Electronic Supplementary Material

Advancing Continuous Flow Techniques in Effective Trimethoprim Oxidation: Combatting Bacterial Resistance in Wastewater

Diana L. Marques,[a] Giusi Piccirillo,[a,b] Fábio M. S. Rodrigues,[a] Rafael T. Aroso,[a] Lucas D. Dias,[a,c] Gabriela Silva,[d] Mário J. F. Calvete *[a] and Mariette M. Pereira *[a]

[a] CQC-IMS, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535, Coimbra, Portugal

[b] Bio4Plas - Biopolímeros, Lda. Zona Industrial Lote 61, 3064-197 Cantanhede, Portugal

[c] Laboratório de Novos Materiais, Universidade Evangélica de Goiás (UniEvangélica), Anápolis, Brazil

[d] Faculty of Pharmacy and Center for Neurosciences and Cell Biology, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

Table of contents

1. Report on the presence of pharmaceuticals in effluents from IPO clinic (IPO = Portuguese Institute of Oncology) in Coimbra, Portugal

ALS LIFE SCIENCES PORTUGAL, S.A.

Data Emissão: 17-03-2022

N.º de Análise:	QH / 4199 / 22
Data Colheita:	-
Data Receção:	25-02-2022
Data Início Ensaio:	26-02-2022
Data Fim Ensaio:	17-03-2022
Código Cliente:	4417

Exmo(s) Sr(s):
Universidade de Coimbra
Rua Larga, R/Ch Esq. do Edifício FMUC
Coimbra
3004-504 Coimbra

Unidade: Universidade de Coimbra

Identificação da Amostra: **64185 / 22**

Produto: *Efluente*
Acondicionamento: frasco
A colheita de amostra não foi efectuada pelo laboratório.

Laboratório Externo

Ensaio/Método	Resultado	U	Unidade
(a) Compostos farmacêuticos por LCMS - DI *CZ_SOP_D06_03_201.A (US EPA 1694)*			
Sulfametoxazol	<0.010 (LQ)		µg/L
Buprenorfina	<0.010 (LQ)		µg/L
Iopromide	707	30.0%	µg/L
Iomeprol	<0.030 (LQ)		µg/L
Hidroclorotiazida	0.074	30.0%	µg/L
Enalapril	<0.010 (LQ)		µg/L
Ciclobenzaprina	2.20	30.0%	µg/L
Capecitabina	<0.010 (LQ)		µg/L
Butorfanol	<0.010 (LQ)		µg/L
Azatioprina	<0.010 (LQ)		µg/L
Anastrozol	0.542	30.0%	µg/L
Zolpidem	<0.010 (LQ)		µg/L
Metronidazol	<0.010 (LQ)		µg/L
Tramadol	1.44	30.0%	µg/L
Oxazepam	0.202	30.0%	µg/L
Salbutamol	<0.010 (LQ)		µg/L
Piroxicam	<0.010 (LQ)		µg/L
Micofenolato de mofetil	<0.010 (LQ)		µg/L
Metoprolol	<0.010 (LQ)		µg/L
Cetoprofeno	<0.010 (LQ)		µg/L
Iopamidol	<0.030 (LQ)		µg/L
Iohexol	<0.030 (LQ)		µg/L
Furosemida	21.7	40.0%	µg/L
Diclofenaco	<0.010 (LQ)		µg/L
Carbamazepina	0.280	35.0%	µg/L
Cafeína	111	40.0%	µg/L
Valsartan	0.142	30.0%	µg/L
Naproxeno	<0.010 (LQ)		µg/L
Thebain	<0.010 (LQ)		µg/L
Paracetamol (acetaminofeno)	4.81	30.0%	µg/L
Flutamida	<0.010 (LQ)		µg/L

Tondela
Zona Industrial Tondela
ZIM II Lote 2 e 6
3460-070 Tondela
Telef.: 232 817 817

Lisboa
Rua das Azenhas, nº 34 B
2730 - 270 Barcarena

Castelo Branco
Zona Industrial
Rua S, Lote 41
6000-459 Castelo Branco
Telef.: 272 347 326

Madeira
Caminho da Igreja nº 35
9125-259 Caniço
Telef.: 291 934 931

Eugénia Silva

Técnica Superior de Laboratório
Eugénia Silva

Mod 201.24 Documento Processado por Computador

Data Emissão: 17-03-2022

N.º de Análise: QH / 4199 / 22
Data Colheita: -
Data Receção: 25-02-2022
Data Início Ensaio: 26-02-2022
Data Fim Ensaio: 17-03-2022
Código Cliente: 4417

Exmo(s) Sr(s):
Universidade de Coimbra
Rua Larga, R/Ch Esq. do Edifício FMUC
Coimbra
3004-504 Coimbra

Unidade: Universidade de Coimbra

Identificação da Amostra: **64185 / 22**

Produto: *Efluente*
Acondicionamento: frasco
A colheita de amostra não foi efectuada pelo laboratório.

Ensaio/Método	Resultado	U	Unidade
Ciprofloxacina	<0.030 (LQ)		µg/L
Ácido clofíbrico	<0.010 (LQ)		µg/L
Citalopram	0.397	30.0%	µg/L
Sertraline	0.046	30.0%	µg/L
Indomethacin	0.012	30.0%	µg/L
Paclitaxel	0.364	35.0%	µg/L
Fluoxetine	<0.010 (LQ)		µg/L
Diazepam	<0.010 (LQ)		µg/L
Loperamida	0.012	35.0%	µg/L
Ifosfamida	<0.010 (LQ)		µg/L
Gabapentin	2.80	30.0%	µg/L
Chloramphenicol	<0.010 (LQ)		µg/L
Lincomycin	<0.010 (LQ)		µg/L
Gemfibrozil	<0.020 (LQ)		µg/L
Sulfamethazine	<0.010 (LQ)		µg/L
Ciclofosfamida	<0.010 (LQ)		µg/L
Atenolol	<0.010 (LQ)		µg/L
Varfarina	<0.010 (LQ)		µg/L
Trimetoprim	0.115	30.0%	µg/L
Terbutalina	<0.010 (LQ)		µg/L
Sotalol	<0.010 (LQ)		µg/L
Propranolol	<0.010 (LQ)		µg/L
Bezafibrate	<0.010 (LQ)		µg/L

Eugénia Silva

Técnica Superior de Laboratório
Eugénia Silva

Data Emissão: 17-03-2022

N.º de Análise:	QH / 4199 / 22
Data Colheita:	-
Data Receção:	25-02-2022
Data Início Ensaio:	26-02-2022
Data Fim Ensaio:	17-03-2022
Código Cliente:	4417

Exmo(s) Sr(s):
Universidade de Coimbra
Rua Larga, R/Ch Esq. do Edifício FMUC
Coimbra
3004-504 Coimbra

Unidade: Universidade de Coimbra

Identificação da Amostra: **64185 / 22**

Produto: *Efluente*
Acondicionamento: frasco
A colheita de amostra não foi efectuada pelo laboratório.

Notas

Lista de abreviaturas: NE- Número estimado; UFC- Unidades formadoras de colónias; LQ – Limite de quantificação; LD – limite de detecção; V.L. – Valor Limite; V.R. – Valor Recomendado; VP - Valor Paramétrico; C - Conforme; A - Aceitável; NC - Não Conforme; Unid. - Unidade; DO - Densidade óptica; Av. C. - Avaliação de Conformidade; NMP- Número Mais Provável; PFC - Propósito de Formação de Colónias; U – Incerteza expandida.

O ensaio assinalado com (s) foi contratado e não é acreditado.
O ensaio assinalado com (a) foi contratado e é acreditado.
Nos resultados assinalados com (k) os microrganismos estão presentes, mas inferiores a 3xdiluição (quando aplicável).
Nos resultados assinalados com (y) os microrganismos estão presentes, mas inferiores a 4xdiluição.
Para os ensaios assinalados por técnicas de cálculo a metodologia seguida pode ser disponibilizada a pedido.

Os dados a *sublinhado e itálico* foram fornecidos pelo cliente e não são da responsabilidade do laboratório.
Este Relatório de Ensaio refere-se apenas às amostras analisadas.

Os resultados deste relatório de ensaio aplicam-se à amostra conforme rececionada.
Incerteza "U" - corresponde à incerteza expandida calculada com um fator de expansão k=2, correspondendo a um nível de confiança aproximadamente igual a 95%. As incertezas apresentadas em % encontram-se em valor relativo e as restantes em valor absoluto.
A incerteza do ensaio não inclui incerteza da colheita. A combinação da incerteza da colheita (quando apresentada) e do ensaio pode ser obtida através da fórmula 1 (*Ucombinada* expressa em %) quando a incerteza do ensaio se encontra apresentada em valor relativo ou através da fórmula 2 (*Ucombinada* expressa em %) quando a incerteza do ensaio se encontra apresentada em valor absoluto. Para colheitas efetuadas segundo a ISO 5667-5:2006 e IGL 16 a incerteza combinada (colheita e ensaio) para o ensaio pH é de 0.27 unidades de pH.

Microbiologia de Alimentos: A incerteza reportada foi estimada de acordo com a ISO19036:2019 e considerada igual ao desvio padrão da reprodutibilidade intralaboratorial e apresenta-se sob a forma de intervalo e com o valor de U em % (quando aplicável).
Microbiologia de Águas: A incerteza reportada foi estimada de acordo com a ISO29201:2012 e apresenta-se sob a forma de intervalo e com o valor de U em % (quando aplicável).
Microbiologia de Atividades médicas, Produtos farmacêuticos, Têxteis e acessórios de vestuário, Antissépticos, Desinfetantes, Sabões, Detergentes e Cosméticos: A incerteza reportada foi estimada de acordo com a ISO 19036:2019 e considerada igual ao desvio padrão da reprodutibilidade intralaboratorial e apresenta-se sob a forma de intervalo.

$$U_{combinada\ (\%)} = \sqrt{(U_{colheita\_relativa})^2 + (U_{ensaio\_relativa})^2} \qquad \text{Fórmula 1}$$

$$U_{combinada\ (\%)} = \sqrt{(U_{colheita\_relativa})^2 + \left(\frac{U_{ensaio\_absoluta}}{resultado\_ensaio} \times 100\right)^2} \qquad \text{Fórmula 2}$$

Eugénia Silva

Técnica Superior de Laboratório
Eugénia Silva

Figure Set S1 - Report on the presence of pharmaceuticals in effluents from IPO clinic (IPO = Portuguese Institute of Oncology) in Coimbra, Portugal.

2. Materials and methods

Chemicals were purchased from Fluorochem, Alfa Aesar or Sigma-Aldrich and used as received. Standard procedures were followed for solvent purification. Solution UV-Vis absorption spectra were registered on a Hitachi U-2010. Solid state UV-Vis absorption spectra were recorded in a Cary 5000 UV–vis-NIR spectrometer. ^{1}H NMR spectra were recorded on a 400 MHz Brucker-AMX apparatus with TMS as standard. Scanning electronic microscopy (SEM) analyses were performed using a FESEM Zeiss–GEMINI II equipment, operating at an accelerating voltage of 2 kV, using secondary electrons detection. Samples were covered with a monometric layer of gold by physical vapor deposition. Thermogravimetric (TG) analyses were carried out using a TG-DSC (differential scanning calorimetry) Perkin-Elmer STA6000 in nitrogen flux at a heating rate of 10 °C/min to a maximal temperature of 900 °C. The ICP-OES measurements were carried out at the University of Aveiro, using a Jobin Yvon Activa M apparatus.

The reactions were checked by HPLC, equipped with a Diode Array (model G1315D from Agilent, USA), using a C18 RP packing column (Agilent® Poroshell 4 μm C18 120 Å, 4.6 x 150 mm, RP) and an automatic injector module (model G1329A, 1200 Infinity Series Agilent, USA) with injected volume of 50.0 μL. The DAD acquisition wavelengths were set at 254.4 nm, 270.2 nm, 210.8 nm, 230.3 nm, 280.2 nm, 220.8 nm and 310.2 nm. The mobile phase was constituted by a mixture of acetonitrile and a buffer solution of ammonium acetate, adjusted to pH 9.0 with ammonium hydroxide. The separation was achieved under gradient elution conditions at 1.000 mL min^{-1} flow rate at 30 °C. In gradient elution, the mobile phase composition from 5 to 20% (v/v) acetonitrile–buffer solution of ammonium acetate for 15 minutes followed by isocratic elution of 20% (v/v) acetonitrile and 80% (v/v) buffer solution until 30 minutes.

For the quantification of TMP, it was used a calibration curve that can be expressed as follow: $Y = 1.0\text{x}10^7X + 12.330$ (R^2=0.999), where X and Y represent the peak area and TMP concentration, respectively. TMP degradation rates were calculated using a calibration curve obtained by HPLC, in which the TMP concentration at the initial (C_0) and final time of the reaction (C_f) were determined and whose degradation rate (%) was calculated according to Equation 1.

$$Degradation\ (\%) = \frac{C_0 - C_f}{C_0} \times 100 \qquad \text{(Equation 1)}$$

The continuous flow reactions were performed in an easy-MedChem Vapourtec® E-Series instrument equipped with 3 Vapourtec® V-3 peristaltic pumps. For the studies carried out in homogeneous medium, a tubular reactor with a capacity of 10 mL Vapourtec® was used. In the studies in heterogeneous setup, fixed-bed reactor of 6.6 mm in internal diameter and 150 mm in length Omnifit® containing the catalyst was used.

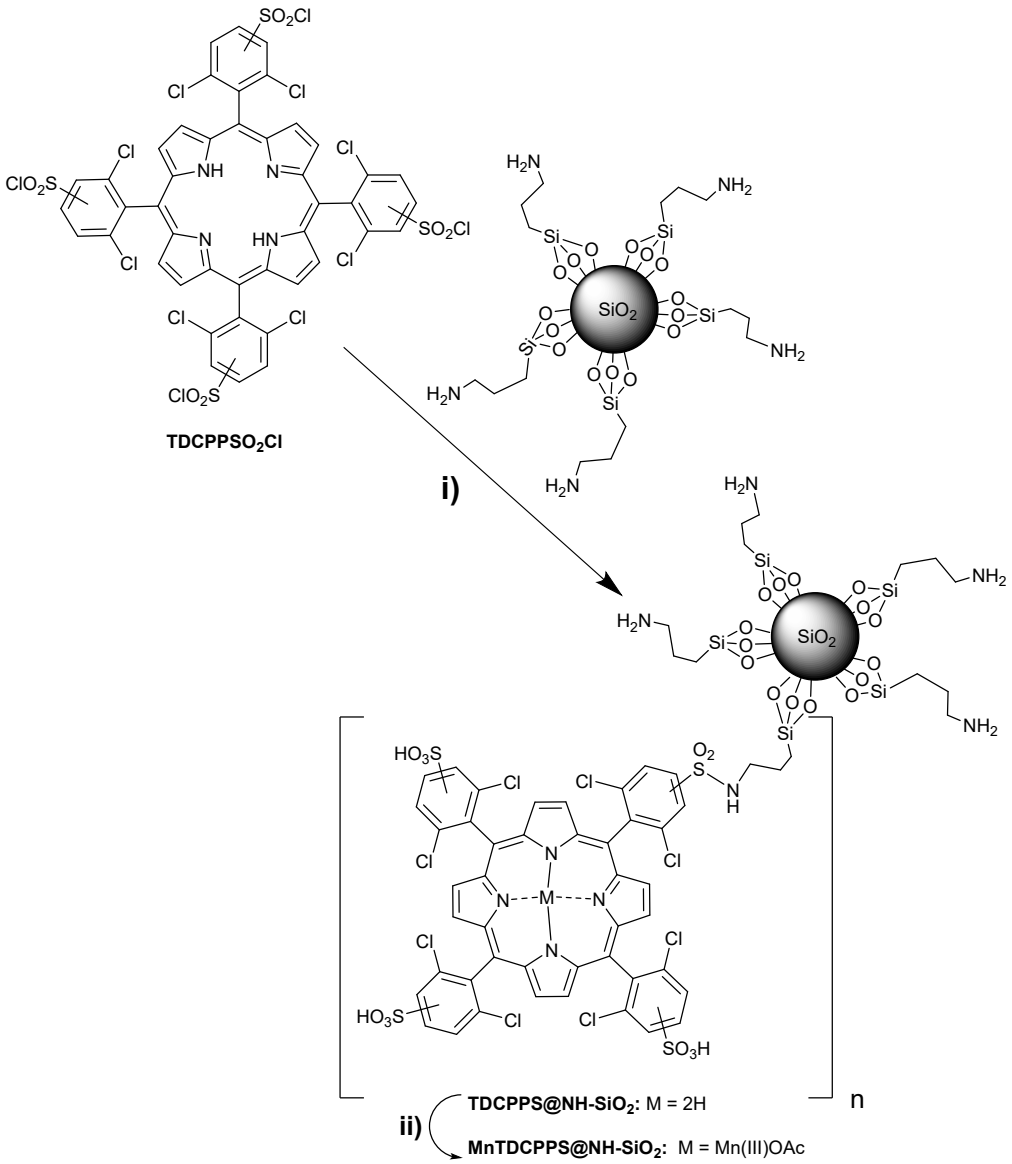
The identification of oxidized products was performed by using an UHPLC-MS system, which was an Ultimate 3000 RSLC UHPLC coupled to an LTQ XL Linear Ion Trap 2D Mass Spectrometer (Thermofisher SCIENTIFIC, San Jose, CA, USA), equipped with an orthogonal electrospray ionization source operating in positive mode. The separation of the compounds was carried out with by using a Hypersil Gold C18 column (100 × 2.1 mm; 1.9 μm) supplied by ThermoFisher (ThermoFisher Scientific, San Jose, CA, USA), using the same conditions for HPLC analysis described above, at a flow rate of 0.15 mL/min. The injection volume in the UHPLC system was 10 μL. The TOC analysis was performed at ITECONS-Coimbra, using an ANALYTIK-JENA equipment, MULTI N/C 3100 model.

3. Catalysts syntheses

meso-tetrakis(2,6-dichloro-3(5)-chlorosulfonyl-phenyl)porphyrin (**TDCPPSO$_2$Cl**)[1] and manganese(III) *meso*-tetrakis(2,6-dichloro-3(5)-sulfonyl-phenyl)porphyrinate acetate (**Mn(III)TDCPPS**) (**CAT 1**)[2,3] were synthesized according to literature (Scheme S1).[4,5] 3-Aminopropyl functionalized silica gel (1 mmol of NH_2 per gram) was commercially acquired with a particle size of 40-63 μm and a pore size of 60 Å and used to prepare the hybrid material **TDCPPS@NH-SiO$_2$**, by modification of our previously reported method (Scheme 1).[6]

In a typical experiment, to a freshly prepared chlorosulfonated porphyrin **TDCPPSO$_2$Cl** (0.23 mmol), dissolved in 100 mL chloroform (in a round bottom 250 mL flask), 3 g (3 mmol NH_2) of 3-aminopropyl functionalized silica suspended in triethylamine (42 mL, 300 mmol) were added in small portions with stirring. The reaction was stirred at room temperature (25 ºC) for 24 h under inert atmosphere. Then, the material was centrifuged (4000 rpm) and the collected solid washed consecutively with distilled water (3× 50 mL), ethanol (4× 50 mL), acetonitrile (3× 50 mL) and acetone (3× 50 mL), until washings were completely clear using each solvent (checked by UV-Vis). The hybrid catalyst **TDCPPS@NH-SiO$_2$** was dried under vacuum and stored in the dark.

Complexation of **TDCPPS@NH-SiO$_2$** with $Mn(OAc)_2$, (OAc = acetate), was carried out by placing 850 mg of **TDCPPS@NH-SiO$_2$** in a 50 mL round bottom flask, in 20 mL of saturated solution containing CH_3COONa in CH_3COOH, under stirring. $Mn(OAc)_2$ was then added (260 mg, 1,8 mmol) and the mixture was refluxed for 24 h at 100 ºC. Upon cooling, the heterogeneous material was washed with isopropanol (300 mL) and dried under vacuum, yielding **Mn(III)TDCPPS@NH-SiO$_2$** (**CAT 2**).



Scheme S1. Preparation of **Mn(III)TDCPPS@NH-SiO$_2$** heterogeneous catalyst. Reagents and conditions: (i) Et_3N, CH_2Cl_2, 25 ºC, 24 h; (ii) $Mn(OAc)_2$, sat. CH_3COONa/CH_3COOH, 100 ºC, 24h.

4. Catalysts Characterization

Solid-state UV-Vis was measured for **Mn(III)TDCPPS@NH-SiO$_2$**, in order to analyze its characteristic absorption bands (Fig. S2).

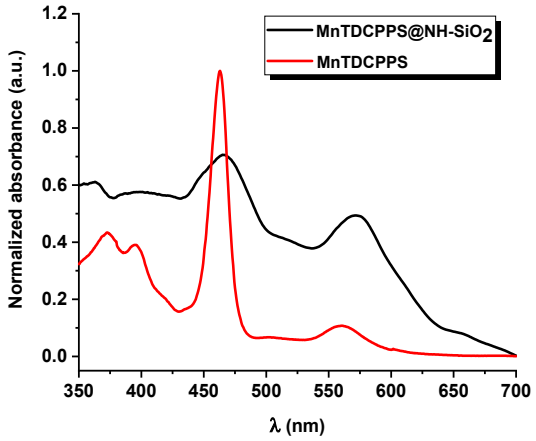


Fig. S2. Solid-state and solution UV-Visible spectra of **Mn(III)TDCPPS@NH-SiO$_2$** (black) and **Mn(III)TDCPPS** (red), respectively. The spectrum of **Mn(III)TDCPPS** was recorded in distilled water and normalized.

The UV-vis spectra of **Mn(III)TDCPPS@NH-SiO$_2$** in solid state and the corresponding metalloporphyrin (**Mn(III)TDCPPS**) in aqueous solution were recorded, and a slight bathochromic shift is observed in the Soret band, from 464 nm for the hybrid catalyst to 466 nm for **Mn(III)TDCPPS**. In the case of the Q bands, these underwent a larger bathochromic shift, from 563 nm (**Mn(III)TDCPPS@NH-SiO$_2$**) to 570 nm (**Mn(III)TDCPPS**). Additionally, the metal to ligand charge transfer (MLCT) and ligand to metal charge transfer (LMCT) bands, which can be observed in the **Mn(III)TDCPPS** UV-Vis profile, are no longer evidenced in the **Mn(III)TDCPPS@NH-SiO$_2$** spectrum.

Mn(III)TDCPPS@NH-SiO$_2$ was also characterized by thermogravimetric analysis (TG) in order to directly quantify the metalloporphyrin that was covalently linked to the functionalized silica (Fig. S3).

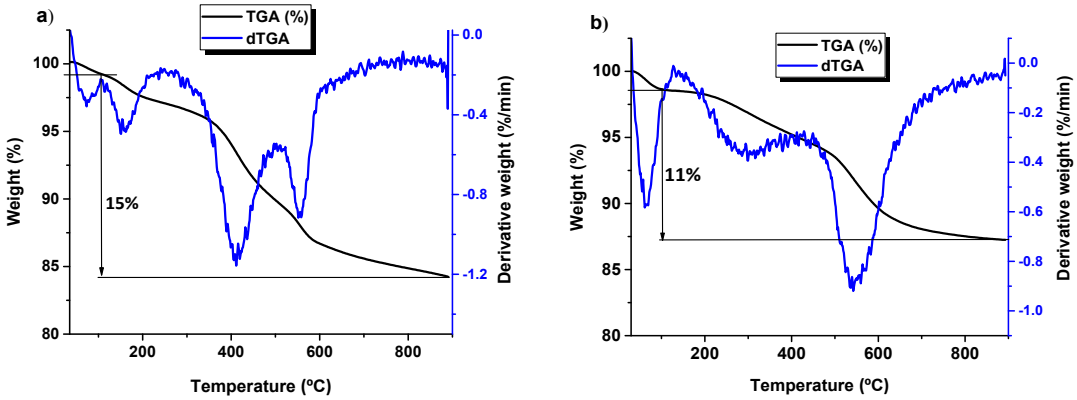


Fig. S3. Thermograms (derivate of weight loss – blue line, weight loss – black line): (a) **Mn(III)TDCPPS@NH-SiO$_2$**, (b) SiO_2-$(CH_2)_3$-NH_2.

The TG analyses were all performed in a heating range from 35 °C to 900 °C with a heating rate of 20 °C/min. Analyzing the thermogram obtained for **Mn(III)TDCPPS@NH-SiO$_2$** (Fig.

S3a), a weight loss is observed between 35 °C and 120 °C, which corresponds to water loss. With increasing temperature, a gradual weight loss is observed by three weight loss steps, resulting in a total weight loss of 15%. The weight derivative curve reveals two first weight loss steps, between 130 °C and 450 °C, and these are attributed to the degradation of the organic part associated with the porphyrin. Observing the thermogram profile of silica functionalized with 3-aminopropyl groups (Fig. S3b), a weight loss associated with the degradation of the aminopropyl groups is observed between 500 °C and 900 °C. Considering the difference between the weight loss of **Mn(III)TDCPPS@NH-SiO$_2$** and that of the functionalized silica, a value of 4% weight loss was obtained. This value corresponds to the weight of porphyrin covalently linked to the inorganic support. From this value and the molecular weight of the organic component, an amount of 3.4×10^{-5} mol of immobilized porphyrin per g of functionalized silica was determined. Considering the sequential preparation of **TDCPPSO$_2$Cl** (~2.3×10^{-4} mol) and its subsequent immobilization reaction with 3 g of amine functionalized silica, in which a theoretical amount of 7.7×10^{-5} mol NH_2 groups per g of inorganic support is assumed, a 44% immobilization yield was determined.

To analyze the morphologic differences between the surfaces of 3-aminopropyl functionalized silica and **Mn(III)TDCPPS@NH-SiO$_2$** catalyst, scanning electron microscopy (SEM) studies were performed, and displayed in Fig. S4.

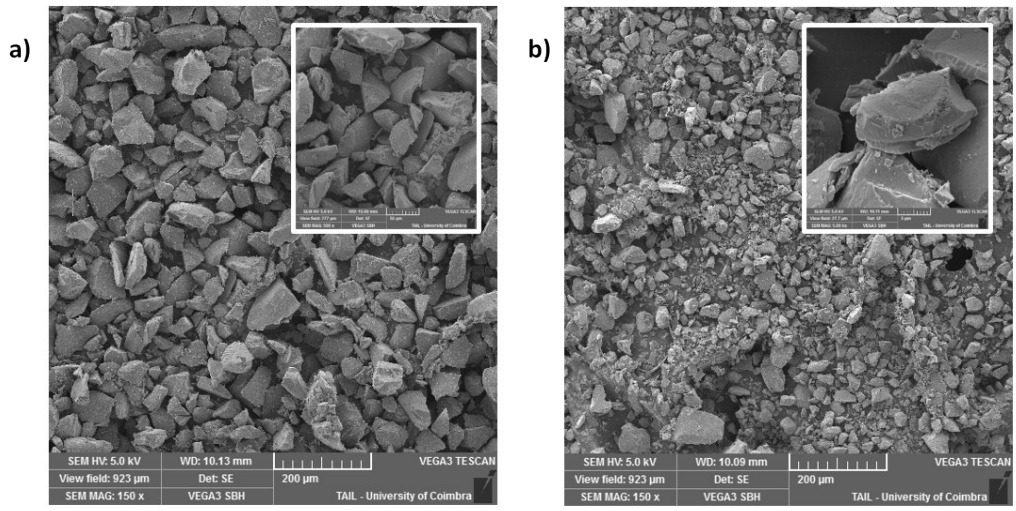


Fig. S4. SEM images of: (a) 3-aminopropyl functionalized silica (obtained with magnification = 150×, zoom was obtained with a magnification of 500×); (b) **Mn(III)TDCPPS@NH-SiO$_2$** catalyst (obtained with magnification = 150×, zoom was obtained with a magnification of 5000×).

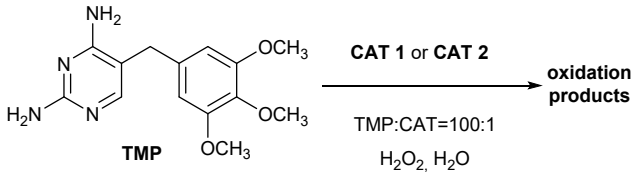
Fig. S4a corroborates vendor's material datasheets, in which the silica particles display sizes between 40 and 60 µm. As for **Mn(III)TDCPPS@NH-SiO$_2$** catalyst (Fig. S4b), a decrease in particle size is observed, with an average Feret's diameter between 5 and 35 µm, which may be associated with some particle fragmentation, caused by the strong magnetic stirring process, during the immobilization reaction.

5. Degradation experiments

5.1. Batch tests

In a typical TMP degradation experiment, in batch conditions, 20 mL of TMP stock solution ([TMP] = 2.3×10^{-4} M, 66.8 μg mL^{-1}) were placed in a 50 mL round bottom flask, protected from light with aluminum foil. Keeping a TMP:**CAT** ratio of 100:1, 40 μL of **Mn(III)TDCPPS** stock solution ([**Mn(III)TDCPPS**] = 1.2×10^{-3} M) was added. The mixture was stirred for 15 min and, after this period, 30 μL de H_2O_2 30% (v/v) were added every 15 minutes, taking one aliquot before each addition, for HPLC analysis.

Table S1. Aqueous room temperature chemical oxidative degradation of TMP using H_2O_2 as oxidant in batch mode. Degradation results are given as average values of reactions performed in triplicate.



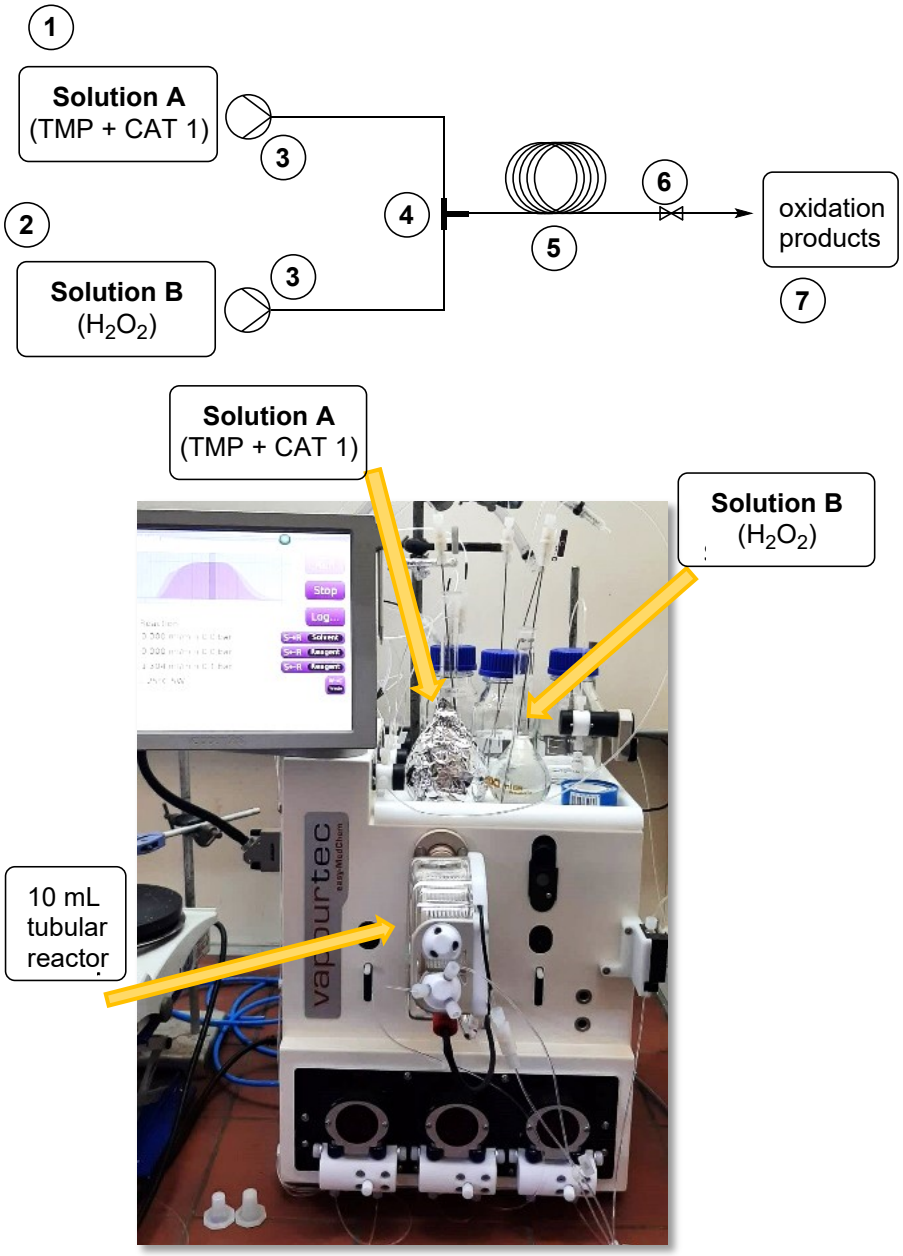
Entry	[TMP] (μg mL^{-1})	Catalyst	Degradation (%)
1 [a)]	66.8	**CAT 1**	94
2 [a)]	66.8	---[f)]	5

[a)] addition of 30 μL of H_2O_2 (30% v/v) every 15 min until total V(H_2O_2) = 300 μL, reaction time = 150 min.

5.2. Homogeneous continuous flow

In the oxidative degradation of TMP, both in homogeneous and heterogeneous conditions, aliquots were collected after reaching the reaction's steady-state and quenched by adding $NaHSO_3$ saturated solution, before HPLC analysis. Stock solutions containing TMP and **CAT 1** with TMP:**CAT 1** ratio = 100:1 were prepared, with [TMP] = 2.3×10^{-5} M (6.68 μg mL^{-1}) and [TMP] = 2.3×10^{-4} M (66.7 μg mL^{-1}). H_2O_2 3% and 0.3% (v/v) stock solutions were also prepared.

The oxidative degradation of TMP in continuous flow homogeneous conditions was performed in a 10 mL tubular reactor from easy-MedChem Vapourtec® E-Series equipment. For each experiment, aliquots were collected after reaching the reaction's steady-state and quenching was performed by adding 300 μL de $NaHSO_3$ solution, when H_2O_2 3% was used and 30 μL de $NaHSO_3$ solution when H_2O_2 0.3% (v/v).



Scheme S2. System set-up of continuous-flow degradation of TMP: (1) Solution A with aqueous solution of TMP and Mn(III)TDCPPS (CAT 1); (2) Solution B with aqueous hydrogen peroxide; (3) V3 pump system; (4) T-mixer; (5) 10 mL tubular reactor; (6) back-pressure regulator; (7) reaction output (collecting valve).

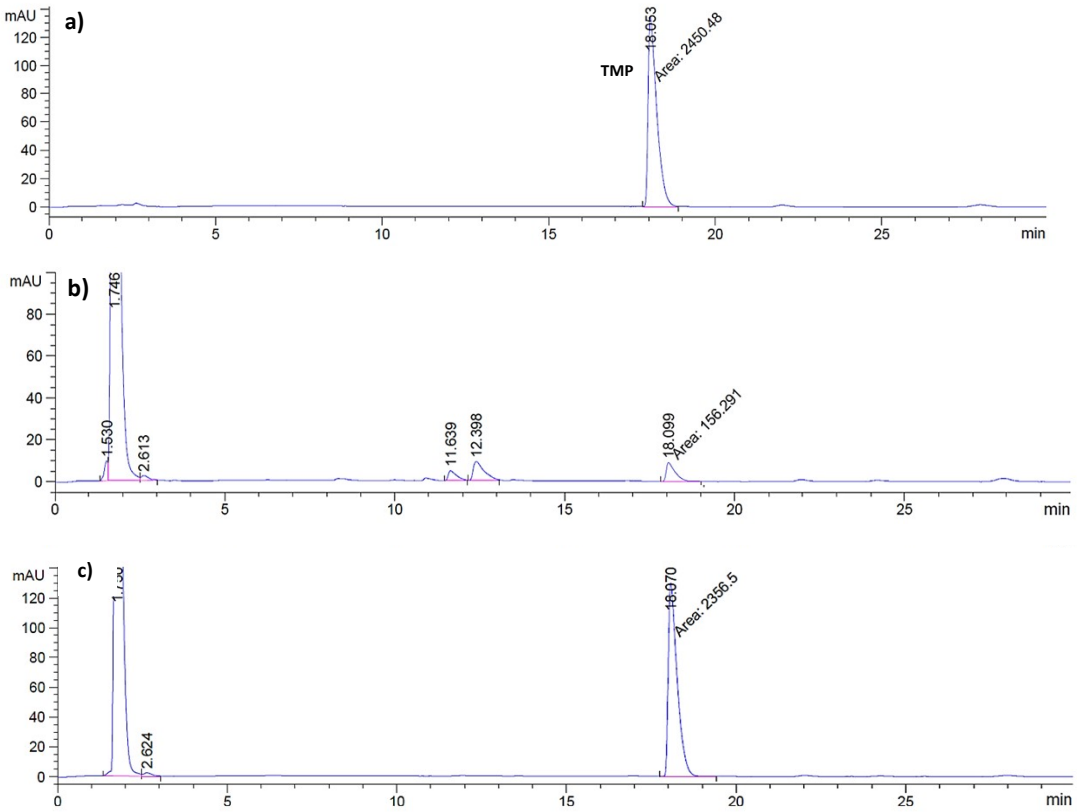


Fig. S5. Selected chromatograms obtained by HPLC for the homogeneous oxidative degradation reactions of TMP in the presence of H_2O_2 as oxidant, under flow conditions: a) at time 0 min; b) RT = 45.5 min with VR = 1:1.2 and H2O2 (3% v/v); c) control reaction, carried out in the absence of catalyst and oxidant. (related to table 1).

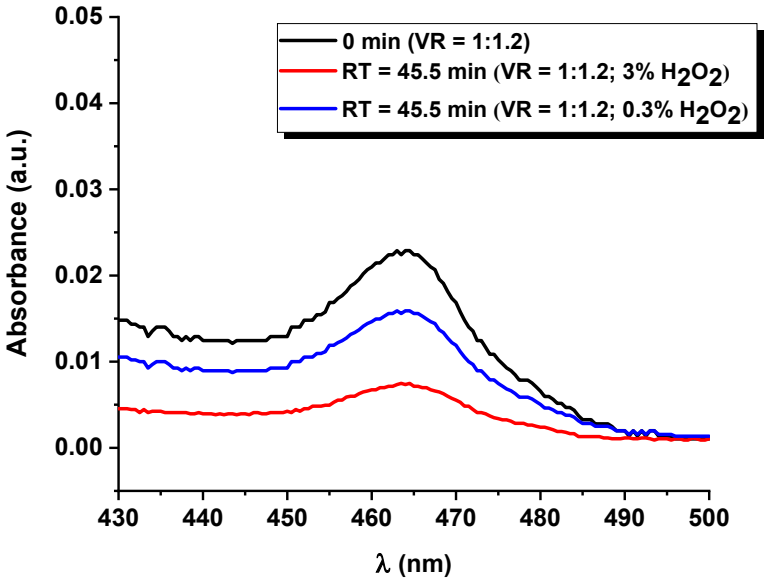
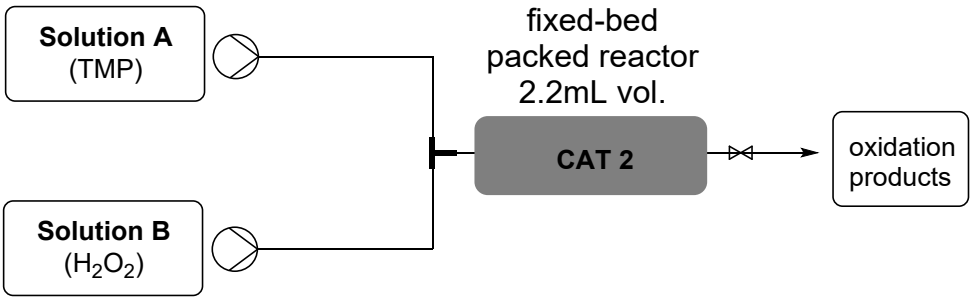


Fig S6. Partial UV-Vis spectra, recorded in distilled water, before reaction (black), and after the oxidation reactions, using H_2O_2 3% (v/v) (red – Entry 8, Table 1) and H_2O_2 0.3% (v/v) (blue – Entry 9, Table 1). In both cases VR = 1:1.2 (A:B) and RT = 45.5 min

5.3. Heterogeneous continuous flow



Scheme S3. Aqueous room temperature chemical oxidative degradation of TMP using H_2O_2 as oxidant in a heterogeneous fixed-bed continuous flow mode (2.2 mL reactor).

Fixed bed reactor packing with CAT 2

The oxidative degradation of TMP in continuous flow heterogeneous conditions was performed in easy-MedChem Vapourtec® E-Series fixed bed reactor equipment. The first task consisted of packing the fixed-bed column with heterogeneous catalyst **Mn(III)TDCPPS@NH-SiO$_2$** (1.5 g, 4.7×10^{-5} mol **Mn(III)TDCPPS – CAT 2**). The column was then weighed to determine the dry column weight. Afterwards, the column was connected to the flow equipment and distilled water was passed through the column in reverse flow, to pack the column and remove any air bubbles present. The packed column was again weighed and the weight of solvent was directly calculated by the difference between dry and wet packed column. A wet packed column volume Vol = 2.2 mL was determined.

Fixed bed reactor adsorption test

Solution A (TMP) was passed through the reactor for 60 min. Each 10 min. an aliquot was collected and analyzed. After 30 min. of continuous flow, the amount of TMP exiting the reactor remained identical to the amount entering.

Fixed bed reactor packing with SiO$_2$ for blank reaction

The same task was carried out for preparing a SiO_2 packed fixed reactor. Commercial 40-63 µm sized SiO_2 (1.5 g) was introduced in the reactor and compacted. The column was connected to the flow equipment and distilled water was passed through the column in reverse flow, to pack the column and remove any air bubbles present. The packed column was again weighed and the weight of solvent was directly calculated by the difference between dry and wet packed column. A wet packed column volume Vol = 2.0 mL was determined.

For each experiment, quenching was performed by adding 30 µL of $NaHSO_3$ solution, when H_2O_2 3% was used and 3 µL de $NaHSO_3$ solution when H_2O_2 0.3% (v/v). During the continuous flow 8 hours experiment, aliquots were collected before reaching the steady-state, between 10 and 40 minutes (every 5 minutes) and then every hour, each one quenched properly and analyzed by HPLC.

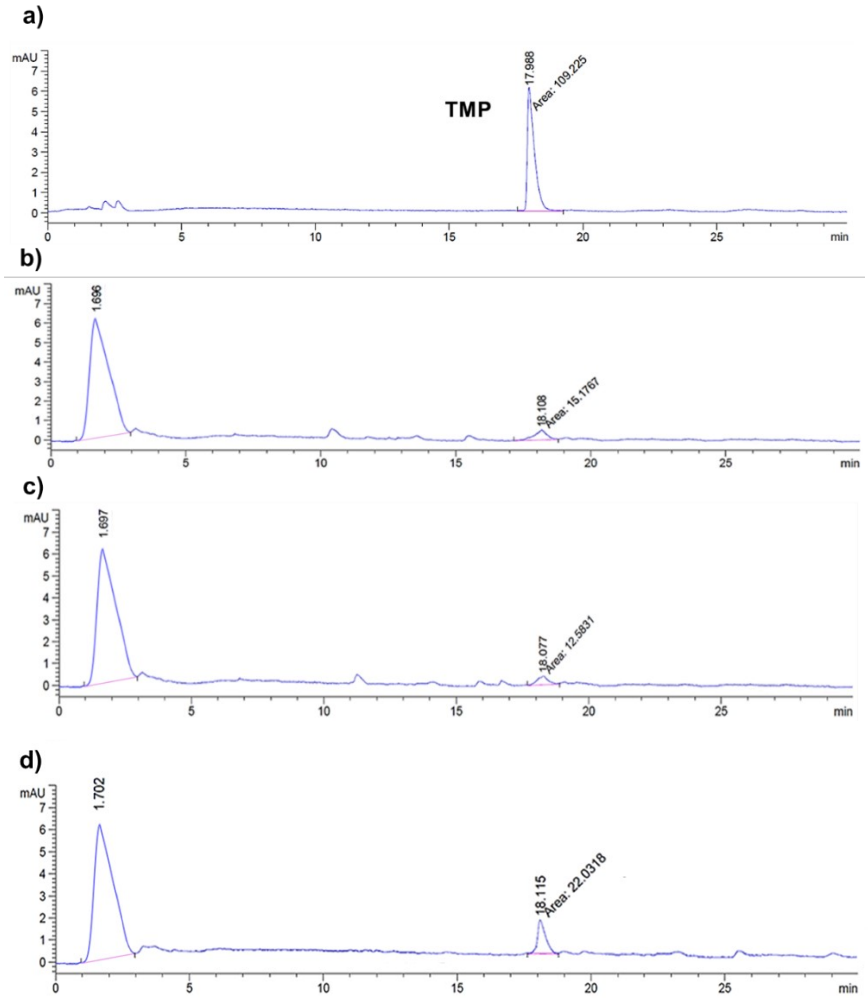


Fig. S7. Chromatograms obtained by HPLC for heterogeneous oxidative degradation reactions of TMP in the presence of **CAT 2** in continuous flow with VR(A:B) = 1:1.2 and [TMP] = 6.68 μg mL^{-1}: (a) at time 0 min; (b) after reaction using 3% H_2O_2 and RT = 10 min; (c) after reaction using 0.3% H_2O_2 and RT = 10 min; (d) after reaction using 0.3% H_2O_2 and RT = 5 min.

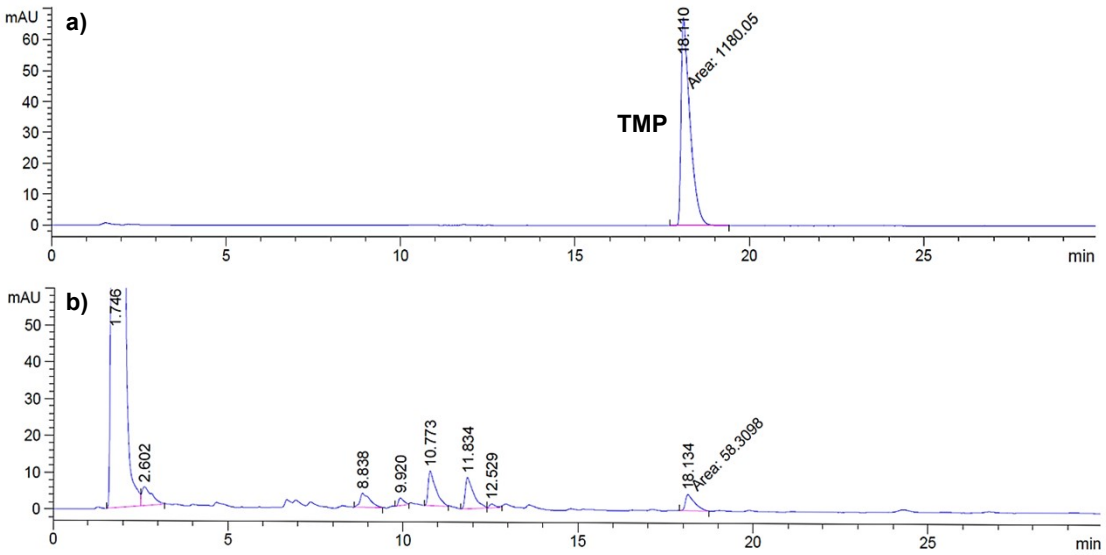


Fig. S8. Chromatograms obtained by HPLC for the oxidative degradation reactions of TMP in the presence of **CAT 2** in continuous flow, using 0.3% H_2O_2 with RV(A:B) = 1:1.2 and [TMP] = 66.8 µg/mL: (a) in time 0 min; (b) after reaction with RT = 10 min.

6. Long run reaction

Initially, aliquots were collected every 5 minutes until a steady-state was reached at 40 minutes. Subsequently, samples were collected every hour to monitor the degradation rate over time.

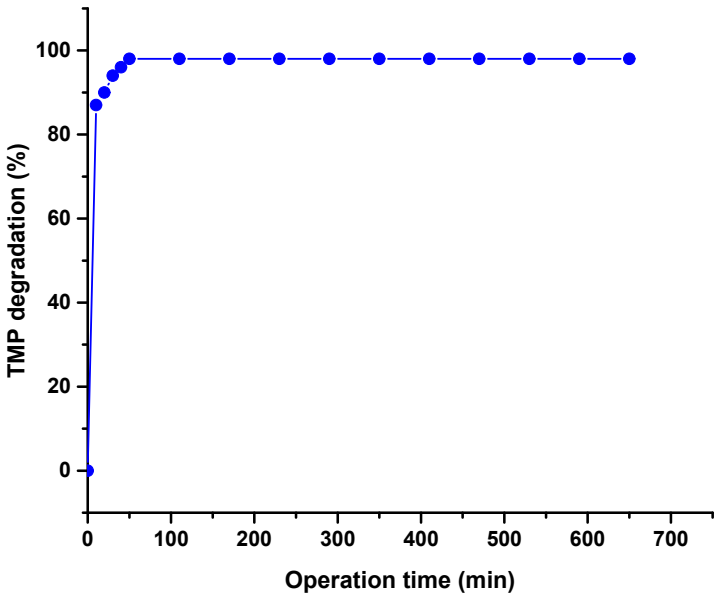


Fig. S9. TMP degradation (%), obtained by HPLC, as a function of reaction time, in minutes. Conditions: Solution A ([TMP] = 6.68 µg/mL); Solution B ([H_2O_2] 0.3% v/v); VR(A:B)=1:1.2; reactor volume = 2.2 mL; flow rate (A) = 0.100 mL/min; flow rate (B) = 0.120 mL/min; room temperature.

7. Microbiology studies

The DPs samples were collected after continuous flow 8 hours experiments (ca. 100 mL), without quenching, lyophilized and stored at 3-5 ºC.

In vitro antibacterial assays were carried out using *Escherichia coli* ATCC 8739, a Gram-negative bacterium. The qualitative antibacterial activity was assessed by the method of Kirby-Bauer (disc diffusion method) and the MIC was determined by broth microdilution assays, using Mueller-Hinton (MH) agar and broth, respectively. The inoculum was prepared by suspending isolated colonies with sterile distilled water to obtain a cell density with a turbidity similar to the 0.5 McFarland standard scale (~ 1-2 × 10^8 CFU mL^{-1}).

General procedure for MIC determination using broth microdilution test. 100 µL of MH broth were added in each microplate of a sterile microplate with 96 round-bottomed wells using an 8-channel pipette. Then, 1 mg mL^{-1} antibiotic stock solution was prepared in distilled water and 100 µL were dispensed into the wells in column 1 and homogenized. After this, 100 µL of was withdrawn from column 1 and added to column 2 and homogenized. This procedure was repeated from until column 12. After the preparation of inoculum suspension, a bacteria suspension was diluted with a factor of 1:20 with distilled water and then 10 µL of diluted bacteria suspension were added into all wells. Appropriate bacterial growth and medium sterility control experiments were conducted. Finally, the plate was placed into the incubator at 37 ºC for 20 h. After the incubation time, MIC was assessed as the lowest concentration where there is no visible growth (white spots on the bottom of the wells or turbidity in the solution). The experiments were performed in triplicate.

The results obtained by this method to assess the MIC of TMP were compared to the breakpoints established by the European Committee on Antimicrobial Susceptibility Testing guidelines.[7]

Resistance development assay

The assay compared the ability of *E. coli* to develop resistance when exposed to:

- Group A: Half the minimum inhibitory concentration (MIC/2) of TMP (~0.2441 µg mL-1).
- Group B: Solution containing 1 mg/mL of degradation products (DPs).

On the 1st day, bacteria of group A were exposed to a concentration of TMP equal to its MIC/2 (~ 0.2441 µg mL^{-1}) value and bacteria of group B, were exposed to a solution of 1 mg of DPs *per* mL. Then, after incubation at 37 °C in MH medium during 20 h, bacterial growth is expectedly observed due to the presence of sub-inhibitory antibiotic concentrations. In the 2nd day, two standard inocula of approximately 5×10^5 CFU/mL were prepared using the bacteria that grew in each group (A and B) and the TMP MIC was determined for each inoculum according to the experimental procedure described below. This cycle of tests was repeated along 10 days. We should highlight that, each day that the TMP MIC value increased, bacteria were exposed to a newly prepared TMP concentration, whose concentration was always equal to the half MIC value.

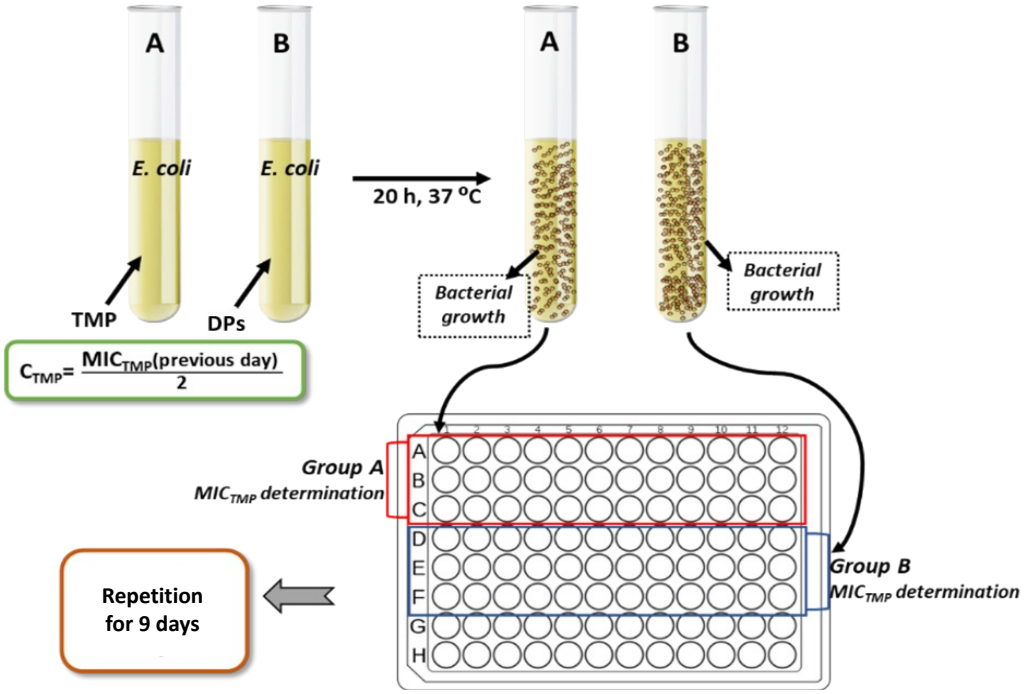


Fig. S10. Schematic representation of *E. coli* resistance assay.

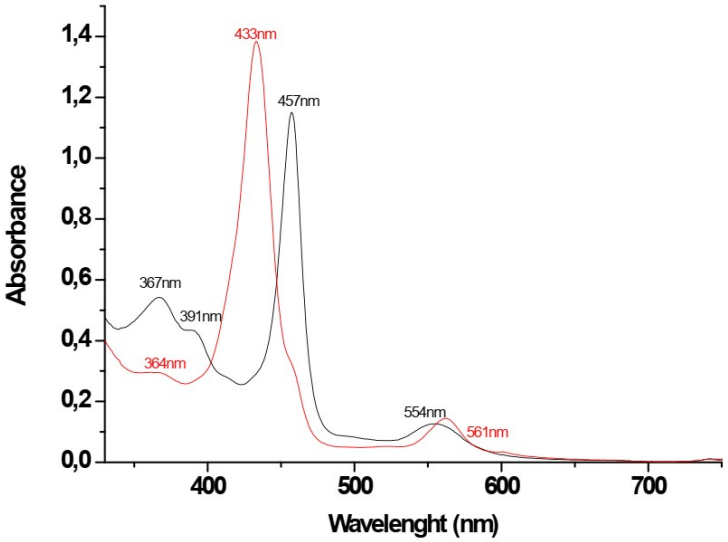


Fig. S11. Typical UV-Vis spectra of a Mn(II) porphyrin (red) and a Mn(III) porphyrin (black).

8. References

1 A. M. D. R. Gonsalves, R. A. W. Johnstone, M. M. Pereira, A. M. P. deSantAna, A. C. Serra, A. J. F. N. Sobral and P. A. Stocks, *Heterocycles*, 1996, **43**, 829. https://doi.org/10.3987/COM-95-7372

2 T.P. Wijesekera and D. Dolphin, in *Metalloporphyrins in Catalytic Oxidations*, ed. R. Sheldon, Marcel Dekker, Inc., New York, 1994, pp. 193.

3 H. Turk and W. T. Ford, *The Journal of Organic Chemistry*, 1991, **56**, 1253. https://doi.org/10.1021/jo00003a058

4 L.J. Boucher, Coord. Chem. Rev., 1972, 7, 289 https://doi.org/10.1016/S0010-8545(00)80024-7

5 L.J. Boucher, J. Amer. Chem. Soc., 1968, 90, 6640 https://doi.org/10.1021/ja01026a014

6 G. Piccirillo, M. Moreira-Santos, M. Valega, M. E. S. Eusebio, A. M. S. Silva, R. Ribeiro, H. Freitas, M. M. Pereira and M. J. F. Calvete, *Appl Catal B-Environ*, 2021, **282**, 195562.

7 EUCAST, *E.coli_Antimicrobial wild type distributions of microorganisms. https://mic.eucast.org/search/?search%5Bmethod%5D=diff&search%5Bantibiotic%5D=-1&search%5Bspecies%5D=261&search%5Bdisk_content%5D=3&search%5Blimit%5D=50*.