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RESEARCH ARTICLE

An eco-friendly and alternative method of forced degradation of fluoroquinolone drugs by microwave irradiation: a new application for analytical eco-scale

Lucas D. Dias[a], Kairo H. E. Gonçalves[b], Jaqueline E. Queiroz[c], Giuliana M. Vila Verde[c] and Gilberto L. B. Aquino[c]

[a]CQC, Depatamento de Química da Universidade de Coimbra, Rua Larga, Coimbra, Portugal; [b]Faculdade de Farmácia, Unidade de Ciências Exatas e Tecnológicas, Universidade Estadual de Goiás, Anápolis, Goiás, Brazil; [c]Laboratório de Pesquisa em Bioprodutos e Síntese, Unidade de Ciências Exatas e Tecnológicas, Universidade Estadual de Goiás, Anápolis, Goiás, Brazil

ABSTRACT

Forced degradation studies are essential to determine the drug stability, elucidate the main degradation routes, and monitor the degradation products in qualitative/quantitative terms. Over the years, the pharmaceutical industry has been using a traditional and conventional method that employs a stove as the heating source; this requires a lengthy process and high energy. Recently, a new forced degradation method using modern microwave reactors has been reported as a greener, more economical and efficient alternative. The present work reports for the first time degradation of fluoroquinolone drugs (levofloxacin and norfloxacin) under microwave irradiation. Also for the first time, it presents the utilization of analytical eco-scale as a novel comprehensive approach to evaluating the greenness of analytical methodology for studies on the forced degradation. The objective of this study was to design and validate a forced degradation method assisted by microwave irradiation, alongside a conventional stress degradation study, and to compare the two methods. Microwave irradiation showed excellent performance as it yielded similar amounts of specific degradation products for both drugs, equivalent to what is produced in the conventional procedure. Therefore, there were some advantages to the new eco-friendly degradation method with respect to the significantly reduced time (72 times), energy (360 times), reagents and waste.

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KEYWORDS
Forced degradation studies; levofloxacin; norfloxacin; microwave irradiation; analytical eco-scale

1. Introduction

Simply stated, 'Green Chemistry is the use of chemistry techniques and methodologies that reduce or eliminate the use or generation of feedstocks, products, by

CONTACT Gilberto L. B. Aquino gilberto.benedito@ueg.br Laboratorio de Pesquisa em Bioprodutos e Sıntese, Unidade de Ciências Exatas e Tecnologicas, Universidade Estadual de Goias, 75132400, Anapolis, Goias, Brazil.
Chemical compounds studied in this article: Levofloxacin (PubChem CID: 149096), Norfloxacin (PubChem CID: 4539).

products, solvents, reagents, etc. that are hazardous to human health or the environment' (Anastas and Warner 1998; Anastas 1999).

The branch of chemistry that contributes most to the use and the generation of hazardous substances is organic synthesis (Welton 2015). In 1999, the term 'green analytical chemistry' was created (Anastas and Warner 1998) by the father of green chemistry, Anastas, who drew attention to the need to develop a green analytical methodology. Since that time, interest in implementing green chemistry principles in analytical chemistry has grown dramatically.

The 12 principles of green chemistry (Anastas and Eghbali 2010) are the basis of guidelines addressed to those who want to follow the green chemistry trend. Normally, the conformity of different analytical issues with the 12 principles of green chemistry refers to reagents, energy, waste, and methods. Method selection is crucial and controls other issues. In modern analytical laboratories, separation and/or identification of a single analyte is made by a set of analytic techniques, such as accurate measurement of high-resolution mass spectrometry (MS), nuclear magnetic resonance (NMR), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), and others (Gałuszka et al. 2012).

In this context, the pharmaceutical industry compared with other sectors of the chemical industry greatly needs green techniques and methodologies (Sanderson 2011) due to the need to obtain a very high-purity product in the multi-stage reactions during which many by-products (waste) are generated and during development/control quality tests (Tobiszewski et al. 2015). Additionally, the production of pharmaceuticals requires the use of high-purity reagents (Dunn et al. 2004).

The forced degradation method (FDM) is an example of a development/control quality test that is executed routinely in a pharmaceutical company to evaluate a drug's chemical stability. Using it also elicits the degradation products, provides an insight into the degradation pathway and the specificity of stability indicating methods (Ngwa 2010; Blessy et al. 2014), using a degradation process under conditions (acid, basic, oxidative, and temperature) that are more severe than accelerated conditions. Remembering that, most of the regulatory guidance documents have defined the concept of forced degradation, but they do not provide detailed information about forced degradation strategies (International Conference on Harmonization [ICH] 2017).

During the last decade, there has been huge interest and challenges to optimize the series of stress time procedures capable of simulating the primary degradation of drugs and medicines and to develop green techniques and methodologies.

The conventional method of heating uses an external source, for instance stove or hot plate (Chilbule and Kakde 2017; Johnsirani et al. 2017; Vishnuvardhan et al. 2017) in which the heat transfer depends upon the thermal conductivity of various materials. This heat initially leads to the increase in the temperature of the reaction vessel and, thereafter, of the reaction mixture. Furthermore, forced degradation studies in solution are performed using reaction volumes between 10 and 100 ml (drug concentration 1–10 mg/ml), applying comparatively moderate temperatures ranging from room temperature up to ~100 °C (reflux conditions) which involves long reaction times (from hours to days), low sample throughput, and a time-consuming sample handling/analytical regime (Lan et al. 2001; Feng et al. 2017).

In this mode, new research related to stress studies has been widely carried out. Among the methods, the use of microwave irradiation in organic synthesis (Pineiro et al. 2016) has been highlighted in experiments of forced degradation as an alternative for heating, accelerating degradation reactions (Sonawane and Gide 2013). As with all microwave-assisted chemistry, proper and reliable control over the reaction parameters (temperature, pressure, stirring) is essential to obtain reproducible results that can be duplicated in other laboratories (Lenardão et al. 2003; Kappe 2004; Herrero et al. 2008; Obermayer et al. 2009; Obermayer and Kappe 2010).

Microwave energy produces efficient internal heating by direct coupling of microwave energy with polar molecules. Accordingly, microwave-assisted reactions are mainly based on the efficient heating of materials by microwave dielectric heating effects (Larhed and Hallberg 2001; Kappe and Dallinger 2006; Sun et al. 2016).

A significant reduction of time has been achieved without the degradation losing the profile and efficiency that has already been observed in exposing the drugs to degrading agents for long periods of time, either at room temperature or in heating through stoves or reflux. In addition, the use of microwave irradiation heating can also considerably reduce the amounts of solvents used as stressing agents, without affecting the efficiency of the study or decreasing the energy; overall, the great advantage is of following the green chemistry principles (Dubé and Salehpour 2014).

Herein, we report the use of microwave irradiation to promote the forced degradation study of norfloxacin and levofloxacin (Figure 1) and the unpublished use of green analytical metrics to evaluate methods for the forced degradation of drugs.

The synthetic antibiotics that are under study are classified as fluoroquinolones, quinolone derivatives of the first generation. They have action against gram negative organisms and are applied in the treatment of infections caused by bacteria that are the causative agents of about 80% of the infections of the genitourinary tract (Bryskier and Chantot 1995; Tillotson 1996; Appelbaum and Hunter 2000; Haraguchi 2000). Furthermore, we also report the use of analytical eco-scale metric to make a comparison between the conventional and the microwave method aiming to present the greener method to able to promote the forced degradation of norfloxacin and levofloxacin.

2. Experimental

2.1. Material and instruments

All reagents and solvents were acquired from Sigma-Aldrich (St. Louis, MO) and used as received without any further purifications. Levofloxacin and norfloxacin

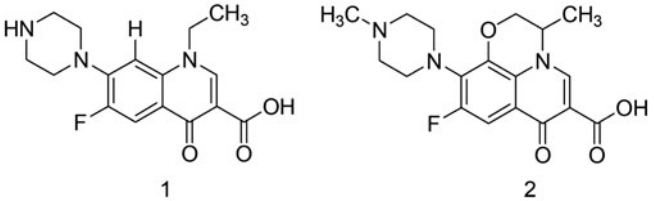

Figure 1. Structures of norfloxacin (1) and levofloxacin (2).

primary standard were purchased from United States Pharmacopeia (USP). Forced degradation studies by microwave irradiation were performed in a *CEM Corporation-Discover S-Class* with the following specifications: heating rate: 2–6 °C/s; internal pressure: ActiVent Self-Venting Technology included for pressure relief during or after reaction; temperature measurement: infrared for volume-independent non-invasive temperature measurement; reaction agitation: electromagnetic stirring with adjustable speeds; weight: 38 lbs (17.3 kg); dimensions: (36.1 cm × 42.9 cm × 28.4 cm). Conventional temperature stress condition was carried out in a stove, *Nova Ética model: 400/3ND*. Liquid chromatography analysis was carried out in a HPLC-*Waters 2695 Separations Module Alliance, Photodiode Array Detector,* and all the filtrations were performed in a syringe filter – *Millex*® 0.45 µm.

2.2. HPLC analysis

2.2.1. Methods and conditions

The HPLC analysis was carried out on a HPLC-*Waters 2695 Separations Module Alliance, Photodiode Array Detector,* and all analyses of the peak areas were integrated automatically by computer using an *Empower 2*® software program (SPSS, Chicago, IL).

Levofloxacin and norfloxacin methods are in accordance with the United States Pharmacopeia (USP) (USP 2012). Levofloxacin analysis was performed using a 10 µl volume of sample, the elution was carried out on a X-Terra Waters C18-reverse phase, with 150 m length × 4.0 mm internal diameter. The column temperature and the sample temperature were 35 and 25 °C, respectively. As the mobile phase, 0.04M NaClO solution (in ammonium acetate buffer pH: 2.2)/acetonitrile (85:15 v/v) was used under isocratic conditions. The flow rate used was of 1.0 ml/min, using photo-diode array (PDA) detection at 294 nm. Norfloxacin analysis used a 10 µl volume of sample, the elution was carried out on a ACE C18-reverse phase, with 250 m length ×4.0 mm internal diameter. The column temperature and the sample temperature were 40 and 25 °C, respectively. As the mobile phase, 0.1% phosphoric acid/aceto-nitrile (85:15 v/v) was used under isocratic conditions. And finally, the flow rate used was of 2.0 ml/min, using PDA detection at 275 nm.

2.2.2. Specificity and adequacy of methods

Five injections of each standard solution determined the adequacy of the methods for norfloxacin (1) and levofloxacin (2). Method specificity was performed by three injections of the diluent solution, mobile phase, levofloxacin, and norfloxacin standard. System suitability parameters were compared with the specifications as shown in Table 1.

Table 1. System suitability specifications (USP 2017).

Parameter	Specification
Relative standard deviation (RSD) or variance coefficient (VC %)	≤2.0%
Theoretical plates (N)	≥2000
Peak symmetry	≤2.0
K′ (capacity factor)	Principal peak $k \geq 2.0$ toward other peaks
Peak purity	Limit angle > peak purity angle

2.3. Preparation of standard solutions – levofloxacin and norfloxacin

An accurate 20 mg of levofloxacin primary standard (USP) was transferred to 100 ml volumetric flask and diluted using H_2O/acetonitrile (85:15 v/v) (Levofloxacin Solution 1). Then, an aliquot of 1 ml was transferred to 10 ml volumetric flask and diluted using H_2O/acetonitrile (85:15 v/v). Levofloxacin concentration = 20 µg/ml.

Norfloxacin standard solution was prepared weighing 200 mg of norfloxacin primary standard (USP) to 100 ml volumetric flask and diluted with 0.1% phosphoric acid/acetonitrile (85:15 v/v) (Norfloxacin Solution 1). At the end, an aliquot of 1 ml was transferred to 10 ml volumetric flask and diluted with 0.1% phosphoric acid/acetonitrile (85:15 v/v). Norfloxacin concentration = 200 µg/ml.

2.4. General procedure of forced degradation studies by conventional method (basic and acid hydrolyses, oxidative, and temperature stress conditions)

An aliquot of levofloxacin solution 1 (1 ml) was transferred to a 10 ml volumetric flask and the respective stress solutions added (0.1 M HCl; 1.0 M NaOH; 0.3% (v/v) H_2O_2) (5 ml) and completed with diluent H_2O/Acetonitrile (85:15 v/v). However, for the temperature stress condition, an aliquot of levofloxacin solution 1 (1 ml) was transferred to a 10 ml volumetric flask and completed with diluent H_2O/Acetonitrile (85:15 v/v). Then, all samples were submitted to heating in a stove for 24 h at 60 °C.

Regarding the norfloxacin, an aliquot of norfloxacin solution 1 (1 ml) was transferred to a 10 ml volumetric flask and the respective stress solutions added (0.1 M HCl; 1.0 M NaOH; 3% (v/v) H_2O_2) (5 ml) and completed with diluent 0.1% phosphoric acid/acetonitrile (85:15 v/v). However, for the temperature stress condition, an aliquot of levofloxacin solution 1 (1 ml) was transferred to a 10 ml volumetric flask and completed with diluent 0.1% phosphoric acid/acetonitrile (85:15 v/v). Then, all samples were submitted to heating in a stove for 24 h at 60 °C. The quantification of degradation products was performed by HPLC.

2.5. General procedure of forced degradation studies by eco-friendly method under microwave irradiation (basic and acid hydrolyses, oxidative, and temperature stress conditions)

An aliquot of levofloxacin solution 1 (1 ml), the respective stress solutions (0.1 M HCl; 0.1 M NaOH; 0.1% (v/v) H_2O_2) (5 ml) and 0.1% phosphoric acid/acetonitrile (85:15 v/v) (4 ml) were transferred to a 35 ml microwave tube (Figure 4(b)). For the temperature stress condition, an aliquot of levofloxacin solution 1 (1 ml) in 0.1% phosphoric acid/acetonitrile (85:15 v/v) (9 ml) was transferred to a 35 ml microwave tube (Figure 4(b)). Then, all samples were submitted to microwave using a *CEM Corporation-Discover S-Class* as energy source, at 100 °C, for 20 min, and at 300 W.

For the norfloxacin, an aliquot of norfloxacin solution 1 (1 ml), the respective stress solutions (0.1 M HCl; 0.1 M NaOH; 0.1% (v/v) H_2O_2) (5 ml), and 0.1% phosphoric acid/acetonitrile (85:15 v/v) (4 ml) were transferred to a 35 ml microwave tube (Figure 4(b)). For the temperature stress condition, an aliquot of levofloxacin solution 1 (1 ml) and 0.1% phosphoric acid/acetonitrile (85:15 v/v) (9 ml) were

transferred to a 35 ml microwave tube (Figure 4(b)). Then, all samples were submitted to microwave using a *CEM Corporation-Discover S-Class* as energy source, at 100 °C, for 20 min, and at 300 W.

2.6. Statistics analysis

The statistics data were analysed by *Graphpad Prism 5.03* (GraphPad Inc., La Jolla, CA) software and the results are shown as a mean ± standard deviation, based on triplicate analysis under stress conditions. Statistical differences were defined from Student *t* test and Anova methods and then by Tukey as multiple comparison, using $p < 0.05$ as minimum significance level.

2.7. Green analytical chemistry metrics – conventional method versus microwave irradiation method

2.7.1. Calculation of energy consumption (E)

The energy consumption (E) was calculated by the following equation (Riese et al. 2014):

$$E = P \times t \tag{1}$$

where E is the energy transferred in kilowatt hours, kWh; P is the power in kilowatts, kW; t is the time in hours, h. The unit of energy is the Joule (J).

The stove (*Nova Ética model: 400/3ND*) and microwave heating (*CEM Corporation-Discover S-Class*) used during the studies has power of 1500 and 300 W, respectively. These powers reported were used as standard to calculate the energy consumption (E).

2.7.2. Calculation of analytical eco-scale

The sum of penalty points for the whole procedure should be included in the eco-scale calculation, according to the following equation (Gałuszka et al. 2012):

$$\text{Analytical eco-scale} = 100 - (\text{total penalty points}) \tag{2}$$

The total penalty points are calculated based on each of analytical procedure parameters (amount of reagents, hazards, energy and waste), penalty points are assigned if it departs from ideal green analysis. Then the total penalty points is calculated by multiplying the sub-total penalty points for a given amount and hazard (Gałuszka et al. 2012). The result of calculation is ranked on a scale, where the score: >75 represents excellent green analysis, >50 represents acceptable green analysis, <50 represents inadequate green analysis (Gałuszka et al. 2012).

3. Results and discussion

3.1. Evaluation of analytical methodology

Forced degradation studies have been reported and evaluated using different chromatography analysis techniques, such as HPLC and UPLC (Gumustas et al. 2014), with

respect to the separation, identification, and quantification of the degradation products. For this, the chromatography methods developed and used must be able to achieve specificity/selectivity conditions (Singh and Bakshi 2002; ICH 2003; USP 2012).

The HPLC method for levofloxacin and norfloxacin used in this work was shown to be specific, since all mobile phase and diluent components (blank) did not show any interference with the drugs' retention time (RT): (levofloxacin RT: 7.90 min and norfloxacin RT: 2.90 min) (Figure 2).

Therefore, based on the absorbance spectra of levofloxacin and norfloxacin (Figure 3), the purity of the peaks was verified (Waters Corporation 2002).

The chromatography parameter studies (CV%, K', peak symmetry and theoretical plates) were evaluated (Table 2) and showed to be satisfactorily in accordance with the ICH guidelines. Furthermore, the peak area values were proportional/linear to levofloxacin at 14–26 μg/ml and a calibration curve $y = 1{,}043{,}159x - 48{,}636.8$ was used; for norfloxacin, peaks ranged from 140 to 260 μg/ml and ($y = 6{,}494{,}872x - 31{,}390.1$) was used as a calibration curve. In the both cases, a correlation coefficient (r^2) > 0.99 was obtained, as stipulated by the Food and Drug Administration (FDA 2017).

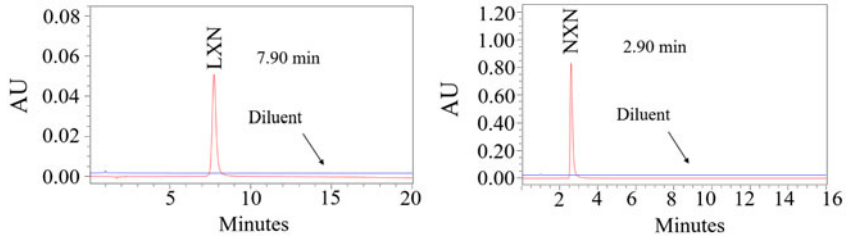

Figure 2. (Left): initial chromatography of levofloxacin standard (20 μg/ml) and its diluent (H_2O/acetonitrile 85:15 v/v); (right): norfloxacin standard (200 μg/ml), and its diluent (0.1% phosphoric acid/acetonitrile 85:15 v/v).

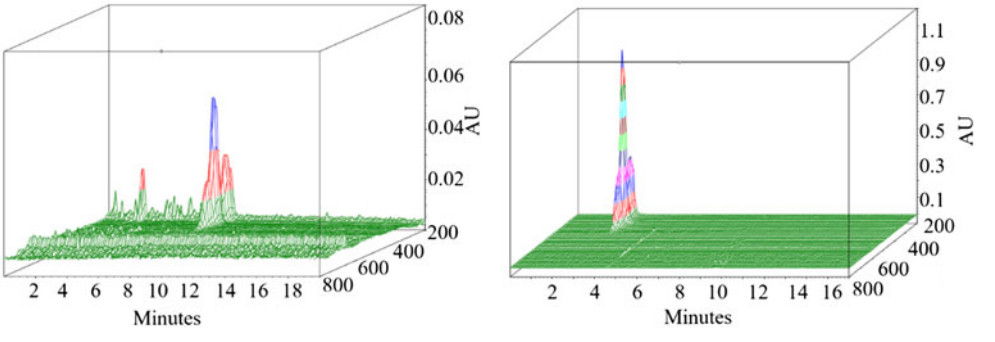

Figure 3. The HPLC-PAD fingerprint 3D spectrum: (left): levofloxacin standard (20 μg/ml); (right): norfloxacin standard (200 μg/ml).

3.2. Forced degradation studies – conventional versus microwave irradiation method

The synthetic antibiotics norfloxacin and levofloxacin (Figure 1) present some differences in structure; for instance, the substituent group is in the *ortho* position in the piperazine ring, and these groups are, more precisely, a methyl and hydrogen group in the two antibiotics, respectively. There is another difference at the eight positions in the quinolone ring, a methoxy and hydrogen group, for levofloxacin and norfloxacin, respectively (Soni 2012). In addition, structural particularities influence the action spectrum, and the pharmacokinetics of the drugs may also be involved in the performance of the compounds in the chemical reactions and consequently in the studies of forced degradation.

Forced degradation studies can help identify possible degradation products of a drug substance, hence giving appropriate information about the inherent stability of the compound. These studies are significant to identify the stability-indicating potential of the proposed analytical procedure (Maher et al. 2017). In this sense, we evaluated the use of microwave heating as an alternative method source to promote the forced degradation study of norfloxacin and levofloxacin, aiming the development of a greener methodology than conventional method (stove or hot plate), according

Figure 4. (a) CEM Corporation-Discover S-Class device; (b) 35 ml CEM reaction vessel.

Table 2. Chromatograph method parameters for levofloxacin and norfloxacin.

Drugs	Concentration ($\mu g\ ml^{-1}$)	CV[a] (%)	Theoretical plates[b]	Symmetry[c]	K′[d]	Retention time	Medium area[e]	Peak purity[f]
Levofloxacin	20.0	1.0	7720.7	1.11	5.36	7.90	980,274.2	TH: 0.644; PA: 0.350
Norfloxacin	200.0	0.8	4891.2	1.24	2.71	2.90	639,046.5	TH: 1.024; PA: 0.650

Reference values stipulated by ICH [34].
[a]CV%$\leq$2.0%.
[b]Theoretical plates $\geq$2000.
[c]Peak symmetry $\leq$2.0.
[d]K′$\geq$2.0.
[e]Average peak area (considering 100% drug) calculated from the calibration curve.
[f]Peak purity (TH > PA).

to green chemistry principles. Regarding the study of forced degradation using microwave as energy source, a *CEM Corporation-Discover S-Class* (Figure 4(a)) and 35 ml CEM reaction vessel (Figure 4(b)) were used.

The degradation studies of norfloxacin and levofloxacin were started in the acid hydrolysis conditions (0.1 M HCl; 60 °C; 24 h) and temperature (60 °C; 24 h) under conventional heating; and in acid hydrolysis conditions (0.1 M HCl; 300 W; 20 min) and temperature (300 W; 20 min; 100 °C) under microwave heating. After this procedure, a decrease on chromatographic peak areas when calculated and compared with the calibration curve was not observed. In addition, the formation of peaks from possible degradation products was observed (Table 3, entries 1 and 4). So, some apparent degradation was not observed, corroborating with Devi's study (Devi and Chandrasekharb 2009) which it was performed degradation/specific studies for levofloxacin using thermolysis conditions (at 100 °C for 5 d) and also some degradation products in response to the stress condition was not observed. In the same way, Chierentin and Nunes Salgado (2013) performed a thermolysis study (at 80 °C for 5 days) for norfloxacin, and no degradation products were observed.

In contrast, in a stress study under extreme acid hydrolysis conditions conducted by Zheng et al. (2014) at a concentration of 0.5 M HCl, it was possible to observe some degradation products from levofloxacin, and the relative areas of the total peaks reached 5% of the total chromatographic area after 20 d of exposure. In this study, the degradation products formed were identified by HPLC coupled with mass spectrum.

In the basic hydrolyses conditions, using the conventional method, 1.0 M NaOH was used for 24 h at 60 °C and no significant content reduction or the formation of degradation peaks from degradation products was observed (Table 3, entry 2). However, the drug levofloxacin was stable at 1.0 M NaOH for 24 h and 60 °C, in the conventional degradation method. Additionally, when the same samples was submitted under microwave irradiation in basic hydrolysis conditions (0.1 M; time: 20 min; potency: 300 W; temperature: 100 °C) degradations products were also not observed

Table 3. Forced degradation studies – conventional method versus microwave irradiation method.

		Levofloxacin		Norfloxacin	
Entry	Stress conditions	Conventional (%)	Microwave (%)	Conventional (%)	Microwave (%)
1	Acid hydrolysis	N/D[a]	N/D[e]	N/D[a]	N/D[e]
2	Basic hydrolysis	N/D[b]	N/D[f]	11.72[b]	10.79[f]
3	Oxidative	26.87[c]	27.22[g]	1.09[c]	1.12[g]
4	Thermolysis	N/D[d]	N/D[h]	N/D[d]	N/D[h]

N/D: nothing detected.
[a]Degradation solution: 0.1 M HCl; temperature: 60 °C (±5 °C); time: 24 h.
[b]Degradation solution: 1.0 M NaOH; temperature: 60 °C (±5 °C); time: 24 h.
[c]Degradation solution: 0.3%(v/v) H_2O_2; temperature: 60 °C (±5 °C); time: 24 h.
[d]Temperature: 60 °C (±5 °C); time: 24 h.
[e]Degradation solution: 0.1 M HCl; time: 20 min.
[f]Degradation solution: 0.1 M NaOH; time: 20 min.
[g]Degradation solution: 0.1% (v/v) H_2O_2; time: 20 min.
[h]Potency: 300 W; temperature: 100 °C; time: 20 min.
[i]Degradation solution: 3.0% (v/v) H_2O_2; temperature: 60 °C (±5 °C); time: 6 h.
[j]Degradation solution: 1.0% (v/v) H_2O_2; temperature: 100 °C; time: 20 dmin.

(Table 3, entry 2), and the same results were observed by Devi and Chandrasekharb (2009), in extreme basic conditions (0.5 M NaOH; time: 7 d; temperature: 70 °C).

Norfloxacin degradation under basic hydrolysis conditions in the conventional method (1.0 M NaOH; 60 °C; 24 h) led to a reduction of the drug peak area of 11.72%, observing the formation of two peaks that are probably derived from possible degradation products (PD1 and PD2) (Figure 5).

In the same way, after optimization conditions, basic hydrolysis conditions under microwave irradiation at 300 W, using pressure limits of 100 psi, at 100 °C for 20 min, provided the highest percentage of norfloxacin degradation, reducing 10.79% of the drug peak area. However, at the times of 5, 10, and 15 min, the norfloxacin peak area was reduced by 1.49, 3.02, and 6.97%, respectively (Figure 6).

The oxidative degradation condition using hydrogen peroxide as an oxidant agent proved to be the most efficient. In this case, the oxidant agent was used at

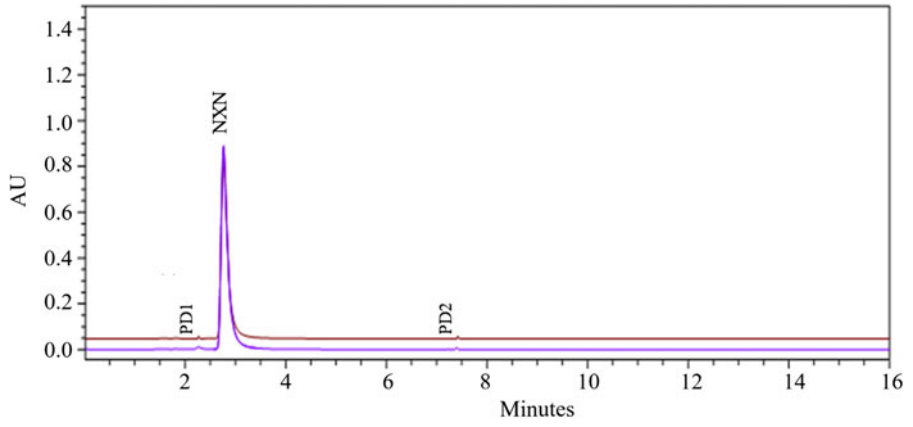

Figure 5. Norfloxacin degradation chromatogram by basic hydrolysis conventional method (1.0 M NaOH; temperature: 60 °C (±5 °C); time: 24 h).

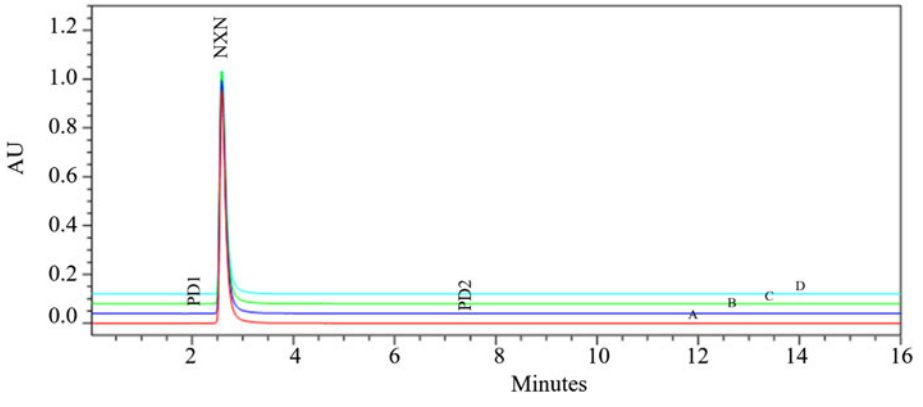

Figure 6. Norfloxacin degradation chromatogram by basic hydrolysis microwave method (0.1 M NaOH; time: 20 min) at the different times (A) 5 min; (B) 10 min; (C) 15 min; (D) 20 min.

concentrations of 0.3% (v/v), 60 °C for 24 h. A decrease in levofloxacin peak area of 26.87% (based on the initial concentration and calculated by calibration curve) was observed by the oxidative conventional method. In addition, there was a formation of a possible degradation peak with greater retention time (∼11.50 min) (Figure 7). Moreover, Zheng et al. (2014) and Chierentin and Nunes Salgado (2013) also demonstrated the formation of degradation products under oxidative conditions, confirming our forced degradation study, which also generated only a degradation product.

When the levofloxacin sample was submitted under microwave irradiation at 300 W, 100 psi pressure limit and 100 °C for 20 min in the presence of 0.1% H_2O_2 (v/v), a reduction was observed in the peak area of 9.05% at 5 min, 14.66% at 10 min, 20.39% at 15 min, and 27.22% at the final time of 20 min of exposure, thus reducing the levofloxacin concentration from 20.0 μg ml^{-1} to 14.6 μg ml^{-1} (calculated based on calibration curve) (Figure 8).

The chromatographic profile of impurities in microwave-assisted degradation processes was no different from that seen under conventional conditions (Table 3). Therefore, in accordance with the results from the microwave forced degradation studies, this method probably does not interfere in the degradation pathway of the molecule, since the chromatographic profile remains similar, despite the considerable reduction in the time of experiments, energy and amounts of reagents and solvents.

Regarding the utilization of microwave radiation as energy source, it is know that microwave is a very polarizing field and may stabilize polar transition states and intermediates in several types of organic reactions (La-Hoz et al. 2005). Moreover, thermal (e.g. hot spots, superheating) and non-thermal (e.g. molecular mobility, field stabilization) effects also have an strong influence on chemical reactions, in this context, both factors may have significant influence on forced degradation pathway with respect to selectivity and ratio of degradation products.

When norfloxacin was exposed to the oxidative condition (0.3% (v/v) H_2O_2 for 24 h under conventional method, a reduction of only 1.09% was observed (calculated based on the calibration curve), although two small signs appeared at ∼2 and 7 min (like those present in the basic hydrolysis condition) (Figure 9).

In both conditions, a peak appeared at 1.2 min, which practically discards the elution of any compound coming from norfloxacin, since the elution occurs in the dead time of the chromatographic column. However, it was considered that this signal is also present in the mobile phase chromatographic run of the norfloxacin mixed oxidative condition (1:1 v/v) without addition of norfloxacin (blank), suggesting that the signal in question is from the diluent itself and not from the drug. The norfloxacin has maximum absorption at 275 nm, allowing detection of this peak. So, in readings made with longer wavelengths (such as levofloxacin at 294 nm), the presence of this peak is not detected (Figure 10).

Compared with the conventional method, the oxidative condition 0.1% (v/v) H_2O_2 in microwave presented a decrease of 1.12% in the area corresponding to the norfloxacin peak within 20 min of exposure.

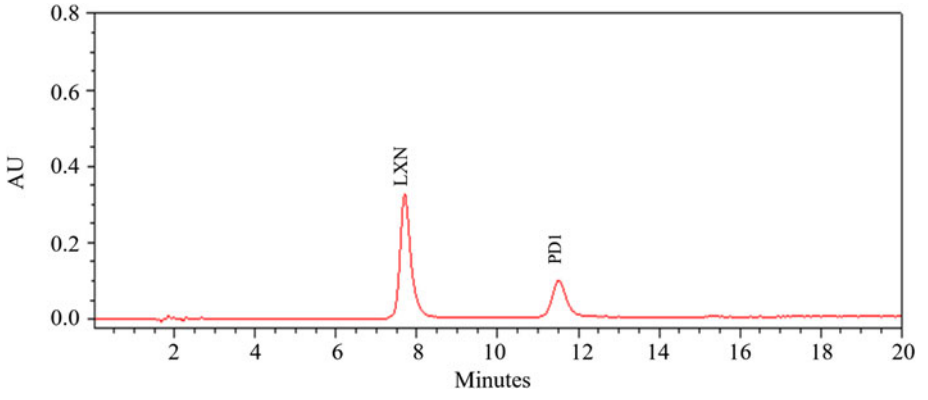

Figure 7. Levofloxacin degradation chromatogram by oxidative conventional method (0.3% (v/v) H_2O_2; 24 h and 60 °C).

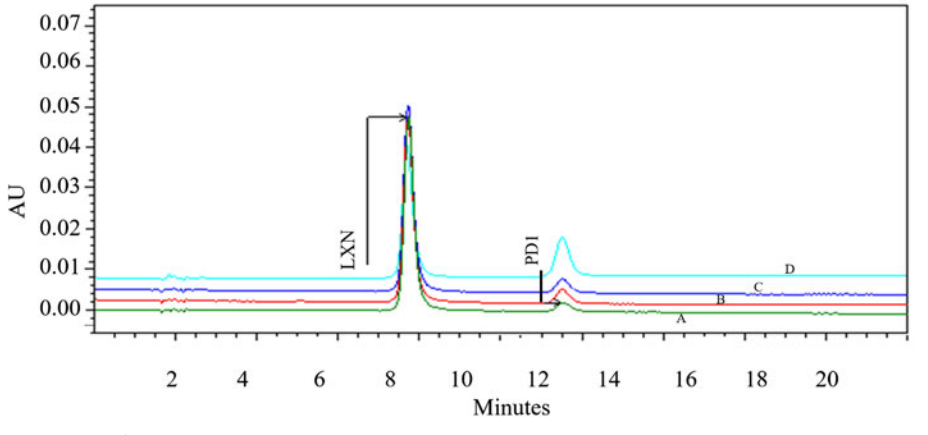

Figure 8. Levofloxacin degradation chromatogram by oxidative hydrolysis microwave method (0.3% (v/v) H_2O_2) at the different times: (A) 5 min; (B) 10 min; (C) 15 minutes; (D) 20 min.

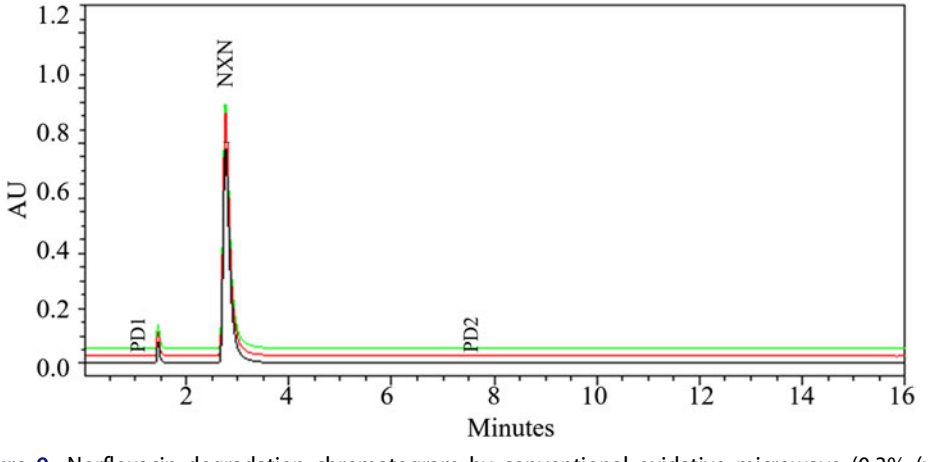

Figure 9. Norfloxacin degradation chromatogram by conventional oxidative microwave (0.3% (v/v) H_2O_2; 24 h and 60 °C).

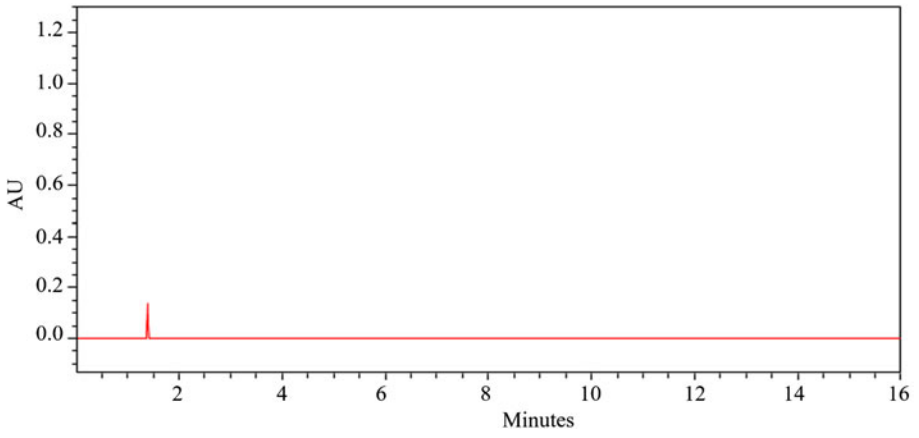

Figure 10. 1.0% (v/v) H_2O_2 diluent initial chromatogram.

3.3. Green analytical chemistry metrics – conventional method versus microwave irradiation method

3.3.1. Calculation of energy consumption (E)

The energy consumption (E) is described in the following table

Heating	Energy consumption (E) ($E = P \times t$), per sample
Conventional	E = 1500 W × 1440 min = 2,160,000 W/min or 36.0 kWh
Microwave irradiation	E = 300 W × 20 min = 6000 W/min or 0.1 kWh

According to the concept of green analytical chemistry (Anastas 1999), an ideal green analysis/method can be characterized by the elimination or minimal use of reagents, minimal energy use, and/or no waste generation. In this study, calculate of energy consumption was performed aiming to show the method that have a minor of energy consumption using Equation (1).

The microwave forced degradation method consumed 360 times less energy than the conventional method, and the time analysis necessary was 72 times less than the conventional method.

3.3.2. Calculation of analytical eco-scale

The basis for our concept of an analytical eco-scale is that the ideal green analysis has a value of 100 (*see supporting information*). Recently, in organic synthesis, the concept of eco-scale values has emerged as a more accurate tool for evaluating the 'eco-friendliness' of a given process, estimating the quality of the organic preparation based on yield, cost, safety, conditions, and ease of workup/purification (Anastas and Warner 1998; Anastas 1999; Gałuszka et al. 2012). But in another case, specifically in the analytical chemistry procedure, in the evaluation based on the amount of reagents, hazards, energy, and waste, penalty points are assigned if it departs from the ideal green analysis method. Because the influence of hazardous substances

depends on their amount, so the total penalty points should be calculated by multiplying the sub-total penalty points by a given amount and hazard.

In this study, analytical eco-scale was evaluated and compared with the conventional and microwave forced method for levofloxacin and norfloxacin in all degradation conditions (basic, acid hydrolysis, oxidative, and temperature) (Tables 4–7).

Comparing all the levofloxacin degradation conditions, the microwave forced degradation method showed to be the greenest methodology. It gained a high ranking on the eco-scale (82, 83, 82, and 85) for basic, acid, oxidative, and temperature conditions, respectively, and used the lowest amount of energy during the degradation process. By contrast, forced degradation using conventional heating had total penalty points (19, 19, 20, and 17) that resulted in the lowest ranking on the analytical eco-scale for basic, acid, oxidative and temperature conditions, respectively, and its use of energy (kWh) was 360 times more than the microwave method, 36 kWh for conventional heating against 0.1 kWh for microwave irradiation.

Norfloxacin-forced degradation using conventional and microwave method also showed that the new microwave method is the greenest. Its high ranking on the

Table 4. The penalty points (PPs) for levofloxacin conventional forced degradation method and analysed by HPLC.

Reagents							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis stove	Penalty points
Standard Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2
HCl (37%): 49.32 mg	2	NaOH: 20 mg	1	H_2O_2 (30%): 0.05 ml	2	H_2O: 92.65 ml	2
H_2O: 100.65 ml	3	H_2O: 101.05 ml	3	H_2O: 101.00 ml	3	–	–
Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.35 ml	4
	11		**10**		**11**		**8**
Instruments							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis Stove	Penalty points
Stove (forced degradation) >1.5 kWh per sample	2	Stove (forced degradation) >1.5 kWh per sample	2	Greenhouse (forced degradation) >1.5 kWh per sample	2	Stove (forced degradation) >1.5 kWh per sample	2
LC	2	LC	2	LC	2	LC	2
Occupation hazard	0	Occupation hazard	0	Occupation hazard	0	Occupation hazard	0
Waste	5	Waste	5	Waste	5	Waste	5
	9		**9**		**9**		**9**
Total penalty points	**19**	Total penalty points	**19**	Total penalty points	**20**	Total penalty points	**17**
Analytical eco-scale score	**81**	Analytical eco-scale score	**81**	Analytical eco-scale score	**80**	Analytical eco-scale score	**83**

Table 5. The penalty points (PPs) for norfloxacin conventional forced degradation method and analysed by HPLC.

Reagents							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis stove	Penalty points
Standard Norfloxacin: 200 mg	1	Norfloxacin: 200 mg	1	Norfloxacin: 200mg	1	Norfloxacin: 200mg	1
HCl (37%): 49.32 mg	2	NaOH: 20 mg	1	H_2O_2 (30%): 0.05 ml	2	–	–
H_2O: 104.48 ml	3	H_2O: 101.05 ml	3	H_2O: 101.00 ml	3	H_2O: 92.65 ml	2
Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.35 ml	4
Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2
	12		**11**		**12**		**9**
Instruments							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis Stove	Penalty points
Stove (forced degradation) >1.5 kWh per sample	2	Stove (forced degradation) >1.5 kWh per sample	2	Greenhouse (forced degradation) >1.5 kWh per sample	2	Stove (forced degradation) >1.5 kWh per sample	2
LC	2	LC	2	LC	2	LC	2
Occupation hazard	0	Occupation hazard	0	Occupation hazard	0	Occupation hazard	0
Waste	5	Waste	5	Waste	5	Waste	5
	9		**9**		**9**		**9**
Total penalty points	**21**	Total penalty points	**20**	Total penalty points	**21**	Total penalty points	**18**
Analytical eco-scale score	**79**	Analytical eco-scale score	**80**	Analytical eco-scale score	**79**	Analytical eco-scale score	**82**

eco-scale (81, 82, 81, and 84) for basic, acid, oxidative, and temperature conditions, respectively, results from the lowest energy used during the degradation process. In contrast, forced degradation using conventional heating had total penalty points (21, 20, 21, and 18) that resulted in the lowest ranking on the analytical eco-scale for basic, acid, oxidative, and temperature conditions, respectively, and its utilization of energy (kWh) was 360 times more than the microwave method, similar to what was seen for levofloxacin degradation conditions. According to their analytical eco-scale rank, both methods represent excellent green forced degradation, but the microwave forced degradation method consumed 360 times less energy than the conventional method and also its analysis took 72 times less time than the conventional method. It is important to recall that the ideal green analysis can be characterized by elimination or minimal use of reagents, minimal energy use, and no waste generation. Therefore, the utilization of the forced degradation method by microwave will provide a greatly reduced analysis time and amount energy used by the pharmaceutical industry; therefore, it will contribute to sustainable analytical development.

Table 6. The penalty points (PPs) for levofloxacin microwave forced degradation method and analysed by HPLC.

Reagents							
Acid hydrolyses	Penalty points	Basic hydrolyses	Penalty points	Oxidative	Penalty points	Thermolysis Stoves	Penalty points
Standard Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2	Levofloxacin: 20 mg	2
HCl (37%): 49.32 mg	2	NaOH: 2 mg	1	H_2O_2 (30%): 0.02 ml	2	–	–
H_2O: 100.65 ml	3	H_2O: 101.05 ml	3	H_2O: 101.00 ml	3	H_2O: 92.65 ml	2
Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.35 ml	4
	11		**10**		**11**		**8**
Instruments							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis stove	Penalty points
Microwave (forced degradation 0.1 kWh per sample)	0	Microwave (forced degradation 0.1 kWh per sample)	0	Microwave (forced degradation 0.1 kWh per sample)	0	Microwave (forced degradation 0.1 kWh per sample)	0
LC	2	LC	2	LC	2	LC	2
Occupation hazard	0	Occupation hazard	0	Occupation hazard	0	Occupation hazard	0
Waste	5	Waste	5	Waste	5	Waste	5
	7		**7**		**7**		**7**
Total penalty points	**18**	Total penalty points	**17**	Total penalty points	**18**	Total penalty points	**15**
Analytical eco-scale score	**82**	Analytical eco-scale score	**83**	Analytical eco-scale score	**82**	Analytical eco-scale score	**85**

4. Conclusion

A new, eco-friendly and alternative method of forced degradation for levofloxacin and norfloxacin by microwave is reported. The possibility of clean, inexpensive, and easy-to-use technology makes it easy to run, but it is just a first step in the search for new technologies. The use of microwave irradiation proposed for stress studies was feasible, since the chromatographic profile was very like those obtained in the results of conventional method. At no time, did the same condition generate different peaks of possible degradation products or impure peaks for the active input when transferred to the microwave reactor, confirming the safety, and reproducibility of the new methodology. It should also be noted that for microwave degradation tests, small amounts of samples were sufficient to perform such tests, proving to be a positive factor, since it provides material savings, reagents and, consequently, emission of waste. For both levofloxacin and norfloxacin, the conditions used were similar and sufficient to generate the desired degradation for both drugs, even for different drugs. The variables of the equipment (power, temperature, pressure, and time) offer a range of possibilities that should vary per the degradation kinetics of each drug. In this way, it is possible to generate libraries of stress studies, facilitating their realization in future research. Finally, among the characteristics of studies of microwave-assisted

Table 7. The penalty points (PPs) for norfloxacin microwave forced degradation method and analysed by HPLC.

Reagents							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis stove	Penalty points
Standard Norfloxacin: 200 mg	1	Norfloxacin: 200 mg	1	Norfloxacin: 200 mg	1	Norfloxacin: 200 mg	1
HCl (37%): 49.32 mg	2	NaOH: 2 mg	1	H_2O_2 (30%): 0.02 ml	2	–	–
H_2O: 104.48 ml	3	H_2O: 104.88 ml	3	H_2O: 104.86 ml	3	H_2O: 99.88 ml	2
Acetonitrile: 16.95ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.95 ml	4	Acetonitrile: 16.35 ml	4
Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2	Phosphoric acid (85%): 0.12 ml	2
	2		**11**		**12**		**9**
Instruments							
Acid hydrolysis	Penalty points	Basic hydrolysis	Penalty points	Oxidative	Penalty points	Thermolysis stove	Penalty points
Microwave (forced degradation 0.1 kWh per sample	0	Microwave (forced degradation 0.1 kWh per sample	0	Microwave (forced degradation 0.1 kWh per sample	0	Microwave (forced degradation 0.1 kWh per sample	0
LC	2	LC	2	LC	2	LC	2
Occupation hazard	0	Occupation hazard	0	Occupation hazard	0	Occupation hazard	0
Waste	5	Waste	5	Waste	5	Waste	5
	7		**7**		**7**		**7**
Total penalty points	**19**	Total penalty points	**18**	Total penalty points	**19**	Total penalty points	**16**
Analytical eco-scale score	**81**	Analytical eco-scale score	**82**	Analytical eco-scale score	**81**	Analytical eco-scale score	**84**

forced degradation, what stands out is the reduction in the time taken (72 times less than conventional method), in the energy used (360 times less than conventional method) and in reagents/solvents needed.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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