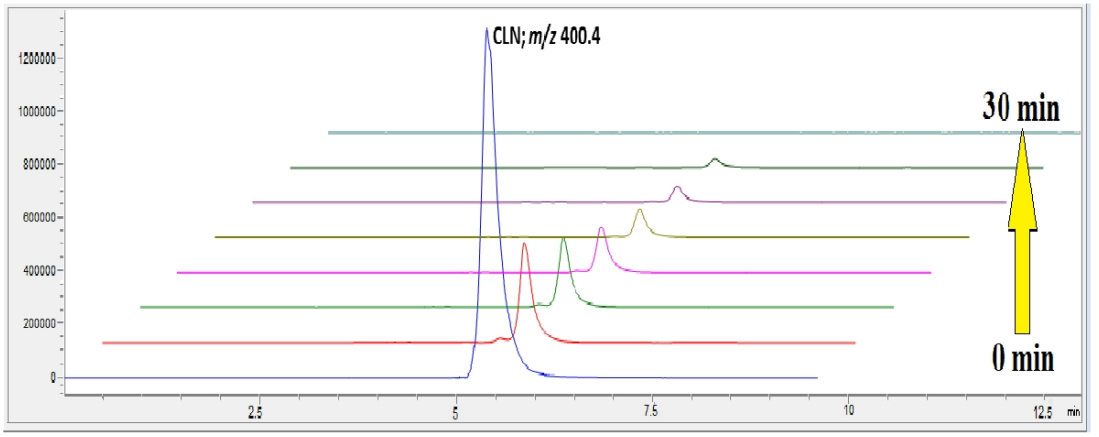
- Ultrasensitive LC/Q-MS method was developed for quantitation of colchicine (CLN).
- It is the first study focusing on the complete degradation of colchicine.
- Heterogeneous Fenton's-like catalysis was utilized for degradation of colchicine.
- 3- demethyl CLN produced during CLN degradation was analyzed by FT-IR and ^{13}C-NMR.
- This study complies with the requirements of green chemistry.

Heterogeneous Fenton's-Like Catalysis for Colchicine Degradation

$$Fe^{3+}_{\textit{(immobilized on the PAN surface)}} + H_2O_2 \leftrightarrow Fe^{2+} + \ ^{\bullet}OOH + H^+$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$





Heterogeneous Fenton's-Like Catalysis for Degradation of Colchicine Coupled with Extraction of Its Biologically Active Metabolite

Noha M. Hosny [a, b*], Katherine Huddersman[b], Noha N. Atia[a], Samia M. El-Gizawy[a]

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

[b] School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, Leicester, UK.

***Corresponding author: Noha M. Hosny**

E-mail: phar_nmh2011@yahoo.com, noha.hosni@pharm.au.edu.eg,

Tel: +2-01144438525

-Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

-Declarations of interest: none.

Abstract

Nowadays, drug pollution; a form of water pollution caused by some pharmaceuticals and their metabolites resulting from consumers, industry and hospitals was reported. Colchicine (CLN) is considered one of the pharmaceutical wastewater contaminants which are not eliminated completely in municipal sewage treatment plants and are discharged into receiving water. Due to the higher toxicity of CLN, a novel heterogeneous Fenton's-like catalysis was established for complete degradation of CLN. So, a highly sensitive and specific liquid chromatographic method with quadrupole mass spectrometry (LC/Q-MS) was developed and validated for estimation of CLN in its pure form and in the presence of its degradation product. Herein, GraceSmart RP C18 column was utilized for separation of the cited drug (Retention time t_R= 5.578 min) using methanol: water (55: 45, v/v) at 1.0 mL/min. Detection was performed by Agilent 6120 Quadrupole MS detector in a positive ionization mode.

Thereafter and for the first time, degradation of CLN by heterogeneous Fenton's-like catalysis using modified polyacrylonitrile (PAN) as a catalyst with H_2O_2 in aqueous acidic medium was performed. This process was firstly optimized by HPLC/UV detection at 248 nm using the aforementioned chromatographic conditions. As a result, CLN degraded completely within 30 min. The only observed degradation product was the biologically active, potent and less toxic antitumor metabolite of CLN (3- demethyl CLN) which was collected, extracted, and analyzed by Fourier Transfer- Infrared Spectroscopy (FTIR) and ^{13}Carbon- Nuclear Magnetic Resonance (^{13}C-NMR).

Finally, this method is eco-friendly and complies with the requirements of the green chemistry. It is suitable for complete removal of CLN and/or its metabolite contaminants from wastewater samples and estimation of the target drug without any

interference from its degradation products. However, further study is required to expand the method applicability to the pharmaceutical wastewater treatment as well the production of 3- demethyl CLN on a large scale.

Keywords

Colchicine; 3- demethyl colchicine; Liquid chromatography-mass spectrometry; Fenton's-like catalysis; Drug pollution.

1. Introduction

Nowadays, drug pollution; a form of water pollution caused by some pharmaceuticals and their metabolites resulting from consumers, industry and hospitals was reported. Pharmaceutical pollutants are not eliminated completely in municipal sewage treatment plants and are discharged into receiving water [1-4]. Drug pollution comes simply from the excreted drugs and/or their metabolites in the urine in addition to the expired or unneeded drugs that are flushed unused down the toilet [3]. Other sources of such pollution include agricultural runoff (because of antibiotic use in livestock) and pharmaceutical manufacturing [1, 3].

Filtration to remove course solids followed by biological treatment and sand filtration are the common processes used for wastewater treatment. Also, Fenton's reagent (H_2O_2 with Fe^{2+} as a catalyst) a form of Advanced Oxidation Process (AOP) is widely utilized to oxidize organic water pollutants to carbon dioxide and water by a powerful hydroxyl radical as following [3, 5];

$$OH \text{ (Hydroxyl radical)} + OH^- \bullet Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} +$$
$$OOH \text{ (Hydroperoxyl radical)} + H^+ \bullet Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} +$$
$$OH \rightarrow \text{Oxidation products} \bullet R \text{ (organic substance)} +$$

But the main limitations of classical Fenton's oxidation are pH modulation, the presence of the catalyst in the final treated samples and the production of sludge formed by the precipitation of Iron (III) oxo/hydoxo species which are insoluble at the pH of the process. This sludge is also likely to be contaminated by non-degraded pollutant [6].

To overcome these demerits, a heterogeneous form of the classical Fenton's catalyst incorporating Fe^{3+} cations in a Fenton's- like catalysis was designed by using modified polyacrylonitrile (PAN) fibres [7-8]. This modification process involves the impregnation of catalytically active transition metal complexes on the functionalized

surface of PAN fibres. Transition metals are preferred because of their partially filled d orbitals making them highly reactive [9]. Studies at De Montfort University has led to further development of a novel PAN catalyst through the immobilization of Fe^{3+} on the surface of the PAN. The presence of chemically highly reactive nitrile groups on the polymer allowed functionalization with a mixture of hydroxylamine and hydrazine salts in alkaline solution to form chelating ligands (carboxylate, amides and oxime) [8, 10]. This indigenous catalyst has been successfully tested for the oxidative decomposition of several waste effluents from pharmaceutical, textile, agrochemical, and other model compounds at laboratory scale in a heterogeneous Fenton-like process using H_2O_2 as an oxidant in a similar cyclic redox mechanism to the aforementioned where the produced hydroxyl radical reacts with the organic pollutant to form oxidation products [11-12].

Colchicine (CLN) is considered one of the pharmaceutical wastewater contaminants [1, 2, 13]. CLN is an alkaloid obtained from different *Colchicum* species (Figure 1). It is an official drug in the European, Chinese, Japanese, and International Pharmacopeias as well USP. It is commonly used to relieve pain and inflammation associated with acute gouty attacks by decreasing the leucocyte mobility [14]. CLN also has an antimitotic action and is widely used in treatment of amyloidosis, Behçet's syndrome, familial Mediterranean fever, idiopathic thrombocytopenic purpura, pericarditis, primary biliary cirrhosis, and pyoderma gangrenosum [15]. Nausea, diarrhea, vomiting and abdominal pain associated with CLN antimitotic action are its main side effects and the first signs of its toxicity. With CLN overdose; anemia, multiple organ failure, bone marrow depression, hepatocellular with muscle damage, renal damage and CNS toxicity have been reported [13-15].

Furthermore, CLN derivatives such as 3-demethyl CLN (Figure 1), 2-demethyl CLN, colchicoside and thiocolchicoside have been reported for their possible application in management of certain forms of leukemia and solid tumors [16]. Unlike CLN, they have potent anti-inflammatory and anti-tumor activity with lower toxicity. But, due to rarity of CLN derivatives through CLN-producing plants, many trials have been made to find alternative routes for their industrial-scale production [16-19]. Biotransformation of CLN employing CYP3A4 enzyme or microbial demethylation were reported for CLN demethylation [17-19].

Concerning literature, liquid chromatography (with UV, MS or Tandem MS detection) [20-28], gas chromatography [29-30], thin layer chromatography (TLC) [31-33], fluorimetric and spectrophotometric methods [34-37] have been reported for estimation of CLN alone or combined with other drugs.

The present work is the first study that focuses on the application of heterogeneous Fenton's-like catalysis for fully degradation of CLN using the modified PAN catalyst with H_2O_2 in aqueous acidic medium. So, a highly sensitive and specific liquid chromatographic method with quadrupole mass spectrometry (LC/Q-MS) for estimation of CLN in pure form and in the presence of its degradation product was developed and validated.

Thereafter, the biologically active, potent and less toxic antitumor derivative of CLN (3- demethyl CLN) produced during CLN degradation was collected, extracted, and analyzed by Fourier Transfer- Infrared Spectroscopy (FTIR) and ^{13}Carbon- Nuclear Magnetic Resonance (^{13}C-NMR).

This work was designed to fulfill the principles required for the green chemistry [38]. So, hazards from chemical toxicity and waste reduction in addition to discovering replacements for hazardous substances were our main targets.

2. Experimental

2.1. Apparatus

Agilent 6120 Quadrupole LC/MS system linked to 1260 Infinity auto-sampler, solvent manager, pump, thermostated column chamber and Quadrupole MS detector (Agilent Technologies, USA) was used. This system was operated with OpenLAB CDS ChemStation Edition C. 01. 06 software. PerkinElmer Series 200 HPLC System (Model LCTURBO, Serial no. ZAAA0646, PerkinElmer® Inc., USA) connected to series 200 degasser, pump and UV/VIS detector was utilized for optimization of the drug degradation process. A rheodyne injection valve (Model 7725i, USA) with a 20 µL loop and 100 µL Hamilton – Bonaduz, Schweiz (Switzerland) sample syringe were used for sample application. The HPLC system was operated with TotalChrom Workstation version 6.3.4 software. The GraceSmart RP C18 encapped column (250 mm length x 4.6 mm inner diameter, 5 µm particle size, 120 Å pore size) was coupled to either LC/MS or HPLC/UV system.

ALPHA FTIR (Serial no. GI004911, operated with OPUS Spectroscopy Software) and AVANCE™- UltraShield 400 NMR (Serial no. GH007300) spectrometers (BRUKER UK Limited, UK) in addition to Thermo Scientific-Evolution 220 UV-VIS spectrophotometer with 1 cm quartz cuvettes *(Waltham*, Massachusetts, USA) were also used. Moreover, analytical series Fisher-Scientific balance-PAS214C (China), Jenway 350 pH meter (China) and ultrasonic cleaner- Kerry PUL 125 (England) were utilized. Furthermore, Nylon 66 membranes (0.45 µm pore size, 47.0 mm diameter) and Fisherbrand PTFE syringe filters (0.45 µm) were obtained from SUPELCO, USA and Fisher Scientific Co., UK; respectively.

Regarding the catalytic oxidation process, Carousel 6 Plus Reaction Station™ set (Radleys, UK) consists of stirring hotplate and 250 mL-round bottom flasks with reflux head was used.

Interflon glass gravity column (200 mm length x 20 mm inner diameter, Sigma-Aldrich Co., UK) and UVGL-55 Handheld lamp (USA) were utilized during extraction of the degradation product. Also, Quantofix® peroxide semi-quantitative test strips (Macherey-Nagel Germany) and Büchi Rotavapor R-300 (UK) were used.

2.2. Chemicals and Solvents

CLN (≥95%) was purchased from Sigma-Aldrich Co., UK. LC/MS grade methanol (99.95%) and water (99.9%) were purchased STRATLAB Laboratory Supplies, UK. HPLC grade Hydrogen peroxide (30% w/w), analytical grade hydrochloric acid (36%), sodium hydroxide, $FeSO_4.7H_2O$ and Acros Organic™ deuterated chloroform for NMR were bought from Fisher Scientific Co., UK.

Modified PAN catalytic mesh was supplied by School of Pharmacy, De Montfort University, Leicester, UK.

TLC aluminium sheets precoated with silica gel G $60F_{254}$ plates (Fluka, 5 x 5 cm, 0.20 mm layer thickness) and Silica gel for column chromatography (200-400 mesh, 60 Å) were obtained from Sigma-Aldrich Co., UK.

2.3. Standard Solutions preparation.

50.0 mg of CLN were dissolved in 50.0 mL water to obtain the stock standard solution (1.0 mg mL^{-1}). This solution was kept in a refrigerator away from light.

The stock solution was firstly diluted with water to prepare 200 ng mL^{-1} followed by serial dilution with the same solvent to prepare the working standard solutions (0.1-50 ng mL^{-1}).

2.4. Analytical procedures.

2.4.1. General Chromatographic Conditions.

10 µL of the working standard or sample solution of the studied drug was injected automatically by the autosampler on Agilent 6120 Quadrupole LC/MS system linked to GraceSmart RP C18 column (250 mm length x 4.6 mm inner diameter, 5 µm particle size, 120 Å pore size; Controlled temperature at 25± 0.8°C). Then, isocratic elution was carried out by methanol: water (55:45, v/v) at flow rate 1.0 mL min^{-1}. The total run time was 10 minutes.

Detection was performed by Agilent 6120 Quadrupole MS detector in a positive ionization mode (Drying gas: N_2 at 12.0 L/min; its Temp. 350°C, Capillary voltage 3000 V, and the main settings: Rough Vacuum 1.38 Torr, Quadrupole Temp. 100°C, Nebulizing Pressure 35 psig, TurboSpd 100%, Fragmentor 70, Gain 1.0, Threshold 150). MS data were collected as either total ion current (TIC, *m/z* 50-500), or selected ion monitoring (SIM) at *m/z* 400.4. CLN mass spectrum showed a prominent molecular ion $[M+H]^+$ peak at *m/z*= 400.2.

2.4.2 Construction of Calibration Curves.

Working standard solutions of CLN equivalent to (0.10, 1.0, 10.0, 20.0, 30.0, 40.0 and 50.0 ng mL^{-1}) were prepared in water. Then, the assay was done as mentioned under the general chromatographic conditions **(Section 2.4.1)**.

The peak area values (µV*Sec) were plotted against the drug final concentration (ng mL^{-1}) to get the calibration graph. Thereafter, the corresponding regression equation was calculated.

2.5. Degradation of Colchicine.

2.5.1. Preparation of PAN Catalyst.

To remove excess iron from the PAN catalyst, washing of the mesh was performed by scrubbing and immersing it in tap water. The colour of water changed from clear to brown which shows leaching off the excess iron from the surface of the catalyst. This process was done until there was no further colouration. The washed PAN was cut to pieces (20*20 cm) and transferred into a beaker with double distilled water then pH was adjusted at 3.0 using 0.1 N hydrochloric acid and/or sodium hydroxide. Upon stabilization of pH (3.0), the PAN mesh was removed away from water and air-dried at room temperature.

2.5.2. Procedure for CLN Degradation.

Working standard solution of CLN (50.0 µg mL^{-1}) was prepared in acidic double distilled water (pH 3.0; adjusted by 0.1 N HCl and/or NaOH). 100 mL of this working solution were transferred into a carousel flask followed by addition of 8 grams of PAN catalyst (cut into 2 cm^2 pieces) and a magnetic stirrer flea. pH of the flask content was checked and readjusted to the desired value (3.0). Carousel 6 Plus Reaction Station™ stirring hotplate was set to 500 rpm at 25°C to ensure a proper mixing **(Figure S1).** Thereafter, the catalytic reaction was initiated by addition of 100 µL of 30% H_2O_2 (corresponding to 333 ppm) to the flask content (CLN+ PAN). Samples were taken at predetermined time intervals of 5 min to monitor the degradation of CLN (the targeted drug degraded completely within 30 min). The first sample (0 min; CLN only) was taken before the introduction of the catalyst. Subsequently, the catalyst and H_2O_2 were added and the samples were pulled every 5 min and filtered through PTFE syringe filters. 10 µL of the collected samples were then injected on LC/MS system. The assay was then carried out in triplicates as

mentioned before **(Section 2.4.1)**. MS data were collected as either TIC at *m/z* 50-500 or SIM at *m/z* 400.4 and 386.4 for CLN and its degradation product; respectively. Mass spectra showed two prominent molecular ion peaks $[M+H]^+$ at *m/z*= 400.2 and 386.1 for CLN and demethylated-CLN; respectively..

Blank experiment was done in the same manner excluding the drug.

Furthermore, two experiments were performed similarly to evaluate the CLN stability in the absence of the PAN catalyst (The first was for CLN in acidic water; while the second one was for CLN in presence of H_2O_2 at pH 3.0).

2.5.3. Isolation of the degradation product.

During the degradation process, CLN degraded to its biologically active, potent and less toxic antitumor metabolite (3- demethyl CLN). It reached a maximum concentration within 5 min of the catalytic oxidation reaction. Due to the product medical importance, we tried to separate and collect it by the aid of TLC and fractional column chromatographic techniques.

The catalytic oxidation reaction was stopped after 5 min via the removal of PAN pieces (by filtration) and evaporation of excess H_2O_2 (by heating on a water bath for 5 min and H_2O_2 content was checked by Quantofix® peroxide semi-quantitative test strips). The degraded solution mixture (CLN and its metabolite) was preliminary separated on silica gel TLC plates using methanol: water (55:45, v/v) and the spots were visualized by portable UV- lamp at 254 nm (Retardation factor (R_f) = 0.61 and 0.13 for CLN and its derivative; respectively).

Subsequently, the fractional column chromatographic method was established to collect each compound separately. Interflon glass column (200 mm length x 20 mm inner diameter) was carefully packed with silica gel and conditioned by methanol then loaded with the degraded solution mixture. Methanol: water (55:45, v/v) was firstly

used as a mobile phase to elute CLN. Then, 3- demethyl CLN was eluted by 100% water. The eluted fractions were checked for their purity by TLC plates and UV/VIS spectrophotometer **(Figure S2)**.

The collected fractions of 3- demethyl CLN were evaporated using Büchi Rotavapor R-300 to obtain a yellowish- white powder of the desired product.

For structure confirmation, CLN and 3- demethyl CLN samples were analyzed by FTIR and NMR spectrometers.

3. Results and Discussion

The main objective of the presented work is to establish an eco-friendly method for fully degradation of CLN by heterogeneous Fenton's-like catalysis for the first time (using modified PAN catalyst with H_2O_2 in aqueous acidic medium). So, a simple, highly sensitive and specific LC/Q-MS method was developed and validated for determination of CLN in its pure form and in the presence of its degradation product. Finally, the isolation and identification of the biologically active metabolite of CLN (3- demethyl CLN) produced during the degradation process were achieved.

3.1. The developed LC/Q-MS Method.

3.1.1. Chromatographic Conditions Optimization.

To achieve a good resolution and fast analysis with symmetric peaks, mobile phase composition and flow rate were tested.

3.1.1.1. Mobile phase

After testing acetonitrile, methanol and ethanol as organic solvents, a good resolution and a sharp peak with higher area was observed when methanol was the organic modifier. While, ethanol and acetonitrile gave asymmetric peak with CLN (broad and tailed). Also, the percentage of the chosen organic solvent was varied (from 10 % to 90 %). Upon increasing the methanol percentage ≥70%, improper resolution between

CLN and its degredant was observed. On the other hand, good separation was achieved within longer run time (>10 min) when the methanol content was decreased ≤40%. So, the chosen mobile phase (55% methanol to 45% water) gave the best resolution and sharp peak of the target drug in presence of its degradation product within reasonable time (6 min). The retention times (t_R) were 5.578 and 4.281 for CLN and 3-demethyl CLN; respectively. The LC/MS chromatograms for the cited drug and its degradation product were shown in Figure 2.

3.1.1.2. Flow rate

The influence of flow rate on the separation of CLN alone or in presence of its degradation product was studied in the range of 0.3-2 mL min^{-1}. In the preliminary studies, overlapping and poor resolution was observed at higher flow rates ≥1.2. But at lower flow rates ≤0.8, good resolution was achieved with broad asymmetric peaks at longer run time (more than 10 min) for the entire elution of these compounds. So, an isocratic mode of flow rate (1 mL min^{-1}) was set to enhance the method's resolution within short separation time (less than 6 min). Thus, the proposed method can successfully applied for rapid analysis of CLN in the presence of its degredant.

3.1.2. Method Validation.

As mentioned in ICH guidelines on the validation of analytical methods ICH Q2 (R1), linearity, detection (LOD) and quantification (LOQ) limits, precision, accuracy, robustness, selectivity and specificity of the proposed LC/MS were evaluated [39].

3.1.2.1. Linearity and sensitivity parameters.

According to the optimal chromatographic conditions, six calibration curves for standard solutions of CLN at seven concentration levels (Figure 3) were constructed by plotting peak area against drug concentration (ng mL^{-1}). The results presented in

Table 1 indicate good linearity over a range of 0.10 – 50.0 ng mL^{-1} with an excellent correlation coefficient (0.9999).

Also, 3 σ/S and 10 σ/S were used for calculation of LOD and LOQ of the studied drugs; respectively (σ is the standard deviation of y-intercept of the regression equation and S is the slope of the calibration curve). The lower LOD and LOQ values obtained (16.4 and 49.8 pg mL^{-1}; respectively) indicate a high sensitivity of the proposed method. In conclusion, the method's simplicity and sensitivity are superior to other reported LC/MS or Tandem MS methods [23-28].

3.1.2.2. Accuracy and precision

Low, medium, and high concentration ranges (0.100, 20.0, and 50.0 ng mL^{-1}) of CLN were analyzed by the developed LC/MS method. After injection of each concentration in six replicates, recovery percentages were calculated. The recoveries were in the range from 100.12 to 100.18% with RSD ≤0.89% indicating good accuracy of the proposed method (**Table 2**). Moreover, the three above-mentioned concentrations were analyzed in triplicates for intra-day precision and gave recoveries of (99.90-100.34%) and (99.67-100.36%) for inter-day precision (n= 9). RSD values were found to be ≤ 0.68 for intra- and inter-day precision (**Table 2**). These results confirm that the acceptable repeatability and accuracy of the proposed method for assay of CLN.

3.1.2.3. Robustness.

Minor changes of the optimal chromatographic parameters were studied. It was found that the slight alteration in flow rate and mobile system composition had no significant influence on the method's performance. The results shown in **Table 3** indicate the reliability of our method during normal usage.

3.1.2.4. Specificity and selectivity.

The proposed method is able to separate the studied drug completely from its degradation product. After injection of the obtained degraded solution (CLN with its derivative), clean chromatograms were observed without any interfering peaks from the catalyst, H_2O_2 or the degradation product especially at the target retention time (Figure 2 & 4). Additionally, the method's specificity was enhanced using MS detection with SIM at the drug's *m/z* 400.4.

3.2. Optimization of CLN Degradation Reaction.

To ensure a complete and efficient degradation process, reaction parameters (PAN catalyst amount, H_2O_2 volume and the effect of pH) were evaluated using HPLC/UV system.

The procedure of CLN degradation (**section 2.5.2)** was carried out and the samples were pulled after 15 min of the reaction time. 20 µL of the sample was then injected manually on HPLC/UV system. As mentioned before **(section 3.1.1)**, methanol: water (55: 45, v/v) at flow rate (1.0 mL min^{-1}) were used as the optimal chromatographic conditions during this study. The UV detector was set at 248 nm (One of two maximum wavelengths of CLN; **Figure S3**).

Figure 4 represents the HPLC - Chromatograms of CLN with and without its degradation product.

3.2.1. PAN Catalyst Amount

The procedure for CLN degradation (**section 2.5.2)** was performed using different weights of PAN mesh (2, 4, 6, 8, 10 g). It was found that 8 g of PAN catalyst gave the lowest peak area of CLN and an efficient degradation (Figure 5A).

3.2.2. Volume of H_2O_2

To study the effect of H_2O_2 concentration on the degradation reaction, different volumes (20, 40, 60, 80, 100, 120, 140 µL) of 30% H_2O_2 were evaluated. As shown in

(Figure 5B), the highest amount of CLN (about 92 %) was degraded within 15 min upon using 100 µL of 30% H_2O_2 (=333 ppm).

On the other hand, calibration curve of H_2O_2 was constructed by plotting peak areas against different concentrations of H_2O_2 (50- 400 ppm) in acidic water (pH 3.0). The obtained linear regression equation was Y= 6088.4 X + 10^6; R^2 = 0.9945 **(Figure S4)**. We found that ~ 85% of the chosen initial concentration of 30% H_2O_2 (100 µL) was consumed on CLN complete degradation. Also, H_2O_2 scavenges hydroxyl radical upon the increase of H_2O_2 concentration.

3.2.3. Effect of pH

Variation of pH of both CLN working standard solution and PAN mesh (during its preparation) was investigated. Chu *et al.* [40] found that the oxidation potential of hydroxyl radicals decreased with increasing pH. Also, degradation rate and efficiency decreased at pH below 3.0 due to the formation of iron complex species $[Fe(H_2O)_6]^{2+}$ which reacts more slowly with H_2O_2 [41]. While; at slightly higher pH >3.0, the activity of Fenton's reagent was reduced due to the presence of an inactive iron oxohydroxides [42]. So, different low pH values (3.0, 3.5, 4.0, 4.5; adjusted by 0.1 N HCl and/or NaOH) were tested. A higher loss of CLN content was achieved at pH 3.0 (Figure 5C). Our finding agrees with many previous studies that recommended the use of pH 3.0 for Fenton's oxidation [4].

3.2.4. Monitoring of Degradation Reaction.

To monitor the degradation of CLN, the samples were analyzed by LC/Q-MS under the optimal reaction parameters at different reaction time (time intervals of 5 min over 0- 30 min). MS data were collected as either total ion current (TIC, *m/z* 50-500), or selected ion monitoring (SIM) at *m/z* 400.4 and 386.4 for CLN and its demethylated product; respectively). As a result, CLN was completely degraded within 30 min

(**Table S1**). One product was observed during this reaction (3- demethyl CLN; t_R= 4.281 min; *m/z* 386.1). Its highest concentration was reached within 5 min of the reaction time.

At 30 min of the reaction time, neither CLN nor its product's peaks were detected (Figure 6). As a result, this method could be applied successfully for the complete removal of CLN and/or its derivative from the wastewater during the treatment process.

3.3. Isolation and Identification of the Degradation Product.

Due to the medical importance of the resulting compound (3- demethyl CLN) during the CLN catalytic oxidation process, we tried to extract and identify it. 3- Demethyl CLN is more polar than CLN. So, it was separated easily with a simple fractional column chromatographic technique. Thereafter, its collected dried fractions were characterized by ALPHA FTIR and AVANCE^{TM}- UltraShield 400 NMR spectrometers in addition to Thermo Scientific-Evolution 220 UV-VIS spectrophotometer. The UV spectra (Figure 7A& A') showed a slight blue shift of 3-demethyl CLN (343 nm) compared to CLN (350 nm) because of the demethylation of CLN. Furthermore, MS spectrum (Figure 2) showed a signal at *m/z* 386.1 corresponding to 3- demethyl CLN. For further structure elucidation, a signal of phenolic hydroxyl group (3657 cm $^{-1}$) was detected on FTIR spectrum of 3- demethyl CLN (Figure 7B'). Also, ^{13}C-NMR spectrum of 3- demethyl CLN (Figure 7C') confirmed that the absence of methoxy group signal at position 3 (56.42 ppm) as compared with the standard CLN spectrum (Figure 7C). Our ^{13}C-NMR data of CLN and 3- demethyl CLN that agree with Dubey *et al.* and Zhang *et al.* findings [16, 19] are summarized in Table S2. Our results ensured that hydroxyl radical (produced from Fenton's- like catalysis) attacks CLN at position no. 3 and not the other positions.

Methoxy group is an electron-donating making the attached carbons more attractive to the electron-seeking hydroxyl radical. Attack at C-3 is preferred over attack at C-1 or C-2 as these sites are sterically hindered [43].

3.4. Stability of CLN.

To evaluate the CLN stability in the absence of PAN catalyst; influence of 100 µL of H_2O_2 and/or acidic aqueous medium (pH 3.0; adjusted by 0.1 N HCl) on CLN (50.0 µg/mL) were studied using HPLC/UV detection. It was observed that the cited drug was stable in H_2O_2 and/or acidic medium for at least 30 min without any detected degradation (Figure 8). On the other hand, CLN disappeared within 30 min in the presence of PAN catalyst with H_2O_2 at pH 3.0 (Figure 6).

4. Conclusion.

Eco-friendly, highly sensitive and specific liquid chromatographic method with quadrupole mass spectrometry (LC/Q-MS) was developed and validated for quantitation of colchicine (CLN) in its pure form and in the presence of its degradation product. MS data were collected as either total ion current (TIC, *m/z* 50-500), or selected ion monitoring (SIM) at *m/z* 400.4 for CLN. A good linearity (r=0.9999) was achieved over a concentration ranging from 0.1 to 50 ng/mL with LOD (16.4 pg//mL).

Additionally and for the first time, heterogeneous Fenton's-like catalysis was established for degradation of CLN using modified polyacrylonitrile (PAN) catalyst with H_2O_2 at pH 3.0. As a result, CLN degraded completely within 30 min.

Thereafter, the biologically active, potent and less toxic antitumor metabolite of CLN (3- demethyl CLN) produced during CLN degradation was collected, extracted, and analyzed by Fourier Transfer- Infrared Spectroscopy (FTIR) and ^{13}Carbon- Nuclear Magnetic Resonance (^{13}C-NMR).

Finally, this work is the first study developed for complete removal of CLN from the water samples and production of less toxic and expensive antitumor drug (3- demethyl CLN). This work has important environmental and pharmaceutical impact to wastewater treatment from pharmaceutical pollutants as well the production of an expensive antitumor drug at the same time. It also complies with the requirements of green chemistry.

However, further study is required to expand the method applicability on the pharmaceutical wastewater treatment as well the large scale production of 3-demethyl CLN.

Conflict of Interest:

"There are no conflicts to declare"

Acknowledgment:

The corresponding author thanks the Egyptian Ministry of Higher Education and Scientific Research in addition to the Egyptian Cultural Affairs & Mission Sector for granting her a short-term mission to De Montfort University, Leicester, the United kingdom to carry out this work.

References

[1] Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., & Buxton, H. T., (2002) . Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ. Sci. Technol., 36(6), 1202-1211. Through https://www.ncbi.nlm.nih.gov/pubmed/11944670

[2] Le Corre, K., Katouli, M., Stratton, H., Ort, C. & Keller, J., (2012). Hospital Wastewater: Urban Water Security Research Alliance Technical Report No. 76. Through https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=37&ved=2ahUKEwiXgZrx4JXgAhV8QhUIHWd_ABM4HhAWMAZ6BAgCEAI&url=http%3A%2F%2Fwww.urbanwateralliance.org.au%2Fpublications%2FUWSRA-tr76.pdf&usg=AOvVaw3u7DRXB9Ipn3uuPsTlBwzc

[3] https://en.wikipedia.org/wiki/Drug_pollution

[4] Dheeaa al deen A. Aljuboury, Puganeshwary Palaniandy, Hamidi Bin Abdul Aziz & Shaik Feroz, (2014). A Review on the Fenton Process for Wastewater Treatment. Journal of Innovative Engineering, 2(3), 4. Through http://www.academia.edu/8594388/A_Review_on_the_Fenton_Process_for_Wastewater_Treatment_Dheeaa_al_deen_A_Aljuboury1_Puganes

[5] https://en.wikipedia.org/wiki/Wastewater_treatment

[6] https://www.degruyter.com/view/j/ijcre.2012.10.issue-1/1542-6580.2913/1542-6580.2913.xml

[7] Li, B., Dong, Y., & Ding, Z., (2013). Heterogeneous Fenton Degradation of Azo Dyes Catalysed by Modified Polyacrylonitrile Fiber Fe Complexes: QSPR

(Quantitative Structure Property Relationship) Study. Journal of Environmental Sciences, 25(7), 1469–1476.

[8] Han Z. B., Dong Y. C., & Dong S. M., (2011). Copper Iron Bimetal Modified Pan Fiber Complexes as Novel Heterogeneous Fenton Catalysts for Degradation of Organic Dye Under Visible Light Irradiation. Journal of Hazardous Materials, 189(1-2), 241-248.

[9] Hagen, J., (2006). Industrial catalysis: A practical approach. Org. Process Res. Dev., 10 (3), 683–684. Through https://pubs.acs.org/doi/abs/10.1021/op050247m

[10] Ishtchenko V. V., Huddersman K. D., & Vitkovskaya R. F., (2003). Production of a Modified PAN. Fibrous Catalyst and its Optimisation towards the Decomposition of Hydrogen Peroxide. Applied Catalysis A, 242(1), 123-137.

[11] Chi, G. T., Churchley, J., & Huddersman, K. D., (2013). Pilot-Scale Removal of Trace Steroid Hormones and Pharmaceuticals and Personal Care Products from Municipal Wastewater Using a Heterogeneous Fenton's Catalytic Process. International Journal of Chemical Engineering, 2013, 1–10.

[12] Chi G. T. & Huddersman, K. D., (2007). Novel Ion Chromatography Technique for the Rapid Identification and Quantification of Saturated and Unsaturated Low Molecular Weight Organic Acids Formed during the Fenton Oxidation of Organic pollutants. Journal of Chromatography A, 1139, 95–103.

[13]https://toxnet.nlm.nih.gov/cgibin/sis/search/a?dbs+hsdb:@term+@DOCNO+3044

[14] Sweetman, S. C. Martindale: The Complete Drug Reference, 38th ed., Pharmaceutical Press, London, 2016.

[15] https://en.wikipedia.org/wiki/Colchicine

[16] Dubey, K. K., Jawed, A., & Haque, S. (2011). Enhanced extraction of 3-demethylated colchicine from fermentation broth of Bacillus megaterium: Optimization of process parameters by statistical experimental design. Engineering in Life Sciences, 11(6), 598-606.

[17] Rosner, M., Capravo, H., Jacobson, A. E., Atwell, L. et al. (1981). Biological effects of modified colchicine. Improved preparation of 2- DMC, 3MC and (1)-colchicine and rearrangement of the position of the double bond in dehydro- 7 deacetamidecolchicine. J. Med. Chem., 24, 257–261.

[18] Tateishi, T., Soucek, P., Caraco, Y., Guengerich, F. P. et al. (1997). Colchicine biotransformation by human liver microsomes: identification of CYP3A4 as the major isoform responsible for colchicine demethylation. Biochem. Pharmacol., 53, 111–116.

[19] Zhang, C., Sun, X., Xu, S. H., Yu, B. Y., & Zhang, J. (2017). Microbial Catalyzed Regio-Selective Demethylation of Colchicine by Streptomyces griseus ATCC 13273. Applied biochemistry and biotechnology, 183(3), 1026-1034.

[20] Joshi, S. A., Jalalpure, S. S., Kempwade, A. A., & Peram, M. R. (2018). Development and Validation of HPLC Method to Determine Colchicine in Pharmaceutical Formulations and its Application for Analysis of Solid Lipid Nanoparticles. Current Pharmaceutical Analysis, 14(1), 76-83.

[21] Çankaya, N., Bulduk, İ., & Çolak, A. M. (2018). Extraction, development and validation of HPLC-UV method for rapid and sensitive determination of colchicine from Colchicum autumnale L. Bulbs. Saudi Journal of Biological Sciences. https://doi.org/10.1016/j.sjbs.2018.10.003

[22] Samanidou, V. F., Sarantis, G. A., & Papadoyannis, I. N. (2006). Development and validation of a rapid HPLC method for the direct determination of colchicine in

pharmaceuticals and biological fluids. Journal of liquid chromatography & related technologies, 29(1), 1-13.

[23] Jiang, Y., Wang, J., Wang, Y., Li, H., Fawcett, J. P., & Gu, J. (2007). Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of colchicine in human plasma. Journal of Chromatography B, 850(1-2), 564-568.

[24] Hamscher, G., Priess, B., Nau, H., & Panariti, E. (2005). Determination of colchicine residues in sheep serum and milk using high-performance liquid chromatography combined with electrospray ionization ion trap tandem mass spectrometry. Analytical chemistry, 77(8), 2421-2425.

[25] Jones, G. R., Singer, P. P., & Bannach, B. (2002). Application of LC-MS analysis to a colchicine fatality. Journal of analytical toxicology, 26(6), 365-369.

[26] Kovvasu, S. P. R., Kunamaneni, P., Yeung, S., & Kodali, B. (2018). Determination of colchicine in human plasma by a sensitive LC-MS/MS assay. World J Pharm Pharmaceu Sci, 7(3), 35-44.

[27] Lo, W. Y., & Krause, G. M. (1987). Simultaneous Determination of Probenecid and Colchicine in Solid Dosage Form by Reversed Phase High Performance Liquid Chromatography. Drug Development and Industrial Pharmacy, 13(1), 57-66.

[28] Hosny, N. M., Huddersman, K., El-Gizawy, S. M., & Atia, N. N. (2019). New approach for simultaneous analysis of commonly used antigout drugs by HPLC/UV method; Application in pharmaceutical and biological analysis. Microchemical Journal, 147, 717-728.

[29] Schreiber, L., Morovič, M., Špacayová, K., & Halko, R. (2018). Colchicine Extract Suicidal Lethal Poisoning Confirmation Using High-Resolution Accurate

Mass Spectrometry: A Case Study. Journal of forensic sciences. Through https://onlinelibrary.wiley.com/doi/abs/10.1111/1556-4029.13977

[30] Clevenger, C. V., August, T. F., & Shaw, L. M. (1991). Colchicine poisoning: report of a fatal case with body fluid analysis by GC/MS and histopathologic examination of postmortem tissues. Journal of analytical toxicology, 15(3), 151-154.

[31] Rahmawati, S. I., Susilowati, A., Yunus, A., & Widyastuti, Y. (2018). Determination colchicine content in aquadest-extracted Gloriosa superba seed from Sukoharjo and Gunung Kidul. In IOP Conference Series: Earth and Environmental Science (Vol. 142, No. 1, p. 012053). IOP Publishing.

[32] Ali, A., Qadir, A., Khan, M. A., Al-Otaibi, F. O., Khan, S., & Abdin, M. Z. (2017). A Simple Validated HPTLC Method for Simultaneous Analysis of Colchicine and Gallic Acid in Polyherbal Formulation: Habb-E-Irqun-Nisha. Food Analytical Methods, 10(9), 2934-2942.

[33] Mohamed, A. M. I., Omar, M. A., Derayea, S. M., Hammad, M. A., & Mohamed, A. A. (2018) Validated thin-layer chromatographic method for alternative and simultaneous determination of two anti-gout agents in their fixed dose combinations. Open Chemistry, 16(1), 496-510.**DOI:** https://doi.org/10.1515/chem-2018-0050

[34] Yongming, L., & Guizhi, L. (2000). Determination of colchicine in Tablet by Fluorimetry. Chinese Journal of Analytical Chemistry, 28(3), 330-332.

[35] Bourdon, R., & Galliot, M. (1976). Determination of colchicine in biological fluids. Annales de biologie Clinique, 34(6), 393-401.

[36] Arslan, M., & Duymus, H. (2007). Spectroscopic studies of charge transfer complexes between colchicine and some π acceptors. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 67(3-4), 573-577.

[37] Hosny, N. M., Atia, N. N., & El-Gizawy, S. M. (2019). A review on: Analysis of certain drugs used in gout treatment. Microchemical Journal, 149, 1-10. https://doi.org/10.1016/j.microc.2019.05.055

[38] https://www.acs.org/content/acs/en/greenchemistry/principles/12-principles-of-green-chemistry.html

[39] ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology, Q2 (R1) Geneva, 2005, http://www.ich.org.

[40] Chu, L., Wang, J., Dong, J., Liu, H., & Sun, X. (2012). Treatment of coking wastewater by an advanced Fenton oxidation process using iron powder and hydrogen peroxide. Chemosphere, 86(4), 409-414.

[41] Pera-Titus, M., García-Molina, V., Baños, M. A., Giménez, J., & Esplugas, S. (2004). Degradation of chlorophenols by means of advanced oxidation processes: a general review. Applied Catalysis B: Environmental, 47(4), 219-256.

[42] Tang, W. Z., & Huang, C. P. (1996). Effect of chlorine content of chlorinated phenols on their oxidation kinetics by Fenton's reagent. Chemosphere, 33(8), 1621-1635.

[43] Huang, Y., Yang, Y., Wang, X., Yuan, X., Pi, N., Yuan, H. & Ni, C. (2018). Heterogeneous Fenton-like degradation of methoxychlor in water using two different FeS@ hydrotalcites (LHDs) and Fe3O4@ LHDs catalysts prepared via an in situ growth method. *Chemical Engineering Journal*, *342*, 142-154.

Figure captions

Figure 1. Chemical structures of the investigated drug and its derivative.

Figure 2. Total ion current (TIC**)** chromatograms and mass spectra of CLN **(A & A')** and its degradation product **(B & B')**.

Figure 3. Three dimensional diagram of LC/MS Chromatograms of CLN in seven concentration levels (0.10 – 50.0 ng mL^{-1}); three replicates for each concentration.

Figure 4. HPLC - Chromatograms of **(A)** standard solution of CLN (50.0 μg mL^{-1}), **(B)** CLN with its degradation product (after 5 min of the reaction time), **(C)** 1.0 mg/mL $FeSO_4.7H_2O$ solution, **(D)** PAN catalyst and **(E)** PAN catalyst with H_2O_2; all solutions were prepared at pH 3.

Figure 5. Effects of **(A)** PAN catalyst amount, **(B)** the volume of 30% H_2O_2 and **(C)** pH on the degradation of CLN (50.0 μg mL^{-1}) (Samples were measured after 15 min of the reaction time).

Figure 6. Influence of the reaction time (0-30 min) on CLN (50.0 μg mL^{-1}) degradation.

Figure 7. Structure elucidation of CLN **(A, B & C)** and 3- demethyl CLN **(A', B' & C')** using UV, FTIR and ^{13}C-NMR; respectively.

Figure 8. The stability of 50.0 μg mL^{-1} CLN in the presence of 100 μL of 30% H_2O_2 and/or acidic aqueous medium (pH 3.0); with/without PAN catalyst.

Table 1. Optimum chromatographic conditions and quantitative parameters of the proposed LC/MS method for determination of CLN.

Studied drug	**CLN**
Mobile phase	Methanol: Water (55: 45, v/v)
Retention time; t_R (min)	5.578
Linearity range (ng mL^{-1})	0.1 – 50
Correlation coefficient (r)	0.9999
Determination coefficient (R^2)	0.9997
Intercept (a) ± SD[a]	$1.50\times10^5 \pm 728.97$
Slope (b) ± SD[a]	$1.46\times10^5 \pm 89.27$
LOD[b]	16.4
LOQ[c]	49.8

[a]Average of six replicates.

[b]Limit of detection (pg mL^{-1}).

[c]Limit of quantitation (pg mL^{-1}).

Table 2. Accuracy, Intra-day and Inter-day precision of the developed LC/MS method for determination of CLN.

Authentic drug	Concentration (ng mL^{-1})	Accuracy		Intra-day precision		Inter-day precision	
		% Recovery ± SD*	RSD %	% Recovery ± SD**	RSD %	% Recovery ± SD***	RSD %
CLN	**0.10**	100.15 ± 0.84	0.84	100.34 ± 0.62	0.62	100.36 ± 0.68	0.68
	20.0	100.18 ± 0.67	0.67	99.90 ± 0.34	0.34	100.29 ± 0.40	0.40
	50.0	100.12 ± 0.89	0.89	100.24 ± 0.12	0.12	99.67 ± 0.22	0.22

* Average of six replicates.

**Average of three replicates.

***Average of nine replicates.

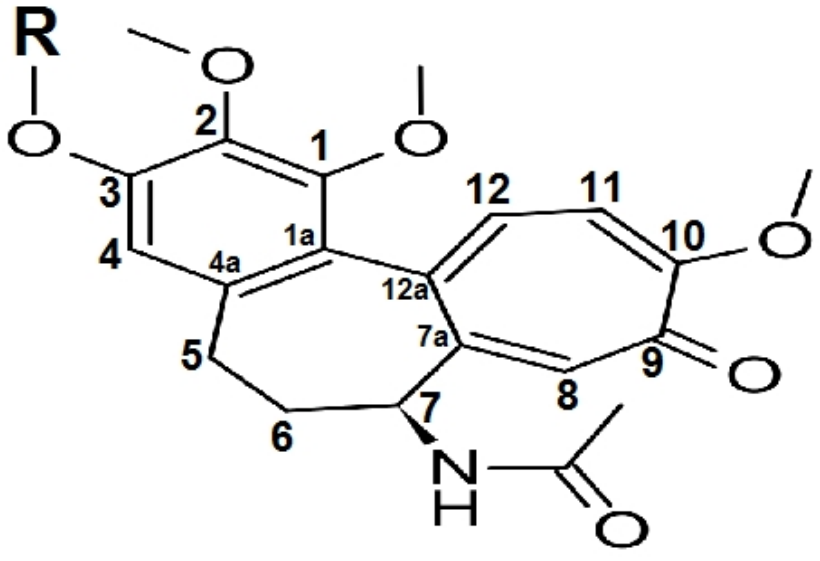
- Results are calculated from standard curve.

Table 3. Robustness of the developed LC/MS method for determination of CLN.

Parameter		% Recovery ± SD*	RSD %
No variation**		100.25 ± 0.77	0.77
Methanol: Water ;	**58:42, v/v**	100.50 ± 0.71	0.70
52:48, v/v		99.45 ± 0.96	0.96
Flow rate;	**+0.1 mL/min**	101.21 ± 1.18	1.17
-0.1 mL/min		100.56 ± 0.49	0.48
Column temperature;	**27°C**	100.51 ± 0.51	0.51
	23°C	99.68 ± 0.42	0.42

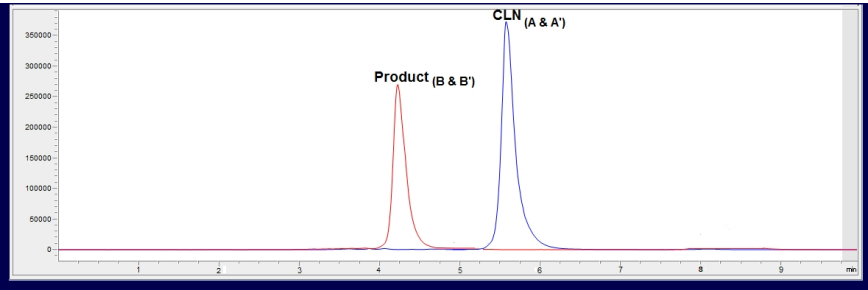
* Average of three replicates.

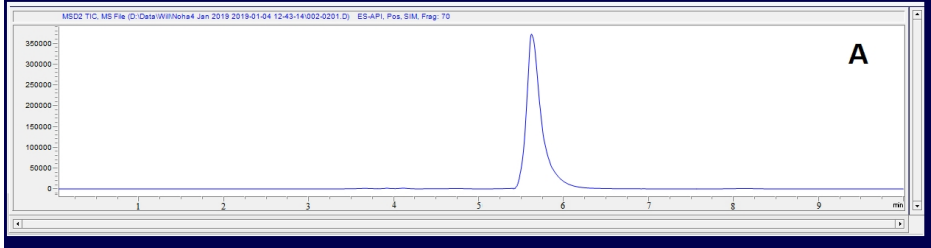
** No variation in the chromatographic conditions of the proposed method. The optimized conditions were [GraceSmart RP C18 column using methanol: water (55: 45, v/v) at 1.0 mL/min, Quadrupole MS detector in a positive ionization mode at total ion current (TIC, *m/z* 50-500), or selected ion monitoring (SIM, *m/z* 400.4)]. (Drug conc. = 20.0 ng mL^{-1}).

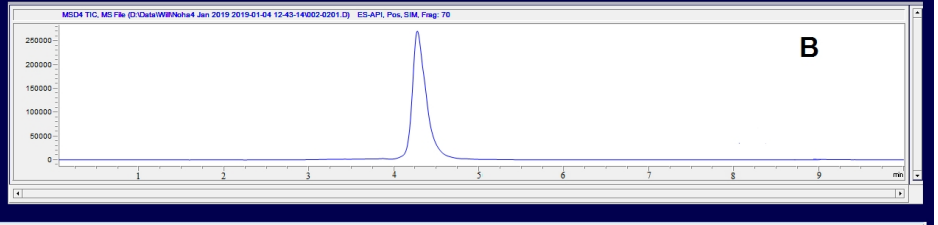


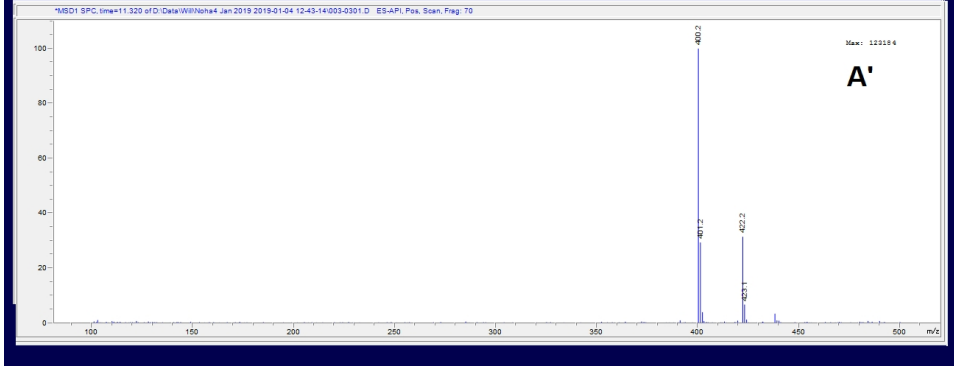
R= -CH_3; Colchicine (CLN); M. Wt.= 399.44

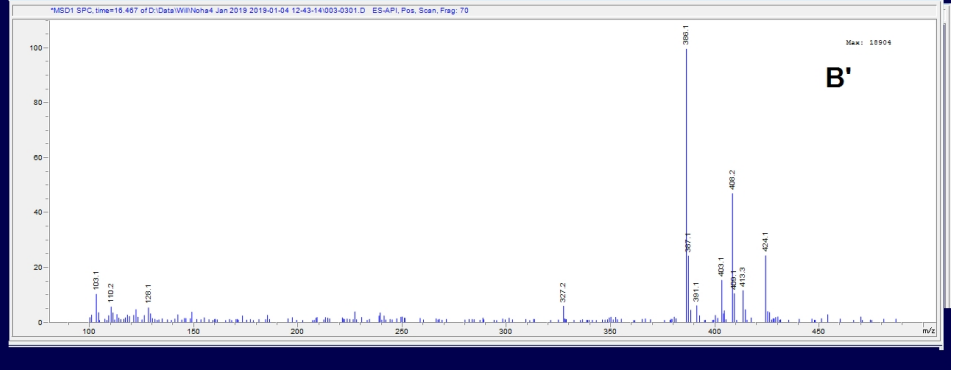
R= -H; 3-demethyl Colchicine (3-demethyl CLN); M. Wt.= 385.42

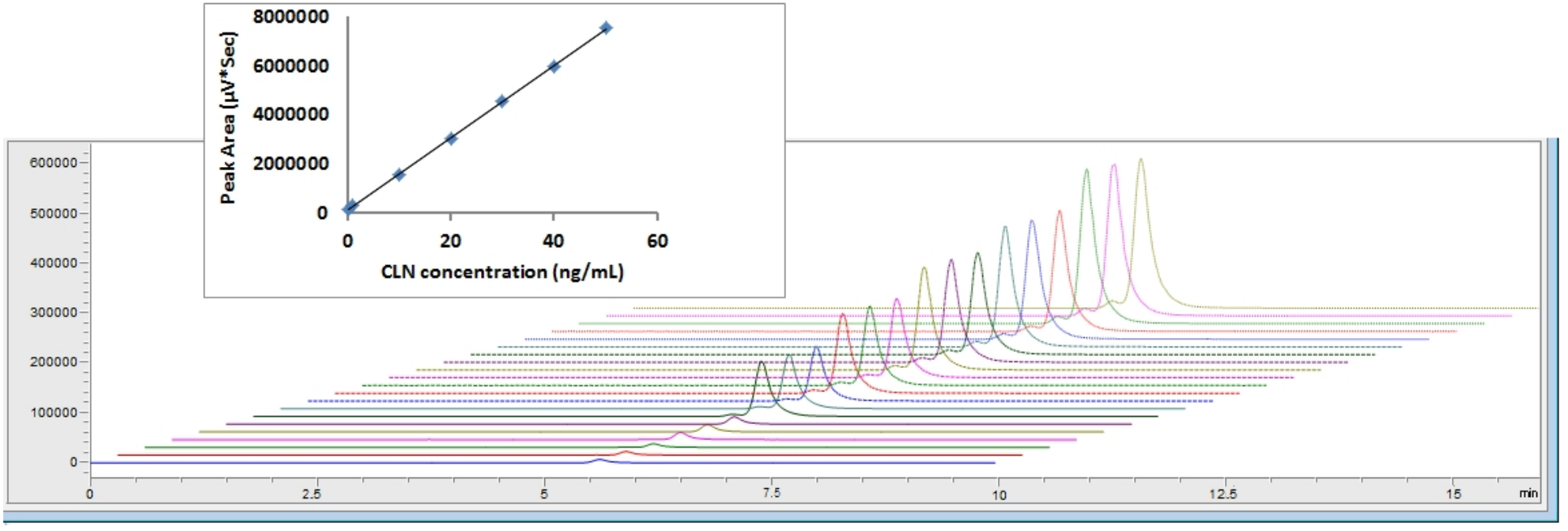

CLN (A & A')
Product (B & B')

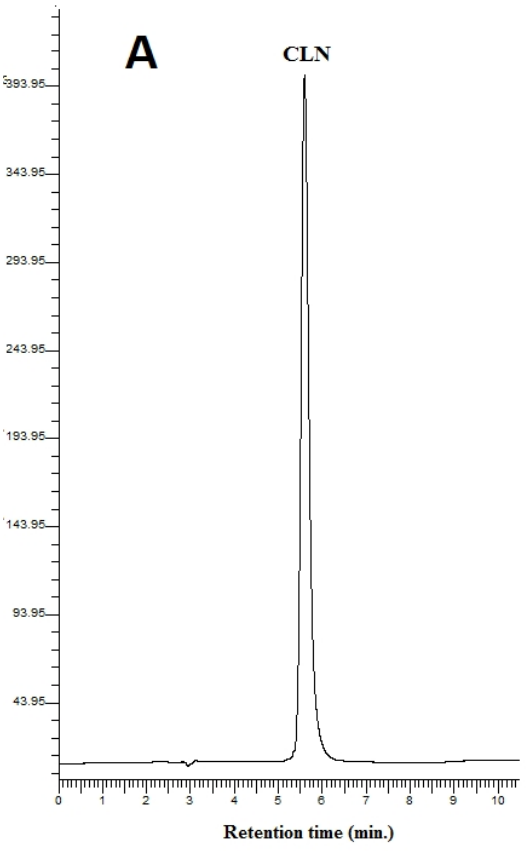

A

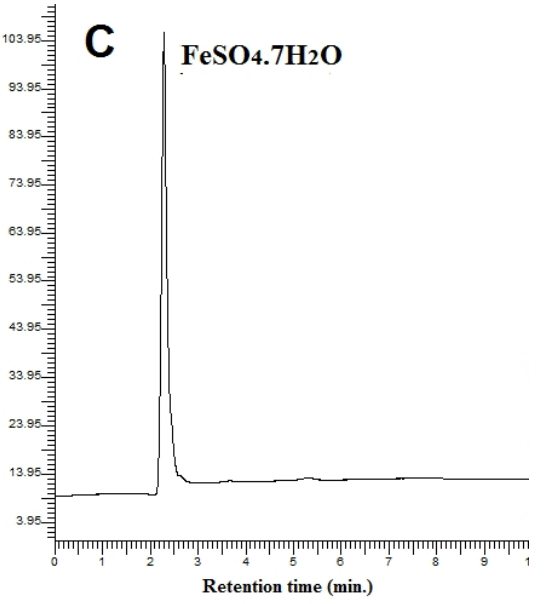

B

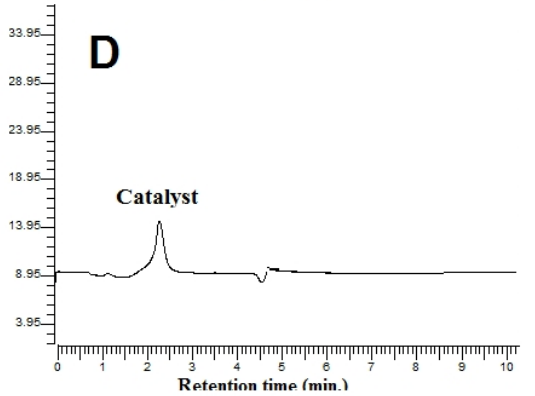

A'

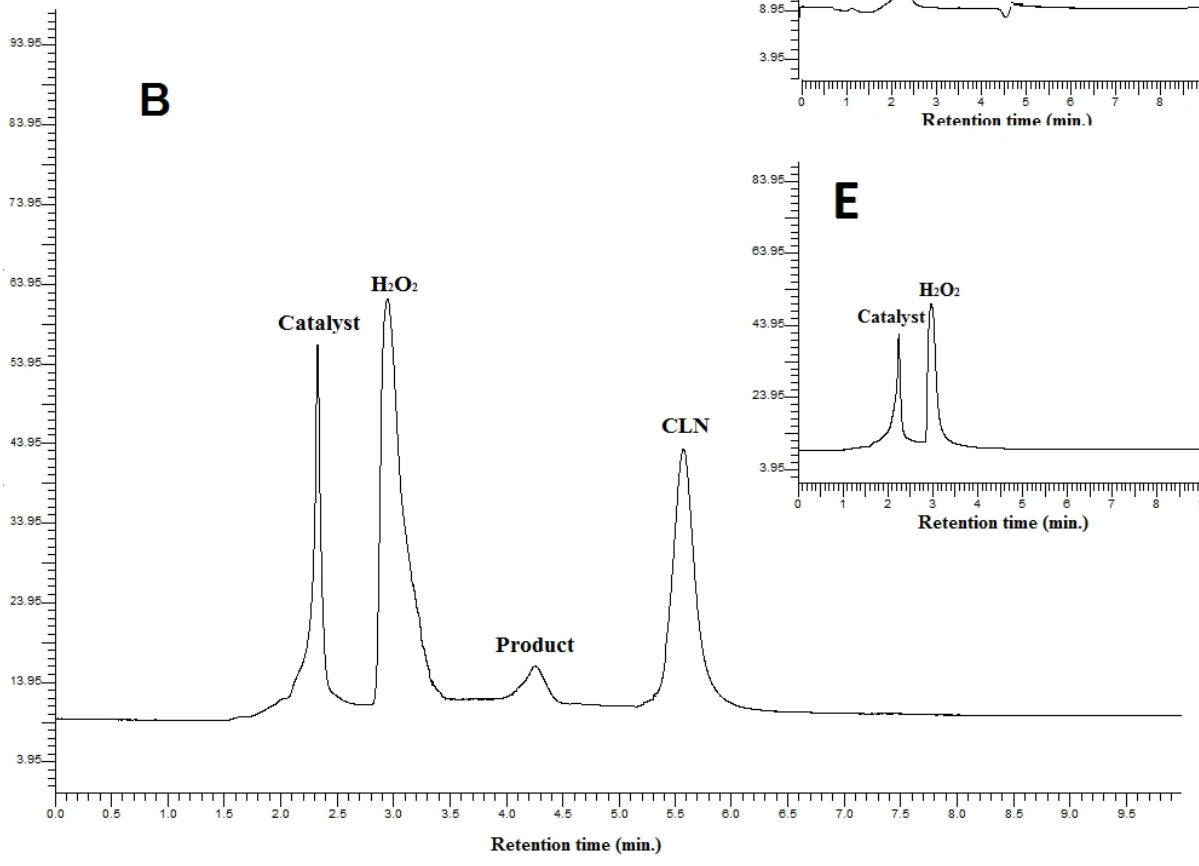

B'


Peak Area (µV*Sec)
8000000
6000000
4000000
2000000
0
0
20
40
60
CLN concentration (ng/mL)
600000
500000
400000
300000
200000
100000
0
0
2.5
5
7.5
10
12.5
15
min

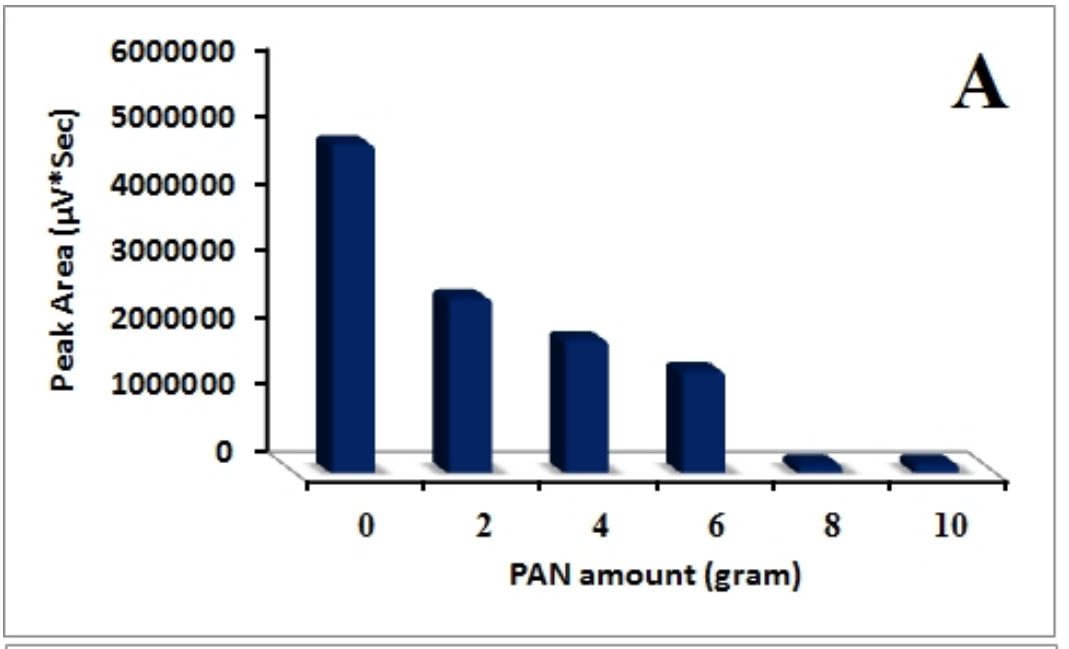

A
CLN
393.95
343.95
293.95
243.95
193.95
143.95
93.95
43.95
0 1 2 3 4 5 6 7 8 9 10
Retention time (min.)

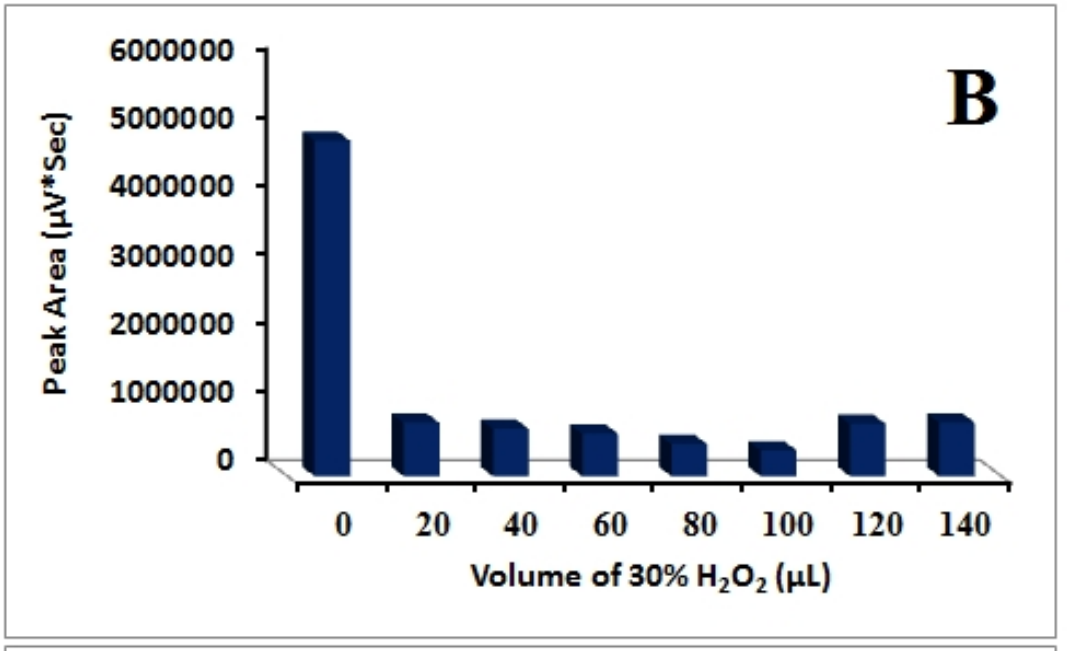

C
FeSO4.7H2O
103.95
93.95
83.95
73.95
63.95
53.95
43.95
33.95
23.95
13.95
3.95
0 1 2 3 4 5 6 7 8 9 1
Retention time (min.)

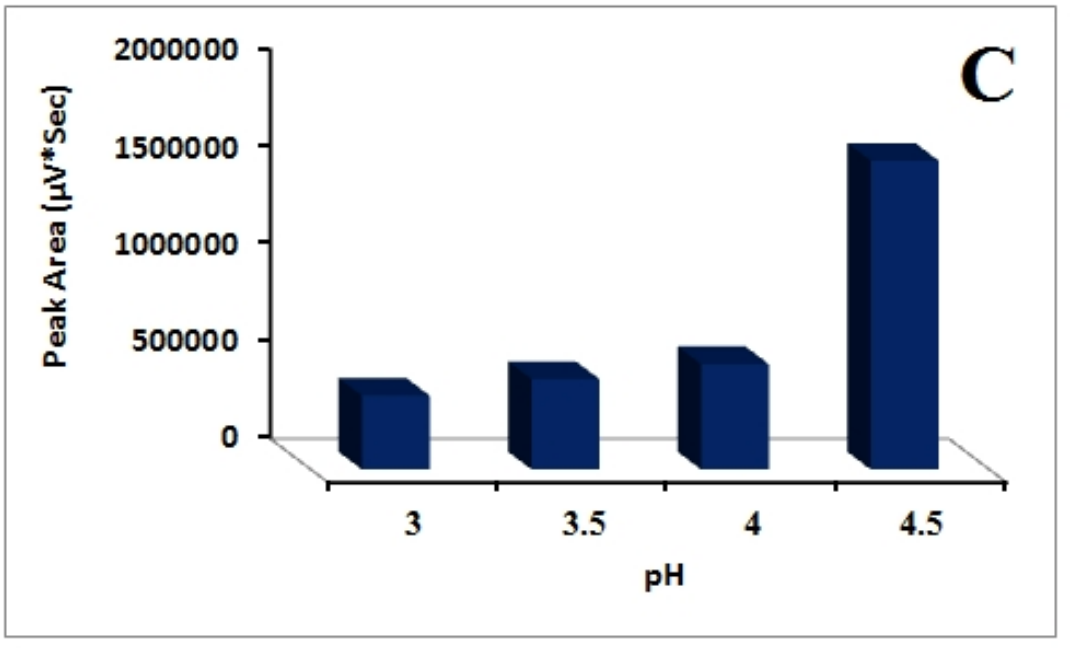

D
33.95
28.95
23.95
18.95
13.95
8.95
3.95
Catalyst
0 1 2 3 4 5 6 7 8 9 10
Retention time (min.)

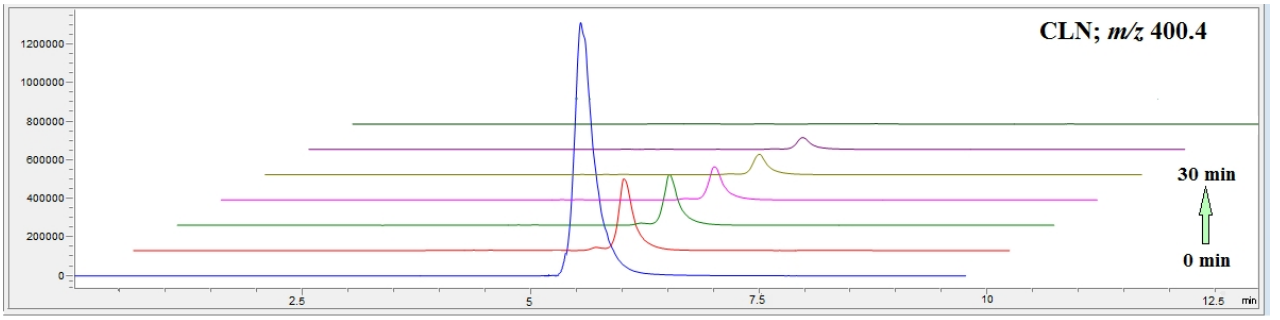

B
93.95
83.95
73.95
63.95
53.95
43.95
33.95
23.95
13.95
3.95
Catalyst
H2O2
Product
CLN
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
Retention time (min.)

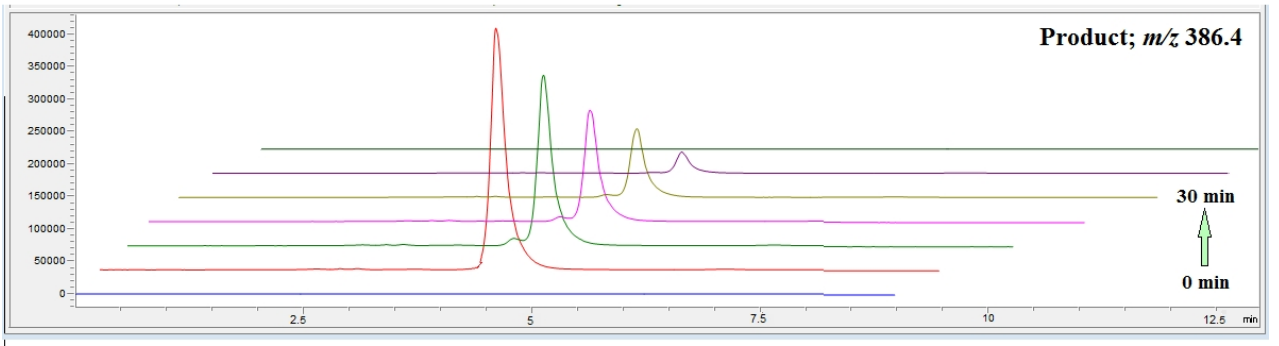
E
83.95
63.95
43.95
23.95
3.95
H2O2
Catalyst
0 1 2 3 4 5 6 7 8 9 10
Retention time (min.)

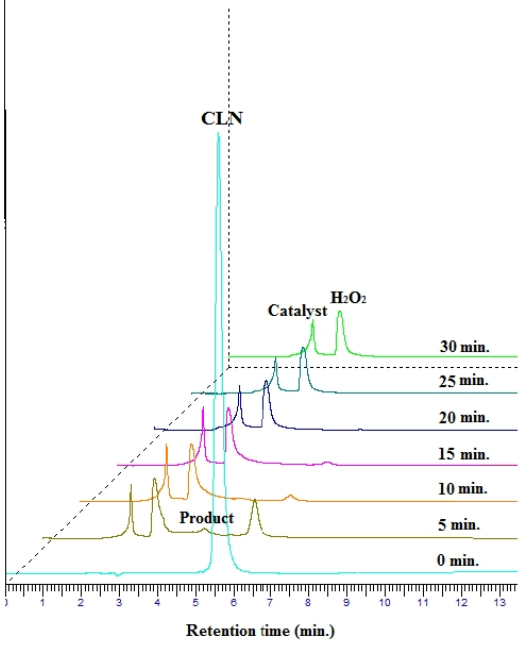

A
Peak Area (µV*Sec)
6000000
5000000
4000000
3000000
2000000
1000000
0
0
2
4
6
8
10
PAN amount (gram)

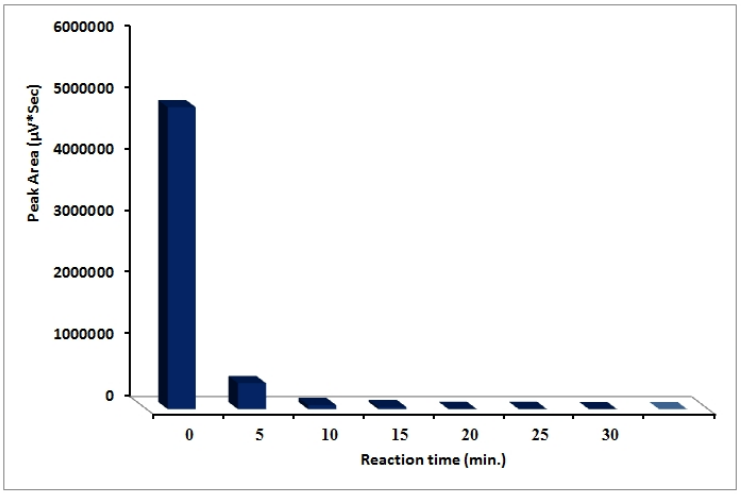

B
Peak Area (µV*Sec)
6000000
5000000
4000000
3000000
2000000
1000000
0
0
20
40
60
80
100
120
140
Volume of 30% H2O2 (µL)


C
Peak Area (µV*Sec)
2000000
1500000
1000000
500000
0
3
3.5
4
4.5
pH

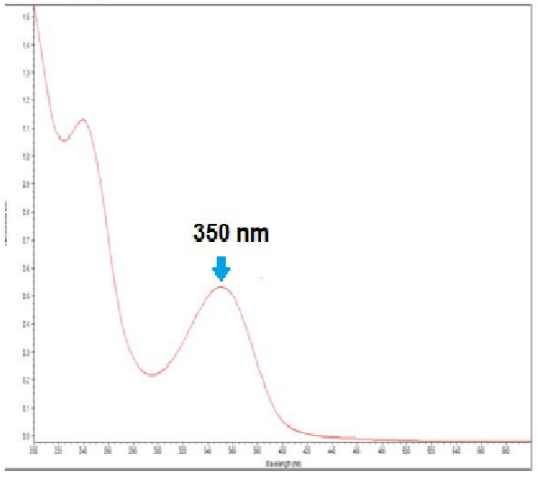

CLN; m/z 400.4
30 min
0 min
min


Product; m/z 386.4
30 min
0 min
min

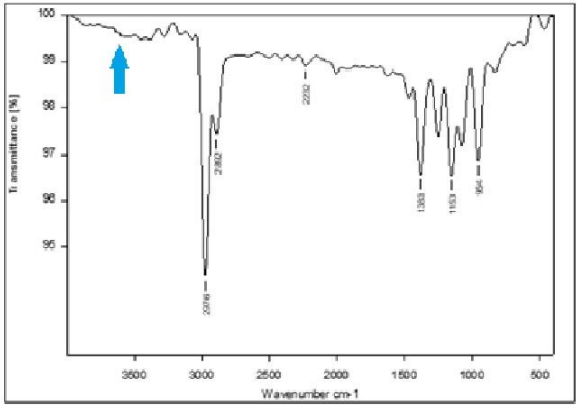

CLN
Catalyst
H2O2
30 min.
25 min.
20 min.
15 min.
10 min.
Product
5 min.
0 min.
Retention time (min.)


Peak Area (µV*Sec)
Reaction time (min.)

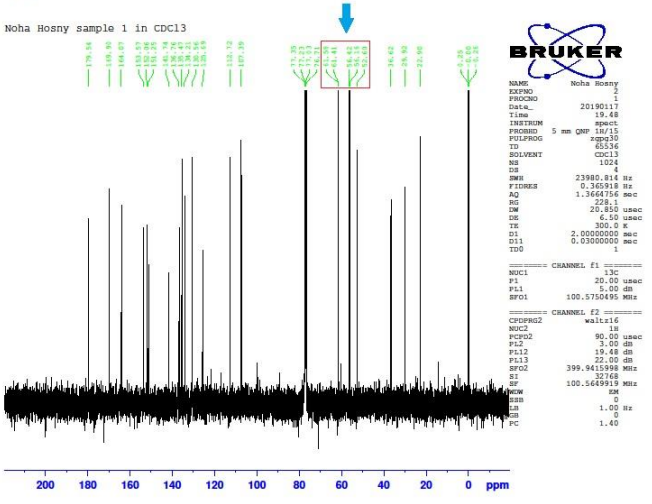
A


350 nm

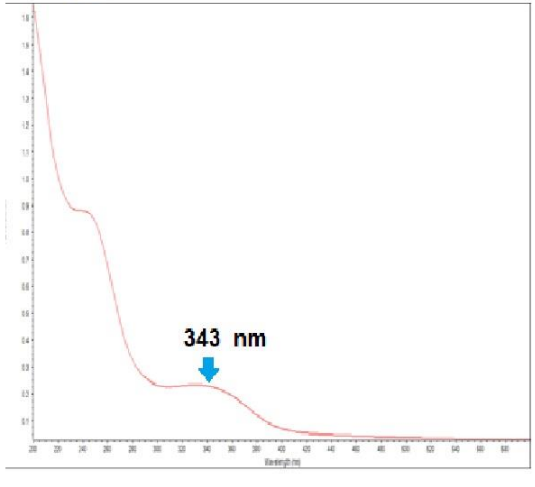
B


Transmittance [%]
Wavenumber cm-1
3500
3000
2500
2000
1500
1000
500

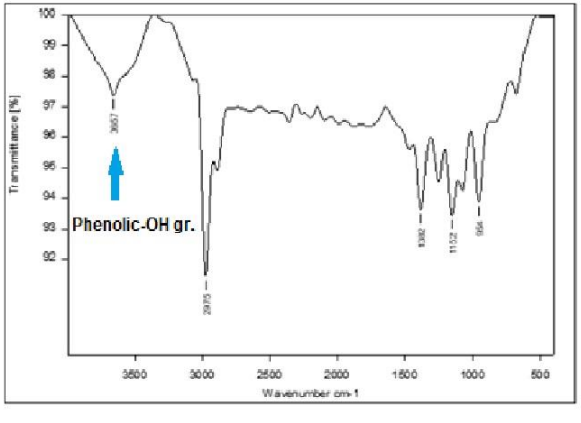
C


Noha Hosny sample 1 in CDCl3
BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

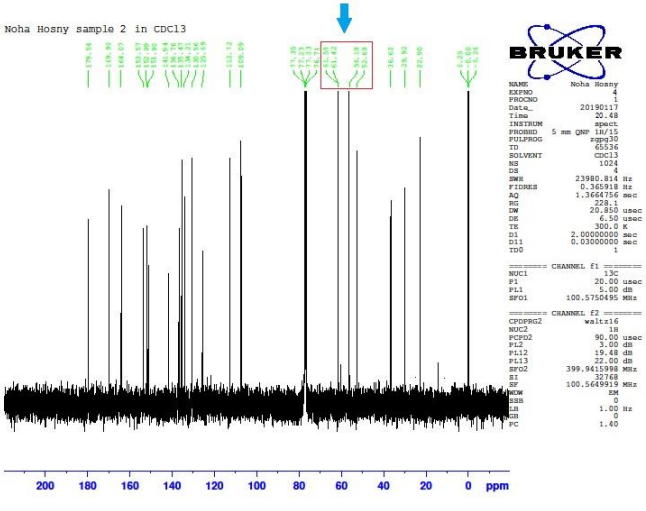
A'

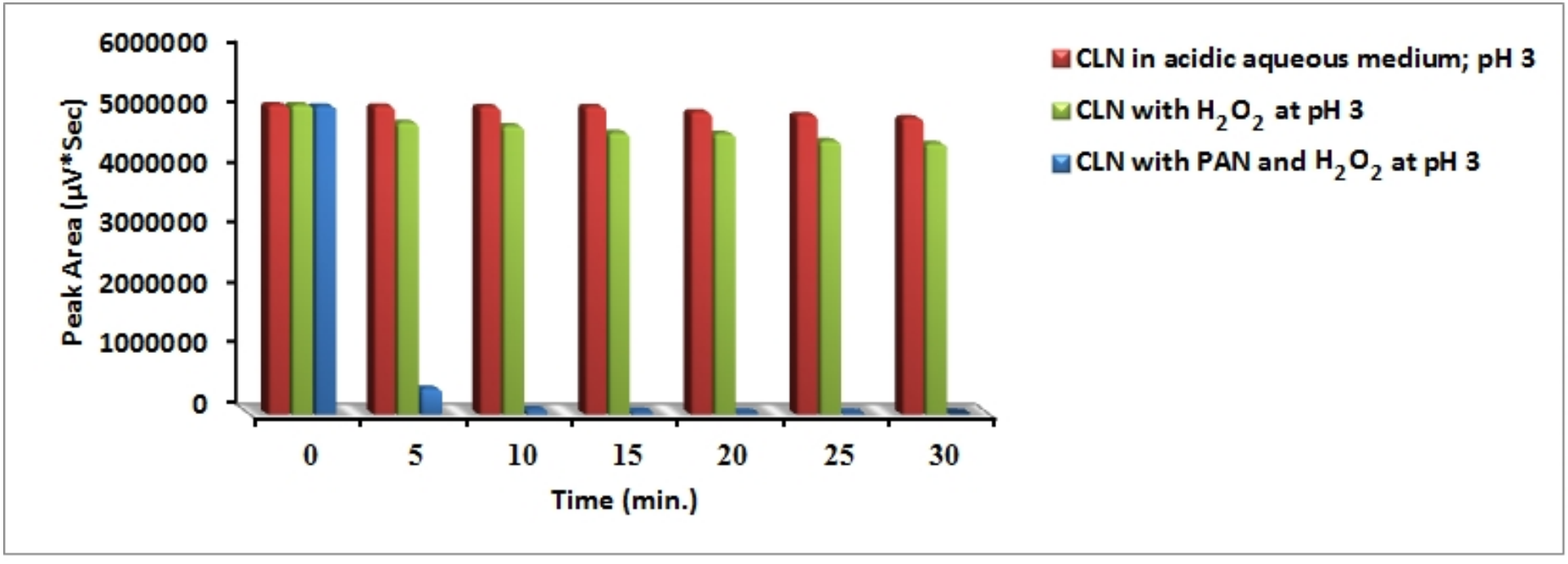

343 nm

B'

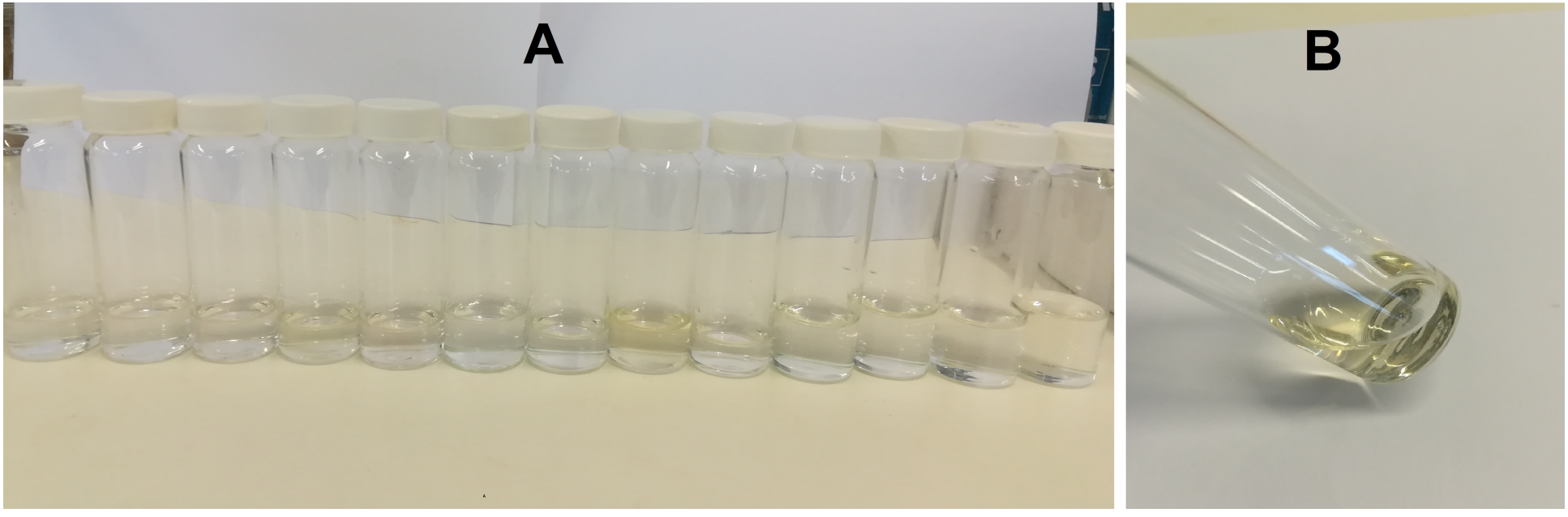

Phenolic-OH gr.
Transmittance [%]
Wavenumber cm-1
3500
3000
2500
2000
1500
1000
500

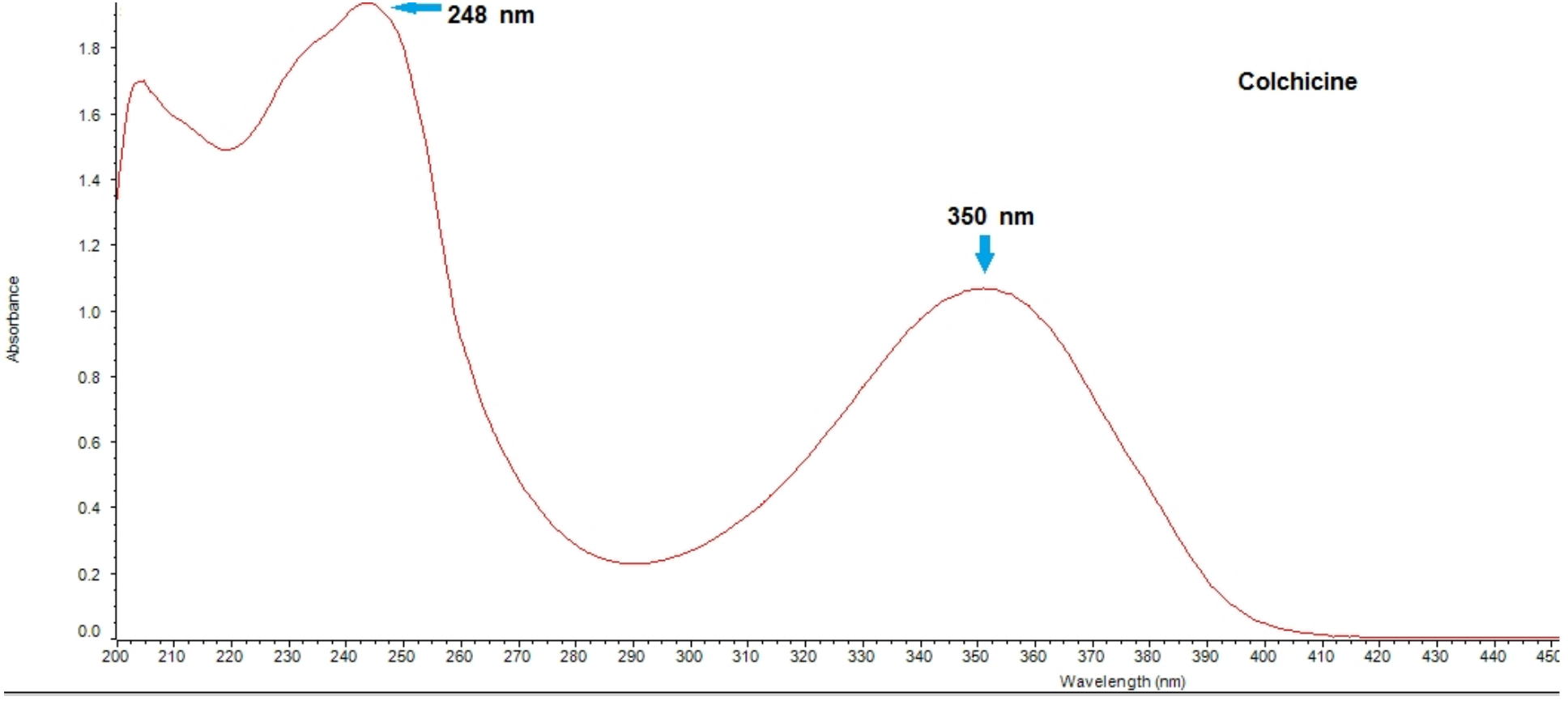
C'

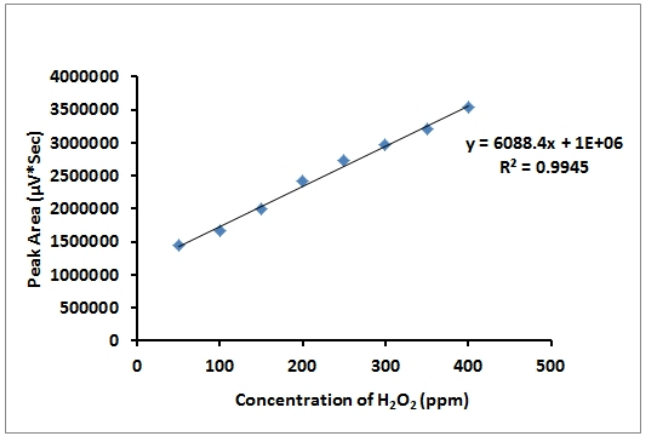

Noha Hosny sample 2 in CDCl3
BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

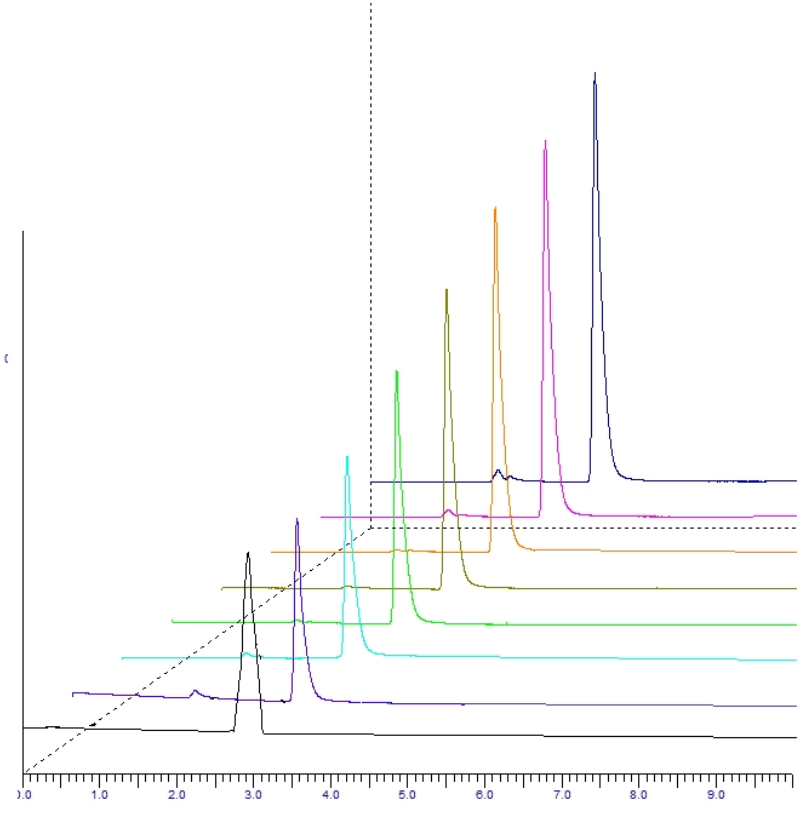

6000000
5000000
4000000
3000000
2000000
1000000
0
Peak Area (µV*Sec)
0
5
10
15
20
25
30
Time (min.)
CLN in acidic aqueous medium; pH 3
CLN with H_2O_2 at pH 3
CLN with PAN and H_2O_2 at pH 3

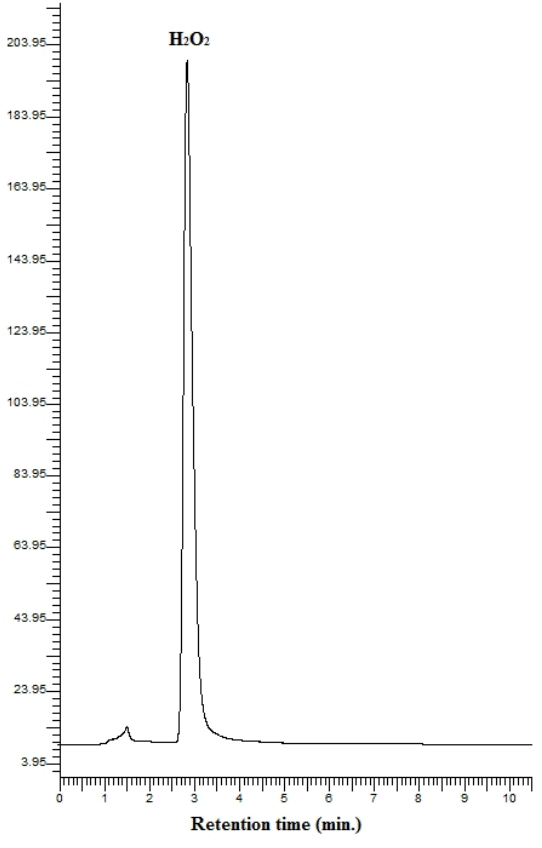

radleys TECH
rpm
°C
500
25


A
B


248 nm
350 nm
Colchicine
Absorbance
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
200
210
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440


4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Peak Area (µV*Sec)
y = 6088.4x + 1E+06
R2 = 0.9945
0
100
200
300
400
500
Concentration of H2O2 (ppm)


1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0


H2O2
203.95
183.95
163.95
143.95
123.95
103.95
83.95
63.95
43.95
23.95
3.95
0
1
2
3
4
5
6
7
8
9
10
Retention time (min.)

Conflict of interest

-Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

-Declarations of interest: All the authors of the paper do not have a direct financial relation with the commercial identity mentioned in the paper and we declare that there is no conflict of interests in our submitted paper.

Heterogeneous Fenton's-Like Catalysis for Degradation of Colchicine Coupled with Extraction of Its Biologically Active Metabolite

Noha M. Hosny [a, b*], Katherine Huddersman[b], Noha N. Atia[a], Samia M. El-Gizawy[a]

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

[b] School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, Leicester, UK.

***Corresponding author: Noha M. Hosny**

E-mail: phar_nmh2011@yahoo.com, noha.hosni@pharm.au.edu.eg, Tel: +2-01144438525

Table S1. Monitoring of the degradation of colchicine by the developed LC/MS method.

Reaction time (min)	**CLN Concentration* (ng mL^{-1})**	**± Standard Deviation (± SD)**
0	50000	1.53
5	58.27	0.95
10	40.39	0.77
15	26.10	1.44
20	15.62	1.36
25	8.520	0.49
30	0.001	1.72×10^{-4}

*Average of three replicates.

Table S2. ^{13}C-NMR spectral data of CLN and 3- demethyl CLN.

Carbon	CLN	3- demethyl CLN
1	153.57	153.57
2	141.74	**141.94**
3	151.25	**151.90**
4	107.39	**109.09**
5	29.92	29.92
6	36.62	36.62
7	52.60	52.60
8	130.56	130.56
9	179.54	179.54
10	164.07	164.07
11	112.72	112.72
12	135.47	135.47
13	169.90	169.90
14	22.90	22.90
1a	125.69	125.69
4a	134.21	134.21
7a	152.06	**152.09**
12a	136.76	136.76
1-OCH_3	61.41	**61.42**
2-OCH_3	61.58	61.58
3-OCH_3	56.42	**------**
10-OCH_3	56.16	**56.10**

- Chemical shift values are expressed in ppm.

Figure captions

Figure S1. Degradation of CLN in a Carousel 6 Plus Reaction Station™ set.

Figure S2. Fractions eluted using the fractional column chromatography **(A)** and the collected product from CLN degradation **(B)**.

Figure S3. Absorption spectrum of standard solution of CLN (20.0 μg/mL prepared in double distilled water).

Figure S4. Calibration of H_2O_2 by HPLC/UV detection at 248 nm using different concentration of H_2O_2 (50- 400 ppm) prepared in acidic water (pH 3.0).