CAPSAICINE IN *Capsicum annuum* CONDENSED EXTRACT DETERMINED BY HPLC

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Red pepper (*Capsicum annuum*) is widely cultivated in Uzbekistan. Preparations of red pepper are effective phytotherapeutics that are widely used in conventional and folk medicine internally for poor digestion, chronic vomiting, and lingual paralysis and externally as an irritant and distractant and for massaging neuralgia, radiculitis, myositis, etc. [1].

The principal active components of the preparations are capsanoid alkaloids, in particular, capsaicine [2]. We previously studied the alkaloid content of *C. annuum* [3].

Our goal was to determine quantitatively the capsaicine content in the condensed extract by HPLC. For this we studied ethanol extracts of red pepper collected in various regions of Uzbekistan (Andizhan, Tashkent, and Fergana districts) and dried at 25-30°C. The resulting extracts (three times, 70% ethanol) were condensed in vacuum to produce a thick extract that satisfied RTD requirements [4, 5].

The capsaicine content in the extracts was determined by HPLC in a Hewlett Packard 1090 liquid chromatograph. The chromatographic system consisted of a Hypersil 5-μ guard column and Hypersil 5-μ (20×2.1 mm) and Hypersil BDS C-18.5-μ (250×4.6 mm) columns. UV detection at 220 nm was used.

The mobile phase was H_2O—CH_3CN (35:65). The flow rate was 1.0 mL/min; analysis time, 25 min.

Preparation of Standard Capsaicine Solution. Standard capsaicine (25 mg, accurate weight, 8-methyl-N-vanillyl-6-nonenamide, Capsaicine, Sigma, USA, ~98%) was dissolved in methanol (65%) in a 100-mL volumetric flask. The solution was adjusted to the mark with methanol (65%). An aliquot (10 mL) of the resulting solution was transfered to a 50-mL volumetric flask, adjusted to the mark with methanol (65%), mixed, and filtered through a 0.45-μ Millipore filter. This solution was used for chromatography. The capsaicine concentration of the standard solution is 0.05 mg/mL.

Preparation of Test Solution. A portion of the condensed extract (1 g, accurate weight) was dissolved in methanol (65%) in a 50-mL volumetric flask and filtered through a 0.45-μ Millipore filter to remove insoluble impurities.

Sample Analysis. Samples of the standard and test solutions (25 μL) were placed on the column. The retention times and peak areas of capsaicine were determined. The retention time of capsaicine for the column and mobile phase described above was 5.6-5.8 min.

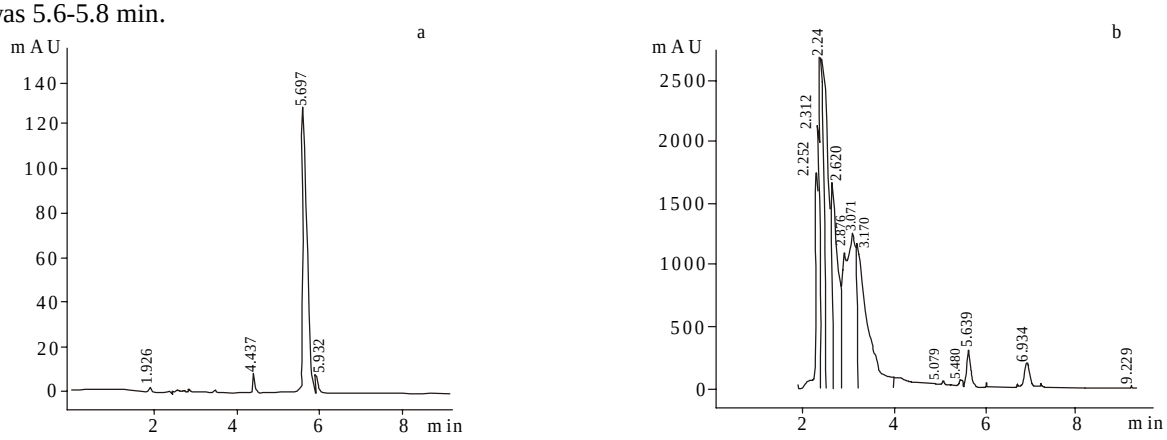

Fig. 1. HPLC chromatogram of standard capsaicine solution (a) and condensed extract (b).

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Figure 1 shows chromatograms for standard capsaicine and the condensed extract obtained from peppers collected in the Fergana valley.

Capsaicine content in extracts was calculated by the usual method using an external standard.

Thus, we found that the amount of capsaicine in the studied samples varied from 0.175 to 0.3246% and depended on the plant habitat. Data for the capsaicine content in the samples of condensed pepper extract are given below.

Collection site, district	Capsaicine content in condensed pepper extract, %
Fergana	0.3246
Andizhan	0.2630
Tashkent	0.175

It can be seen that samples of condensed extract from peppers collected in Fergana District have the highest capsaicine content.

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