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Quantification of reactive carbonyl compounds in icodextrin-based peritoneal dialysis fluids by combined UHPLC-DAD and -MS/MS detection

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ABSTRACT

During heat sterilization of peritoneal dialysis (PD) fluids, the glucose component is partially degraded. The formed glucose degradation products impair biocompatibility and limit the long-term application of PD fluids. As an alternative to glucose, icodextrin, a polyglucose, is used as osmotic agent in PD fluids. After targeted screening for reactive carbonyl compounds, NMR- and MS-analyses very recently revealed 4-deoxyglucosone (4-DG), 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), 3,4-dideoxypentosone (3,4-DDPS), and 5-hydroxymethylfurfural (5-HMF) as main polyglucose degradation products (pGDPs) in icodextrin-based PD fluids. Now, the present study established and validated a UHPLC method with DAD as well as a UHPLC-MS/MS method for the first-time quantification of those five major pGDPs in commercial icodextrin PD fluids after derivatization with *o*-phenylenediamine. Thus, 4-DG was identified to be the main degradation product (in concentrations up to 20 μM). In contrast to the values measured in glucose-based products, the concentration of 3-DGal (≤16 μM) was higher than the concentration of 3-DG (≤7 μM) indicating different reaction pathways starting from polyglucose compared to glucose. The compounds 3,4-DDPS and 5-HMF were present in minor quantities (≤0.3 μM each).

1. Introduction

Patients suffering from renal failure may undergo different renal replacement therapies, mainly hemodialysis or peritoneal dialysis (PD). While the former is a clinical-based extracorporal blood purification method, PD provides intracorporal treatment at home. During PD, the patient's peritoneal cavity is filled with a highly osmotic fluid, which is discharged after the filtration process. Diffusion and osmosis along an osmotic pressure gradient drive uremic waste like urea or creatinine and excessive water from the blood stream into the PD fluid, while the peritoneal lining functions as semipermeable membrane [1,2]. For the removal of water, PD fluids must contain an osmotic agent. The most commonly employed agent, glucose, however, has certain disadvantages: Its rapid absorption leads to a loss of osmotic pressure gradient already after a short dwell time [3]. Secondly, glucose is partially degraded during the heat sterilization of PD fluids, which results in the formation of glucose degradation products (GDPs) [4]. GDPs are highly reactive substances, which can modify biomolecules like proteins or DNA. This leads to the formation of advanced glycation endproducts [5–8], impairing cellular and peritoneal membrane functions, for example by reducing enzyme activity [9–13]. Furthermore, GDPs show cytotoxic effects on peritoneal mesothelial cells and interfere with cell signaling [13–18]. Thus, GDPs reduce the biocompatibility of PD fluids and may cause the discontinuation of therapy. GDPs in glucose-containing PD fluids have already been investigated in detail. To date, the following GDPs were identified and quantified in glucose-based PD fluids: monocarbonyls like 5-hydroxymethylfurfural (5-HMF; in concentrations ≤69 μM), acetaldehyde (≤420 μM), 2-furaldehyde (≤3 μM), and formaldehyde (≤11 μM) as well as α-dicarbonyl-GDPs like

Abbreviations: 3-DG, 3-deoxyglucosone; 3-DGal, 3-deoxygalactosone; 3,4-DDPS, 3,4-dideoxypentosone; 3,4-DGE, 3,4-dideoxyglucosone-3-ene; 4-DG, 4-deoxyglucosone; 5-HMF, 5-hydroxymethylfurfural; DTPA, diethylene triamine pentaacetic acid; GDP, glucose degradation product; KET, keto-enol tautomerization reactions; MGO, methylglyoxal; MRM, multiple reaction monitoring; OPD, *o*-phenylenediamine; PD, peritoneal dialysis; pGDP, polyglucose degradation product.

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Table 1
Calibration curves for parallel UHPLC-DAD analysis of 3-deoxyglucosone, 3-deoxygalactosone, and 5-hydroxymethylfurfural in polyglucose-based PD fluids after derivatization with *o*-phenylenediamine. Linear regression models of a standard series of 3-(quinoxalin-2′-yl) propane-1-diol (3,4-$DDPS_{qx}$) and peritoneal dialysis (PD) fluids spiked with 3,4-$DDPS_{qx}$. Analysis of 3,4-$DDPS_{qx}$ was performed by UHPLC-MS/MS.

	Linear regression	R^2	Concentration [μM]
3-Deoxyglucosone	$0.0497x - 0.0126$	0.9998	0.54–35.03
3-Deoxygalactosone	$0.0359x - 0.0103$	0.9998	0.44–35.08
5-Hydroxymethylfurfural	$2.0452x - 0.1685$	0.9997	0.10 –10.09
3,4-$DDPS_{qx}$	$5.67 \times 10^6 x + 1.76 \times 10^4$	0.9998	0.01–0.52
PD fluid lot A[a]	$4.93 \times 10^6 x + 9.76 \times 10^5$	0.9960	0.05–0.52
PD fluid lot B[a]	$5.02 \times 10^6 x + 1.04 \times 10^6$	0.9960	0.05–0.52
PD fluid lot C[a]	$5.40 \times 10^6 x + 1.60 \times 10^6$	0.9996	0.05–0.52

[a] Spiked with different concentrations of 3,4-$DDPS_{qx}$.

glucosone (≤41 μM), 3-deoxyglucosone (3-DG; ≤540 μM), 3-deoxygalactosone (3-DGal; ≤137 μM), glyoxal (≤23 μM), methylglyoxal (MGO; ≤14 μM), and 3,4-dideoxyglucosone-3-ene (3,4-DGE; ≤18 μM) [5,15,19–28].

To avoid the drawbacks of glucose-based PD fluids, osmotic agents such as icodextrin are used as an alternative. Icodextrin is a starch-derived, water-soluble polyglucose, where the monomers are linked mainly via 1,4-glycosidic bonds [3]. The glucose polymers with an average molecular weight of 17 kDa cannot be absorbed as fast as glucose monomers. Consequently, the osmotic pressure gradient and, thus, the mass transfer across the peritoneum remain stable for a longer time [29,30]. Furthermore, additional calorie uptake is reduced [31]. Some studies also indicate that the concentration of GDPs is lower compared to conventional glucose-based PD fluids [15,22]. However, 1,4-glycosidically linked saccharides are subject to other degradation pathways than monosaccharides, resulting in different degradation products [32–36]. Therefore, the formation of degradation products in icodextrin-based PD fluids would supposedly be underestimated, if analysis aimed only for glucose-derived degradation products. Consequently, profiling of reactive degradation products in polyglucose-based PD fluids was achieved by NMR- and MS-analyses after targeted screening [37]. Thus, 3-DG, 3-DGal, 4-deoxyglucosone (4-DG), 3,4-dideoxypentosone (3,4-DDPS), and 5-HMF could be identified as major polyglucose degradation products (pGDPs) in icodextrin-based PD fluids. Among those, 4-DG and 3,4-DDPS had not been detected in glucose-containing products so far, indicating that both structures were typical of polyglucose-based fluids.

The present study developed and validated a method for the quantification of 3-DG, 3-DGal, 4-DG, 3,4-DDPS, and 5-HMF, combining UHPLC-DAD and UHPLC-MS/MS after derivatization with *o*-phenylenediamine (OPD) to yield quinoxaline or benzimidazole derivatives, respectively. Finally, the method was applied to measure pGDP levels for the first time in icodextrin-containing PD fluids.

2. Materials and methods

2.1. Reagents and samples

Unless otherwise noted, all chemicals and reagents were at least of analytical grade and purchased from Acros (Geel, Belgium), AppliChem (Darmstadt, Germany), Fluka (Steinheim, Germany), or Sigma–Aldrich (Steinheim, Germany). 3-DG (purity >95%) was obtained from Chemos (Regenstauf, Germany) and 5-HMF from SAFC (St. Louis, MO, USA). 3-DGal as well as 3-(quinoxalin-2′-yl) propane-1-diol (3,4-$DDPS_{qx}$) were synthesized as reported previously [37,38]. The present study investigated three lots of a typical commercial PD fluid containing 7.5% icodextrin.

Table 2
Validation parameters of the UHPLC-DAD method analyzing 3-deoxyglucosone, 3-deoxygalactosone, and 5-hydroxymethylfurfural in icodextrin-containing peritoneal dialysis fluids. Recovery rates from six independent measurements ± coefficient of variation are shown.

3-Deoxyglucosone	
Recovery (34.4 μM) in %	101.9 ± 0.6
Recovery (16.0 μM) in %	100.4 ± 0.9
Recovery (0.7 μM) in %	102.5 ± 3.5
LOD in nM	2.8
LOQ in nM	4.3
3-Deoxygalactosone	
Recovery (34.6 μM) in %	103.0 ± 0.8
Recovery (15.9 μM) in %	100.6 ± 1.6
Recovery (0.7 μM) in %	101.4 ± 3.0
LOD in nM	2.6
LOQ in nM	5.1
5-Hydroxymethylfurfural	
Recovery (9.5 μM) in %	100.7 ± 1.0
Recovery (1.5 μM) in %	98.7 ± 1.6
Recovery (0.2 μM) in %	98.4 ± 1.0
LOD in nM	7.6
LOQ in nM	11.6

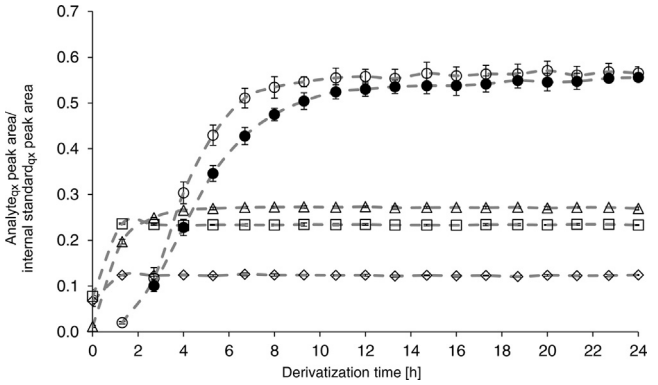

Fig. 1. Effect of derivatization time on the formation of (5-(1*H*-benzo[*d*]imidazol-2-yl) furan-2-yl) methanol (5-HMF_{bfm}, ○), (*S*)-(3-(3-hydroxymethyl) quinoxalin-2-yl) propane-1,2-diol (4-DG_{qx}, Δ), (2*R*,3*R*)-4-(quinoxalin-2-yl) butane-1,2,3-triol (3-$DGal_{qx}$, □), and (2*R*,3*S*)-4-(quinoxalin-2-yl) butane-1,2,3-triol (3-DG_{qx}, ◊) as well as the derivatization progress of 5-HMF in presence of diethylene triamine pentaacetic acid (DTPA, ●). Mean ± SD of three independent measurements of a representative PD sample spiked with 4 μM 5-HMF are shown.

2.2. UHPLC-DAD

The applied Dionex UltiMate 3000RS UHPLC-DAD system (Thermo Fisher Scientific, Dreieich, Germany) included a pump with degasser, autosampler, temperature-controlled column compartment, and diode array detector. Data acquisition and processing was carried out by Chromeleon 6.80 software. An ACQUITY UPLC® BEH phenyl column (1.7 μM particle size; 2.1 × 100 mM, Waters, Eschborn, Germany) equipped with a Waters VanGuard ACQUITY UPLC® BEH phenyl precolumn (1.7 μM particle size;