*Journal of Chromatography*, 111 (1975) 242–245


CHROM. 8344

## Note

# Determination of 5-fluorouridine and 5-fluorocytidine in RNA hydrolysates using high-performance ion-exclusion chromatography

IVAN I. KAISER*

*Division of Biochemistry and Department of Chemistry, University of Wyoming, Laramie, Wyo. 82071 (U.S.A.)*

and

PATRICIA A. YOUNG

*Division of Biochemistry, University of Wyoming, Laramie, Wyo. 82071 (U.S.A.)*

(Received April 1st, 1975)

The 5-fluorouridine and 5-fluorocytidine content of RNA hydrolysates may be rapidly determined by using high-performance ion-exclusion chromatography[1,2]. The method described resolves nanomolar amounts of 5-fluorouridine, pseudouridine, uridine (U), 5-fluorocytidine, and cytidine (C) in less than 15 min at room temperature. Peak height is proportional to concentration, making quantitation a relatively simple matter. The use of an isocratic eluting buffer (0.02 *M* Tris–formic acid, pH 7.6) also eliminates the need for column regeneration following each analysis.

## EXPERIMENTAL

The liquid chromatograph used was a Waters Ass. (Milford, Mass., U.S.A.) Model ALC/GPC-202, equipped with a Model 6000 pumping system, a U6K injector valve, and ultraviolet (UV) detector (254 nm). All runs were at room temperature (*ca.* 23°) at a flow-rate of 1.0 ml/min (2000–2500 p.s.i.), with a full-scale UV-deflection equal to 0.16 absorbancy units. The photometer output (10 mV) was fed into a Varian (Walnut Creek, Calif., U.S.A.) A-25 strip-chart recorder. The stainless-steel column (60 cm × 3.5 mm I.D.) contained 2-$\mu$m stainless-steel frits and was a slurry packed with Aminex A-6, 17.5-$\mu$m particle size (Bio-Rad, Richmond, Calif., U.S.A.). Degassed, 0.02 *M* Tris–formic acid (pH 7.6) was used as the eluting buffer. All injections (25 $\mu$l) were made with a 0–25 $\mu$l Pressure-Lok liquid syringe (Precision Sampling Co., Baton Rouge, La., U.S.A.).

Calibrating solutions of uridine (Sigma, St. Louis, Mo., U.S.A.), 5-fluorouridine (Calbiochem, La Jolla, Calif., U.S.A.), cytidine (Schwarz/Mann, Orangeburg, N.Y., U.S.A.), and 5-fluorocytidine (kindly supplied by Dr. W. E. Scott of Hoffmann-La Roche, Nutley, N.J., U.S.A.), were prepared by dissolving milligram amounts in water. Concentrations of each were determined by dilution into either 0.1 *M* potassium phosphate (pH 7.0) or 1.0 *M* hydrochloric acid (pH $\approx$ 1) and measuring absorbancies

* To whom all correspondence should be addressed.

in a Gilford (Oberlin, Ohio, U.S.A.) Model 240 spectrophotometer at the appropriate wavelengths. The molar extinction coefficients and wavelength maxima used were as follows: uridine, $E_{262\ nm} = 10.1 \cdot 10^3$ (pH 7.0)[3]; 5-fluorouridine, $E_{268\ nm} = 8.95 \cdot 10^3$ (pH 1.0)[4]; cytidine, $E_{271\ nm} = 9.1 \cdot 10^3$ (pH 7.0)[3]; and 5-fluorocytidine, $E_{281\ nm} = 8.06 \cdot 10^3$ (pH 7.0)[5]. Aliquots (25 $\mu$l) of serially-diluted stock solution mixtures were chromatographed and the nucleoside peak heights determined. A representative chromatographic separation of one of these mixtures is shown in Fig. 1.

Preparation of 5-fluoropyrimidine-containing tRNA and total enzymic digestion of the tRNAs to nucleosides was as previously described[6,7].

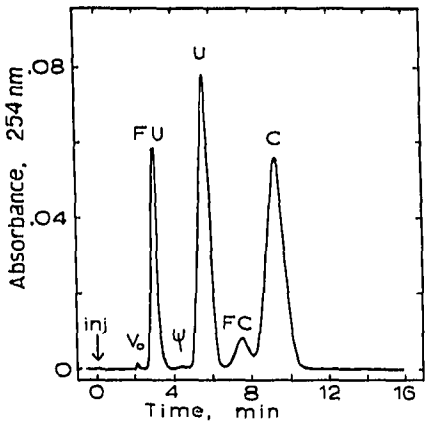
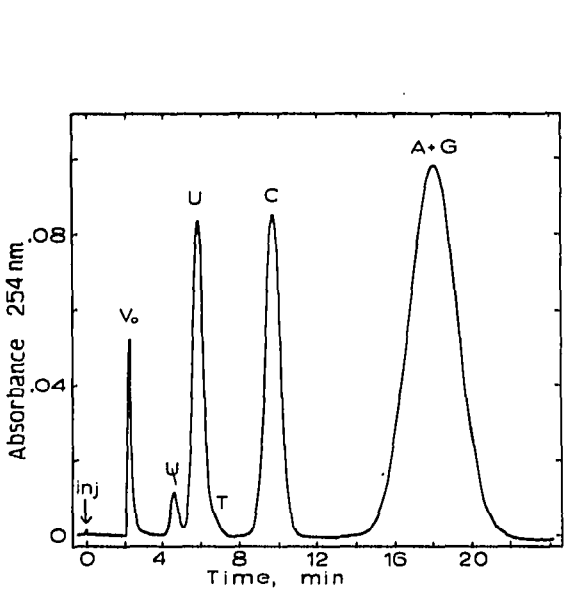



Fig. 1. Representative separation of 3.16 nmoles of 5-fluorouridine (FU), 3.94 nmoles of uridine (U), 0.841 nmoles of 5-fluorocytidine (FC), and 3.08 nmoles of cytidine (C). Peak $V_0$ is unidentified material eluting with the column void volume. Peak $\psi$ is contaminating pseudouridine derived from the uridine standard.

## RESULTS AND DISCUSSION

Figs. 2 and 3 show chromatograms obtained by ion-exclusion chromatography of nucleosides from enzymic digests of normal and 5-fluoropyrimidine-containing tRNAs. Fig. 2 corresponds to normal, unfractionated *Escherichia coli* B tRNA and Fig. 3 to unfractionated, 5-fluorouridine- and 5-fluorocytidine-containing tRNAs from the same organism enriched in the fluoropyrimidines by DEAE-cellulose chromatography[6]. Under these chromatographic conditions, no interfering compounds were noted to elute in the normal tRNA hydrolysate at the positions of 5-fluorouridine and 5-fluorocytidine. Ribothymidine (Fig. 2) appears as a shoulder on the uridine peak and is not resolved at the pH used here[2]. By increasing the pH, resolution of ribothymidine and uridine is possible, although 5-fluorouridine then elutes earlier and the void-volume peak may interfere with it. Adenosine and guanosine are also unresolved under these conditions (pH 7.6) and elute together.

The photometer output is linear in absorbancy units, with the peak height proportional to concentration. The standard curves (Fig. 4) were constructed by analyzing samples containing known amounts of uridine, 5-fluorouridine, cytidine, and 5-fluorocytidine (0.10–7.9 nmoles per 25 $\mu$l) and then plotting the resultant peak



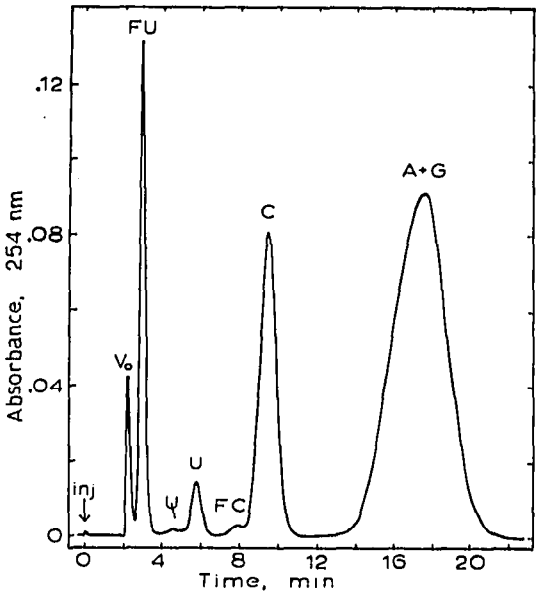




Fig. 2. Chromatogram of a normal tRNA hydrolysate. A + G = Mixture of adenosine and guanosine; T = ribothymidine; further peak identification as in Fig. 1.

Fig. 3. Chromatogram of a 5-fluoropyrimidine-containing tRNA hydrolysate. Peak identification as in Fig. 2.

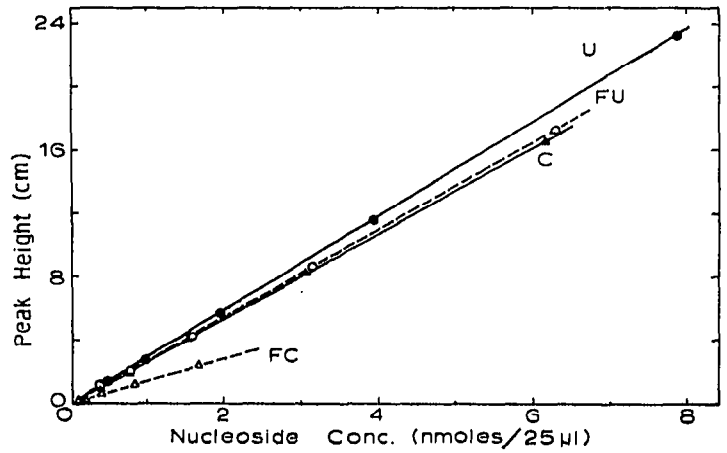



Fig. 4. Plot of concentration (nmoles per 25 $\mu$l) of nucleoside *vs.* peak height (cm). See Experimental section for details.

heights against the concentration. The percentage of 5-fluorouridine replacement of uridine ([5-fluorouridine/5-fluorouridine + uridine] · 100) and 5-fluorocytidine replacement of cytidine ([5-fluorocytidine/5-fluorocytidine + cytidine] · 100) in the analog-containing tRNA sample shown in Fig. 3, was found to be 90.9 $\pm$ 0.22% and 6.52 $\pm$ 0.47% ($\pm$ standard deviation, $n$ = 3) respectively. These percentage replace-

ments compare favorably with previously reported values in tRNA isolated from *E. coli* treated with 5-fluorouracil[6,8,9]. The described method is rapid and sensitive. With slight modification it may also prove to be a suitable analytical procedure for monitoring fluoropyrimidine levels in biological fluids.

ACKNOWLEDGMENTS

This work was supported by a Grant from the U.S.P.H.S., GM 20326. Journal paper No. 744 of the Wyoming Experiment Station.

REFERENCES

1 R. P. Singhal and W. E. Cohn, *Biochim. Biophys. Acta*, 262 (1972) 565.
2 R. P. Singhal, *Arch. Biochem. Biophys.*, 152 (1972) 800.
3 G. H. Beaven, E. R. Holiday and E. A. Johnson, in E. Chargaff and J. Davidson (Editors), *The Nucleic Acids*, Vol. 1, Academic, New York, 1955, Ch. 14, p. 493.
4 N. C. Yung, J. H. Burchenal, R. Fechner, R. Duschinsky and J. J. Fox, *J. Amer. Chem. Soc.*, 83 (1961) 4060.
5 I. Wempen, R. Duschinsky, L. Kaplan and J. J. Fox, *J. Amer. Chem. Soc.*, 83 (1961) 4755.
6 I. I. Kaiser, *Biochemistry*, 8 (1969) 231.
7 M. Jacobson, J. F. O'Brien and C. Hedgcoth, *Anal. Biochem.*, 25 (1968) 363.
8 I. I. Kaiser and L. Kwong, *FEBS Lett.*, 32 (1973) 281.
9 J. Horowitz, C. N. Ou, M. Ishaq, J. Ofengand and J. Bierbaum, *J. Mol. Biol.*, 88 (1974) 301.