Optimized Extraction of Oleoresin Capsicum and Analytical Method Validation for Capsaicin using HPLC

Shubham Sharma¹, Ranjit Singh Kushwaha¹, Subh Naman¹, Umesh Kumar Patil², Ashish Baldi¹,*

¹Department of Pharmaceutical Sciences and Technology, Maharaja Ranjit Singh Punjab Technical University, Bathinda, Punjab, INDIA.
²Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, INDIA.

ABSTRACT

Background: Capsaicin, the main active constituent of oleoresin capsicum present in capsicum fruits, is responsible for their pungency and color. Capsaicinoids is an important phytoconstituents and hence exported for various benefits. Hypothesis of work: The process of extraction of oleoresin capsicum by reflux method was to be optimized using terms of their percentage yield. Further ICH compliant analytical method for quantitative analysis of capsaicin using HPLC was developed and validated in present study. Methods: Critical extraction parameters like extraction time, temperature and solvent were optimized to develop an efficient method for extraction of oleoresin from capsicum fruits. A simple, rapid and stable HPLC method of capsaicin was developed using C¹₈ (2) HPLC column, 250x4.5 (mm), particle size 5 μm and validated using various parameters viz., linearity, accuracy, precision and LOD and LOQ using methanol and HPLC grade water (65:35v/v) as mobile phase. The elution was performed at 280 nm with run time of 10 min and flow rate of 1 mL/min. Results: The optimized conditions for oleoresin extraction were found to be 40°C (temperature), 5 hr (extraction time) and acetone as a solvent with highest percentage yield of (3.7% w/w). Furthermore, the developed HPLC method showed linear response over a concentration range of 1-9 μg/mL with standard regression equation (y=4614.9x+5344.1.9) with a coefficient of correlation value, of R²=0.9974. The validation parameter evaluated using this method showed effective and satisfactory results as per ICH Q2 (R¹) guidelines and percentage recovery ranged from (98%-99.71%) indicated good accuracy. The inter and intraday precision with relative standard deviations of capsaicin < 1%, while the LOD and LOQ 1.04 to 3.03 μg/mL respectively. The oleoresin capsicum extract was analyzed for the content of capsaicin using HPLC showing the same retention time with highly pungency value. The percentage of capsaicin in the extract was 730%. Conclusion: The developed extraction and analytical method for capsaicin and oleoresin capsicum was accurate, precise, stable and may be useful for routine analysis of capsaicin content in capsicum fruits as well as various pharmaceutical and food preparations. Key words: Capsaicin, HPLC, Oleoresin capsicum, Reflux method.

INTRODUCTION

Oleoresin capsicum (OC) is the natural lipophilic irritant and the pungent principle of capsaisinoids present in the capsicum fruits. It is an oily, deep red colored mixture of many compounds, extracted from the dried ripe fruit of different pungent varieties of capsicum plant by solid-liquid extraction.¹ ² Extraction is the significant advance for the recuperation and purging of active elements of plant materials are generally founded on the right selection of solvents and the utilization of heat or agitation to build the dissolvability of materials and the pace of mass exchange.³ The selection of solvents for extraction is confined to a couple of solvents of characterized virtue permitted by national and worldwide food laws in the handling of food materials.⁴ The main pungent principle of oleoresin capsicum is mainly dependent upon at least five capsaisinoids i.e. capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homcapsaicin.⁵ Above five capsaicin, dihydrocapsaicin and nordihydrocapsaicin contribute 98% of capsaisinoids concentration and pungency of pepper. (Figure 1) summarises the chemical structure of different capsaisinoids.⁶ There are many carotenoids, which provide color to oleoresin capsicum. 5,6-epoxide and capsorubin are carotenoids, which produce red color to paprika; while β-carotene, zeraxanthin, violoxanthin, antheroxanthin, b-cryptoxanthin and cucurbitaxanthin provide yellow color to these spices. Oleoresin capsanthin in ripe fruit is the major carotenoid contributing up to 60% of the total amount.⁷ ⁹ Capsaicin produces a sensation of intense burning and inflammation due to the binding of transient receptor potential vanilloid 1 (TRPV1), the release of substance P and other cytokines present on the sensory neurons. These are also used against inflammation, analgesic against arthritis, neurogenic inflammation, antimicrobial, hypertension, ischemic heart disease, reduction of cholesterol levels and obesity. It has also been reported against the anticancer effect. Capsaicin and its analogue have provided many pharmacological effects on the gastrointestinal tract, cardiovascular, respiratory as well as sensory and thermoregulation systems.⁴⁰ Exposure to OC produces inflammation, burning, swelling of the nose, throat and mucous membrane due to the binding of transient receptor potential vanilloid 1 (TRPV1).¹² OC is biodegradable, non-toxic, non-carcinogenic produce more inflammation and onset of action as compared to tear gases.

Correspondence: Ashish Baldi, Professor, Pharma Innovation Lab, Department of Pharmaceutical Sciences, Maharaja Ranjit Singh Punjab Technical University, Bathinda Punjab, INDIA. Phone No: +91 8968423848; E-mail: baldiashish@gmail.com

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chloroacetophenone (CN) and o-chlorobenzylidene malononitrile (CS). Scoville heat unit is used to measure the pungency of capsaicinoids. Scoville scale was developed by pharmacologist Wilbur Scoville in 1912 to measure the heat levels of chilies. The scale ranges from 0 (bell pepper) to 16,000,000 (pure capsaicin). The pungency of capsaicin fruits is classified into five levels 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), Very High pungent (>80,000SHU). Capsicum species have a critical function in cardiovascular diseases, gastrointestinal infection and so on. In Mayan medicine, capsaicin is utilized for the treatment of bacterial and fungal contamination. Today as the country growing in a multi-dimensional manner, the crime rate is also increasing rapidly. This includes sexual harassment, rape against women, drug trafficking, kidnapping, murder, money laundering and other valuable throughout the world. However, frequent use of chemical irritants; accomplish self-protection against crime by causing temporary blindness, pain and irritation to skin and mucous membrane. In the present study, extraction of oleoresin capsicum from Capsicum frutescens was done by optimization of time, temperature and solvent using the reflux method. Additionally, HPLC method for quantitative analysis of capsaicin was developed as per ICH Q2 (R1) guideline viz. linearity, accuracy, precision, robustness, LOD and LOQ. Furthermore, validation of oleoresin capsicum extract was done by comparing peak, area, retention time, Scoville heat unit and content of capsaicin in the extract.

**Materials and Methods**

**Sample processing:** Capsicum frutescens fruits were purchased from Bathinda (Punjab) (Figure 2). The fruits were sun-dried for 3 days, ground and cut into small pieces and kept in an airtight container until further process. The standard capsaicin (purity 99%) was purchased from Sigma Aldrich USA. Methanol of HPLC grade was purchased from Finar Chemicals, Ahmedabad. Chloroform, ethanol and acetone were purchased from Loba Chemicals, Mumbai. Ultrapure Type 1 water was obtained from Millipure (India), installed at our department.

**Extraction and percentage yield of capsaicinoids using different solvent**

The effect of four different solvents (methanol, ethanol, acetone and chloroform) on percentage yield of capsaicinoids was assessed using the reflux method. The pre-treatment of dried red paprika (Capsicum frutescens) was done by drying triturated sample in a hot air oven at 60°C for 1 hr. The extraction of paprika was carried out using different solvents under the solid-liquid ratio. Accurately weighed (100 gm) of the paprika sample was mixed with selected solvent i.e. methanol, ethanol, acetone and chloroform in 1:4 ratio. Furthermore, the extraction parameter was studied at different temperatures (30, 40, 50 and 60°C) using a thermostatic water bath. The effect of extraction time on the analyte of interest was studied for 5 hr (optimization data not shown here). After extraction for selected time under different temperatures, the extract was filtered and the solvent was evaporated under a rotary vacuum evaporator at 50°C. Then the extract was collected in a round bottom flask and washed with 1% of hydrochloric acid for removal of impurities. Oleoresin capsicum was then collected in beaker and weighed to determine its percentage yield. The extraction procedure at the same operating conditions was performed in duplicate to determine the percentage yield. Figure 3 illustrates the flow chart of the extraction of oleoresin capsicum from Capsicum frutescens.

The percentage yield of oleoresin capsicum under different experimental setup was calculated using the following formula:

\[
\% \text{ Yield} = \left( \frac{\text{Weight of oleoresin extractive}}{\text{Weight of Capsicum frutescens taken}} \right) \times 100 
\]

**Quantitative analysis of capsaicin using HPLC system**

Chromatographic separation was carried out on an isocratic HPLC system (Waters 1525 system) attached with a binary HPLC pump and Waters 2489 UV-visible detector. Data were recorded and processed using EMPOWER-3 software. The separation was performed on a C18 (2) column (particle size 5 μm; 250 mm x 4.5 mm). Water purification system for obtaining HPLC grade ultrapure type 1 water (Millipore, USA) and ultrasonicator (Amar Enterprises) were used during the chromatographic study.

**Conditions**

Methanol and HPLC grade water (65:35 v/v) was used as a mobile phase for the best separation of with flow rate at 1 mL/min of capsaicin. The temperature of the column was maintained at 37°C during the chromatographic separation and elution was detected at 280 nm for a
run time of 10 min. Before injecting the drug solution, the column was equilibrated for at least 45 min. with the mobile phase.

**Standard solutions**

Stock working solutions of standard capsaicin (1-9 μg/ml) were prepared by diluting the stock solution with the desired volume(s) with methanol. All samples were passed through a 0.45 μm membrane filter and the mixture was degassed using a bath sonicator. At first, blank (methanol) was injected and the chromatogram was recorded and then the standard solution(s) of capsaicin was injected separately.

**HPLC method development and validation**

**Specificity**

The specificity of the HPLC method was evaluated with the injection of blank methanol in the HPLC system and peak area was measured.

**Linearity**

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations of the analyte in the sample. The calibration curve was constructed at the concentration range of 1-9 μg/mL by plotting concentration on X-axis and area under curve.

**Accuracy**

The accuracy of the analytical method can be predicted to a measured value close to the real value. Accuracy studies were carried out in three different levels corresponding to 80%, 100% and 120% of target concentration i.e. (7, 9 and 11 μg/mL) of the standard drug. The mean value of experiment concentration was statistically analyzed using the following formula.

\[
\text{Mean recovery} = \frac{\text{Recovered concentration}}{\text{Injected concentration}} \quad \text{(2)}
\]

and % recovery was calculated by multiply with 100 \(\text{----------(3)}\)

**Precision**

Precision, of the developed method, was assessed by assessing three different quality control levels of capsaicin at LQC (low-quality control), MQC (medium quality control) and HQC (High-quality control) at different time-intervals on the same day (i.e., intraday) and by application on subsequently day (i.e., interday) and % RSD was calculated.

**Robustness**

Robustness of the developed analytical method was evaluated by minor modifications in the HPLC condition of a developed method such as a change in mobile phase ratio, temperature and flow rate and the result was expressed in % RSD.

**Limit of detection (LOD) and limit of quantification (LOQ)**

The sensitivity of the method was determined by LOD and LOQ using the standard deviation of the response (σ) and slope from the calibration curve \(s\) using the following formula:

\[
\text{LOD} = 3.3 \sigma / s \quad \text{(4)}
\]

\[
\text{LOQ} = 10 \sigma / s \quad \text{(5)}
\]

\(\sigma\) = Standard deviation of the response,

\(s\) = Slope of relative calibration curve.

**Sample preparation**

HPLC method for determination of capsaicin in oleoresin capsicum

Accurately weighed (50 gm), triturated and dry samples of Capsicum frutescens and transferred into 1 L boiling flask. Then the volume was made up to 1 L with methanol. The extraction of the sample was carried out by using the reflux method. The sample was gently refluxed for 5 hr at 90°C by attaching the condenser to avoid the evaporation of the solvent and cooled for a sufficient period. The extract was then transferred into a beaker and filtered to remove the impurities. Then the solvent was evaporated using a hot plate by heating at 40-50°C and used for capsaicin analysis.

Sample solution (oleoresin capsicum) was prepared by dissolving 10 mg of oleoresin capsicum into 10 mL of methanol to give 1000 μg/ml concentration. The resultant solution was then sonicated for 10 min.

This stock solution was further diluted with methanol to get a 20 μg/ml concentration of oleoresin capsicum and passed through a 0.45 μm membrane filter. The mixture was degassed using a bath sonicator for 10 min. At first, blank (methanol) was injected and a chromatogram was recorded. Then sample solution of oleoresin capsicum was injected after passing through a 0.45 μm membrane filter and the sample was analyzed using the same condition of the HPLC method of standard capsaicin.

Scoville Heat Unit of Capsaicinoids

The spicy strength of oleoresin capsicum was calculated by measuring the peak areas of respective capsaicinoids from duplicate injection with the average peak area of standard capsaicin. Scoville heat unit (SHU) was calculated according to the following formula:

\[
\text{Capsaicin SHU} = \frac{(C/A)(C/WX)(HC/RC)}{(HC/RC)} \quad \text{(6)}
\]

Where: \(A\), average peak area of standard;

\(C\), average peak area of respective capsaicinoids from duplicate injections

\(C_s\), concentration of standard in mg/mL.

\(WX\), weight of sample in mg/mL.

\(HC\), accepted heat factor of respective capsaicinoids relative to standard (HC=16.0 E + 06)

\(RC\), response factor of respective capsaicinoids relative to standard (RC=0.89).\[19\]

Percentage of capsaicin content

The percentage of capsaicin content was calculated by the following formula:

\[
\text{Capsaicin content} (%) = \frac{\text{Total SHU}}{16 \text{ al SHU}} \quad \text{(7)}
\]

**RESULTS**

Percentage yield of capsaicinoids

The Capsicum frutescens extracted using a solid-liquid extraction process using different solvents by the reflux method as shown in Figure 3. The oleoresin capsicum extracted with acetone showed the highest yield of oleoresin capsicum. The percentage yield of oleoresin capsicum with different solvents was shown in Table 1.

HPLC method of standard capsaicin

A representative chromatogram of 20 μg/mL capsaicin sample run at previously mentioned chromatographic condition and respective chromatogram and the standard plot is given in Figure 4 and Figure 5, respectively. Table 2 summarises the selected conditions for method development. The average retention time of capsaicin was found to
be 8.138 min. Run time of 10 min was fixed for all the analysis. The calibration curve was prepared within a range of 1-9 μg/mL by plotting concentration on X-axis and peak area on Y-axis. Table 3 summarises the average retention time with the average peak area of capsaicin. It indicated a linear relationship between the tested concentrations of analyte and areas of the corresponding peaks.

Validation parameters
Validation of developed analytical method was done on the basis of International Conference on Hormonization (ICH) Guidelines Q2 (R1).[20]

Specificity
Specificity of the HPLC method was shown there is no interaction of capsaicin with methanol.

Linearity
Five different concentration levels (1 μg/mL, 3 μg/mL, 5 μg/mL, 7 μg/mL and 9 μg/mL) were prepared from the standard solution (20 μg/mL) in a triplicate manner and analysis was carried out at 280 nm, which gave linear relationship over the concentration range of 1-9 μg/mL for capsaicin. From the regression analysis, the standard curve equation was found to be $y = 4614.9x + 5344.1$ with a coefficient of correlation value, of 0.9974.

Accuracy
Accuracy of the method was evaluated by the recovery test of the samples at three different levels, viz., 7, 9 and 11 μg/mL each with three replicates as per the ICH guidelines.[20] Both peak area and average peak area were calculated and the result was expressed in % recovery. The observations, as summarized in Table 4 revealed the high accuracy of the developed method.

Precision
Precision, of the developed method, was assessed by analyzing three different quality control levels of capsaicin at LQC (low-quality control), MQC (medium quality control) and HQC (High-quality control) at different time-intervals on the same day (i.e., intraday) and by application on the next day (i.e., interday). The results depicted the analytical method as sufficiently repeatable, as indicated by the corresponding % RSD values Table 5 illustrates inter-day and intra-day data for various quality control samples of capsaicin, with the value of % RSD, during interday (0.60-0.94) and intraday (0.56-1.01) precision studies, indicated low variability and high precision of the method performance.

Robustness
Parameters like observed concentration and % RSD showed no appreciable difference and % RSD remained within the limit, i.e. < 1% indicate that the developed method was robust. Table 6 shows the result obtained from the robustness of capsaicin.

| Table 1: Percentage yields of oleoresin capsicum using different solvent and extraction conditions. |
|-------------------------------------------------|-------------------------------|------------------------------|-----------------|-----------------|
| Extraction method | Weight of sample | Extracting solvent (200 mL) | Temperature (°C) | Time (hr) | Yield (%w/w) |
|-------------------|------------------|-----------------------------|------------------|---------|--------------|
| Reflux            | 50 gm            | Ethanol                     | 30               | 5       | 2.57         |
|                   |                  |                              | 40               |         | 2.63         |
|                   |                  |                              | 50               |         | 2.67         |
|                   |                  |                              | 60               |         | 2.72         |
| Reflux            | 50 gm            | Chloroform                  | 30               | 5       | 2.48         |
|                   |                  |                              | 40               |         | 2.51         |
|                   |                  |                              | 50               |         | 2.54         |
|                   |                  |                              | 60               |         | 2.57         |
| Reflux            | 50 gm            | Methanol                    | 30               | 5       | 2.99         |
|                   |                  |                              | 40               |         | 3.02         |
|                   |                  |                              | 50               |         | 3.07         |
|                   |                  |                              | 60               |         | 3.13         |
| Reflux            | 50 gm            | Acetone                     | 30               | 5       | 3.3          |
|                   |                  |                              | 40               |         | 3.7          |
|                   |                  |                              | 50               |         | 3.11         |
|                   |                  |                              | 60               |         | 3.17         |

Figure 3: Extraction of oleoresin capsicum from *Capsicum frutescens*. 

Figure 4: Chromatogram of standard capsaicin.
The LOD and LOQ for the developed HPLC method were found to be 1.04 and 3.03 μg/mL respectively.

**HPLC Method for Determination of Capsaicin in Oleoresin capsicum Extract**

The oleoresin capsicum was determined according to the same instrumentation and operating condition of standard capsaicin evaluated using the identification of peak at the same retention time. Figure 6 illustrates the chromatogram of extracted capsaicinoids showing the peak of capsaicin at the same retention time (8.179) as that of standard capsaicin (8.136).

**Scoville Heat Unit of Capsaicinoids and capsaicin content**

The pungent principle of oleoresin capsicum was determined in the category of very highly pungent \[^{[13]}\] with a Scoville heat unit of 1.16 ×10⁶. The percentage content of capsaicin was found to be 7.30% w/w.

**DISCUSSION**

The content of capsaicin present in different varieties of capsicum fruits mainly depend upon many factors such as geographical origin, environmental condition, soil characteristics as well as extraction conditions. The current study showed fast, cheap, efficient and reproducible method for extraction of oleoresin capsicum from *Capsicum frutescens*. The main advantages of this study were the minimum of extraction time and increased percentage yield as

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**Table 2: Selected condition for method development.**

| Parameters          | Instrument conditions                                      |
|---------------------|------------------------------------------------------------|
| Column              | C₁₈ (2) HPLC column, 250×4.5 (mm), particle size 5μm      |
| Mobile phase        | Methanol and water (65:35 v/v)                             |
| Flow rate           | 1mL/min                                                    |
| Detection wavelength| 280 nm                                                     |
| Retention time      | 8.138 min                                                  |
| Injection volume    | 20 μL                                                      |
| Run time            | 10 min                                                     |
| Temperature         | 37°C                                                        |

**Table 3: Average retention time with average peak area of capsaicin.**

| S. No | Retention Time | Average Retention Time | Area     | Average Area |
|-------|----------------|------------------------|----------|--------------|
| 1.    | 8.136          | 8.138                  | 142133   | 142175       |
| 2.    | 8.138          |                        | 142181   |              |
| 3.    | 8.142          |                        | 142212   |              |

**Table 4: Accuracy data of capsaicin.**

| Predicted concentration. (μg/mL) | Peak Area | Mean Peak Area | Mean Recovery | (%) Recovery |
|---------------------------------|-----------|---------------|---------------|--------------|
| 7                               | 37611     | 37670         | 6.98          | 99.71        |
|                                 | 37687     |               |               |              |
|                                 | 37712     |               |               |              |
| 9                               | 47137     | 47479         | 8.82          | 98           |
|                                 | 47189     |               |               |              |
|                                 | 48112     |               |               |              |
| 11                              | 62144     | 62495         | 10.81         | 98.27        |
|                                 | 63112     |               |               |              |
|                                 | 62229     |               |               |              |

**Table 5: Intraday and intraday precision data of capsaicin.**

| Standard concentration (µg/mL) | Recovered concentration (µg/mL) | S.D   | % RSD |
|--------------------------------|---------------------------------|-------|-------|
| Intra-day precision            |                                 |       |       |
| LQC:7                          | 6.72                            | 0.04509 | 0.67 |
| MQC:9                          | 9.16                            | 0.05567 | 0.60 |
| HQC:11                         | 10.81                           | 0.10263 | 0.94 |
| Inter-day precision            |                                 |       |       |
| LQC:7                          | 6.70                            | 0.03785 | 0.56 |
| MQC:9                          | 9.18                            | 0.05859 | 0.63 |
| HQC 11                         | 10.83                           | 0.11015 | 1.01 |
Table 6: Robustness data of capsaicin.

| Robustness data with change in composition of mobile phase | Methanol: water (v/v) | Run 1 (µg/mL) | Run 2 (µg/mL) | Run 3 (µg/mL) | Mean conc. | S.D | % RSD |
|----------------------------------------------------------|----------------------|---------------|---------------|---------------|------------|-----|-------|
|                                                          | 64:36                | 4.94          | 4.88          | 4.91          | 4.91       | 0.03| 0.61  |
|                                                          | 65:35                | 4.96          | 4.98          | 5.02          | 4.98       | 0.03| 0.60  |
|                                                          | 66:34                | 5.01          | 4.99          | 5.03          | 5.01       | 0.02| 0.39  |

| Robustness data with change in temperature |
|-------------------------------------------|
| Temperature | Run 1 (µg/mL) | Run 2 (µg/mL) | Run 3 (µg/mL) | Mean conc. | S.D | % RSD |
| 35          | 5.01          | 4.99          | 5.00          | 5.00       | 0.01| 0.2  |
| 37          | 4.95          | 4.97          | 4.93          | 4.95       | 0.02| 0.40 |
| 39          | 4.89          | 4.91          | 4.97          | 4.92       | 0.04| 0.81 |

| Robustness data with change in flow rate |
|-----------------------------------------|
| Flow rate | Run 1 (µg/mL) | Run 2 (µg/mL) | Run 3 (µg/mL) | Mean conc. | S.D | % RSD |
| 0.9        | 5.03          | 5.01          | 5.04          | 5.02       | 0.01| 0.19 |
| 1          | 4.97          | 4.99          | 5.01          | 4.99       | 0.02| 0.40 |
| 1.1        | 4.87          | 4.83          | 4.80          | 4.83       | 0.03| 0.62 |

Table 7: Validation parameters of capsaicin.

| Validation parameters | Values |
|-----------------------|--------|
| Linearity range       | 1-9 µg/mL |
| Standard regression equation | Y=4614.9x + 5344.1 |
| Correlation coefficient (R²) | 0.9974 |
| Accuracy (% recovery) | 98 to 99.71 |
| Precision             | Intraday (% RSD 0.67 to 0.94) | Interday (% RSD 0.56 to 1.01) |
| Robustness            | Change in composition of mobile phase (% RSD 0.39 to 0.61) |
|                       | Change in temperature (% RSD 0.2 to 0.81) |
|                       | Change in flow rate (% RSD 0.19 to 0.62) |
| LOD                   | 1.04 µg/mL |
| LOQ                   | 3.03 µg/mL |

as reflected by the highest percentage yield of capsaicinoids present in the oleoresin capsicum. The extraction efficiency increases with the increase in extraction time and temperature ranges. Increasing time will not affect the extraction after equilibrium of the solute is reached inside and outside the solid materials, various researchers,[24,25] performed similar study on the extraction of capsaicinoids using reflux and other extraction methods with (methanol, acetonitrile, acetone, ethanol and chloroform). Whereas, acetonitrile and ethanol were the best solvents for the extraction of capsaicin from fresh pepper fruits, while acetone was a better solvent for dried pepper fruits for high yield. Extraction of capsaicinoids from (Capsicum frutescens) using supercritical fluid extraction assisted by ultrasound, with CO₂ as a solvent. But the method used was sophisticated costly and difficult to operate optimized extraction method for high percentage yield.[26-28] As reported in present study, the reflux extraction method was more efficient than other extraction methods (Percolation, maceration and sonication). Hence reflux method can be successfully used to extract capsaicinoids from oleoresin capsicum (Capsicum frutescens) in contrast to other extraction methods because of safety, ease of operation, less requirement of solvent and highest extraction efficiency. The extracted oleoresin capsicum was highly pungent due to presence of 7.30% of capsaicin.

In present study, the analytical HPLC method to quantify capsaicin was developed at 280 nm. The validation parameters of reported analytical method for capsaicin is summarized in Table 7. The constructed calibration curve (1-9 µg/mL) showed a linear response with a coefficient of determination (R²) (0.9974), indicating a linear relationship between the concentration of analyte and the area of the corresponding peak. The result of accuracy and precision were found to be within the limit of ICH Q2 (R1) guidelines. The result of robustness studies with a minor change in method conditions, such as the composition of the mobile phase, flow rate and temperature, was robust within acceptable limits. The LOD and LOQ of the developed method were 1.04 µg/mL and 3.03 µg/mL respectively. Identification of capsaicin using the same operating condition by comparison of the peak at the same retention time found satisfactory with that of standard capsaicin. Various analytical methods for quantification of phytochemicals and active pharmaceuticals have also been reported by our research group on similar lines.[29-33] Thus, the developed HPLC method can be successfully applied for routine compared to similar studies.[21,22] Various types of extraction methods for the extraction of capsaicinoids have been used for the last decades.[23] Designing of extraction technique, selection of solvent, the volume of solvent, solubility, cost, safety profile, sample quantity, time and temperature was the main factor to achieve high extraction efficiency.[21] Moreover, ethanol, acetone and methanol are the universal solvents in the solvent extraction of phytochemical investigation.[21] High temperatures increases the solubility as well as extraction efficiency.[22] Moreover, the temperatures that are very high above the boiling point of the solvent may cause solvents to be lost, leading to extracts of undesirable impurities and decomposition of components.[21] In this study, oleoresin capsicum was successfully extracted from Capsicum frutescens (Red paprika) using the reflux method by polar solvents such as methanol, ethanol, chloroform and acetone for 5 hrs and at different temperature (30-60°C) conditions. The extracted profile of oleoresin capsicum mainly depends upon the solvent polarity, physiochemical properties of the particular solvent and different temperature conditions. Therefore oleoresin capsicum extracted using acetone as compared to other solvents for 5 hr at 30-60°C had confirmed the effect of polarity on extraction efficiency.
analysis of capsaicin in different species of capsicum fruits and also used to
determine the other minor capsaicinoids once suitable standards are
available.

CONCLUSION
This study highlights the optimum conditions for extraction of oleoresin
capsicum from Capsicum frutescens. The reflux method developed is safe,
easy to operate, utilizes less solvent volume and gives the highest
percentage yield as compared to other reported extraction methods. The
developed HPLC method for capsaicin and extracted oleoresin capsicum
was accurate, precise, stable and may be useful for routine analysis of
capsaicin content in capsicum fruits as well as various pharmaceutical
and food preparations. The Scoville heat unit and capsaicin content of
extracted oleoresin capsicum were also evaluated in this study to
determine the pungency and capsaicin content in Capsicum frutescens
extract.

The oleoresin capsicum extracted in this study can be used as coloring,
flavoring agents in foods and beverages, formulation of insect, pest
repellents and personnel self-defense spray.

As well as can be substituted with various capsaicin based formulations
for analgesic, antifungal, anti-psoriatic activity (creams and ointments),
for treatment of neuropathic pain (patch) and non-allergic rhinitis (nasal
spray).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

%- Percentage; AUC: Area Under Curve; Cm: Centimetre; Conc.: Concentration; C max : Maximum Concentration; °C: Degree Celsius; Mg:
Microgram; %w/w: per cent weight by volume; FDA: Food and Drug
Administration; Fig.: Figure; GC: Gas Chromatography; GC-MS:
Gas Chromatography-Mass Spectroscopy; Gm: Gram; H:O: Water;
HCl: Hydrochloric Acid; Hr: Hours; ICH: International Council
for Harmonisation; IP: Indian Pharmacopoeia; IR: Infra-Red; IU:
International Unit; LD 50 : Lethal Dose; LOD: Limit of Detection; LOQ:
Limit of Quantification; Mg: Milligram; MP: Melting point; mL:
Millilitre; PPM: Parts Per Million; QC: Quality Control; R 2 :
Coefficient of Correlation; SCU: Scoville Colouring Unit; SD:
Standard Deviation; Sec: Seconds; SHU: Scoville Heat Unit; UV:
Ultraviolet; %w/w: per cent weight by weight; Nm: Wavelength NaOH: Sodium Hydroxide; OG: Oleoresin Capsicum; HPLC:
High Pressure Liquid Chromatography; HPTLC: High-Performance Thin Layer Chromatography; max.: maximum; mm:
millimetre; MT: Metric ton; NMPB: National Medicinal
Plants Board; R f: Retention factor; TCM: Traditional Chinese Medicine;
TLC: Thin Layer Chromatography; UV: Ultraviolet; WHO: World
Health Organization.

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**ABOUT AUTHORS**

**Shubham Sharma** is a M. Pharm Scholar at Department of Pharmaceutical Sciences & Technology, MRSPTU, Bathinda, Punjab.

**Ranjit Singh Kushwaha** is currently working as Research Fellow in a research project funded by National Medicinal Plant Board, Ministry of AYUSH, Govt. of India under the guidance of Prof. Ashish Baldi at Department of Pharmaceutical Sciences & Technology, MRSPTU, Bathinda, Punjab.

**Subh Naman** is currently DST-SERB Junior Fellow and also pursuing PhD at Department of Pharmaceutical Sciences & Technology, MRSPTU, Bathinda, Punjab under the guidance of Prof. Ashish Baldi. With several best presentation and research award, he is presently working on quality certification of spices using machine learning.

**Dr. Umesh Kumar Patil**, is currently working as a Professor at Dr. Harisingh Gour University, Department of Pharmaceutical Sciences and Technology Sagar, Madhya Pradesh, India. He has more than 200 national and international publications with > 3300 cumulative citations. With several awards and scientific acclaims, he is an active researcher in herbal drugs technology and product development.

**Dr. Ashish Baldi**, is Professor and founder head of Department of Pharmaceutical Sciences &Technology, MRSPTU, Bathinda, Punjab. Recently he was featured in top 2% best scientists in ‘Pharmacy and Pharmacology’ as per survey of Stanford University, USA. He has 05 patents, 03 technology transfers, 06 books, 4 special issues with Bentham Science Publishers along with over 125 national and international publications with cumulative impact factor of >110. He has presented more than 150 papers at conferences/seminars in India and abroad, and has several best paper awards to his credit. He has completed several research projects for various government agencies and is currently supervising 6 government funded projects including DST, ICMR, ICSSR, Ministry of AYUSH, etc.