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TIME-RELEASE FORMULATION OF ROSMARINIC ACID AS A POTENTIAL APPLICATION FOR SLEEP DISORDERS

Pharmaceutical Science

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ABSTRACT

Rosmarinic acid (RA) exhibits potential as a beneficial ingredient for promoting sleep-related advantages. The half-life of RA has been reported to vary within the range of 0.75 to 1 hour. The very brief duration of RA's half-life may provide challenges in fully capitalising on its sleep-related benefits. The aim of this study was to develop time-release formulations of RA by utilising various ratios of excipients. Additionally, the in-vitro release profiles of these formulations were assessed in order to identify one formulation for immediate release and another for delayed release. The granules with a concentration of 5% rosmarinic acid were prepared by using rosemary extract with a rosmarinic acid content of 20% as a starting material. These granules were formulated as both immediate release and delayed release formulations utilising the Extrusion and Spheronization process. Four different formulations of RA, each exhibiting immediate release and delayed release patterns, were developed by the use of different excipient systems in varying quantities. Based on the results obtained from the in-vitro dissolution studies, a specific formulation of immediate release granules was selected as a potential candidate, aiming to achieve full release of the active ingredient, RA, during the initial two hours following consumption. Additionally, a specific formulation of delayed release granules was selected for consideration based on its release profile, aiming to produce a delayed release within the time frame of 0-2 hours and a release rate of over 90% up to 6 hours following the ingestion of the RA granules. The utilisation of both immediate release and delayed release granules of RA could prove beneficial in sustaining optimal sleep quality for a duration of 7-8 hours. The utilisation of this particular combination would effectively enable the decrease of high doses of RA in human subjects, hence enhancing patient adherence to the prescribed treatment regimen. Further clinical investigations may be necessary to validate the efficacy of these formulations in relation to sleep.

KEYWORDS

Rosmarinic acid, Rosemary extract, Sleep, Time-release formulation

INTRODUCTION

Rosmarinic acid (RA) is a reddish-orange powder that is soluble in most organic solvents but insoluble in water. It is an ester of caffeic acid and 3, 4-dihydroxyphenyl lactic acid[1]. Rosmarinic acid is present in various botanical plants belonging to the Lamiaceae family, including rosemary, lemon balm, salvia, and oregano[1]. The literature has documented many biological activities associated with RA, including anti-depressant, sleep promoting effects, antioxidant, anti-inflammatory, anti-aging, and antiviral benefits[2].

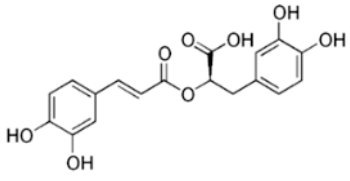

Figure 1: Structure Of Rosmarinic Acid

Plants containing high amounts of RA have been utilised as food and medicine for thousands of years. Several dietary supplements containing the active component of RA are available on the market, including extracts of Salvia rosmarinus, Melissa officinalis, and Perilla frutescens[3-5]. The reported oral bioavailability of RA ranges from 0.91% to 1.69%[6]. Based on a pharmacokinetic study in rats, the half-life of RA given orally was 63.68 minutes[7]. Another study reported that the analgesic effects of RA were diminished over time, showing that this molecule has a short half-life of about 45 minutes[8].

Rosmarinic acid has been used orally for insomnia in the dose range of 200 – 500 mg per day[9]. Further, the reported half-life of RA in humans is about 45 minutes. Due to the short half-life of RA, large doses are required to show therapeutic effects. Additionally, the short half-life of RA could also be responsible for the diminishing sleep effects after a few hours of sleep and therefore lack of efficacy. As a result of the short half-life, RA is a good candidate for the modified release dosage form to improve its resident time in the body which could increase the efficacy of RA in maintaining a positive impact on overall sleep efficiency and quality throughout the sleep duration of 7-8 hours.

Seldom attempts have been made to improve resident time of RA in the body. Extrusion and spheronization is the most widely used pelletization technique for controlled-release formulations[10].

The objective of this research was to develop time-release formulations of RA by utilising various ratios of excipients. Additionally, the in-vitro release profiles of these formulations were assessed in order to identify one formulation for immediate release and another for delayed release.

MATERIALS AND METHODS

Rosemary extract containing not less than 20% of rosmarinic acid was procured from Xi'An Imaherb Biotech Company Limited, China. According to the certificate of analysis, the actual concentration of rosmarinic acid determined by HPLC analysis was 21.85%. This was the raw material from which immediate and delayed release granules of 5% rosmarinic acid were manufactured. To arrive at a safe, stable, efficacious, and cost-effective formulation of rosmarinic acid granules using Extrusion & Spheronization technology, systematic experimentation was conducted. Firstly, pre-formulation studies were designed and carried out which included: (a) Physicochemical characterization of rosmarinic acid, (b) Study of physical properties (c) Analysis of rosmarinic acid and (d) Rosmarinic acid - excipients compatibility study. In the second phase of the study, experiments were designed and carried out to develop, formulate and evaluate immediate and delayed release granules of 5% rosmarinic acid.

Following steps were followed for making immediate and delayed release granules containing NLT 5% rosmarinic acid:

1. Rapid mixing granulator (RMG) was loaded with rosemary extract powder 20%, microcrystalline cellulose, and croscarmellose sodium for immediate release granules, and rosemary extract powder 20% and microcrystalline cellulose for delayed release granules as per formulas stated in Table 4 and 6.

The dry mixing process was conducted at an impeller speed of 200 revolutions per minute (rpm) for a duration of 5 minutes, resulting in the formation of a homogeneous powder.
2. The required quantity of water and ethanol was weighed as per the formula.
3. In the initial stage, half quantity of water and ethanol combination was gradually introduced into the RMG for a duration of 5 minutes. This process was carried out at an impeller speed of 200 rpm and a chopper speed of 300 rpm.
4. Following the addition of water and ethanol combination, the rotational speed of the chopper was increased to 400 rpm, and the mixing process was sustained for an additional duration of 5 minutes.
5. The mixing process was stopped and the wet mass was manually extracted from the walls. Subsequently, the mixture was mixed for an additional duration of 2 minutes, with the impeller speed set at 200 rpm and the chopper speed set at 400 rpm, in order to achieve a homogeneous wet mass.
6. The remaining half quantity of the water and ethanol combination from step 2 was added gradually to step 5 over a period of 5 minutes. The mixture was then mixed for an additional 5 minutes at an impeller speed of 200 rpm and a chopper speed of 400 rpm.
7. The wet mass was transferred from the RMG into a polythene bag that was clean, dry, and food grade. Subsequently, the wet mass was subjected to the subsequent E&S procedure.
8. The wet mass that was collected in a clean and dry polythene bag during step 7 was afterwards processed through an extrusion machine equipped with a 0.5 mm radial screen.
9. The screw speed was maintained within the range of 40-50 rpm in order to provide a consistent and uniform extrude.
10. All extrude were collected in a tray in preparation for the subsequent Spheronization process.
11. A quantity of roughly 0.2 kg of extrude was placed onto a Spheronizer plate with a pitch of 2.0 mm. Spheronization was initiated at 500 to 700 rpm for a duration of 2 to 3 minutes, with the objective of obtaining spherical granules.
12. The granules were removed from the Spheronizer and subsequently subjected to drying in a tray drier using an oven for a duration of 2-3 hours. The drying process was carried out at a controlled temperature of 60±5°C until the desired level of moisture content, as indicated by the loss on drying (LOD), was reduced to below 5%.
13. The dry granules were sieved using a 30# sieve attached to the Vibro sifter.
14. The fines of the dried granules were separated by passing them through an 80# sieve fitted on the Vibro sifter. The material that was kept on the 80# sieve was collected in a polythene bag that was food grade. This collected material was labelled as Granule 1, which was an immediate release granule containing no less than 5% rosmarinic acid.
15. The material that did not pass through the 80# sieve was discarded.
16. Following the process of sifting, the collected samples were next subjected to assay and in-vitro dissolution testing.

Additional steps for preparing delayed release granules containing NLT 5% rosmarinic acid:

17. The coating process was conducted using granules with a mesh size ranging from #30 to #80. The granules were coated according to a specified formula in Table 6 using a Fluid Bed Coater machine (FBC) at a temperature range of 25-30°C.
18. Once the coating process was finished, the dry granules were sieved using a 25# sieve that was put onto the Vibro sifter.
19. Following the process of filtration, the collected samples were then evaluated for assay and in-vitro dissolution testing.

Analytical studies were conducted to evaluate the content of rosmarinic acid in immediate and delayed release granules as against the set assay of 5% and to study the release profiles using in-vitro dissolution studies with suitable dissolution media. The immediate release granules were evaluated for in-vitro release in 0.1 N HCL (1-2 hrs) whereas delayed release granules were evaluated in 0.1 N HCL (1-2 hrs), pH 5.5 (2-4 hrs) and pH 6.8 (5-6 hrs) phosphate buffer. USP type II (Paddle) apparatus was used with temperature set at 37° C and dissolution media volume of 500 ml. Following HPLC conditions were used to determine the content of rosmarinic acid in immediate and delayed release granules:

Wave length	:	330 nm
Flow Rate	:	0.5 mL per minute
HPLC Column	:	Zorbax Eclipse plus C18; 4.6 nm X 150 mm, 5µm
Column Temperature	:	30°C
Injection Volume	:	50 µl
Run time	:	15 minute
Mobile Phase Preparation	:	Transfer 700 mL of 0.1% formic acid and 300 mL acetonitrile, sonication for 5 minutes.
Diluent	:	30% Acetonitrile in water

Additionally, accelerated stability studies were conducted on the shortlisted immediate and delayed release formulations by keeping the granules in aluminium pouch, temperature: 40° C, relative humidity: 75% for 1 month.

RESULTS

The results of pre-formulation studies are reported as follows:

a. Physico-chemical characterization

The rosemary extract containing NLT 20% rosmarinic acid was available in the powder from, brown yellow in color, had characteristic odor and was bitter in taste.

b. Study of physical properties

Physical properties of rosemary extract powder containing NLT 20% rosmarinic acid was studied with following observations to decide the formulation strategies.

i. Bulk Density (gm/ml)

Bulk density of rosemary extract powder containing 20% rosmarinic acid was found to be 0.45 gm/ml.

ii. Tap Density (gm/ml)

Tap density of rosemary extract powder containing 20% rosmarinic acid was found to be 0.65 gm/ml.

iii. Particle size (micron)

Particle size of rosemary extract powder containing 20% rosmarinic acid was measured using Malvern particle size analyser and was found to be as follows:

Table 1: Particle Size Of Rosemary Extract Powder Containing 20% Rosmarinic Acid

Mean diameter	Particle size (micron)
D10	5.09
D50	24.3
D90	50.2

iv. Angle of Repose

Angle of rosemary extract powder containing 20% rosmarinic acid was found to be 42.13º suggesting very poor flowability.

Table 2: Angle Of Repose Of Rosemary Extract Powder Containing 20% Rosmarinic Acid

Angle of Repose	Flowability
< 25	Excellent
25 – 30	Good
30 – 40	Passable
> 40	Very poor

Analysis of Rosmarinic Acid

Conducting an analysis of rosmarinic acid was a crucial aspect of pre-formulation investigations. The analysis of samples for assay and dissolution studies was conducted using a high-performance liquid chromatography (HPLC) analytical method available from the literature.

Compatibility Studies of Excipients at 40°C/75%RH

The objective of conducting the compatibility studies was to investigate potential interactions between the rosemary extract powder containing 20% rosmarinic acid (RA) and the inactive ingredients. In order to investigate the compatibility of RA with inactive ingredients, physical mixtures were produced using the specified ratios as presented in the Table 3. These mixtures were then placed in sealed glass vials and subjected to chemical stability evaluation. The evaluation involved storing the compatibility blends in stability ovens at a temperature of 40°C and a relative humidity of 75% for a duration of four weeks. A range of excipients were chosen for the purpose of screening, taking into account their respective functionalities. These excipients were subsequently subjected to drug excipient compatibility testing. The tabulated data below presents the initial results and subsequent data of the blend, which was stored for a duration of four weeks at a temperature of 40°C and a relative humidity of 75%, both in open and closed conditions.

Table 3: Physical Observation Of Compatibility Studies Of RA

Sr. No.	Ingredients	Ratio	Initial Observation	2 weeks, 40°C/75% RH	4 weeks, 40°C/75% RH
1	RA: Microcrystalline Cellulose	1:1	Brownish white powder	Brownish white powder	Brownish white powder
2	RA: Microcrystalline Cellulose	1:2	Brownish white powder	Brownish white powder	Brownish white powder
2	RA: Croscarmellose sodium	1: 0.5	Brownish white powder	Brownish white powder	Brownish white powder
3	RA: Kollicoat 30%	1: 0.5	Brownish white powder	Brownish white powder	Brownish white powder
4	RA: Hydroxypropyl methylcellulose	1:2	Brownish white powder	Brownish white powder	Brownish white powder
5	RA: Talc	1: 0.3	Brownish white powder	Brownish white powder	Brownish white powder
6	RA: Colloidal silicon dioxide (Aerosil 200 Pharma)	1: 0.2	Brownish white powder	Brownish white powder	Brownish white powder

Preparation Of Immediate Release Granules Containing NLT 5% Rosmarinic Acid

As per the formula mentioned in the Table 4, four different formulations were made for the immediate release granules containing NLT 5% rosmarinic acid using different quantity of excipients using the Extrusion and Spheronization (E&S) process.

Table 4: Formula For Preparing Immediate Release Granules Of NLT 5% Rosmarinic Acid

Sr. No.	Excipients	Role	Formulation 1	Formulation 2	Formulation 3	Formulation 4
1	Rosemary extract 20%	API	30%	30%	30%	30%
2	Microcrystalline cellulose	Diluent	70%	69.5%	68.5%	67%
3	Croscarmellose sodium	Disintegrant	-	0.5%	1.5%	3%
4	Ethanol	Vehicle	qs	qs	qs	qs
5	Treated RO Water	Vehicle	qs	qs	qs	qs
	Total		100%	100%	100%	100%

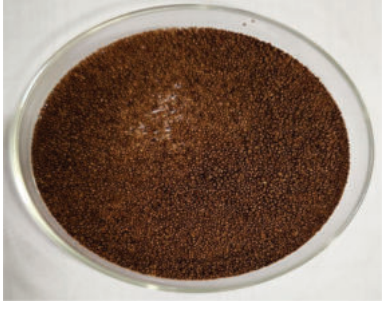

Figure 2: Immediate Release Granules Of Rosemary Extract Powder Containing NLT 5% Rosmarinic Acid

The assay content of rosmarinic acid was determined using an HPLC based method (Figure 3: Chromatogram of rosmarinic acid).

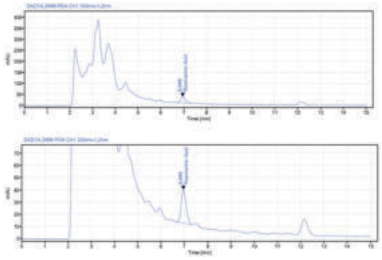

Figure 3: Chromatogram Of Rosmarinic Acid

Table 5: Assay Content Of Immediate Release Granule Formulations

Assay	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Assay content of rosmarinic acid	5.1%	5.3%	5.4%	5.3%

Observation: It was observed that all the four immediate release granules contained NLT 5% rosmarinic acid.

In-vitro Dissolution Studies On Immediate Release Granules Of Rosmarinic Acid 5%

All the four formulation trials were taken and the immediate release granules were studied for their release in 0.1 N HCL using in-vitro dissolution studies. Based on the release profile of immediate release granules, Formulation 4 was shortlisted for the project work considering the requirement for immediate release of rosmarinic acid from the granules. The other formulations showed either slow release at the first hour or an incomplete release at 2 hours which was not desirable (Figure 4: In-vitro release profile of immediate release granules of rosmarinic acid).

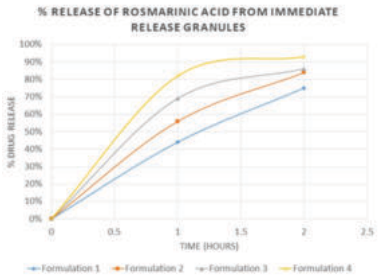

Figure 4: In-vitro Release Profile Of Immediate Release Granules Of Rosmarinic Acid

Preparation Of Delayed Release Granules Containing NLT 5% Rosmarinic Acid

As mentioned in the Table 6, different formulations were made for the delayed release granules of rosemary extract powder with different quantity of excipients using the Extrusion and Spheronization (E&S) process mentioned below and further coating these core granules with delayed release polymer was performed using Fluid Bed Coater.

Table 6: Formula For Preparing Delayed Release Granules Of NLT 5% Rosmarinic Acid

Sr. No.	Excipients	Role	Formulation 1	Formulation 2	Formulation 3	Formulation 4
1	Rosemary extract 20%	API	30%	30%	30%	30%
2	Microcrystalline Cellulose	Diluent	60%	55%	50%	45%
4	Kollicoat 30%	Delayed release polymer	10%	15%	20%	25%
5	Ethanol	Vehicle	qs	qs	qs	qs
6	Treated RO Water	Vehicle	qs	qs	qs	qs
	Total		100%	100%	100%	100%

Figure 5: Delayed Release Granules Of Rosemary Extract Powder Containing NLT 5% Rosmarinic Acid

The assay content of rosmarinic acid was determined using an HPLC based method (Figure 2: Chromatogram of rosmarinic acid).

Table 7: Assay Content Of Delayed Release Granule Formulations

Assay	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Assay content of rosmarinic acid	5.3%	5.4%	5.1%	5.2%

Observation: It was observed that all the four delayed release granules contained NLT 5% rosmarinic acid.

All the four formulation trials were taken and the delayed release granules were studied for their release in 0.1 N HCL (1-2 hrs), pH 5.5 (2-4 hrs) and pH 6.8 (5-6 hrs) phosphate buffer using in-vitro dissolution studies. Based on the release profile of delayed release granules, Formulation 4 was shortlisted for the project work considering the requirement for a delayed release (<10%) in 2 hours and >90% release of rosmarinic acid in 6 hours from the granules. The other formulations showed either faster release in acidic pH or showed incomplete release in pH 5.5 condition and pH 6.8 conditions (Figure 6: In-vitro release profile of delayed release granules of rosmarinic acid).

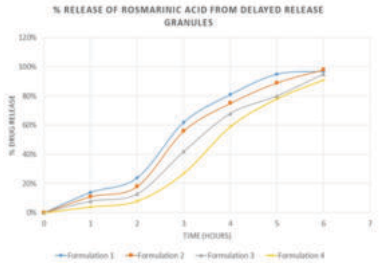

Figure 6: In-vitro Release Profile Of Delayed Release Granules Of Rosmarinic Acid

Based on the shortlist of granule formulation 4 for the immediate release and delayed release formulations, the final release profile of the shortlisted formulation of rosemary extract granules containing NLT 5% rosmarinic acid was as per (Figure 7: In-vitro release profile of combination of immediate and delayed release granules of NLT 5% rosmarinic acid).

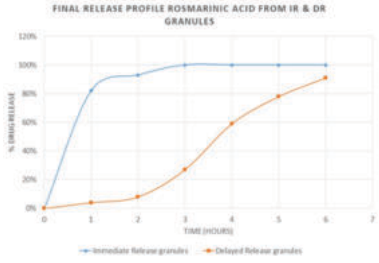

Figure 7: In-vitro Release Profile Of Combination Of Immediate And Delayed Release Granules Of NLT 5% Rosmarinic Acid

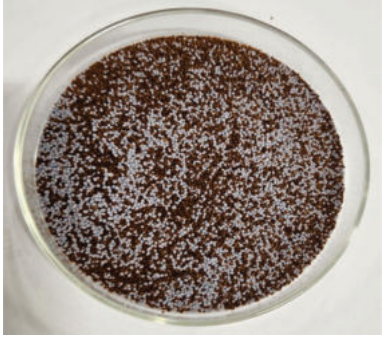

Figure 8: Combination Of Immediate And Delayed Release Granules Of NLT 5% Rosmarinic Acid

Table 8: Assay Of Immediate & Delayed Release Granules Of Rosmarinic Acid 5% Based On Accelerated Stability Studies

Product Details	Initial assay	1 month assay	% drop
Immediate release granule	5.30%	5.15%	2.8%
Delayed release granule	5.20%	5.06%	2.7%

Observation: Both immediate and delayed release granules of rosmarinic acid 5% were found to be stable with <3% drop in the assays values under accelerated storage conditions of Temperature: 40° C and Relative Humidity: 75%.

DISCUSSION

Rosmarinic acid has been reported to possess several biological properties like sleep promoting activity, antidepressant, antioxidant, anti-inflammatory, anti-aging, antiviral, and antibacterial effects.

The short half-life and poor bioavailability of rosmarinic acid in the treatment of sleep related disorders remains one of the major drug delivery concerns of nutraceutical manufacturers and researchers. Further, the short half-life of RA could be responsible for the diminishing sleep effects after a few hours of sleep and therefore lack of efficacy.

As a result of the short half-life, rosmarinic acid was a good candidate for the modified release dosage form to improve its half-life in the body which could increase the efficacy of rosmarinic acid in maintaining a positive impact on overall sleep efficiency and quality throughout the sleep duration of 7-8 hours.

Few formulations have been evaluated experimentally to effectively deliver RA like nanoparticles loaded with RA using poly(lactic-co-glycolic acid) polymeric matrix which showed excellent encapsulation efficiency and release of RA in a rate-controlled manner[11]. In another study, esterification with the right length of alkyl chains (C1-C4) proved to be a promising method to increase the in vivo bioavailability of rosmarinic acid[12]. A different study using transdermal administration of RA-loaded ethosomes (ETHs) and liposomes (LPs) showed that ETHs were more efficacious than LPs for the transdermal administration of RA[13]. Further, a study evaluated the bioaccessibility of a rosmarinic acid-phospholipid complex (RA-PLC) utilising the TNO dynamic intestinal model-1 (TIM-1) which showed that complexation by phospholipids decreased the bioaccessibility of RA in the jejunum compartment while maintaining bioaccessibility in the ileum compartment[14]. Another study attempted the use of liposomes to efficiently encapsulate rosmarinic acid (RA) and carvacrol (CA) to address its poor bioavailability[15]. However, none of these approaches used the concept of time-release formulations which was the basis of our research work. To our knowledge, this is the first time making time-release granules of rosmarinic acid in experiments, as it has not been attempted and reported in the public domain.

Based on the physical observations of drug-excipients complex at the initial stage and after 4 week exposure at 40°C/75%RH conditions, no change was observed in the color of drug-excipients complex suggesting a good compatibility between drug and excipients for use in the formulation development.

Four formulations of immediate release granules were made using Extrusion and Spheronization technology with rosemary extract containing 20% rosmarinic acid, and different percentages of excipients like microcrystalline cellulose, and croscarmellose sodium to shortlist one formulation. The granules showed different release profiles for 2 hours in 0.1 N HCL. Formulation 4 was shortlisted for the project work considering the requirement for an immediate release of rosmarinic acid from the granules. Formulation 4 showed this faster release probably due to the presence of optimal amounts of disintegrant which helped in faster disintegration of these granules in 0.1 N HCL that was desired to show a quick release to provide an instant effect to induce sleep by reducing sleep latency. The other formulations showed either slow release at the first hour or an incomplete release at 2 hours which was not desirable.

Similarly, four formulations of delayed release granules were made using Extrusion and Spheronization technology and fluid bed coating with rosemary extract containing 20% rosmarinic acid, and different percentages of excipients like microcrystalline cellulose, and Kollicoat 30% polymer to shortlist one formulation. The granules showed different release profiles for 2 hours in 0.1 N HCL, up to 4 hours in pH 5.5 buffer and up to 6 hours in pH 6.8 buffer. Formulation 4 was shortlisted for the project work considering the requirement for a delayed release (<10%) in 2 hours and >90% release of rosmarinic acid in 6 hours. Formulation 4 showed this release probably due to better coating of the granules which demonstrated drug release more specifically in pH 5.5 and 6.8 that was desired to show a longer release

to provide better sleep quality through 8 hours of sleep. The other formulations showed either faster release in acidic pH or showed incomplete release in pH 5.5 condition and pH 6.8 conditions which was undesirable considering the scope of the project. Further, both immediate and delayed release granules of rosmarinic acid 5% were found to be stable with <3% drop in the assays values under accelerated storage conditions of Temperature: 40° C and Relative Humidity: 75%.

The limitation of this research is that we could not obtain 100% pure rosmarinic acid. Instead the experiments were carried out using rosemary extract containing 20% rosmarinic acid. Nevertheless, the experiments performed using E&S technology would replicate the release profiles if performed on 100% rosmarinic acid as the starting material.

CONCLUSION

Four different formulations of rosmarinic acid each with immediate release and delayed release profiles were prepared by using different types of excipient systems in varying proportions. Based on the in-vitro dissolution studies, immediate release granules were selected so as to achieve complete release of rosmarinic acid in the first 2 hours of consumption. Further, delayed release granules were shortlisted from based on the release profile to achieve a delayed release between 0-2 hours and >90% release up to 6 hours after consumption of rosmarinic acid granules. A combination of immediate release and delayed release granules of rosmarinic acid would be helpful to maintain effective sleep quality for 7-8 hours of sleep duration. Use of this combination would also facilitate reduction in large dose size of rosemarinic acid to proposed dose of 100-200 mg for humans to improve patient compliance. Further clinical studies may be needed to confirm the usability of these formulations on sleep.

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