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Journal of Chromatography A, 1034 (2004) 133–137
JOURNAL OF CHROMATOGRAPHY A
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Separation and detection of oxidation products of fluorodeoxyglucose and glucose by high-performance liquid chromatography–electrospray ionisation mass spectrometry

E. Búriová [a,*], M. Medová [a], F. Macášek [b], P. Brúder [a]

[a] *Department of Nuclear Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska Dolina CH-1, SK-84215 Bratislava, Slovak Republic*
[b] *Cyclotron Centre of the Slovak Republic, Slovak Office of Standards, Metrology and Testing, P.O. Box 76, SK-81005 Bratislava, Slovak Republic*

Received 23 October 2003; received in revised form 26 January 2004; accepted 29 January 2004

Abstract

2-Deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F] FDG), the most popular positron emitting radiopharmaceutical, may oxidise by autoradiolysis in aqueous solution. The aim of this work was to use LC–MS for determination of the oxidation products of fluorodeoxyglucose and glucose (Glc) obtained by oxidation with Fenton's reagent. Asahipak NH2P-50 polyamide silica column and acetonitrile–0.025% aqueous ammonium formate (80:20 (v/v)) eluent were utilised with an Agilent 1100 HPLC–MS instrument. Ten major oxidation products of FDG and Glc were separated and identified by mass spectrometry: 2-fluorogluconic acid, 2-fluoroglucuronic acid, 2-oxoerythronic acid, arabinose, arabonic acid, araburonic acid, erythrose, erythrulose, gluconic acid, and glucuronic acid. The most intensive electrospray ionisation signals were found in the negative ion spectra and were due to $HCOO^-$ adducts, the other acids being in their lactone forms.

Keywords: Fluorodeoxyglucose; Glucose; Carbohydrates; Organofluorine compounds

1. Introduction

Carbohydrates are some of the most difficult biomolecules to separate and measure at low levels. Traditional methods of analysis include separation and detection by gas chromatography, however, derivatisation often results in incomplete transformation of a species to a complex mixture of volatile derivatives. Therefore, GC is not suitable for pharmaceuticals quality control and thin-layer chromatography is preferred [1–3]. The first work on analysis of carbohydrates by HPLC was performed by Lawrence [4]. Now, several reviews and monographs appeared on the topic, see [5–7] for example. Usually, good separation of mono- and oligosaccharides and their fluorinated derivatives is achieved on cation-exchange resin using water as eluent [8–13] or by ion-exclusion chromatography [14]. Recently, excellent separation of monosaccharides was achieved using concanavalin immobilised on Sepharose. Small differences between monosaccharides in terms of affinity interaction with a lectin-based stationary phase were successfully exploited to give good resolution with isocratic elution [15].

The organic acids, which are assumed to be oxidation products of glucose (Glc) and 2-deoxy-2-fluoro-D-glucose (FDG), were also analysed by HPLC and UV-Vis. A Spherisorb column was used with sulphuric acid as the eluent [16,17].

With ion-exchange HPLC, refractometric index detection lacks adequate sensitivity, and eluents containing non-volatile buffers or other salts are not suitable for the atmospheric pressure electrospray ionisation (ESI) source for MS analysis. Therefore, analysis and characterisation of carbohydrates using a combination of HPLC and MS [18,19] and ESI of carbohydrates has been described [20,21]. We have applied the HPLC–ESI-MS to the development of an analytical method for the radiopharmaceutical [^{18}F] FDG [22] and have published detailed ESI mass spectra of FDG and Glc [23].

In previous studies on the oxidation of saccharides by Fenton's reagent [24], separation of glucose oxidation

* Corresponding author. Fax: +421-2-60294748.
E-mail address: e_buri@hotmail.com (E. Búriová).

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doi:10.1016/j.chroma.2004.01.060

products was done only by paper chromatography [25–27]. No works deal with detection and separation of the oxidation products of fluorodeoxyglucose, although the presence of transient cytotoxic species of unknown nature in the [^{18}F] FDG stock solutions [28] may encourage such work. In the present article, we use polyamine silica and MeCN–ammonium formate eluent for complex FDG analysis [22].

2. Experimental

2.1. Reagents and methods

Pure HPLC-grade water (Acros Organics) and HPLC-grade acetonitrile (99.98%) (Scharlau) were used for eluent preparation. Ammonium formate, arabinose, FDG, gluconic acid (all from Sigma–Aldrich), glucose (Lachema, Czech Republic), and glucuronic acid (Fluka) were of analytical grade.

Chromatography was performed at a constant temperature in the range of 10–45°C on a polyamide-bonded silica-base Shodex Asahipak NH2P-50 2D 150 mm × 4 mm column (Showa Denko, Japan) using a SGXNH$_2$ 40 mm × 4 mm (Tessek, Czech Republic) guard column. The mobile phase, MeCN–0.025% (4 mM) ammonium formate varied from 60:40 to 90:10 in composition and was used at a constant flow rate of 0.2 ml/min.

Resolution $R_{j,i}$ of the ith and jth solute was calculated according to:

$$R_{j,i} = \frac{2(t_{R,j} - t_{R,i})}{Y_j + Y_i} \quad (1)$$

where t_Rs are the corresponding retention times and Ys are the base widths of chromatographic peaks.

An Agilent 1100 LC–MS system operated by Windows NT based ChemStation software was used. The HPLC equipment was used with UV-Vis diode array detection (DAD) and mass spectrometric (MS) detection. The ESI source of the MS system worked with a nebulizing nitrogen gas pressure of 35 p.s.i. (0.25 MPa), a capillary voltage of 3000 V and a fragmentation voltage of 70 V. Drying nitrogen gas at 250 °C was used at a flow rate of 10 l/min.

Both positive and negative ions in the 80–450 *m/z* range were registered in the conventional scanning mode or in the single ion measurement (SIM) mode. Mass spectra were evaluated only in the negative mode because of superior sensitivity.

2.2. Oxidation of saccharides with Fenton's reagent

To 400 μl of a 0.40 g/l solution of FDG or glucose (pH adjusted to 4–5 with concentrated sulphuric acid) were added 20 μl of 2.77 g/l solution of $FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$. At room temperature were added 25 μl of 20% (v/v) H_2O_2 and after 1.5 h another portion of hydrogen peroxide. After 3 h, iron was removed from the reagent mixture using a small plastic syringe loaded with about 300 mg of Dowex 50-X8 (NH_4^+) cation exchanger. The solution was passed through the column, which was afterwards washed with 400 μl of pure water. The washed solution was diluted using an acetonitrile–0.025% ammonium formate solution (80:20 (v/v)), in volume ratio 1:4. A 5 μl sample of this solution was applied to the chromatographic column.

3. Results and discussion

To find optimal performance, chromatographic resolution of substrates and their oxidation products at various temperatures was tested on the FDG–Glc, FDG–gluconic acid and gluconic acid–glucuronic acid pairs (Table 1). The retention times of FDG, GLC, gluconic and glucuronic acid was examined at various eluent compositions (Table 2). It is not possible to get generally valid rules about the influence of temperature on HPLC separations. At increased temperature the performance of a column often increases because of the decrease of mobile phase viscosity, which improves mass transfer. The optimal temperature was the same as used before for the [^{18}F] FDG analysis [22], and the same eluent composition MeCN–0.025% ammonium formate (80:20) was found suitable. Moreover, an advantage of using MS is that the detection of analytes can be achieved either by reconstructing the characteristic ion chromatograms for FDG and Glc (Figs. 1 and 2), from the scanning experiment, or by using SIM detection of the analyte of particular interest.

Oxidation products were sought because hydrogen peroxide in the presence of Fe^{2+} salts induces chain shortening

Table 1
Resolution (R)[a] of the substrate–oxidation product pairs as a function of column temperature

Column temperature (°C)	R (gluconic acid–glucuronic acid)	R (FDG–gluconic acid)	R (Glc–FDG)
10	0.78 ± 0.28	0.16 ± 0.17	4.01 ± 0.80
15	0.89 ± 0.29	0.18 ± 0.18	4.70 ± 0.82
20	1.07 ± 0.30	0.15 ± 0.17	4.79 ± 0.83
25	1.04 ± 0.30	0.22 ± 0.19	5.18 ± 0.85
30	1.21 ± 0.31	0.37 ± 0.20	5.63 ± 0.90
35	1.07 ± 0.30	0.35 ± 0.20	5.47 ± 0.88
40	1.01 ± 0.30	0.18 ± 0.18	5.23 ± 0.87

[a] See Eq. (1).

Table 2
Retention times (t_R, min) of FDG, Glc, gluconic and glucuronic acids as a function MeCN–0.025% ammonium formate eluent composition

Composition MeCN–ammonium formate	FDG	Glc	Gluconic acid	Glucuronic acid
60:40	6.0 ± 0.1	6.9 ± 0.2	5.9 ± 0.2	4.4 ± 0.1
70:30	6.2 ± 0.1	8.8 ± 0.2	6.2 ± 0.2	4.8 ± 0.1
80:20	6.3 ± 0.1	9.5 ± 0.2	6.3 ± 0.2	5.1 ± 0.1
90:10	6.6 ± 0.2	15.2 ± 0.2	6.4 ± 0.2	5.3 ± 0.1

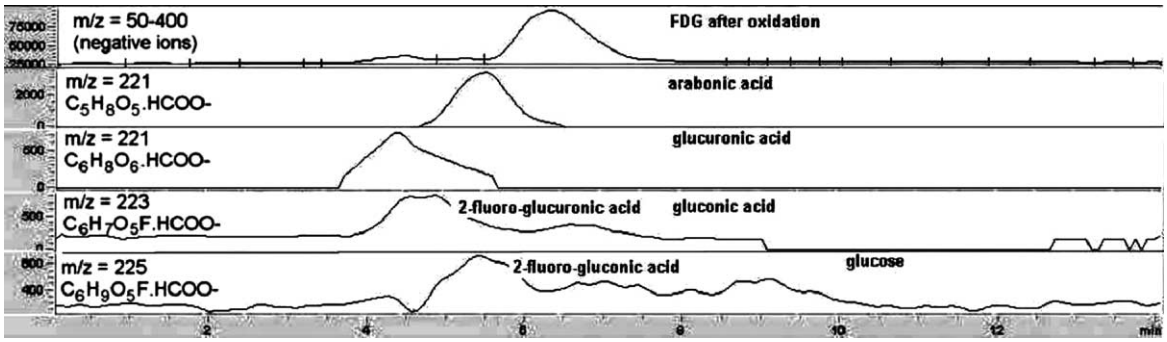

Fig. 1. Chromatograms of oxidation products of FDG.

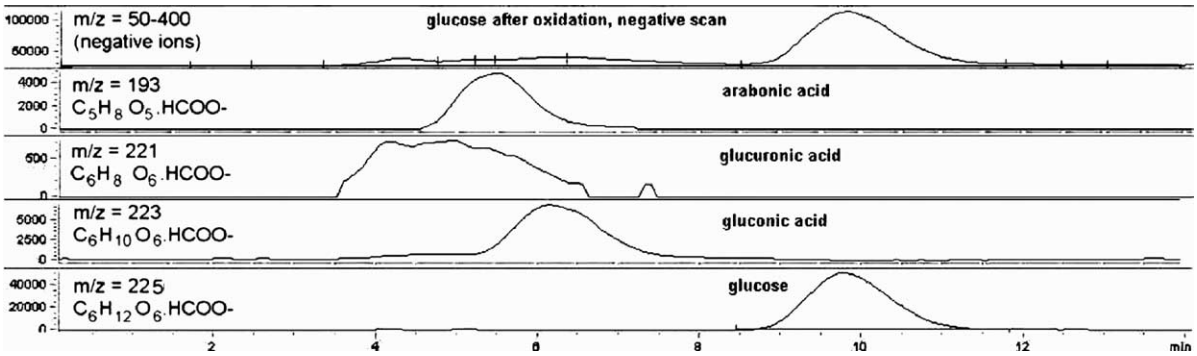

Fig. 2. Chromatograms of oxidation products of Glc.

which proceeds via loss of a hydrogen atom at the β-carbon [29], as shown in Fig. 3. Common processes in the oxidation of hexoses are the formation of hexonic acids and ring-scission reactions, which lead to aldehydic fragments, for example formaldehyde and arabinose [30].

Samuelsson and Thede [31] have studied oxidative degradation of glucose in details and shown the presence of a series of by-products as 2-deoxyerythro-pentonic acid, gluconic and glucuronic acid, arabonic acid, erythronic acid, etc. Following these expected products we analysed the chromatographic peaks and mass spectra, and has acquired corresponding values of the retention times and the ion forms in which these products occurred.

The calibration curves of FDG and Glc were constructed using SIM registration of *m/z* 227 and 225 signals for FDG and glucose correspondingly, which relate to M·HCOO$^-$ anion [23]. When y is a peak area [imp/s] and x is a weight of analyte, then $y = 17343 + 1296x$ ($r^2 = 0.9974$) for FDG, and $y = 12195 + 1102x$ ($r^2 = 0.9973$) for glucose. According to these data, oxidative decomposition of the substrates was about 47–49%.

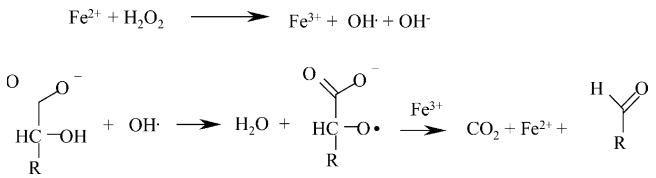

Fig. 3. Chain shortening of saccharides by oxidation processes [29].

The most intensive signal of each oxidation product was found in the negative ion spectra as the associates with the HCOO$^-$ anions. No signals related to the anions of the acids occurred, and the acids were identified only in their lactones forms associated with formate anion. In Figs. 4 and 5, the three-dimensional negative-ion spectra of chromatograms of fluorodeoxyglucose and glucose solution,

Table 3
Percentage yields of the oxidation products of FDG and Glc

Oxidation products	Yield (%)
Fluorodeoxyglucose	
Erythrose	1 ± 0.1
Erythrulose	1.8 ± 0.2
2-Oxoerythronic acid	0.8 ± 0.1
Arabinose	3.4 ± 0.2
Arabonic acid	14.5 ± 2.5
Araburonic acid	1.1 ± 0.2
2-Fluorogluconic acid	3.9 ± 0.1
2-Fluoroglucuronic acid	2.7 ± 0.2
Gluconic acid	1.8 ± 0.4
Glucose	2.7 ± 0.2
Glucose	
Erythrose	1.2 ± 0.1
Erythrulose	5.9 ± 0.7
2-Oxoerythronic acid	3.3 ± 0.3
Arabinose	7.1 ± 0.5
Arabonic acid	23 ± 2.1
Araburonic acid	2.1 ± 0.4
Gluconic acid	12.1 ± 2.3
Glucuronic acid	5.5 ± 0.5

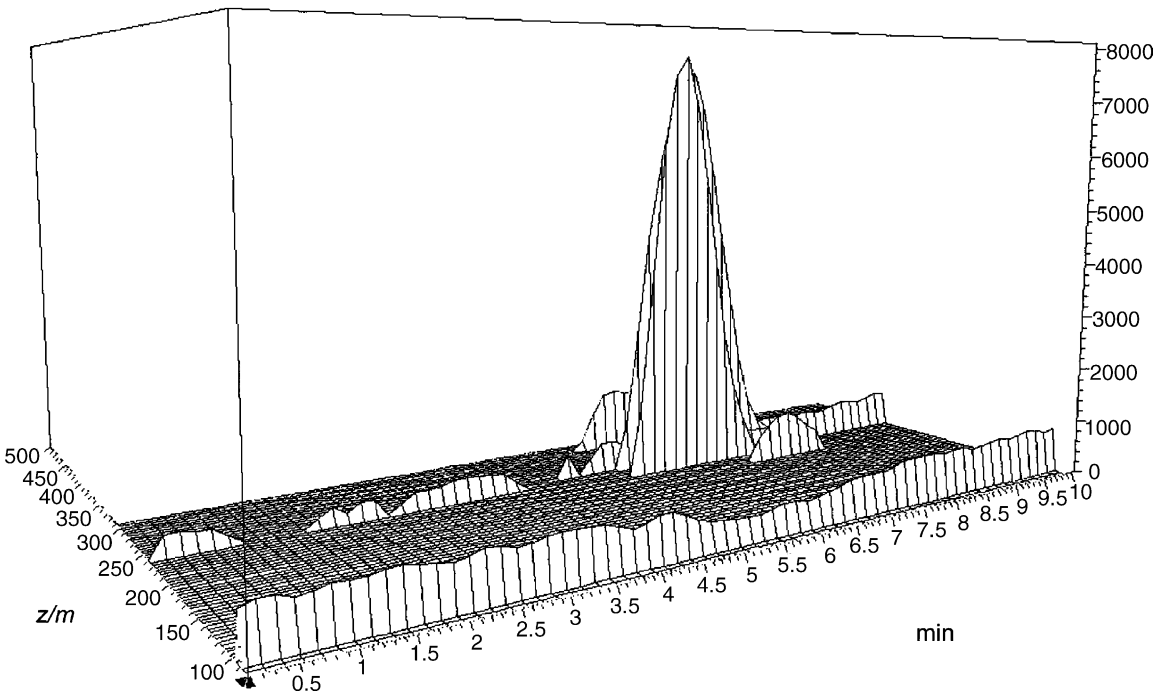

Fig. 4. Three-dimensional negative-ion spectra of FDG and its oxidation products.

respectively, are displayed. The LC–MS chromatograms and spectra show the presence of some unknown components. Table 3 shows the oxidation products of FDG and Glc, which were identified by MS, with their percentage yields. Table 4 shows details of the oxidation products of fluorodeoxyglucose, retention time and corresponding *m/z* value of the characteristic signal, and interpretation of the specific ion form.

Most important from the point of view of the [^{18}F] FDG decomposition is that 2-fluorogluconic and 2-fluoroglucuronic acid are the primary products of oxidation of FDG. They were determined in its lactones' form by signal at the *m/z* 225 $(C_6H_{11}O_6{}^{19}F - H_2O + HCOO^-)^-$ and *m/z* 223 $(C_6H_9O_6{}^{19}F - H_2O + HCOO^-)^-$. Isobar signals of FDG·$HCOO^-$ or Glc·$HCOO^-$ can not interfere with the previous ones because they are well resolved from the FDG

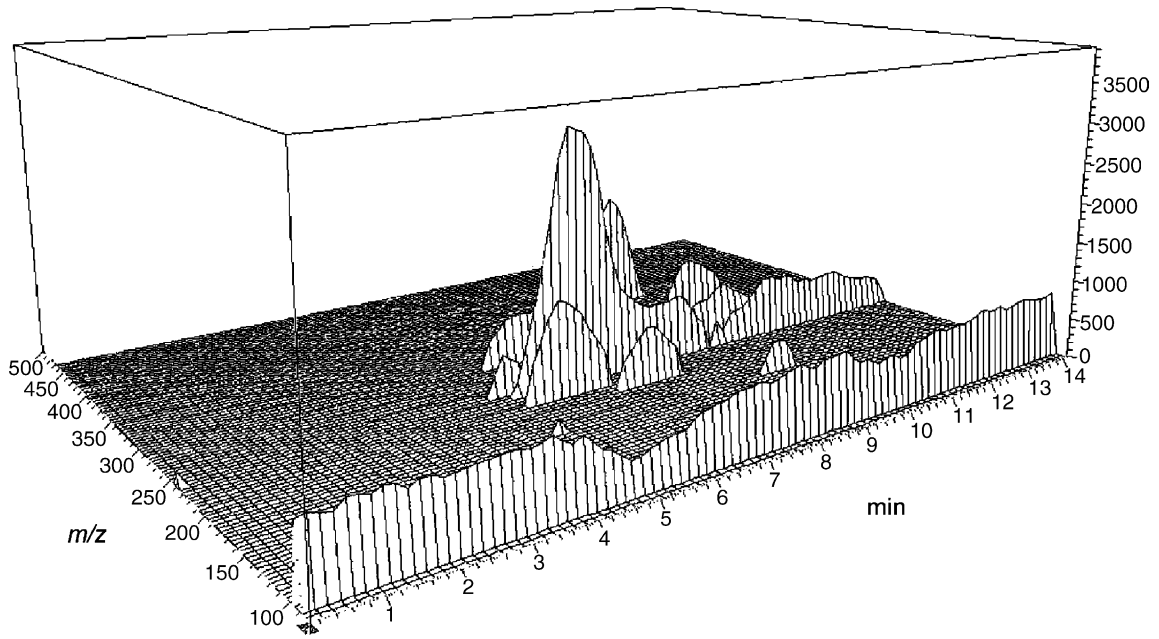

Fig. 5. Three-dimensional negative-ion spectra of Glc and its oxidation products.

Table 4
Characterisation of FDG and its oxidation products in ESI negative ion spectra

Oxidation products of FDG	Ionic forms	Retention time (min)	*m/z*
2-Fluoroglucuronic acid	$(C_6H_9O_6{}^{19}F - H_2O + HCOO^-)^-$	4.6 ± 0.1	223
Erythrulose	$(C_4H_6O_4 + HCOO^-)^-$	4.9 ± 0.1	163
Glucuronic acid	$(C_6H_{10}O_7 - H_2O + HCOO^-)$	5.1 ± 0.1	221
2-Oxoerythronic acid	$(C_4H_6O_5 - H_2O + HCOO^-)^-$	5.1 ± 0.2	161
Araburonic acid	$(C_5H_8O_6 - H_2O + HCOO^-)^-$	5.2 ± 0.2	191
Erythrose	$(C_4H_8O_4 + HCOO^-)^-$	5.3 ± 0.2	165
Arabonic acid	$(C_5H_{10}O_6 - H_2O + HCOO^-)^-$	5.4 ± 0.2	193
2-Fluorogluconic acid	$(C_6H_{11}O_6{}^{19}F - H_2O + HCOO^-)^-$	5.7 ± 0.3	225
Gluconic acid	$(C_6H_{12}O_7 - H_2O + HCOO^-)^-$	6.3 ± 0.3	223
FDG	$(C_6H_{11}O_5{}^{19}F + HCOO^-)^-$	6.3 ± 0.1	227
Arabinose	$(C_5H_{10}O_5 + HCOO^-)^-$	7.4 ± 0.2	195
Glucose	$(C_6H_{12}O_6 + HCOO^-)^-$	9.5 ± 0.2	225

and Glc peaks. (Table 4). These two fluorinated derivatives are formed in relatively high yield due to a primary oxidation of FDG by hydroxyradicals, and the corresponding autoradiolysis products of [^{18}F] FDG can be expected in long-stored (expired) radiopharmaceutical solutions. 2-Fluoro-2-deoxy-3,4-dioxopenturonic acid signal at *m/z* 207 ($C_5H_3O_5F \cdot HCO_2{}^-$?!) was a suspect in expired sample [22] but its presence was not confirmed in the model systems above. It is evident, however, that all such admixtures may decrease the noise at the positron emission tomography scanning.

As the oxidation products of minor abundance and importance were found arabinose, arabonic and araburonic acid. Small amounts of erythrose and its oxidation products erythrulose and 2-oxoerythronic acid are found as well.

Acknowledgements

Experiments were performed on HPLC–DAD–MS instrumentation of the Cyclotron Centre of the Slovak Republic supplied by the International Atomic Energy Agency within a grant of the Technical Co-operation Project SLR/2/002.

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