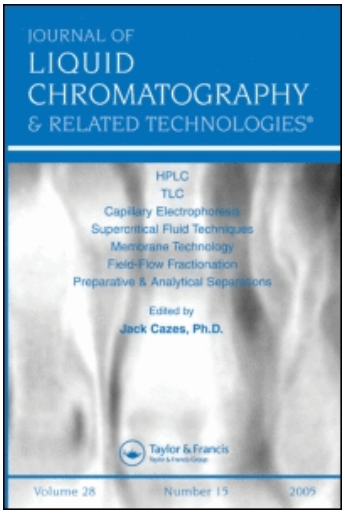



**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# High Performance Liquid Chromatography of an New Antineoplastic: (1-γ diethyl amino propyl amino)-5-methyl-dipyrido [4,3-b] [3,4-f] Indole (BD 40)

D. Blais[a]; B. Gourmel[a]; M. Marty[a]; C. Gisselbrecht[a]; E. Bisagni[b]; M. Boiron[a]; C. Dreux[a]
[a] Groupe de Pharmacologie Clinique des anticancéreux, Hôpital Saint-Louis, PARIS cedex 10 [b] Fondation Curie, Institut du radium, section Biologie, ORSAY, FRANCE

**To cite this Article** Blais, D. , Gourmel, B. , Marty, M. , Gisselbrecht, C. , Bisagni, E. , Boiron, M. and Dreux, C.(1983) 'High Performance Liquid Chromatography of an New Antineoplastic: (1-γ diethyl amino propyl amino)-5-methyl-dipyrido [4,3-b] [3,4-f] Indole (BD 40)', Journal of Liquid Chromatography & Related Technologies, 6: 5, 939 — 949
**To link to this Article: DOI:** 10.1080/01483918308067016
**URL:** http://dx.doi.org/10.1080/01483918308067016

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(5), 939-949 (1983)

# HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF A NEW ANTINEOPLASTIC : (1-δ diethyl amino propyl amino)-5-methyl-dipyrido [4,3-b] [3,4-f] indole (BD 40)

D. BLAIS*, B. GOURMEL*, M. MARTY*, C. GISSELBRECHT*,
E. BISAGNI**, M. BOIRON*, C. DREUX*

* Groupe de Pharmacologie Clinique des anticancéreux,
Hôpital Saint-Louis, 75475 PARIS cedex 10.
** Fondation Curie, Institut du radium, section Biologie,
91405 ORSAY, FRANCE.



## ABSTRACT

An HPLC method for determination and quantitation of BD 40, a dipyrido indole derivative with potential antitumor activity is described. Samples obtained after ethyl acetate extraction have been chromatograph ed on a reverse phase column with a counter ion mobile phase. The molecule tested beeing detected by spectrofluorimetry and sensibility, specificity and reproduc ibility of the



 0148-3919/83/0605-0939$3.50/0

method are satisfactory enough to allow for pharmacokinetics studies in human.

## INTRODUCTION

Recent developments in oncology chemotherapy research have shown that DNA intercalating agents take a major place among various drugs proposed for cancer treatment (1, 2, 3, 4).

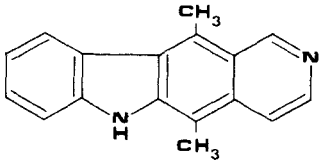
Newly synthetized dipyrido [4,3-b][3,4-f] indole compounds differ from ellipticins (pyridocarbazol basic formular) by the presence of a nitrogen at position 9 (figure 1). Furthermore, an original procedure for the synthesis allows for the addition of a lateral chain $\gamma$ diethyl amino propyl amino at position 1 for the most intersting compound in this series (5, 6).

This additional chain confers to the molecules an increased activity against L 1210 leukemia, TG 180 and moloney sarcoma, Lewis $C_3H$ mammary carcinomas (7, 8, 9).

As with ellipticins, the antitumor activity of this drug may be releated to intercalating binding to DNA.

Preliminary data in man have suggested intersting antitumor activity (10). In order to study the pharmacokinetics of the agent in conjonction with the phase I clinical trials in our Institut, we have developed an analytical method using HPLC for determination of plasma and urinary levels of BD 40.

a) ELLIPTICINE



b) BD 40

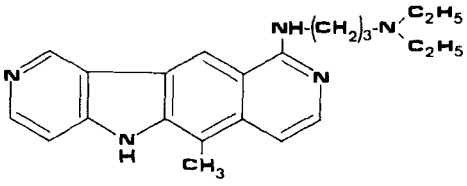


Figure 1 : a) Ellipticin : 5,11-dimethyl-6H-pirydo [4,3-b] carbazole.

b) BD 40 : (1-γ-diethyl amino propyl amino)-5-methyl-dipyrido [4,3-b] [3,4-f] indole.

## MATERIALS

### Apparatus

HPLC apparatus consisted of a 6000 A pump (Waters Associated, Milford, Mass. 01757), WISP automatic sampler injector (Waters Ass.) with 300 µl limited volumeinserts, SFM 23 B spectrofluorescent detector (Kontron, Roche électronique, 78140 Velizy-Villacoublay, France). Excitation wavelenght was set at 304 nm and emission at

404 nm. Cell volume was 20 µl. Data were registered and processed using data processor chromatopac CR 18 (Shimadzu corporation, Kyoto, Japon). Column was custom packed in our laboratory with Partisil ODS 10 (Whatman) in a stainless steel tube 4 mm ID x 250 mm under 6000 PSI in 50 : 50 (v : v) methanol water solution.

Chemical reagents

BD 40 was kind ly provided by E. BISAGNI. The original compound consists in a trimaleate salt cristalized with one water molecule (M.W 709). The stock solution ($10^{-3}$ M) in diluent solution methanol : water 50 : 50 (v : v) has been stored at - 18 °C. Nor harmane, (9 H pyrido [3,4-b] indole, figure 2 b) (Aldrich-Europe Division, Belgium), has been used as an internal standard. The stock solution is prepared and stored in the same conditions.

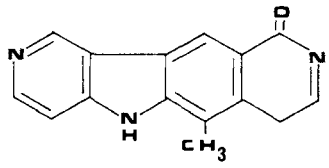
Biological samples

Blood samples were collected in dry tubes, centrifuged and sera were stored at - 18 °C until assay.

Urine samples were collected as voided and stored in same conditions.

Overloaded samples : an aqueous solution 250 nmol of BD 40 trimaleate salt has been diluted in pool of 100 ml of serum from healthy subjects not taking drugs. A standard protein solution (2,5 µM) was thus obtained and stored frozen (- 18 °C) as 1.5 ml aliquots.

a) BD 38



b) NOR HARMANE

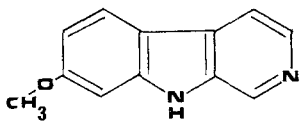


Figure 2 : a) BD 38 : 1-oxo-5 methyl-dipyrido [4,3-b] [3,4-f] indole.

b) Nor Harmane : 9H pyrido [3,4-b] indole.



## HPLC reagent

A methanol-water 75 : 25 (v : v) solution containing sodium dodecyl sulfate (6.47 mM) was used as a counter ion mobile phase. The pH of the aqueous SDS solution has been adjusted to 1.80 by perchloric acid. Methanol was then added and the solution filtered (Gelman filter 0.45 µ) under vacuum and sonicated for five minutes.

## METHODS

### Extraction procedure

BD 40 extraction from plasma (1 ml) or urine (2 ml) samples was performed by ethyl acetate after alkalinization with 100 µl of NaOH 1N. Samples were vortexed for 2 mn. Aqueous phase was frozen in dry ice-acetone mixture and organic phase transferred in a conic flask and evaporated to dryness under nitrogen flow. The dry residue was diluted with 250 µl of mobile phase and centrifuged (Beckman Spincomicrofuge) for 1 mn to discard any particles.



### Standard curve

The standard curve was constructed after measurement of eight standard protein solutions (1, 2.5, 5, 6.5, 7.5, 10, 12.5 and 15 µM of BD 40) each point was injected twice.

### Chromatography

50 µl of each extract were injected under the following conditions : mobile phase 2.0 ml/mn (P = 1500 PSI), fluorimetric signal was integrated and area ratio BD 40 : internal standard was calculed. BD 40 concentration was obtained by reference to standard curve.

## RESULTS AND DISCUSSION

The following chromatograms (figure 3) were obtained. The first peak (1) with 2.0 mn retention time is the internal standard.

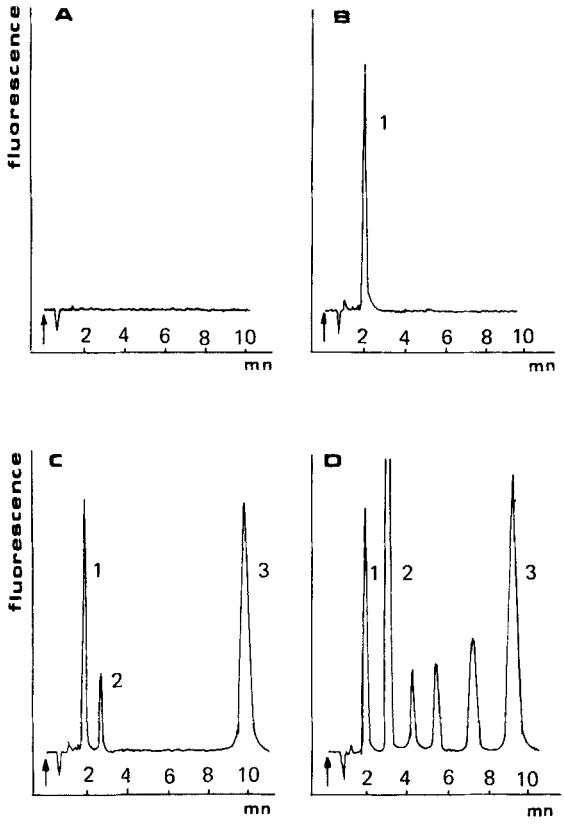



Figure 3 : a) Blank serum

b) Serum with internal standard Nor Harmane

c) Patient's serum immediately after a 30 minutes perfusion 600 mg/m2

d) Patient's urine 60 mn after perfusion.

The second peak (2) with 3.5 mn retention time, has been caracterized by its spectra emission and retention time as BD 38, 1 oxo-5 methyl-dipyrido [4,3-b] [3,4-f] indole, an oxo derivative of BD 40 (figure 2 a) which has lost the diethyl amino propyl

amino lateral chain. This compound is a by pass contamination occuring during the drug synthesis. The level of this impurty is less than 3 per cent of the BD 40 amount. The third peak (3) with 9.3 mn retention time is BD 40.

In order to achieve a better separation, we have studied the retention time shift in relation with the pH of the mobile phase (figure 4). A pH 1.80 was chosenfor better identification and calibration of the previous described peaks.

BD 40 recovery after extraction procedure, measured with tritiated molecules, was 78 $\pm$ 5 per cent.



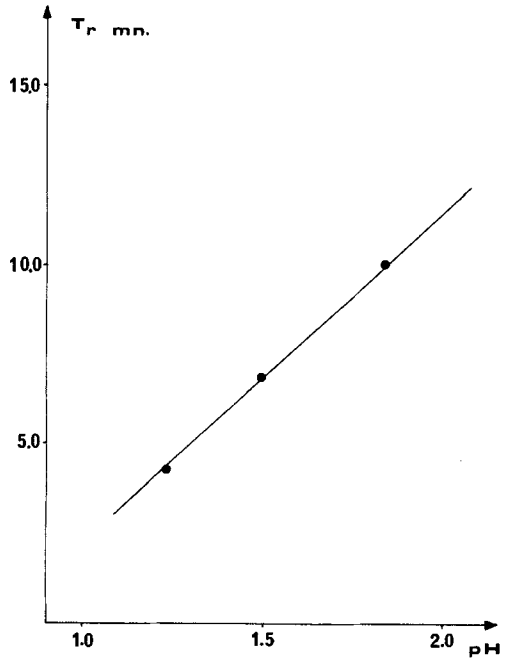



Figure 4 : Retention time in relation with pH of the mobile phase.

Reproduc ibility of this method was tested in consecutive 12 extractions of two overloaded sera. The coefficient of variation was 6.0 % with the 14 µM solution and 4.0 % with the 4.66 µM solution.

Using this methodology a perfectly linear standard curve was obtained in overloaded plasma samples for 1 to 20 µM of BD 40 in relation with the area ratio BD 40/Nor Harmane (r = 0.994) (figure 5). The lower limit of detection was 0.25 nmol/ml with a

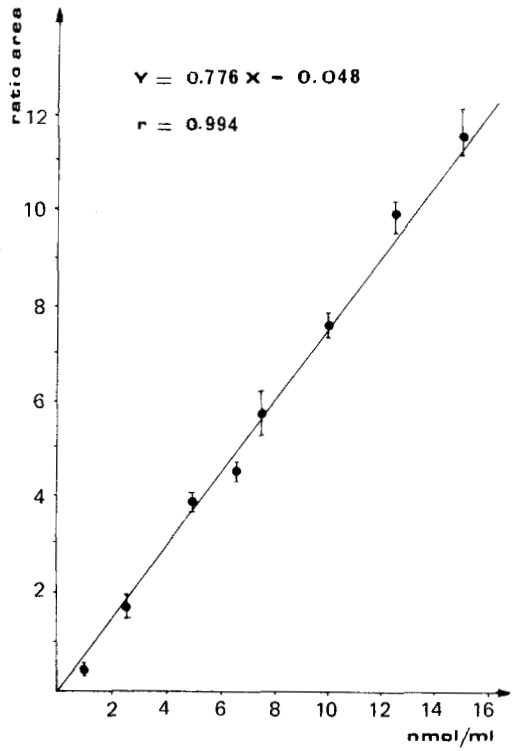



Figure 5 : Standard curve of BD 40 in nmol/ml in relation with area ratio BD 40/Nor Harmane.

signal to baseline noise ratio of 10. The sensitivity is given by the standard deviation as 0.55 nmol/ml. Precision was calculated from the standard curve at the 0.01 level is 4.73 per cent.

Therefore the described HPLC detection allows for quick accurate and reproduc ible determination of BD 40 in plasma and urine.

Such method allows for accurate which of BD 40 pharmacokinetics in animals and human (in preparation). Furthermore, as similar results are obtained with other dipyrido indole derivatives, detection of BD 40 metabolites could be achieved using the described method.



## ACKNOWLEDGEMENT

This work was supported in part by DGRST grant-81-S 0211. We would like to thank "Institut Pasteur" for technical support. We would also like to thank miss C. COURTOIS for typing this manuscript.

## REFERENCES

1. LE PECQ J.B., Chimiotherapie anticancéreuse . Mécanisme d'action des substances antitumorales. In Ed. HERMANN Paris 1978.
2. KOHN K.N., WARING H.J, GLAUBIGER D., FRIEDMAN C.A., Intercalative binding of ellipticin to DNA. Cancer Res., 35, 71-76,1975.
3. LE PECQ J.B., DAT XUONG N., GOSSE C., PAOLETTI C., A new antitumoral agent : 9 hydroxy ellipticin possibility of a rational design of anticancerous drugs in the series of DNA intercalating drugs. Proc. Nat. Acad. Sci. (USA), 71, 5078-5082, 1974.

4. MULLER W., CROTHERS M., Interactions of heteroaromatic compounds with nucleic acids. 1. The influence of heteroatoms and polarizability on the base specificity of intercalating ligands. Eur. J. Biochem., 54, 267-277, 1975.

5. DUCROCQ C., BISAGNI E., RIVALLE C., LHOSTE J.M., Synthesis of 1 substituted dipyrido [4,3-b] [3,4-f] indoles. J. Chem. Soc. (Perkin I), 142-145, 1979.

6. RIVALLE C., DUCROCQ C., BISAGNI E., Synthesis of 5,11-dimethyl dipyrido [4,3-b] [3,4-f] indole, a new area analog of ellipticin. J. Chem. Soc. (Perkin I), 138-141, 1979.

7. CHERMANN J.C., GRUEST J., MONTAGNIER L., WENDHUF F., TAMBOURIN P., PERRIN M., POCHON F., DUCROCQ C., RIVALLE C., BISAGNI E., Action cytotoxique et antitumorale de dérivés d'une nouvelle série hétérocyclique : les dipyrido [4,3-b] [3,4-f] indole. C.R. Acad. Sc. Paris, 285, série D, 945-948, 1977.

8. LIDEREAU R., CHERMANN J.C., GRUEST J., MONTAGNIER L., DUCROCQ C., RIVALLE C., BISAGNI E., Antitumoral activity of dipyrido [4,3-b] [3,4-f] indole on L 1210 leukemia. Bull. cancer (Paris), 67, 1, 1-8, 1980.

9. CHERMANN J.C., LIDEREAU R., MONTAGNIER L., Antitumoral effect of dipyridoindoles. Current Chemotherapy, 1200-1201, 1978.

10. MARTY M., JASMIN C., POUILLARD P., GISSELBRECHT C., GOUVENIA G. MAGDALAINAT H., Phase I studies with diethyl N-amino propyl dipyrido indole (B 40 M). 17th Annual Meeting of the American Society of Clinical Oncology, C 108, 1981.