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Galactosamine limit analysis in total hexosamines in injectable porcine sodium heparin samples

Análise de limite de galactosamina em hexosaminas totais em amostras injetáveis de heparina sódica suína

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ABSTRACT

Introduction: Research, one of the core areas of health surveillance, has its importance justified by its search for answers to various health problems. Heparin, a biological product with anticoagulant and antithrombotic properties, has been related to adverse events between the years 2007–2008. Because of that, the official compendiums updated the monograph for raw material. However, there is a lack of monographs that evaluate the final product. **Objective:** The goal of this study is to propose a physicochemical method of analysis of the limits of galactosamine in total hexosamine from the finished product of porcine sodium heparin. **Method:** We developed an analytical method from the ion-exchange high performance liquid chromatography with amperometric detection, with the appropriate evaluations of the system suitability for posterior analysis of three samples of porcine sodium heparin previously submitted to the sample preparation protocol through the Micro Bio-Spin column. **Results:** The samples were compared to the solution of the system suitability and raw material of porcine sodium heparin. We could detect the presence of galactosamine in one of the three analyzed samples in lower amounts than the limit stipulated by the American pharmacopeia. **Conclusions:** We concluded that the aforementioned method is efficient for the analysis of the finished product and that is the reason why it will be suggested to the Brazilian pharmacopeia.

KEYWORDS: Heparin; Quality Control; High Pressure Liquid Chromatography; Health Surveillance

RESUMO

Introdução: A pesquisa, uma das vertentes da vigilância sanitária, tem sua importância justificada pela busca de respostas aos diversos prejuízos relativos à saúde. A heparina, produto biológico com propriedades anticoagulantes e antitrombóticas, esteve relacionada com eventos adversos entre 2007 e 2008. Diante do ocorrido, os compêndios oficiais atualizaram a monografia para matéria-prima. No entanto, há uma deficiência de monografias para avaliação do produto final. **Objetivo:** Propor método físico-químico de análise de limite de galactosamina em hexosaminas totais a partir do produto acabado de heparina sódica suína. **Método:** Foi desenvolvido método analítico a partir da cromatografia líquida de alta eficiência por troca iônica e detecção amperométrica, com as devidas avaliações de adequação do sistema para posterior análise de três amostras de heparina sódica suína, as quais foram previamente submetidas ao protocolo de preparo de amostra através da coluna Micro Bio-Spin™. **Resultados:** As amostras foram comparadas com a solução de adequação do sistema e matéria-prima de heparina sódica suína, sendo possível detectar presença de galactosamina em uma das três amostras analisadas em quantidade inferior ao limite estipulado pela Farmacopeia Americana. **Conclusões:** Conclui-se que o método é eficiente para análise do produto acabado e, por isso, será sugerido à Farmacopeia Brasileira.

PALAVRAS-CHAVE: Heparina; Controle de Qualidade; Cromatografia Líquida de Alta Pressão; Vigilância Sanitária

INTRODUCTION

The role of health surveillance is not limited to monitoring compliance with sanitary legislation and reprimands. Research is not widespread and there is little investment, but its importance is justified in the articulation of cross-sector actions to produce knowledge and in the search for answers to various health-related issues[1]. When a given product has its credibility and reputation jeopardized, regulatory bodies turn their attention to the subject in order to investigate the sanitary damage[2]. This is the case of heparin, a biological product and one of the most complex linear polysaccharides of the glycosaminoglycan family, which also includes chondroitin sulfate (CS) and dermatan sulfate (DS)[3]. Heparin has anticoagulant and antithrombotic properties that have been widely known for over six decades. The commercial harvest comes from the intestinal mucosa of swine or the lung tissue of bovines, however, they are not equivalent drugs. At the end of 2007 and early 2008, the reliability of this biological product was affected after the episodes reported by the Food and Drug Administration (FDA) of about 350 cases of adverse events due to the use of porcine heparin manufactured by Baxter Healthcare Corporation in the United States[4]. Investigation of the composition of suspect samples found that there was a 20% content of supersulfated chondroitin (SSC) and more than 1% by weight of DS. Authors point out that exposure to certain swine viruses triggers the production of small amounts of SSC, but nothing compared to the large amount found in these batches[5,6]. The structure of SSC, composed of disaccharide units of D-glucuronic acid and N-acetylgalactosamine, β-linked (1 ® 3), analogous to chondroitin sulfate, was elucidated by means of nuclear magnetic resonance (NMR) spectroscopy, but with a peculiar pattern of sulfation found in positions 2 and 3 of glucuronic acid and in 4 and 6 of N-acetylgalactosamine[7]. It is known that CS is composed of D-glucuronic acid β-linked (1 ® 3) to N-acetylgalactosamine; usually its structure has a sulfate group at the 4- or 6-position in the N-acetylgalactosamine portion. While the DS has in its structure L-iduronic acid linked β (1 ® 3) to N-acetylgalactosamine, with sulfate groups present in carbon 2 of uronic acid and carbon 4 of N-acetylgalactosamine, heparin consists primarily of L-iduronic acid and N-sulfoglucosamine β-linked (1 ® 4), highly replaced with O-sulfate residues on carbon 6 of the glucosamine residues and carbon 2 of uronic acid residues[8]. At the time, the Brazilian Health Surveillance Agency (Anvisa) issued a report in which Baxter Healthcare notified that the batches involved in the American cases had not been distributed or marketed in Brazil[9]. However, it became clear that there was a need to look for safe methods that could more accurately attest to the quality of the product offered to the population, leading the official compendiums to update their heparin monographs. In this context, the 5th ed. of the Brazilian Pharmacopoeia contains monographs for calcium heparin[10] and sodium heparin[11], whose active principle and purpose are the same, but both are intended for raw material and not for the finished product. We observed that the monographs belonging to the 5th ed. of the Brazilian Pharmacopoeia[10,11] are similar to the 8th ed. of the European Pharmacopoeia[12,13], in which there are no methods for the injectable form. In contrast, the US Pharmacopoeia USP39-NF34 is more comprehensive with regard to monographs for heparins with different dosage forms: raw material[14] and injectable[15]. Among the chromatographic methods approached for the raw material, is the limit analysis of galactosamine in total hexosamines, high performance liquid chromatography (HPLC) with ion exchange column and pulsed amperometric detection, also studied by Itoh et al.[16]. For injectable heparin, in the US Pharmacopoeia USP39-NF34[15], there is a lack of recommended chromatographic methods. This fact showed us the importance of developing methods, so that there is scope for quality control in both the raw material and the finished product. The finished product has peculiarities that need to be evaluated, such as the composition of the fillers. In this case, swine sodium heparin samples have as fillers sodium chloride, hydrochloric acid, sodium hydroxide, benzyl alcohol and water for injection, of which the salt is the most problematic ingredient when it comes to analysis through ion exchange chromatography. This filler may compete with the analyte for the site of interaction of the stationary phase, thus interfering with the analysis, so the samples need to undergo a desalination treatment to remove the salts from the formulation. The objective of this study was to propose a method of physico-chemical analysis of the limit of galactosamine in total hexosamines from the finished product of porcine sodium heparin, as well as to optimize sample preparation.

METHOD

Standards and reagents

Galactosamine and glucosamine supplied by the United States Pharmacopeia (USP). Sodium acetate, analysis grade, 37% hydrochloric acid and 50% sodium hydroxide solution; supplied by Merck. Potassium hydroxide, analysis grade provided by Fluka. Water was deionized by the Milli-Q purification system and filtered through Millipak® Express 40 filter (MPGP04001).

Chromatographic conditions

The equipment consists of a high-performance liquid chromatograph containing a DIONEX ICS-5000 + SP quaternary pump with coupled degasser, a pulsed amperometric detector next to the DIONEX ICS-5000 + DC column oven, a DIONEX-AS AP automatic injector and Chromeleon software version 7.0, all from Thermo Fisher Scientific. Adaptations in the chromatographic conditions of Itoh et al.[16], where the mobile phase was composed of 8 mM sodium hydroxide (isocratic elution for 11 min), the cleaning step was done with 60% 8 mM sodium hydroxide and 40% 1 M sodium acetate, and the balancing step occurred with 10 min of elution phase between injections, all steps remaining at a constant rate of 1.0 mL/min. The samples were eluted with a volume of 5 µL in a system composed of: Amino-Trap™ pre-column, with dimensions of 3 x 30 mm; Borate-Trap™ pre-column, with dimensions of 4 x 50 mm; CarboPac™ PA1 ion exchange column, dimensions 4 x 250 mm, maintained at 30° C. Column and pre-columns were provided by Dionex. The pulsed amperometric detection followed the schedule in Table 1.

Table 1. Programming of the silver/gold amperometric cell specific for polysaccharides.

Level	Time (min)	Potential (V)	Integration
1	0,00	+0,1	-
2	0,20	+0,1	Begins
3	0,40	+0,1	Ends
4	0,41	-2,0	-
5	0,42	-2,0	-
6	0,43	+0,6	-
7	0,44	-0,1	-
8	0,50	-0,1	-

min: minutes; V: volts.

Samples and raw material

We used three injectable samples and a raw material of porcine sodium heparin, purchased from the manufacturers, with the assistance of the Technical Center of Biological Drugs of the National Institute of Quality Control of the Oswaldo Cruz Foundation. The samples were identified, cataloged and stored under refrigeration between 6° C and 10° C until the time of the tests.

Preparation of system suitability solution

Two solutions were made for the preparation of the system suitability solution: USP standard solution of 1.6 mg/mL glucosamine in 5 N hydrochloric acid and USP standard solution of 1.6 mg/mL galactosamine in 5 N hydrochloric acid. Equal volumes of the glucosamine and galactosamine solutions were poured into a screw tube. We called this final solution the "system suitability solution". Finally, 5 mL of the suitability solution were hydrolyzed for 6 hours at 100° C. Upon cooling, that was diluted with purified Type I water in a ratio of 1:100.

Raw material preparation

About 2.4 mg of porcine sodium heparin were weighed, diluted in 1.0 mL of 5 N HCl and hydrolyzed for 6 hours at 100° C and, after cooling, diluted with Type I purified water in a ratio of 1:100.

Sample preparation

Desalination protocol

To prepare the injectable heparin samples, we used the Micro Bio-Spin™ 6 column, commonly used to remove salts from a given sample since it retains molecules with molar mass smaller than 6 kDa. The buffer packed column requires a step for removing this solution and a step for adding the analyte to rinse the column so that there is no contamination with the sample. This column saturates with 75 µL of added volume, so the desalination process must occur at least 11 times so that there is enough volume to proceed with the analysis.

Sample hydrolysis

The previously desalinated solution was poured into a vessel and then dried in a SpeedVac for 4 hours, without heating, with a vacuum of 0.1 vac and trap temperature of -90° C. After drying, the tubes were weighed and the dilution was done in order to meet the ratio of USP39-NF34[14], which is 12.0 mg of sodium heparin to 5 mL of 5 N hydrochloric acid. These solutions were hydrolyzed for 6 hours at 100° C and, upon cooling to room temperature, filtered on Milipore Millex 0.22 mm membrane, then diluted with purified Type I water in a ratio of 1:100.

System suitability

Resolution (Rs)

Parameter defined as a quantitative measure of the separation of two successive peaks. Two factors are required for the calculation: the distance between their retention times (Tr) and the width of the base (W)[17] (Equation 1).

$$Rs = \frac{2(Tr)_A - (Tr)_B}{(W_A + W_B)} \quad \text{Equation 1}$$

Efficiency or number of theoretical plates (N)

Parameter defined as equilibrium of solute distribution between the two phases: mobile and stationary. The amplitude that the signal undergoes during the passage of the analyte through the system is measured, and there is a constant value for each peak in a chromatogram with a certain set of operating conditions. This parameter is calculated according to Equation 2, where Vn is the peak elution volume and W is the peak width at the baseline[18].

$$N = \left[16.\left(\frac{Vn}{W}\right)\right]^2 \quad \text{Equation 2}$$

Peak asymmetry factor (As)

The parameter measures the asymmetry of the peak, because this parameter is inversely proportional to the accuracy of the quantification, making it difficult to determine the time and the beginning and ending position of the peak[18]. Equation 3 shows the calculation for asymmetry factor in which $W_{0.05}$ is the width of the peak at 5% of height and f is the distance from the vertical line of the maximum signal to the vertical line and perpendicular to the baseline that intercepts the trace at 5% of height.

$$As = \frac{W_{0,05}}{2f} \quad \text{Equation 3}$$

RESULTS AND DISCUSSION

The presence of SSC and the high content of DS in a sample of heparin indicate poor quality and its consequent disposal. For the evaluation of the presence or absence of SSC and DS, the galactosamine limit method can be used in total hexosamines. In this method, the sample is hydrolyzed and converted into monosaccharides and we expect to find only glucosamines from the heparin structure, since galactosamine comes from impurities such as DS and adulterants such as SSC. Relatively, the galactosamine content present in the sample can be quantified from the glucosamine signal. The chromatographic separation occurs through the ion exchange phenomenon and the detection is performed by oxidation reactions occurring between the analyte and the pulsed amperometric detector cell from the potential difference used in the analysis. It should be emphasized that

the standard needs to pass through the same hydrolysis process in order to be submitted to the same sample conditions. The method contained in the USP39-NF34 US Pharmacopoeia did not obtain results that met the criteria of adequacy of the system of the compendium itself, so they were not shown in this work. The method discussed by Itoh et al.[16] has similarities to that of the USP39-NF34[14] in regard to the preparation of the sample, which in both cases refers to the raw material. Table 2 lists the main differences between the methods, which basically involve the mobile phase and the chromatographic column.

The chromatographic columns in question are indicated for high resolution separations of monosaccharides and are functionalized with ammonium salt anion exchange resin. However, Carbo Pac PA1[20] has a larger particle length, diameter and size than Carbo Pac PA20[19]. In the case of highly substituted monosaccharides, interaction with larger particles is more stabilized than with smaller ones, thus facilitating chromatographic separation. According to the manufacturer of Thermo Fisher Scientific[21] liquid chromatograph, which reproduced the method of USP39-NF34[14], the replacement of potassium hydroxide with sodium hydroxide would not cause changes in the results.

Table 2. Main differences between the methods for raw material of sodium heparin present in the American Pharmacopoeia and in the work of Itoh et al.[16].

Sodium heparin method		
Differences	American Pharmacopoeia USP39-NF34[14]	Work by Itoh et al.[16]
Chromatographic column	Carbo Pac PA20	Carbo Pac PA1
Elution phase	14 mM potassium hydroxide	8 mM sodium hydroxide
Cleaning step	100 mM potassium hydroxide	60% 8 mM sodium hydroxide and 40% 1 mM sodium acetate

Therefore, the success of this method can be attributed to the chromatographic column and the washing step. Figure 1 shows the comparison between five injections of the system suitability solution with the method adapted from Itoh et al.[16], in which we observed the retention times of approximately 7.75 and 9.50 min for galactosamine and glucosamine, respectively. In some injections, an unidentified signal at the time of 1.60 min could be detected. The data was treated by the Grubbs test and injection 3 was discarded because it had aberrant values ($\alpha = 0.05$).

The parameters of suitability of the system for the method in question followed those addressed in the American Pharmacopoeia, in order to evaluate if the chromatographic system is suitable for the analysis. Table 3 summarizes the data for evaluation.

For all evaluated criteria, the method was approved and therefore able to be tested for the samples. Figure 2 shows the comparison between blank, system suitability solution, raw material and analyzed samples, in which we can observe that the time peak of 1.60 min is still detected, raising the hypothesis that it may be related to the hydrochloric acid used for the hydrolysis, since it is found not only in the standard, raw material and in samples, but also in the blank. This peak is not detected in the study of Itoh et al.[16], but signals near the dead volume were observed in the studies of Thermo Fisher Scientific[21] and Restaino et al.[22]. In the results of Thermo Fisher Scientific[21], in which the American Pharmacopoeia USP39-NF34[14] method was reproduced, as well as in Restaino et al.[22], in order to study the chromatographic behavior of other species of hydrolyzed polysaccharides, the hydrolysis occurred under the same conditions of the present study, so the detection of signals near the dead volume, such as that seen at 1.60 min, can undoubtedly be attributed

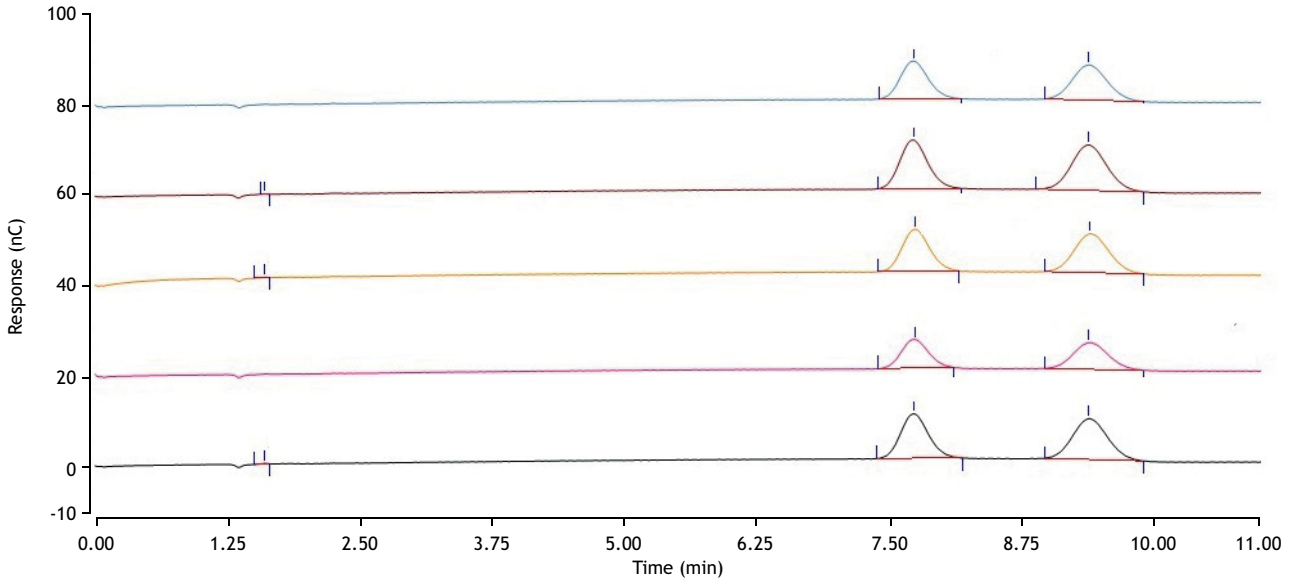

Figure 1. Result of five injections of system suitability solution, where the signal at 7.75 min refers to galactosamine and the signal at 9.50 min refers to glucosamine.

Table 3. Results of the system suitability parameters for the galactosamine limit method in total hexosamines.

System suitability parameters	Acceptance criterion	Results obtained for the proposed method	Note
Resolution	≥ 2 between galactosamine and glucosamine peaks	3.01	Approved
Number of theoretical plates	≥ 2,000 for peak glucosamine	3.754	Approved
Asymmetry factor	Between 0.8 and 2.0 for the galactosamine and glucosamine peaks	1.13 and 1.10 for galactosamine and glucosamine, respectively	Approved

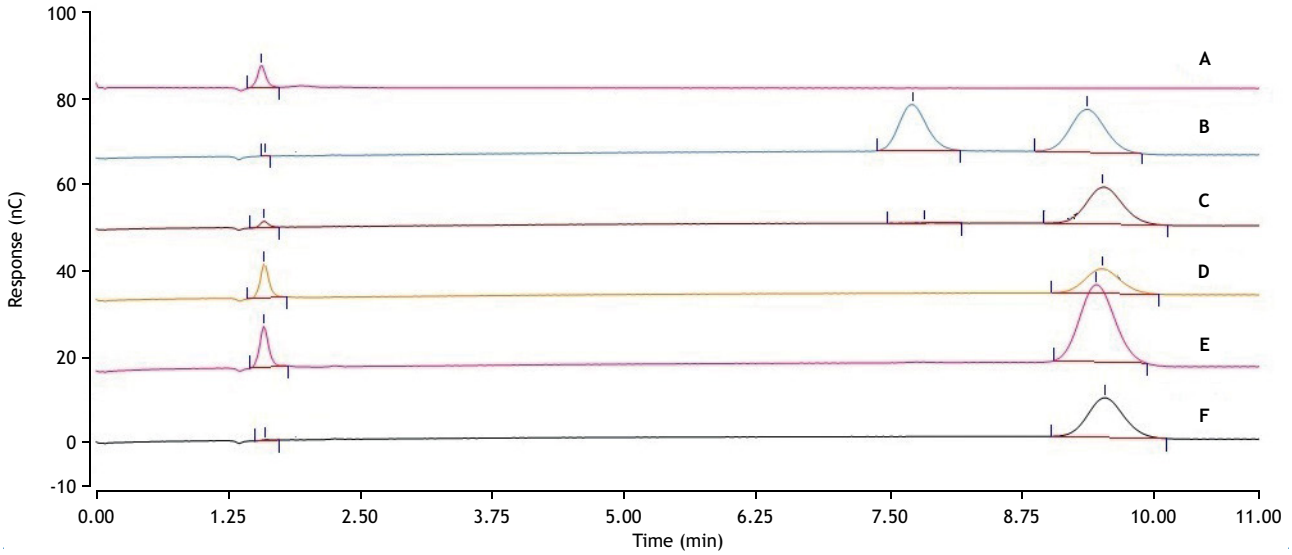

Figure 2. Comparison between (A) Blank; (B) System suitability solution; (C) Sample 1; (D) Sample 2; (E) Sample 3; (F) Raw material of porcine sodium heparin.

to hydrochloric acid. The glucosamine peak remains at the retention time of about 9.5 min in all cases. In sample 1, galactosamine peak was detected at 7.75 min and in the subsequent samples no peak at the galactosamine retention time was detected, therefore galactosamine could not be found within the detection limit of the method, which is 0.1 mg/ml.

Following the acceptance criteria in USP39-NF34[14] of ≤ 1% galactosamine limit in total hexosamines, it was possible to evaluate sample 1, in which the results of five injections were treated by Grubbs test (α = 0.05) and no aberrant value was found. The data used in Equations 4 and 5 were the results of the mean of the injections, thus, *PGALACTOSAMINE / PGLUCOSAMINE* would be the ratio between the galactosamine and glucosamine response in the system suitability solution; $PA_{GALACTOSAMINE}$ is the galactosamine peak area of the system suitability solution; $PM_{GALACTOSAMINE}$ refers to the galactosamine mass of the standard solution; $PM_{GLUCOSAMINE}$ would be the mass of glucosamine in the standard solution; $PA_{GLUCOSAMINE}$ is the area of the glucosamine peak of the system suitability solution; $AA_{GALACTOSAMINE}$ refers to the area of the galactosamine peak in the sample solution, and finally $AA_{GLUCOSAMINE}$ is equal to the area of the glucosamine peak in the sample solution.

$$PGALACTOSAMINE/PGLUCOSAMINE = \left(\frac{PAGALACTOSAMINE}{PMGALACTOSAMINE}\right) \times \left(\frac{PAGLUCOSAMINE}{PMGLUCOSAMINE}\right)$$

$$\left(\frac{2,67}{0,8}\right) \times \left(\frac{3,17}{0,98}\right) = 3,38 \times 3,26 = 10,80$$

Equation 4

$$\text{Resultado} = \left\{\left(\frac{AAGALACTOSAMINE}{(PGALACTOSAMINE/PGLUCOSAMINE)}\right) \Big/ \left(\frac{AAGALACTOSAMINE}{(PGALACTOSAMINE/PGLUCOSAMINE)}\right) + AAGLUCOSAMINE\right\} \times 100$$

$$\left\{\left(\frac{0,03}{10,80}\right) \Big/ \left[\left(\frac{0,03}{10,80}\right) + 4,58\right]\right\} \times 100 = 0,06\%$$

Equation 5

Therefore, by calculation, we can state that the sample has a galactosamine content within the acceptable limit. According to the promising results seen in this study and the importance of developing physico-chemical analysis methods for heparin in injectable form, we can suggest that one of the paths for implementation in official compendia would be the proposal adapted from the work of Itoh et al.[16] for the finished product.

CONCLUSIONS

We conclude that the chromatographic method proposed for physico-chemical analysis of injectable porcine heparin samples has parameters of suitability of the system within the acceptance criteria established by the American Pharmacopoeia. The analysis of three samples of porcine sodium heparin evidenced the presence of galactosamine in only one of the presentations, with a content below that established by the compendium. Since it considered simple and economical, the method will be submitted to the Brazilian Pharmacopoeia as a monograph suggestion for the finished product.

REFERENCES

1. Fonseca EP. Novos rumos para a pesquisa em vigilância sanitária no Brasil. Vigil Sanit Debate. 2013;1(2):22-6.
2. Gemal AL, Teixeira CRRR, Carmo EH, Vital NC. (In) Definições sobre o componente laboratorial de vigilância sanitária no Brasil. Vigil Sanit Debate. 2016;4(4):5-12. https://doi.org/10.22239/2317-269x.00772
3. Rabenstein DL. Heparin and heparan sulfate: structure and function. Nat Prod Rep. 2002;19:312-31. https://doi.org/10.1039/B100916H
4. Cavalheiro Filho C, Chamone DAF, Rached RA, Maffei FH. Heparinas: momento atual. Rev Assoc Med Bras. 2008;54(6):471-86. http://dx.doi.org/10.1590/S0104-42302008000600001
5. Kemsley, J. Tainted heparin's link to reactions. Chem Eng News. 2008[acesso 19 jul 2017];86(49):9. Disponível em: http://cen.acs.org/articles/86/i49/Tainted-Heparins-Link-Reactions.html
6. Keire DA, Trehy ML, Reepmeyer JC, Kolinski RE, Ye W, Dunn J et al. Analysis of crude heparin by ^{1}H NMR, capillary electrophoresis, and strong-anion-exchange-HPLC for contamination by over sulfated chondroitin sulfate. J Pharm Biomed Anal. 2010;51(4):921-6. https://doi.org/10.1016/j.jpba.2009.10.017
7. Guerrini M, Beccati D, Shriver Z, Naggi AM, Bisio A, Capila I et al. Oversulfated chondroitin sulfate is a contaminant in heparin associated with adverse clinical events. Nat Biotechnol. 2008;26(6):669-75. https://doi.org/10.1038/nbt1407
8. Rosenfeld L, Prior MT, Girardi LM. Comparison of the separation of bovine heparin by strong anion exchange and by gel filtration chromatography. Thromb Res. 1991;64(2):203-11. https://doi.org/10.1016/0049-3848(91)90119-H
9. Agência Nacional de Vigilância Sanitária - Anvisa. Heparina da Baxter retirada nos Estados Unidos não foi comercializada no Brasil: o medicamento foi retirado por estar relacionado a reações alérgicas. Informe SNVS/Anvisa/GFARM nº 2, de 19 de fevereiro de 2008[acesso 19 abr 2018]; Disponível em: http://portal.anvisa.gov.br/resultado-de-busca?p_p_id=101&p_p_lifecycle=0&p_p_state=maximized&p_p_mode=view&p_p_col_id=column-1&p_p_col_count=1&_101_struts_action=%2Fasset_publisher%2Fview_content&_101_assetEntryId=401931&_101_type=content&_101_groupId=33868&_101_urlTitle=informe-snvs-anvisa-gfarm-n-2-de-19-de-fevereiro-de-2008&inheritRedirect=true
10. Heparina cálcica: heparinum calcium. In: Agência Nacional de Vigilância Sanitária - Anvisa. Farmacopeia brasileira. 5a ed. Brasília, DF: Anvisa; 2010. v. 2, p. 1017-22.
11. Heparina sódica: heparinum natricum. In: Agência Nacional de Vigilância Sanitária - Anvisa. Farmacopeia brasileira. 5a ed. Brasília, DF: Anvisa; 2010. v. 2, p. 1022-27.
12. Heparina cálcica: heparinum calcium. In: Council of Europa. European pharmacopoeia: suplemente 8.5. 8a ed. Strasbourg: Council of Europe; 2016. p. 4339-40.
13. Heparina sódica: heparinum natricum. In: Council of Europa. European pharmacopoeia: suplemente 8.5. 8. ed. Strasbourg: Council of Europe, 2016. p. 4341-3.
14. Heparin sodium. In: U.S. Pharmacopeia. The United States Pharmacopeia 39. National Formulary 34. Rockville, MD: U.S. Pharmacopeia; 2016. v. 2, p. 4188-93.
15. Heparin sodium injection. In: U.S. Pharmacopeia. The United States Pharmacopeia 39. National Formulary 34. Rockville, MD: U.S. Pharmacopeia, 2016. v.2, p. 4188-93.
16. Itoh S, Hiruta Y, Hashii N, Fujita N, Natsuga T, Hattori T et al. Determination of galactosamine impurities in heparin sodium using fluorescent labeling and conventional high-performance liquid chromatography. Biologicals. 2013;41(6):355-63. https://doi.org/10.1016/j.biologicals.2013.06.002.
17. Lanças FM. Cromatografia líquida moderna: HPLC/CLAE. Campinas: Átomo; 2009.
18. Pallastrelli MB. Desenvolvimento e validação de métodos analíticos enantiosseletivos para separação e determinação do esmolol e sotalol [dissertação]. São Paulo: Universidade de São Paulo; 2013.
19. Thermo Fisher Scientific. Dionex™ CarboPac™ PA20 Carbohydrate Column. 2017[acesso 19 jul 2017]. Disponível em: https://www.thermofisher.com/order/catalog/product/060142
20. Thermo Fisher Scientific. Dionex™ CarboPac™ PA1 Carbohydrate Column. 2017[acesso 19 jul 2017]. Disponível em: https://www.thermofisher.com/order/catalog/product/057178
21. Hurum D, Rohrer J. Determination of galactosamine-containing organic impurities in heparin by HPAE-PAD. Application Note. 2016;233.
22. Restaino OF, Finamore R, Diana P, Marseglia M, Vitiello M, Casillo A et al. A multi-analytical approach to better assess the keratan sulfate contamination in animal origin chondroitin sulfate. Anal Chim Acta . 2017;958:59-70.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.