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Lean and eco-efficient method for evaluating the potency of tinidazole in tablets

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Tinidazole (TIN), an amoebicide and giardicide, does not present microbiological methods in official compendia for evaluating the potency of final products. The objective of this work is to develop and validate an effective, lean and eco-efficient microbiological turbidimetric method by National Environmental Method Index (NEMI) and Eco-Scale Assessment (ESA) to evaluate the potency of TIN-based tablets. The microbiological method was performed using *Escherichia coli* ATCC 25922 at 1 % in BHI broth, TIN solution in purified water and ethanol (90:10, v/v) at concentrations of 40, 60 and 90 μg mL^{-1}, shaker at 80 rpm, 4 hours of incubation, quartz cuvette and 530 nm. The method was linear from 40 to 90 μg mL^{-1} with a correlation coefficient of 0.9991, selective, precise (RSD < 4 %), accurate with 99.65 % recovery and robust to changes in culture medium volume, culture medium brand, inoculum percentage and shaker rotation speed. The potency of TIN tablets was 98.50 %. The greenness of the method was evaluated and NEMI presented 3 green quadrants and the ESA score was 93, showing an excellent green analysis option. This work presents a lean and eco-efficient proposal for evaluating the potency of TIN tablets for chemical-pharmaceutical laboratories around the world.

Keywords: microbiological method, antimicrobial potency, tinidazole, tablets, Green Analytical Chemistry.

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Introduction

Tinidazole (TIN, Figure 1A) is a drug derived from the imidazole compound replaced by 5-nitroimidazole, has amoebicidal and giarcidal activity and is used against anaerobic bacteria. It has the formula $C_8H_{13}N_3O_4S$ and molecular weight 247.27 g mol^{-1}. TIN appears as colorless crystals, has a melting range between 125 and 128 °C, has low solubility in water <0.1 g/L and is more soluble in organic solvents such as ethanol, propanol, butanol and methanol. Complete absorption of TIN occurs rapidly after oral administration with a half-life of approximately 12 to 14 hours, being excreted mainly in urine and little in feces in unchanged form or in the form of metabolites (1-3).

Chemical and biological methods can be used to quantify TIN in tablets, and the United States Pharmacopeia (4) determines that the dosage of TIN tablets should be performed by high-performance liquid chromatography (HPLC).

Microbiological methods for TIN are not described in the official compendia, but the advantage of these methods is that they are used to determine both potency and bioactivity against microorganisms, because although subtle changes in antimicrobial activity can be demonstrated by chemical methods, the loss of antibiotic activity against microorganisms can only be determined by standard methods, such as the microbiological test (4). Furthermore, they do not use toxic reagents, following Green Analytical Chemistry (GAC).

Currently, analysis methods follow the line of GAC, being fast, without using or reducing the use of toxic solvents, with miniaturized samples, reduction in the number of steps and pre-treatments (5-13). However, the analytical methods for evaluating TIN present in the literature (14-29) and official compendia (4, 30-33) do not show a green and sustainable analytical method by National Environmental Method Index (NEMI) and Eco-Scale Assessment (ESA) for quantifying TIN tablets or even any microbiological method by agar diffusion or turbidimetry.

NEMI and ESA are tools to objectively evaluate the greenness of an analytical method. NEMI is a semi-quantitative tool represented by 4 quadrants and each one corresponds to a purpose (toxicity of reagents, chemicals used, pH and waste generated). ESA is a quantitative tool represented by an equation that penalizes choices that do not comply with GAC (reagents used, quantity used, risk words assigned to them, energy used, occupational risks and waste generation) (34-39).

Therefore, this work focuses on the development and validation of an effective, lean and eco-efficient microbiological method by turbidimetry, which follows the requirements of GAC for the evaluation of the potency of TIN in tablets in order to contribute to a sustainability cycle, in which the guarantee of product quality and analytical awareness about time, waste generation and cost coexist.

Experimental section

Material

The reference was TIN, with a content of 100 % (USP) and the samples were TIN tablets containing 500 mg (labeled content).

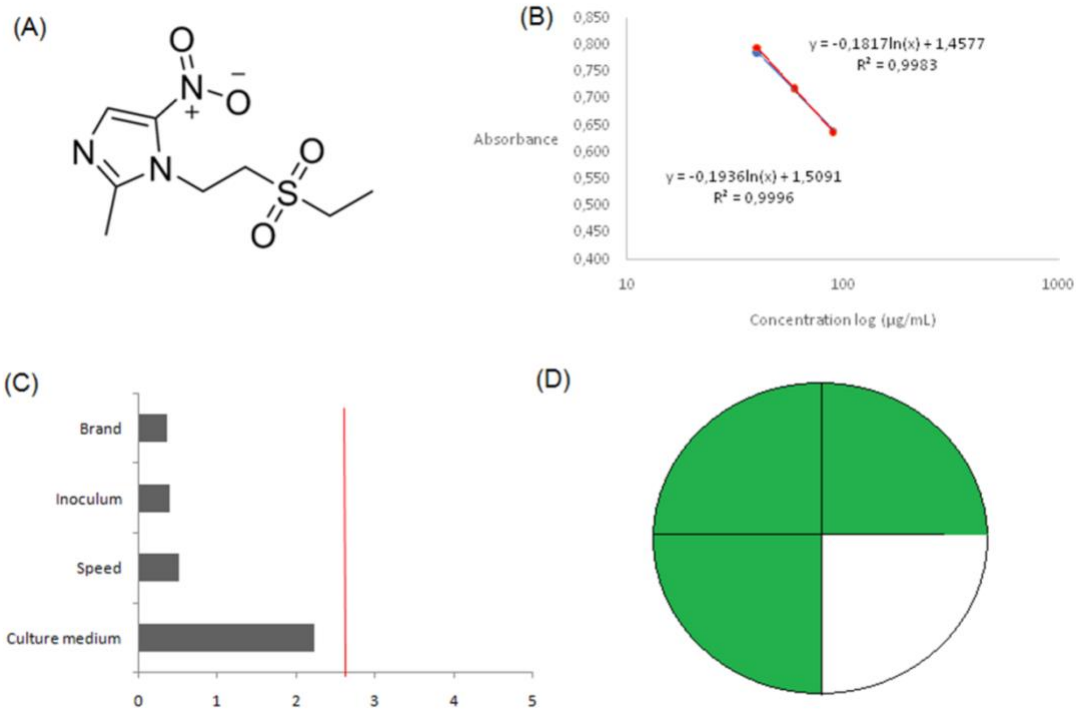

Figure 1. (A) Chemical structure of tinidazole (CAS 19387-91-8). (B) Linear dependence of TIN reference (blue) and TIN sample (red) at concentrations of 40, 60 and 90 µg mL^{-1}. (C) Pareto chart for studying the robustness of the method. (D) NEMI result.

Equipment

Analytical balance (Radwag®, Poland), vertical autoclave (Phoenix Luferco®, Brazil), drying and sterilization oven (Fanem®, Brazil), incubation oven for microbial growth (Quimis®, Brazil), shaker incubator (Tecnal®, Brazil). The readings were taken using quartz cuvettes (Qualividros®, Brazil) at a wavelength of 530 nm in a spectrophotometer (Spectrumlab®, New Zealand).

Microorganisms

Microorganisms *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were tested. They were cultured in tilted tubes containing 7 mL of BHI agar (Kasvi®, Paraná), prepared according to the manufacturer's instructions. The culture media were distributed into the tubes using a manual pipettor and then the tubes were subjected to wet sterilization in a vertical autoclave. After the sterilization process, the tubes were tilted and left to cool. So, the microorganism was transferred to the tubes using a laminar flow and then taken for incubation in a bacteriological oven at a controlled temperature of 35 ºC ± 2 ºC, for a period of 24 hours, to allow growth. After the end of incubation, the tubes were kept refrigerated for preservation.

Every 30 days, a new culture was carried out by transferring it to tubes with recently prepared tilted agars, thus ensuring the maintenance and continuity of the culture.

Culture medium

BHI broth culture medium (Himedia®, India) was prepared with purified water, following the manufacturer's instructions. After preparation, the culture medium was submitted to wet sterilization in an autoclave at 121 ºC at 1 atm for 15 minutes.

TIN solutions

A stock solution of TIN at a concentration of 200 µg mL^{-1} was prepared. 10 mg were weighed to prepare the reference TIN and 13.1 mg of a sample pool (equivalent to 10 mg of TIN considering the average weight of 654.83 mg) was weighed to prepare the sample TIN and transferred to a 50 mL volumetric flask and the volume was completed with purified water and ethanol (90:10, v/v). The assessment of the impact of 10 % ethanol was carried out previously in the development of the method and showed no activity. Different concentrations were prepared from this stock solution, 40, 60 and 90 µg mL^{-1}.

Turbidimetric method

The design used was 3x3, in which 3 concentrations of reference solution and 3 concentrations of sample solution are used. Concentrations used were 40, 60 and 90 µg mL^{-1}. In each analysis, 20 tubes were prepared, 3 for the concentrations of the reference solution in triplicate (10 mL culture medium + 100 µL inoculums + 200 µL TIN solution), 3 for the concentrations of the sample solution in triplicate (10 mL culture medium + 100 µL inoculums + 200 µL TIN solution), 1 for the positive control (10 mL culture medium + 100 µL inoculum) and 1 for the negative control (10 mL culture medium).

The inoculum was prepared 24 hours before use. BHI broth was used together with a thin slide of microorganism. After the activation and proliferation period, the inoculum was standardized to 25 % ± 2 % transmittance in a spectrophotometer.

The tubes were incubated in a shaker at 35 ºC ± 2 ºC for 4 hours and after this time, the turbidity was read using a spectrophotometer at 530 nm.

Method development

Some conditions were tested to find the most appropriate response of percentage and type of microorganism, drug concentrations, type of culture medium, shaker rotation and temperature, as shown in Table 1.

Table 1. Parameters tested in method development

Parameters	Description
Microorganisms	*Staphylococcus aureus* ATCC 25923 *Escherichia coli* ATCC 25922
Culture medium	BHI, Tioglicolato, Caseína soja, Ágar *Sabouraud*
Diluent solutions	Purified water BHI broth
Inoculum concentration (%)	1 %; 5 %; 10 %
TIN concentrations (µg mL^{-1})	2, 10, 50; 10, 20, 40; 40, 60, 90; 50, 100, 200; 90, 180, 360; 50, 150, 450; 50, 200, 800; 25, 75, 225

Method validation

The validation of the method was proven by linearity, selectivity, precision, accuracy and robustness parameters (33, 40-43).

Linearity and selectivity

Linearity and selectivity, differently from physicochemical methods, were performed concomitantly using reference and sample TIN solutions. The analytical curves were performed on 3 different days and in triplicate using concentrations 40, 60 and 90 µg mL^{-1}. The turbidity values of each tube were obtained in absorbance. The data were evaluated using the least squares method, correlation coefficient and ANOVA.

Precision

Precision was proven at 3 levels in sextuplicate: intraday (same analyst, same day and same analytical conditions), interday (same analyst, different days and same analytical conditions) and interanalyst (different analysts, different days and same analytical conditions). The response of the 60 µg/mL concentration was evaluated by relative standard deviation (RSD %).

Accuracy

Accuracy was proven by standard recovery test, in which known amounts of standard are added and recovered from the sample. 3 levels in triplicate were used, 80 % (48 µg mL^{-1}), 100 % (60 µg mL^{-1}) and 120 % (72 µg mL^{-1}).

From a stock solution at 200 µg mL^{-1}, known volumes of the standard solution of 0.4, 1 and 1.6 mL were added to a 10 mL flask containing the 2 mL volume of sample solution to obtain the concentrations of 48 µg mL^{-1} corresponding to the 80 % level, a concentration of 60 µg mL^{-1} corresponding to the 100 % level and a concentration of 72 µg mL^{-1} corresponding to the 120 % level. From the absorbance value obtained, the accuracy was analyzed through the recovery values.

Robustness

Robustness was studied by the impact of small modifications to the method. The modifications made were: volume of culture medium in the tube = 9.8 mL (modified) and 10 mL (normal); shaker rotation speed = 78 rotations per minute (modified) and 80 rotations per minute (normal); inoculum percentage = 1.2 % (modified) and 1 % (normal); BHI broth brand = Oxoid® (modified) and Kasvi® (normal).

Product potency analysis

The potency of TIN tablets was evaluated during the linearity and selectivity parameters, comparing the absorbance values obtained. Potency values were obtained in triplicate on three different days.

Method greenness assessment

NEMI parameters (a) persistent, bio-accumulative and toxic (PBT); (b) hazardous; (c) corrosive and (d) waste were evaluated.

ESA penalty points (PP) were calculated, according to the Equation 1.

$$ESA = (100 - PP) \qquad \text{Equation 1}$$

PP = (chemical reagents pictogram x quantity of reagents x signal words) + energy + ocupacional hazard + (waste amount x waste characteristic)

Results and Discussion

Method development

The combinations shown in Table 1 were tested and the only one that presented a correlation coefficient greater than 0.99 and non-significant results for Parallelism, Quadratic and Quadratic difference and significant for Regression was the combination: *Escherichia coli* ATCC 25922 at 1 % in BHI broth, TIN solution at concentrations of 40, 60 and 90 µg mL^{-1}, shaker at 80 rpm and 35 ºC ± 2 ºC.

Method validation

Linearity and selectivity

The equations of the line for TIN reference and sample were, respectively, y = -0.1817ln(x)+1.4577 with correlation coefficient (r) 0.9991 and y = -0.1936ln(x)+1.5091 with r 0.9998 (Figure 1B). The Analysis of Variance (ANOVA) was calculated from the data of the analytical curves and the method can be considered linear and selective, since the linearity deviation and parallelism parameters were not significant (Table 2).

Precision

The precision of the method at the 3 levels, shown in Table 3, shows RSD less than 15 %, which proves the parameter (33, 40).

Accuracy

The accuracy of the method was proved by the standard recovery test. The results are presented in Table 4 and are within the specification of 98 to 102 %, according to legislation for pharmaceutical analysis (40-41).

Table 2. Assessment of the linearity of the method by ANOVA

Sources of Variation		GL	SQ	QM	F_{cal}	$F_{tab.}$
Linearity deviation	**Preparation**	1	0.00	0.00	1.36	4.96
	Regression	1	0.07	0.07	2961.52*	4.96
	Parallelism	1	0.00	0.00	2.99	4.96
	Quadratic	1	0.00	0.00	2.84	4.96
	Quadratic difference	1	0.00	0.00	0.27	4.96
	In between doses	5	0.07	0.01	593.80*	3.33
	Between wells	2	0.00	0.00	1.88	4.10
	Inside (error)	10	0.00	0.00	……	……
	Total	17	0.07	……	……	……

*Significant at a 95 % confidence level

Table 3. Values obtained in the study of the precision of the method

Level	Absorbance						RSD (%)
	1	2	3	4	5	6	
Intraday	0.747	0.731	0.721	0.726	0.797	0.753	3.75
Interday	0.742	0.721	0.792	0.783	0.771	0.782	3.71
	0.737	0.716	0.756	0.745	0.714	0.725	
Interanalyst	0.747	0.731	0.721	0.726	0.797	0.753	3.45
	0.756	0.707	0.735	0.709	0.717	0.755	

Table 4. Standard recovery test result to evaluate the accuracy of the method

Level	Recovery*	Recovery average (%)	RSD (%)**
Recovery 1 (80 %)	99.73		
Recovery 2 (100 %)	99.79	99.65	1.03
Recovery 3 (120 %)	99.44		

*Average of 3 determinations; **RSD = relative standard deviation

Robustness

The method was robust to the proposed modifications, as shown in Figure 1C. Volume of culture medium in the tube, shaker rotation speed, inoculum percentage and change in the brand of BHI broth presented $t_{calculated}$ of 2.22, 0.51, 0.38 and 0.36, respectively, which are smaller than the $t_{tabulated}$ of 2.78.

Product potency analysis

The potency result of TIN tablets was 98.50 % (Table 5). However, it cannot be compared with any monograph specification, since the TIN in tablets does not have a monograph described in official compendia to evaluate the potency of the final product by microbiological method (4, 30-33). In this context, it is important to state the obvious, for antimicrobial products, physicochemical methods are not always sufficient, which makes microbiological methods essential when making decisions about the quality of these products. These facts make the developed and validated method even more pertinent for the scientific community and chemical-pharmaceutical laboratories around the world, which evaluate the quality of TIN in tablets.

Table 5. Application of the method developed to evaluate the potency of TIN tablets

Day	Potency (%)*	Average (%)	RSD (%)**
1	100.06		
2	97.90	98.50	1.38
3	97.54		

*Average of 3 determinations; **RSD = relative standard deviation

Method greenness assessment

National Environmental Methods Index (NEMI) and Eco-Scale Assessment (ESA)

NEMI (Figure 1D) showed 3 green quadrants, (a) because it does not use persistent, bio-accumulative and toxic reagents, (b) because it does not use harmful chemicals and (c) because the average pH of the solutions is in the range of 2 to 12. As it is a microbiological method, culture media are used and the residue generated ends up being greater than 50 g.

According to ESA, the greenness of an analytical process is classified into the following categories: > 75 represents excellent Green analysis; > 50 represents acceptable Green analysis; < 50 represents inadequate Green analysis. The proposed method is considered an excellent green analysis, since the greenness of the analytical process was 93, as shown in the Equation 1.

$$ESA = 100 - [(1 \text{ x } 1 \text{ x } 2) + 0 + 0 + (5 \text{ x } 1)]$$

$$ESA = 100 - 7 = 93$$

In addition to proving the validation and greenness of the proposed method, it also adds advantages such as (i) less waste generation, (ii) optimized and clean sample preparation (purified water and ethanol, 90:10, v/v) (iii) no need to use formaldehyde as it is generally used in the

turbidimetric method, (iv) lower volume of glassware used compared to agar diffusion and (v) shorter incubation time compared to agar diffusion, which requires 24 hours.

Conclusions

The turbidimetric microbiological method was developed and validated to evaluate the potency of TIN tablets in a lean and reliable way. The method was linear (40 to 90 µg mL^{-1}), selective, precise (RSD < 4 %), accurate (99.65 %), robust and eco-efficient by NEMI and ESA (93). The method developed is an excellent option for use in routine analyzes in chemical-pharmaceutical laboratories for evaluating final TIN products.

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Conflict of interest

The authors declare no conflicts of interest.

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Not applicable.

Supplementary sections

Not applicable.

References

1. Abiose PA, Olupitan SB, Yousuf M. Tinidazole in the treatment of amoebic liver abscess. CTR 1976; 20:32-35.
2. Dickey LJ, Nailor MD, Sobel JD. Guidelines for the treatment of bacterial vaginosis: focus on tinidazole. Ther Clin Risk Manag 2009; 5:485-489.
3. Sousa JC, Kogawa AC. Overview of analytical methods for evaluating tinidazole. J AOAC Int 2023; 106:309-315.
4. United States Pharmacopoeia (USP), USP 43-NF 38, The United States Pharmacopeial Convention, Rockville, 2020.
5. Anastas PT. Green chemistry and the role of analytical methodology development. Crit Rev Anal Chem 1999; 29:167-175.
6. Kogawa AC, Salgado HRN. Golden Age of Green Chemistry. EC Microbiol 2017: 12:52-54.
7. Kogawa AC, Salgado HRN. Analytical Methods: Where do we stand in the current environmental scenario? EC Microbiol 2017; 13:102-104.
8. Marco BA, Rechelo BS, Totoli EG, Kogawa AC, Salgado HRN. Evolution of green chemistry and its multidimensional impacts: A review. Saudi Pharm. J. 2019; 27:1-8.
9. Pedroso TM, Kogawa AC, Salgado HRN. Validation and routine application of a versatile bioassay for quantification of ertapenem sodium. Res. Rev.: J. Pharm. Toxicol. Stud. 2018; 6:1-9.
10. Nascimento PA, Kogawa AC, Salgado HRN. Turbidimetric method: a new, ecological and fast way for evaluation of vancomycin potency. J AOAC Int 2020; 103:1582-1587.
11. Correa CBM, Kogawa AC, Chorilli M, Salgado HRN. Miniaturized microbiological method to determine the potency of rifaximin in tablets. J AOAC Int 2021; 104:1049-1054.
12. Richardi JF, Kogawa AC, Belavenuto EGT, Chorilli M, Salgado HRN. Ecological and miniaturized biological method for analysis of daptomycin potency. J AOAC Int 2021; 104:466-471.
13. Ferreira RGL, Da Silva Júnior JR, Torres IMS, Kogawa AC. Fast and new microbiological method for evaluating the potency of marbofloxacin-based tablets. J AOAC Int 2023; 106:690-694.
14. Nagaraja P, Sunitha KR, Vasantha RA, Yathirajan HS. Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations. J Pharm Biomed Anal 2002; 28:527-535.
15. Salo JPK, Salomies H. Two stability-indicating UV spectrophotometric methods for the analysis of hydrolyzed tinidazole. J Pharm Biomed Anal 2003; 31:523-536.
16. Adegoke O, Umoh O. A new approach to the spectrophotometric determination of metronidazole and tinidazole using p-dimethylaminobenzaldehyde. Acta Pharm 2009; 59:407-419.
17. Meshram DB, Bagade SB, Tajne MR. Simple HPLC method for simultaneous estimation of fluconazole and tinidazole in combined dose tablet. J Chromatogr Sci 2009; 47:885-888.
18. Abou-Taleb NH, El-Sherbiny DT, El-Wasseef DR, El-Enin MAA, El-Ashry SM. Digitally enhanced thin layer chromatography for simultaneous determination of norfloxacin and tinidazole with the aid of Taguchi orthogonal array and desirability function approach: Greenness assessment by analytical Eco-Scale. Int J Biomed Sci 2011; 7:137-144.
19. Singh L, Nanda S. Method for determination of tinidazole using direct UV-visible spectrophotometry and differential spectrophotometry in pure and tablet dosage forms. East Cent Afr J Pharm Sci 2011; 14:75-80.
20. Lotfy HM, Abdel-Monem HM. Comparative study of novel spectrophotometric methods manipulating ratio spectra: An application on pharmaceutical ternary mixture of omeprazole, tinidazole and clarithromycin. Spectrochim Acta A 2012; 96:259-270.
21. Darwish KM, Salama I, Mostafa S, El-Sadek M. Extractional spectrophotometric analysis of metronidazole, tinidazole, ornidazole and secnidazole bases through acid-dye complexation using bromothymol blue dye. J Chromatogr Sci 2013; 51:566-576.

22. Yin Z, Suying MA, Wang J, Shang X. Simultaneous determination of matrine and tinidazole in compound lotion by RH-HPLC method. J Anal Methods Chem 2013; 2013:1-5.
23. Lotfy HM, Hegazy MA, Mowaka S, Mohamed EH. Validated spectrophotometric methods for simultaneous determination of omeprazole, tinidazole and doxycycline in their ternary mixture. Spectrochim Acta A 2016; 153:321-332.
24. Kamal AH, El-Malla SF. Mixed micellar liquid chromatographic method for simultaneous determination of norfloxacin and tinidazole in pharmaceutical tablets. Microchem J 2019; 150:104-151.
25. Naguib IA, Abdelaleem EA, Hassan ES, Ali NW. HPTLC method for simultaneous determination of norfloxacin and tinidazole in presence of tinidazole impurity. J Chromatogr Sci 2019; 57:81-86.
26. Dadhich MB, Rakesh GMR, Agarwal D. Simultaneous determination tinidazole, clarithromycin and lansoprazole of in tablets by HPLC. IJARW 2020; 2:9-14.
27. Hemdan A, Mahmoud EN, Tawfik ED, Ibrahim EH. Digitally enhanced thin layer chromatography for simultaneous determination of norfloxacin and tinidazole with the aid of Taguchi orthogonal array and desirability function approach: Greenness assessment by analytical Eco-Scale. J Sep Sci 2020; 43:1195-1202.
28. Ayhan DG, Aslan SS. Simultaneous determination of tinidazole and lidocaine in pharmaceutical preparations by HPLC with photodiode array detection. J Pharm Res Int 2021; 33:10-16.
29. Dos Santos Galvão NS, Kogawa AC. Eco-friendly method by Eco-Scale Assessment for quantification of tinidazole in tablets. Green Anal Chem 2024; 10:100139.
30. European Pharmacopeia 2019, Council of Europe, Strasbourg, 2019.
31. British Pharmacopoeia 2021, The Stationery Office, London, 2021.
32. Japanese Pharmacopeia 2021, Tokyo Society of Japonese Pharmacopoeia, Tokyo, 2021.
33. Farmacopeia Brasileira, 6th ed.; ANVISA: Brasília, 2019.
34. Gałuszka A, Migaszewski ZM, Konieczka P, Namieśnik J. The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices. TrAC 2012; 37:61-72.
35. Tobiszewski M, Marc M, Gatuska A, Namiesnik JG. Green chemistry metrics with special reference to green analytical chemistry. Molecules 2015; 20:10928-10946.
36. Mohamed D, Fouad MM. Application of NEMI, Analytical Eco-Scale and GAPI tools for greenness assessment of three developed chromatographic methods for quantification of sulfadiazine and trimethoprim in bovine meat and chicken muscles: Comparison to greenness profile of reported HPLC methods. Microchem J 2020; 157:104873-104886.
37. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE-Analytical GREEnness metric approach and software. Anal. Chem. 2020; 92:10076-10082.
38. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. TrAC 2021; 138:116223.
39. Sinzervinch A, Torres IMS, Kogawa AC. Tools to evaluate the eco-efficiency of analytical methods in the context of green and white analytical chemistry: a review. Curr.Pharm.Des. 2023; 29:2442-2449.
40. Horwitz W, Kamps LR, Boyer KW. Quality assurance in the analysis of foods for trace constituents. J AOAC Int 1980; 63:1344-1354.
41. Association of Official Analytical Chemists: Official Methods of Analysis, 15th ed., AOAC: Gaithesburg, 2002.
42. Agência Nacional de Vigilância Sanitária (ANVISA); Resolução da Diretoria Colegiada (RDC) No. 27, de 17 de maio de 2012, Dispõe sobre os requisitos mínimos para a validação de métodos bioanalíticos empregados em estudos com fins de registro e pós-registro de medicamentos; Diário Oficial da União (DOU), Brasília. [https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0027_17_05_2012.html] accessed in September 2024
43. Lustosa IA, Gil ES, Kogawa AC. Analytical aspects for evaluation of pharmaceutical products: a mini-review. Curr Pharm Anal 2022; 18:909-918.