BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 45, n. 2, abr./jun., 2009

Article

Development and validation of an electroanalytical methodology for determination of isoniazid and rifampicin content in pharmaceutical formulations

Katia Christina Leandro*[1], Juliana Machado de Carvalho[1], Luiz Fernando Giovanelli[1], Josino Costa Moreira[2]

[1]National Institute for Health Quality Control, Oswaldo Cruz Foundation/FIOCRUZ, [2]Center of Health Studies of Worker and Human Ecology, Oswaldo Cruz Foundation/FIOCRUZ

Tuberculosis remains a major public health problem, especially in developing countries. Brazil presents the largest number of cases in Latin America and is among the 22 countries considered priorities by the World Health Organization (WHO). The Rio de Janeiro state has the largest number of cases registered in the country. The treatment of patients, commonly, makes use of the drugs isoniazid and rifampicin for six months. This study aimed to develop and validate an electroanalytical methodology, using the technique of differential pulse voltammetry for the determination of these drugs in the associated form, in order to evaluate the quality of medicines distributed in the state of Rio de Janeiro. The potential reduction for the isoniazid and rifampicin were -1.10 and -0.90 V. The developed and validated electroanalytical method presented a linear range of 0.25 to 1.25 mg/L to isoniazid, limits of detection and quantification of 0.05 and 0.14 mg/L, and recovery of 98.2 ± 0.4%; a tracking linear of 0.40 to 2.00 mg/L for rifampicin, with limits of detection and quantification of 0.07 and 0.19 mg/L and recovery of 95.8 ± 0.6%. Six lots of medicines from two pharmaceutical companies were analyzed. Only one of the samples showed unsatisfactory levels of rifampicin.

Uniterms: Isoniazid. Rifampicin. Tuberculosis/treatment. Tuberculostatics/quantitative analysis. Differential pulse voltammetry.

A tuberculose continua sendo um importante problema de saúde pública, especialmente em países em desenvolvimento. O Brasil apresenta o maior número de casos da América Latina, estando entre os 22 países considerados prioritários nas ações de controle da doença pela Organização Mundial da Saúde (OMS). No Brasil, o Rio de Janeiro é o estado com o maior número de casos registrados no país. O tratamento de doentes com tuberculose faz uso dos fármacos isoniazida e rifampicina durante seis meses. O presente trabalho objetivou desenvolver e validar metodologia eletroanalítica, utilizando a técnica de voltametria de pulso diferencial, para a determinação desses dois princípios ativos na forma associada e avaliar a qualidade dos medicamentos distribuídos no estado do Rio de Janeiro. Os potenciais de redução para a isoniazida e rifampicina foram respectivamente -1,10 e -0,90 V. O método eletroanalítico desenvolvido e validado apresentou para a isoniazida faixa linear de 0,25 a 1,25 mg/L, limites de detecção e quantificação de 0,05 e 0,14 mg/L e recuperação de 98,2 ± 0,4%; para a rifampicina faixa linear de 0,40 a 2,00 mg/L, limites de detecção e quantificação de 0,07 e 0,19 mg/L e recuperação de 95,8 ± 0,6%. Foram analisados 6 lotes de medicamentos de dois laboratórios farmacêuticos. Apenas uma das amostras apresentou teor de rifampicina insatisfatório.

Unitermos: Isoniazida/determinação. Rifampicina/determinação. Tuberculostáticos/análise quantitativa. Tuberculose/tratamento. Voltametria de pulso diferencial/utilização.

***Correspondence:** K. C. Leandro. Instituto Nacional de Controle de Qualidade em Saúde, Fundação Instituto Oswaldo Cruz. Avenida Brasil, 4365–Manguinhos – 21090-000 - Rio de Janeiro, RJ, Brasil. E-mail: katia.leandro@incqs,fiocruz.br

INTRODUCTION

The World Health Organization (WHO), since 1993, stated alert status in the world respecting to tuberculosis, which represents a great challenge in terms of world public health. A third of human population is infected by *Mycobacterium tuberculosis* and great proportion of them could develop and transmit the disease for the community (Kritisk *et al*., 2007). According to World Health Organization, it is estimated that about 100 million of people are yearly infects, all over the world, out of which eight to ten million will develop the disease along their lives; a half of infected will present the contagious form and three million of them will die, every year (Freire, Bonametti, Matsuo, 2007). The tuberculosis is one of the main causes of death for infectious diseases, being responsible for 1.6 million of deaths all over the world, in 2005. This situation is aggravating since de appearance of AIDS (WHO, 2007).

Brazil occupies the 13th place in the ranking of 22 countries concentrating 80% of tuberculosis cases in the world. According to official data from the Ministry of Health, in Brazil there are, currently, about 50 million people infected with the Koch bacillus, but without disease development; with a contamination of more than 1 million people every year, due to contact with sick persons. Yearly in Brazil, approximately, 111thousand new cases appear and 6 thousand deaths occur, being Rio de Janeiro the state with higher number of registered cases yearly, approximately 17,000; which corresponds to 20% of registered cases in the country (Souza, Vasconcelos, 2005). In the state of Rio de Janeiro, the disease was declared as priority since 1999 and the Program of Tuberculosis Control was been working hardly in the last years, in order to reverse this picture (Secretaria, 2008).

The treatment of tuberculosis patients is done with short duration chemotherapy during six months, including isoniazid, rifampicin and pyrazinamide during the first two months, followed by isoniazid and rifampicin (Figure 1) during the next four months. The use of these last two drugs is justified, based on their activity on the intracellular slow growing bacilli, as well as on those of intermittent growing present on caseous necrosis, which is essential for the successful sterilization of tissular lesions (Dalcolmo, Andrade, Picon, 2007).

The voltammetry technique is based on the study of potential-current curves (voltammograms) resulting from processes of electrons transference. These processes occur in the surface of a work electrode, always as a process of electrons transference (reduction or oxidation of electrochemically active species) is observed, as a function of applied potential.

The analytical importance of voltammetry is originated from two characteristics of obtained voltammograms. Firstly, the position of analytical signals respecting to applied potential could be utilized as a useful tool, in the identification of electroactive species. Secondly, under specific experimental conditions, the limiting current is governed by the concentration of electroactive species in the analyzed solution (Bard, Faulkner, 2001), which allows the use of this technique for quantitative analysis.

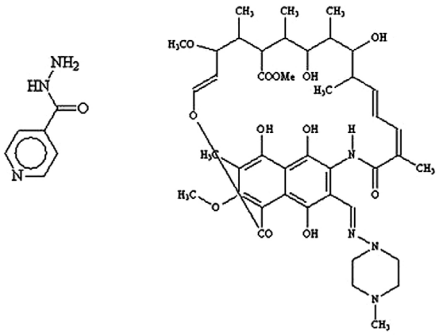
The electroanalytic methodology for the determination of isoniazid and rifampicin in the medications utilized for tuberculosis treatment was developed, utilizing the differential pulse voltammetry. The isoniazid and rifampicin posses electroactive site and are reduced on the work electrode surface (Hanging Mercury Drop Electrode – HMDE).

Studies based in electroanalytical techniques are reported in the literature, as for the determination of isoniazid, as for the characterization of its electrochemical behavior. Lund (1963), employing the polarography, has evidenced that isoniazid presents, in acid medium, two reduction waves. The first one is attributed to amide group reduction, after transference of two electrons; the second corresponds to reduction of pyridinic ring involving the same number of electrons. Sulaiman and Hameed (1988) reported the determination of isoniazid and other hydrazides using the polarography of differential pulse, which was applied in the determination of a ternary mixing with other hydrazides. Rodrigues-Mellado *et al.* (1993) have studied the oxidation of isoniazid on mercury electrode, in a pH interval between 6 and 13. Angulo *et al.* (1993) reported that polarographic reduction of isoniazid is characterized by two waves in pH <9.5. The first one is referred to reduction of C=N binding present in the pyridinic ring of the molecule and the second results from the reduction of hydrazide group, which leads to ammonia formation. Tong *et al.* (1997) described the voltammetric determination of isoniazid on vitreous carbon electrode. Ghoneim *et al.* (2003) reported the utilization of a procedure of voltammetry of adsorptive redissolution, employing a mercury electrode for the determination of isoniazid in pharmaceutical formulations and in biologic fluids. Wahdan (2005) and Liang *et al.* (2007) studied the voltammetric behavior of rifampicin oxidation, reporting that the mechanism involves the same typical number of electrons and protons as for hydroquinones.

Hammam *et al.* (2004), by adsorptive redissolution voltammetry, and Wahdan (2005), by cyclic voltammetry and linear scanning, developed methodologies for the determination of isoniazid and rifampicin, in pharmaceutical formulations and biologic fluids, employing electrode of

carbon paste. Lomillo *et al.* (2001) reported the utilization of a mathematic model to obtain a voltammetric resolution of ternary mixings containing rifampicin, isoniazid and pyrazinamide, employing polarography of differential pulse.

In this sense, the proposal of this work was to develop and validate an electroanalytical methodology for the determination of isoniazid and rifampicin drugs in the associated form in medications utilized for tuberculosis treatment, and evaluate the quality of these medications distributed in Rio de Janeiro state.



a b

FIGURE 1 – Molecular structures of isoniazid (a) and rifampicin (b).

MATERIAL AND METHODS

Equipment and laboratory glasses

The voltammograms in differential pulse were registered utilizing the voltammetric system of Metrohm model 757 VA Computrace coupled to an electrochemical cell composed by three electrodes: hanging mercury drop electrode (HMDE) as work electrode, Ag/AgCl, KCl (3 mol/L) as reference electrode and platinum as auxiliary electrode.

All volumetric flasks and pipettes were calibrated.

Reagents and samples

All solutions were done utilizing ultrapure water from the Milli-Q system (Millipore).

All utilized reagents were of analytic grade (Merck).

The support electrolyte was the monoacid sodium phosphate 0.2 mol/L and citric acid 0.1 mol/L (buffer McIlvaine pH 7.0).

The reference chemical substances isoniazid and rifampicin, utilized as standards, were obtained from Brazilian Pharmacopoeia.

Four lots of capsules of isoniazid and rifampicin (1, 2, 3 and 4), coming from Laboratory A, were analyzed. According to this laboratory, such capsules contained 200 mg of isoniazid and 300 mg of rifampicin. Two other lots (5 and 6) of tablets, from Laboratory B, were also analyzed. According to the package of lot 5, the content was 200 mg of isoniazid and 300 mg of rifampicin; and respecting lot 6, the content was 100 mg of isoniazid and 150 mg of rifampicin.

The electroanalytical methodology for the determination of isoniazid and rifampicin, in medications utilized for tuberculosis treatment, was developed utilizing the differential pulse voltammetry.

The work, reference and auxiliary electrodes were the hanging mercury drop electrode (HMDE), Ag/AgCl, (KCl 3 mol/L) and platinum, respectively.

The analytical curves were built adding in the electrochemical cell, 5 consecutive portions of 50 µL of isoniazid (50 mg/L) and rifampicin (80 mg/L) standards and varied amounts of buffer McIlvaine pH 7, in order to obtain the cell total volume of 10.0 mL. The final concentrations, therefore, were of 0.25, 0.50, 0.75, 1.00 and 1.25 mg/L for the isoniazid and 0.40, 0.80, 1.20, 1.60 and 2.00 mg/L for the rifampicin. The solution was deaired for 5 minutes with nitrogen for the removal of oxygen and, next, static for some seconds before the step of scanning in the cathodic sense. Table I presents the optimized operational parameters for determination of these active principles, in medication samples in form of tablets or capsules.

All measurements were done in 10.0 mL of solution and in triplicate, as in the analytical curve preparation, as in the analyzed samples.

TABLE I – Experimental parameters for the determination of isoniazid and rifampicin in medicines by differential pulse voltammetry

Voltammetric Parameters	Optimized Value
Initial potential	-0.80 V
Final potential	-1.20 V
Scanning velocity	10 mV/s
Pulse amplitude	50 mV
Balance time	5 s
Scanning direction	cathodic (negative)

The developed electroanalytical methodology was validated according the document of INMETRO (INMETRO, 2007). The evaluated parameters were: linearity (work range), detection and quantification limits, precision (repeatability and intermediate precision), accuracy (recovery) and robustness. For the parameter robustness,

electrolyte pH and scanning velocity were evaluated. The software Statistic 8.0® (Statsoft) was used to determine the limits of detection and quantification.

The methodology developed was also compared with the official method (high performance liquid chromatography) professed in The United States Pharmacopeia (USP 31, 2008).

After the methodology validation, the determinations of associated isoniazid and rifampicin, in form of capsules and tablets medications produced by two Pharmaceutical Companies (A and B), were developed. The samples mass was calculated and weighted utilizing the parametric values professed by the manufacturers, and dissolved in 1 mL of methanol and buffer McIlvaine pH 7 within a volumetric flask of 50 mL, in order to locate the obtained concentration around the middle of analytical curve.

RESULTS AND DISCUSSION

The isoniazid and rifampicin were determined by differential pulse voltammetry, according to experimental conditions described at Table I. In the voltammogram of isoniazid, two peaks were found, meaning two different electroactive sites in the respective molecule, in -0.98 V and in -1.10 V; with the latter one presenting the higher current. Because of that, the peak in -1.10 V was chosen for the building of analytical curve. For the rifampicin, it was found a single peak around -0.90 V (Figure 2).

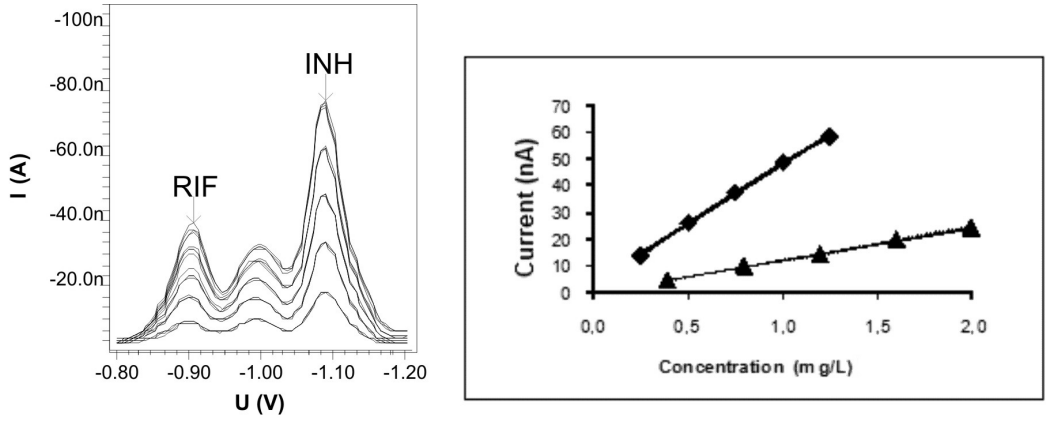
The work ranges were of 0.25 to 1.25 mg/L (y (na) = 45.29 (mg/L) + 2.752; r^2 of 0.9990) for the isoniazid (INH) and of 0.40 to 2.00 mg/L (y (na) = 12.64 (mg/L) – 0.9380; r^2 of 0,9992) for the rifampicin (RIF).

To evaluate the two determination methods of associated isoniazid and rifampicin, Differential Pulse Voltammetry (DPV – our developed method) and high performance liquid chromatography (HPLC – official method – UPS 31, 2008) were used. The bilateral test F, with 95% of confidence interval and ($F_{6.6}$) grades of liberty was applied. To express the results accuracy, the standard deviation and relative standard deviation of every conjunct of determinations referring to sample of lot 5, coming from Pharmaceutical Company B, were calculated. Through the obtained results, with was possible to verify that the methods do not present statistically significant differences and the calculated values for the relative standard deviation were below 2% (Haswell, 1992), demonstrating so accuracy in both methods. For the calculations of isoniazid and rifampicin tenor in the analyzed medication, the values professed by its manufacturer were adopted. The method accuracy was evaluated by repeatability and intermediate accuracy (the same previous determinations, but in different days), as described at Table II.

The developed electroanalytical methodology was validated according the parameters described in the orientation document from INMETRO (INMETRO, 2007).

During the pH study, it was verified a cathodic displacement of peak potential with an increase of pH, as for INH, as for RIF; however, between the pH 6.5 and 7.5 such displacements were practically absent. Respecting to pH, the method was robust for this narrow pH range. The obtained validation results are described at Table III.

After the validation of developed electroanalytical method, the medication samples from Pharmaceutical Companies A and B containing isoniazid and rifampicin in the associated form, were analyzed taking as a base the analytical curves presented at Figure 2. The obtained results are described at Table IV.



2 (a) 2 (b)

FIGURE 2 – Voltammograms (a) and analytical curves (b) of INH (◆) 0.25; 0.50; 0.75; 1.00 and 1.25 mg/L and of RIF (▲) 0.40; 0.80; 1.20; 1.60 and 2.00 mg/L with buffer McIlvaine pH 7.0 as support electrolyte, in the conditions described at Table I.

TABLE II – Comparison of 'high performance liquid chromatography' and 'differential pulse voltammetry' methods for determination of both, isoniazid and rifampicin, associated into a pharmaceutical formulation

Method of determination	Tenor (%) *		Repeatability RSD (%)		Intermediate Accuracy RSD (%) (n=14)
	First day (n=7)	Second day (n=7)	First day (n=7)	Second day (n=7)	
			ISONIAZID		
HPLC	98.4 ± 0.2	98.0 ± 0.1	0.4	0.3	0.5
DPV	97.6 ± 0.3	98.2 ± 0.2	0.6	0.7	0.7
			RIFAMPICIN		
HPLC	97.0 ± 0.4	97.6 ± 0.3	0.8	0.9	1.1
DPV	96.4 ± 0.5	96.8 ± 0.5	0.6	0.7	0.9

* Composition of formulation: 200 mg/capsule of isoniazid and 300 mg/capsule of rifampicin.

TABLE III – Evaluated parameters in the validation of electroanalytical methodology, according to document of INMETRO, 2007

Evaluated parameters	**Found results**	
	Isoniazid	**Rifampicin**
Linearity (work range)	(0.25 - 1.25) mg/L	(0,40 – 2,00) mg/L
Detection limit	0.05 mg/L	0,07 mg/L
Quantification limit	0.14 mg/L	0,19 mg/L
Recovery	98.2 ± 0.4 %	95,8 ± 0,6 %
Robustness respecting to pH	pH between 6.5 e 7.5	

TABLE IV – Results found in medications from Pharmaceutical Companies A and B, using differential pulse voltammetry, under the conditions described at Table I

Sample (Lot) (*)	Pharmaceutical Company	Isoniazid		Rifampicin	
		Recovery (%) (n=7)	Accuracy RSD (%)	Recovery (%) (n=7)	Accuracy RSD (%)
1	A	96.1 ± 0.8	1.3	94.3 ± 0.7	1.0
2	A	94.2 ± 0.4	1.0	89.0 ± 0.9	1.4
3	A	98.3 ± 0.6	1.1	91.7 ± 0.6	1.2
4	A	100.9 ± 0.5	0.5	101.0 ± 0.8	0.2
5	B	97.6 ± 0.6	0.8	96.4 ± 0.9	1.4
6	B	98.4 ± 0.8	1.1	96.7 ± 0.9	1.3

* Values professed by manufactures: Lots 1, 2, 3 and 4 - 200 mg of isoniazid and 300 mg of rifampicin per capsule. Lot 5 - 200 mg of isoniazid and 300 mg of rifampicin per tablet. Lot 6 - 100 mg of isoniazid and 150 mg of rifampicin per tablet.

To evaluate the obtained results of isoniazid and rifampicin tenors in the analyzed samples, the acceptable ranges at USP 31, of 90 to 110% for isoniazid and 90 to 130% for rifampicin, both respecting to the value professed by manufacturer, were consulted.

Both, as the samples from Pharmaceutical Company A, as those from Pharmaceutical Company B, presented satisfactory results respecting the isoniazid tenor. For the rifampicin tenor, only one sample (Lot 2) presented a non-satisfactory result.

CONCLUSIONS

Although the technique professed at The United States Pharmacopeia (USP 31) for the determination of isoniazid and rifampicin in medications is high perfor-

mance liquid chromatography, such active principles could also be determined through differential pulse voltammetry (DPV), the technique developed and validated in this work.

Through the electroanalytical methodology, it was possible to determine simultaneously the isoniazid and rifampicin. The method was validated, being obtained satisfactory results, and presenting some advantages respecting to official method (USP 31), such as lower analysis time (approximately three times lower), operation practicability of equipment, higher easiness in preparation of samples and standards, and analyses cost estimated as approximately three times lower.

Respecting to voltammetric methods employing electrode of carbon paste, the proposed method presents better repeatability, due to perfect reproduction in the formation of mercury drop at every determination.

All the evaluated parameters are compatible with the recommendations established at the INMETRO document of orientation for validation, and the methodology could be utilized for quantitative and routine analyses of pharmaceutical formulations containing isoniazid and rifampicin in the associated form, in the format of both, tablets and capsules.

According to USP 31, the acceptability ranges for tenors of isoniazid and rifampicin are of, respectively, 90 to 110% and 90 to 130% of the value professed by manufacturer. Out of the all samples analyzed, only a single lot from Pharmaceutical Company A presented a result below the range described at USP 31 for rifampicin. For isoniazid, all obtained results were satisfactory.

ACKNOWLEDGMENT

The authors thank to National Institute for Health Quality Control/FIOCRUZ by the motivation to developed researches, as well as to FAPERJ and CNPq by financial support.

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Received for publication on 28th august 2008
Accepted for publication on 10th december 2008