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Use of different matrices to construct the standard curve for the paracetamol dosage by visible spectrophotometry

Uso de diferentes matrizes na construção da curva padrão para a dosagem de paracetamol por espectrofotometria visível

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ABSTRACT

Introduction: Acute paracetamol poisoning is confirmed by the determination of its serum level and allows assessing the risk of hepatotoxicity, which can be monitored by the Rumack-Matthew nomogram for the administration of the N-Acetylcysteine antidote, as well as for the prognosis of intoxication. **Objective:** Because of its analytical importance, we evaluated the influence of different matrices (ultrapure water, serum, and plasma) on the construction of the paracetamol calibration curve, aiming to reduce the analytical cost and facilitate its implementation in clinical and emergency laboratories. **Material and methods:** A standard stock solution of paracetamol of 1 mg ml^{-1} was obtained, from which appropriate dilutions originated the following concentrations 20, 50, 100, 150, 200, 250, and 300 mg l^{-1} in the different matrices, in triplicate, reading at complete after 430 nm in spectrophotometer and reproduced after three months. The results were statistically analyzed ($p < 0.05$). **Results and discussion:** Good laboratory practices include remaking the calibration curve when stock reagents are remade aiming to readjust the line equation indicated by a measuring instrument. The biological samples indicated as matrices on a calibration curve are usually serum and plasma. However, these biological products, when commercially purchased, are of high cost. Ultrapure water can replace serum and plasma in the paracetamol calibration curve according to the linearity of the curve, which showed the same trend line for the three matrices. **Conclusion:** The three matrices can be used in the construction of the paracetamol calibration curve, but the use of ultrapure water reduces the analysis costs.

Key words: analgesics drugs; anti-inflammatory drugs; antipyretics; acetaminophen; UV-Vis spectrophotometry.

RESUMO

Introdução: *A intoxicação aguda pelo paracetamol é confirmada pela determinação de seu nível sérico e permite avaliar o risco de hepatotoxicidade, que pode ser monitorado pelo nomograma Rumack-Matthew para a administração do antídoto N-acetilcisteína, bem como para o prognóstico da intoxicação.* ***Objetivos:*** *Diante de sua importância analítica, avaliamos a influência de diferentes matrizes (água ultrapura, soro e plasma) na construção da curva de calibração do paracetamol, visando diminuir o custo analítico e facilitar a sua implantação em laboratórios clínicos e de urgência.* ***Material e métodos:*** *Obtivemos uma solução estoque padrão de paracetamol de 1 mg ml^{-1}, da qual originaram diluições apropriadas para se obter as concentrações de 20, 50, 100, 150, 200, 250 e 300 mg l^{-1} com as diferentes matrizes, em triplicata, com leituras em espectrofotômetro a 430 nm, sendo reproduzidas após três meses. Os resultados foram analisados estatisticamente* ($p < 0,05$). ***Resultados e discussão:*** *Nas boas práticas de laboratório, inclui-se o refazimento da curva de calibração quando os reagentes estoques são refeitos visando ao reajuste da equação de reta indicado por um instrumento de medição. As amostras biológicas indicadas como matrizes em uma curva de calibração são, usualmente, soro e plasma. Porém, esses produtos biológicos quando adquiridos comercialmente são de*

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custo elevado. A água ultrapura pode substituir soro e plasma na curva de calibração do paracetamol em função da linearidade da curva, a qual mostrou a mesma linha de tendência para as três matrizes. ***Conclusão:*** *As três matrizes podem ser utilizadas na construção da curva de calibração do paracetamol, mas o uso de água ultrapura diminui os custos da análise.*

Unitermos: *analgésicos; anti-inflamatórios; antipiréticos; acetaminofeno; espectrofotometria UV-Vis.*

RESUMEN

Introducción: *La intoxicación aguda por paracetamol se confirma mediante la determinación de su nivel sérico y permite evaluar el riesgo de hepatotoxicidad, que puede ser monitorizado mediante el nomograma de Rumack-Matthew para la administración del antídoto N-acetilcisteína, así como para el pronóstico de intoxicación.* ***Objetivos:*** *Por su importancia analítica, se evaluó la influencia de diferentes matrices (agua ultrapura, suero y plasma) en la construcción de la curva de calibración del paracetamol, con el objetivo de reducir el costo analítico y facilitar su implementación en laboratorios clínicos y de emergencia.* ***Material y métodos:*** *Se obtuvo una solución madre del estándar (stock) de paracetamol de 1 mg ml^{-1}, de la cual se originaron diluciones adecuadas para obtener las siguientes concentraciones de 20, 50, 100, 150, 200, 250 y 300 mg l^{-1} con las diferentes matrices, por triplicado, con lectura a 430 nm en epectrofotômetro, reproduciéndose a los tres meses. Los resultados se analizaron estadísticamente ($p < 0,05$).* ***Resultados y discusión:*** *Las buenas prácticas de laboratorio incluyen rehacer la curva de calibración cuando se rehacen los reactivos del estándar con el fin de reajustar la ecuación lineal indicada por un instrumento de medición. Las muestras biológicas indicadas como matrices en una curva de calibración suelen ser suero y plasma. Sin embargo, estos productos biológicos cuando se compran comercialmente son de alto costo. El agua ultrapura puede reemplazar el suero y el plasma en la curva de calibración de paracetamol de acuerdo con la linealidad de la curva, que mostró la misma línea de tendencia para las tres matrices.* ***Conclusión:*** *Las tres matrices pueden usarse en la construcción de la curva de calibración de paracetamol, pero el uso de agua ultrapura reduce los costos de análisis.*

Palabras clave: *analgésicos; antiinflamatorios; antipiréticos; paracetamol; espectrofotometría UV-Vis.*

INTRODUCTION

Paracetamol or acetaminophen is a non-steroidal anti-inflammatory (NSAID) inhibitor of cyclooxygenase (COX), non-selective for COX-1 and COX-2 and selective for COX-3; it is expressed in greater quantities in the cerebral cortex and in the heart[1]. It has high antipyretic action, medium analgesic action, and low anti-inflammatory action. It is recommended to replace acetylsalicylic acid (ASA) in cases of viruses, to avoid Reye's syndrome[2], and in pregnant women to avoid teratogenesis, except in cases of high risk of developing preeclampsia[3].

Paracetamol is widely used by the population, however, due to its effect of hepatotoxicity it is one of the analgesics that most causes intoxication, also because it is an over-the-counter drug, with no control and surveillance in its commercialization[4]. The toxicity of this drug is related to its pharmacokinetics. Its absorption in the gastrointestinal tract is rapid and reaches the peak in plasma after 40-60 minutes (30 minutes in liquid preparations). In cases of overdose, most are absorbed in 2 hours, but the plasma peak is not reached before 4 hours[5,6].

The main route of biotransformation (more than 90% of the amount absorbed) is in the liver, which occurs through three metabolic mechanisms: conjugation with glucuronic acid (40% to 67%), sulfation (20% to 46%, mainly in children), and oxidation (5% to 15%). Oxidation occurs even at therapeutic doses by microsomal enzymes to form a reactive metabolite called N-acetyl-p-benzoquinone imine (NAPBQI). The conjugation of NAPBQI to the water-soluble antioxidant molecule glutathione (GSH), during the phase II reaction of biotransformation, results in its elimination, with no harmful consequences to the organism. However, in cases of overdose, large amounts of NAPBQI are covalently bound to GSH, whose depletion causes hepatotoxicity[5,7].

The analysis of the serum concentration of paracetamol confirms the diagnosis of acute intoxication. The result not only has diagnostic certainty value, but also assesses the risk of hepatotoxicity, indicating the use of the specific antidote

(N-acetylcysteine), which is hydrolyzed in body cysteine and replenishes glutathione levels[8].

The Rumack-Matthew nomogram (**Figure 1**), used to monitor the severity of intoxication, aims to indicate whether the antidote should be administered when assessing the serum concentration of paracetamol in the interval between the ingestion of the drug and blood collection[9]. According to the nomogram (Figure 1), serum levels of paracetamol above the straight lines[10], known as lines of probable toxicity, are predictive of hepatotoxicity[5].

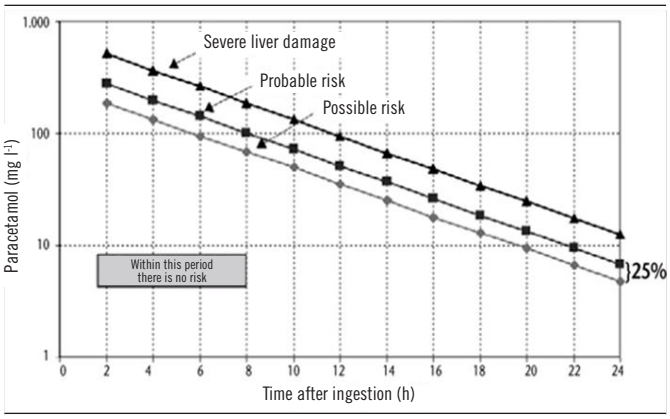

FIGURE 1 – *Rumack-Matthew Nomogram*[11]

In this context, the quantification of paracetamol is essential, whether in pharmaceutical formulations aiming at reliable quality control results in the pharmaceutical industry[11] or in biological samples[5, 12]. The analytical methods usually employed are spectrophotometric[13, 14], spectrofluorimetric[15], chromatographic[16-18], and voltammetric methods[19, 20]. Ultraviolet-visible spectrophotometry is an efficient analytical method for determining organic and inorganic compounds, such as the identification of a pharmaceutical asset. This technique is based on molecular absorption and identifies functional groups, in addition to quantifying compounds that contain absorbent groups[21], as in the paracetamol case.

The achievement of a reliable result, such as the serum concentration of paracetamol, is preceded by the calibration of the spectrophotometer to be used. Calibration is performed using a set of standards that show an amount of the analyte of interest, an analytical step known as the calibration curve. Therefore, the instrument's response to each standard is measured, establishing the relationship between the instrument's response and the analyte concentration[22].

The objective of this work was to test ultrapure water as a matrix in the construction of the paracetamol calibration curve, and compare it with the serum and plasma matrices[5], which are ideal biological samples for the quantification of paracetamol to monitor acute intoxication, aiming at decreasing the analytical cost.

MATERIAL AND METHODS

Matrix samples

Plasma, serum, and ultrapure water were used as matrices in the construction of the calibration curve.

Reagents and equipment

Acetaminophen (99%, catalog number A7085) and sodium nitrite (99%, 563218) were purchased from the Sigma laboratory (St. Louis, USA), while trichloroacetic acid (99%, 01A1066.01.AE) and sodium hydroxide (98%, 01H2000.01.AG), from the Synth laboratory (Diadema, Brazil).

Plasmas and serums were obtained from blood collected with and without anticoagulant, respectively, from a donkey at the Veterinary Hospital of the University of Sorocaba [Universidade de Sorocaba (Uniso)] and the collected samples were donated by the Coordination of the Veterinary Medicine Course at the Uniso for this study (Official Letter n. 02/2020). Blood collection has a therapeutic or prophylactic purpose when performed on the animal, and does not require authorization from the Ethics Committee on the Use of Animals (CEUA-Brazil).

We obtained ultrapure water from the Milli Q water purification system, Model Direct 8 (Merck Millipore, Billerica, Massachusetts, USA). The equipment used was the PerkinElmer spectrophotometer (Perkin Elmer from Brazil, São Paulo, Brazil), and readings (in triplicate) were performed using quartz cuvettes with 10 mm optical path.

Standard stock solution

For the standard stock solution, we weighed about 10 mg of paracetamol, which was transferred to a 10 ml volumetric flask and dissolved with purified water, obtaining a 1 mg l^{-1} standard stock solution. Appropriate dilutions of the stock solution with the different matrices (ultrapure water, plasma, and serum) were used to construct a calibration curve at the following concentrations: 20, 50, 100, 150, 200, 250, and 300 mg ml^{-1} (**Table 1**).

TABLE 1 – Amount of the dilution from the stock SS in the different matrices (ultrapure water, serum, and plasma)

[Total] (mg l^{-1})	SS (ml)	Matrix (ml)
20	0.1	4.9
50	0.25	4.75
100	0.5	4.5
150	0.75	4.25
200	1	4
250	1.25	3.75
300	1.5	3.5

SS: standard solution.

Principle of the method

The method is based on the reaction of acetaminophen (n-acetyl-p-aminophenol), resulting from sample deproteinization, with sodium nitrite, forming 2,4-nitro-4-acetaminophenol, which changes to a yellow color in alkaline medium[5].

Analytical procedure

In conical test tubes (Falcon type) we add 500 µl of sample (plasma, serum, or ultrapure water), deproteinized by the addition of 5 ml of 3% trichloroacetic acid (TCA); then we stir in the mixer for 30 seconds. After centrifugation for 10 minutes at 3000 rotations per minute (rpm), 2 ml of the supernatant was transferred to another test tube with a pipette, in which we added 0.5 ml of freshly prepared 0.07 M sodium nitrite; the tubes were shaken in a mixer for 5 seconds. After 10 minutes of incubation in a water bath at 37°C ± 1, we add 100 µl of 8 M NaOH for the yellow color appearance. After stirring in a mixer for 30 seconds, the absorbance of the samples, in triplicate, were obtained in a spectrophotometer with 430 nm wavelength. Using the same equipment, however, remaking new standard solutions, we repeated the analysis after three months to verify the impact of reagent remaking.

Data analysis

Through the absorbance obtained from the triplicates of seven concentration levels (20, 50, 100, 150, 200, 250, and 300 mg l^{-1}), the respective means and the linear regression equation were obtained, using the data adjustment by the least-squares method. The equation of the line is expressed by absorbance = a × paracetamol (mg l^{-1}) with a correlation coefficient of at least 0.98[23]. The results obtained from the two calibration curves were statistically analyzed ($p < 0.05$) using the Student's *t* test and Anova one-way (OriginPro8 software, OriginLab Corporation, Northampton, MA, USA).

RESULTS

Analytical curves of the averages obtained from sample triplicates were performed for each matrix (**Table 2**). This reading was replicated in a three months interval for reproducibility test, read successively to the procedure.

TABLE 2 – Results of the readings obtained from the different matrices

Total concentration (mg l^{-1})	Average absorbance 1	SD	Average absorbance 2	SD
Ultrapure water*				
20	0.056	7.22	0.0427	6.93
50	0.0903	8.09	0.0877	5.38
100	0.1743	2.29	0.1746	1.4
150	0.2681	6.39	0.2477	5.15
200	0.3459	4.96	0.3347	7.7
250	0.4174	10.96	0.4031	6.47
300	0.5058	9.29	0.5007	2.64
Plasma*				
20	0.0594	10.72	0.0522	4.65
50	0.1086	4.82	0.093	1.56
100	0.1772	2.97	0.1756	4.5
150	0.2632	19.26	0.2584	6.53
200	0.3408	11.7	0.3248	5.91
250	0.4023	4.94	0.3952	4.5
300	0.4926	16.2	0.4567	6.98
Serum*				
20	0.0962	21	0.0586	2.18
50	0.1238	8.67	0.1097	5.04
100	0.197	6.1	0.1819	3.1
150	0.2531	8.7	0.2492	4.32
200	0.3479	8.13	0.3367	5.01
250	0.4429	6.46	0.4066	5.43
300	0.5099	8.58	0.4949	6.86

*Statistical analysis: ultrapure water – there was no significant difference between the values of the first and the second analysis (p > 0.05); plasma – there was no significant difference between the values of the first and second analysis (p > 0.05); serum – there was no significant difference between the values of the first and second analysis (p > 0.05). *when ultrapure water, plasma and serum were analyzed, there was no significant difference between the matrices (p > 0.05); SD: standard deviation.*

There was no statistically significant difference between the three matrices ($p > 0.05$) in the first analysis, in the studied concentrations. Nor was there any significant difference in the second analysis, three months later. We also compared statistically the biological samples between the two analyzes and there was no significant difference for water, plasma and serum.

The following line equations were obtained for each matrix:

- ultrapure water

(A): y = 0.01859 + 0.00161x; r = 0.99727, and (B): y = 0.01017 + 0.00161x; r = 0.99888;

• plasma

(A): y = 0.03219 + 0.00149x; r = 0.99856, and (B): y = 0.02317 + 0.00150x; r = 0.99673;

• serum

(A): y = 0.04295 + 0.00158x; r = 0.99636, and (B): y = 0.02862 + 0.00153x; r = 0.99915.

The calibration curves of the three matrices are shown in **Figure 2**, for analyzes carried out at different times, *A* for pure water; *B* for plasma; *C* for serum; and *D* curves of the three matrices plotted together.

For a better analysis, **Figure 3** illustrates the standard deviations (SD) of the different matrices. Note that in this case, the curves of the three matrices, even at different times, flatten due to the scale of the *y*-axis; serum and plasma are the matrices with the largest deviations in some concentrations of paracetamol. In general, from the SD perspective, we demonstrate that the three matrices can be used in the construction of a standard curve for the analytical determination of paracetamol.

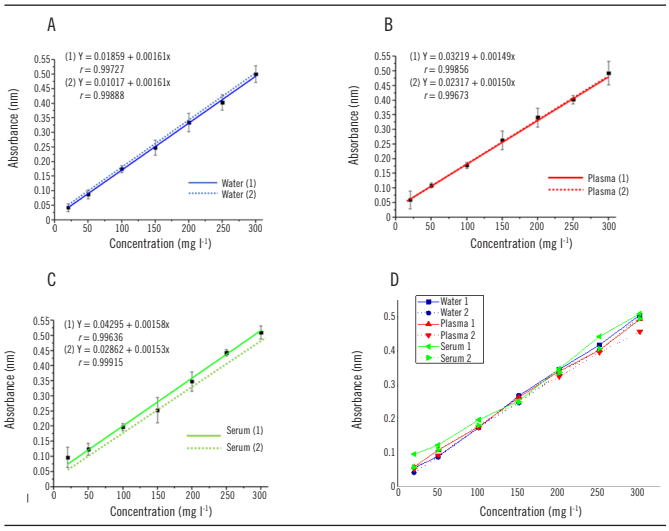

FIGURE 2 – *Comparison between the calibration curves at different times (1), and three months after (2) for the following matrices: A) pure water* (n = *3); B) plasma* (n = *3); C) serum* (n = *3); and D) all curves together*

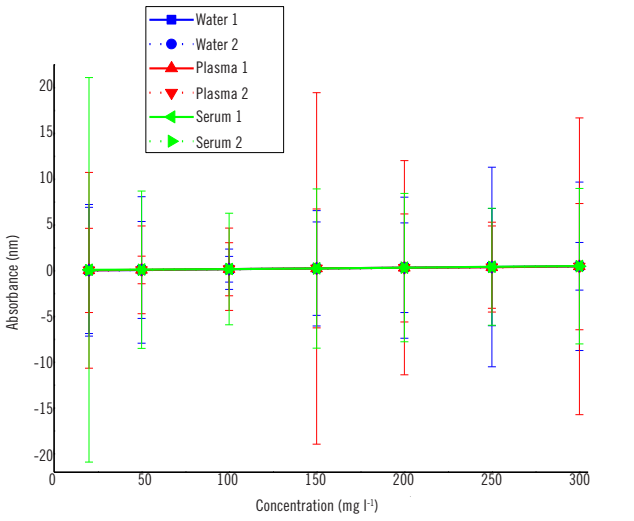

FIGURE 3 – *Standard deviation of the different matrices*

DISCUSSION

All drugs can produce beneficial or harmful effects, depending on the dose. The nature of the harmful effects belongs to two categories associated with the mechanisms of action: 1. pharmacodynamics – effects related to the pharmacological action of the drug (hypotension due to the action of an antihypertensive, bleeding due to an anticoagulant, for example); and 2. toxicological – effects not related to the main pharmacological action of the drug[24]. For example, taking a single dose of 10-15 g paracetamol in adults, and up to 150 mg/kg in children, may cause hepatotoxicity[1], an effect that is not related to its pharmacological effect (antipyretic > analgesic > anti-inflammatory), but with a toxicological effect.

The therapeutic dose of paracetamol in adults should not exceed 4 g per day and in chronic alcoholics, 2 g/per day. In children, however, doses of up to 50 mg/kg/day can be administered[1]. It is clear, therefore, that hepatotoxicity may occur at intakes of 2-4 times greater than the therapeutic dose and, therefore, it is essential that the urgency and emergency laboratories are prepared to perform the quantification of paracetamol aiming at the biomonitoring of intoxication, according to the recommendations of the Rumack-Matthew nomogram to assist in the treatment and prognosis of the intoxicated patient.

Analytical methods for the detection and quantification of drugs in biological samples are important tools for the diagnosis of intoxications, justifying the need to rely on well-characterized, validated and, therefore, reliable methods[5]. The spectrophotometric method suggested by Sebben *et al.* (2010)[5] brought advantages in their use, replacing chromatographic techniques, such as high-performance liquid chromatography – considered a high cost analysis of instrumentation and operation, which takes a relatively long time of analysis and requires experience in handling the equipment and in the treatment of samples[25], as well as the operator's exposure to toxic reagents such as phenol[12].

Sebben *et al.* (2010)[5] suggest serum or plasma as matrices for the standard addition, paracetamol, a method known as the

standard addition method (SAM), frequently used in environmental and biochemical systems, and in the analysis of ultratraces in general[26]. Barros Neto *et al.* (2002)[26] report that, when matrix effects exist, or are expected, and if calibration samples with a similar matrix are not available, SAM may be the most suitable. By adding standard solutions to the sample, a behavior similar to that of the sample is produced in the calibration series, as long as the analyte in the added standard is identical to the sample. This model is based on the prerequisite that the blank has no statistically different value than zero or that this value can be eliminated.

By the fact that in the work by Sebben *et al.* (2010)[5] there was no report on the behavior of paracetamol using water as a matrix, in the present work we performed the same procedure as these authors, using ultrapure water for the preparation of standard solutions with different concentrations of paracetamol compared to the SAM performed with plasma and serum. In line with good laboratory practices and as an internal quality standard, it is ideal to remake the calibration curve whenever any of the parameters that were used as beacons undergo any modification, such as the remake of reagents kept in stock (standard stock solution of 1 mg ml^{-1} paracetamol, TCA 3%, or 8 M NaOH) or when a new batch of a chemical product is purchased and should ideally be compared with the previous batch, or even for checking the equipment[27].

In the methodology used by Sebben *et al.* (2010)[5], the amount of plasma or commercial serum required for the construction of the curve with various concentrations, in triplicate, makes the method more expensive due to the market value of these biological products. In this context, due to the results obtained, the calibration curve using ultrapure water as a matrix was linear to serum and plasma in the analyzes performed. Moreover, the remaking of the reagents from the same batches, after three months, showed little variability in the curves with the same equipment, but it is reasonable to point out that the results could be resounding if batches of reagents from different laboratories or different equipment were used. Remaking the calibration curve and obtaining the line equation readjusted to the new reagents as equipment calibrators is an important record form for the laboratory's internal quality control[27].

In this study, in all tests we observed the same trend line for the three matrices tested. However, although the composition of the serum is similar to that of blood plasma, the former does not have fibrinogen and other factors that were consumed in the coagulation process[28, 29]. This difference indicates that, when choosing the ultrapure water matrix for the construction of the calibration curve, the most suitable sample for the determination of paracetamol is serum. This measure can be easily adopted by the laboratory, advising not to use the anticoagulant during patient's blood collection.

CONCLUSION

We can assert that there is linearity between the serum and plasma matrices with ultrapure water, both statistically and by the correlation coefficients obtained. The use of ultrapure water in the construction of the calibration curve to quantify the serum level of paracetamol is advantageous in relation to serum or plasma due to the significant reduction in the cost of analysis, as it does not require commercial acquisition of lyophilized plasma and serum.

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REFERENCES

1. Brayner AAS, Almeida FR. O risco do uso irracional do paracetamol na população brasileira e seus efeitos na hemostasia. Rev Cient FASETE. 2018; 1: 138-53.

2. Pesaro AE, D'Amico E, Aranha LF. Dengue: manifestações cardíacas e implicações na terapêutica antitrombótica. Arq Bras Cardiol. 2007; 89(2): e12-e15.

3. Ferreira SS, Martins AC, Magalhães AC, Martins H. Ácido acetilsalicílico na prevenção da pré-eclâmpsia: uma revisão baseada na evidência. Rev Port Med Geral Fam. 2017; 33(2): 118-32.

4. Farias PO. Aspectos epidemiológicos das intoxicações por analgésicos não opioides e anti-inflamatórios não esteroides em hospital de urgência e emergência da rede pública do Brasil. Rev Med Minas Gerais. 2016; 26(Supl 5): S11-S15.

5. Sebben VC, Lugoch RW, Schlinker CS, Arbo MD, Vianna RL. Validação de metodologia analítica e estudo de estabilidade para quantificação sérica de paracetamol. J Bras Patol Med Lab. 2010; 46(2): 143-48.

6. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Farmacologia. 7 ed. Rio de Janeiro: Elsevier; 2012.

7. Oshima-Franco Y, Franco LM. Biotransformação: importância e toxicidade. Saúde Rev. 2003; 5(9): 69-76.

8. Castro PLP. Farmacocinética do paracetamol [thesis]. Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Fernando Pessoa; 2014.

9. Munné P, Saenz Bañuelos JJ, Izura JJ, Burillo-Putze G, Nogué S. Intoxicaciones medicamentosas (II): analgésicos y anticonvulsivantes. Anales Sis San Navarra. 26(Suppl 1): 65-97.

10. American Academy of Pediatrics. Acetaminophen toxicity in children. Pediatrics. 2001; 108(4): 1020-24.

11. Junior EJAG, Roeder JS, Oliveira KBL, Ferreira MP, Silva JG. Validação de método analítico para a quantificação de paracetamol em solução oral por espectrofotometria no UV. Rev Virtual Quim. 2017; 9(4): 1747-59.

12. Frings CS, Saloom JM. Colorimetric method for the quantitative determination of acetaminophen in serum. Clin Toxicol. 1978; 15(1): 67-73.

13. Pandya EJ, Kapupara P, Shah KV. Development and validation of simultaneous estimation of diclofenac potassium, paracetamol and serratiopeptidase by first order derivative UV spectroscopy method in pharmaceutical formulation. J Chem Pharm Res. 2014; 6(5): 912-24.

14. Khanage SG, Mohite PB, Jadhav S. Development and validation of UV-visible spectrophotometric method for simultaneous determination of eperisone and paracetamol in solid dosage form. Adv Pharm Bull. 2013; 3(2): 447-51.

15. Moreira AB, Dias ILT, Neto GO, Zagatto EAG, Kubota LT. Simultaneous spectrofluorimetric determination of paracetamol and caffeine in pharmaceutical preparations in solid-phase using partial least squares multivariate calibration. Anal Letters. 2006; 39(2): 349-60.

16. Dewani AP, Bakal RL, Shelke PG, et al. An gradient HPLC-DAD determination of phenylepherine, paracetamol, ambroxol and levocetrizine in pharmaceutical formulation. Indian J Chem Technol. 2016; 23(5): 419-24.

17. Topkafa M, Ayyildiz HF, Memon FN, Kara H. New potential humic acid stationary phase toward drug components: development of a chemometric-assisted RPHPLC method for the determination of paracetamol and caffeine in tablet formulations. J Sep Sci. 2016; 39(2): 2451-58.

18. Clausen DN, Oliveira FM, Casarin J, Sartori ER, Tarley CRT. Development of HPLC method for quantification of orphenadrine, paracetamol, and caffeine in pharmaceutical formulations. Rev Virtual Quim. 2014; 7(6): 2066-79.

19. Sipa K, Socha E, Skrzypek S, Krzyczmonik P. Electrodes modified with composite layers based on poly (3,4-ethylenedioxythiophene) as sensors for paracetamol. Anal Sci. 2017; 33(3): 287-92.

20. Kalambate PK, Srivastava AK. Simultaneous voltammetric determination of paracetamol, cetirizine and phenylephrine using a multiwalled carbon nanotubeplatinum nanoparticles nanocomposite modified carbon paste electrode. Sens Actuators B Chem. 2016; 233(5): 237-48.

21. Saran LM. Fundamentos de espectrofotometria molecular UV-visível. Jaboticabal: Faculdade de Ciências Agrárias e Veterinária, Unesp; 2012.

22. Prichard L, Barwick V. Preparation of calibration curves – a guide to best practice. LGC/VAM/2003/032. Available at: http://www.lgcgroup.com/our-science/national-measurement-laboratory/publications-and-resources/good-practice-guides/preparation-of-calibration-curves-a-guide-to-best/ [accessed on: 26 Mar 2020].

23. Brasil. Agência Nacional de Vigilância Sanitária. Resolução da diretoria colegiada – RDC nº 166, de 24 de julho de 2017. Brasília (DF): Diário Oficial da União; 2017.

24. Rang HP, Dale MM. Toxicidade de drogas. In: Rang HP, Dale MM, editors. Farmacologia. Guanabara Koogan SA; 1993. pp. 550-51.

25. Clausen DN, Oliveira FM, Casarin J, Sartori ER, Tarley CRT. Desenvolvimento de método por CLAE para quantificação de orfenadrina, paracetamol e cafeína em formulações farmacêuticas. Rev Virtual Quim. 2015; 7(6): 2066-79.

26. Barros Neto B, Pimentel MF, Araújo MCU. Recomendações para calibração em química analítica – parte I. Fundamentos e calibração com um componente (calibração univariada). Quim Nova. 2002; 25(5): 856-65.

27. Séries Anvisa. Laboratório. Guia para qualidade em química analítica uma assistência à habilitação. Seleção, uso e interpretação de programas de ensaios de proficiência (EP) por laboratórios. 2000. Eurachem Nederland Laboratory of the Government Chemist of United Kingdom. Tradução: Anvisa. Brasília: SENAI/DN; 2005.

28. Bozzini CE, Molinas F. Hemostasia. In: Houssay AB, Cirgolani HE. Fisiologia humana de Houssay. 7 ed. Artmed: Porto Alegre; 2004.

29. Carlos MML, Sousa Freitas PDF. Study of blood coagulation cascade and the reference values. Acta Vet Bras. 2007; 1(2): 49-55.

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