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Short communication

# Separation of capsaicin from capsaicinoids by simulated moving bed chromatography

Feng Wei *, Yingxian Zhao

*Ningbo Institute of Technology, Zhejiang University, Ningbo 315100, China*

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**Abstract**

Capsaicinoids were separated from capsicum oleoresin by solvent extraction and adsorption separation on macropore resin, and used directly as the feed of simulated moving bed. Using a mobile phase of methanol/water (75/25, v/v) and ODS columns, the two key components, capsaicin and dihydrocapsaicin, were separated completely, while part of minor weak impurities were discarded by forcing them to leak into zone I. The amount of discarded impurities increases with decreasing flow rate in zone I so that the purity of capsaicin in raffinate stream could be improved.


*Keywords:* Capsaicin; Capsaicinoids; Simulated moving bed; ODS

## 1. Introduction

Capsaicin, an active compound in chilli peppers, is currently used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia, and psoriasis [1]. Recently, it was found that capsaicin is able to kill prostate cancer cells by causing them to undergo apoptosis [2].

Besides capsaicin (C), there also exist several analogues, including dihydrocapsaincin (DHC), nordihydrocapsaicin (NDHC), and homodihydrocapsaicin (HDHC), etc., in chilli peppers. These compounds with similar structures are called as capsaicinoids. Since simulated moving bed (SMB) is suitable for the separation of binary mixtures, such as enantioseparation [3–9], the separation of capsaicin with high purity from multi-component capsaicinoids seems not to be carried out through a single SMB process. However, as the elution order on C18 column with the mobile phase of methanol/water are NDHC, C, DHC and HDHC simulated moving bed may be able to separate the multi-component mixture into NDHC + C (in raffinate) and DHC + HDHC (in extract). Furthermore, as the original content of NDHC is small compared with C, the purity of C in raffinate stream could be improved by making part of NDHC leak into zone I.

In this work, capsaicinoids were separated from capsicum oleoresin by extraction and adsorption on resin column, and then a simulated moving bed was used to separate capsaicin from capsaicinoids. The effects of flow rates in zone I and IV on the purity and solvent consumption were investigated.

## 2. Experimental

### 2.1. Preparation of capsaicinoids from capsicum oleoresin

Capsicum oleoresin (40% capsaicinoids, Guizhou Wubeizi, Guiyang, China) was saponified with a 4% sodium hydroxide solution. The saponified solution was conditioned to have a pH value of 9.57 by the addition of hydrochloric acid and extracted with diethyl ether two times. The obtained organic phase was extracted with a sodium carbonate solution (5%), separated from the water phase, and then extracted with a sodium hydroxide solution (2%). The water phase was conditioned to slight opaque by the addition of 10% hydrochloric acid, and then fed into a column packed with ADS-8 macropore adsorption resin (Polystyrene, Naikai Hecheng, Tianjin, China). After the bed was saturated, water, methanol/10% (w/w) hydrochloric acid (30/70, v/v), methanol/water (30/70, v/v) were in turn eluted to discard impurities. Then a solution of methanol/water (75/25, v/v) was eluted to give a capsaicinoids solution with a purity of 97.8%, based on the percentage of peak area of capsaicinoids. The impurity is an unknown compound (UNC) eluted before the

* Corresponding author. Tel.: +86 57487219109.
*E-mail address:* weifeng@nit.net.cn (F. Wei).

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NDHC. The solution was used as the feed of simulated moving bed without additional treatment.

### 2.2. Chromatography and SMB

A chromatography system (K501 pump, K2501 UV detector, Knauer, Berlin, Germany) was used to analyze the purities of products from SMB at 280 nm. A column, Agilent TC-C18(150 mm × 4.6 mm, 5 μm, Agilent Technologies, Santa Clara, USA), was used to analyze the product from simulated moving bed with mobile phase of methanol/water (70/30, v/v).

A SMB system (Pilot System CSEP C916) made by Knauer was used for the SMB separation experiments. The column (100 mm × 10 mm) packed with Sinochrom ODS-BP (10 μm) was purchased from Elite, Dalian, China. The mobile phase is methanol/water (75/25, v/v). The operation temperature is 30 °C.

Triangle theory [10–14] was an effective method to design simulated moving bed process. One needs to determine the column voidage and the adsorption isotherms of solutes in advance. The voidage $\varepsilon$ was calculated as follows:

$$\varepsilon = \frac{V_0}{V_{\mathrm{Col}}} = \frac{Ft_0}{V_{\mathrm{Col}}} \tag{1}$$

where $V_0$ is the retention volume of uracil and $V_{\mathrm{Col}}$ the volume of column, $F$ is the flow rate of mobile phase, $t_0$ the retention time of uracil. The simple linear adsorption isotherm was used. The adsorption equilibrium constant, $K$, was calculated as follows:

$$K = \frac{t_{\mathrm{R}} - t_0}{t_0}\left(\frac{\varepsilon}{1-\varepsilon}\right) = k'\frac{\varepsilon}{1-\varepsilon} \tag{2}$$

where $t_{\mathrm{R}}$ is the retention time of solute. These parameters are measured and listed in Table 1. The resolution of NDHC and C is poor so that the two peaks could not be detected separately.

It could be found that the columns performances are significantly different. In order to reduce the effect on the separation by simulated moving bed, the columns were connected in series in the order of 3-6-1-8-4-5-7-2. In such way, the migration of solutes will be relatively constant during half of a switch.

In addition, the voidages measured in our lab are larger than those given by the manufactory although both use the uracil as non-retained compound. This difference may be caused by the inaccurate flow rate of the pump used in this work. According to the definition of adsorption equilibrium constant in Eq. (2), the capacity factors, $k'$, is independent of flow rate of mobile phase, thus the $t_0$ and $t_{\mathrm{R}}$ measured in our laboratory should be used to calculate the capacity factors, $k'$, while the $\varepsilon$ given by manufactory was used to calculate $K$ and design-simulated moving bed. The results of the simulated moving bed separations show that such treatment is reasonable.

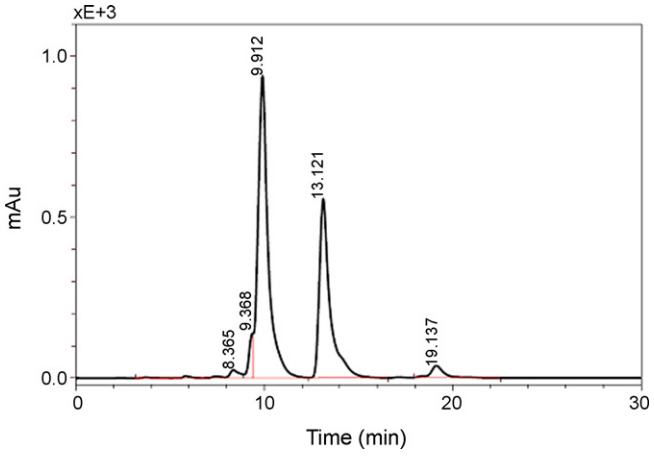


Fig. 1. The chromatogram of capsaicinoids prepared in this work. According to the elution order, the compounds are unknown compound (2.12%), NDHC (3.09%), C (56.53%), DHC (35.31%), HDHC (2.96%), respectively.

## 3. Results and discussion

After being treated with the adsorption resin column, the purity of capsaicinoids reached 97.8%. In Fig. 1, the peaks corresponding to capsaicinoids were characterized by comparing with the chromatogram reported by Cooper et al. [15] The minor impurities could be completely discarded by crystallization, which will require additional processes such as evaporation, crystallization and decrease the recovery of capsaicinoids, thus the solution was used as the feed of simulated moving bed directly.

If the mixture is cut into UNC + NDHC + C and DHC + HDHC, the highest purity of C in raffinate stream is 91.56%. While the separation of key components, C and

Table 1
Column voidage and adsorption equilibrium constants for all components in capsaicinoids

| Column number | Column voidage (%) | | Adsorption equilibrium constant, $K$ | | | |
|---|---|---|---|---|---|---|
| | Given by the manufactory | Measurement | UNC | NDHC + C | DHC | HDHC |
| 1 | 63.18 | 68.92 | 3.07 | 3.89 | 5.73 | 9.03 |
| 2 | 65.73 | 69.83 | 3.22 | 4.12 | 6.07 | 9.56 |
| 3 | 62.68 | 69.45 | 3.12 | 3.98 | 5.88 | 9.27 |
| 4 | 64.20 | 69.83 | 3.25 | 4.12 | 6.05 | 9.56 |
| 5 | 67.77 | 74.55 | 3.91 | 5.02 | 7.35 | 11.58 |
| 6 | 68.28 | 74.57 | 3.97 | 5.02 | 7.38 | 11.67 |
| 7 | 64.20 | 70.29 | 3.17 | 4.00 | 5.86 | 9.23 |
| 8 | 68.28 | 73.71 | 3.84 | 4.89 | 7.18 | 11.32 |
| Average | 65.54 | 71.39 | 3.44 | 4.38 | 6.44 | 10.15 |