

# Comparison of formulation characteristics of drugs and dietary supplements containing alpha-lipoic acid relevant to therapeutic efficacy

M.N. LALIĆ-POPOVIĆ<sup>1,2</sup>, M.M. VUKOVIĆ<sup>1</sup>, J.N. JOVIČIĆ-BATA<sup>1</sup>,  
J.M. ČANJI-PANIĆ<sup>1</sup>, N.B. TODOROVIĆ<sup>1</sup>

<sup>1</sup>Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia

<sup>2</sup>Centre for Medical and Pharmaceutical Investigations and Quality Control (CEMPHC), Faculty of Medicine, University of Novi Sad, Novi Sad, Republic of Serbia

**Abstract. – OBJECTIVE:** Healthcare professionals lack the knowledge about the impact of formulations on treatment effectiveness. This is further complicated by the existence of dietary supplements containing the same active pharmaceutical ingredients (API) as drug formulations [e.g., alpha-lipoic acid (ALA)], to which the strict formulation testing requirements do not apply. This research aimed to compare ALA-containing drugs and dietary supplements through the determination of uniformity of content, disintegration time and dissolution rates.

**MATERIALS AND METHODS:** A total of seven different ALA formulations (5 dietary supplements, 2 drugs) were tested for uniformity of content, disintegration time and dissolution rates. All tests were performed in accordance with the 10<sup>th</sup> European Pharmacopoeia. ALA was determined spectrophotometrically.

**RESULTS:** Uniformity of content testing revealed larger variations of ALA content in three formulations of dietary supplements. Dissolution curves generated at 50 and 100 rpm differed significantly. Testing requirements were met only by one dietary supplement at 50 rpm, and one drug and two dietary supplements at 100 rpm. Disintegration testing showed limited impact on the release kinetic of ALA, as opposed to formulation type.

**CONCLUSIONS:** Considering the lack of regulation on dietary supplement formulations and the variable success of them conforming to pharmacopoeial requirements, it is an imperative for stricter regulations on the dietary supplements' formulations to be imposed globally.

*Key Words:*

Alpha-lipoic acid, Dietary supplements, Dietary supplements regulation, Formulation characterization.

## Introduction

The availability of multiple products of drugs and dietary supplements with similar ingredients, different formulations and lack of health care professionals' understanding of the formulation impact on treatment efficacy is a problem in many countries worldwide<sup>1,2</sup>. Dietary supplements formulations are more diverse in comparison to drugs. In addition, supplements are available without prescription, but some contain active pharmaceutical ingredients (API) which are also present in existing drugs. There are regulatory requirements that allow for the same active substance in the same dose to be registered as a medicine and as a dietary supplement<sup>3</sup>. As there are no equal quality control requirements for these two product groups, the choice of appropriate product for or by patients/consumers may be wrong. *In vitro* methods like disintegration and dissolution testing in some pharmacopoeias, e.g., USP<sup>4</sup>, are adapted for dietary supplement products. However, in order to compare the quality of products with the same API that are registered as dietary supplements as well as medicines in the same dose, equal *in vitro* tests should be conducted.

Alpha-lipoic acid (ALA, synonym: thioctic acid, ATC code: A16AX01) is formulated both, as a medicine and a supplement, with no evident difference in dosage form. ALA is a potent natural antioxidant that is synthesized by lipoic acid synthase from octanoic acid and a sulphur source<sup>5</sup>. It acts as a cofactor for mitochondrial respiratory enzymes<sup>6,7</sup>, a powerful antioxidant<sup>8</sup>, and an anti-inflammatory agent<sup>9-11</sup>. ALA has a strong negative redox potential, and it can recycle other

antioxidants. ALA reduces oxidative stress, a pathogenic element in a variety of diseases, thus the use of ALA-containing dietary supplements has considerably grown in the past 2 years. ALA is particularly suitable for the prevention and/or treatment of diabetes complications<sup>5,12</sup>. It is synthesized by the human body but in insufficient amount to fulfil the energy requirements of the cell. Thus, it is mostly obtained from the diet, especially from meat and vegetables. Fruits are also a source of this acid<sup>13</sup>.

ALA is a relatively small, amphiphilic molecule with a dithiolane ring and carboxyl group in its structure. It is a weak acid with pKa reported in different sources to be between 4.52 and 5.3<sup>14,15</sup>. ALA is sparingly soluble in water<sup>16</sup>, but soluble in organic solvents<sup>16,17</sup>. It has two enantiomeric forms, S and R. The R isomeric form is present naturally, while the S isomer is prepared through chemical processes<sup>18</sup>. Various studies<sup>19,20</sup> suggested that ALA is rapidly absorbed in the gut, taken up into various tissues in which it is metabolized, and then excreted. The acidic pH of the stomach favors the gastric absorption of a weak acid such as ALA<sup>20-22</sup>. However, instability of ALA in the stomach, its low solubility, and hepatic degradation lead to small bioavailability of ALA, of about 30%<sup>23</sup>. Transporter-mediated transport is the major mechanism for intestinal absorption of ALA<sup>20,22,24</sup>, but it is still not clear which transporter is involved in the absorption process. Based on current research<sup>20,22,24</sup>, monocarboxylic acid transporter (MCT)<sup>20</sup> and sodium-dependent multivitamin transporter (SMVT)<sup>22,24</sup> are proposed. These characteristics of ALA resulted in a vast variety of pharmaceutical formulations. ALA is commonly formulated in various solid dosage forms such as immediate-release tablets, soft and hard capsules and delayed-release gastro-resistant tablets. Dissolution and disintegration testing are used in the development, optimization and quality control in drug products, however in many countries these tests are not required for dietary supplements as the latter are often regulated as foods and not pharmaceutical products<sup>25</sup>. Thus, many quality control tests for dietary supplements are not legally required during the registration process<sup>26</sup>. In the case of ALA, impact of formulation on release kinetic could affect the efficacy of supplementation, since ALA is considered to be class II substance in the biopharmaceutical system of classification, thus formulation of solid dosage form has a great impact on its dissolution profi-

le. Limited and/or decreased rate of ALA release from its formulations in correlation with its pharmacokinetic characteristics might affect its efficacy.

Considering the ongoing research<sup>13,19,23,27-30</sup> on ALA's potential uses in a variety of different fields, from obesity to gynecology, it can be expected for the interest in ALA supplements to rise in the following years. Therefore, the aim of this study was to compare dietary supplements and formulations of medicines that contain ALA through determination of uniformity of content, disintegration time and dissolutions rate. Additionally, all dosage forms were tested under lower and higher hydrodynamic conditions to determine the stability of release rate of ALA from various formulations. This study aimed to underline the importance of knowledge about similarities and differences among different solid dosage forms of drug and dietary supplements among health care professionals.

## Materials and Methods

ALA powder was purchased from Farmalabor, Canosa di Puglia, Italy. Methanol (Lachner, Neratovice, Czechia). Dissolution medium was 0.5% water solution of sodium lauryl sulphate (Lachner, Neratovice, Czechia). This medium was chosen to increase the solubility of ALA so that dissolution profiles are dependent only on the formulation factors.

Study was conducted in 2021. At that time, seven different ALA formulations were available at the Serbian market. Samples of each formulation were obtained from local pharmacies in Novi Sad, Serbia. Two formulations were registered as medicines (A, B) and the other five were ALA dietary supplements (C, D, E, F and G). The compositions of all seven formulations are shown in the Table I. Formulation C had the same dose of ALA as drug formulations. Formulations B, C and D are tablets, formulations A and F are soft gelatin capsules, and formulations E and G are hard hypromellose capsules.

### *Ultraviolet and Visible (UV-Vis) Spectrophotometry*

Concentration of ALA was determined using previously published UV-Vis spectrophotometric method<sup>31</sup>. The absorbance of ALA was measured at 333 nm using Agilent Technologies UV-Vis spectrophotometer (model 8453, Santa Clara, CA,

## Formulation comparison of drugs and dietary supplements of alpha-lipoic acid

**Table 1.** Characteristics of the analyzed ALA formulations.

Formulation	Pharmaceutical form	Labelled content of ALA [mg/dosage unit]	Excipients: Fill/Core	Excipients: Shell/Coating
A	Capsule, soft	600	Solid fats, medium chain length triglycerides (Vehicle/Diluent)	Gelatin (Coating agent/ Vehicle); Sorbitol, liquid (non-crystallizing), Glycerol (Moisturizing agent/Plasticizer); Carmine lacquer (E 120), Titanium dioxide (E 171) (Coloring and opacifying agent)
B	Film-coated tablet	600	Microcrystalline cellulose, Lactose monohydrate (Vehicle/Diluent); Hypromellose (binder); Colloidal anhydrous silica, Talc, Magnesium stearate, Dimethicone (Glidant/Lubricant/Anti-adhesive agent); Cross-linked sodium carboxymethylcellulose (Disintegrant)	Macrogol 6,000, Hypromellose, Talc (Coating agent/Vehicle); Sodium lauryl sulphate (Surfactant)
C	Tablet (double-layered)	600	Microcrystalline cellulose (Vehicle/Diluent); Hypromellose (binder); Magnesium stearate (Glidant/Lubricant/Anti-adhesive agent)	
D	Film-coated tablet	300	Microcrystalline cellulose, Dicalcium phosphate (Vehicle/Diluent); Magnesium salts of fatty, Silicon dioxide (Glidant/Lubricant/Anti-adhesive agent); Cross-linked sodium carboxymethylcellulose (Disintegrant)	Hypromellose (Coating agent/Vehicle); Polyethylene glycol (Moisturizing agent/Plasticizer); Titanium dioxide (E 171) (Coloring and opacifying agent)
E	Capsule, hard	300	Microcrystalline cellulose (Vehicle/Diluent); Hypromellose (binder); Magnesium stearate, Silicon dioxide, Talc (Glidant/Lubricant/Anti-adhesive agent)	Hypromellose (Coating agent/Vehicle)
F	Capsule, soft	300	Fatty acid triglycerides, Magnesium stearate, polyglyceryl oleate, soy lecithin in soybean oil (Vehicle/Diluent)	Yellow iron oxide (E 172), Titanium oxide (E 171) (Coloring and opacifying agent)
G	Gastro-resistant capsule, hard	300	Gellam gum (Vehicle/Diluent)	Hypromellose (Coating agent/Vehicle)

USA). Concentrations were calculated based on the calibration curve that was linear over the range 0.08-2.4 mg/mL ( $R^2 = 0.9996$ ).

### **Uniformity of Content**

Uniformity of content of single-dose preparations was tested in accordance with the requirements of 10<sup>th</sup> European Pharmacopoeia (2.9.6)<sup>17</sup>.

The individual content of ALA in 10 dosage units, randomly chosen from each product, was determined. Hard-shell and soft-shell capsules were opened, and capsule fill was transferred into 100 ml volumetric flask, tablets were crashed and homogenized then transferred to 100 ml volumetric flask. Extraction was performed using HPLC grade methanol (J.T. Baker, Radnor, PA, USA).

The content of ALA substance was determined spectrophotometrically. The dosage form was considered to comply with the test if not more than one individual content is outside the limits of 85% to 115% of the average content and none is outside the limits of 75% to 125% of the average content.

### Disintegration Test

Disintegration of tablets and capsules was performed in accordance with the 10<sup>th</sup> European Pharmacopoeia (2.9.1.), using a disk and 0.5% sodium lauryl sulphate water solution as immersion fluid and an apparatus with six cylinders (Erweka ZT54, Langen, Germany). Test A was performed using six randomly selected dosage units (tablets or capsules). Test was performed until all six dosage forms disintegrated<sup>17</sup>.

### Dissolution Test

Dissolution protocol was designed in accordance with the requirements of 10<sup>th</sup> European Pharmacopoeia (2.9.3.) for solid dosage forms using a dissolution tester (Erweka DT 600, Langen, Germany). Apparatus 1 was used for capsules, and Apparatus 2 for tablets. For every product tested, dissolution was performed using two rotation speeds (50 and 100 rpm) and 900 mL of 0.5% sodium lauryl sulphate water solution as a dissolution medium. This medium and two rotation speeds (low and high hydrodynamic conditions) were chosen as conditions suitable to determine the effect of formulation on dissolution rate. Sampling of 5 mL of dissolution medium was performed at 5<sup>th</sup>, 15<sup>th</sup>, 25<sup>th</sup>, 35<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> minute, with compensation

of the taken volume. The content of the released substance was determined spectrophotometrically. The measurements were taken for six dosage units of each formulation.

Compliance of the obtained results with the requirements of the 10<sup>th</sup> European Pharmacopoeia was observed in accordance with the guidelines<sup>17</sup> given in Chapters 5.17.1 and 2.9.3. Specifically, the test requirements have been met if all six of the tested conventional-release forms released at least 80% of the content within 45 minutes of the test or less (level S1). All formulations were tested under the same conditions to obtain comparable data.

### Statistical Analysis

The data were presented as mean value  $\pm$  standard deviation, where suitable. Dissolution profiles were compared using ANOVA test (post-hoc Tukey HSD). Statistical comparison was used to detect differences between profiles within the same formulation after 50 rpm vs. 100 rpm. Differences were considered statistically significant if  $p < 0.05$ . Comparisons were performed within the same type of solid formulations (tablet with tablet and capsule with capsule).

## Results

Content of ALA in all formulations was within 85-115% of average content. Uniformity of content is presented in Table II and III. Larger standard deviations of content were measured in

**Table II.** Content of ALA [mg] in formulations with dose of 600 mg per unit [average  $\pm$  standard deviation (SD)] with per cent to average content.

Formulation	A		B		C	
	mg of ALA	%	mg of ALA	%	mg of ALA	%
1	610.33	100.7	589.37	100.2	661.35	113.5
2	602.11	99.3	590.20	100.3	565.61	97.1
3	606.32	100.0	591.80	100.6	523.51	89.9
4	607.96	100.3	580.14	98.6	665.35	114.2
5	603.87	99.6	579.14	98.5	572.79	98.3
6	604.53	99.7	589.08	100.1	528.51	90.7
7	609.01	100.5	592.72	100.8	610.36	104.8
8	607.58	100.2	593.37	100.9	662.64	113.7
9	608.32	100.4	590.59	100.4	531.91	91.3
10	601.93	99.3	586.12	99.6	571.64	98.1
Average	606.19	100.0	588.25	100.0	589.37	101.2
SD	2.94	0.5	4.98	0.8	57.02	9.8

## Formulation comparison of drugs and dietary supplements of alpha-lipoic acid

**Table III.** Content of ALA [mg] in formulations with dose of 300 mg per unit [average  $\pm$  standard deviation (SD)] with per cent to average content.

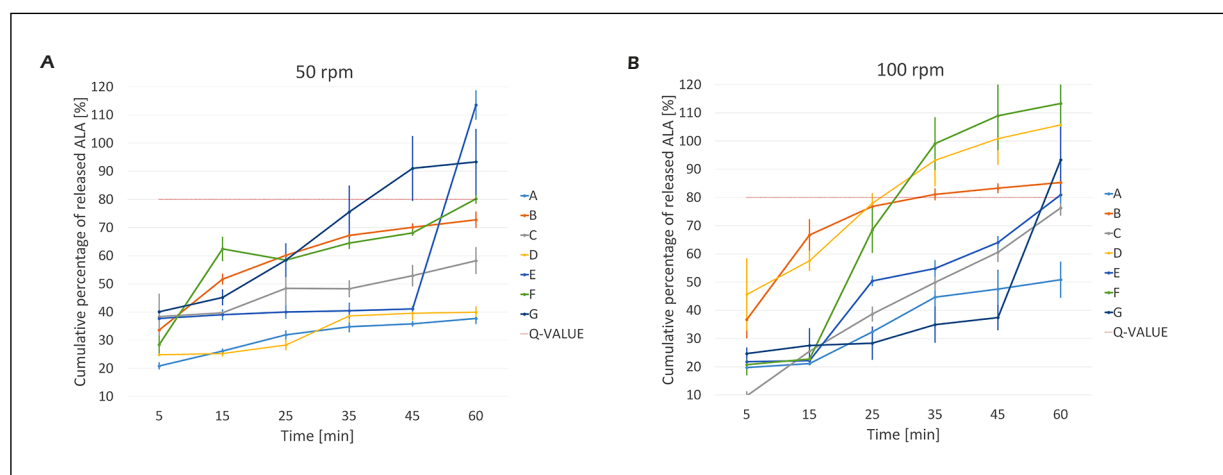
Formulation	D		E		F		G	
	mg of ALA	%	mg of ALA	%	mg of ALA	%	mg of ALA	%
1	303.21	99.3	286.17	97.3	308.42	104.8	338.46	105.9
2	306.65	100.4	306.66	104.2	312.95	106.4	322.58	100.9
3	308.01	100.8	288.21	98.0	311.81	106.0	299.94	93.8
4	299.99	98.2	292.54	99.4	304.23	103.4	333.64	104.4
5	305.45	100.0	297.81	101.2	310.41	105.5	314.58	98.4
6	307.25	100.6	291.07	98.9	307.48	104.5	343.93	107.6
7	301.64	98.7	295.81	100.6	305.47	103.8	331.93	103.8
8	308.54	101.0	300.56	102.2	315.00	107.1	289.67	90.6
9	304.75	99.8	304.78	103.6	313.95	106.7	303.72	95.0
10	309.06	101.2	283.40	96.3	309.25	105.1	318.93	99.8
Average	305.46	100.0	294.70	100.2	309.90	105.3	319.7	100.0
SD	3.05	1.0	7.82	2.7	3.59	1.2	17.8	5.6

formulations C, E and G, all three dietary supplements, though all had content variation in a range of pharmacopeial requirements.

Dissolution rate curves of ALA are presented in Figure 1a and 1b. There was a significant difference in dissolution rate curves generated at 50 and 100 rpm of rotation speed. At the speed of 50 rpm, only formulation G released 80% of content after 45 minutes. At the rotation speed of 100 rpm, formulations B, D and F released more than 80% of their content. Also, formulation G had slower release rate at speed 100 rpm than 50 rpm, whereas all other formulations showed increased dissolution rates. Generally, dissolution kinetic was altered with the increase of rotation speed. Most significant difference in dissolution

profiles at 50 and 100 rpm was noticed in formulation D (Figure 1a-1b).

Formulations B, C and D are tablet formulations, where formulation B is a medicine and formulation C is a dietary supplement, but it contains the same amount of ALA. Formulation B and D are film coated tablets, while formulation D is a double layered tablet. Formulation B is more complex than formulations D and C. In lower hydrodynamic conditions (at 50 rpm), formulations B and D had parallel dissolution profiles, but at 100 rpm there was no statistically significant differences between released ALA at investigated time points. Formulation C had parallel and different dissolution profile to formulation B and D (Table IV).



**Figure 1.** Dissolution rate curves of ALA formulations at 50 rpm (A) and 100 rpm (B).



**Table IV.** Statistical comparison (*p*-values) between released cumulative percentage of ALA from tablet formulations in investigated time points for dissolution profiles at 50 and 100 rpm (*p* < 0.05 statistical significance).

Time [min]	5		15		25		35		45		60	
	50 rpm											
Formulation	C	D	C	D	C	D	C	D	C	D	C	D
B	.590	.065	.000	.000	.010	.000	.001	.000	.007	.000	.054	.000
C	/	.003	/	.000	/	.000	/	0.113	/	.041	/	.011
100 rpm												
B	.001	.551	.000	.000	.000	0.998	.000	.269	.013	.994	.702	.040
C	/	.000	.076	/	/	.000	/	.000	/	.000	/	.003

Formulations A and F are both soft gelatin capsules, but they had completely different dissolution profiles in the same medium. Formulation E demonstrated better release of ALA at 100 rpm, whereas formulation G showed better release at 50 rpm. Formulation F and G had parallel profiles. At 100 rpm in the first 15 minutes, they had similar release and after this time point, the released amounts were statistically different (Table V).

When tablets were tested, formulation B had the fastest disintegration time, whereas formulation C disintegrated after one hour and formulation D after more than half an hour. Soft capsules disintegrated faster than hard capsules. Soft capsule formulation A had the fastest disintegration time, while formulation F had the highest dissolution rate (Figure 2).

## Discussion

Health care professionals like physicians and pharmacists often choose between drugs and dietary supplements that contain the same active ingredient, such as ALA formulations investigated in this research.

There is evidence that pharmacists are aware that formulation factors might affect the effects of drugs, but their knowledge of the subject is still unsatisfactory<sup>32</sup>. Such information on medical doctors is unavailable. There are, however, implications that medical doctors consider formulations from a more simplistic point of view - when prescribing drugs or advising the use of dietary supplements, they consider the ease of use as well as comfort of their patients and therefore, often choose gelatin capsules

(USA-based medical doctors) or solid forms such as tablets or dragées (Germany-based doctors)<sup>33</sup>.

Apart from API and formulation properties, physiological conditions in the gastrointestinal tract (e.g., hydrodynamics) can affect the drug/dietary supplement dissolution rates and absorption levels<sup>34</sup>. This is especially significant in certain diseases such as diabetes where delayed gastric emptying and gastroparesis have been repeatedly reported<sup>35-38</sup>. Consequently, dissolution rates and absorption from various formulations among diabetic patients can significantly differ from expected and result in unsatisfactory therapeutic/supplementation success. However, USP testing conditions do not reflect the myriad of factors altering the dissolution and absorption *in vivo*<sup>34</sup>.

Dissolution testing at different rotation speeds might prove to be a simple, yet effective method to test for differences in formulations response to high and low hydrodynamic conditions (as shown in the results section) which would be of great use, especially for diabetic patients, who are, indeed, frequent ALA users.

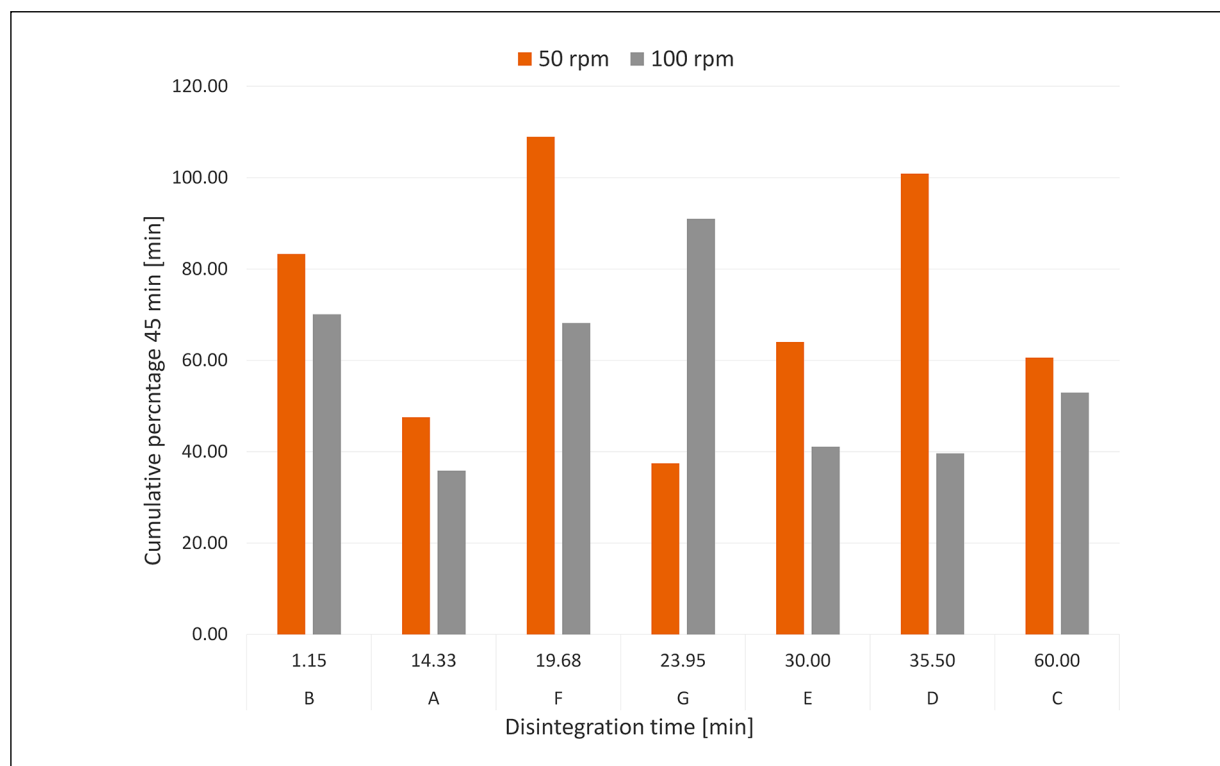
In recent years, the increase in demand for ALA products brought on the rise in the number of ALA products offered at the global market. Two drug formulations as well as five dietary supplements available at the Serbian market were tested and no differences in content were found, but disintegration and dissolution properties were different between the same type of formulations.

Drug formulations (A and B) and dietary supplement formulations (C, D, E, F and G) had within 85-115% of the average content and were compliant to pharmacopoeial requirements. The larger standard deviations were observed in for-

Formulation comparison of drugs and dietary supplements of alpha-lipoic acid

**Table V.** Statistical comparison (*p*-values) between released cumulative percentage of ALA from capsule formulations in investigated time points for dissolution profiles at 50 and 100 rpm (*p*<0.05 statistical significance).

Time [min]	5			15			25			35			45			60		
<b>50 rpm</b>																		
Formulation	E	F	G	E	F	G	E	F	G	E	F	G	E	F	G	E	F	G
A	.000	.140	.000	.000	.000	.000	.109	.000	.000	.607	.000	.000	.797	.000	.000	.000	.000	.000
E	/	.047	.971	/	.000	.040	/	.000	.000	/	.000	.000	/	.000	.000	/	.000	.005
F	/	/	.010	/	/	.000	/	/	.997	/	/	.053	/	/	.000	/	/	.000
<b>100 rpm</b>																		
Formulation	E	F	G	E	F	G	E	F	G	E	F	G	E	F	G	E	F	G
A	.996	.998	.963	.997	.998	.452	.005	.000	.918	.560	.000	.608	.164	.000	.642	.006	.000	.861
E	/	.0997	.995	/	.988	.517	/	.004	.000	/	.000	.020	/	.000	.004	/	.001	.024
F	/	/	.979	/	/	.634	/	/	.000	/	/	.000	/	/	.000	/	/	.000



**Figure 2.** Disintegration times of ALA formulations in relation to the cumulative percent of ALA released at 45 min.

mulations C, E, F and G. This is in correlation with previous findings<sup>39-41</sup> that showed high variability of the content of active pharmaceutical ingredient in dietary supplement products. Two different studies<sup>42,43</sup> of dietary supplements of ALA also showed significant variability of content in relation to the labelled content of ALA - from 87% to 110%<sup>42</sup>, and from 53% to 100%, respectively<sup>43</sup>. As an extreme example, an analysis<sup>44</sup> of vitamin D dietary supplements found that supplements contained 8-177% of the declared vitamin D content.

In the Republic of Serbia, as in many different countries worldwide, regulatory requirements considering obligatory testing of dosage forms for dietary supplements is non-existent. The requirements which dietary supplements need to meet before being placed on the market in Serbia are rather administrative (entry in the database of the Ministry of Health) and are related to health safety (absence of chemical and microbiological contamination). Proof of effectiveness and specifics on the characteristics of the formulation are not required<sup>25,26</sup>. Dissolution rate testing is important for formulations where API has low solubility. ALA is very slightly soluble in water<sup>16</sup> and has

liposoluble molecular properties and belongs to Class II of the Biopharmaceutical Classification System (BCS).

Formulations B, C and D are tablet formulations with immediate release, thus similar profile of release is expected. Additionally, formulation C is a dietary supplement that has the same dose of ALA as drug formulation B. At lower speed of rotation all three formulations had different profiles, but at higher rotation speeds, formulations B and D had similar profiles. Differences between profiles of formulations B and D at lower speed rotation (or during mild hydrodynamic conditions) might be due to differences in coating, as formulation B contains sodium lauryl sulphate which improves solubility. Also, formulation B has a mixture of microcrystalline cellulose and lactose monohydrate in its core, whereas formulation D has a mixture of microcrystalline cellulose and dicalcium phosphate, which tends to create a less porous tablet and prevents water from entering the tablet core. The purpose of that is to decrease the dissolution rate. However, at 100 rpm formulation D had faster release rate probably due to dimethicon in formulation B, which prevents wetting and slows dissolution.



Rapid disintegration occurred in formulation B, but not in D where it would also be expected since both formulations contain croscarmellose sodium. Croscarmellose sodium is a cross-linked polymer of carboxymethylcellulose sodium. It is a super disintegrant with rapid swelling properties whose positive effects on disintegration and thus, the dissolution of drugs with low water solubility, have been confirmed in various studies<sup>45-47</sup>.

Formulation A and F are soft gelatin formulations with solid fats as fillers and thus similar dissolution profiles were expected. Formulation F released more ALA in each time point at 50 and 100 rpm. Increased hydrodynamic condition (100 rpm) led to complete release of ALA from formulation F, but not formulation A. The main difference in these two formulations is that formulation F has had glycerol in its shell as a moisturizing agent and formulation A had sorbitol.

Formulation F has soy lecithin listed as a filler ingredient which might contribute to its faster dissolution in comparison to formulation A. A study<sup>48</sup> on human bioavailability and pharmacokinetic profile of formulation F was conducted in 2012. Scientists reported that ALA solubility may be increased by vehiculation in an amphiphilic matrix such as lecithin, a good emulsifier. After rapid dissolution there was a good solubilization by lecithin - an inexpensive method that was very simple to use<sup>48</sup>.

Formulation E and G are hard hypromellose capsules and have different fillers. Formulation E has microcrystalline cellulose and hypromellose, while formulation G is listed as gastroresistant and filled with gellan gum pellets. At lower rotation speed, pellets had time to completely disintegrate, release ALA and thus dissolution rate of ALA was higher at 50 rpm compared to 100 rpm, which is a paradoxical situation. However, *in vivo* conditions might affect the effective release of ALA at small intestine area where absorption is expected.

Amenta et al<sup>49</sup> evaluated pharmacokinetics of three formulations of ALA in healthy volunteers. Three formulations had different rates of absorption, two with a claimed high absorption rate and one with a claimed prolonged absorption rate. In view of the elapse of time taken by cells to regenerate cellular stores of glutathione after ALA uptake, the most rational approach for eliciting antioxidant activity at the cellular level seemed to be through the use of a formulation allowing the compound to reach its target at the highest concentrations and in the shortest time<sup>49</sup>.

In accordance with these findings, the release of ALA from formulation B was superior in comparison to other formulations.

Different ways of increasing the solubility of ALA have been reported<sup>50,51</sup>, including ALA complexation with cyclodextrins. Complexation with various cyclodextrins improved not only solubility, but also the stability of ALA. The pH value has a great influence on the solubility of weak acids in water. With the increase of the pH value of the solution, their solubility can be increased. The inclusion complex prepared by co-grinding ALA with hydroxypropyl- $\beta$ -cyclodextrin and alkalizer to enhance the solubility and stability of ALA showed significant improvement in the solubility and stability of ALA<sup>48</sup>. However, complexes and composites based on cyclodextrins, or other polymers require costly processes that are often difficult to carry out and do not ensure complete complexation of the active ingredient<sup>48</sup>.

## Conclusions

Tablet formulation of ALA registered as a drug had the best disintegration time and stable kinetic in both dissolution speed, whereas tablet formulations of dietary supplements (C and D) had low disintegration time and variable dissolution kinetic depending on the dissolution speed. Soft capsules had better disintegration time but slower dissolution release rate while formulation A (drug formulation) had incomplete dissolution, which might be improved by altering the dissolution medium. Formulation F had high dissolution rate due to soy lecithin present in the formulation. Formulation E and G, hard hypromellose capsules, had unstable release kinetic, highly dependent on dissolution hydrodynamic conditions. Formulation G had lower dissolution rate at higher speed. All these differences could be correlated with patients' differences such as the amount of gastric fluid, peristaltic motility and the amount of secreted bile. Formulations with unstable kinetic tend to have variable effects dependent on the status of human organism. Thus, knowledge about the impact of formulation on enhancing the API bioavailability, providing alternative routes of administration, and increasing patient convenience is important in maximizing the treatment efficiency. Stricter regulations on dietary supplements should be imposed worldwide, especially in the terms of formulation quality as this would lead to better efficacy of dietary supplementation.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgements

This study was supported by The Ministry of Education, Science and Technological Development, Republic of Serbia (project 451-03-68/2022-14/200114).

### Informed Consent

Not applicable.

### Ethics Approval

Not applicable.

### Authors' Contribution

Conceptualization: M.L.P. and J.J.B.; methodology: M.L.P. and J.J.B.; Data curation: J.C.P. and N.T.; investigation: M.V. and J.C.P.; formal analysis: N.T.; writing-original draft: M.L.P. and M.V.; writing-review and editing: J.J.B. All authors have read and agreed to the published version of the manuscript.

### Funding

This research received no external funding.

### ORCID ID

Mladena Lalić-Popović: 0000-0001-8354-3868; Milana Vuković: 000-0003-1829-8434; Jelena Jovičić-Bata: 0000-0002-0540-6654; Jelena Čanji-Panić: 0000-0002-9558-7902; Nemanja Todorović: 0000-0002-1519-5643.

## References

- 1) Lesar TS. Prescribing errors involving medication dosage forms. *J Gen Intern Med* 2002; 17: 579-587.
- 2) Castañeda-Hernández G, Caillé G, du Souich P. Influence of drug formulation on drug concentration-effect relationships. *Clin Pharmacokinet* 1994; 26: 135-143.
- 3) U.S. Food and Drug Administration. FDA. Available at: <https://www.fda.gov/food/cfsan-constituent-updates/fda-releases-draft-guidance-enforcement-discretion-certain-nac-products> (accessed on 18 July 2022).
- 4) The United States Pharmacopeia. The National Formulary. United States Pharmacopeial Convention, 1979.
- 5) Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. *J Nutr* 2001; 131: 369S-373S.
- 6) Robinson JR, Klein SM, Sagers RD. Glycine metabolism. *J Biol Chem* 1973; 248: 5319-5323.
- 7) Reed LJ, Debusk BG, Gunsalus IC, Hornberger CS Jr. Crystalline alpha-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. *Science* 1951; 114: 93-94.
- 8) Kagan VE, Shvedova A, Serbinova E, Khan S, Swanson C, Powell R, Packer L. Dihydrolipoic acid—a universal antioxidant both in the membrane and in the aqueous phase. *Biochem Pharmacol* 1992; 44: 1637-1649.
- 9) Suzuki YJ, Aggarwal BB, Packer L. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochem Biophys Res Commun* 1992; 189: 1709-1715.
- 10) Dos Santos SM, Romeiro CFR, Rodrigues CA, Cerqueira ARL, Monteiro MC. Mitochondrial dysfunction and alpha-lipoic acid: Beneficial or harmful in Alzheimer's disease? *Oxid Med Cell Longev* 2019; 2019: 8409329.
- 11) Salinthon S, Yadav V, Schillace RV, Bourdette DN, Carr DW. Lipoic acid attenuates inflammation via cAMP and protein kinase A signaling. *PLoS One* 2010; 5: e13058.
- 12) Melhem MF, Craven PA, Liachenko J, DeRubertis FR. Alpha-lipoic acid attenuates hyperglycemia and prevents glomerular mesangial matrix expansion in diabetes. *J Am Soc Nephrol* 2002; 13: 108-116.
- 13) Brufani M. Acido  $\alpha$ -lipoico farmaco o integratore. Una panoramica sulla farmacocinetica, le formulazioni disponibili e le evidenze cliniche nelle complicanze del diabete. *Prog Nutr* 2014; 16: 62-74.
- 14) Swain M. Chemicalize.org by ChemAxon Ltd. *J Chem Inf Model* 2012; 52: 613-615.
- 15) Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem* 2004; 11: 1135-1146.
- 16) Wiley-VCH. Ullmann's encyclopedia of industrial chemistry, 7th ed. Wiley-VCH, 2011.
- 17) European Pharmacopoeia Commission; European Directorate for the Quality of Medicines & Healthcare. European pharmacopoeia, 10th ed. Council of Europe, 2019.
- 18) Brookes MH, Golding BT, Howes DA, Hudson AT. Proof that the absolute configuration of natural  $\alpha$ -lipoic acid is R by the synthesis of its enantiomer [(S)-(-)- $\alpha$ -lipoic acid] from (S)-malic acid. *J Chem Soc Chem Commun* 1983; 19: 1051-1053.
- 19) Bustamante J. A-lipoic acid in liver metabolism and disease. *Free Radic Biol Med* 1998; 24: 1023-1039.
- 20) Takaishi N, Yoshida K, Satsu H, Shimizu M. Trans epithelial transport of alpha-lipoic acid across human intestinal Caco-2 cell monolayers. *J Agric Food Chem* 2007; 55: 5253-5259.
- 21) Brufani M, Figliola R. (R)- $\alpha$ -lipoic acid oral liquid formulation: pharmacokinetic parameters and

- therapeutic efficacy. *Acta Biomed* 2014; 85: 108-115.
- 22) Vadlapudi AD, Vadlapatla RK, Mitra AK. Sodium dependent multivitamin transporter (SMVT): A potential target for drug delivery. *Curr Drug Targets* 2012; 13: 994-1003.
  - 23) Salehi B, Berkay Yilmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, Akram M, Riaz M, Capanoglu E, Sharopov F, Martins N, Cho CW, Sharifi-Rad J. Insights on the use of  $\alpha$ -lipoic acid for therapeutic purposes. *Biomolecules* 2019; 9: 356.
  - 24) Zehnpfennig B, Wiriyasermkul P, Carlson DA, Quick M. Interaction of  $\alpha$ -lipoic acid with the human Na<sup>+</sup>/multivitamin transporter (hSMVT). *J Biol Chem* 2015; 290: 16372-16382.
  - 25) Binns CW, Lee MK, Lee AH. Problems and prospects: Public health regulation of dietary supplements. *Annu Rev Public Health* 2018; 39: 403-420.
  - 26) Regulation on dietary supplements. *Official Gazette of RS*. 45/2022.
  - 27) Canepa P, Dal Lago A, De Leo C, Gallo M, Rizzo C, Licata E, Anserini P, Rago R, Scaruffi P. Combined treatment with myo-inositol, alpha-lipoic acid, folic acid and vitamins significantly improves sperm parameters of sub-fertile men: a multi-centric study. *Eur Rev Med Pharmacol Sci* 2018; 22: 7078-7085.
  - 28) Abdel Hamid DZ, Nienaa YA, Mostafa TM. Alpha-lipoic acid improved anemia, erythropoietin resistance, maintained glycemic control, and reduced cardiovascular risk in diabetic patients on hemodialysis: a multi-center prospective randomized controlled study. *Eur Rev Med Pharmacol Sci* 2022; 26: 2313-2329.
  - 29) Malvasi A, Tinelli A, Lupica G, Vimercati A, Spyropoulou K, Dellino M, Mynbaev O. Effects of a combination of resveratrol and alpha-lipoic acid on body weight and adipose composition in women with PCOS: a preliminary pilot study. *Eur Rev Med Pharmacol Sci* 2022; 26: 6578-6582.
  - 30) Menichini D, Imbrogno MG, Basile L, Monari F, Ferrari F, Neri I. Oral supplementation of  $\alpha$ -lipoic acid (ALA), magnesium, vitamin B6 and vitamin D stabilizes cervical changes in women presenting risk factors for preterm birth. *Eur Rev Med Pharmacol Sci* 2022; 26: 8879-8886.
  - 31) Korićanac Z, Čakar M, Tanasković S, Jovanović T. Spectrophotometric determination of thioctic ( $\alpha$ -lipoic) acid in water and pharmaceutical preparations. *J Serb Chem Soc* 2007; 72: 29-35.
  - 32) Ramadan M, Yousef B. Knowledge, attitude, and practice of community pharmacists toward dispensing drugs available in different delivery systems at Khartoum locality. *Int J Health Allied Sci* 2021; 10: 36.
  - 33) Which drug form is preferred by doctors and patients? Available at: <https://cpsl-web.s3.amazonaws.com/kc/library/which-drug-form-is-preferred-by-doctors-and-patients.pdf?mtime=20170701121847> (accessed on 18 July 2022).
  - 34) Mudie DM, Amidon GL, Amidon GE. Physiological parameters for oral delivery and in vitro testing. *Mol Pharm* 2010; 7: 1388-1405.
  - 35) Yarandi SS, Srinivasan S. Diabetic gastrointestinal motility disorders and the role of enteric nervous system: current status and future directions. *Neurogastroenterol Motil* 2014; 26: 611-624.
  - 36) Kamiya T, Fukuta H, Hagiwara H, Shikano M, Kato T, Imaeda K. Disturbed gastric motility in patients with long-standing diabetes mellitus. *J Smooth Muscle Res* 2022; 58: 1-10.
  - 37) Chiu YC, Kuo MC, Rayner CK, Chen JF, Wu KL, Chou YP, Tai WC, Hu ML. Decreased gastric motility in type II diabetic patients. *Biomed Res Int* 2014; 2014: 894087.
  - 38) Intagliata N, Koch KL. Gastroparesis in type 2 diabetes mellitus: prevalence, etiology, diagnosis, and treatment. *Curr Gastroenterol Rep* 2007; 9: 270-279.
  - 39) Lockwood GB. The quality of commercially available nutraceutical supplements and food sources. *J Pharm Pharmacol* 2011; 63: 3-10.
  - 40) Hilleman DE, Wiggins BS, Botorff MB. Critical differences between dietary supplement and prescription omega-3 fatty acids: A narrative review. *Adv Ther* 2020; 37: 656-670.
  - 41) Boniglia C, Carratù B, Gargiulo R, Giammarioli S, Mosca M, Sanzini E. Content of phytoestrogens in soy-based dietary supplements. *Food Chem* 2009; 115: 1389-1392.
  - 42) Sittou A, Schmid MG, Gūbitz G, Aboul-Enein HY. Determination of lipoic acid in dietary supplement preparations by capillary electrophoresis. *J Biochem Biophys Meth* 2004; 61: 119-124.
  - 43) Ravanić N, Filipić S, Nikolić K, Popović G, Vovk I, Simonovska B, Agbaba D. Analysis of  $\alpha$ -lipoic acid in drug formulations and dietary supplement preparations. *Acta Chromatogr* 2009; 21: 433-441.
  - 44) Verkaik-Kloosterman J, Seves SM, Ocké MC. Vitamin D concentrations in fortified foods and dietary supplements intended for infants: Implications for vitamin D intake. *Food Chem* 2017; 221: 629-635.
  - 45) Bolhuis GK, Zuurman K, te Wierik GHP. Improvement of dissolution of poorly soluble drugs by solid deposition on a super disintegrant. II. The choice of super disintegrants and effect of granulation. *Eur J Pharm Sci* 1997; 5: 63-69.
  - 46) Sangalli ME, Giunchedi P, Colombo P, Conte U, Gazzaniga A, La Manna A. Cross-linked sodium carboxymethylcellulose as a carrier for dissolution rate improvement of drugs. *Boll Chim Farm* 1989; 128: 242-247.
  - 47) Gissinger D, Stamm A. A comparative evaluation of the properties of some tablet disintegrants. *Drug Dev Ind Pharm* 1980; 6: 511-536.

- 48) Mignini F, Nasuti C, Gioventu G, Napolioni V, Di Martino P. Human bioavailability and pharmacokinetic profile of different formulations delivering alpha lipoic acid. *J Clin Cell Immunol* 2012; 1: 418.
- 49) Amenta F, Traini E, Tomassoni D, Mignini F. Pharmacokinetics of different formulations of tiotic (alpha-lipoic) acid in healthy volunteers. *Clin Exp Hypertens* 2008; 30: 767-775.
- 50) Zheng M, Tang W, Kong R, Zhu X. Inclusion complex of alpha-lipoic acid containing alkalizer for improving the solubility and stability prepared by co-grinding. *Indian J Pharm Sci* 2017; 79: 544-552.
- 51) Takahashi H, Bungo Y, Mikuni K. The aqueous solubility and thermal stability of  $\alpha$ -lipoic acid are enhanced by cyclodextrin. *Biosci Biotechnol Biochem* 2011; 75: 633-637.