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Simultaneous quantification of Curcumin, Piperine and Capsaicin by HPTLC in *Rasam*, a polyherbal soup

Research Article

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Abstract

Traditionally *rasam* is consumed for the treatment of cold and cough. The major chemical constituents present in the spices required to prepare the *rasam* are known but, the chemical composition of the prepared *rasam* are unknown. The preparation involves, boiling the spices in water and oil, this may create an opportunity for an altered chemical profile of the prepared *rasam*. As a part of our chemical investigation in *rasam*, curcumin, piperine and capsaicin were chosen as marker compounds based on their literature for exhibiting potent activity against upper and lower respiratory tract infections (URTI & LRTI). The current work was planned to identify and quantify curcumin, piperine and capsaicin simultaneously by HPTLC in *rasam*. Silica gel $60F_{254}$ TLC plates were used for the study. The optimized mobile phase used was toluene and ethyl acetate (7:3, v/v) and the densiometric scanning was done at 254 nm. The R_f values of curcumin, piperine and capsaicin were found to be 0.26, 0.40 and 0.47 respectively. The calibration graph of peak area versus concentration graph of curcumin, piperine and capsaicin was found to be linear in the range of 2 to 7 µg $spot^{-1}$. The amount of curcumin, piperine and capsaicin in 100 mL of *rasam* was found to be 0.49, 0.66 and 0.41% w/v respectively. The developed method was validated as per ICH guideline parameters. The current study shows that the developed TLC method is simple, precise, specific, robust and accurate. This method will also be useful to identify or quantity any polyherbal formulation containing curcumin, piperine and capsaicin as chemical constituents.

Key Words: *Saaru, Chaaru, Turmeric, Black pepper, Chili pepper.*

Introduction

South Indian traditional cuisine is not complete without *rasam,* a polyherbal spice soup. It is also called as *saaru* or *chaaru* in Malayalam, Kannada and Telugu. In Sanskrit, *rasam* means "the essential products of digestion" (1). *Rasam* is prepared traditionally by incorporating spices such as turmeric, coriander, black pepper, garlic, chilli pepper, curry leaves, cumin, mustard, and asafoetida in tamarind juice. Traditionally *rasam* is consumed for the treatment of cold and cough. There are also reports of *rasam* being used in diabetes, mineral deficiency and general health maintenance (2). There is evidence that regular consumption of traditional foods with functional ingredients may be prophylactic for numerous diseases and disorders (3). The major chemical constituents present in the spices required to prepare the *rasam* are known but, the chemical composition of the prepared *rasam* are unknown. The preparation involves, boiling the spices in water (>100 °C) and oil, this may create an opportunity for an altered chemical profile of the prepared *rasam*. Loss of active ingredient/s or breakdown of inactive ingredient/s to an active one or new chemical entities (NCEs) is possible hence, a thorough phytochemical investigation is required to profile the chemical constituent/s in *rasam*.

In our earlier studies, the ingredients used, their quantity, and the process involved in the preparation of *rasam* have been standardized (4). Other studies reported on *rasam* include, antimicrobial (5), cytotoxic, antimitotic, antiproliferation (6) and *in vivo* breast cancer studies (7). Recently, the physicochemical properties of *rasam* have been reported (8). As a part of our chemical investigation in *rasam*, curcumin, piperine and capsaicin were chosen as marker compounds based on their literature for exhibiting potent activity against upper and lower respiratory tract infections (URTI & LRTI) (9-13). The current work was planned to identify and quantify curcumin, piperine and capsaicin simultaneously by HPTLC in *rasam*.

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Materials and Methods

Ingredients and chemicals

Ingredients required for preparing *rasam* were procured from the local market, Vadodara, Gujarat, India. Standard curcumin, piperine and capsaicin were procured from Yucca Enterprises, Mumbai,

Maharashtra, India. Silica gel $60F_{254}$ TLC plates were procured from Merck, Darmstadt, Germany for the chromatographic studies. Chemicals and solvents of analytical grade were used for the study.

Chromatography

Camag TLC system equipped with Camag Linomat V (an automatic TLC sample spotter) and glass twin trough chamber (Camag, 20X10 cm) were used for the analysis. Chromatographic studies were performed by using Merck silica gel $60F_{254}$ TLC plates (20X10 cm; 250 µm layer thickness). Standards and all samples were applied as 8 mm wide bands with Linomat V under N_2 gas flow and 15 mm as the distance between each band. Development of TLC plates was carried out linearly in a twin trough chamber after saturation with 20 mL of the mobile phase. The saturation time was 20 min and room temperature was 25±2 °C with 40% relative humidity. The TLC plates were developed till a pre-marked 8 cm under the prescribed conditions and after development, it was dried by blowing hot air with a hair dryer. The dried TLC plates were evaluated with Camag scanner 4 (visions CATS 3.1 integration software). Densitometric scanning of the plates were carried out in absorption-reflection mode (slit width 6X0.45 mm; resolution 100 µm; scanning speed 100 mm/s).

Method validation

Developed TLC method was validated as per ICH guideline parameters like linearity, precision, accuracy, LOD, LOQ and robustness (14). Specificity was determined by analyzing the spots in the sample track with the standard spots. The spot in the sample parallel to the standard spot was confirmed by R_f values and spectral overlay. Peaks at the start, apex and end of the spectra were assessed for peak purity.

Preparation of *rasam*

The ingredients used for the preparation of *rasam* are as shown in table 1. *Rasam* was prepared as per earlier reports (4).

Table 1. Quantity of the ingredients used in the preparation of *rasam*

Ingredients	Quantity
Tamarind	6.88 g
Turmeric	0.4 g
Sea salt	4 g
Tomato	82.44 g
Chili pepper	0.82 g
Cumin	2.67 g
Garlic	9.63 g
Black pepper	1.33 g
Indian sesame oil	4 mL
Black mustard	0.82 g
Chili pepper	1.53 g
Curry leaves	0.61 g
Potable water	500 mL
Coriander	1.50 g
Asafoetida	0.05 g

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 same procedure was repeated again with another 50 mL of diethyl ether. The combined 150 mL of ether layer was collected and concentrated to 30 mL in a rotary evaporator under vacuum.

Preparation of curcumin, piperine and capsaicin standard solution

Accurately weighed 10 mg of curcumin, piperine and capsaicin were dissolved separately in 10 mL of methanol to yield their respective the stock solution (1000 µg/mL). Working solutions were prepared by appropriate dilution of the stock solution with methanol. A standard mixture solution was prepared by mixing all three standard solutions together for simultaneous estimation. The prepared stock and working solutions were protected from light and stored in a refrigerator at 2 to 4 °C.

Preparation of calibration curve

For preparing the standard curves, curcumin, piperine and capsaicin solutions were applied in the range of 2 to 7 µg $spot^{-1}$ on the TLC plate. The calibration curve was plotted with peak area versus concentration.

Identification and quantification of curcumin, piperine and capsaicin in *rasam*

Identification was carried out by external method, using pure curcumin, piperine and capsaicin as standards. The sample solution was applied to the TLC plate and developed in the mobile phase toluene and ethyl acetate in a ratio of 7:3 (v/v). Densitometric scanning was carried out at 254 nm in absorption-reflection mode. Peak areas were recorded and the amount of curcumin, piperine and capsaicin was quantified using the calibration curve.

Results and Discussion

Identification of curcumin, piperine and capsaicin

The R_f value of three bands in samples were matching with standard R_f values of curcumin, piperine and capsaicin (Figure 1, 2). The R_f values of curcumin, piperine and capsaicin were found to be 0.26, 0.40 and 0.47 respectively. Comparison of the spectra for the peaks of standard compounds (curcumin, piperine and capsaicin) with that of the matching peaks in the sample confirmed the presence of curcumin, piperine and capsaicin. Symmetrical and reproducible peaks with good resolutions were obtained.

System suitability

Three replicates of standard mixture were applied over developed chromatographic condition to verify the system suitability. %RSD (relative standard deviation) were less than 2% showing an excellent system suitability for the developed method.

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Figure 1. Identification of curcumin, piperine and capsaicin in *rasam* sample (1-capsaicin; 2-piperine; 3-curcumin)

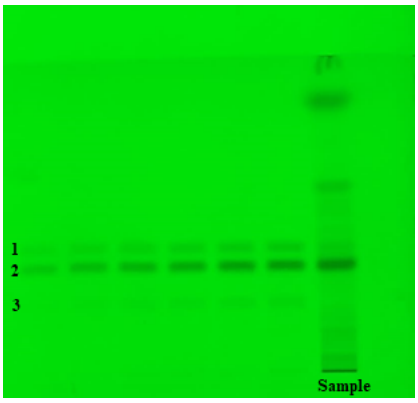
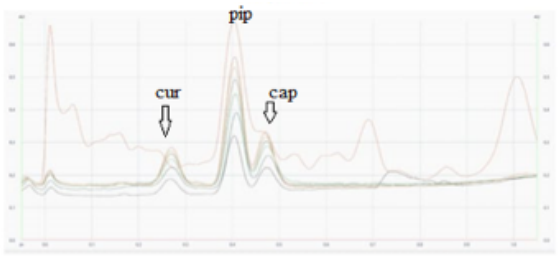


Figure 2. Chromatogram showing the curcumin, piperine and capsaicin peaks in *rasam* sample (cur-curcumin; pip-piperine; cap-capsaicin)



Specificity

Specificity was determined by comparing the R_f value and spectra of the standard band with the corresponding band in *rasam* sample. There was no interference in any of the peaks.

Linearity and detection limit

Correlation coefficient and regression equation for curcumin, piperine and capsaicin indicated good linearity (Table 2). The calibration graph of peak area versus concentration graph was found to be linear in the range of 2 to 7 µg spot^{-1} for curcumin, piperine and capsaicin. The limit of detection for curcumin, piperine and capsaicin was 3.98, 3.75 and 3.13 µg respectively. The limit of quantification for curcumin, piperine and capsaicin was 12.05, 11.36 and 9.49 µg respectively (Table 2).

Table 2. Linearity regression data for estimation of curcumin, piperine and capsaicin

Parameter	Curcumin	Piperine	Capsaicin
R_f	0.26	0.40	0.47
Linearity range (µg spot^{-1})	2-7	2-7	2-7
Equation	y = 8E-07x + 0.0021	y = 2E-06x + 0.0029	y = 8E-07x + 0.0021
Correlation coefficient	0.9965	0.9971	0.9964
Specificity	Specific	Specific	Specific
LOD (µg)	3.98	3.75	3.13
LOQ (µg)	12.05	11.36	9.49

LOD-limit of detection; LOQ-limit of quantification

Precision studies

Precision studies for repeatability was done on the same day for three times and intraday precision was calculated. Similarly, interday precision was determined for consecutive three days and the % RSD was calculated (Table 3).

Table 3. Precision data for curcumin, piperine and capsaicin

Parameter	Standard	Concentration (µg spot^{-1})	% RSD*
Instrumental precision	Curcumin	2	1.94
		7	0.92
	Piperine	2	0.62
		7	0.54
	Capsaicin	2	1.46
		7	0.76
Interday precision	Curcumin	2	0.54
		7	0.30
	Piperine	2	0.17
		7	0.15
	Capsaicin	2	0.65
		7	0.28
Intraday precision	Curcumin	2	1.04
		7	1.15
	Piperine	2	0.43
		7	0.12
	Capsaicin	2	0.36
		7	0.33
*n=3			

Robustness studies

Robustness study was done by varying scanning wavelength by ±2% change. The calculated % RSD was within limits, assuring the robustness of the developed method.

Simultaneous quantification and recovery studies

Currently developed TLC method was used for the simultaneous quantification of curcumin, piperine and capsaicin using standard mixture. The amount of curcumin, piperine and capsaicin in 100 mL of *rasam* was found to be 0.49, 0.66 and 0.41% w/v respectively. The recovery studies were performed by adding curcumin, piperine and capsaicin at 80, 100 and 120% to preanalyzed samples and then quantified (Table 4).

Table 4. Recovery studies data of curcumin, piperine and capsaicin

% level	Standard	Amount of standard in sample (µg)	Amount spiked (µg)	Mean % recovery*
80%	Curcumin	3.71	2.96	101.48
	Piperine	4.97	3.97	100.18
	Capsaicin	3.1	2.48	99.96
100%	Curcumin	3.71	3.71	101.45
	Piperine	4.97	4.97	99.93
	Capsaicin	3.1	3.1	100.24
120%	Curcumin	3.71	4.45	100.24
	Piperine	4.97	5.96	99.25
	Capsaicin	3.1	3.72	100.62

*n=3

Conclusion

The developed TLC method is simple, precise, specific, robust and accurate in quantification of curcumin, piperine and capsaicin. This method can be used effectively for identification and quantification of curcumin, piperine and capsaicin in *rasam*. The amount of curcumin, piperine and capsaicin in 100 mL of *rasam* was found to be 0.49, 0.66 and 0.41% w/v respectively. The developed TLC method can also be used to identify or quantity any polyherbal formulation containing curcumin, piperine and capsaicin as chemical constituents.

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