ELSEVIER

Journal of Chromatography B, 702 (1997) 245–248

JOURNAL OF CHROMATOGRAPHY B

Technical Note

# Preparation of (*R*)-*N*-methylsalsolinol via reversed-phase high-performance liquid chromatography using β-cyclodextrin as a mobile-phase additive

Chiyoko Minami[a], Yulin Deng[a], Fumi Urano[a], Toyo Kaiya[c], Kohfuku Kohda[c], Masao Kawai[a], Makoto Naoi[b,*]

[a]*Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466, Japan*
[b]*Department of Biosciences, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466, Japan*
[c]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya 467, Japan*

Received 28 April 1997; received in revised form 24 June 1997; accepted 7 July 1997

**Abstract**

A new preparation method was devised using β-cyclodextrin as a mobile-phase additive in a reversed-phase high-performance liquid chromatography system. Preparative separation of the biologically active (*R*)-enantiomer was achieved from racemic *N*-methylsalsolinol. β-Cyclodextrin was removed completely in good yield by acid extraction and solid-phase extraction. By a slight modification, this method will be applicable to the isolation of various types of biologically important enantiomers. © 1997 Elsevier Science B.V.

*Keywords:* (*R*)-*N*-Methylsalsolinol

## 1. Introduction

Recently, we found that the chirality of endogenous alkaloids, 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline [salsolinol, Sal] derivatives, is essentially involved in their biological and toxic properties [1,2]. For the direct separation of Sal enantiomers, we tried several commercially available high-performance liquid chromatography (HPLC) columns, such as bovine serum albumin-bonded or cellulose derivatives. The Sal derivatives were successfully separated only by use of a β-cyclodextrin (β-CD)-bonded column [3], or an HPLC system using β-CD and a counter-ion, sodium 1-heptanesulfonate (SHS) as additives in a mobile phase with a reversed-phase column [4]. The results using the latter method suggest that this system may be applicable to the preparation of the selective enantiomer of a Sal derivative, (*R*)-*N*-methylsalsolinol [(*R*)-NMSal], a potent dopaminergic neurotoxin [1].

This article reports the preparative separation of (*R*)-NMSal from the racemic isoquinoline and a method to remove β-CD completely from the purified sample. These results are discussed in relation to the further application of this method to

*Corresponding author.


*PII* S0378-4347(97)00374-5

prepare biologically and pharmacologically active enantiomers from racemic compounds.

## 2. Experimental

NMSal was produced by the Pictet-Spengler condensation [5] of epinine with acetaldehyde. The product was concentrated and precipitated from the methanol solution with ether, and used as a starting material.

Synthesized racemic NMSal was applied to the preparative separation by HPLC using β-CD as a mobile-phase additive. The chromatography was performed using a reversed-phase Partisil ODS-10 column (250×22.2 mm I.D., GL Science, Tokyo, Japan). The mobile phase consisted of 25 m*M* sodium phosphate buffer, pH 3.0, containing 12 m*M* β-CD, 1 m*M* SHS and 2% acetonitrile [6], and the flow-rate was 4 ml/min. The elution was monitored by measurement of the absorbance at 280 nm.

The fractions exhibiting UV absorption were subjected to HPLC analysis with a chiral column for the determination of the enantiomeric composition. The separation was performed using a Nucleodex β-OH column (200×4.0 mm I.D., Macherey-Nagel, Düren, Germany) [3]. The mobile phase was 0.1 *M* sodium phosphate buffer, pH 3.6, containing 5% methanol. The flow-rate was 0.6 ml/min. NMSal was detected by measuring the absorbance at 280 nm. The fractions containing only (*R*)-NMSal were collected and lyophilized. To the lyophilized sample (100–200 mg dry weight), 1 ml of 0.01 *M* HCl was added, the sample was vortex-mixed for 10 min, centrifuged at 22 000 g for 10 min, and the supernatant was collected. This acid extraction was repeated twice.

To remove β-CD completely, a PSA (primary and secondary amine) and a PBA (phenyl boronic acid) cartridge were connected together in series. The conditioning of the columns and the purification procedure were as described previously [3]. The NMSal enantiomer in each eluate was analyzed by chiral HPLC and the β-CD content was determined by silica gel TLC, stained with 0.1% 1,3-dihydroxynaphthalene-ethanol–$H_2O$–$H_2SO_4$ (200:157:43, v/v/v) solution.

## 3. Results and discussion

Racemic NMSal (184 μg) was applied to a reversed-phase HPLC system with β-CD as an additive in the mobile phase, as shown in Fig. 1. Fig. 1A shows the elution profile of NMSal, monitored by the absorbance at 280 nm, and Fig. 1B shows the enantiomeric composition of NMSal in the eluate as

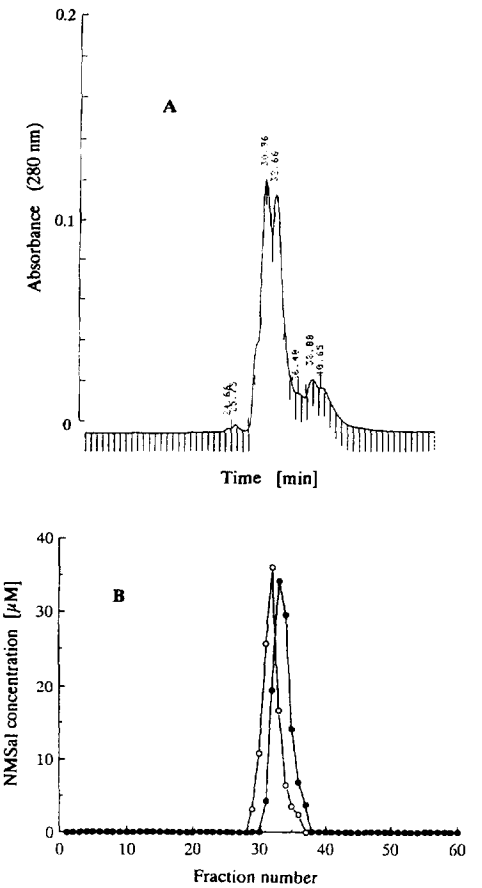


Fig. 1. Elution profile of racemic NMSal by reversed-phase HPLC. Racemic NMSal (184 μg) was applied to a reversed-phase Partosil ODS-10 column and eluted with 25 m*M* sodium phosphate buffer, pH 3.0, containing 12 m*M* SHS and 2% acetonitrile, at a flow-rate of 4 ml/min. The elution was monitored by measurement of the absorbance at 280 nm, and fractions were collected at 1 min intervals. (A) Elution profile measured at an absorbance of 280 nm; (B) Chiral composition of (*R*)- and (*S*)-NMSal in the fraction as measured by chiral HPLC–UV detection. ○=(*R*)-NMSal; ●=(*S*)-NMSal.

determined by HPLC with a chiral column. As shown in Fig. 1A–B, (*R*)-NMSal was eluted first, followed by (*S*)-NMSal. Fig. 1B indicates that only (*R*)-NMSal was contained in the first fractions of the initial peak. Thus, the (*R*)-form was successfully separated from the (*S*)-form. The elution profile shown in Fig. 1A indicates that there were also contaminants in the starting material.

However, the (*R*)-NMSal fractions that were collected and lyophilized were found to contain β-CD. To remove β-CD, the NMSal was extracted with hydrochloric acid twice, and the recovery yield of the combined first and second extractions was 99%.

To remove β-CD completely, solid-phase extraction of (*R*)-NMSal was performed. The final eluate extracted with 0.1 *M* HCl was not contaminated with β-CD. The yield of pure (*R*)-NMSal from the solid-phase extraction was 82.7%.

The yield of (*R*)-NMSal was very low because only the first few fractions of the preparative HPLC, containing only (*R*)-NMSal, were used for further purification, while other fractions that were contami-

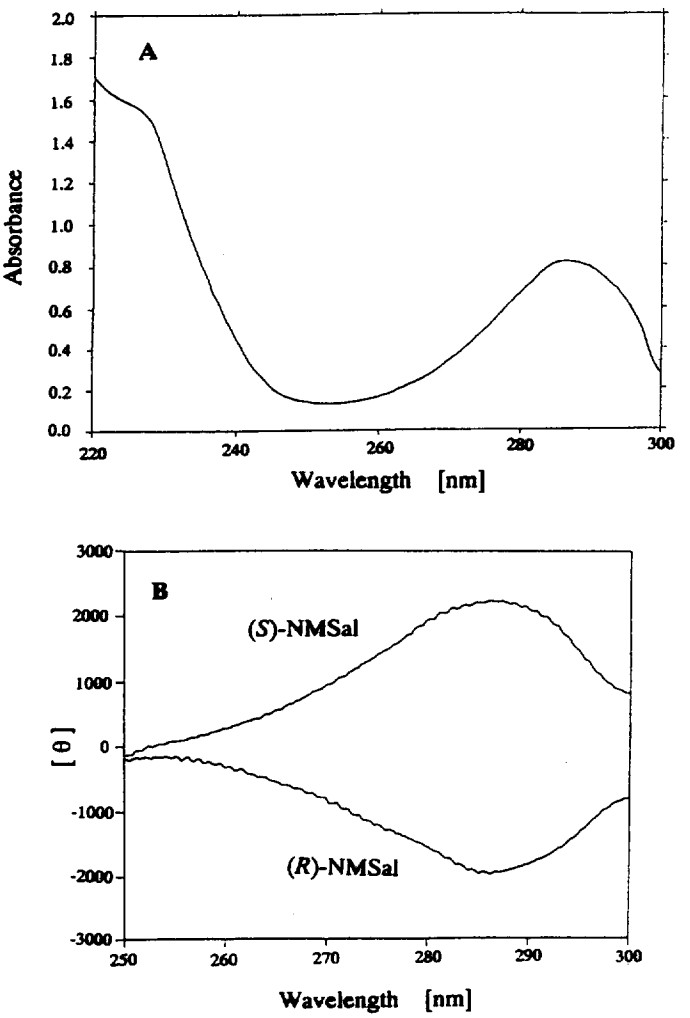


Fig. 2. Absorbance and CD spectra of standard (*S*)-NMSal and of prepared (*R*)-NMSal after the solid-phase extraction. (A) UV spectrum of NMSal prepared by the solid extraction procedure. (B) CD spectrum of (*R*)- and (*S*)-NMSal.

nated with the (*S*)-enantiomer were not subjected to further purification. When 184 μg of racemic NMSal were applied, the yield of purified (*R*)-NMSal was 11%. With racemic NMSal samples of up to 5 mg, a similar elution pattern and yield were obtained. If the fractions containing (*R*)-NMSal that is contaminated with some (*S*)-NMSal are also subjected to further separation, the yield will be improved. To scale up the amount of the preparation, a larger sized reversed-phase column, which is now available commercially, should be used.

The UV and circular dichroism (CD) spectra of standard (*S*)-NMSal and of the prepared (*R*)-NMSal were measured to examine the optical purity of the prepared sample. The UV spectrum of the purified NMSal sample is given in Fig. 2A, which shows a peak at around 285 nm and a shoulder peak at 225 nm. Fig. 2B shows the CD spectra of prepared (*R*)-NMSal and standard (*S*)-NMSal. The peaks were found at 286 nm in the spectra of the (*R*)- and (*S*)-enantiomers, and the $[\theta]$ values were $-2000$ and $+2200$, respectively. The CD spectra of (*R*)- and (*S*)-NMSal were antipodal and the absolute value of $[\theta]$ in the (*R*)-NMSal sample is almost the same as that of the standard (*S*)-form.

This method, using a chiral additive in the mobile phase, is very convenient, because enantiomers can generally be separated using a commercially available reversed-phase column. β-CD added in the mobile phase can be removed completely by acid extraction and solid-phase extraction. This method will be applied in future to develop a new series of cyclodextrin derivatives, in combination with various types of HPLC columns for the separation of enantiomers. This method can not only be used on a large scale, but to isolate an enantiomer from a small amount of precious sample.

## Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Area (M.N.) from the Ministry of Education, Science and Culture, of Japan.

## References

[1] W. Maruyama, M. Naoi, T. Kasamatsu, Y. Hashizume, T. Takahashi, K. Kohda, P. Dostert, J. Neurochem., 69 (1997) 322.
[2] M. Naoi, W. Maruyama, P. Dostert, Y. Hashizume, D. Nakahara, T. Takahashi, M. Ota, Brain Res. 709 (1995) 285.
[3] S. Sällström Baum, H. Rommelspacher, J. Chromatogr. B 660 (1994) 235.
[4] Y. Deng, W. Maruyama, H. Yamamura, M. Kawai, P. Dostert, M. Naoi, Anal. Chem. 68 (1996) 2826.
[5] C. Schöpf, H. Bayerle, Liebigs Ann. Chem. 513 (1934) 190.
[6] Y. Deng, W. Maruyama, M. Kawai, P. Dostert, H. Yamamura, T. Takahashi, M. Naoi, J. Chromatogr. B 689 (1997) 313.