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Note

Chromatographic separation of some small molecules from their halogenated analogues

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The semi-preparative scale separation of tyrosine, uracil, cytosine, uridine and histidine from their bromo- and iodo-analogues was investigated using reversed-phase high-performance liquid chromatography (HPLC). Successful separations were achieved using pure water as eluent.

Small molecules such as amino acids, pyrimidines and related compounds are frequently labelled with radioisotopes of iodine or bromine for studies in biochemical or medicinal research[1–5]. In general the labelling techniques used produce a mixture of the parent compound and the halogenated product which requires separation on a preparative or semi-preparative scale before the halogenated material is available for use. The most commonly used method of achieving such separations has been some form of ion-exchange chromatography[5], although this has the disadvantage that the desired product is obtained in a solution of the salts used for buffering.

We have developed semi-preparative, reversed-phase HPLC separations for a number of small biomolecules and the non-radioactive iodinated and brominated analogues, which can be used for the isolation of the halogenated material in aqueous solution free from the buffering agents mentioned above. We report here on the separations of tyrosine, uracil, cytosine, uridine and histidine from their monoiodo- and monobromo-analogues.

EXPERIMENTAL

Materials

Uridine (99%), histidine (98%), 5-bromouridine and 5-bromocytosine were obtained from Aldrich Milwaukee, Wisc., U.S.A.; 5-bromouracil and 3-iodo-L-tyrosine were obtained from Sigma, St. Louis, Mo., U.S.A.; tyrosine (chromatographic grade) and cytosine (98%) were obtained from BDH Poole, Great Britain; uracil was obtained from Halewood Chemicals, Staines, Great Britain; All the above materials were found to be sufficiently pure on chromatographic analysis for our purposes and were used without further purification.

Samples of the other halogenated materials were obtained by warming the halogen and the parent compound in NaOH solution, following the method of Johnson and Johns[6]. The reaction mixtures, or mixtures produced by mixing samples of

parent compound and a halogenated analogue, were then analysed on a semi-preparative scale by HPLC.

Apparatus

The chromatographic separations were performed on an Altex Model 300 chromatograph fitted with a UV (254 nm) biochemical monitor. The column was 25 × 1 cm I.D., packed with 10-μm Spherisorb ODS (Phase Separations, Queensferry, Great Britain) and supplied by Anachem. The eluent was distilled water or water–methanol mixtures as described below. Samples were of fixed volume (0.5 ml) and were loaded using an Altex Model 905 sample injection valve. Elutions were performed at a column pressure of 460 p.s.i. which gave a flow-rate (of water) of *ca.* 10 ml/min.

RESULTS AND DISCUSSION

The individual compounds or preparation mixtures were chromatographed in water or water–methanol eluents by loading 0.5-ml samples (*ca.* 50 μg of compound in water) onto the column. In most cases an eluent flow-rate of *ca.* 10 ml/min was used, and in all cases the column was operated at room temperature (*ca.* 20°). After calibrating the system by obtaining retention volumes for the individual materials the separations were checked on samples containing both the parent compound and its halogenated analogue.

TABLE I

RETENTION VOLUMES OBTAINED USING DISTILLED WATER ELUENT

Flow-rate *ca.* 10 ml/min; column 25 × 1 cm Spherisorb ODS, 10 μm.

*Compound**	*Retention volume (ml)*
Tyrosine	45
3-Bromotyrosine	82
3-Iodotyrosine	220
Uracil	25
5-Bromouracil	44
5-Iodouracil	60
Uridine	26
5-Bromouridine	84
5-Iodouridine	35
Cytosine	30
5-Bromocytosine	82
5-Iodocytosine	80
Histidine**	35
5-Iodohistidine	55

* Samples *ca.* 50 μg in 0.5 ml water.

** Obtained by fraction collection and UV absorption measurements at 200–210 nm.

Chromatographic separations using water eluent

Since our primary interest was in obtaining the halogenated compounds in aqueous solution, most of our experiments were performed using distilled water as the eluent. The retention volumes obtained for each individual compound studied are collected in Table I. Most of the separations of mixtures occurred readily and the quantities of material involved could be increased where the solubilities allowed. The most difficult separation was that involving uracil and bromo- or iodouracil, and the chromatogram obtained for a uracil–iodouracil–iodide ion mixture is shown in Fig. 1. The peak shapes obtained for the uracil-containing components are much broader than was observed for other samples with similar retention volumes. In spite of this the separations were sufficient to allow collection of the halogenated product.

Variation of retention with methanol concentration

The retention volumes of all compounds studied decreased as methanol was added to the eluent. Typical of the effect of added methanol was the variation of retention volume of iodocytosine with eluent composition, shown in Fig. 2. In practice most of the separations were acceptable using methanol–water up to about 20% methanol, and were found particularly useful for iodotyrosine separations during radiolabelling experiments.

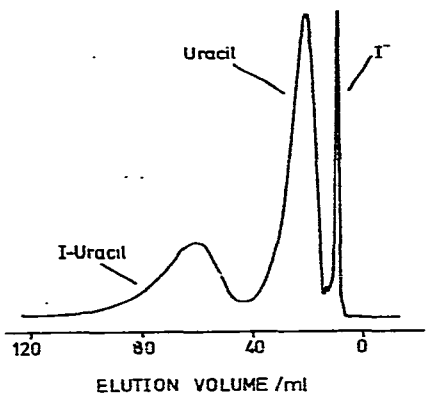

Fig. 1. Separation of I^-, uracil and 5-iodouracil on Spherisorb ODS column using water as eluent. Flow-rate *ca.* 10 ml/min.

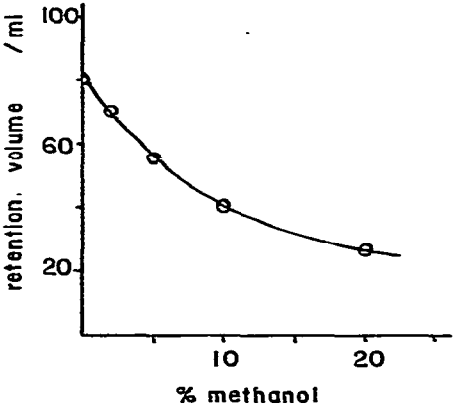

Fig. 2. Variation of retention volume for iodocytosine with methanol concentration using methanol–water as eluent.

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