International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue: 6 2014 Pages:184-193
(pISSN: 2348-5213; eISSN: 2348-5221)

RESEARCH ARTICLE

# QUALITATIVE ANALYSIS OF CAPSAICIN FROM CHILLIES AND CHILLI POWDER BY H.P.L.C METHOD.

**P. SRIDHARA BABU[1], M.GURAVAIAH[2*], I. HATTI[3] AND K.SRIKANTH[4]**
[1]Department of Chemistry, J.K.C College, Guntur-6, Andhra Pradesh, India
[2]Department of Biotechnology(P.G), J.K.C College ,Guntur-6,Andhra Pradesh, India.
[3]Department of Chemistry, J.K.C College, Guntur-6, Andhra Pradesh, Indi .
[4]Department of Chemistry, Vivek Vardhini day college, Hyderabad, Telangana state, India
*Corresponding author: *gurubt1980@gmail.com*

**Abstract**

Capsaicinoids are the compounds that are responsible for the pungency, aroma and flavor of the hot chili peppers. Chilli peppers are generally known as ripen fruits of various species of genus capsicum. They play an important role as one of the most commercial crops used both as condiment or culinary supplement and as vegetable. Capsaicinoids are soluble in moderate polar organic solvents e.g. chloroform, acetone, ethyl acetate, methylene chloride, methanol, ethanol, acetonitrile, and among others (Duarte *et al*., 2004; Santamaria *et al.*, 2000). There are five levels of pungency classified using Scoville heat units (SHU): non-pungent (0–700 SHU), mildly pungent (700–3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU) and very highly pungent (>80,000 SHU). The regarding with HPLC as mentioned above it is currently the most popular and reliable technique for the analysis of capsaicinoids. Capsaicin has been recognized as anti-cancer agent for many years (Surh, 2002).
.

**Keywords:** Capsaicinoids, Chilli, Scoville heat units (SHU), HPLC and anti-cancer.

## 1. Introduction

Capsaicinoids are the compounds that are responsible for the pungency, aroma and flavor of the hot chili peppers. Capsaicin is the most abundant capsaicinoid found in chili peppers. There are many varieties of capsaicinoids like nordihydrocapsaicin, hydrocapsaicin, homocapsaicin, nornorcapsaicin, nornornorcapsaicin, nonivamide (Barbero *et al.,* 2006). 90% of the total capsaicinoids are capsaicin and dihydrocapsaicin and capsaicin makes about 71% in total (Ravishankar *et al*., 2003; geandFuruta, 1970). The commercial quality of hot peppers is solely determined by amount of capsaicin (hotness) present in them (Jarret *et al*., 2007). There concentrations in different capsaicin fruits is regulated by factors such as light intensity, age of fruit and plant's growing temperature.

**Synthesis of Mannich base, PBN**

Benzaldehyde, piperazine and nicodinamide were taken in 1:1:1 mole ratio. Piperazine 0.9mL (10mM), nicodinamide 1.22g (10mM) and then 1mL of benzaldehyde (10mM) was added and kept under microwave for 2min. As usual workup followed by purification under thin layer chromatography gave the compound, PBN. Yield: 79%., m.p. 169$^0$C.

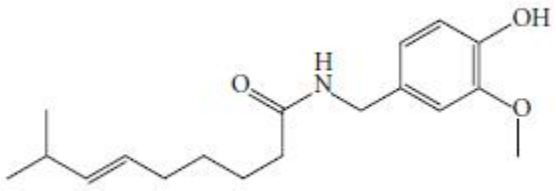



FIGURE 1: Molecular structure of capsaicin.

**Synthesis of Mannich base, PBB**

Benzaldehyde, piperazine and benzamide were taken in 1:1:1 mole ratio. Piperazine 0.9mL (10mM), benzamides 1.2g (10mM) and then 1mL of benzaldehyde (10mM) was added and kept under microwave for 3min. As usual workup followed by purification under thin layer chromatography gave the compound, PBB. Yield: 79%., m.p. 167$^0$C. All the compounds (PBA, PBN, and PBB) gave satisfactory spectral data like IR and 1H-NMR.

Chilli peppers are generally known as ripen fruits of various species of genus capsicum. They play an important role as one of the most commercial crops used both as condiment or culinary supplement and as vegetable. Commonly, their hot sensory taste is due to capsaicinoids as the major group of organic compounds which is closely related to the family of alkaloids, and are known to be biosynthesized and accumulated in the placenta of Capsicum fruits (Pruthi, 1976; Tapia *et al*., 1993; Prasad *et al*., 2006). Capsaicinoids are soluble in moderate polar organic solvents e.g. chloroform, acetone, ethyl acetate, methylene chloride, methanol, ethanol, acetonitrile, and among others (Duarte *et al*., 2004; Santamaria *et al.*, 2000). The major capsaicinoids present in most varieties of the chilli pepper are capsaicin (tran-8-methyl-N-vanillyl-6-nonenamide)and dihydrocapsaicin (8-methyl-N-vanillylnonanamide).

The levels of capsaicin in tip and ovaries are high and in seeds its concentration is low (Supalkavo *et al*., 2007). 90% of the capsaicinoids are produced in placenta portion. Seeds are not source of capsaicinoids but absorb them from placenta (Andrews, 1984).

The determination of capsaicinoids in chilli peppers, topical cream (Kaale *et al*., 2002), self-defense weapons (Reilly *et al*., 2001a) and aerosol defense sprays (Spicer and Almirall, 2005) has been of increasing interest for many reasons. Extraction methods of the capsaicinoids from chilli pepper sample have been conducted using various extraction techniques including liquid-liquid extraction (LLE) (Tapia *et al*., 1993; Kaale *et al*., 2002; Spicer and Almirall, 2005), enzymatic extraction (Santamaria *et al.*, 2000), supercritical fluid extraction (SFE) (Sato *et al*., 1999; Gnayfeed *et al*., 2001; Kaale *et al.*, 2002; Duarte *et al.,* 2004; Uzunalic *et al*., 2004), pressurized liquid extraction (PLE) (Barbero *et al.,* 2006a), magnetic stirring extraction (MSE) (Kaale *et al*., 2002), solid-phase microextraction (SPME) (Spicer and Almirall, 2005), reflux heating (Peusch *et al.*, 1997), microwave assisted extraction (MAE) (Barbero *et al*., 2006b), maceration (Titze et al., 2002), Soxhlet extraction (Korel *et al.*, 2002) and ultrasonic assisted extraction (UAE) (Karnka et al., 2002). The extraction of capsaicinoids in Capsicum plants is, in fact, influenced by their chemical nature, extraction method, sample particle size, storage time as well as the presence of interfering substances. Thus, the extracts of the plants are always a mixture of different classes of organic compounds that are soluble in the solvents used. Methanol, ethanol, propanol, acetone, ethyl acetate, n-butyl chloride, acetonitrile, dichloromethane, hexane and their combinations are frequently used for the extraction of capsaicinoids. Peusch *et al*. (1997) reported that SFE of capsaicinoids in some pepper samples led to a six-fold higher extraction yield compared to organic solvent extraction using various solvents. However, SFE is cost effective equipment and is not common in routine laboratories. Besides, an ordinary extraction method is still adopted with relative ease to carry out by using magnetic stirring solvent system (Kaale *et al.*, 2002). But it takes time, and then the extract containing the analyte is subjected for centrifugation and or filtration.

Previously, determination of total capsaicinoids have relied on direct measurement of ultraviolet absorption or colorimetric method using Folin-Ciocalteu reagent by UV-Visible spectrometry (Bajaj and Kaur, 1979; Perucka and Oleszek, 2000).

The regarding with HPLC as mentioned above it is currently the most popular and reliable technique for the analysis of capsaicinoids. The technique has been mainly associated with UV absorption detection (Hoffman *et al*., 1983; Betts, 1999; Perucka and Oleszek, 2000; Santamaria *et al.*, 2000; Kaale *et al.*, 2002; Karnka *et al*., 2002; Kim *et al.*, 2002; Korel *et al*., 2002; Kurian and Starks, 2002; Duarte *et al.,* 2004; Materska and Perucka, 2005; Lui *et al.*, 2007), fluorescence detection (Gnayfeed *et al.*, 2001; Titze *et al*., 2002; Barbero *et al*., 2006a), photodiode array (PDA) (Cooper *et al*., 1991; Constant *et al*.,1995; Padilla and Yahia, 1998; Estrada *et al*., 1999 & 2002) and or coupled with fluorescence detection (Cooper *et al*., 1991; Peusch *et al*., 1997; Uzumalic *et al*., 2004; Barbero *et al.,* 2006b).

The amount of capsaicin in a given variety can vary depending on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit on the plant. The first test developed to measure pungency was the Scoville test, first developed in 1912 by Wilbur Scoville. There are five levels of pungency classified using Scoville heat units (SHU): non-pungent (0–700 SHU), mildly pungent (700–3,000 SHU), moderately pungent (3,000–25,000 SHU), highly pungent (25,000–70,000 SHU) and very highly pungent (>80,000 SHU)

### Anticancer activity:-

Capsaicin has been recognized as anti-cancer agent for many years (Surh, 2002). In an experiment capsaicin was seen to hinder the movement of breast cancer cells and to retard growth of prostate cancer cells. Also, in this experiment, dihydrocapsaicin was reported to kill HCT116 human cancer cells (Oh *et al*., 2008; Thoennissen et al., 2010; Yang et al., 2010). Another study showed that leukemic cell's growth can be retarded by natural capsaicin (Ito *et al*., 2004). Capsaicin attacks the abnormally dividing cells and hinders the proliferation of these malignant cells by cycle arrest, apoptosis, autophagy or through deactivating the cellular metabolism (Choi *et al*., 2010; Ghosh and Basu, 2010 and Thoennissen *et al*., 2010). Isoform of enzyme cytochrome P450 is inhibited by capsaicin. This enzyme detoxifies many low molecular weight carcinogens (Singh *et al*., 2001). Capsaicin identifies malignant cell lines and specifically attaches the immortal dividing cancerous cells and retards their growth (Kim and Moon, 2004). Studies show that cancer mutation in the DNA can be caused by the activity of metabolites of capsaicin (Baez *et al*., 2010).

## 2. Materials and Methods

### 2.1 Collection samples:-

A total of 12 Chilies samples were collected from Agricultural market yard, Guntur and chili powder were collected from Sri Lakshmi Ganapathi Chilli powder Industries, Guntur. samples were collected between January 2014 to April 2014. These samples were selected randomly and purchased in amounts greater than 0.5 kg. The samples were maintained at $04^0$ C until arrival at the laboratory, where all samples were ground into powder and stored in plastic bags at $4^0$C until the onset of the analysis. The collected samples were different varieties namely 4884 , 273, 341, Teja Talu, Dabhi, Teja, 248, 334, Medium Teja Talu, Byadgi, 243 and 341 Talu

### 2.2 Method for measuring Capsaicin in chillies and chilli powder:- (ASTA ANALYTICAL MANUAL METHOD No. 21.3)

### A. Apparatus

1. Standard flasks, 50 mL,100 mL & 200 mL capacity
2. Heating mantle of 500 mL capacity, with regulator
3. HPLC system with accessories as mentioned under Instrument conditions
4. Micro litre syringe capable of injecting 1 - 20 µL.
5. Pipette, 10 mL
6. Balance, readable to 0.01 g.
7. Glass beads.
8. Boiling flask, 500 mL.
9. Whatman No. 1 filter paper (90 mm)/ syringe filter 0.45 µm.
10. Sample powdering mill or equivalent
11. Water cooled condensor.

**B. Reagents:**

1. Rectified spirit, (chromatographically pure): Whenever fresh batch is purchased check for purity by gas chromatography and if necessary distil the same before use for analysis.
2. Acetonitrile HPLC grade
3. Water HPLC grade
4. Acetic acid HPLC grade.
5. Acetone -HPLC grade.
6. Standard - N - Vanillyl - n - nonanamide, 97% (Sigma or equivalent).

**Standard stock solution** - Weigh accurately 0.1 g of the above standard and dissolve and make upto 100 mL with rectified spirit. Keep this solution as stock solution (1000 ppm) in standard flask wrapped in black cover. Shelf life is one year under refrigeration.

**Working standard 100 ppm** - From the stock solution pipette 10 mL to the 100 mL standard flask and make up to the mark with rectified spirit. Keep under refrigeration in standard flask wrapped in black cover. Shelf life is six months under refrigeration.

**C. Procedure :**

**Sample preparation :**

**Whole chillies:** After mixing & quartering, powder 100 g of the sample and pass Through the sieve ASTM No. 20. (850 m).

**Entire sample:-**

1. Weigh accurately 25.0 g of the above sample in duplicate into 500 mL boiling flask.
2. Add 200 mL rectified spirit and then several glass beads to aid boiling.
3. Reflux gently for 5 hours.
4. Allow to cool and then filter 3 to 4 mL through whatman no.1 filter paper pre- wetted with rectified spirit or 0.45µm syringe filter into stoppered test tubes.

**Oleoresins:** Accurately weigh 1 to 2 g oleoresin (increase sample size if total Cpsaicinoid concentration is below 1%) into 50 mL volumetric flask, being sure not to allow any

oleoresin to coat the sides of the flask. Add 5 mL of acetone to flask and swirl acetone until sample is completely dispersed as evidenced by observing no oleoresin coating bottom of flask with neck at 45° angle. Slowly add rectified spirit, mixing completely by swirling with acetone while adding. Continue adding and mixing until solution becomes cloudy. Dilute to volume and mix well. Filter 3 to 4 mL through Whatman no. 1 filter paper pre-wetted with rectified spirit or 0.45 µm syringe filter into stoppered test tubes.

**Instrument condition:**

HPLC System - Water -TM 600 system controller Waters TM 486 tunable absorbance detector

* Waters 746 Data module / Waters millennum 32 software
* Waters U6K injector / Rheodyne injector
*Waters 717 plus Autosampler.

HPLC Column - 5 µm Waters Symmetry TM C18 (4.6x250 mm) steel column

**Note :** Use either degassed eluent or sparge with Helium before run. All solvent should be HPLC grade. Eluent used - Freshly prepared 60 % acetonitrile + 40 % water with 1 % acetic acid at 0.8 mL / minute flow.

**Absorbance** - 280 nm

**Volume for injection** - 5 to 10 µL

1. Switch on the instrument.
2. Wait for system configuration .
3. Press the set up function key to display the pump set up screen.
4. Enable sparging if necessary (appropriate reservoirs A/B/C/D)
5. Press the direct function key and sparge helium at 100 mL/min. for first 20 mts then reduce flow to 30 mL / min. for acetonitrile + water system. (If helium is used for sparging
6. Vent the eluent by keeping the small glass bottle under the open vent tubing (injection port) to cut on the vented eluent.
7. Turn the load/ inject handle to the inject (left) position and then the sample loading plug handle to the open ( vertical) position to open the vent port.
8. From the direct function key at the % A field, type a value of 100 to set the flow to 100 % for eluent reservoir A.
9. Increase the flow by 0.1 mL / min upto 0.8 mL / min .

10. Attach the priming syringe, open the eluent drain - off valve (turn the knurled knob counter clockwise) and draw off 10 mL of eluent.
11. Repeat the above step, if air bubble is trapped in the delivery system (tube).
12. Close the eluent draw - off valve and remove the primary syringe keep an empty bottle at the eluent outlet waste tube.
13. Keep the flow of eluent (0.8 mL / min) through the HPLC column
14. When the pressure indicated in direct control screen becomes steady, the system is ready for injection.
15. Load the sample ( 5 to 10 µL) into waters U6K manual injector or rheodyne injector (with 5 or 20 µL sample loops), using micro litre syringe after rinsing in pure rectified spirit not less than 10 times.
16. Turn the load / inject handle to the load (right) position and turn the sample loading plug handle to the open (vertical) position.
17. Remove the sample loading plug.
18. Insert the syringe with sample / standard into the sample loading port until the syringe bottoms and empty the syringe file the loop.
19. Remove the syringe, replace the sample loading plug and turn the sample loading plug handle to the closed (horizontal) position.
20. Chromatograms are stored in respective BINS of data module.
21. Make a file with run time parametrs.
22. Reprocess the chromatograms of samples and take the report. (Calculation is made based on area of standard capsaicin.)
23. Run the system in pure acetonitrile at least half an hour before switching off the system. Always prime the system when eluent is changed.
24. Switch off the detector, system controller at the unit and then main power of the above. Keep the plastic plunger to the eluent draw off valve.
25. Switch off the data module at the main power.
26. Discard the eluent from the waste bottle.
27. Change the prepared eluent from the
28. reservoirs only when the system is used next time.

* **Autosampler** :-The autosampler is switched on and the sample extracts taken in the vials are loaded in the carousel. In the Millenium32 software, the system is selected and the functions to be carried out are entered in samples set of the run mode.

**D. Calculations :**

1.Scoville Heat Units (SHU) are the sum of SHU of three major capsaicinoids.
Calculate SHU as follows:

a) Nordihydrocapsaicin, SHU = (N/A) x (Cs/WX) x (HN/RN).
b) Capsaicin, SHU = (C/A) x (Cs/WX) x (HC/RC).
c) Dihydrocapsaicin, SHU = (D/A) x (Cs/WX) x (HD/RD).

Where: A = average peak area of standard;
N, C, and D = average peak areas for respective capsaicinoids from duplicate injections;
Cs = concentration of std in mg/mL;
WX = wt of sample in mg/mL;
HN, HC, and HD = heat factors for respective capsaicinoids;
RN, RC, and RD = response factors of respective capsaicinoids relative to standard.

2.Accepted heat factors and response factors:

| | | |
|---|---|---|
| Nordihydrocapsaicin | - HN = 9.3 E + 06; | RN = 0.98 |
| Capsaicin | - HC = 16.0 E + 06; | RC = 0.89 |
| Dihydrocapsaicin | - HD = 16.0 E + 06; | RD = 0.93 |

3. Relative retention times:

| | |
|---|---|
| Nordihydrocapsaicin | 0.90 |
| N-vanillyl-n-nonanamide | 1.00 |
| Capsaicin | 1.00 |
| Dihydrocapsaicin | 1.58 |

4. Capsaicin content in percentage is calculated as follows:

$$\text{Capsaicin content (\%)} = \frac{\text{Total SHU}}{16 \times 10000}$$

## E. Result and Reporting :

Result is given in SHU or in percentage as requested in laboratory information sheet.

During the reporting in scoville heat unit correct the value to the nearest hundred and for percentage, correct the value to 2 decimals.

## G. Environmental aspects:

1. N-Vanillyl-n-nonanamide is an extreme irritant - handle with care - Do not inhale.

## 3. Results and Discussion

The samples were collected from agricultural market yard and S.L.C, Guntur, India .The samples were shown very highly pungent and highly pungent.

**Table: 01 Collection of samples and content of pungent (Capsaicin) in Scoville Heat Unit (S.H.U)**

| S.NO | DATE | NAME OF VARIETY | RESULTS (IN S.H.U) |
|---|---|---|---|
| 1. | 04/01/2014 | 4884 (Chilies) | 74400 |
| 2 | 05/01/2014 | 273 (Chilies) | 95671 |
| 3 | 07/01/2014 | 341 (Chilies) | 64893 |
| 4 | 04/02/2014 | TEJA TALU (Chilies powder) | 53002 |
| 5 | 15/02/2014 | DABHI (Chilies powder) | 68907 |
| 6 | 17/02/2014 | TEJA (Chilies) | **99698** |
| 7 | 24/03/2014 | 248 (Chilies powder) | 76890 |
| 8 | 05/03/2014 | 334 (Chilies powder) | 76549 |
| 9 | 07/03/2014 | MEDIUM TEJA TALU (Chilies) | 56897 |
| 10 | 14/03/2014 | BYADGI (Chilies powder) | 85670 |
| 11 | 15/04/2014 | 243 (Chilies powder) | 73490 |
| 12 | 17/04/2014 | 341 TALU (Chilies) | **48762** |

## 3.2 Analysis of Capsaicin by HPLC Method:-

The HPLC was standee, after inject the blank (Acetonitrile). The acetonitrile was given only one peak. the peaks were analyzed with standard .

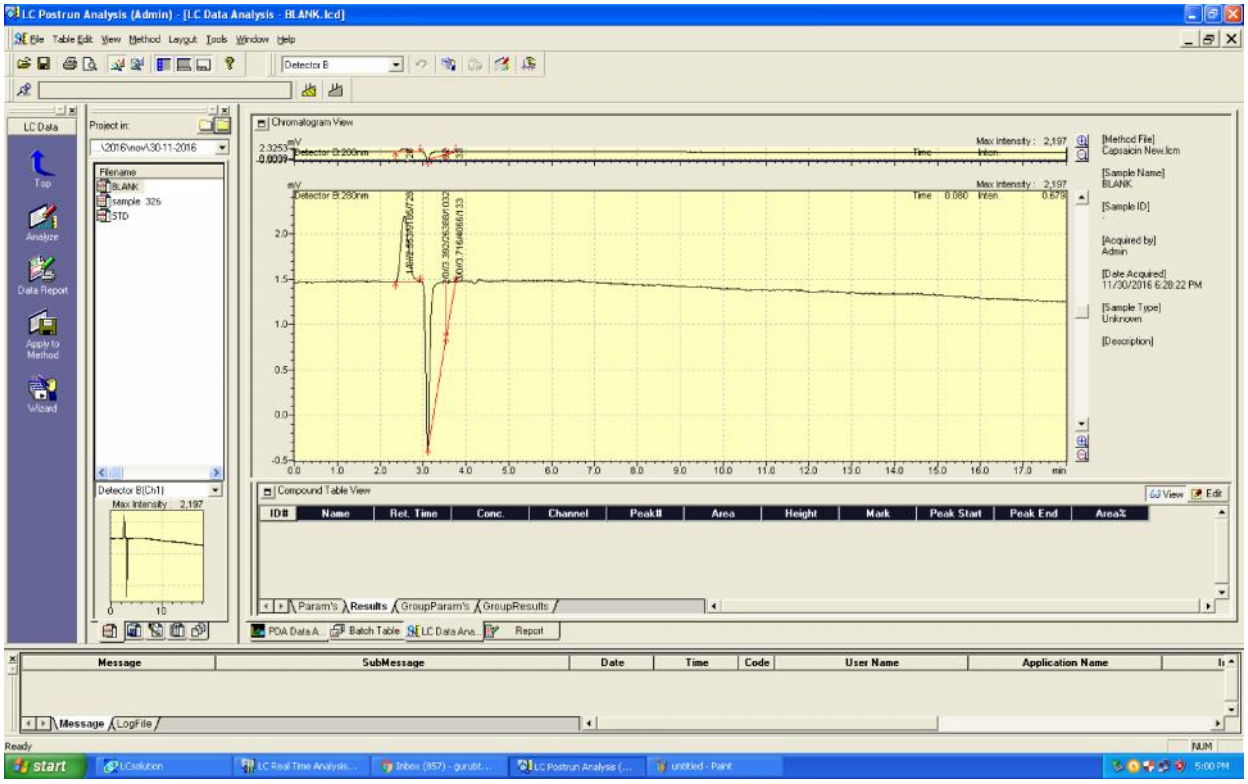
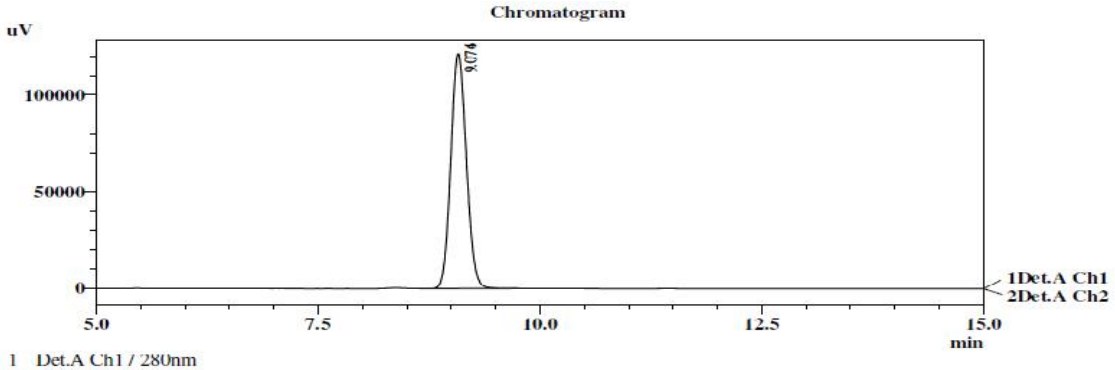



**Figure:-02 Blank chromatogram**

The blank (Acetonitrile) peaks were collected, after inject the standard .the standard were given only one peak (Figure:-03)




PeakTable

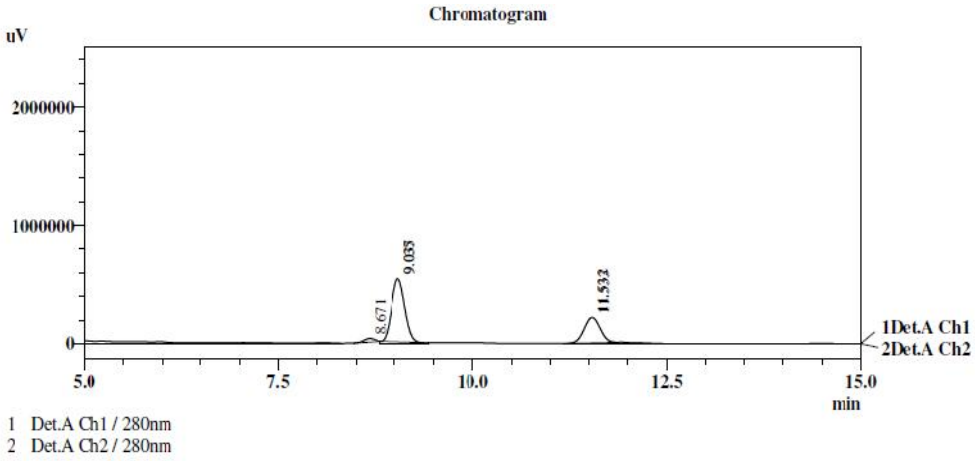
Detector A Ch1 280nm

| Peak# | Name | Ret. Time | Height | Area | Area % |
|---|---|---|---|---|---|
| 1 | NVNA | 9.074 | 121233 | 1479326 | 100.000 |
| Total | | | 121233 | 1479326 | 100.000 |

**Figure:-03 Capacin Standard Chromatogram**

The standard peaks were collected ,after inject the sample .the sample were given three peaks (Figure:-04)




PeakTable

Detector A Ch1 280nm

| Peak# | Name | Ret. Time | Height | Area | Area % |
|---|---|---|---|---|---|
| 1 | NHC | 8.671 | 30789 | 288602 | 2.988 |
| 2 | CAP | 9.035 | 535549 | 6362592 | 65.881 |
| 3 | DHC | 11.532 | 215574 | 3006489 | 31.131 |
| Total | | | 781912 | 9657682 | 100.000 |

**Figure:- 04 Sample Chromatogram**

In the present study Capsaicin concentration were based on pungent and moisture content .the Teja variety was given high pungent (**99698 S.H.U )** and 341 Talu were given low pungent (**48762 S.H.U).** Any Talu variety was given low pungent **so** the Talu verities were affected diseased **.**

Finally the exporters and house holders will purchase only dried red chillies .the dried chilles will not have any aflotoxins . if aflotoxins level is high it affect cancer.

## Conclusion

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for extraction of capsaicin from Chillies and chilli powder . The method development and validation process for the analysis of extraction of capsaicin from Chillies and chilli powder and its estimation by HPLC has been investigated in the study. The mobile phase is simple to prepare and it's economical. The optimized method showed appropriate retention time for the respective peaks and good system suitability. The parameters which are validated for the developed method offered satisfactory results within acceptable limits, which reveal that developed method is valid table, transferable, robust, reliable, accurate and precise.

## Acknowledgements

The author would like to thank Sri Lakshmi Ganapathi Quality Evaluation Laboratory, Guntur, India and Sri Lakshmi Ganapathi Industries, Guntur, India for providing Analysis work and given chilli powder samples.

## References

1. Andrews, J. 1984. Peppers: The Domesticated Capsicums, University of Texas Press, Austin, Texas.
2. Asta Analytical Manual Method No. 21.3
3. Bajaj, K. L. and Kaur, G. 1979. Colorimetric determination of capsaicin in Capsicum fruits with the Folin-Ciocalteu reagent. Mikrochimica Acta 1: 81-86.
4. Barbero, G. F., M. Palma and G. Barroso. 2006. Determination of capsaicinoids in peppers by microwave assisted extraction, high-performance liquid chromatography

with fluorescence detection, Anal. Chim.
5. Baez, S, Y. Tsuchiya , A. Calvo, M. Pruyas, ,K. Nakamura, C. Kiyohara, M. Oyama ,M. Yamamoto. 2010. Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women. World J. Gastroenterol. 16: 372–378.
6. Barbero, G. F., Palma, M. and Barroso, C. G. 2006b. Determination of capsaicinoids in peppers by microwave-assisted extraction-high performance liquid chromatography with fluorescence detection. Analytica Chimica Acta 5: 227-233.
7. Choi, C. H., Y. K. Jung, S. H. Oh. 2010. Selective induction of catalasemediated autophagy by dihydrocapsaicin in lung cell lines. Free Radic. Biol. Med. 49: 245–257
8. Constant, H. L., Cordell, G. A., West, D. P. and Johnson, J. H. 1995. Separation and quantification of capsaicinoids using complexation chromatography. Journal ofNatural Products 58: 1925-1928.
9. Duarte, C., Martins, M. M., Gouveia, A. F., da Costa, S. B., Leitao, A. E. and Gil, M. G. B. 2004. Supercritical fluid extraction of red pepper (*Capsicum frutescens L.*). Journal of Supercritical Fluids 30: 155-161.
10. Gnayfeed, M. H., Daood, H. G., Illes, V. and Biacs, P. A. 2001. Supercritical CO2 and subcritical propane extraction of pungent paprika and quantification of carotenoids, tocopherols, and capsaicinoids. Journal of Agricultural and Food Chemistry 49: 2761-2766.
11. Ghosh, A. K., S. Basu. 2010. Fas-associated factor 1 is a negative regulator in capsaicin induced cancer cell apoptosis. Cancer Lett. 287: 142–149.
12. Hoffman, P. G., Lego, M. C. and Galetto, W. G. 1983. Separation and quantitation of red pepper major heat principles by reverse-phase high-pressure liquid chromatography. Journal of Agricultural and Food Chemistry 31: 1326-1330.
13. Ito, K. T., Nakazato, K. Yamato, Y. Miyakawa, T. Yamada, N. Hozumi, K. Segawa, Y. Ikeda, M. Kizaki. 2004. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Res. 64: 1071–1078.

Acta, 578: 227-233.
14. Jarret R.L., E. Baldwin, B. Perkins, R. Bushway , K. Guthrie. 2007. Diversity of fruit quality characteristics in Capsicum frutescens. Hort. Sci. 42: 16–19.
15. Kaale, E., Schepdael, A. V., Roets, E. and Hoogmartens, J. 2002. Determination of capsaicinoids in topical cream by liquid-liquid extraction and liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 30:1331-1337
16. Karnka, R., Rayanakorn, M., Watanesk, S. and Vaneesorn, Y. 2002. Optimization of high-performance liquid chromatographic parameters for the determination of capsaicinoid compounds using the simplex method.Analytical Sciences 18:661-665.
17. Kim, S., A. Moon. 2004. Capsaicin-induced apoptosis of H-ras-transformed human breast epithelial cells is Rac-dependent via ROS generation. Arch. Pharm. Res. 27: 845–849.
18. Kim, S., Park, J. B. and Hwang, K. 2002. Quality attributes of various varieties of Korean red pepper powders (*Capsicum annuum L.*) and color stability during sunlight exposure. Journal of Food Sciences 67: 2957-2961.
19. Korel, F., Bagdatlioglu, N., Balaban, M. O. and Hisil, Y. 2002. Ground red peppers: capsaicinoids content, scoville scores, and discrimination by an electronic nose. Journal of Agricultural and Food Chemistry 50: 3257-3261.
20. Kurian, A. L. and Starks, A. N. 2002. HPLC analysis of capsaicinoids extracted from whole orange habanero chili peppers. Journal of Food Sciences 67: 956-962
21. Lui, X., Ardo, S., Bunning, M. Parry, J., Zhou, K., Stushnoff, C., Stoniker, F., Yu, L. and Kendall, P. 2007. Total phenolic content and DPPH• radical scavenging activity of lettuce (*Lactuca sativa L.*) grown in Colorado. LWT-Food Science and Technology 40: 552-557.
22. Oh, S. H., Y. S. Kim, S. C. Lim, Y. F. Hou, I. Y. Chang, H. J. You. 2008. Dihydrocapsaicin (DHC), a saturated structural analog of capsaicin, induces autophagy in human cancer cells in a catalase-regulated manner. Autophagy. 4: 1009–1019 .
23. Padilla, M. C. and Yahia, E. M. 1998. Changes in capsaicinoids during development, and senescence of chile

peppers and relation with peroxidase activity. Journal of Agricultural and Food Chemisyry 46: 2075-2079.

24. Perucka, I. and Oleszek, W. 2000. Extraction and determination of capsaicinoids in fruits of hot pepper *Capsicum annuum L*. by spectrophotometry and high performance liquid chromatography. Food Chemistry 71: 287-291
25. Peusch, M., Seitz, E. M., Muller, M. A. and Anklam, E. 1997. Extraction of capsaicinoids from chillies (*Capsicum fruitescens L*.) and paprika (*Capsicum annuum L.*) using supercritical fluids and organic solvents. Z Lebensm-Unters- Forsch A 204: 351-355.
26. Prasad, B. C. N., Gururaj, H. B., Kumar, V., Giridhar, P. and Ravishankar, G. A. 2006. Valine pathway is more crucial than phenyl propanoid pathway in regulating capsaicin biosynthesis in *Capsicum frutescens* mill. Journal of Agricultural and Food Chemisyry 54: 6660-6665.
27. Pruthi, J. S. 1976. Spices and Condiments. National Book Trust, New Delhi, India. pp.269
28. Ravishankar, G. A., B. Suresh, P. Giridhar, S. Ramachandra Rao, T. Sudhakar Johnson. 2003. Biotechnological studies on capsicum for metabolites production and plant improvement. Capsicums: 97-122.
29. Reilly, C., Crouch, D. and Yost, G. 2001b. Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products. Journal of Forensic Sciences 46(3): 502-509.
30. Santamaria, R. I., Reyes-Duarte, M. D., Barzana, E., Fernando, D., Gama, Moto, M. and Lopez-Munguim, A. 2000. Selective enzyme-mediated extraction of capsaicinoids and carotenoids from chili guajillo puya (*Capsicum annuum L*.) using ethanol as solvent. Journal of Agricultural and Food Chemistry 48: 3063-3067.
31. Santamaria, R. I., Reyes-Duarte, M. D., Barzana, E., Fernando, D., Gama, Moto, M. and Lopez-Munguim, A. 2000. Selective enzyme-mediated extraction of capsaicinoids and carotenoids from chili guajillo puya (*Capsicum annuum L*.) using ethanol as solvent. Journal of Agricultural and Food Chemistry 48: 3063-3067.
32. Sato, K., Sasaki, S. S., Goda, Y., Yamda, T., Nunomura, O., Ishikawa, K. and Maitani, T. 1999. Direct connection of supercritical fluid extraction and supercritical fluid chromatography as a rapid quantitative method for capsaicinoids in placentas of Capsicum. Journal of Agricultural and Food Chemistry 47: 4665-4668.
33. Singh, S., S. F. Asad, A. Ahmad, N. U. Khan, S. M. Hadi. 2001. Oxidative DNA damage by capsaicin and dihydrocapsaicin in the presence of Cu (II). Cancer Lett. 169: 139–146.
34. Spicer, O. J. and Almirall, J. R. 2005. Extraction of capsaicin in aerosol defense sprays from fabrics. Talanta 67: 377-382.
35. Scoville, W.L. Note on *Capsicum*. *J. Am. Pharm. Assoc.* 1912, *1*, 453-454.
36. Supalkova V, H. Stavelikova,S. Krizkova, V. Adam, A. Horna, L. Havel, P. Ryant, P. Babula, R. Kizek. 2007. Study of capsaicin content invarious parts of pepper fruit by liquid chromatography withelectrochemical detection. ActaChimSlov. 54: 55-59.
37. Surh, Y. J. 2002. More than spice: capsaicin in hot chili peppers makes tumor cells commit suicide. J. Natl Cancer Inst. 94: 1263– 1265 .
38. Tapia, J. C., Garcia, R., Eleazar, M., Calva, G. and Rocha, J. A. 1993. Capsaicin recovery from a cell culture broth. Indian Engineering and Chemical, Research 32: 2242-2246.
39. Thoennissen, N. H., J. O'Kelly, D. Lu, G. B. Iwanski, D. T. La, S. Abbassi, A. Leiter, B. Karlan, R. Mehta, H. P. Koeffler,. 2010. Capsaicin causes cellcycle arrest and apoptosis in ER-positive and -negative breast cancer cells by modulating the EGFR/HER-2 pathway. Oncogene. 29: 285–296.
40. Titze, P. K., Seitz, E. M. and Petz, M. 2002. Pungency in paprika (*Capsicum annuum L*.). 2. Heterogeneity of capsaicinoid content in individual fruits from one plant. Journal of Agricultural and Food Chemistry 50: 1264-1266.
41. Uzunalic, A. P., Skerget, M., Weinreich, B. and Knes, Z. 2004. Extraction of chilli pepper (*var. Byedige*) with supercritical CO2 : Effect of pressure and temperature on capsaicinoid and color extraction efficiency. Food Chemistry 87: 51-58.

42. Yang, Z. H., X. H. Wang, H. P. Wang, L.Q. Hu, X. M. Zheng, S. W. Li. 2010. Capsaicin mediates cell death in bladder cancer T24 cells through reactive oxygen species production and mitochondrial depolarization. Urol. 75: 735–741.