Capsaicin and Dihydrocapsaicin

Method number	PV2177
Control number:	T-PV2177-01-9408-CH
Target concentration:	1 mg/m^3
Procedure:	Samples are collected by drawing a known volume of air through a 37-mm Glass Fiber Filter (GFF). The samples are extracted with methanol for 30 minutes on a shaker and then analyzed by high performance liquid chromatography (HPLC) using an ultraviolet (UV) detector.
Recommended air volume and sampling rate:	100 minutes at 1 L/min (100 L)
Detection limit of the overall procedure:	0.01 mg/m^3
Status of method:	Partially validated. This method has been subjected to the established evaluation procedures and is presented for information and trial use.

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Mary Eide

Organic Service Branch 1
OSHA Salt Lake Technical Center
Salt Lake City UT

1 General Discussion

1.1 Background

1.1.1 History

The OSHA Salt Lake Technical Center received requests for a sampling procedure for air borne capsaicin, and had received bulk samples requesting analysis for capsaicin. Attempts to analyze bulks by GC-Mass Spec, using various capillary columns, were not successful. Analysis using a direct insertion probe on Mass Spec did detect the capsaicin. Based on the difficulties encountered in getting capsaicin through a gas chromatography column in the analysis by Mass Spec, analysis by liquid chromatography was tried. The analytical standard, obtained from Sigma Aldrich, was a mixture of capsaicin and dihydrocapsaicin. For our purposes, we are assuming equal response factors. Both the capsaicin and dihydrocapsaicin occur in plants together, and have similar irritating properties and toxicology. For this study, it was assumed that both would occur together in the exposures encountered. Collection on glass fiber filters was found to have good extraction, retention, and storage properties. The target concentration for this study was 1 mg/m^3. The air volume was 100 liters and the sampling rate was 1.0 L/min. The reliable quantitation limit was 0.01 mg/m^3.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.) (Ref. 5.1)

Capsaicin and dihydrocapsaicin are severe eye, respiratory, skin, and mucous membrane irritants. Large doses of capsaicin can cause muscle spasms, vomiting, diarrhea, and respiratory distress. In mice, oral doses of 3318 mg/kg for 5 weeks caused gastrointestinal tumors. In humans, the effects of being sprayed in the eyes with pepper mace, in which capsaicin and dihydrocapsaicin are the active ingredients, include massive production of tears, swelling of the eyelids, and reddening of the eyes, and can last up to eight hours.

1.1.3 Workplace exposure (Ref. 5.2)

Capsaicin and dihydrocapsaicin are naturally occurring products of pepper plants; they are among the ingredients in peppers which supply the "heat" in hot peppers. Workers in the food industry, such as plants making pepper sauces and Mexican sauces, may be exposed during the pepper chopping or grinding operations. A mixture of both compounds is used as an animal repellant. Capsaicin and dihydrocapsaicin are the major active ingredients in pepper mace, a personal protective spray.

1.1.4 Capsaicin

Physical properties and other descriptive information (Ref. 5.3)

<u>Capsaicin</u>

Synonyms:	Capsaicine; Capsicum; trans-8-Methyl-N-vanillyl-6-nonenamide; (E)-N-[(4-Hydroxy-3-methoxyphenyl)-methyl]-8-methyl-6-nonenamide
CAS #:	404-86-4
RTECS:	RA8530000
IMIS:	R206
Molecular weight:	305.42
Boiling point:	210-220 ^{0}C
Melting point:	65 ^{0}C

Odor:	pungent burning
Color:	white crystals
Molecular formula:	$C_{18}H_{27}N0_3$
Structural formula:	

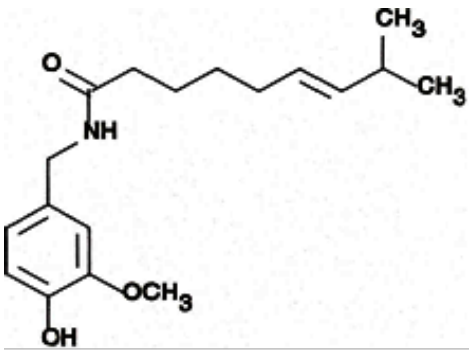


Figure 1. The structure of capsaicin.

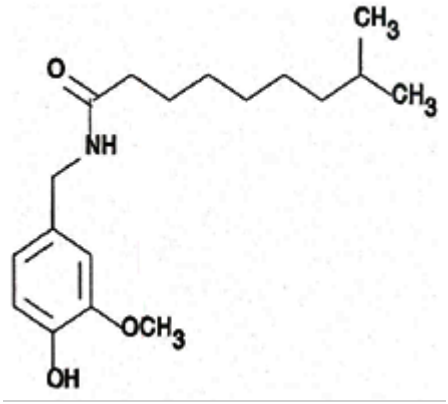


Figure 2. The structure of dihydrocapsaicin.

Dihydrocapsaicin

Synonyms:	Dihydrocapsaicine
Molecular weight:	307.42
Boiling point:	215-220 ^{0}C
Melting point:	65 ^{0}C
Odor:	pungent burning
Color:	white crystals
Molecular formula:	$C_{18}H_{29}N0_3$

The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations listed in ppm are reference to 25 °C and 101.3 kPa (760 mmHg)

1.2 Limit defining parameters

1.2.1 Detection limit of the overall procedure (DLOP)

The detection limit of the overall procedure is 1 µg per sample (0.01 mg/m^3). This is the amount of analyte spiked on the sampler that will give a response that is significantly different from the background response of a sampler blank.

1.2.2 Reliable quantitation limit (RQL)

The reliable quantitation limit is 1 µg per sample (0.01 mg/m^3). This is the amount of analyte spiked on a sampler that will give a signal that is considered the lower limit for precise quantitative measurements.

2 Sampling Procedure

2.1 Apparatus

2.1.1 Samples are collected using a personal sampling pump calibrated, with the sampling

device attached, to within ±5% of the recommended flow rate.

2.1.2 Samples are collected on 37-mm diameter glass fiber filters, type A/E. These are placed into two-piece cassettes.

2.2 Technique

2.2.1 Immediately before sampling, remove the caps from the cassette.

2.2.2 Attach the cassette to the pump with flexible tubing.

2.2.3 Air being sampled should not pass through any hose or tubing before entering the cassette.

2.2.4 Attach the cassette, in the worker's breathing zone, and positioned so it does not impede work performance or safety.

2.2.5 After sampling for the appropriate time, remove the sample and replace the end caps. Wrap each sample end-to-end with a Form OSHA-21 seal.

2.2.6 Submit at least one blank sample with each set of samples. Handle the blank sampler in the same manner as the other samples except draw no air through it.

2.2.7 Record sample volumes (in liters of air) for each sample, along with any potential interference.

2.2.8 Ship any bulk samples separate from the air samples.

2.2.9 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples in a refrigerator.

2.3 Extraction efficiency

The extraction efficiencies of capsaicin and dihydrocapsaicin were determined by liquid spiking the glass fiber filters with the analytes at 0.1 to 1 times the target concentration. The loadings on the filters were 10, 50, and 100-µg total of capsaicin and dihydrocapsaicin, or 6.5, 32.5, and 65-µg capsaicin and 3.5, 17.5, and 35 µg of dihydrocapsaicin. These samples were stored overnight at ambient temperature, then extracted, and analyzed. The average extraction efficiency over the studied range was 99.2% for capsaicin, and 98.9% for dihydrocapsaicin.

Capsaicin
Extraction Efficiency

filter #	% recovered		
	6.5 µg	32.5 µg	65 µg
1	100	100	98.7
2	100	99.2	98.4
3	99.4	96.8	98.3
4	99.8	99.4	99.0
5	100	99.5	98.8
6	99.0	99.7	99.1
average	99.7	99.1	98.7

overall average = 99.2%
standard deviation = ±0.805

Dihydrocapsaicin
Extraction Efficiency

filter #	% recovered		
	3.5 µg	17.5 µg	35 µg
1	98.6	99.2	97.9
2	98.8	99.2	98.5
3	96.8	97.6	97.9
4	99.1	99.9	98.8
5	99.8	99.6	98.8
6	99.9	99.7	99.3
average	98.8	99.2	98.5

overall average = 98.9%
standard deviation = ±0.852

2.4 Retention efficiency

The glass fiber filters were spiked with 100 µg (1 mg/m^3) total (65 µg capsaicin and 35 µg dihydrocapsaicin), and allowed to equilibrate overnight. They were placed in series with a new glass fiber filter, and had 100 L humid air (87 % RH at 22 ^{0}C) pulled through them at 1 Lpm. They were opened, extracted, and analyzed by HPLC-UV. The retention efficiency averaged 98.4% for capsaicin and 99.4% for dihydrocapsaicin. There was no capsaicin or dihydrocapsaicin found on the backup filters.

Table 2.4
Capsaicin and Dihydrocapsaicin
Retention Study 100 L @ 1 Lpm 87% RH @ 22 ^{0}C

tube #	% recovered capsaicin			tube #	% recovered dihydrocapsaicin		
	front filter	backup filter	total		front filter	backup filter	total
1	98.6	0.0	98.6	1	100	0.0	100
2	97.9	0.0	97.9	2	98.5	0.0	98.5
3	98.3	0.0	98.3	3	100	0.0	100
4	98.2	0.0	98.2	4	99.2	0.0	99.2
5	98.8	0.0	98.8	5	99.8	0.0	99.8
6	98.5	0.0	98.5	6	98.8	0.0	98.8
	average = 98.4%				average = 99.4%		

2.5 Sample storage

Six glass fiber filters were each spiked with 100-µg total (65-µg capsaicin and 35-µg dihydrocapsaicin). Six more filters had 100 liters of humid air (90% RH at 24 ^{0}C) drawn through them before they were spiked with 100 µg total (65 µg capsaicin and 35 µg dihydrocapsaicin). They were sealed and stored at room temperature. Three samples of each type were analyzed after 7 days and the remaining three samples of each type after 14 days. The amounts recovered, corrected for extraction efficiency, indicate that there was no effect on storage by humidity or time, and had an average recovery of 98.5% (humid) and 99.2% (dry) for capsaicin and 98.0% (humid) and 99.1 % (dry) for dihydrocapsaicin.

Table 2.5
Capsaicin and Dihydrocapsaicin
Storage Study 100 L @ 1 Lpm 90% RH @ 24 ^{0}C

time (days)	% recovered capsaicin		time (days)	% recovered dihydrocapsaicin	
	humid	dry		humid	dry
7	98.3	99.0	7	98.7	98.6
7	100	99.0	7	96.7	99.6
7	98.9	99.4	7	98.6	99.8
14	98.3	100	14	98.3	99.7
14	98.0	99.3	14	97.5	98.2
14	97.2	98.3	14	98.0	98.8
ave	98.5%	99.2%	ave	98.0%	99.1%

2.6 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 100 L air samples should be collected at a sampling rate of 1.0 L/min.

2.7 Interferences (sampling)

2.7.1 It is not known if any compounds will severely interfere with the collection of capsaicin and dihydrocapsaicin on the glass fiber filter. In general, the presence of other contaminant particulates in the air will reduce the flow though the filter affecting the ability of the glass fiber filters to collect capsaicin and dihydrocapsaicin.

2.7.2 Suspected interferences should be reported to the laboratory with submitted samples.

2.8 Safety precautions (sampling)

2.8.1 The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.8.2 All safety practices that apply to the work area being sampled should be followed.

3 Analytical Procedure

3.1 Apparatus

3.1.1 The instrument used in this study was a liquid chromatograph equipped with an ultraviolet detector, specifically a Waters 600E controller, 490E ultraviolet detector, and 717 autosampler.

3.1.2 An LC column capable of separating the analyte from any interference. The column used in this study was a Supelco 25-cm x 4.6-mm i.d. with 5 µm LC-8-DB.

3.1.3 An electronic integrator or some suitable method of measuring peak areas.

3.1.4 Four milliliter vials with PTFE-lined caps.

3.1.5 A 100-µL syringe or other convenient size for sample injection.

3.1.6 Pipets for dispensing the extracting solvent. A Repipet® dispenser was used in this study.

3.1.7 Volumetric flasks, 10-mL, and other convenient sizes for preparing standards.

3.2 Reagents

3.2.1 Capsaicin, mixture of 65% capsaicin and 35% dihydrocapsaicin from Sigma Aldrich

3.2.2 Deionized Millipore water

3.2.3 Methanol, HPLC grade

3.2.5 The mobile phase was 0.1:20:80 of H_3P0_4:methanol:water

3.3 Standard preparation

3.3.1 At least two separate stock standards are prepared by diluting a known quantity of capsaicin and dihydrocapsaicin with methanol. The concentration of these stock standards was 650 µg/mL capsaicin and 350 µg/mL dihydrocapsaicin.

3.3.2 Dilutions of these stock standards were prepared to bracket the samples. The range of the standards used in this study was from 0.65 to 65 µg/mL capsaicin and 0.35 to 35 µg/mL dihydrocapsaicin.

3.4 Sample preparation

3.4.1 Sample cassettes are opened and the glass fiber filter is placed in a 4-mL vial.

3.4.2 The filter is extracted with 3 mL of methanol.

3.4.3 The vials are sealed immediately and allowed to extract for 30 minutes with constant shaking.

3.5 Analysis

3.5.1 Liquid chromatography conditions.

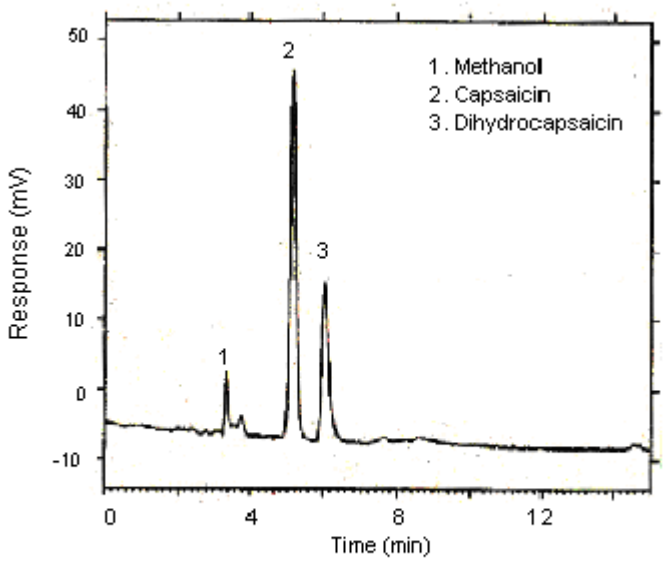


Figure 3.5 A chromatogram of 65 ug/mL Capsaicin and 35 ug/mL Dihydrocapsaicin in methanol.

Injection size:	10 µL
Column:	Supelco 25-cm x 4.6-mm i.d. with 5 µm LC-8-DB
Mobile phase:	1 mL/min of 0.1:20:80 of H_3P0_4:methanol:water
Detector:	UV at 280 and 220-nm

3.5.2 Peak areas are measured by an integrator or other suitable means.

3.6 Interferences (analytical)

3.6.1 Any compound that produces a response and has a similar retention time as the analyte is a potential interference. If any potential interferences are reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate interference from the analyte.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by mass spectrometry or by another analytical procedure.

3.7 Calculations

3.7.1 The calibration curve was made from at least four standards at different concentrations bracketing the samples.

3.7.2 The values for the air samples and blanks are obtained from the calibration curve.

3.7.3 Values (µg) obtained for blanks are subtracted from the air samples.

3.7.4 To calculate the concentration of analyte in the air sample, the following formulas are used:

$$mass\ of\ analyte,\ \mu g = \frac{(\mu g / mL)(desorption\ volume,\ mL)}{(desorption\ efficiency,\ decimal)}$$

$$moles\ of\ analyte = \frac{(mass\ of\ analyte,\ \mu g)(1\ g)}{(molecular\ weight)(10^6\ \mu g)}$$

$$volume\ of\ analyte = (moles\ of\ analyte)(molar\ volume)$$

$$ppm = \frac{(volume\ of\ analyte)(10^6)}{(air\ volume,\ L)}*$$

* All units must cancel.

$$ppm = \frac{(\mu g / mL)(DV)(24.46)}{(100\ L)(DE)(MW)}$$

Where:

µg/mL = concentration of analyte in sample
24.46 = Molar volume (liters/mole) at 25 °C and 760 mmHg
MW = Molecular weight (g/mole)
DV = Desorption volume, 3 mL
100 L = Air volume, L
DE = Desorption efficiency, decimal

3.7.5 The above equations can be consolidated to form the following formula. To calculate the mg/m^3 of analyte in the sample based on a 100-liter air volume:

$$mg/m^3 = \frac{(\mu g/mL,\ blank\ corrected)(desorption\ volume,\ mL)}{(air\ volume,\ L)(desorption\ efficiency,\ decimal)}$$

3.7.6 This calculation is done for each filter.

3.8 Safety precautions

3.8.1 Avoid skin contact and inhalation of all chemicals.

3.8.2 Wear safety glasses, gloves and a lab coat at all times while in the laboratory areas.

4 Recommendations for Further Study

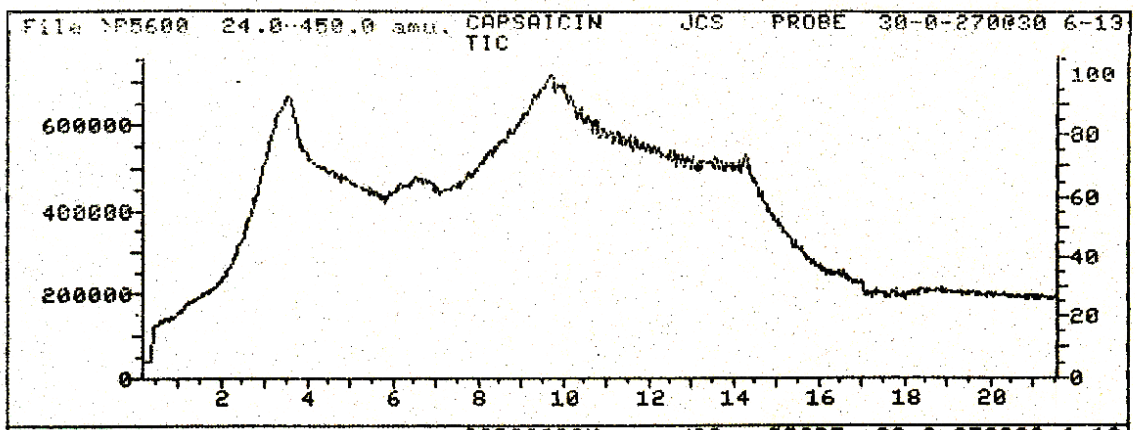
Collection studies need to be performed. Method needs to have validation completed.

5 References

5.1 Sweet, D., "Registry of Toxic Effects of Chemical Substances," 1994 Edition, U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, NIOSH, CD-ROM, 1994, Vol. A94-1.

5.2 Grayson, M., "Kirk Othmer Encyclopedia of Chemical Technology", Third Edition, John Wiley & Son, N.Y., 1981, Vol. 10, p. 795, Vol. 11 p. 210, and Supplement, p. 802.

5.3 Windholz, M., "The Merck Index," Tenth Edition, Merck & Cp., Rahway N.J., 1983, p. 243.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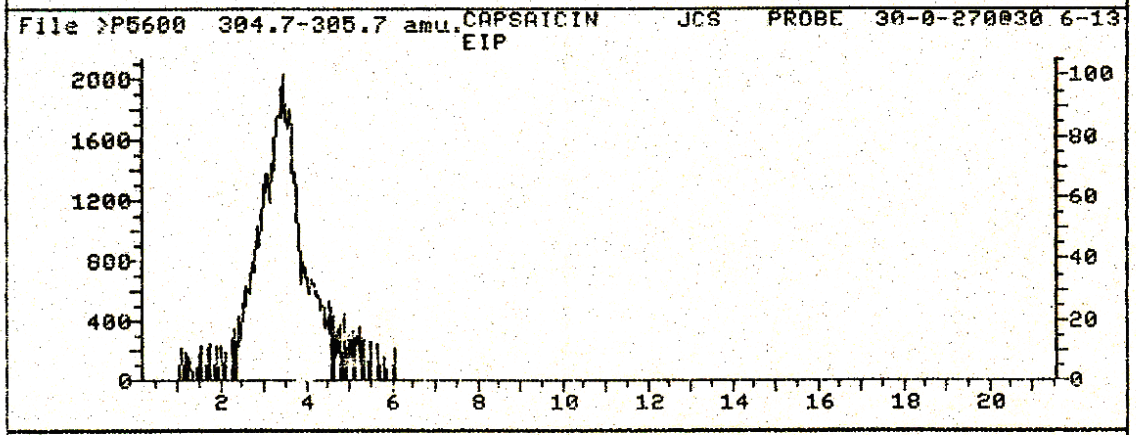
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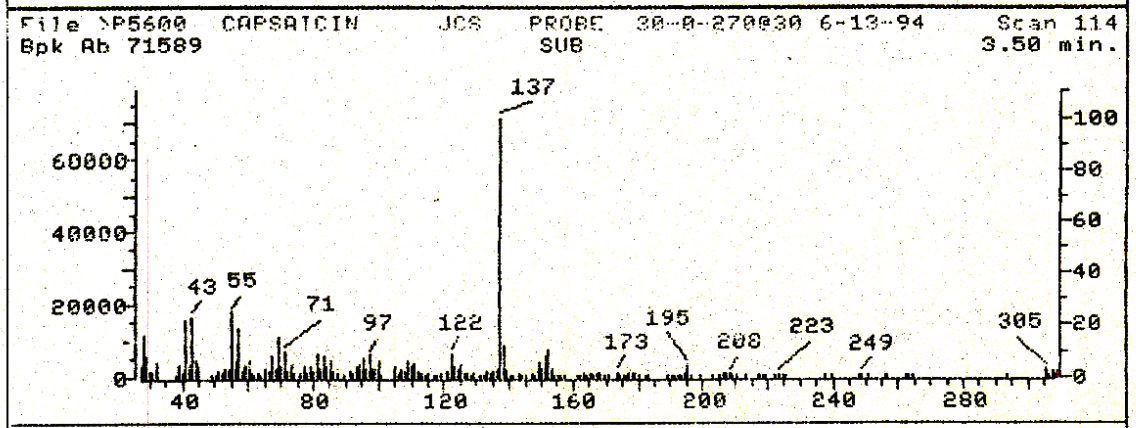
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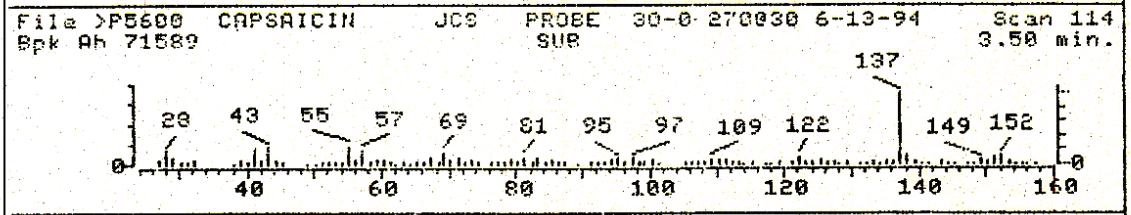
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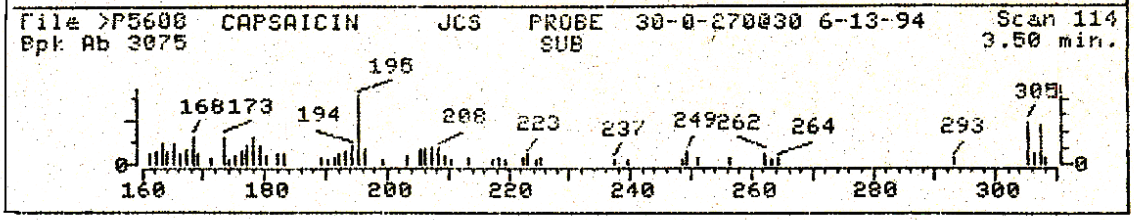
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