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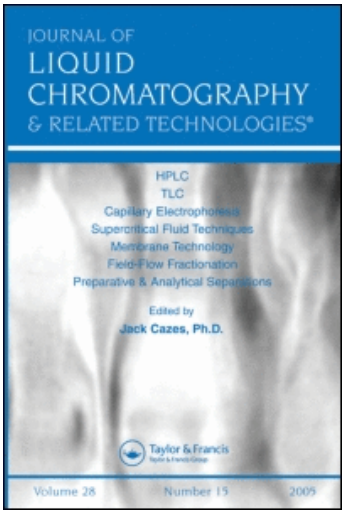

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RAPID QUANTIFICATION OF CAPSAICIN AND DIHYDROCAPSAICIN IN HUMAN SKIN EXTRACTS AFTER DERMAL ADMINISTRATION USING HPLC-ESI-MS

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ABSTRACT

An HPLC electrospray mass spectrometric method for the specific and sensitive quantification of Capsaicin [404-86-4] and Dihydrocapsaicin [19408-84-5] in extracts from human skin is presented.

A 2 mm RP_{18} column was used with a mobile phase of methanol/water/acetic acid 90/9/1 at a flow rate of 0.2 mL/min. The reached limit of detection was 500 pg/mL. MS/MS and MS^3 experiments were performed using an ion trap mass spectrometer. The fragmentation pattern in the positive mode is explained. The method was applied to study Capsaicin penetration from different pharmaceutical preparations.

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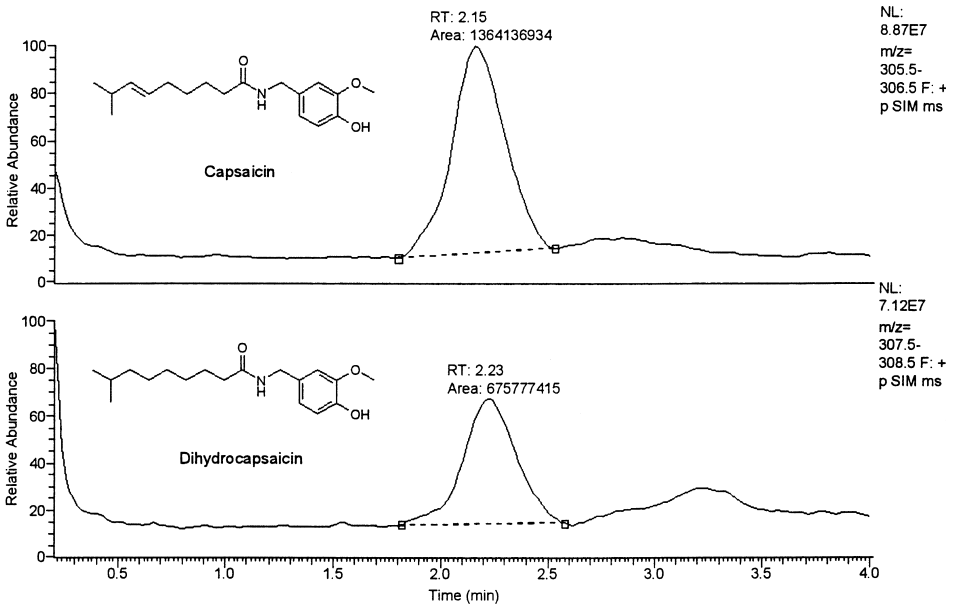

Figure 1. Typical chromatogram of capsaicin and dihydrocapsaicin in a skin extract.

INTRODUCTION

Capsaicin and Dihydrocapsaicin (see Fig. 1) are the most important ingredients of the fruit placenta of Capsicum species, e.g., Capsicum frutescens, which cause the extraordinary hot taste known from chili or cayenne pepper. In the field of pharmacy Capsaicin is used topically in the form of plasters and ointments, especially to treat rheumatic diseases. Recently its successful administration in the treatment of psoriasis and diabetic neuropathia was reported, too.[1,2,3] Capsaicin produces a hyperemia in the skin and relieves pain by inhibiting the pouring out of the undecapeptide substance P.[4]

Although Capsaicin has been used for a long time, no data have been reported concerning its penetration and permeation behaviour into and through the human skin. A reason for that fact are analytical difficulties in quantifying small amounts of capsaicin and dihydrocapsaicin in different human skin layers. A number of studies reported the determination of the Capsaicin content in plants of the Capsicum genus by HPLC with UV detection, which was correlated to the hot taste measured in Scoville units.[5,6,7] The limit of detection was approximately 3 µg/mL.

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In a further interesting paper the analysis of Capsaicin by HPLC-MS with the moving belt ionization interface was described.[8] This interface could not provide the sensitivity and reliability of the modern ESI interfaces.

Therefore, it was the goal of the presented work to develop a rapid and reproducible HPLC-MS method for the quantification of Capsaicin and Dihydrocapsaicin in extracts from human skin layers. The method should enable the quantification of amounts smaller than 100 ng/mL. Furthermore, comparative studies on the penetration and permeation behaviour of different topical Capsaicin preparations were performed.

EXPERIMENTAL

Chemicals and Pharmaceutical Preparations

Methanol (gradient grade) which was used for sample extraction and as a component of the mobile phase was purchased from J.T. Baker (Deventer, The Netherlands). For chromatographical purposes doubly distilled water was applied. A mixture of Capsaicin (60%) and Dihydrocapsaicin (approx.30%) was obtained from Sigma (Deisenhofen, Germany); the balance consists of other naturally occuring capsaicinoids.

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Sample Preparation

The penetration studies were performed with human skin sheets using the diffusion cell according to Franz.[9] The procedure of the preparation of samples representing the stratum corneum, the epidermis, and dermis was described by Schmalfuß et al.[10] The aqueous acceptor medium was evaluated, too. The extraction of Capsaicin and Dihydrocapsaicin from the samples was performed with methanol and directly subjected to HPLC-MS analysis without further preparation.

HPLC-MS

HPLC operations were carried out with a Waters 600 E system (Waters GmbH, Eschborn, Germany) with a Waters WISP 712 autosampler. Chromatographic separations were performed on an ET 125/2 Nucleosil®120-3C_8 column (125 x 2 mm I.D., 3 µm particle size; Macherey-Nagel GmbH, Düren, Germany) without precolumn.

The HPLC was coupled to a single quadrupole mass spectrometer Finnigan SSQ 710C (Finnigan MAT GmbH, Bremen, Germany) with an electrospray ionization interface, from which the quantitative data were obtained. MS-MS and MS^n were performed on an ion trap mass spectrometer Finnigan LCQ (see above). The sample solution (10 µg/mL of the Capsaicinoid mixture in methanol/water/acetic acid 90:9.5:0.5) was introduced via a syringe pump (flow rate 15 µL/min).

As mobile phase a mixture of methanol/water/acetic acid (90:9:1; v/v/v; degassed with helium) was used. 200 µL of the sample and 10 µL of 20 µg/mL n-Dodecyl β-D-glucopyranoside, which was used as a standard to control ESI stability (RSD<7% was tolerated), were placed into each of the autosampler vials, from which 5 µl were injected. A flow of 0.2 mL/min was applied. Electrospray ionization was carried out with a voltage of 4.5 kV and a temperature of 250°C at the heated capillary. Detection was performed in the positive mode. For quantification of Capsaicin and Dihydrocapsaicin the SIM mode (Selected Ion Monitoring) was chosen because of its higher sensitivity. A peak width of 0.5 amu and a scan time of 0.25 seconds per scan were adjusted. The parameters of the lenses of the ion optics were optimized for Capsaicin and Dihydrocapsaicin.

The quantification was performed using external standards. Calibration curves with 13 concentrations between 0.005 and 2 µg/mL for the Capsaicinoid mixture were set up. To control the electrospray stability, a quality standard (20 µg/mL n-dodecyl β-D-glucopyranoside) was used (10 µl + 200 µl skin extract) and a calibration sample was set after every 7th measurement.

Quantification referred to peak areas which were evaluated using Microsoft Excel 7.0 software on an IBM type personal computer equipment.

RESULTS AND DISCUSSION

HPLC-MS

The mobile phase (see experimental) was optimized with respect to short analysis times and to the sensitivity achieved in ESI-MS. The goal of the HPLC method was to reach a separation of the Capsaicinoids from matrix components. The chromatographical separation of Capsaicin and Dihydrocapsaicin was not necessary. The detection in the Selected Ion Monitoring (SIM) mode with positive ionization provided the best results concerning sensitivity. Figure 1 shows a typical chromatogram of Capsaicin/Dihydrocapsaicin determination in a skin extract.

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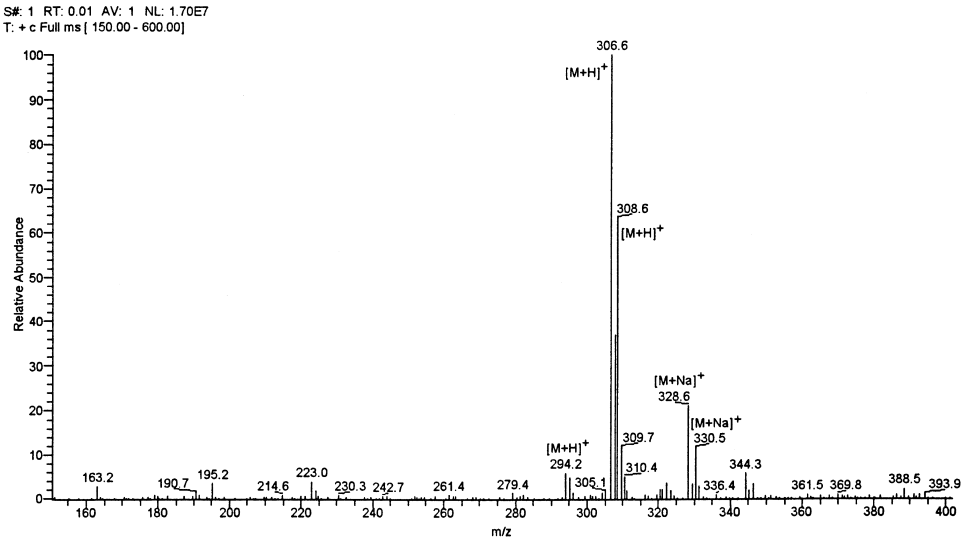

Figure 2. ESI mass spectrum of the capsaicinoid mixture, positive mode.

The analysis of Capsaicin and Dihydrocapsaicin shows, that even coelueting substances with a mass difference of only 2 amu can be separately quantified without interference, using the SIM mode (see figure 1). Under the given conditions ESI as a soft ionization technique generates in the positive mode a $[M+H]^+$ peak and a $[M+Na]^+$ peak, which can be used for quantification. The m/z= 306.2 represents the $[M+H]^+$ of Capsaicin, the m/z= 328.6 its sodium adduct. Dihydrocapsaicin shows m/z of 308.6 and 330.5, respectively (see figure 2). The peak at m/z= 294.2 refers to 7-Methyl-N-vanillyl-octanamide, known as Nordihydrocapsaicin, which occurs in an amount of 5-7%.

The analytical parameters of the presented method were:

Limit of detection: 250 pg/mL (mixture) and 500 pg/mL (single substances), respectively,
Lower limit of quantitation: 5 ng/mL, and
Higher limit of quantitation (in SIM mode): 5 µg/mL.

The wide range of quantification and an excellent limit of detection indicate the analytical power of the method described. The relative standard deviation calculated from 50 measurements of n-Dodecyl β-D-glucopyranoside was 6.6%. This quality standard has been well proven in previous works and was chosen to control the general ESI stability.[11]

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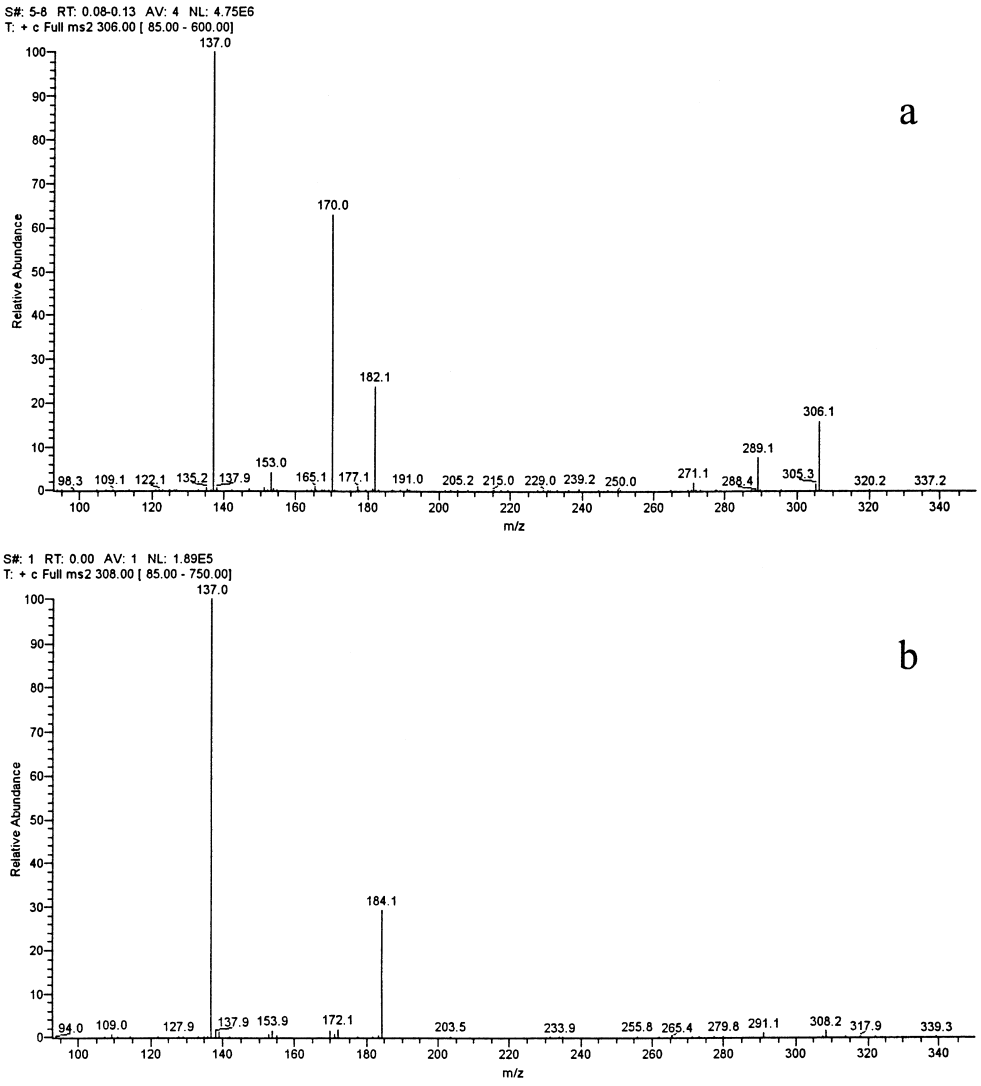

Figure 3. MS/MS spectra of capsaicin (a) and dihydrocapsaicin (b).

MS/MS

For a further improvement of detection specificity and sensitivity the application of MS-MS and MS^n is possible. Although we have not yet coupled this technique to HPLC, we performed additional investigations on an ion trap

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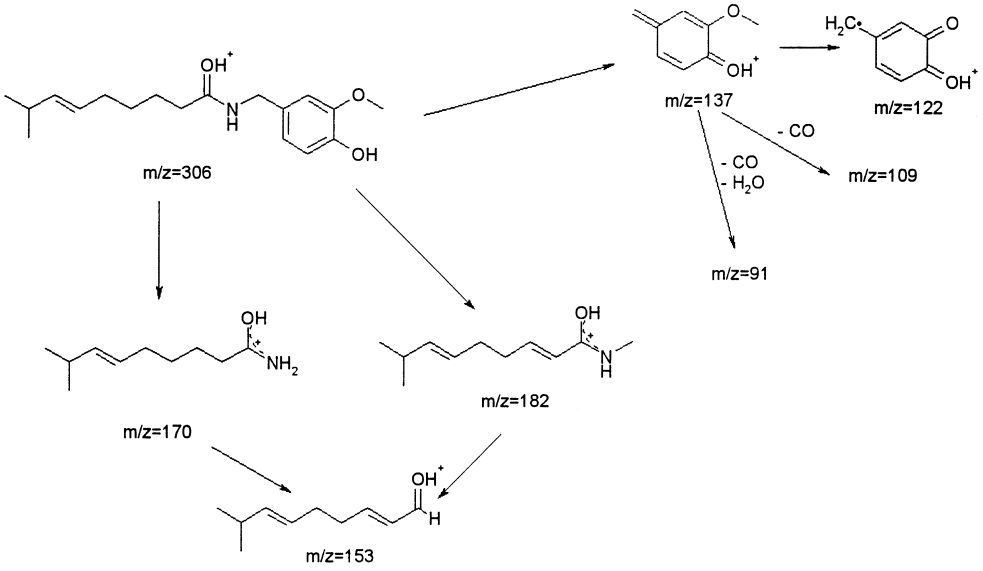

Figure 4. Proposed pathways of fragmentation of capsaicin in the positive ionization mode (MS/MS and MS^3)- see text for further explanation.

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mass spectrometer in the positive as well as in the negative mode. (See Figure 3.) The fragmentation was performed by isolating the parent ions (peak width m/z=1.4) and applying collision energy (12-30%) in the ion trap. The scheme in Fig. 4 shows the proposed pathways of fragmentation for the positive mode. The fragments can be divided into two groups, one which refers to the acid amide structure, and a second one which refers to the vanillyl moiety. This classification can very simply be confirmed by comparing the m/z of Capsaicin and Dihydrocapsaicin. The vanillyl moiety is identical for both compounds and yielded a fragment of m/z=137 by MS/MS. MS^3 of this ion resulted in ions with m/z of 122, 109 and 91, respectively. The acid amide structure provided in MS/MS, ions of m/z=170 and 182 for Capsaicin and m/z=172 and 184 for Dihydrocapsaicin. It is interesting that the ratio of 170 to 182 in the case of Capsaicin is much greater than the ratio of 172 to 184 for Dihydrocapsaicin. This is supposed to be due to the better ionization ability caused by the double bond in pos. 6. MS^3 resulted in m/z=153 for Capsaicin and m/z=155 for Dihydrocapsaicin.

MS/MS in the negative ionization mode yielded only the $[M-H]^-$ ions of 8-Methyl-6-nonenamide (m/z=168) in the case of Capsaicin, and of 8-Methyl-nonanamide (m/z=170) for Dihydrocapsaicin.

Penetration of Capsaicin and Dihydrocapsaicin from Pharmaceutical Preparations

The analytical method described above was successfully applied to a comparative study on the penetration and permeation behaviour of different pharmaceutical preparations. This investigation yielded, that capsaicin/ dihydrocapsaicin generally showed a good penetration into the skin layers, as well as, a good permeation through the skin into an aqueous acceptor medium. Nevertheless, remarkable differences were observed between the different preparations. A paper discussing the penetration studies from the pharmaceutical point of view is in preparation.[12]

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