Journal of Chromatography B, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Development and application of novelty pretreatment method for the concurrent quantitation of eleven water-soluble B vitamins in ultrafiltrates after renal replacement therapy

Dorota Wirkus [a], Aleksandra Jakubus [a], Radosław Owczuk [b], Piotr Stepnowski [a], Monika Paszkiewicz [a,*]

[a] University of Gdansk, Faculty of Chemistry, Department of Environmental Analysis, ul. Wita Stwosza 63, 80-308 Gdansk, Poland
[b] Department of Anaesthesiology and Intensive Therapy, Medical University of Gdansk, ul. Mariana Smoluchowskiego 17, 80-214 Gdansk, Poland

ARTICLE INFO

Article history:
Received 3 April 2016
Received in revised form 9 September 2016
Accepted 14 September 2016
Available online xxx

Keywords:
Water-soluble vitamins
Ion exchange resins
LC–MS
High volume continuous veno-venous haemofiltration
Ultrafiltrates

ABSTRACT

Continous renal replacement therapy (CRRT) is particularly recommended for septic shock patients in intensive care units. The CRRT technique used most frequently is high volume continuous veno-venous haemofiltration. It provides a high rate of clearance of uremic toxins and inflammatory cytokines. However, it should also be taken into account that substances important for homeostasis may be concurrently unintentionally removed. Accordingly, water-soluble vitamins can be removed during continuous renal replacement therapy, and the estimate of the loss is critical to ensure appropriate supplementation. The aim of this work was to develop a simple methodology for a purification step prior to the LC–MS/MS determination of water-soluble vitamins in ultrafiltrate samples. For this purpose, two types of resin and a mix of resins were used as sorbents for the purification step. Moreover, parameters such as the amount of resin and the extraction time were optimized. The LC–MS/MS method was developed and validated for final determination of 11 vitamins. The results demonstrated the high purification capability of DEAE Sephadex resin with recoveries between 65 and 101% for water-soluble vitamins from ultrafiltrate samples. An optimized method was applied to assess the loss of B-group vitamins in patients after 24 h of renal replacement therapy. The loss of vitamins B2, B6 pyridoxamine, B6 pyridoxal, B7, B1, and B5 in ultrafiltrates was similar in all patients. In the native ultrafiltrates, vitamins B6 pyridoxine, B9 and B12 were not detected.

1. Introduction

Continuous renal replacement therapies became widely used in intensive care units in the 90 s of the XX century [1–5]. Hemofiltration is particularly recommended for septic shock patients in intensive care units. It belongs to the continuous renal replacement therapy techniques, and on the basis of convective transport, dissolved substances are removed with a stream of water [1–5]. This mechanism increases with the difference in pressure on both sides of the hemofilter's membrane, where the reduced pressure (typically 200–500 mm Hg) is on the outside of the membrane [4,5]. This process mimics the natural filtration in the kidney glomerulus [3]. Continuous renal replacement therapy implies the effective removal of uremic toxins and inflammatory cytokines. The electric charge, lipophilicity and protein binding have a significant influence on the removal of proinflammatory and antiinflammatory mediators as a result of the filtration process [6]. Due to the relatively large pore diameter of high-flux membranes, the risk of loss of the nontoxic substances, including proteins, amino acids, vitamins and trace elements, increased [1,2]. During renal replacement therapy, the loss of micronutrients, particular trace elements and water-soluble vitamins was also observed [7–9]. Hemodialysis patients may suffer from vitamin deficiency [10–12]. Until now, a reduction in the concentration of vitamins in the serum of patients after renal replacement therapy was established [10–12]. A slight loss of water-soluble vitamins can be seen from the first days of hemodialysis [10]. The loss of vitamins such as: ascorbic acid, folic acid and pyridoxine was shown in most of the patients after hemodialysis. [7,9]. The loss of vitamins depends on the molecular weight of the particle, the treatment time and the type of dialysis membrane [6,10,13]. Vitamin C, due to good water-solubility and particle size, can be easily removed with dialysate [9,10,13,14].

* Corresponding author.
E-mail address: monika.paszkiewicz@ug.edu.pl (M. Paszkiewicz).

http://dx.doi.org/10.1016/j.jchromb.2016.09.021
1570-0232/

Hitherto, the loss of B-group vitamins in ultrafiltrates during renal replacement therapy has not been shown [10,13,15].

Therefore, it is essential to assess the loss of these substances during renal replacement therapy in order to select the appropriate dose of supplementation. Qualitative and quantitative analysis of vitamins is a difficult task of research. A major analytical problem is that the vitamins belong to different groups of organic compounds and thus differ in chemical properties. The techniques most widely used are HPLC with different detection, like UV–VIS with variable wavelength or diode array detection (DAD) [16–19], UV–MS [20], fluorescence (FLD) [21] and capillary electrophoresis (CE) [22,23]. There are also several reports describes the use of LC–MS technique for the determination of water-soluble vitamins [20,24–30]. Moreover, there are no preconcentration methods dedicated to the isolation of water-soluble vitamins from ultrafiltrates obtained after haemofiltration. Due to the complex matrix and the large sample volume of ultrafiltrates, the sample preparation step is extremely important prior to the final analysis. Moreover, the composition of ultrafiltrates is variable and depends on the patients' diseases, applied therapy and pharmacological or renal replacement. In available literature it can be found that ion exchange resins are useful for the initial treatment of urine samples and food samples [31–37]. The authors report effectiveness and simplicity as the main advantages of sample preparation with a mixed ion exchange resin. Cedli et al. [31] used the same commercial resin for desalting urine prior to the determination of monosaccarides. They recommended using 500 mg of resin to 1 mL of urine as an optimal ratio. A lower amount of resin compromised the purification, whereas a higher reduced sugar recovery. In another work, a smaller amount of resin, 250 mg per 1 mL, was used for the purification of urine [33]. Such an amount of resin ensures both desalting of the urine specimens and the highest recoveries of sugars. For all of the analytes, analytical recoveries were close to 100% after the purification step.

Considering the similarity of the urine and the ultrafiltrate matrix it was decided to check the usefulness of ion exchange resins for the purification of ultrafiltrates samples prior to the LC–MS determination of vitamins. Due to the expected low concentrations of vitamins, the complex matrix of ultrafiltrates and the large sample volume, the main objective of this work was to develop a simple methodology for 11 vitamins determination in ultrafiltrate samples. At first, the sorption rate of vitamins on ion exchange resin was checked and then the amount of resin and contact time was optimized. The LC–MS/MS method was developed and validated for final determination of 11 vitamins. The developed method was applied to determine the vitamins in ultrafiltrate samples from patients of the Medical University of Gdansk.

2. Materials and methods

2.1. Chemicals

The vitamin standards (analytical standard, >97%) including thiamine hydrochloride (B1) riboflavin (B2), nicotinamide (B3 amide), nicotinic acid (B3 acid), calcium pantothenate (B5), pyridoxal hydrochloride (B6 pyridoxal), pyridoxine hydrochloride (B6 pyridoxine), pyridoxamine dihydrochloride (B6 pyridoxamine), biotin (B7), folic acid (B9), cyanocob(III)alamin (B12) were purchased from Sigma-Aldrich (Steinheim, Germany). The stock solution containing individual vitamins were prepared at a concentration 0.5 $mgmL^{-1}$, and kept at 4 °C in darkness. Then a working solution at concentration of 0.1 $mgmL^{-1}$ was prepared by mixing suitable volumes of the stock solutions containing individual vitamin. DEAE Sephadex (Diethylaminoethyl Sephadex) and Amberlite XAD-2 were obtained from Sigma-Aldrich (Steinheim, Germany). Gradient grade methanol, potassium dihydrogen phosphate (pure p.a.), phosphoric acid(V) (pure p.a.) was purchased from POCh (Gliwice, Poland).

2.2. LC–MS/MS analysis

The LC–MS/MS system consisted of an Agilent 1200 Series LC system (Agilent Technologies Inc., Santa Clara, USA) and an HCT Ultra ion trap mass spectrometer (Brucker Daltonics, Bremen, Germany) equipped with an electrospray ionization source. EsquireControl software was used for data acquisition. Analytes were separated on a Hypersil Gold aQ C18 polar endcapped column (150 cm × 4.6 mm, 5 μm pore size, Thermo Scientific) at a temperature of 25 °C. Mobile phase A was a 5 mM HCOOH aqueous solution, pH 3.00 and mobile phase B was 100% MeOH. The gradient elution was as follows: from 0 to 10 min isocratic elution with 0% of mobile phase B, then the mobile phase B content was increased linearly to 80% in 10 min, and after that the conditions were isocratic (20% phase A and 80% of phase B, 8 min). From 28 to 33 min solvent B was decreased to 0% and then maintained for another 2 min for column re-equilibration. The flow rate was 0.3 mL min^{-1}, the injection volume was 50 μL and the analytical wavelength was 270 nm. The LC flow was introduced directly into the MS. The source temperature was 300 °C. The system was operated in multiple reaction monitoring mode (MRM). The optimized MS/MS conditions are included in Table 1.

2.3. Validation procedure

The validation procedure for simultaneously determining 11 vitamins using LC–MS/MS analysis was undertaken [38,39]. Several parameters, such as linearity, the regression coefficients (R^2), precision, accuracy, the quantification limit (LOQ), and the detection limit (IDL) were established. The linearity was designated by triplicate measurements of the calibration samples containing 11 vitamins at concentration ranging from 0.5 μgL^{-1} to 3000 μgL^{-1}. The lowest instrumental detectable (LOD) and quantifiable (LOQ) limit were calculated as the concentration levels that provides a signal-to-noise ratio of 3 and 10, respectively. Precision were evaluated by means of five consecutive injections in the same day of a standard mixture of the vitamins at three levels of concentration (1 μgL^{-1}, 100 μgL^{-1}, 1000 μgL^{-1}). The accuracy was determined according to equation: accuracy = $C_{measured}/C_{known}) \times 100\%$ for five concentration (1 μgL^{-1}, 10 μgL^{-1}, 100 μgL^{-1}, 1000 μgL^{-1}, 2000 μgL^{-1}).

2.4. Sorption rate of water-soluble vitamins onto the ion exchange resin

Before use, the ion-exchange resin was prepared according to the following procedure: the resin was washed with 50 mL of deionized water, followed by 30 mL of methanol and then once again 50 mL of deionized water. 10 mL of deionized water was spiked with the vitamin B mixture solution to a final concentration of 1 μgmL^{-1}. Then, the ion exchange resin Amberlite XAD 2, DEAE Sephadex (300 mg per 10 mL of water sample) or a mixture of resins (300 mg per 10 mL of water sample, Amberlite XAD 2:DEAE Sephadex 2:1 w/w) was added. The contact time between water sample and resin was 30 min. Then, 1 mL of the sample was filtered using a syringe filter with a pore size of 0.45 μm and analyzed by LC–MS/MS. All experiments were performed in triplicate.

2.5. Purification of ultrafiltrate samples using DEAE sephadex resin

200 mL of the ultrafiltrate sample was taken and lyophilized. Then, the lyophilizate was dissolved in 10 mL of deionized water

Please cite this article in press as: D. Wirkus, et al., Development and application of novelty pretreatment method for the concurrent quantitation of eleven water-soluble B vitamins in ultrafiltrates after renal replacement therapy, J. Chromatogr. B (2016), http://dx.doi.org/10.1016/j.jchromb.2016.09.021