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Short communication

Liquid chromatography–electrospray mass spectrometry method to separate and detect N-*tert*.-butoxycarbonyl peptides

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Abstract

A method to separate and detect peptides was developed. A mixture of 16 pentapeptides was chromatographically separated on two different optically active chiral stationary phases. The peptides were modified with an N-*tert*.-butoxycarbonyl group at the amino end and a hydrazide group at the acid end. Because of the difficulties of dissolving the peptides in polar solvents, a non-polar one was used. Normal-phase chromatography conditions were used. By adding water in a post-column manner, the ionisation efficiency was increased and useful mass spectra were obtained.

Keywords: Chiral stationary phases, LC; Peptides

1. Introduction

To obtain pure enantiomers e.g., in the pharmaceutical industry is of great importance. Chiral separations are therefore necessary in drug production and research. If the wrong enantiomer reaches a receptor in the body, unwanted side effects could appear as in the case of thalidomide [1]. The goal of this work is to obtain a chromatographic system with good separation factor (α value) for the peptides.

For peptide work soft ionisation such as electrospray ionisation (ESI) is useful [2]. Most of the liquid chromatography (LC) separations that utilise the LC–ESI-mass spectrometry (MS) techniques are run by reversed-phase chromatography. Because of the difficulties in dissolving N-*tert*.-butoxycarbonyl peptides (*N*-t-Boc) in polar solvents, there is a need for using normal-phase chromatography. To increase the ionisation efficiency in LC–ESI-MS, it is common to add a small amount of sodium or potassium salt in the eluent [3,4]. This will not work under normal-phase conditions, however by adding water in a post-column manner, we were able to increase the ionisation efficiency of the electrospray.

2. Experimental

2.1. Instrumentation

A VG ZabSpec mass spectrometer (Fisons Instruments, UK) with electrospray was used. The drying and nebulizing gases were nitrogen. The mass spectrometer was calibrated with a poly(ethylene glycol) (PEG) mixture. Data were acquired in the scan mode from m/z 200 to 2000 in 4 s. The electrospray (ES)

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needle voltage was set to 8000 V, counter electrode to 5200 V and the sampling cone to 4200 V. A Phoenix pump (Carlo Erba, Italy) was used for the LC mobile phase. The flow was 400 μl/min and a Rheodyne 8125 manual injector with a fixed loop of 5 μl was used. A post-column flow of 30 μl/min of water, delivered by another Phoenix pump, was mixed with the flow from the LC column by a stainless steel T-piece and a polyether ether ketone (PEEK) tube of 10 cm length. To reduce the flow to 200 μl/min before it was led into the electrospray source, a post-column splitter was used (see Fig. 1).

2.2. Chemicals

The solvents were analytical-reagent grade tetrahydrofuran (THF) (Merck) dichloromethane (LabScan, analytical-reagent quality), acetic acid (Riedle-de Haen, 99.8%) and water (double-distilled). The peptide mixture was synthesised following the Merrifield method. The peptides were dissolved in dichloromethane to approx. 4 mg/ml.

2.3. Columns and mobile phases

Two optically active columns (250×2.1 mm), one *N*-t-Boc-L-phe immobilised on 3-aminopropylsilica (Kromasil 5 μm 100 Å, EKA Nobel, Sweden) and the other *N*-t-Boc-D-Phe immobilised on 3-aminopropylsilica (Kromasil 5 μm 100 Å, EKA Nobel) were prepared. Except that diisopropylcarbodiimide was used (not dicyclohexylcarbodiimide) the preparation followed the method Hara and Dobashi [5] describe to prepare *N*-acetyl-L-valyl-aminopropylsilanazed silica. All amino groups on the silica were end-capped with acetic anhydride. The eluent mixtures were dichloromethane–THF (4:1) for the D-column and (40:1) for the L-column. In both cases 1% of acetic acid was added to reduce tailing.

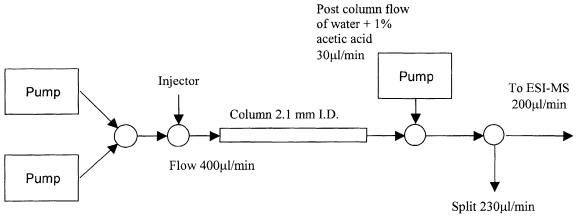

Fig. 1. Schematic diagram of the LC system.

3. Results and discussion

Boc-Peptides are difficult to dissolve in polar solvents. Instead, the peptides were dissolved in dichloromethane. The mobile phase polarity was changed with different concentrations of THF from 2.5–25%. The Boc-peptides had a shorter retention time with higher concentration of THF. With these non-polar solvents, no peptide-related ions were detected by the MS system. We could not use a post-column flow of inorganic salt solution, commonly used to increase the ionisation efficiency. However, by adding a post-column flow of water with 1% of acetic acid this problem was solved.

Three different *N*-t-Boc-L-Phe columns were made and tested with eluents of different polar strength. No changes were detected in the chromatographic order in which the peptides were eluted. This indicates that the polarity of the eluent or the interactions with the stationary phase do not affect the chromatographic order. Thus, changes in the chromatographic order will mainly be dependent on chiral interactions. Other interactions of the stationary phase are reduced by end-capping of the aminopropyl groups with acetic anhydride.

3.1. Results

Mass chromatograms were constructed (see Fig. 2). A significant change in the chromatographic order was detected (see Table 1). By looking at Fig. 2 is it clear that the retention order of the two different peptides with masses 444 and 754 have switched. The difference in retention order indicates that the two columns have different chiral interactions with the Boc-peptides. It is important to notice that if the THF content in the eluent is reduced from 25% to 2.5% the retention time of the peptides are increased.

3.2. Conclusions and outlook

A new technique to separate and detect *N*-t-Boc-peptides in normal-phase chromatography conditions

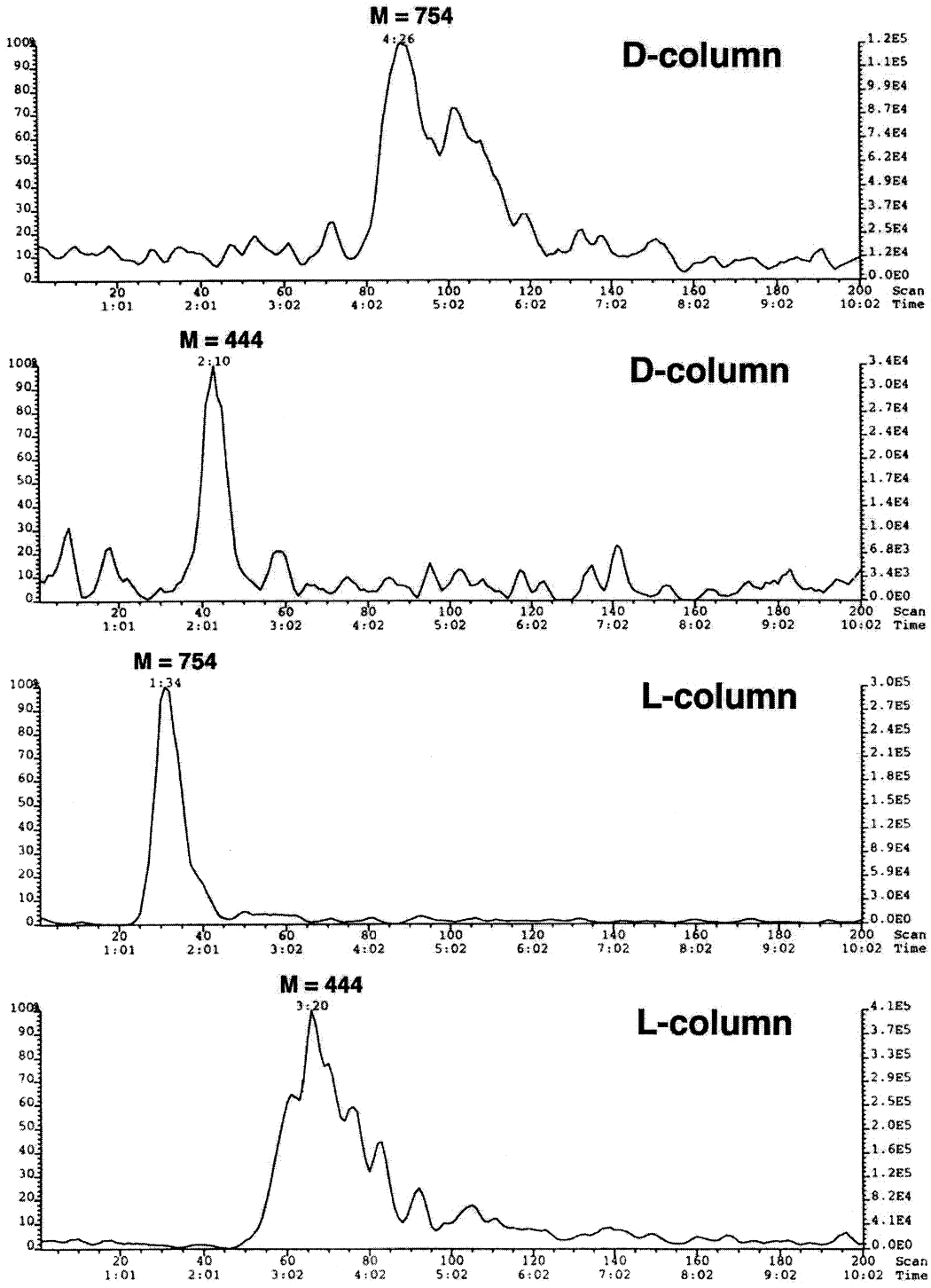

Fig. 2. Mass chromatograms of two different peptides run on the D-column (top) and on the L-column (bottom).

Table 1
Retention times (t'_R, min) for nine pentapeptides on D- and L-columns

Peptide	M_r	Monitored $\pm 1/2$ m/z	t'_R	
			D-Column	L-Column
Ile–Gly–Ala–Gly–Leu–NH–NH_2	444	445	2.1	3.2
Boc–Ile–Gly–Ala–Gly–Leu–NH–NH_2	544	545	8.02	3.15
x-pep	570	571	3.23	1.43
Boc–Ile–Gly–Ala–Ser(OBzl)–Leu–NH–NH_2	664	665	7.14	2.07
Boc–Ile–Gly–Ala–Tyr(OBzl)–Leu–NH–NH_2	740	741	7.2	2.32
Boc–Ile–Ser(OBzl)–Ala–Phe–Leu–NH–NH_2	754	755	4.26	1.34
Boc–Ilu–Tyr(OBzl)–Ala–Phe–Leu–NH–NH_2	830	831	5.05	1.31
Boc–Ile–Tyr(OBzl)–Ala–Ser(OBzl)–Leu–NH–NH_2	860	861	4.02	1.31
Boc–Ile–Tyr(OBzl)–Ala–Tyr(OBzl)–Leu–NH–NH_2	936	937	4.32	1.34

The eluent was 25% THF in dichloromethane for the D-column and 2.5% THF in dichloromethane for the L-column. Bzl=Benzyl.

has been evaluated. Two optical active columns one *N*-t-Boc-L-Phe immobilised on 3-aminopropylsilica and the other *N*-t-Boc-D-Phe immobilised on 3-aminopropylsilica, were tested. The retention order of the peptides is clearly altered which show the chiral effect of the columns. The system includes a post-column flow of water that increases the ionisation efficiency considerably.

To further improve the separation between enantiomers, the peptide with the greatest difference in retention time on the two columns will be immobilised on silica and tested as a new chiral stationary phase.

References

[1] S. Allenmark, Chromatographic Enantioseparation, Ellis Horwood, Chichester, 2nd ed., 1991.
[2] J.R. Chapman, Practical Organic Mass Spectrometry, Wiley, New York, 2nd ed., 1994.
[3] M.W.F. Nielen, Rapid Commun. Mass Spectrom. 10 (1996) 1652.
[4] A. Roda, A.M. Gioacchini, C. Cerrè, M. Baraldini, J. Chromatogr. B 665 (1995) 281.
[5] S. Hara, A. Dobashi, J. Liq. Chromatogr. 2 (1979) 883.