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# A HPTLC method for the quantitative determination of Piperine and Capsaicin in Rasam, A South Indian spice soup

**Research Article**

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## Abstract

*Rasam* is a popular spice soup of South Indian traditional food. *Rasam* is prepared traditionally by incorporating spcies such as turmeric, coriander, black pepper, garlic, chili pepper, curry leaves, cumin, mustard, and asafoetida in tamarind juice. All ingredients used in the preparation of *rasam* are known for various medicinal uses which, makes *rasam* a traditional food with a lot of functional ingredients. There are reports to suggests that a regular chronic consumption of traditional foods with functional ingredients may prevent numerous diseases. There was no scientific literature available on the phytochemical composition of *rasam*. We, herein aim to estimate the quantity of piperine and capsaicin present in the standardized *rasam* by HPTLC method. *Rasam* was prepared as per reported method and the sample solution was prepared by extracting *rasam* with diethyl ether. The mobile phase used was toluene: ethyl acetate (7:3, v/v). Densitometric scanning was performed in absorption-reflection mode at at 527 nm. Linear range was 1 to 5 µg for both piperine and capsaicin. The amount of piperine and capsaicin from the standardized *rasam* was found to be 0.234 and 0.335 % w/v respectively. This TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for piperine and capsaicin in *rasam*.

**Key Words:** Black pepper, Chili pepper, *Saaru, Chaaru, Standardized rasam.*

## Introduction

*Rasam* is a popular spice soup of South Indian traditional food. It is consumed regularly by most of the South Indians. It is also called as *rasam* or *saaru* or *chaaru* in South Indian languages like Tamil, Telugu, Malayalam and Kannada. The word, *rasam* in Sanskrit means "the essential products of digestion" (1). *Rasam* is prepared traditionally by incorporating spcies such as turmeric, coriander, black pepper, garlic, chili pepper, curry leaves, cumin, mustard, and asafoetida in tamarind juice. *Rasam* consumption periodically is considered to be good for health. Several reports have claimed rasam for the treatment of cold, cough, diabetes, mineral deficiency and health maintenance (2). All ingredients used in the preparation of *rasam* are known for various medicinal uses which, makes *rasam* a traditional food with a lot of functional ingredients. There are reports to suggests that a regular chronic consumption of traditional foods with functional ingredients may prevent numerous diseases (3). In our earlier studies, the ingredients used, their quantity, and process involved in the preparation of *rasam* has been standardized (4). Other studies reported on standardized *rasam* includes, antimicrobial (5), cytotoxic, antimitotic, antiproliferation (6) and *in vivo* breast cancer studies (7). Recently, the physicochemical properties of standardized *rasam* have been reported (8). There was no scientific literature available on the phytochemical composition of *rasam*. Piperine is known for its potential to affect bioavailability of other chemical constituents in food and dietary supplements. Capsaicin has shown beneficial effects in clinical study related to obesity, diabetes, cancer and cardiovascular diseases. Considering the therapeutic value of these functional ingredients in *rasam* and also in continuation with the further studies in *rasam*, we herein aim to estimate the quantity of piperine and capsaicin present in the standardized *rasam* by HPTLC technique.

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## Materials and Methods

### Ingredients, chemicals and reagents

All the ingredients required for the preparation of standardized *rasam* was procured from local market, Vadodara, Gujarat, India. Standard piperine and capsaicin were procured from Yucca Enterprises, Mumbai. Solvents and chemicals used were of analytical grade. Merck silica gel $60F_{254}$ TLC plates were used for the chrompatographic studies.

**Chromatogrpahy**

Camag TLC system equipped with Camag Linomat V (an automatic TLC sample spotter) and Camag glass twin trough chamber (20 X 10 cm) were used for the analysis. Chromatographic studies were performed by using Merck silica gel $60F_{254}$ TLC plates (20 X 10 cm; layer thickness 250 µm). The plates were pre-activated before each analysis at 60 °C for 5 min. Standards and samples were applied on the plate as 8 mm wide bands with Linomat V under a flow of $N_2$ gas, 10 mm from the bottom and 10 mm from the side. The distance between two spots were maintained as 15 mm. Linear ascending development of the TLC plates were carried in a twin trough chamber saturated with 20 mL mobile phase for 20 min at room temperature (25±2 °C and 40% relative humidity). The plates were developed up to 8 cm at chamber saturation conditions. The developed TLC plates were dried in hot air with the help of a hair dryer. The dried plate was subjected to post chromatographic derivatization. The quantitative evaluations of the plates were performed with Camag scanner 4 (visionCATS 3.1 integration software). Densitometric scanning was performed in the absorption-reflection mode using a slit width of 6 X 0.45 mm and data resolution 100 µm step and scanning speed 100 mm/s.

**Method validation and specificity**

The method was validated for precision, accuracy and repeatability as per standard protocol (9). Specificity was determined by analyzing the samples with the standard. The corresponding spot in the sample was confirmed by comparing the $R_f$ and the spectra with that of the standard spot. The peak purity was assessed by comparing the densitogram at three different levels, i.e., peak start, peak apex and peak end position of the spot.

**Preparation of *rasam***

The standardized *rasam* was prepared as per the reported procedure of Devarajan and Raja, 2017 (4). Dried tamarind fruit pulp (6.88g) was soaked in 450 mL of water for 10 min. Then it was crushed in hand for 45 times and further strained and to which dried rhizome powder of turmeric (0.4g) turmeric powder and sea salt (4g) was added. In a separate container, fresh riped fruit of tomato (82.44g) was crushed in hand 60 times. In a mortar and pestle, the dried long pepper chilli (1.33g) was crushed 85 times, to which dried cumin fruits was added and crushed (2.67g) for 100 times, then pepper (0.82g) was added and crushed for 50 times, followed by cloves of garlic (9.63g) and crushed for 90 times. The crushed tomato mixture and spice mixture tamarind was mixed together. The whole mixture was rinsed with 5ml water. In a stainless-steel bowl, Indian sesame oil (4ml) was heated at 60°C for 2 min. After which, dried mustard seeds (0.82g), whole chilli pepper (1.53g) and fresh curry leaves (0.61g) were added with an interval of 5s, 3s and 2s respectively. The whole mixture was added and rinsed with 20ml of water. The entire mixture was heated till frothing and fresh coriander leaves (1.50g) and asafoetida powder (0.05g) was added and finally the heat source was terminated.

**Preparation of sample solution**

50 mL of the prepared *rasam* was shaken with 50 mL of diethyl ether in a separating funnel. The ether layer was collected and another 50 mL was added for extraction. The combined 100 mL ether layer was filtered and concentrated to 30 mL in a rotary evaporator under vacuum.

**Preparation of piperine and capsaicin standard solution**

Accurately weighed 10 mg each of piperine and capsaicin was transferred to 10 mL volumetric flask and the volume was madeup with methanol (HPLC grade) separately to yield a concentration of 1000 µg/mL. Working solutions were prepared by appropriate dilution of the stock solution with the same solvents The prepared stock and working solutions were protected from light and stored in refrigerator at 2 to 4 °C.

**Preparation of calibration curve**

Standard piperine and capsaicin solutions in the range of 1 to 5 µg $spot^{-1}$ were applied on TLC plate for preparation of calibration curve of peak area versus concentration.

**Estimation of piperine and capsaicin in rasam**

Quantification was performed by external standard method, using pure piperine and capsaicin as standard. 5 µL of the sample solution (ether extract of *rasam*) was applied in triplicate on the TLC plate and developed with the mobile phase toluene: ethyl acetate (7:3, v/v). The post chromatographic derivatization was carried out with anisaldehyde-sulphuric acid placed in a dipping chamber (CAMAG) followed by heating in an oven at 105 °C for 5–10 min (10). Densitometric scanning was performed in absorption-reflection mode at at 527 nm. Peak areas were recorded and the amount of piperine and capsaicin was calculated using the calibration curve.

## Results and Discussion

**HPTLC separation optimization**

The ether extract of *rasam* when subjected to HPTLC as per the methods described above, showed the presence of piperine and capsaicin. The densitometric scanning was performed in absorption-reflection mode at 342 nm for piperine and at 366 nm for capsaicin respectively. $R_f$ value of the bands in sample was matched with standard $R_f$ value (Figure 1 and 2) of piperine and capsaicin. A comparison of the spectral characteristics of the peaks for standard compounds (piperine and capsaicin) and that of the sample further confirmed the identity of piperine and capsaicin present in the samples. Good resolutions with symmetrical and reproducible peaks were obtained.

**Figure 1. Overlay of piperine standard and *rasam***

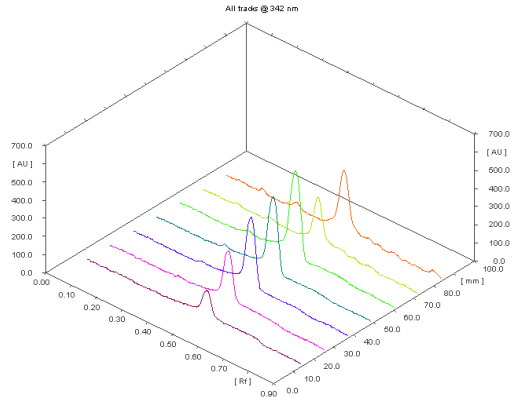
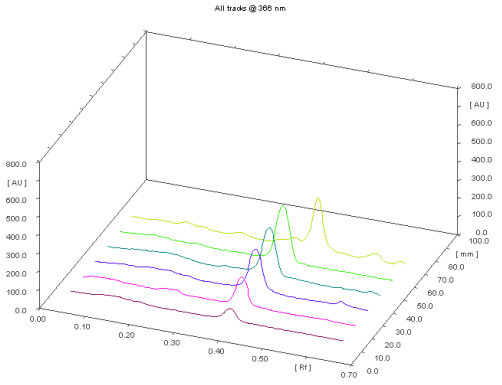



**Figure 2. Overlay of capsaicin standard and *rasam***




**System suitability test**

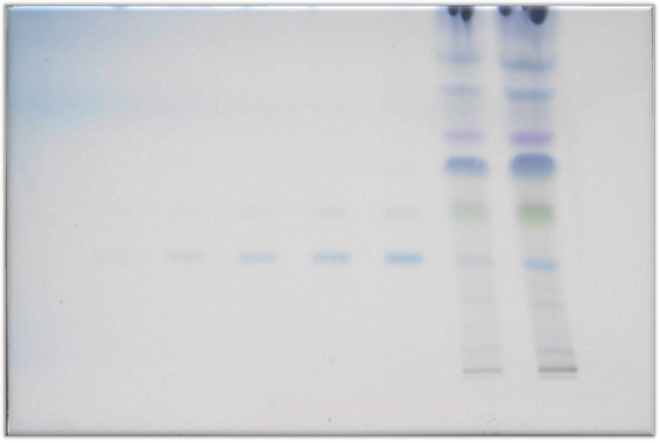
**Linearity and detection limit**

The peak area versus concentration graph were found to be linear in the range of 1 to 5 µg spot$^{-1}$ for both piperine and capsaicin. The regression equation and correlation coefficient for both piperine and capsaicin indicated good linearity (Table 1). The limit of detection for piperine and capsaicin were 23.57 and 7.67 ng respectively. The limit of quantification was 76.84 and 25 ng for piperine and capsaicin respectively (Table 1).

**Table 1. Linearity regression data for quantification of piperine and capsaicin using proposed TLC**

| Linearity regression data | | |
|---|---|---|
| **Parameter** | **Piperine** | **Capsaicin** |
| **$R_f$** | 0.51 | 0.40 |
| **Dynamic range (µg spot$^{-1}$)** | 1-5 | 1-5 |
| **Equation** | $Y$= 2.7033$x$+1297.3 | $Y$= 1.4148$x$-39.89 |
| **Slope** | 2.7033 | 1.4148 |
| **Intercept** | 1297.3 | 39.89 |
| **Limit of detection** | 23.57 ng | 7.67 ng |
| **Limit of quantification** | 76.84 ng | 25 ng |
| **Linearity (correlation coefficient)** | 0.9981 | 0.9976 |
| **Specificity** | Specific | Specific |
| **Amount of compound quantified (% w/v)** | 0.234 | 0.335 |

**Figure 3. TLC plate of standard mixture and *rasam***



***Precision studies***

Instrumental precision was checked by repeated scanning of the same spots of piperine and capsaicin three times and % RSD values were calculated (Table 2). To determine the precision of the methods, standards were analyzed three times inter-day and intra-day (Table 2).

**Table 2. Precision studies data for quantification of piperine and capsaicin using proposed TLC densitometric method**

| Precision studies | | | | |
|---|---|---|---|---|
| **TLC method** | **Concentration (µg spot$^{-1}$)** | **Instrumental precision (% RSD)** | **Method precision (% RSD)** | |
| | | | **Intra-day** | **Inter-day** |
| **Piperine** | 1 | 0.71 | 1.15 | 1.21 |
| | 5 | 0.40 | 0.72 | 1.02 |
| **Capsaicin** | 1 | 0.46 | 1.04 | 1.33 |
| | 5 | 0.56 | 0.75 | 0.85 |

*Sample analysis and recovery studies*

The amount of piperine and capsaicin from the standardized *rasam* was found to be 0.234 and 0.335 % w/v (Table 1 and Figure 3). The recovery studies were performed by adding piperine and capsaicin at 80, 100 and 120% to preanalyzed samples and their quantitative analysis were estimated (Table 3).

**Table 3. Recovery studies data for quantification of piperine and capsaicin using proposed TLC densitometric method**

| Recovery studies | | | | |
|---|---|---|---|---|
| **TLC method** | **Amount in the sample (µg)** | **Amount added (µg)** | **Amount found (µg)** | **Recovery (%)** |
| **Piperine** | 7.5 | 6 | 13.2 | 97.78 |
| | 7.5 | 7.5 | 14.8 | 98.67 |
| | 7.5 | 9 | 16.2 | 98.18 |
| **Capsaicin** | 2.6 | 2.1 | 4.8 | 102.13 |
| | 2.6 | 2.6 | 5.0 | 96.15 |
| | 2.6 | 3.1 | 5.8 | 101.75 |

## Conclusion

The TLC methods are rapid, simple, specific and effective in quantification of piperine and capsaicin, should prove to be a useful alterative under circumstances where the other slower and more costly chromatographic methods are not appropriate. This TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for piperine and capsaicin in *rasam*.

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