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Note

High-performance liquid chromatographic determination of glucose-1-phosphate and glucuronic acid-1-phosphate

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Oxidation of glucose-1-phosphate [α-D-glucopyranosyl phosphate (I)] by means of oxygen in the presence of a platinum catalyst[1] results in the formation of glucuronic acid-1-phosphate [α-D-glucupyranuronic acid 1-phosphate (II)]. Hydrolysis products, which are subsequently oxidized to the corresponding acids (gluconic and glucaric acid), are also formed. Hydrolysis also gives rise to orthophosphate (III), which may, *inter alia*, also be present in I. In the course of our study of the catalytic oxidation of I[2], we needed a rapid method for the quantitation of I and II in the presence of the hydrolysis products.

Chromatographic separations of phosphate esters, using anion-exchange resins or gels as the stationary phase, are well known[3–6]. These methods, however, generally employ complex salts and pH gradients which require post-column colorimetric detection. Ion-moderated partioning chromatography[7] has been applied to the analysis of I and simple carbohydrates[8]. However, we have been unable to resolve I and II by this method.

As the potential of ion-pair reversed-phase chromatography for the separation of moderately strong acids is being increasingly recognized[9], we decided to investigate the application of this method to the determination of I and II. Because of the low pK_a values of I–III (1.1–2.1 for the first ionization[10]), pH values of the mobile phase down to 1.5 might be required, which is within the capabilities of Nucleosil C_{18}[11]. We selected the tetrabutylammonium ion as the lipophilic cation and hydrogen sulphate–sulphate as the buffer system.

EXPERIMENTAL

Reagents

Glucose-1-phosphate (dipotassium salt) and tetrabutylammonium hydroxide (40% aqueous solution) were obtained from Janssen Chimica (Beerse, Belgium). Glucuronic acid-1-phosphate (tripotassium salt) was prepared in our laboratory[2]. All other chemicals were obtained from Merck (Darmstadt, F.R.G.).

Chromatography

Chromatographic analyses were carried out using a Waters Assoc. M 6000A

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TABLE I

CAPACITY FACTORS (k') ON NUCLEOSIL C_{18} OF ORTHOPHOSPHATE, GLUCOSE-1-PHOSPHATE AND GLUCURONIC ACID-1-PHOSPHATE AT DIFFERENT pH VALUES OF THE MOBILE PHASE

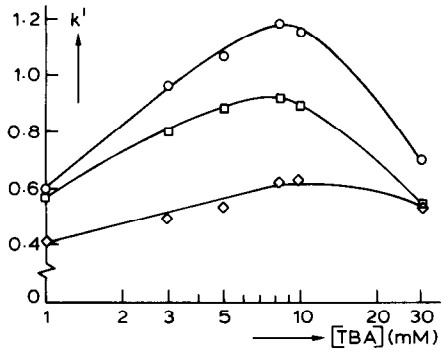
Chromatographic conditions: 10 m*M* TBA; flow-rate, 1.0 ml/min; temperature, 25°C.

Component	k'		
	pH 1.8	*pH 2.2*	*pH 3.0*
Exclusion peak	−0.13	−0.13	−0.13
Orthophosphate (III)	0.44	0.93	1.23
Glucose-1-phosphate (I)	0.73	1.07	1.27
Glucuronic acid-1-phosphate (II)	0.97	2.10	3.24

pump, a Rheodyne 7125 injection valve, a Waters Assoc. custom-packed 100 × 8 mm I.D. 10-μm Nucleosil C_{18} Radial-Pak cartridge in an RCM 100 radial compression unit and a Waters Assoc. R401 refractive index detector. The mobile phase was prepared from tetrabutylammonium hydroxide and the pH was adjusted with 96% sulphuric acid. The mobile phase was filtered and degassed by sonication under low pressure before use. The tetrabutylammonium concentration and the pH of the samples were made equal to those of the mobile phase by addition of a concentrated tetrabutylammonium solution with a low pH.

RESULTS AND DISCUSSION

Initially, the tetrabutylammonium ion (TBA) concentration was kept at 10 m*M*, whilst the pH was varied from 1.8 to 3.0. The retention of I–III decreased with



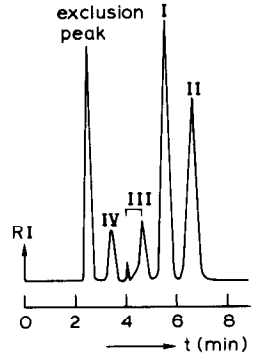


Fig. 1. Capacity factors (k') of glucose-1-phosphate (□), glucuronic acid-1-phosphate (○) and orthophosphate (◇) at different TBA concentrations on Nucleosil C_{18}. Chromatographic conditions: pH, 1.9; flow-rate, 1.0 ml/min; temperature, 25°C.

Fig. 2. Chromatographic separation of glucose-1-phosphate (I), glucuronic acid-1-phosphate (II), orthophosphate (III) and glucaric acid (IV) on Nucleosil C_{18}. (The orthophosphate peak is disturbed by the presence of a system peak.) Chromatographic conditions: 8.3 m*M* TBA; pH, 1.9; flow-rate, 1.0 ml/min; temperature, 25°C.

TABLE II

CAPACITY FACTORS (k') OF SEVERAL COMPOUNDS ON NUCLEOSIL C_{18}

Chromatographic conditions: 8.3 m*M* TBA; pH, 1.9; flow-rate, 1.0 ml/min; temperature, 25°C.

Component	k'	*Component*	k'
Exclusion peak	−0.13	Glucose-1-phosphate	0.87
Glucose	0.07	Glucuronic acid-1-phosphate	1.20
Gluconic acid	0.13	Succinic acid	1.53
Glucaric acid	0.13	Malonic acid	2.07
Orthophosphate	0.60	Oxalic acid	2.60

increasing pH, which reflects the suppression of the ionization of the phosphate groups at low pH (Table I). The selectivity was also pH dependent: at pH > 2.2 I and III were not resolved; at pH < 1.8 the resolution of I and II was unsatisfactory. A pH of 1.9 was selected as the optimum value for further experiments.

When the concentration of TBA was increased from 3 to 30 m*M*, the retention of I–III showed a maximum at a TBA concentration of 8.3 m*M* (Fig. 1). Presumably, the surface concentration of TBA is then at a maximum[12] and decreases at higher concentrations in the mobile phase because of micelle formation.

The capacity factors of I–III and several carboxylic acids as measured at a TBA concentration of 8.3 m*M* and pH 1.9 are given in Table II. A baseline separation of I–III is obtained under these conditions, as shown in Fig. 2. Quantitative determinations of I and II were carried out with a relative error of 3% at concentrations of *ca.* 30 m*M* in the samples.

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