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Note

High-performance liquid chromatography method for the separation of the halogenated pyrimidine 5-bromo-2′-deoxyuridine from its metabolites

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The halogenated pyrimidine 5-bromo-2′-deoxyuridine (BrdUrd) has a number of interesting biological effects including teratogenesis, mutagenesis and termination of differentiation[1]. Its widest use, derived from its action as a thymidine analogue, is in the visualization of the induction of sister chromatid exchanges (SCE), an important biological measure of genotoxicity[2]. Assessment of BrdUrd levels is complicated by its instability due to debromination and deribosylation *in vivo*[3]. Methods of analysis reported in detail are fairly tedious[3] or insensitive[4].

This paper describes a high-performance liquid chromatographic (HPLC) system to quantitate and separate rapidly BrdUrd and its common breakdown products.

MATERIALS AND METHODS

Analytical-grade chemicals were purchased from Sigma (St. Louis, MO, U.S.A.) except where noted. The different reagents were dissolved in distilled water to 1 mg/ml (bromouracil and uracil to 0.2 mg/ml) and these stock solutions diluted with distilled water and used in various combinations. Chromatography was performed on a Beckman Model 334 density gradient chromatograph using a reversed-phase Altex 25 cm × 4.6 mm ODS-Ultrasphere column. A water–methanol (87:13) mobile phase using HPLC grade methanol (Fisher, Pittsburgh, PA) and glass-distilled water at a flow-rate of 1.5 ml/min was used. UV absorbance was measured at 279 nm.

Radioactivity measurement

As a marker, 100 nCi of 5-[2-^{14}C]bromodeoxyuridine (44 mCi/mmol sp.act., New England Nuclear, MA, U.S.A.), 95% pure, was added. Fractions were collected from various metabolite peaks, incubated at 50°C until dryness, reconstituted to 1 ml with distilled water and vortexed. A 10-ml volume of Biofluor (New England Nuclear) was added to each, and the samples were counted on a Searle Analytic 92 liquid scintillation counter.

RESULTS AND DISCUSSION

The material from fractions containing the BrdUrd peak co-chromatograph with pure BrdUrd dissolved in 13% methanol at a number of different retention

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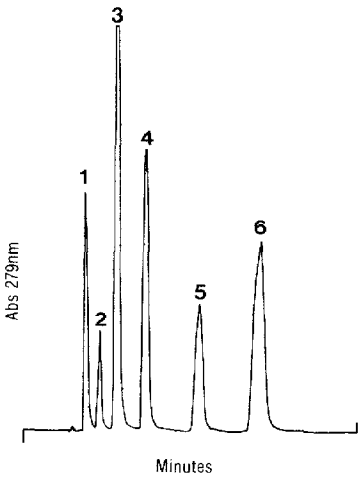

Fig. 1. Liquid chromatogram of BrdUrd and its metabolites. Column: 25 cm × 4.6 mm ODS Ultraspheres. Eluent: water–methanol (87:13). Flow-rate 1.5 ml/min. Sample: 20 μl of a solution containing 1 μg each of uracil (1), uridine (2), deoxyuridine (3), 5-bromouracil (4), 5-bromouridine (5) and BrdUrd (6). Abcissa full scale is ten min.

times, which were varied by altering the solvent composition from 6 to 18 % methanol and by varying the flow-rate. The UV absorption spectrum was also similar[1]. Approximately 99 % of the radioactivity was recovered from the column and 95 % was contained in the BrdUrd peak. Estimates of BrdUrd levels from radioactivity measurements and from integrated UV absorbance differed by less than 1 %.

The HPLC profile clearly differentiates the major *in vivo* metabolites of BrdUrd, uracil and bromouracil[3], from the parent compound as well as from other nucleosides (Fig. 1). Retention times of the various compounds are given in Table I for 8 determinations of each. Of the compounds tested, BrdUrd is the easiest to detect at 279 nm. The separation of the peaks is a result of a combination of the addition of the sugar, the deoxy sugar and the halogenation to the pyrimidine since progressive alterations of uracil lengthen the retention times in this methanol–water system. The halogenation is the most significant factor. The magnitude of the combined shift is enough to allow easy determination of the blood BrdUrd levels *in vivo* during SCE visualization[1] using a minor variation of this same technique with minimal treatment

TABLE I

RETENTION TIMES FOR BrdUrd AND OTHER NUCLEOSIDES USING A REVERSED-PHASE SYSTEM WITH WATER–METHANOL (87:13) AS MOBILE PHASE

Compound	*Retention time (min)*
Uracil	1.9
Uridine	2.3
Deoxyuridine	2.9
Bromouracil	3.8
Bromouridine	5.6
BrdUrd	7.6

of the blood sample. This permits rapid and sensitive measurement of biologically significant amounts of the nucleoside and quantification of its derivatives for a better understanding of its metabolism for elucidation of its biological effects.

REFERENCES

1 A. Turturro, N. P. Singh, J. Bazare, Jr. and R. W. Hart, *Proc. Arkansas Acad. Sci.*, in press.
2 P. E. Perry, in A. Hollaender and F. de Serres (Editors), *Chemical Mutagens*, Vol. 6, Plenum Press, New York, 1979, pp. 1–39.
3 J. P. Kriss and L. Revesz, *Cancer Res.*, 22 (1962) 254.
4 B. Matz, *J. Chromatogr.*, 187 (1980) 453.