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Determination of raffinose and lactobionic acid in ViaSpan® by anion exchange chromatography with pulsed amperometric detection

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Abstract

An anion exchange chromatographic method has been developed to quantify raffinose and lactobionic acid in ViaSpan®, an organ preservation product. Separation was accomplished using an aqueous sodium hydroxide/acetate solvent system (pH 13) on a Carbopac PA1 column. Detection was performed using a pulsed amperometric detector equipped with a gold working electrode. The method was able to resolve raffinose, lactobionic acid and other ingredients in the ViaSpan® product. Validation testing of the method for routine use produced excellent linearity, precision and accuracy. The limits of detection for raffinose and lactobionic acid were 1.0 ng (1.7×10^{-12} mol, $S/N = 3$) and 2.0 ng (5.6×10^{-12} mol, $S/N = 3$) respectively. The total analysis time is less than 15 min.

Keywords: Anion exchange; Lactobionic acid; Pulsed amperometric detection; Raffinose; ViaSpan®

1. Introduction

ViaSpan® is an approved medical device used in extending the viability of specific donor organs for transplantation. Its matrix consists of a 5% w/v hydroxyethyl starch base, the carbohydrates raffinose and lactobionic acid, adenosine, allopurinol and glutathione. A rapid, quantitative method for the raffinose and lactobionic acid was needed for the release and stability testing of this product.

Traditional methods of carbohydrate analysis include chromatographic and non-chromatographic techniques. These techniques have been the subject of previous reviews [1,2]. Non-chromatographic methods such as enzymatic and other specific chemical methods were found to be less suitable for rapid and routine applications. Chromatographic methods for carbohydrates include ligand exchange, amino bonded phase chromatography, borate complexes and ion exchange. The majority of these were found not to be selective, sensitive or rapid enough for routine use. Only anion exchange chromatography (HPAEC),

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combined with pulsed amperometric detection, was found to meet the above criteria for this analysis.

Pulsed amperometric detection (PAD) was first reported for carbohydrate analysis by Hughes and Johnson [3]. The technique involved the use of a triple waveform to quickly detect, clean and reactivate the electrode surface. This detector allowed for real-time monitoring of eluting bands during chromatographic analysis. The specific HPAEC/PAD technique for carbohydrates was first reported by Rocklin and Pohl [4]. This technique was applied to a series of sugar alcohols, saccharides (including raffinose) and oligomers of maltose. A review of PAD detection for liquid chromatography offers an excellent description of the theory and practice of this specific technique [2,5].

Raffinose and lactobionic acid have been analyzed by reversed-phase HPLC, ion exchange HPLC and adsorption chromatography on porous graphite and porous polymer columns [6–9]. Detection was accomplished using either refractive index or PAD. Here the use of the HPAEC/PAD technique for the determination of raffinose and lactobionic acid in ViaSpan® organ transplantation solution is reported. The results from the validation of the method are presented to illustrate the sensitivity, selectivity, precision and accuracy of the method.

2. Experimental

2.1. Materials and reagents

Raffinose pentahydrate (Wako Pure Chemicals) and lactobionic acid (Fluka Chemika) were used as supplied. ViaSpan® product was used as manufactured by the DuPont Merck Pharmaceutical Company. The ingredients contained in the formulation are listed in Table 1. 50% sodium hydroxide and sodium acetate trihydrate (J.T. Baker) were both analytical reagent grade and used as supplied. The water used for all preparations was deionized and further purified by a Milli-Q (Millipore Corp.) reverse osmosis filtration system. Carbon dioxide was removed from the water used for chromatography by sparging with helium.

2.2. Cyclic voltammetry

A Bioanalytical Systems Model CV-1B cyclic voltammograph was used with a gold working electrode and a Ag/AgCl reference electrode. Voltammograms were obtained between −0.9 V and 0.7 V with a sweep rate of 100 mV s^{-1}. Solutions of raffinose and lactobionic acid were prepared at 3 mM and 10 mM respectively in 0.1 N NaOH.

2.3. Chromatography

A Dionex 4000 gradient pump module (GPM) attached to a Perkin-Elmer LC-600 autosampler with a Rheodyne 7010 injection valve equipped with a 50 μl sample loop was used. A Dionex pulsed amperometric detector equipped with a gold electrode and a Ag/AgCl reference electrode was used for detection. The detector pulse duration settings were $T_{det} = 300$ ms, $T_{oxd} = 120$ ms and $T_{red} = 300$ ms. The detector applied potentials were $E_{det} = 0.05$ V, $E_{oxd} = 0.06$ V and $E_{red} = -0.80$ V. A Fisons Multichrom lab data system was used to acquire all chromatographic data.

All tested ViaSpan® samples were diluted in water to prepare solutions of 17.8 μg ml^{-1} raffinose and 35.8 μg ml^{-1} lactobionic acid. Standards of raffinose and lactobionic acid were prepared in water at the same concentrations as the

Table 1
The ingredients contained in the ViaSpan® formulation

Ingredient	Concentration (g l^{-1})
Adenosine	1.340
Allopurinol	0.136
Glutathione	0.922
Hydroxyethyl starch	50.000
Lactobionic acid	35.830
Magnesium sulfate	1.230
Potassium hydroxide	5.610
Potassium phosphate, monobasic	3.400
Raffinose	1.230
Sodium hydroxide	1.000

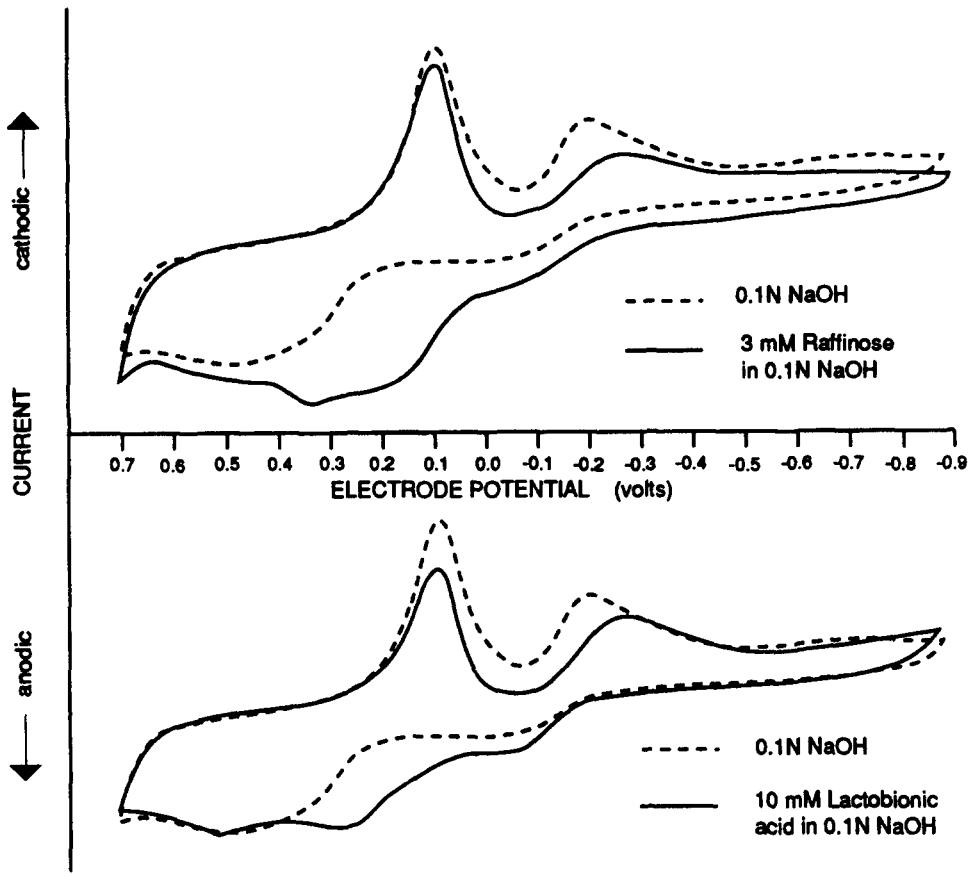

Fig. 1. Cyclic voltammograms of raffinose and lactobionic acid in aqueous 0.1 N NaOH.

samples. These solutions were analyzed at ambient temperature.

The mobile phase was prepared by first sparging the water with helium to remove all carbon dioxide. To 1 l of water, 8.0 ml of 50% sodium hydroxide and 6.81 g of sodium acetate trihydrate were added. The solution was mixed and filtered through a 0.5 μm filter. The solution was stored in plastic containers and continually sparged with helium.

The column was a Carbopac PA1 column (Dionex Corp., P/N 35391) used with a Carbopac PA1 guard column (Dionex Corp., P/N 37141). The flow rate of the mobile phase was 1.3 ml min^{-1}.

3. Results and discussion

3.1. Detection

Current–potential voltammetric response curves were used to screen for potential detector operation conditions. These voltammograms are illustrated in Fig. 1. The oxidation at the gold electrode surface by each carbohydrate occurred between 0.0 and 0.4 V. The reference solution (0.1 N NaOH) shows significantly less anodic current in this region. The electrochemical response of the reference solution was believed to be due to formation of surface oxide at $E > +0.2$ V and oxygen evolution at $E > +0.65$ V [5,10]. The cyclic

voltammetric data indicated that liquid chromatography with pulsed amperometric detection of raffinose and lactobionic acid should be operated with the E_{det} potential set between 0.0 and +0.4 V.

The detector response was then used to optimize the amperometric response of the PAD unit. At a starting potential of 0.0 V, the detector was zeroed and the background response was measured as a function of increasing E_{det}. Using standard solutions of raffinose and lactobionic acid, the response of each compound was measured as a function of potential E_{det}. Fig. 2 shows a plot of background, raffinose and lactobionic acid responses versus the measurement potential, E_{det}. The maximum difference between background response and the response for both compounds occurred at approximately 0.05 V. This was the voltage used for E_{det} in the final method conditions.

3.2. Chromatography

The mobile phase consisted of 0.1 N NaOH with sodium acetate added at 0.05 N as a retention modifier. The concentration of NaOH influenced the detector response (including background response) as well as the retention of the carbohydrates. The detector response effects are due to pH dependence of the rates of the reaction steps leading to the oxidation. Increasing the hydroxide concentration leads to increases in both detector

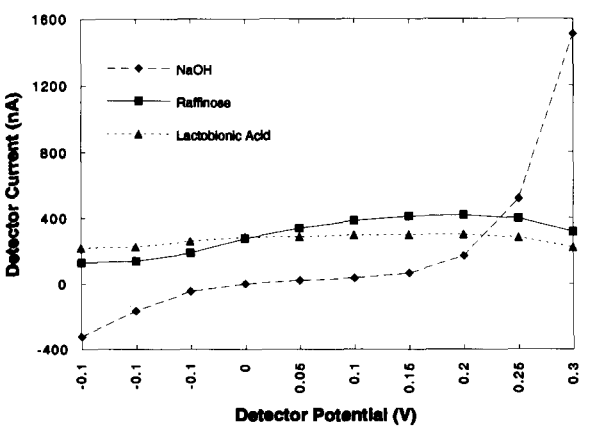

Fig. 2. Plot of detector response (nA) as measurement potential (V) is varied.

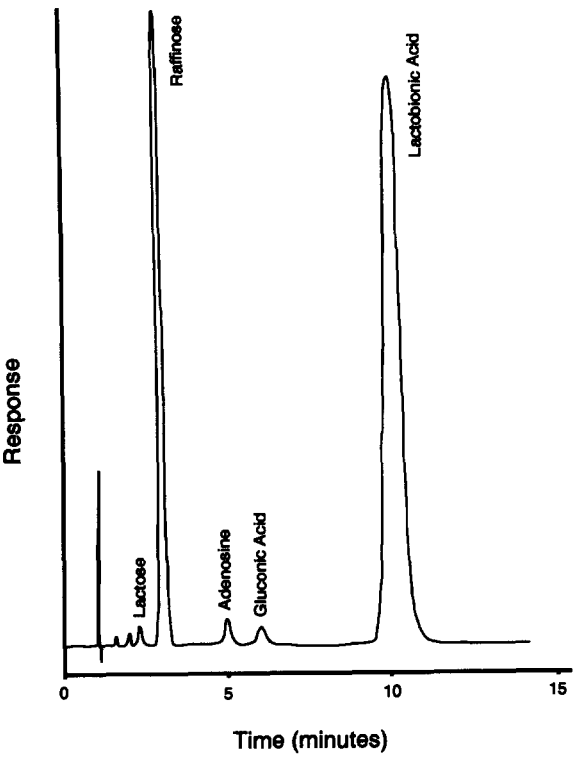

Fig. 3. Representative chromatogram of a ViaSpan® solution.

background levels and detector response to the analytes. Since raffinose and lactobionic acid act as weak acids, the pH will also affect the degree of ionization, leading to changes in retention. In contrast, the concentration of the sodium acetate influenced mainly the retention. The combination of both ingredients resulted in excellent sensitivity and selectivity for the desired compounds in ViaSpan® solutions. A representative chromatogram of a ViaSpan® sample is shown in Fig. 3. Compounds related to lactobionic acid by degradation or synthesis, gluconic acid and lactose, were also observed in the sample chromatograms along with one of the ingredients, adenosine.

3.3. Linearity

The linearity of the method was demonstrated from 10–120% of the analyte concentrations in prepared samples from ViaSpan® solutions. This range corresponded to 0.72–8.80 μg ml^{-1} for raffinose and 1.35–16.15 μg ml^{-1} for lactobionic

acid. Correlation coefficients were both greater than 0.999 and the Y intercepts corresponded to less than 0.7% of the 100% theoretical concentrations of raffinose and lactobionic acid in ViaSpan®. At concentrations corresponding to greater than 140% of the analyte concentration, the peak shape of lactobionic acid begins to deteriorate.

3.4. Precision

System precision (RSD), determined from 12 injections, was found to be less than 0.5% for both raffinose and lactobionic acid. The method precision was determined from the analysis of six bags of ViaSpan® obtained from the same lot of manufactured product. The method precision was calculated as 1.06% for raffinose and 0.78% for lactobionic acid.

3.5. Sensitivity

The limits of detection ($S/N = 3$) for raffinose and lactobionic acid were found to be 0.05 μg ml^{-1} and 0.10 μg ml^{-1} for raffinose and lactobionic acid respectively. The limits of quantitation ($S/N = 10$) were found to be 0.17 μg ml^{-1} and 0.33 μg ml^{-1} for raffinose and lactobionic acid respectively.

3.6. Specificity

The specificity of the method was demonstrated by its ability to resolve raffinose and lactobionic acid from all potential degradation products, impurities and other ViaSpan® constituents. A sample chromatogram of a ViaSpan® sample containing common impurities is shown in Fig. 3.

Raffinose can degrade by hydrolysis into the disaccharides melibiose and sucrose. It can also hydrolyze completely into the monosaccharides galactose, glucose and fructose. Lactobionic acid can degrade by hydrolysis into the monosaccharides galactose and gluconic acid. It can also contain small amounts of lactose as a synthetic impurity. All of these potential impurities and the ViaSpan® constituents adenosine, glutathione and hydroxyethyl starch were either resolved from raffinose and lactobionic acid or showed no response with the PAD detector.

4. Conclusion

Anion exchange liquid chromatography with pulsed amperometric detection is an excellent method for the quantitation of raffinose and lactobionic acid in the ViaSpan® organ preservation solution. The method is suitable for product testing in a manufacturing quality control environment.

References

[1] J.D. Olechno, S.R. Carter, W.T. Edwards and D.G. Gillen, Am. Biotechnol. Lab., 5 (1987) 38–50.
[2] Y.C. Lee, Anal. Biochem., 189 (1990) 151–162.
[3] S. Hughes and D.C. Johnson, Anal. Chim. Acta, 132 (1981) 11–22.
[4] R.D. Rocklin and C.A. Pohl, J. Liq. Chromatogr., 6 (1983) 1577–1590.
[5] D.C. Johnson and W.R. LaCourse, Anal. Chem., 62 (1990) 589A–597A.
[6] L.A. Th. Verhaar, H.E.J. Hendricks, W.P.Th. Groenland and B.F.M. Kuster, J. Chromatogr., 549 (1991) 113–125.
[7] M. Stefansson and B. Lu, Chromatographia, 35 (1993) 61–66.
[8] W.K. Herber, K. Robinett and R.S. Robin, J. Chromatogr., 676 (1994) 287–295.
[9] G. Palla, Anal. Chem., 53 (1981) 1966–1967.
[10] W.R. LaCourse, D.A. Mead, Jr. and D.C. Johnson, Anal. Chem., 62 (1990) 220–224.