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Microwave assisted extraction of curcumin by sample–solvent dual heating mechanism using Taguchi L_9 orthogonal design

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Abstract

The present work reports on a novel extraction method using microwaves based on solvent–sample duo-heating synergism, for the extraction of curcumin from *Curcuma longa* L. The duo-heating mechanism is based on simultaneous heating of sample matrix and extracting solvent under microwave energy. Methanol soaked plant material was used as a modifier to bring about selective and effective heating of the sample under microwave. Acetone was used as the extracting solvent, which has excellent curcumin solubilizing capacity and heats up under microwave owing to its good dissipation factor. Extraction conditions, namely microwave power, irradiation time, particle size and modifier volume were optimized using Taguchi design approach and curcumin was quantified using high performance thin layer chromatography. The optimum conditions as obtained from signal-to-noise ratio analysis and interaction studies between factors were as follows: 20% microwave power, 4 min irradiation time, particles screened through sieve 20 and 8 ml of modifier. Microwave assisted extraction (MAE) under the influence of dual heating mechanism showed better precision and dramatically higher yield with significant reduction in extraction time under optimum extraction conditions, when compared to conventional approaches.

Keywords: Microwave assisted extraction; Curcumin; Taguchi approach; High performance thin layer chromatography; Signal-to-noise ratio

1. Introduction

The rhizomes of *Curcuma longa* L. (Zingiberaceae) provide a yellow and flavorful powder when dried and ground. As a powder called 'Turmeric', it has been valued worldwide as a functional food because of its health promoting properties [1]. The coloring principle curcumin (diferuloylmethane, $C_{12}H_{20}O_6$) and two other related compounds, viz. demethoxy curcumin and bis-(demethoxy) curcumin, together known as curcuminoids, are the active phytoconstitutents present in *C. longa* species [2]. There are several reports in the literature indicating a variety of pharmacological activities of curcuminoids such as anti-inflammatory, antibacterial, antifungal, antiparasitic and antimutagenic and as modest inhibitors of HIV 1 and HIV 2 proteases [2–6]. The most conventional method for extraction of curcumin has been Soxhlet extraction with heating time ranging as long as up to 12 h [7,8]. The Soxhlet extraction process is time consuming, laborious and makes use of bulk amount of organic solvents. As the heating process continues for long hours, the approach possibly involves high risk of thermal decomposition of target molecules [9,10]. Soxhlet extraction and other conventional methods operate through cell permeation followed by solubilizing the active constituents by the extracting solvent. Curcumin present inside the oleoresin cells which in turn is covered by tightly packed cork cells probably makes the entry route for the solvent even tougher and time consuming.

MAE operates through cell bursting due to localized internal superheating followed by leaching out of the active constituents. Cell bursting phenomenon probably facilitates entry of the extracting solvent to solubilize out the target compound, thus lead to faster and efficient extraction. As compared to other conventional techniques like Soxhlet and maceration, MAE provides considerable reduction in extraction time, solvent consumption with improved extraction rate and to some extent selective extraction. Some applications of MAE for biologically active compounds have appeared in the literatures, such as extraction of camptothecin from *Nothapodytes foetida* [11],

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extraction of tanshinones from *Salvia miltiorrhiza* [12], extraction of glycyrrhizic acid from licorice [13], and so on.

Heating mechanism in MAE differs widely from conventional Soxhlet heating. Heating in MAE is dependent on the dielectric properties of the solvent and matrix [14]. Different heating mechanisms have been reported with MAE [14–16]. This paper offers a new MAE method for rapid and efficient extraction of curcumin using a synergistic heating mechanism where the solvent and powdered drug samples were heated simultaneously. The extraction conditions were optimized using Taguchi L_9 orthogonal array design. The interaction among the factors and repeatability of the method was also investigated. Finally the MAE results were compared with conventional extraction techniques.

2. Experimental

2.1. Plant materials

Dried rhizomes of *C. longa* were provided as gift sample from herbal exporter M/S Ram Traders (Mumbai, India) and were used as received without any pretreatment. Rhizomes were milled to homogenous 40, 20, 10 mesh powder (selected by sieve), immediately before the experiment.

2.2. Reagents

Acetone and methanol used in the experimental for extraction purpose were all of analytical grade from Merck (India). Chloroform and methanol used in HPTLC analysis were all of HPLC grade. Precoated silica gel $60F_{254}$ plates for HPTLC analysis were from E. Merck (Darmstadt, Germany). Curcumin standard of 99% (w/w) purity was obtained as a gift sample from Centre for Cellular and Molecular Biology (Hyderabad, India).

2.3. Apparatus

The extraction system comprised of a microwave extractor (CATA R) manufactured by Catalyst Systems (Pune, India) equipped with a magnetron of 2450 MHz with a nominal maximum power of 700 W, a reflux unit, 10 power levels, time controller, exhaust system, beam reflector and a stirring device (Fig. 1). The whole system was open and run at atmospheric pressure. A Camag (Switzerland) HPTLC system was used for quantification of curcumin.

2.4. Conventional extraction techniques

Three conventional extraction techniques as given below were used for comparison with MAE. Soxhlet extraction was considered as the reference extraction technique and at each step of MAE; extraction efficiency was reported by comparison with Soxhlet data.

2.4.1. Soxhlet extraction

Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with accurately weighed 2 g of the drug powder (screened through sieve 40) for 24 h. Extraction was performed with acetone as the extracting solvent. The extraction was conducted for 8 h/day for 3 days. Every day, the previous day's extract was removed and fresh 100 ml acetone was added, finally all the three extracts were combined and evaporated under vacuum. The dried residue was dissolved in 10 ml methanol, from which 100 μl was transferred to a 5 ml volumetric flask, and after making up the volume with methanol, 2 μl was subjected for quantification by HPTLC. Percentage extraction of curcumin (w/w) present in 24 h acetone extract was found to be 4.37.

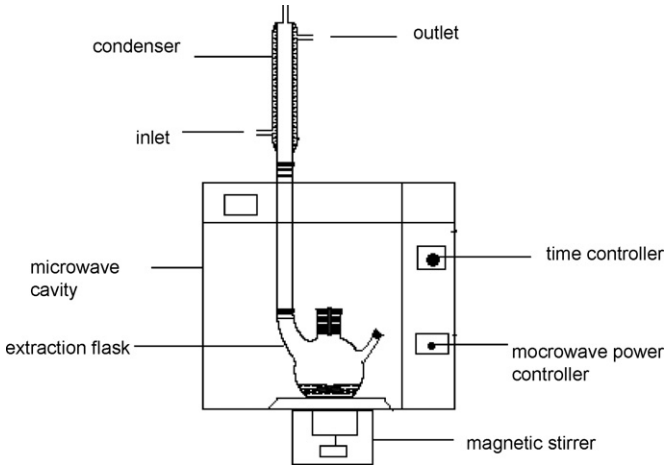

Fig. 1. Schematic diagram of microwave assisted extraction apparatus.

2.4.2. Maceration and Stirring extraction

Maceration was carried out in a closed conical flask for 24 h. Stirring extraction was carried out by continuous stirring for 24 h with the help of a magnetic stirrer in a closed conical flask. In both the cases 2 g powdered drug sample (screened through sieve 40) and 40 ml acetone was used as the extracting solvent. Heat was not applied in either of the cases. The suspension after maceration/stirring was centrifuged and the supernatant evaporated under reduced pressure, dissolved in methanol for HPTLC analysis as described earlier.

2.5. Microwave assisted extraction (MAE)

For MAE accurately weighed 2 g of the homogenous 40, 20, 10 mesh drug powder was used. The samples were mixed thoroughly with a suitable modifier (methanol) in accordance with the experimental design. A saturation time of 10 min was allowed for the powdered drug to absorb methanol. Methanol soaked powdered drug was then placed into the extraction vessel, followed by the addition of 40 ml of the extracting solvent (acetone). MAE was carried for different time of irradiation with the microwave extractor operating at different power levels. The sample was treated under microwave irradiation in an intermittent way, i.e. irradiation–cooling–irradiation. The irradiation time was kept for 1 min and 1 min was taken to cool the sample solution between two irradiations. After extraction, the samples were centrifuged at 4000 rpm ($3520 \times g$) for 10 min.

Table 1
Factors and levels for the orthogonal design (A–D are the respective codes for each factor)

Levels	Microwave power (%) A	Irradiation time (min) B	Sieve number C	Modifier volume (ml) D
1	60	1	10	2
2	40	2	20	4
3	20	4	40	8

The supernatant was filtered, concentrated under vacuum, dissolved in methanol for quantification by HPTLC as described earlier. In the present work, extraction efficiency (%) for MAE is defined as follows.

$$\text{Relative extraction efficiency (\%)} = \frac{\text{Percentage extraction of curcumin (w/w) obtained from MAE} \times 100}{\text{Percentage extraction of curcumin (w/w) obtained from 24 h of exhaustive Soxhlet extraction}}$$

2.6. HPTLC analysis

The samples were spotted (2 μl) in the form of bands of width 8 mm, positioned 10 mm from the bottom of the plate, with a Camag microlitre syringe on precoated silica gel aluminum plate $60F_{254}$ (20 cm × 10 cm). The mobile phase consisted of chloroform: methanol (98:2, v/v, 20 ml) [17]. Linear ascending development was carried out in a twin trough glass chamber pre-saturated with mobile phase for 30 min at room temperature (25 ± 2 °C) at relative humidity of 55 ± 5%. The height of the solvent (mobile phase) front was 80 mm. Quantification was done in absorbance/reflectance mode of a Camag TLC scanner III at 366 nm. Standard solution (0.05 mg/ml in methanol) volumes of 2–10 μl was used for the preparation of a 5-point calibration curve corresponding to an amount of 100–500 ng.

2.7. Taguchi design

The Taguchi-based optimization technique was adapted for the process optimization of MAE of *C. longa* through dual heating mechanism. Taguchi-based optimization technique is a unique and powerful optimization discipline that allows optimization with minimum number of experiments [18]. Thus by this method, it is possible to reduce the time and cost for experimental investigations and improve the performance characteristics. In the present study, three levels are defined for each of the factors as summarized in Table 1. A L_9 orthogonal array scheme was adapted which needs 9 experiments to complete the optimization process [19]. The extraction results performed under orthogonal design conditions are shown in Table 2. The sequence in which the experiments were carried out was randomized to avoid any kind of personal or subjective bias. All the results at each step of the design are expressed as the mean of three experiments. After conducting the experiments, the results were converted into signal-to-noise (S/N) ratio data [20].

3. Results and discussion

3.1. S/N ratio analysis

The S/N ratio analysis was computing the signal-to-noise-ratio for each level of process parameters. Regardless of the category of the quality, the-lower-the-better, the-higher-the-better and the-nominal-the-better, a larger S/N ratio corresponds to better quality characteristics [20]. In other words, the optimal level of the process parameters is the level with the greatest S/N ratio. This is the foundation for the decision of the optimum level for each factor. Since the current study takes the percentage extraction of curcumin (w/w) as the quality characteristics, the higher-the-better criterion was applied when evaluating the S/N ratios of the various extraction parameters. A parameter effects plot was then generated from the results of the analysis of means (ANOM) test after conducting the S/N ratio calculations (Fig. 2).

3.2. Choice of extracting solvent

In all the conventional extraction approaches used in this work, acetone was used as the extracting solvent. Literature reveals that acetone has been frequently used for extraction

Table 2
The results of orthogonal test L_9 (3^4)

Tests	A	B	C	D	Percentage curcumin (w/w)	S/N ratio for percent curcumin (w/w)	Relative extraction efficiency (%)
1	1	1	1	1	1.37	7.50	36.35
2	1	2	2	2	4.29	17.78	98.16
3	1	3	3	3	4.09	17.00	93.59
4	2	1	2	3	4.00	16.81	91.53
5	2	2	3	1	3.19	14.84	72.99
6	2	3	1	2	3.39	15.37	77.57
7	3	1	3	2	3.12	14.65	71.39
8	3	2	1	3	4.03	16.87	92.21
9	3	3	2	1	4.98	18.72	113.95

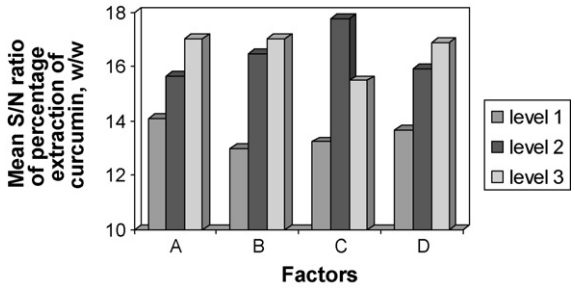

Fig. 2. Response graph illustrating the variation of the average S/N ratios plotted against the various extraction parameters. (A) Microwave power [level 1 = 60%, level 2 = 40%, level 3 = 20%]. (B) Irradiation time [level 1 = 1 min, level 2 = 2 min, level 3 = 4 min]. (C) Sieve number [level 1 = 10, level 2 = 20, level 3 = 40]. (D) Modifier volume [level 1 = 2 ml, level 2 = 4 ml, level 3 = 8 ml].

of curcumin from *C. longa* because of its greater solubilizing capacity for curcumin [8,17,21]. Acetone also heats up to a good extent under microwave effect due to its better dissipation factor ($\tan\delta = 0.5555$) so it was decided to use acetone as the extracting solvent in MAE. Methanol which has a very high dissipation factor ($\tan\delta = 0.6400$) was used in less quantity to improve the microwave absorbing capacity of the plant material.

3.3. Interaction of factors during extraction of curcumin from C. longa

Since there may be some interaction among parameters during MAE, the influence of this interaction on extraction needed to be considered when the conditions were optimized. For this purpose, graphs of the following variable pair were constructed: (a) the highest value for two factors; (b) the lowest value for two factors; (c) the highest and the lowest values for each pair of factors. In this method, interaction graphs for all pair of factors were obtained.

3.3.1. Interaction of irradiation time with other factors

Fig. 3a shows the effect of the interaction between irradiation time and microwave power on extraction efficiency of curcumin. At 20% microwave power and 4 min irradiation time extraction efficiency obtained was 41.8% more than 1 min irradiation time at the same power level. On the contrary, when 60% microwave power was used, the shorter the irradiation time, the higher the extraction efficiency. Interaction graph of irradiation time and particle-grinding degree (Fig. 3b) shows a different behavior for the tested compound. Particles screened through sieve 10 showed 30.6% higher extraction efficiency in 4 min than in 1 min. A similar type of response was seen with particles screened through sieve 40, which produced 20.9% higher extraction efficiency with 4 min irradiation than with 1 min.

Fig. 3c represents the interaction between irradiation time and modifier volume. It was seen that with increase in modifier volume a steady increase in the extraction efficiency takes place at both the irradiation time.

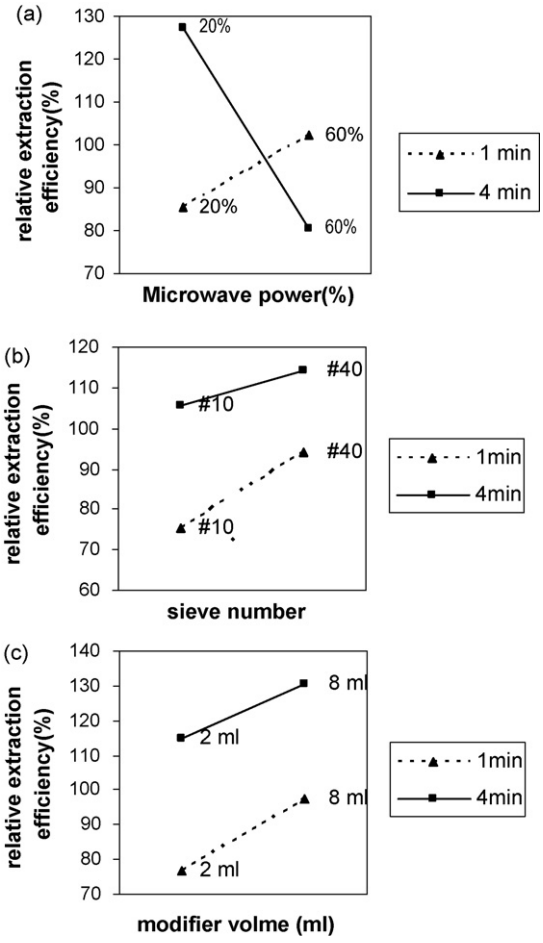

Fig. 3. The influence of the interaction of irradiation time with microwave power (a), sieve number (b) and modifier volume (c) on the extraction efficiencies of curcumin.

3.3.2. Influence of interaction of grinding degree and microwave power

Interaction is shown in Fig. 4a. Change in microwave power from 60% to 20% did not show much change in extraction efficiency with particles screened through sieve 10. At 20% microwave power, extraction efficiency was found to increase when particle-grinding degree was increased from sieve 10 to sieve 40. From the S/N ratio (Fig. 2) and from the interaction graph (Fig. 3a) it is quite evident that highest extraction efficiency can be produced by particles screened through sieve 20. On the other hand, at 60% microwave power extraction efficiency was found to decrease when particle-grinding degree was increased from sieve 10 to sieve 40.

3.3.3. Interaction between modifier volume and microwave power

Interaction between microwave power and modifier volume (Fig. 4b) had a great influence on the entire MAE. With the maximum volume of modifier used a 34.72% higher extraction efficiency was obtained using 20% microwave power than 60% microwave power. At 20% microwave power, the higher the modifier volume, the higher the extraction efficiency. However

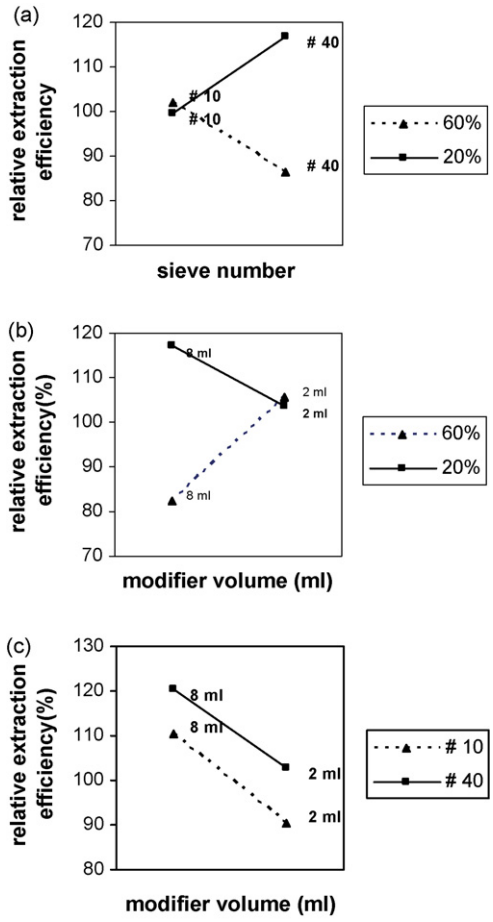

Fig. 4. (a) The effect of the interaction of grinding degree with microwave power on extraction efficiency of curcumin. (b) The effect of the interaction of modifier volume with microwave power on extraction efficiency of curcumin. (c) The effect of the interaction of modifier volume with grinding degree on extraction efficiency of curcumin.

at 60% microwave power, the lower the modifier volume the higher the extraction efficiency.

3.3.4. Interaction between modifier volume and grinding degree (sieve size)

According to the interaction graph between microwave power and grinding degree (Fig. 4c) extraction efficiency was found to increase with the modifier volume at the same sieve size. Increase in extraction efficiency by 10% was observed with 8 ml of modifier when particles were made finer.

3.4. Effect of modifier on the MAE process

Methanol, which has a very high dissipation factor ($\tan\delta = 0.6400$), will make the sample more vulnerable to microwave heating. In order to prove the effectiveness of modifier, the experiment using L_9 orthogonal design was performed but without the modifier column, i.e. no modifier was added. Fig. 5 shows the graphical representation of the variation in percentage extraction of curcumin (w/w) obtained under orthogonal design conditions, both in presence and absence of modifier. The difference in percentage extraction of curcumin (w/w) was

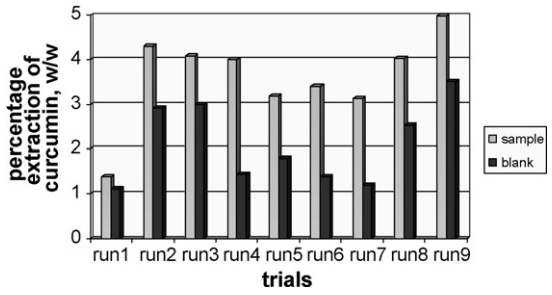

Fig. 5. Variation in percentage extraction of curcumin (w/w) obtained under orthogonal design conditions by MAE, using modifier (sample) and without modifier (blank).

found to be statistically significant (Student's t test, $p < 0.001$). Pretreatment of the powdered sample with methanol allows heating of the extraction system to proceed by at least two synergistic mechanisms: (a) direct heating from the interaction of microwaves with acetone, which also as a good dissipation factor ($\tan\delta = 0.5555$) and (b) from the diffusion of excess heat resulting from the interaction of the microwaves with the pretreated sample. Thus, microwave energy absorbed by the plant material generates a sudden increase in temperature inside the cells. The higher temperature attained by the cell wall, during MAE, causes dehydration of cellulose and reduces its mechanical strength, which allows the solvent to gain an easy entry inside the oleoresin cells and solubilize out curcumin in a much shorter time.

Thus, the optimum condition for MAE of curcumin as obtained from S/N ratio analysis and interaction studies was found to be, 20% microwave power, 4 min irradiation time, particles screened through sieve 20 and 8 ml of modifier.

3.5. Reproducibility and recovery of the MAE process

To determine the reproducibility of the novel extraction method five samples of the same weight (2 g) were processed under the optimum extraction conditions as obtained from the Taguchi design. The mean percentage extraction of curcumin (w/w) obtained under the optimized conditions was found to be 5.55, which was 27% more efficient than the conventional Soxhlet extraction. The calculated R.S.D. value was 3.5%, which shows that the proposed method has an acceptable precision. To estimate the extraction losses, some samples were spiked with a known quantity of standard curcumin. The percent loss was calculated on the basis of recovery of added curcumin by subtracting the values from unspiked samples. The results showed that recoveries were generally better than 93%. The repeatability of the chromatographic process was also considered. An amount of 2 g sample was processed under the optimal MAE conditions. The sample was analyzed repeatedly for five times under the same chromatographic conditions. The percentage extraction of curcumin (w/w) obtained was 5.57, 5.46, 5.49, 5.53, and 5.55. Hence, the R.S.D. of the chromatographic analysis was 0.81%. The recovery rates of the chromatographic process were investigated by adding known concentration of stock solutions of standard curcumin, to a pre-analyzed crude extract. Recovery values of curcumin obtained were 98.35, 96.86 and 99.13%.

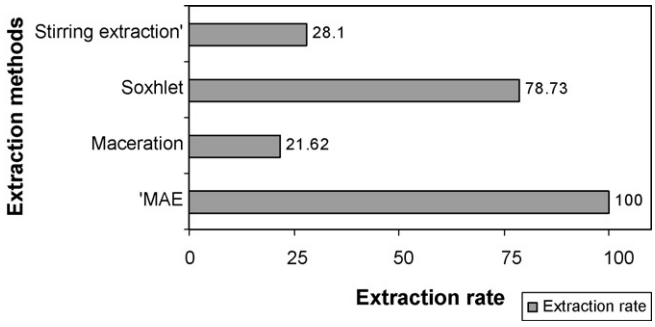

Fig. 6. Extraction rates (%) for different conventional extraction approaches, with respect to MAE yield obtained under optimum conditions. Extraction rate (%) for conventional approaches was calculated by considering the percentage extraction of curcumin (w/w) obtained from MAE as 100%.

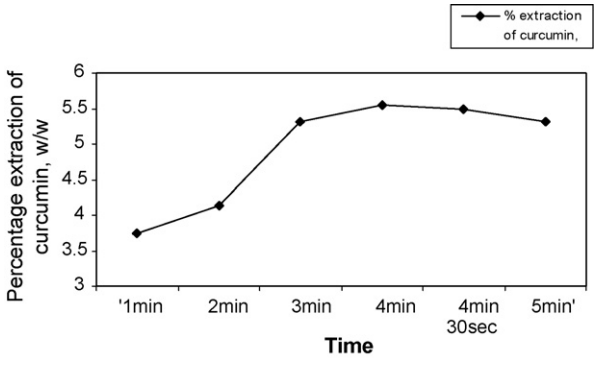

Fig. 7. Effect of extraction time on percentage extraction of curcumin. Extraction conditions: 20% microwave power, 4 min irradiation time, particles screened from sieve 20 and 8 ml of modifier.

3.6. Comparison of MAE with other conventional techniques

Comparison with the three conventional extraction techniques reveals that MAE can reach much higher yield in 4 min. The R.S.D. values for Soxhlet extraction, maceration and stirring extraction was found to be 8.4, 14.5 and 12.1 respectively; this indicates that the proposed MAE has better precision than other conventional approaches. Fig. 6 shows the extraction rate (%) obtained for different conventional approaches, calculated with respect to the highest yield obtained from MAE under optimum conditions.

4. Conclusion

The proposed MAE for curcumin showed drastic reduction in extraction time with much better precision, when compared to conventional extraction methods. The main mechanism responsible for extraction efficiency enhancement was the dual heating phenomenon of solvent and sample matrix, which resulted in effective rupture of plant cell wall. The simultaneous heating of the solvent and the sample further increased the solubility for curcumin. In addition, it was observed that when irradiation time was increased to 5 min (Fig. 7), dual heating proved detrimental as it resulted in reduction of percentage extraction of curcumin. This shows that dual heating is effective only when other supportive extraction parameters are set at the optimum level. The concept can be applicable to all natural products and if explored properly, can prove to be an efficient tool for sample preparation and large-scale industrial application.

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