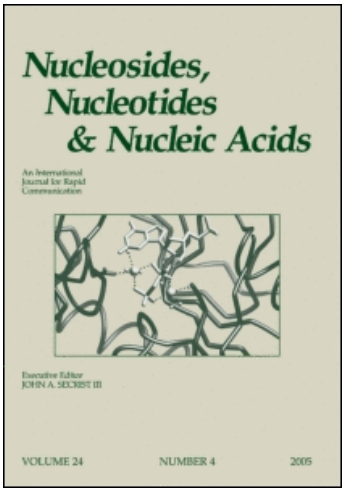

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Determination of 5-Fluorouracil and its Main Metabolites by Ion-Pair Liquid Chromatography on a Microbore Column

M. Barberi[a]; J. L. Merlin[a]; B. Weber[a]
[a] Centre Alexis Vautrin, Vandoeuvre-Les-Nancy, FRANCE

To cite this Article Barberi, M. , Merlin, J. L. and Weber, B.(1990) 'Determination of 5-Fluorouracil and its Main Metabolites by Ion-Pair Liquid Chromatography on a Microbore Column', Nucleosides, Nucleotides and Nucleic Acids, 9: 3, 435 — 436
To link to this Article: DOI: 10.1080/07328319008045162
URL: http://dx.doi.org/10.1080/07328319008045162

NUCLEOSIDES & NUCLEOTIDES, 9(3), 435-436 (1990)

DETERMINATION OF 5-FLUOROURACIL AND ITS MAIN METABOLITES BY ION-PAIR LIQUID CHROMATOGRAPHY ON A MICROBORE COLUMN.

M. Barberi, J.L. Merlin*, B. Weber
Centre Alexis Vautrin, 54511 Vandoeuvre-Les-Nancy, FRANCE

Abstract

5-Fluorouracil (5-Fu) and 5-Fluorodeoxyuridine (FdUrd) are substances widely used in the chemotherapy of cancers. The exploration of the metabolic pathways, is of great interest when they are combined with drugs which are likely to alter their biological response. The method described consists in the detection with ion-pair microbore HPLC of minimum amounts, ranging from 100 to 500 pg.

Material & methods

The HPLC system used consists of a computer-monitored BECKMAN GOLD PC apparatus, an automatic injector (WATERS WISP 512) and a 5 µm C18 column (BECKMAN ULTRASPHERE ODS, 15 x 0.2 cm I.D.). The mobile phase, made up of a phosphate/methanol buffer mixture (95:5 v/v) is eluted at 0.3 ml/mn. The column eluent is monitored at 280 nm using a UV detector. Optimisation of the mobile phase composition was carried out by adjusting capacity factors (k') of each compound to the polarity of the mobile phase, the respective tetrabutylammonium phosphate (TBA), the monopotassic phosphate concentrations and to the pH.

Results

In order to optimize elution defined as optimal retention per time unit of the three non-ionizable compounds, we have tried to obtain capacity factors values (k') ranging between 1 and 5 depending on the polarity of the mobile phase illustrated by the water / methanol ratio .The water/methanol mixture 95:5 (v/v) yielding results which were the closest to the limits we had set, was retained for our analysis. When eluted with this mixture, FdUMP had a k' value of 0.33, too close to the dead volume of the column.

The incorporation of polyvalent cations such as tetrabutylammonium ions (TBA) leads to the development of apolar ion pairs, which are hardly soluble in the mobile phase and are hence eluted later.

For the above reasons we have decided to use the ion-pair chromatography and to incorporate TBA ions to the mobile phase.

The composition of the mobile phase shall be determined as follows: 95% phosphate buffer TBA 10^{-4}M , KH_2PO_4 2.10^{-2}M, pH 5.9; 5% methanol.

The retention times for FU, FUrd, FdUrd, and FdUMP are 1.9±0.2; 3.6±0.4; 4.6±0.5; 6.4±0.6 minutes respectively corresponding to the optimal k' values ranged between 0.7 and 5.9. The detection limit corresponds to the smallest identifiable peak for a 10 µl injection: 5-FU 10 ng/ml (i.e 0.1 ng), FUrd 30 ng/ml (i.e. 0.3 ng), FdUrd 30 ng/ml (i.e. 0.3 ng), FdUMP 50 ng/ml (i.e. 0.5 ng). Calibration curves were established between 0.05 and 5 µg/ml for each compound.

Discussion

The use of ion-pair chromatography is obviously advantageous where assessment of FdUMP is concerned, since it is possible to "position" the elution of that compound between the peaks of the other fluoropyrimidines . Such a shifting should be particularly useful in the presence of interference peaks from the plasma. It should specified that the sensitivity limit described (50 ng/ml) is obtained in the most unfavorable position (late elution).

Finally, even if the use of a microbore column (2 mm I.D) permits the sensitivity threshold of the assessment to be reduced, the extraction method of the biological fluids will have to be improved in order to reduce as much as possible the presence of parasite peaks and of substances adsorbing onto the top of the column, which is a main drawback of that microbore method.

Acknowledgements

This study was supported by a grant from the Comité Départemental de Meurthe et Moselle de la Ligue Nationale contre le Cancer.