Communications to the Editor

[Chem. Pharm. Bull. 30(6)2287—2290(1982)]

A DIRECT INJECTION METHOD OF PLASMA SAMPLES ONTO A REVERSE PHASE COLUMN FOR THE DETERMINATION OF DRUGS

Hisanobu Yoshida,* Ikue Morita, Tsutomu Masujima and Hideo Imai

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734, Japan

A reverse phase HPLC method, which can be applied to the direct analysis of drugs in plasma omitting deproteinizing, is proposed. It was found that an ODS column treated with human plasma no longer adsorbed plasma proteins from an aqueous solution, but still held the characteristics of the reverse phase column for lipophilic small molecules. This column was used in HPLC analyses and proved to be useful to determine drugs in plasma. The procedure was simple and rapid, and the recoveries of both drugs and proteins were almost quantitative(99-101%) with a good reproducibility(c.v.; less than 2%, within-run). The results obtained for procainamide, 6-mercaptopurine, methotrexate, doxorubicin, theophylline and propranolol in human plasma are summarized.

KEYWORDS—protein-coated ODS column; direct injection of plasma samples; reproducible deproteinizing; direct drug monitoring in plasma; HPLC

High performance liquid chromatography(HPLC) is now widely used in many fields, including routine work for clinical drug monitoring. In drug monitoring in plasma by HPLC, as a separation mode, reverse phase chromatography is most commonly used. In these cases, the direct injection of biological samples containing proteins supposedly causes the deterioration of efficiencies or characteristics of the column, although there is little experimental evidence. Accordingly, deproteinizing or the solvent extraction is the usual first step for HPLC analyses. For simplicity and reproducibility, it is desirable to develop a method which can directly analyze biological samples. Few reports have appeared on the direct analysis of plasma samples by HPLC. One example which has been reported is analysis of flucytosine in plasma by ion-exchange chromatography,[1] but it is not clear whether this method can be applied to successive analyses or to many kinds of drugs.

In this communication we present a method which has a wide range of application for drug analyses by direct injection of plasma samples onto a reverse phase column.

An HPLC apparatus with a stepwise elution system was assembled in our laboratory. The detector used was a UV-8(Toyo Soda, Tokyo, Japan) or a RF 500 LC(Shimadzu, Kyoto, Japan). Drugs used as model compounds were procainamide, 6-mercaptopurine, methotrexate, theophylline, doxorubicin and propranolol; the first four drugs were detected by UV spectrophotometry and the latter two by fluorometry.

Table I. Summary of Experimentals and Results

Drug	Experimentals: Bounding to Plasma Proteins (%)	Drug Concentration (μg/ml)	Analyzed Plasma (μl)	Detection	Elution[b]	Results: Recovery, % (C.V., %) Present Method n=8	5% TCA[g] n=5	50% CH_3CN[h] n=5
Procainamide	14-16[3)a)]	5.4	20	290nm	1[c)] PBS[d)] ② [c)] PB[e)] : AN[f)] = 97 : 3	100.2 (0.4)	88.6 (1.8)	98.6 (2.0)
6-Mercaptopurine	15-30[4)]	0.3	20	326nm	① PB	99.3 (1.9)	43.0 (3.2)	90.4[i)] (2.0)
Methotrexate	33-57[5)]	2.0	50	310nm	1 PBS ② PBS : AN = 92 : 8	100.8 (1.0)	63.4 (1.1)	101.0[i)] (1.2)
Doxorubicin	34-53[6)]	0.6	100	Ex.475nm Em.580nm	1 PB ② PB : AN = 75 : 25	99.7 (1.9)	29.3 (2.3)	90.3 (2.1)
Theophylline	53-65[7)]	9.0	10	280nm	1 PBS ② PBS : AN = 94 : 6	98.7 (1.9)	71.0 (2.1)	98.6 (2.0)
Propranolol	90-96[8)]	0.3	100	Ex.287nm Em.340nm	1 PBS 2 PBS : AN = 65 : 35 ③ PB : AN = 80 : 20	100.1 (0.9)	25.9 (4.0)	101.0 (3.2)

a) The superscript numbers in this column are the reference numbers. b) Flow rate was 1.5ml/min, at 30°C.
c) A stepwise elution method was used, except for 6-mercaptopurine, and a drug was eluted from the column with the last buffer, which indicated by a circle (O).
d) Phosphate saline(pH 7.4). e) Phosphate buffer(pH 3.0). f) Acetonitrile(CH_3CN).
g) The plasma sample was mixed with an equal volume of 10% TCA(trichloroacetic acid), and the neutralized supernatant was analyzed.
h) The plasma sample was mixed with an equal volume of acetonitrile, and the supernatant was analyzed, except in those cases with superscript i).
i) After the addition of acetonitrile, the supernatant was dried in vacuo and re-dissolved in water. This aqueous solution was analyzed.

An ODS column(4 x 60mm of TSK LS-410, 20-32μm, 80Å average pore size, Toyo Soda) was equilibrated with phosphate saline(pH 7.4), and human plasma was injected to the column(elution; phosphate saline) until the quantitative recovery of 50μg of bovine serum albumin(BSA) was reached from the column. The total volume of human plasma required was around 300μl for the 4 x 60mm column.

The column treated with human plasma eluted 50μg of BSA near the void volume quantitatively(99.2% as calculated from the recovery of absorbancy at 280nm, c.v.; 1.1%, n=4), and the native ODS column adsorbed 50μg of BSA[2] and yielded almost no recovery when eluted with phosphate saline. However, the treated column still held characteristics similar to the native ODS column for small molecules, though k' values for 6-mercaptopurine(elution; phosphate buffer, pH 3.0) and for procainamide (elution; phsphate buffer, pH 3.0, containing 3% acetonitrile and 0.5% trichloroacetic acid) were slightly reduced(ca. 5%) and theoretical plate numbers for both drugs were also reduced by 20-30% in the treated column. Treatment of an ODS column with either human plasma or 6% BSA solution yielded similar effects. This results led us to speculate that, in the ODS column treated with human plasma, proteins were adsorbed to the outside surface of the ODS resins, and that the inside surface of the porous resins was not affected by proteins due to a molecular sieve effect. More detailed characteristics of this column are under the study. However, in practice, the present column was stable enough for routine work and could be applied to direct drug determination in human plasma.

For practical drug monitoring, a drug was added to the pooled human plasma in the concentrations shown in Table I. In principle, human plasma spiked with a drug was directly injected onto the column, and the initial solvent eluted plasma proteins

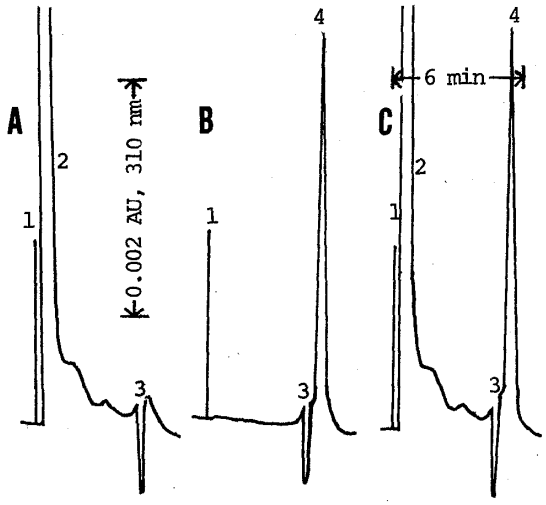

Fig. 1. Chromatograms of Methotrexate Analysis

column ; 4 x 60 mm (20-32 μm)
elution ; as shown in Table I
sample ;
A, human plasma (50 μl).
B, methotrexate (2.0 μg/ml, 50 μl).
C, human plasma spiked with methotrexate (2.0 μg/ml, 50 μl).
signals ;
1, injection marker.
2, proteins and others.
3, buffer change drift.
4, methotrexate.

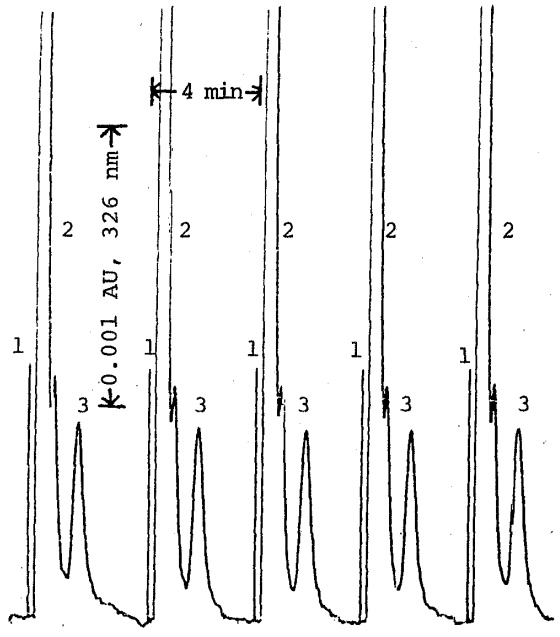

Fig. 2. Successive Analyses of 6-Mercaptopurine in Plasma

column ; 4 x 60 mm (20-32 μm)
elution & sample ; as shown in Table I
signals ;
1, injection marker.
2, proteins and others.
3, 6-mercaptopurine.

near the void volume; then the drug was eluted by the reverse phase mode, and detected in a flow cell. The recoveries of drugs tested were almost quantitative(99-101%), and this result indicated that drugs bound to plasma proteins were also transferred to the inside of the porous ODS resins at high speed and with high efficiency. It was noteworthy that the recovered drug was the total amount in plasma, regardless of whether it was free or bound to plasma proteins. As shown in Table I, recovery of drugs by the present method was superior with good reproducibilities(c.v.; less than 2%) to common HPLC analyses, which included the deproteinizing step by either trichloroacetic acid(TCA) or acetonitrile. These features might be important for accurate drug determination in plasma, as well as having the advantage of simplicity.

Typical chromatograms of human plasma are shown in Fig. 1. The peak width or retention time of a drug was identical whether it was dissolved in human plasma or phosphate saline. This result indicated that plasma components including proteins did not deteriorate the separation and also indicated again that the transfer of the drug from the bound proteins to the ODS resins was completed in a few seconds, even for a drug of high affinity to plasma proteins, such as propranolol.

As shown in Fig. 2, the present method was reproducible in successive analyses of plasma samples; all the results summarized in Table I were obtained by a single column.

In conclusion, the ODS column treated with human plasma was useful for direct drug determination in plasma in terms of accuracy, reproducibility and simplicity. Using finer resins, monitoring of the practical drug and its metabolites in plasma is in progress in our laboratory.

ACKNOWLEDGMENT This research was supported in part by a Grant-in-Aid for Scientific Research from the Iatro Chemical Foundation.

REFERENCES

1) A.D. Blair, A.W. Forrey, B.T. Meijsen and R.E. Culture, J. Pharm. Sci., 63, 1334 (1975).
2) R.V. Lewis, A. Fallon, S. Stein, K.D. Gibson and S. Udenfriend, Anal. Biochem., 104, 153 (1980).
3) K. Karlsson, Clin. Phamacokinet., 3, 97 (1978).
4) J.E. Finbel, Anal. Biochem., 21, 362 (1967).
5) S.H. Wan, D.H. Huffman, D.L. Azarnoff, R. Stephens and B. Hoogstraten, Cancer Res., 34, 3487 (1974).
6) T. Terasaki, T. Iga, Y. Sugiyama and M. Hanano, The Proc. of 13th Symp. on Drug Metab. and Action, ed. by Pharmaceutical Society of Japan, p. 73 (1981).
7) K.M. Paifsky, D.S. Stair, R.E. Rangno and R.I. Ogilvie, New Engl. J. Med., 296, 1495 (1977).
8) T.P. Moyer and S.G. Sheps, Therapeut. Drug Monit., 3, 217 (1981).

(Received May 8, 1982)