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Direct determination of polyglucose metabolites in plasma using anion-exchange chromatography with pulsed amperometric detection

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Abstract

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE–PAD) was evaluated for the quantitation of polyglucose metabolites (DP2–DP7) in human plasma. The method was investigated for accuracy, precision, specificity, linearity, range and analyte stability. Samples were prepared by dilution into the standard range (0.1–10 μg/ml) followed by deproteinization using a 30 000 molecular mass cut-off filtration device. The limit of detection was 0.05 μg/ml for all metabolites. Method precision for DP2–DP7 varied from approximately 2% R.S.D. in the upper range to approximately 15% R.S.D. at the limit of quantitation. Samples were stable following one or two freeze–thaw cycles and, after preparation, they could be refrigerated for up to 72 h. Application of this method to clinical plasma samples from continuous ambulatory peritoneal dialysis (CAPD) patients administered one daily night-time intraperitoneal exchange of 2 l of 7.5% polyglucose solution for four weeks indicated that plasma levels of DP2, DP3 and DP4 increased from baseline levels of <0.01 g/l to steady-state levels of 1.2±0.3, 1.2±0.3 and 0.4±0.1 g/l (mean±S.D.), respectively. These steady state plasma levels for DP2 and DP3 are comparable to previously reported levels in patients administered daily overnight 7.5% polyglucose dialysis solution.

Keywords: Polyglucose; Glucose

1. Introduction

Peritoneal dialysis is a renal replacement therapy that utilizes an intraperitoneally administered solution containing an osmotic agent, generally glucose, to effect transport of water and metabolic waste products from the blood to the peritoneal cavity. Polyglucose (PG), a soluble polymeric form of glucose that is primarily linked α1–4, has been demonstrated to be an effective osmotic agent for peritoneal dialysis in end stage renal disease (ESRD) patients [1,2]. Clinical studies using PG with a weight average molecular mass of approximately 14 000–20 000 (DP86–DP120) have shown that only 20–30% of the polyglucose is absorbed and its metabolism is not complete [2]. The absorbed polymer is hydrolyzed by circulating α-amylase, resulting in elevated steady state levels of the metabolites maltose (DP2), maltotriose (DP3) and maltotetraose (DP4), and relatively minor elevations of maltopentaose (DP5), maltohexaose (DP6) and maltoheptaose (DP7).

Previous studies have reported maltose and maltotriose to be the primary metabolites following poly-

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glucose administration [1,2]. The analytical method employed was gel-permeation chromatography on BioGel P2 with a modified Jelco 6AH automatic carbohydrate analyzer and an orcinol–sulfuric acid detection system [3]. This method requires long analysis times (9–10 h) and is relatively insensitive, due to peak broadening.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE–PAD) has been demonstrated to be a sensitive and specific method for carbohydrate analysis, enabling the separation of oligosaccharides differing in linkage position [4,5]. HPAE–PAD has been successfully utilized in a variety of analytical applications for the analysis of carbohydrates in complex sample matrices [5–7]. This report describes the validation and application of a method for the quantitation of DP2–DP7 in plasma from ESRD patients administered peritoneal dialysis solution containing polyglucose. The method involves sample deproteinization and dilution, separation on an ion-exchange column, and pulsed amperometric detection. This method has high selectivity without interferences from plasma components.

2. Experimental

2.1. Materials

Maltose (DP2), maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6) and maltoheptaose (DP7), with purities of 92–99%, were purchased from Sigma (St. Louis, MO, USA). Sodium hydroxide 50% (w/w) solution (J.T. Baker, Phillipsburg, NJ, USA), sodium acetate trihydrate, ultrapure (J.T. Baker) and MilliQ water (Millipore, Bedford, MA, USA) were used. Centrifree Micropartition System filter units (Amicon, Beverly, MA, USA), centrifuged in a GPR Centrifuge, GA24 fixed-angle rotor (Beckman Instruments, Palo Alto, CA, USA), were used for sample deproteinization.

2.2. Apparatus and chromatographic conditions

The chromatography system (DX-500, Dionex, Sunnyvale, CA, USA) consisted of a Model GP40 gradient pump, a Model ED40 electrochemical detector and a Model 717 injector (Waters, Milford, MA, USA). Data were acquired and integrated using a Waters 860 Data system running ExpertEase v3.1 chromatography software. The analytical column was a CarboPac PA-1 (25×0.4 cm) with a CarboPac PA-1 Guard column (2.5×0.4 cm) supplied by Dionex. Metabolites were separated using the gradient shown in Table 1. Detection, using integrated amperometry, was performed on a Gold electrode using a Ag–AgCl reference. PAD settings were as follows: E1+0.05 V (0.4 s), E2+0.75 V (0.2 s), E3−0.15 V (0.4 s), integrate (0.2–0.4 s).

2.3. Preparation of standard and samples

Since the purity of commercially available maltooligosaccharides (DP2–DP7) varied from 92–99%, standards were prepared individually and assayed to assess cross-contamination from other DPs. Small interfering cross contaminant peaks were observed near DP5 and DP6. Accurate quantitation of DP5 and DP6 was achieved, however, by using the pump gradient (shown in Table 1) to partially resolve the contaminant peaks and by using peak height instead of area for quantitation. Following corrections for cross contaminants, purity and water content of individual maltooligosaccharide standards, a stock standard containing DP2–DP7, each at a concentration of 100 μg/ml, was prepared and aliquots were frozen for subsequent analyses. Dilutions of the combined DP2–DP7 standard were prepared daily to yield concentrations of 10, 5, 2.5, 1, 0.5 and 0.1 μg/ml.

Pooled plasma blanks were prepared by diluting

Table 1
Pump gradient

Time (min)	Eluent 1 (%)	Eluent 2 (%)
Initial	95	5
0.0	95	5
10.0	95	5
29	73	27
35	60	40
35.1	40	60
40	40	60
40.1	95	5
60	95	5

Eluent 1: 100 m*M* Sodium hydroxide.
Eluent 2: 0.5 *M* Sodium acetate in 100 m*M* sodium hydroxide.

1:100 with MilliQ water. Spiked pooled plasma was prepared (n=4) on three separate days by the addition of stock standard DP2–DP7 to the diluted plasma, yielding spike concentrations of 7.5, 1 and 0.1 μg/ml. Approximately 1 ml of each sample was transferred to a Centrifree filter unit and protein was removed by centrifuging at 1500 g for 15 min at approximately 4°C. A 0.25-ml volume of filtrate was transferred to an autosampler vial and maintained at 4°C until injection.

Samples from clinical patients were prepared by diluting 1:200 with MilliQ water followed by deproteinization as described previously.

3. Results and discussion

Initial sample preparation involved dilution of samples (1:10, v/v) in MilliQ water prior to injection. Although adequate separation of DP2–DP7 from plasma components was achieved, a progressive loss in detector response occurred over the course of an analytical run (ten–fifteen samples), contributing to unacceptable method accuracy and precision. Membrane deproteinization, in combination with dilution (1:10, v/v), was incorporated into the sample preparation procedure in an effort to stabilize detector response; however, the progressive loss in detector response was still observed.

An internal standard (cellotriose), eluting between DP3 and DP4, was also evaluated to compensate for the loss in detector response. Since detector response varies across DPs, cellotriose was only useful in correcting detector response for peaks having similar detector response (DP3, DP4). Therefore, the use of an internal standard was discontinued because it failed to improve method accuracy and precision.

After reviewing our efforts to correct the loss in detector response, increasing sample dilution from 1:10 to 1:100 followed by membrane deproteinization was chosen as the best sample preparation procedure. Increasing the sample dilution reduced the method's limit of quantitation by a factor of ten; however, it resulted in a stable detector response, leading to acceptable method accuracy and precision. Although the limit of quantitation was increased to 0.01 g/l in undiluted plasma, the potential impact on the analysis of clinical samples would be minimal because this level represents only 1–2% of the plasma steady-state metabolite levels for DP2–DP4. Following validation as described below, this method was applied to the analysis of clinical samples.

3.1. Standard response range

Linear and quadratic response models were evaluated for lack of fit testing and residual plots. A quadratic regression model provided the best fit of response to component concentration for each DP, with correlations of >0.999 for all DP peaks. Therefore, all subsequent standard calibrations were performed using a six-point standard curve ranging from 0.1 to 10 μg/ml, fit to a quadratic polynomial. A typical integrated standard chromatogram at 5 μg/ml and calibration curves for DP2–DP4 are shown in Fig. 1a,b, respectively.

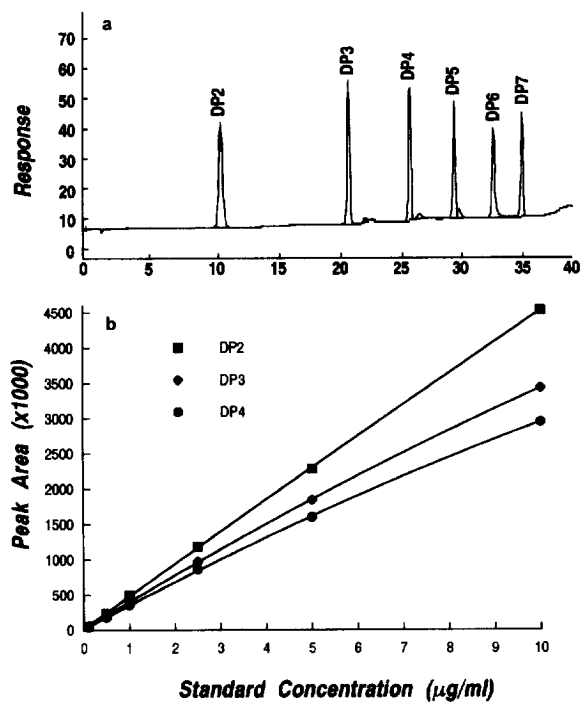

Fig. 1. (a) Chromatogram of 5 μg/ml PG metabolite standard. (b) Typical standard curves for DP2–DP4. Lines represent the best estimate of assay values across concentrations using a quadratic model. DP2: $y=-1.07x^2+461x+20.7$; DP3: $y=-5.04x^2+392x+13.1$; DP4: $y=-5.24x^2+347x+9.09$.

Table 2
Inter-day precision and accuracy of PG metabolite method.

Concentration added (μg/ml)	*n*	Mean concentration found (μg/ml)	Precision [R.S.D. (%)]	Accuracy [Recovery (%)]
7.50				
DP2	12	7.46	1.2	99–100
DP3	12	7.53	1.3	99–101
DP4	12	7.58	1.4	99–101
DP5	12	7.51	1.6	98–101
DP6	12	7.58	1.9	97–102
DP7	12	7.64	2.7	99–103
1.00				
DP2	12	1.02	5.3	97–105
DP3	12	1.04	2.8	102–104
DP4	12	1.01	3.0	97–103
DP5	12	1.00	2.8	97–104
DP6	12	0.99	2.4	97–105
DP7	12	0.99	3.0	98–105
0.10				
DP2	12	0.11	15.4	92–115
DP3	12	0.11	6.2	105–117
DP4	12	0.09	17.2	81–103
DP5	12	0.10	10.8	99–109
DP6	12	0.10	12.9	87–92
DP7	12	0.10	26.7	91–105

3.2. Precision and accuracy

A summary of results obtained by injection for all peaks DP2–DP7 is presented in Table 2. These results were assessed based on acceptance criteria of not more than 15% R.S.D. for precision [20% at the limit of quantitation (LOQ)] and not more than 15% deviation from the nominal value for accuracy (20% at LOQ) [8,9].

Accuracy acceptance criteria of not more than 15% deviation from the nominal value (20% at LOQ) determined as percent recovery were met for all peaks at all spike levels (Table 2). Inter-day sample precision acceptance criteria of not more than 15% R.S.D. (20% at LOQ) were met for all metabolites at all levels, except for DP7 at 0.1 μg/ml (26.7% vs. 20% R.S.D. limit).

3.3. Limit of quantitation (LOQ)

The LOQ was defined as the lowest detectable standard concentration with a precision below 20% R.S.D. All DP peaks, with the exception of DP7, met these LOQ criteria at a concentration of 0.1 μg/ml, with precision and accuracy ranging from 6.2–17.2 and 81–117%, respectively.

3.4. Specificity

Human plasma samples from six different patients with ESRD were prepared in duplicate and assayed. Baseline separation was observed between DP peaks and all other peaks. In addition, the analysis of clinical baseline plasma samples ($n=20$) showed no observed interferences with DP2–DP7.

3.5. Stability

No significant change in PG metabolite concentrations was detected in human plasma samples stored at −70°C for one month nor after two freeze–thaw cycles. Following sample preparation, analytes proved to be stable when stored at 4°C for up to 72 h.

3.6. Clinical sample analysis

During a seven-week clinical study, peritoneal dialysis patients received one daily night-time intraperitoneal exchange of either 4.25% dextrose Dianeal (Baxter Healthcare, Deerfield, IL, USA) at weeks 1, 6 and 7 or 7.5% PG containing dialysis solution at weeks two–five [10]. Weekly blood samples were collected and plasma samples were separated and frozen at −70°C until analysis. Fig. 2 shows typical chromatograms of a pooled plasma blank, a pooled plasma spike and a typical sample from a patient at week 5. Results from patients administered 7.5% PG solution intraperitoneally for four weeks indicated that plasma levels of DP2, DP3 and DP4 increased from baseline levels of <0.01 g/l to steady state levels of 1.2±0.3, 1.2±0.3 and 0.4±0.1 g/l (mean±S.D.), respectively. Following discontinued administration of the 7.5% PG-containing dialysis solution, all metabolite concentrations returned to baseline levels of less than 0.01 g/l.

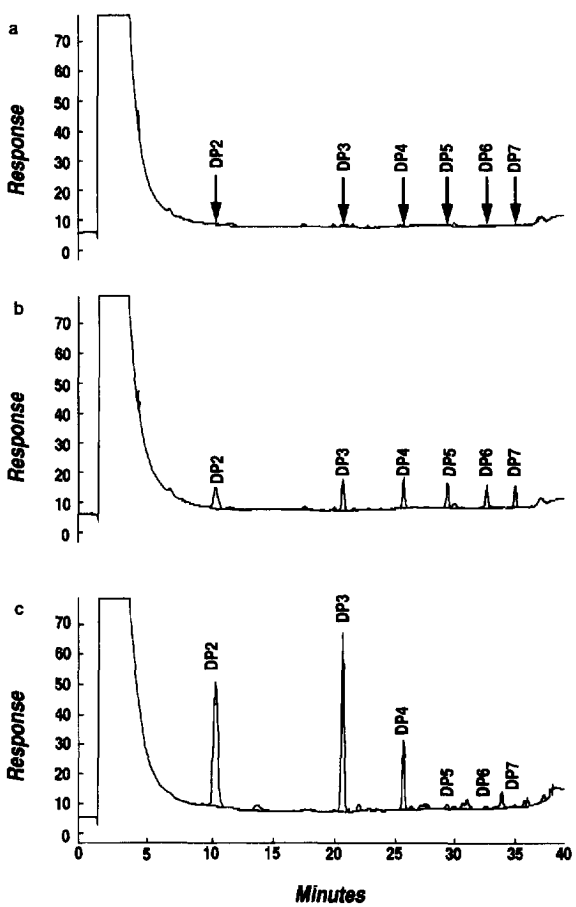

Fig. 2. Representative chromatograms of PG metabolites in plasma. (a) Pooled plasma blank. The arrows indicate retention times of DP2–DP7. (b) Pooled plasma spiked with 1 μg/ml PG standard. (c) Week 5 plasma sample (diluted 1:200) from a patient following four weeks of daily intraperitoneal administration of 7.5% PG-containing peritoneal dialysis solution.

4. Conclusions

HPAE–PAD provides a rapid, sensitive and direct determination of the major PG metabolites (DP2–DP4) in human plasma. A minimum sample dilution of 1:100 and deproteinization prior to injection are required to maintain detector stability. The application of this method to the analysis of clinical samples provides a significant advantage over the previously reported method [3], by reducing run time and increasing specificity for the quantitation of PG metabolites in human plasma.

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