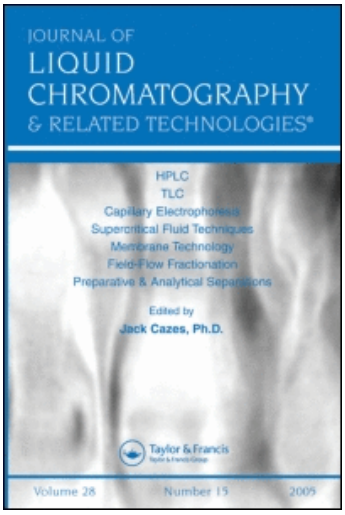


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Reverse Phase High Performance Liquid Chromatography of Human Bence Jones Proteins

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**To cite this Article** Kametani, Fukuyi , Toshima, Toshiyuki , Tonoike, Hiroshi , Hoshi, Akihiko , Shinoda, Tomotaka and Okuyama, Tsuneo(1985) 'Reverse Phase High Performance Liquid Chromatography of Human Bence Jones Proteins', Journal of Liquid Chromatography & Related Technologies, 8: 10, 1845 — 1852

**To link to this Article: DOI:** 10.1080/01483918508074099

**URL:** http://dx.doi.org/10.1080/01483918508074099

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(10), 1845-1852 (1985)

# REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF HUMAN BENCE JONES PROTEINS

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ABSTRACT

A high performance liquid chromatography system is presented for analytical and preparative separations of human Bence Jones proteins. The method utilizes 5-7 um macroreticular polystylene resin with bonded hydroxymethyl functional groups, and the proteins are eluted with a linear gradient of an increasing concentration of acetonitorile(10-60%, V/V) in 0.1 % (V/V) trifluoroacetic acid, pH 2.1. By this elution condition, seven λ type Bence Jones proteins with molecular weights of 23,600 (monomer)-47,000(dimers) daltons(216-434 amino acids) were eluted within 80 min with the yields of 78%-98%. The method allows a rapid and sharp elution of Bence Jones proteins.

## INTRODUCTION

Recent reverse phase high performance liquid chromatography (RP-HPLC) has greatly facilitated a rapid and high resolution

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 0148-3919/85/0810-1845$3.50/0

separation of peptides and proteins. Such applications still seem to be limited for proteins with relatively low molecular weights(14-28Kd)(1-3), because of characteristics of the supports that give poor resolutions and recoveries with proteins of higher molecular weights. Because of its great versatility over the classical chromatographic techniques, HPLC is becoming increasingly popular as a purification technique of proteins. Thus, a variety of supports including porous, microparticulate, chemically bonded alkylsilicas(3) have been introduced. However, in contrast to its higher efficiency in peptide purifications, these supports have dificiencies that mast be solved for succesful separations of many proteins.



Among the supports currently introdued into RP-HPLC, a macroreticular (5-7 um) polystylene resin with bonded hydroxymethyl groups, Hitachi Gel 3013-O, has certain advantages over more popular suppots, such as alkylsilicas. First, because it is a synthetic polymer, reproducible production may be easier than for silica supports. Second, chemically stable polymer supports can be regenerated by washing with aggressive solvents, such as 2 N NaOH in 20 % 2-propanol or 20 % acetone. This is an advantage in practical applications. Third, polymer supports have a much higher capacity than C-18 silica supports. Fourth, concentration and desalting of dilute proteins in buffer solutions can be possible with a column of polymer supports. Lastly, initial applications show separations of proteins of

medium molecular weights up to ca. 50K daltons with yields of 78-98%.

This communication describes an application of a hydroxymethyl macroreticular porous polymer support for the separation and purification of human λ type Bence Jones proteins, which achieves a rapid, sensitive and sharp elution in preparative yields from nanogram to milligram quantities. Bence Jones proteins, structurally related to the light chains of antibodies, are a group of proteins secreted into urine by patients with multiple myeloma(a form of cancer of antibody-secreting cells), and are important for elucidation of the principles of antibody structure and of specificity and its genetic control.



## MATERIALS AND METHODS

### Chemicals

A macroreticular polystylene resin with bonded hydroylmethyl groups(Hitachi Gel 3013-O, Hitachi Ltd., Tokyo, Japan) had a particle size of 5-7 um. Acetonitrile of chromatography grade and trifluoroacetic acid (TFA) of sequanal grade were purchased from Wako Pure Chemicals (Tokyo, Japan). Water was distilled, passed through a mixed-bed ion exchange resin, and redistilled prior to each use.

### Apparatus

Hitachi Gel 3013-O resin was suspended in 50% acetonitrile in water and packed into a stainless-steel column (250 x 4.6 mm

i.d.) at a pressure of 30 kg/cm$^2$(414 psi). The column was operated with a pump(NSP-800-3, Kyowa Seimitsu Co., Tokyo, Japan), and the eluents monitored at 210 nm in a 1 cm light path (Schoeffel SF770 spectrophotometer, Schoeffel Instrument Corp., Westwood, N.J.).

Chromatography

Sample protein on the column were eluted at room temparature (ca. 27$^{o}$C) with a linear gradient of an increasing concentration of acetonitrile(10-60 %, V/V) in 0.1 % (V/V) TFA at a flow rate of 0.5 ml/min. After the completion of one analytical run, the column was equilibrated for 30-60 min with the initial solvent for the next operation. After several run, the column may have to be regenerated with a solvent of 2N NaOH in 20 % (V/V) 2-propanol or 20 % acetone for 30 min under the operational conditions.



Proteins

The λ type Bence Jones(BJ) proteins, NIG-51, -64, -58, -68, -11, -8, -48(numbers correspond individual specimen code in our stocks), were purified from the urine of patients with multiple myeloma according to the described procedures(4-9). The amino acid sequences of these proteins have been published from our loboratory(4-9).

## RESULTS AND DISCUSSION

We have employed seven λ type BJ proteins which have different amino acid compositions and sequences. Uppon mobile

phase gradient of acetonitrile in 0.1 % TFA, all proteins were eluted as sharp peaks from the column within 80 min. The chromatograms are shown in Fig. 1(A-G).

As summarised in Table 1, all the proteins were eluted at the retention time between 54 min and 65 min under the conditions. The resolutions between BJ proteins are incomplete. However, the proteins do elute as sharp peaks far from the origin and some group separations would be possible, e.g. those separated by 1.5 min or more, since the monomer and dimers resolved from each others in the case of NIG-64. A separation between monomer and dimers can be obtained with as much as 8 mg of NIG-64(Fig. 2). The recoveries of these proteins were between 78 % and 98 % as summarized in Table 1.



TABLE 1. A List of Bence Jones Proteins Subjected to RP-HPLC

| protein | amino acid residues | molecular weight | retention time(min) | amount (ug) | yield (%) |
|---|---|---|---|---|---|
| NIG-51 | 432 | 44,600 | 57.5 | 80 | 93 |
| NIG-64 | 216 | 22,600 | 57.0 | 50 | 99 |
| | 432 | 45,200 | 58.5 | | 78 |
| NIG-58 | 430 | 45,600 | 58.5 | 40 | 93 |
| NIG-68 | 426 | 45,400 | 56.0 | 120 | 79 |
| NIG-11 | 426 | 46,600 | 55.0 | 50 | 98 |
| NIG- 8 | 432 | 45,000 | 54.0 | 50 | 86 |
| NIG-48 | 434 | 46,600 | 57.0 | 150 | 78 |

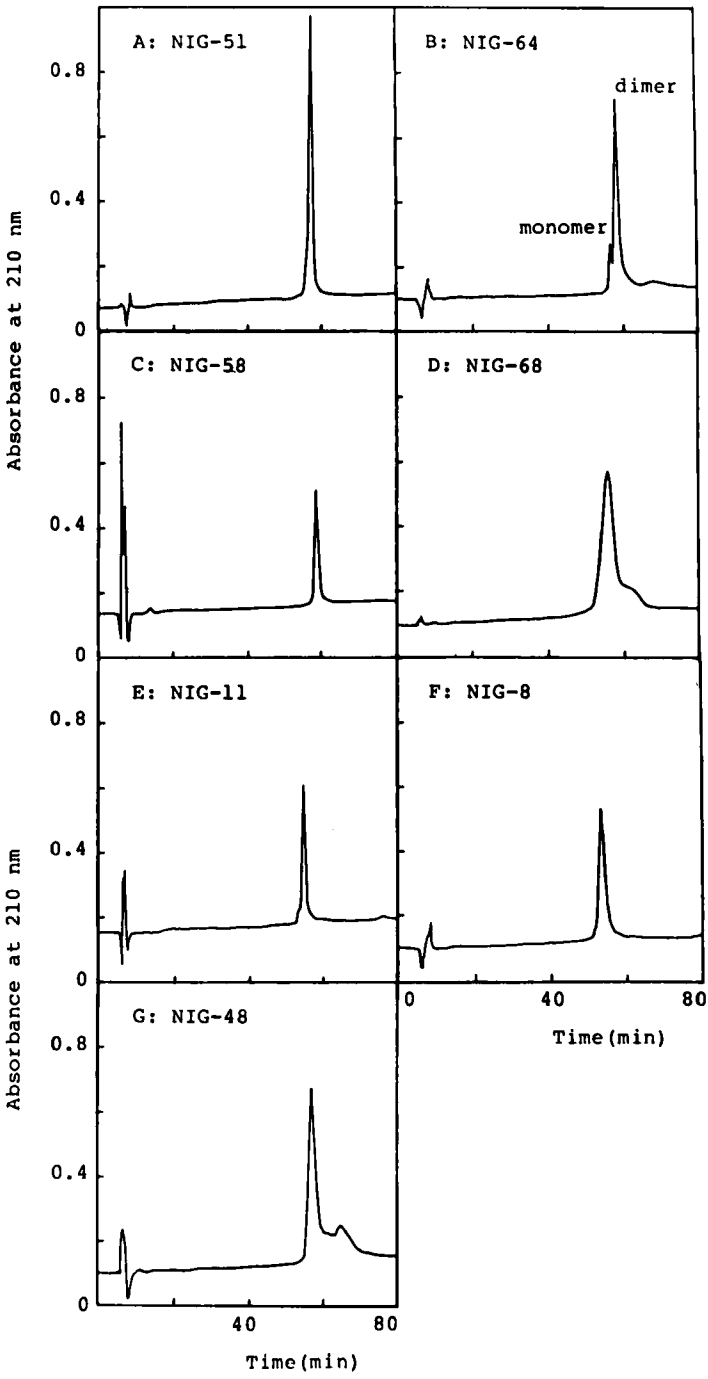


FIGURE 1. Elution profile of Bence Jones protien in RP-HPLC. Each protein of known amount(Table 1) was chromatographed under the conditions described in Materials and Methods.

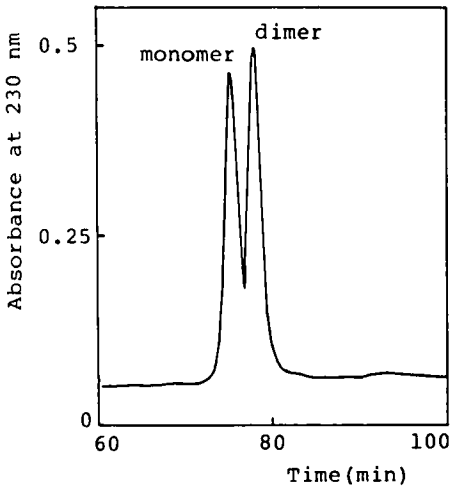


FIGURE 2. Preparative separation of NIG-64. Protein(8 mg) was chromatographed under the same conditions, except that a rate of gradient was decreased by 66 % of the standard condition.



## Conclusion

The present column of macroreticular polystylene resin with hydroxymethyl functional groups was demonstrated to give sharp peaks with high recovery for human λ BJ proteins. This material should also be applicable to other proteins of similar molecular weights. Applications to peptide separations have already been described by the present authours(10, 11).

## ACKNOWLEDGEMENTS

The authors wish to thank, Drs. A. Shimizu, H. Tomioka, T. Isobe for generous gifts of proteins, and Hitachi Ltd. for the resins. This work was supported in part by grants-in-aid from the Ministry of Health and Welfare Primary Amyloidosis Research

Committee and the Ministry of Education, Science and Culture of Japan.

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