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Short communication

Determination of capsaicin and zucapsaicin in human serum by high-performance liquid chromatography with fluorescence detection

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Abstract

A reversed-phase high-performance liquid chromatographic (HPLC) assay was developed to analyze capsaicin and zucapsaicin (civamide) in human serum at concentrations from 1 to 100 ng/ml. Human serum specimens were extracted twice with hexane–methyl *tert.*-butyl ether (1:1). The chromatographic separation was carried out on a C_{18} column at 40°C using a mobile phase consisting of 40% acetonitrile in water with 5% tetrahydrofuran and 1% acetic acid. The concentration of the eluting compounds was monitored by a fluorescence detector with excitation at 270 nm and an emission cutoff of 300 nm. No interferences were observed from the extract of blank serum. The standard curves were linear in the detection range. The relative standard deviation of the assay was better than 8.4%. The limit of detection was 0.5 ng/ml. © 1997 Elsevier Science B.V.

Keywords: Capsaicin; Zucapsaicin

1. Introduction

Capsaicin (Cap, Fig. 1) is one of the major components found in capsicum fruits such as red hot pepper. It is used not only as a flavoring agent, but also as a therapeutic drug. Numerous studies [1–3] have shown that capsaicin can be used as a topical analgesic in painful conditions such as postherpetic neuralgia, diabetic neuropathy and osteoarthritis. Recent studies of capsaicin have focused on its effect on the nervous system [4–6] and toxicity [7–9]. Zucapsaicin (Zucap, Fig. 1), also known as civamide, is a synthetic isomer (*cis*-) of capsaicin (*trans*-). It has similar activity and potency to capsaicin. However, there are no studies reported in the literature on the pharmacological effect and pharmacokinetics of zucapsaicin. To better understand the pharmacokinetic properties of capsaicin and zucapsaicin in order to ensure their safe and effective use, a simple, fast and accurate analytical method is necessary.

A variety of GC [10,11] and HPLC [12–19] methods for the determination of capsaicin and its analogues have been reported. The GC methods usually require tedious sample preparation procedures. HPLC methods with ultraviolet (UV) [12–17], fluorescence [14,16,18] and electrochemical (EC) detection [19] have been frequently used to analyze capsaicin and its analogues. However, there has not been any method reported for the determi-

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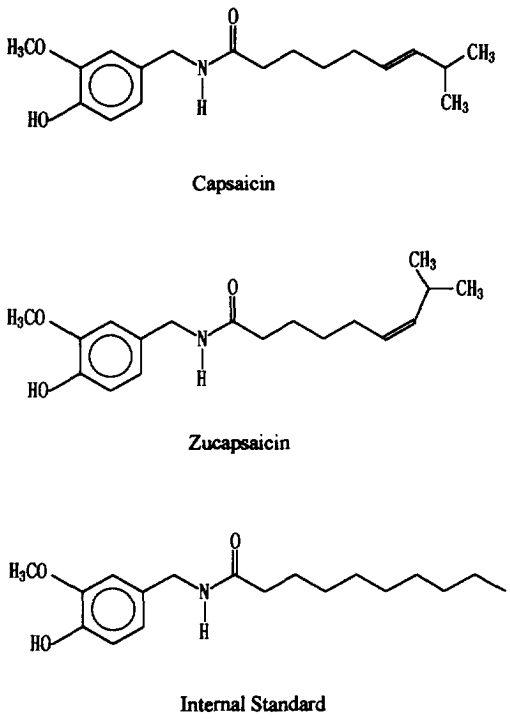

Fig. 1. Chemical structures of capsaicin, zucapsaicin and the internal standard.

nation of zucapsaicin in serum samples. The aim of this study was to develop an HPLC method for the determination of capsaicin and zucapsaicin in human serum with fluorescence detection. The quantitation limit was 1 ng/ml.

2. Experimental

2.1. Reagents and material

Capsaicin (purity 99.4%) and zucapsaicin (purity 98.6%) were obtained from Macfarlan Smith (Edinburgh, UK). Methanol (HPLC grade), hexane (optimum grade), acetonitrile (HPLC grade), tetrahydrofuran (HPLC grade) and glacial acetic acid (reagent grade) were from Fisher Scientific (Pittsburgh, PA, USA). Methyl *tert.*-butyl ether (High purity) was obtained from Baxter Healthcare (McGaw Park, IL, USA). 4-Hydroxy-3-methoxy benzyl amine hydrochloride and decanoyl chloride were obtained from Aldrich (Milwaukee, WI, USA). Deionized water was obtained as needed from a Barnstead Nanopure II System (Fisher Scientific, Pittsburgh, PA, USA). Pooled human serum was collected from normal volunteers on site and stored at −20°C.

2.2. Instrumentation

The HPLC system consisted of two M-510 pumps, an M-600E pump controller, a WISP-712 autosampler (Waters Associates, Milford, MA, USA) and a 980 programmable fluorescence detector (Applied Biosystems, Foster City, CA, USA). An HP-3359 laboratory automation system (Hewlett-Packard, Palo Alto, CA, USA) was used for data acquisition. Instruments of equivalent performance may be substituted for any of the above listed equipment.

2.3. Preparation of the internal standard

Decanoyl 4-hydroxy-3-methoxybenzyl amide. A 200 mg amount of 4-hydroxy-3-methoxybenzyl amine hydrochloride was dissolved in 3 ml of 1 *M* NaOH. Decanoyl chloride was added into the solution dropwise until a maximum white precipitate formed. The white precipitate was filtered through a Whatman 9 cm No. 1 filter paper and washed with cold water. The precipitate was dissolved in 1 ml of 1 *M* HCl and extracted with 5 ml hexane. The hexane layer was transferred to a clean tube and back extracted with 2 ml of methanol. After evaporating the methanol to dryness, the final product was dried at room temperature and stored in a desiccator at 4°C. The product was found to be >99% pure by HPLC.

A stock solution was prepared by dissolving 1 mg decanoyl 4-hydroxy-3-methoxybenzyl amide in 20 ml of methanol.

2.4. Preparation of standard solutions

A stock solution of capsaicin or zucapsaicin was made by dissolving 10 mg of the drug in 100 ml methanol. Working solutions were prepared by dilut-

ing the stock solution to desired concentrations with water.

2.5. Chromatographic conditions

The mobile phase consisted of water–acetonitrile–tetrahydrofuran-glacial acetic acid in a ratio of 55:40:5:1. It was mixed and filtered through a 0.45-μm nylon filter. A YMC-Pack ODS-A HPLC column (3 μm, 150×4.6 mm, YMC, Wilmington, NC, USA) maintained at 40°C was used for the chromatographic separation. The flow-rate was 0.8 ml/min. The run time was 30 min. After the internal standard eluted, the concentration of acetonitrile in the mobile phase was increased to 90% over 15 min to eliminate late eluting peaks from the column. It was returned to the original mobile phase in 5 min and the mobile phase was allowed to equilibrate for 5 min before the next injection. The eluting compounds were monitored by fluorescence detection with excitation at 270 nm and an emission cutoff of 300 nm.

2.6. Extraction procedures

After addition of the internal standard (decanoyl 4-hydroxy-3-methoxy-benzyl amide), human serum samples (2 ml each) were extracted with 8 ml of hexane–methyl *tert.*-butyl ether (1:1, v/v). The organic layer was transferred to a clean tube and evaporated under a stream of nitrogen at 40°C. The aqueous layer was extracted a second time with 8 ml of the same solvent and this was combined with the first extract and evaporated to dryness. The residue was reconstituted in 120 μl methanol–water (1:1) and a 100 μl aliquot was injected onto the HPLC column.

3. Results and discussion

3.1. Extraction of capsaicin isomers from serum samples

To ensure the highest recovery and least interferences, the following solvents were tested: hexane, heptane, ethyl ether, methyl *tert.*-butyl ether and methylene chloride. Ethyl ether and methyl *tert.*-butyl ether gave high recovery (~80%), but also introduced interference peaks. Hexane and heptane had lower recovery (~50%) and a relatively clean chromatogram. A combination of hexane–ethyl *tert.*-butyl ether (1:1) was found to give high recovery (~70%) without any interference peaks to either capsaicin or zucapsaicin. A serum sample was extracted 3 times with the recoveries of each extraction as 70%, 20% and 1%, respectively. Therefore, a multiple extraction procedure is necessary to achieve the expected sensitivity of the assay.

3.2. Separation of capsaicin isomers

Fig. 2 shows a chromatogram of capsaicin, zucapsaicin and the I.S. in an aqueous solution. All three compounds were well separated. Tetrahydrofuran was used in the mobile phase to improve peak shape and resolution of capsaicin and zucapsaicin. No interferences were found in blank serum, nor from the I.S. solution. The column was flushed at each injection after elution of the I.S. to ensure a clean baseline for the next injection. Representative chromatograms from blank serum, serum samples spiked with 1.0 ng/ml of capsaicin and zucapsaicin are shown in Fig. 3. The retention time was approximately 14 min for zucapsaicin, 15 min for capsaicin and 25 min for the I.S.

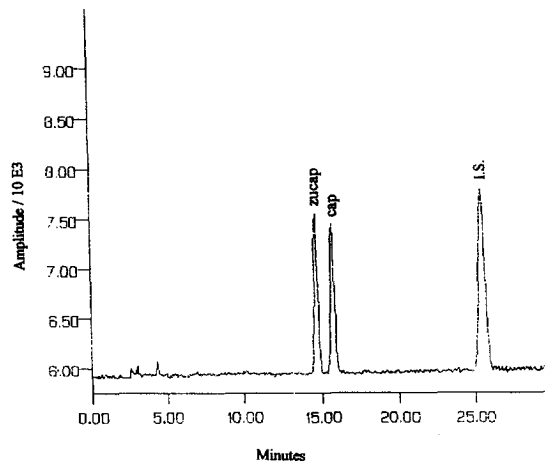

Fig. 2. Typical HPLC chromatogram of capsaicin, zucapsaicin and the I.S. in an aqueous solution.

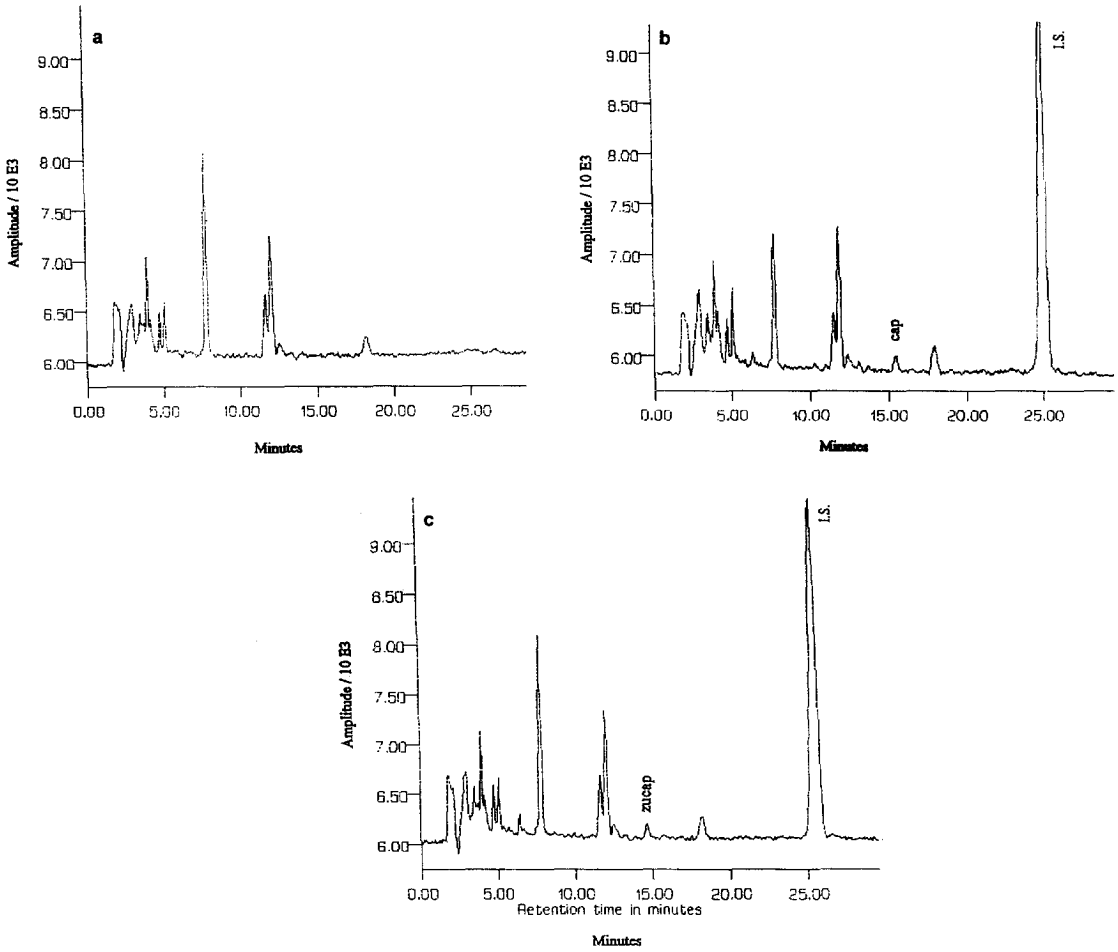

Fig. 3. (a) Chromatogram of extract of blank serum. (b) Chromatogram of extract of serum spiked with 1.0 ng/ml of capsaicin. (c) Chromatogram of extract of serum spiked with 1.0 ng/ml of zucapsaicin.

3.3. Quantitation of capsaicin isomers

Quantitation of capsaicin and zucapsaicin was achieved by comparison of peak area ratios (cap/I.S. or zucap/I.S.) to samples of known concentration. The detector response was linear over the range of 1.0 ng/ml to 100 ng/ml for both capsaicin and zucapsaicin. Correlation coefficients were all greater than 0.99 for the individual standard curves. The mean of slope ($n=5$) was 0.0213 (S.D.±0.0004) for capsaicin and 0.0197 (S.D.±0.001) for zucapsaicin. The y-intercepts were determined to be not significantly different from zero ($t<2.57$, d.f.$=5$, $p>0.05$). The limit of quantitation (the lowest capsaicin or zucapsaicin concentration at which percent error and R.S.D. were <20%) for both isomers was 1 ng/ml. The limit of detection ($S/N>2$) was 0.5 ng/ml. The assay accuracy and precision results are summarized in Table 1. These compounds are stable in serum when stored at −20°C for at least 60 days. There is no decrease in the amount of either compound in reconstituted samples during analysis for at least 48 h.

In summary, this assay provides a sensitive,

Table 1
Accuracy and precision for the analysis of capsaicin and zucapsaicin in serum

	Concentration (μg/ml)			R.S.D. (%)	Accuracy (%)
	Nominal	Determined (Avg.)	S.D.		
Capsaicin					
Within-run	45.0	48.3	2.7	5.7	107.3
(n=7)	8.71	9.04	0.4	4.1	103.8
	2.01	2.15	0.1	6.7	107.0
Between-run					
(n=5)	45.0	45.3	3.0	6.6	100.7
	8.71	8.77	0.4	4.2	100.7
	2.01	2.08	0.2	8.4	103.5
Zucapsaicin					
Within-run	45.4	48.2	0.4	0.8	106.2
(n=7)	8.79	9.06	0.3	3.5	103.0
	2.03	1.92	0.07	3.5	94.6
Between-run					
(n=5)	45.4	47.0	1.9	4.1	103.6
	8.79	8.94	0.3	3.6	101.7
	2.03	2.09	0.1	6.4	102.9

accurate and reproducible method for the determination of capsaicin and/or zucapsaicin (civamide) in serum.

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