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# Development of level A, B and C in vitro–in vivo correlations for modified-release levosimendan capsules

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**Abstract**

The aim of this study was to investigate the possibility of developing different levels of correlation between in vitro release and in vivo absorption rate for four modified-release levosimendan capsule formulations. Differences and similarities in the in vitro dissolution curves were compared with pharmacokinetic parameters describing absorption rate. Formulations F, G, H and I differed in the amounts of the delaying excipients alginic acid and HPMC. In vitro release rate was studied by the USP basket method using the following conditions: pH 5.8 or 7.4 and a rotation speed of 50 or 100 rpm. In vivo bioavailability was tested in nine healthy male volunteers and the fractions absorbed were calculated by the Wagner–Nelson method. Dissolution conditions pH 5.8 and a rotation speed of 100 rpm predicted best the similarities and differences in absorption rates among different formulations, and levels C and B correlation coefficients were 0.85 and 0.97, respectively. For formulation H level A correlation ($r = 0.997$) was found when in vitro lag time was 0.2 h and time scale factor 1.9. This study indicated that dissolution tests developed can be used as a surrogate for human bioequivalence studies, for development processes of final commercial products, to ensure batch to batch bioequivalence and in the future in possible scale-up and post approval change cases for modified-release levosimendan formulation H. 

*Keywords:* Modified-release; Levosimendan; In vitro–in vivo correlation; Wagner–Nelson; Weibull

## 1. Introduction

Levosimendan is a novel calcium sensitizer having positive inotropic and peripheral vasodilatory effects (Lilleberg et al., 1995). It has been developed for the treatment of congestive heart failure. Levosimendan is absorbed rapidly and the elimination half-life is short, about 1 h both after intravenous infusion and oral administration (Sandell et al., 1995; Sunberg et al., 1998). Levosimendan is therapeutically active as such but it has also an active metabolite OR-1896. Levosimendan is metabolised first to inactive metabolite OR-1855, and thereafter to OR-1896, which has similar pharmacological effects as the parent

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compound (Takahashi et al., 2000). The amine metabolite OR-1855 is mainly formed at the lower parts of the intestine (Antila et al., 1999) and the type of formulation controls the formation of the metabolities after oral administration (Sunberg et al., 1998). Reduced bioavailability of levosimendan and four times higher metabolite concentrations have been observed after a slow release tablet compared to immediate release tablet and capsule formulations (Sunberg et al., 1998).

Since levosimendan has a short half-life a modified release formulation might be desirable to maintain therapeutic drug levels in the blood for a reasonable time. However, drug release should not be prolonged so much that a remarkable part of the dose will be released at the lower parts of the intestine forming therapeutically too high OR-1855 and OR-1896 concentrations.

For the development of an ideal formulation it would be highly desirable to have an appropriate dissolution method which could predict the in vivo release rate of levosimendan. It is possible only if the in vitro–in vivo correlation (IVIVC) can be determined. IVIVC can be established if dissolution rate is the rate-limiting step in the absorption process (Cardot and Beyssac, 1993). In order to evaluate the possibilities of determining the IVIVC for the product, the Biopharmaceutics Classification System (BCS) of the drug substances was compiled (Amidon et al., 1995). Drug substances are divided into four classes according to their solubility and permeability characteristics. Class I substances are highly permeable in the intestine and highly soluble at physiological pH. In class II the permeability is high and solubility low, in class III the permeability is low and solubility high and in class IV both permeability and solubility are low. A drug substance is considered to be highly permeable if extent of absorption is determined to be $>90\%$ or more and highly soluble if the highest single dose is soluble in 250 ml of water or less over the pH range of 1–7.5. According to these criteria, levosimendan belongs to class I with a relative bioavailability of 84–85%, which indicates rather high intestinal permeability and high solubility within the pH range of 1–7.5. In theory, IVIVC can be established most obviously for drugs belonging to class II or class I, if the release rate is modified. Levosimendan modified-release formulations differed in the amounts of the delaying excipients alginic acid and hydroxypropyl methylcellulose.

In the present study, slightly modified-release hard gelatin capsules of levosimendan were investigated. The absorption of the products was tested in healthy volunteers. The in vitro dissolution conditions were changed in order to find a method, which would predict the in vivo absorption rate of levosimendan. The biowaiver dissolution method can be used as a surrogate for human bioequivalence studies, in the development of a final commercial product, to ensure batch-to-batch bioequivalence and in the future in possible scale-up and post approval changes, if IVIVC is found or drug product is rapidly dissolving and drug substance belongs to the BCS class I (Skelly et al., 1990).

Table 1
Compositions of modified-release levosimendan formulations F, G, H and I

| Ingredient (mg) | Formulations | | | |
|---|---|---|---|---|
| | F | G | H | I |
| Levosimendan | 2.0 | 2.0 | 2.0 | 2.0 |
| Alginic acid | 18.0 | 23.0 | 28.0 | 33.0 |
| HPMC | 37.0 | 46.0 | 56.0 | 66.0 |
| Stearic acid | 2.1 | 1.5 | 2.4 | 2.5 |
| Microcrystalline cellulose | 84.0 | 69.5 | 56.0 | 43.0 |

## 2. Material and methods

### 2.1. Dosage forms

The compositions of the modified-release capsule formulations F, G, H and I are presented in Table 1. The initial powder mass consisted of a homogeneous mixture that included the half of amount of levosimendan (Fermion, Finland) needed and varying amounts of alginic acid (Ladyburn Works, UK), hydroxypropylmethyl cellulose (HPMC) (Methocel K100LV, Colorcon Limited, UK) and part of the stearic acid (Merck KgaA, Germany, Croda Chemicals, UK). This

mixture was manufactured to granules by bricketing. Granules were sieved and the fraction 0.75–1.7 mm was mixed with the other half of levosimendan and varying amounts of microcrystalline cellulose (Avicel PH 101, FMC International, Ireland) and with the rest of the stearic acid. The mixture was dispensed into size 3 hard gelatin capsules (S.A Capsugel, Belgium). The amounts of alginic acid and HPMC increased gradually from formulation F to I. The amount of microcrystalline cellulose decreased gradually from formulation F to I. Differences among formulations were expected to lead to a sustained release and absorption rate of levosimendan increasing from capsule F to I.

Oral solution was used as a reference. The solution was prepared by diluting levosimendan concentrate, 1 mg of levosimendan in 1 ml of ethanol, in 200 ml of water.

### 2.2. Dissolution tests

The release rate of levosimendan was studied using the basket method described in USP 24 (apparatus: Dissolutest 07, Prolabo, France). The dissolution medium (500 ml at $37 \pm 0.5$ °C) was either pH 5.8 or 7.4 phosphate buffer (USP 24). The basket rotation speed was either 50 or 100 $min^{-1}$. The dissolution apparatus was connected to a flow-through spectrophotometer (Ultrospect II, LKB Biochrom Ltd, UK) via a peristaltic pump. Absorbance at 210 nm was recorded automatically by a computer running Tablet dissolution software (TDS™, LKB Biochrom Ltd, UK). Similarities of the dissolution curves were compared with similarity factor $F_2$ (Moore and Flanner, 1996).

### 2.3. Bioavailability studies

The study was an open, single-dose, randomised study with a cross over design carried out in accordance with the guidelines of the Declaration of Helsinki (World Medical Assembly 1964) as revised in Tokyo (1975). Nine healthy male volunteers participated in the study. The ages of the volunteers varied from 21 to 30 years and weight from 68 to 100 kg. Before the studies the subjects underwent physical examination where the status of the heart, lungs and blood circulation, as well as medical history were recorded. The volunteers were informed about possible risks and adverse effects of taking the drug, and written consent was obtained. The study protocol and the subject information leaflet were submitted to Ethical Committee of the Deaconess Hospital in Helsinki. The dose of levosimendan in each formulation was 2 mg. The drug capsule was taken with 200 ml of water in the morning of the study day (at about 08:00 h) into an empty stomach after 10 h fast. The oral solution was administrated to the subjects immediately after the dilution. No food or drink was allowed until 4 and 9 h after drug intake when the subjects were served a standard lunch and dinner, respectively. A snack was served 11 h after the drug intake. Supine position was forbidden during the 4 h after the drug intake. Blood samples (3 ml) for the determination of levosimendan concentrations in plasma were drawn from an upper arm vein at the following time points: 0 min (before drug intake), 15, 30, 45, 60 min, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10 and 12 h after drug intake. A washout period of at least 1-week was allowed between the study days.

### 2.4. Bioanalytics

Levosimendan concentrations in plasma were determined by High Performance Liquid Chromatography (HPLC) with UV detection (Karlsson et al., 1997). The metabolites OR-1855 and OR-1896 concentrations in plasma were determined by liquid chromatography-tandem mass spectrometry (Antila et al., 1999).

The methods were linear in the concentration range 5–250, 0.5–10 and 0.2–10 ng/ml for levosimendan, OR-1855 and OR-1896, respectively. The quantitation limit was 5 ng/ml for levosimendan. The corresponding limits for OR-1855 and OR-1896 were 0.5 and 0.2 ng/ml.

### 2.5. Pharmacokinetic parameters

The pharmacokinetic parameters were calculated by non-compartmental methods using the

pharmacokinetic software, BIOPAK (Clin Trials Inc., Lexington, USA). The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule from time zero to the last observed concentration. Maximum plasma concentration ($C_{max}$) and time to peak concentration ($T_{max}$) were registered from the observed plasma concentration–time data. Mean residence time (MRT) was also determined. Mean and standard deviations were calculated for the pharmacokinetic parameters. Analysis of variance (ANOVA) was completed by SAS 6.08 (SAS Institute Inc., Cry, NC) with and without logarithmic transformation for $C_{max}$, $T_{max}$, AUC and MRT values.

### 2.6. In vitro–in vivo correlation

Three levels, A, B and C, of in vitro–in vivo correlation (USP 24) were studied. Level C IVIVC represents single-point correlation between one dissolution time point and one pharmacokinetic parameter, for example between the time, when 80% had released in vitro ($T_{80\%}$) and $C_{max}$. Level B IVIVC bases on statistical moment analysis. The mean in vitro dissolution time ($MDT_{in\ vitro}$), calculated by Weibull function, was compared with MRT in vivo. Level A IVIVC represents point-to-point relationship between in vitro cumulative released and in vivo cumulative absorbed curves. The in vivo concentration versus time curve for each volunteer was initially transformed to the cumulative percents absorbed at each time point, comparable with cumulative released in vitro curve, using the method of Wagner and Nelson (1964).

In vitro cumulative released and in vivo cumulative absorbed fractions were fitted to the Weibull model:

$$F(t) = 1 - e^{-\left[\left(\frac{t}{t_d}\right)^{\beta}\right]}$$

where $F(t)$ = fraction dissolved at time $t$, $t_d$ = MDT, $\beta$ = empirical exponential factor (shape factor).

If there is level A IVIVC, these curves should be directly superimposable or can be made to be superimposable by using the time scaling factor and/or the constant offset value (Brockmeier et al., 1983):

$$t_{in\ vivo} = a + b \cdot t_{in\ vitro}$$

where $t_{in\ vivo}$ = time in vivo, $t_{in\ vitro}$ = time in vitro, $a$ = lag time in vivo and $b$ = time scale factor. The scale factor takes into account the fact that in vitro release and in vivo absorption do not always follow the same time scales. Calculations were carried out with pharmacokinetic software, Kinetica 2000 (Innaphase, France) and Excel 97 (Microsoft, USA).

## 3. Results

### 3.1. In vitro dissolution studies

Preliminary dissolution tests were performed at pH 1 and 0.1 N HCl was used as a dissolution medium. Dissolution was too rapid and that's why tests were interrupted. The effect of the amounts of alginic acid and HPMC on drug release was evaluated at pH levels 5.8 and 7.4 and with rotation speeds 50 and 100 rpm. The dissolution curves obtained are seen in Figs. 1 and 2 and the MDT and $T_{80\%}$ values are given in Table 2. At pH 7.4 and 100 rpm it was impossible to find out any difference between the formulations (Fig. 1 right), but at lower rotation speed (50 rpm), small differences were noted (Fig. 1 left). At pH 5.8, the differences between the formulations were more evident; as expected, the higher the amounts of polymers, the lower the dissolution rate. The best discrimination was achieved at pH 5.8 and 50 rpm (Fig. 2 left). The calculated similarity factors ($F_2$) confirmed the conclusion (Table 3). At pH 7.4 and 100 rpm, all $F_2$ values were higher than 50, which is the limit for considering the curves as similar. At pH 5.8 and 50 rpm, on the contrary, all $F_2$ values were lower than 50, indicating dissimilarity between curves.

### 3.2. Pharmacokinetics

The plasma concentrations of all four modified-release formulations as well as levosimendan solution are seen in Fig. 3. The pharmacokinetic parameters, without logarithmic transformation, used for further analyses are given in Table 4 and

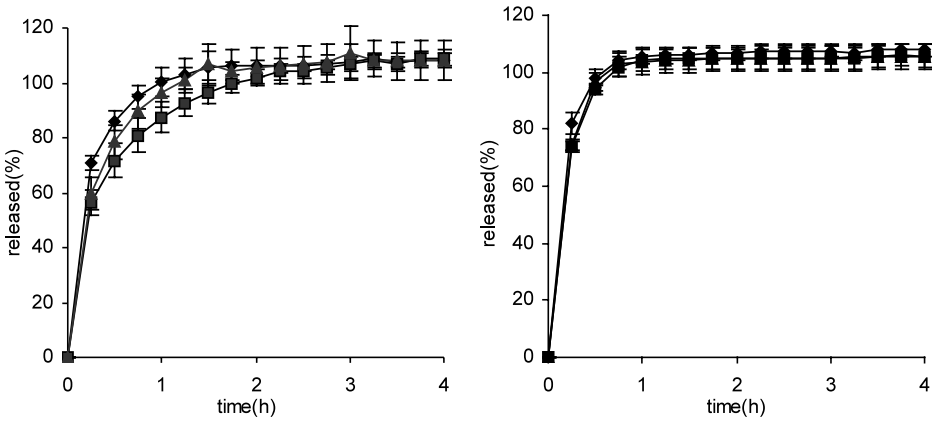


Fig. 1. Dissolution of levosimendan from modified-release capsules (F–H): ◆ = F, ■ = G and ▲ = H. Dissolution conditions: pH 7.4 and a rotation speed of 50 rpm (left) and 100 rpm (right), means ± S.D., $n = 5$-6.

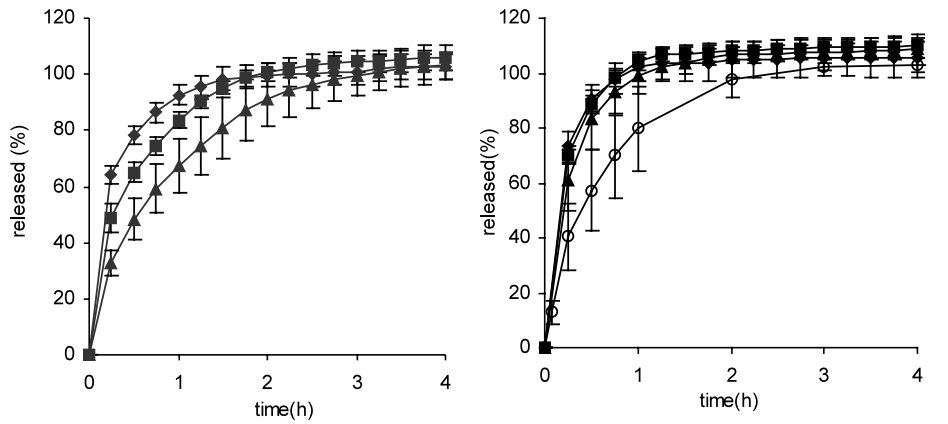


Fig. 2. Dissolution of levosimendan from modified-release capsules (F–I): ◆ = F, ■ = G, ▲ = H and ○ = I. Dissolution conditions: pH 5.8 and a rotation speed of 50 rpm (left) and 100 rpm (right), means ± S.D., $n = 5$–6.

Table 2
In vitro MDT* (h) and $T_{80\%}$** (h) parameters of levosimendan modified-release formulations F, G, H and I

| Formulation | pH 5.8, 50 rpm | pH 5.8, 100 rpm | pH 7.4, 50 rpm | pH 74, 100 rpm |
|---|---|---|---|---|
| F | 0.26*, 0.55** | 0.19*, 0.35** | 0.21*, 0.28** | 0.17*, 0.24** |
| G | 0.45*, 0.90** | 0.21*, 0.38** | 0.36*, 0.73** | 0.20*, 0.33** |
| H | 0.81*, 1.46** | 0.27*, 0.47** | 0.30*, 0.53** | 0.20*, 0.31** |
| I | – | 0.58*, 1.0** | – | – |

their statistical comparison in Table 5. Logarithmic transformation of pharmacokinetic parameters did not change the results of the statistical analysis. Plasma concentrations of OR-1855 and OR-1896 could be detected only in few subjects.

### 3.3. In vitro-in vivo relationship

There were no statistically significant differences in the pharmacokinetic parameters, $T_{max}$ and MRT, describing the absorption rate between

Table 3
$F_2$ similarity factors for levosimendan capsules, when compared different formulations, F, G, H and I in different dissolution conditions

| pH | Rotation speed (rpm) | Formulation | $F_2$ |
|---|---|---|---|
| 5.8 | 50 | F vs. G, F vs. H, G vs. H | 45, 29, 40 |
| 5.8 | 100 | F vs. G, F vs. H, F vs. I, G vs. H, G vs. I, H vs. I | 82, 53, 29, 58, 29, 35 |
| 7.4 | 50 | F vs. G, F vs. H, G vs. H | 45, 54, 58 |
| 7.4 | 100 | F vs. G, F vs. H, G vs. H | 63, 67, 87 |

$F_2 > 50$ indicates similarity and $F_2 < 50$ indicates dissimilarity.

formulations F versus G, F versus H and G versus H (Table 5). Formulation I differed from all other formulations in being significantly more slowly absorbed. There was no rank order correlation between pharmacokinetic and in vitro parameters at pH 7.4 either at a rotation speed of 100 rpm or 50 rpm. Dissolution conditions pH 5.8 and a rotation speed of 50 rpm were too discriminative, indicating differences between the absorption rates of all formulations. Dissolution results at pH 5.8 and 100 rpm predicted the in vivo results best. The similarity factors were higher than 50, indicating the same bioavailabilities for formulations F, G and H. Formulation I differed from all other formulations, which was indicated by similarity factors below 50. According to this study, the best dissolution method for the modified-release levosimendan capsule series was pH 5.8 and a rotation speed of 100 rpm.

### *3.4. Evaluation of the in vitro–in vivo correlation*

Level C in vitro–in vivo correlation was investigated for $T_{80\%}$ versus $C_{max}$. Correlation coefficients varied from 1 to 0.52 (Table 6). Level B in vitro–in vivo correlation was investigated for $MDT_{in\ vitro}$ versus $MRT_{in\ vivo}$. Correlation coefficients varied from 0.98 to 0.74. Correlation coefficients were considerably better at pH 5.8 than at 7.4. The best correlation coefficient was obtained with pH 5.8 and a rotation speed of 50 rpm. As mentioned earlier, that method was too discriminative. Thus pH 5.8 and with a rotation speed of 100 rpm was the best method for the levosimendan capsule series for predicting in vivo absorption behaviour.

Level A in vitro–in vivo correlation was represented for modified-release levosimendan formulation H, which was the most promising formulation from clinical point of view. With a time-scale factor ($b$) 1.9 and a lag time ($a$) 0.2 h there was a high degree of level A in vitro–in vivo correlation ($r = 0.997$) between in vivo absorbed and in vitro released fractions (Fig. 4). As can be seen from Fig. 4 the profile was slightly sigmoidal. The dissolution method with pH 5.8 phosphate buffer and 100 rpm had a high level in vivo predictability for the modified-release levosimendan formulation H.

## 4. Discussion

The results indicated that the amount of alginic acid and HPMC retarded the levosimendan release rate in vitro and even more in vivo. Increas-

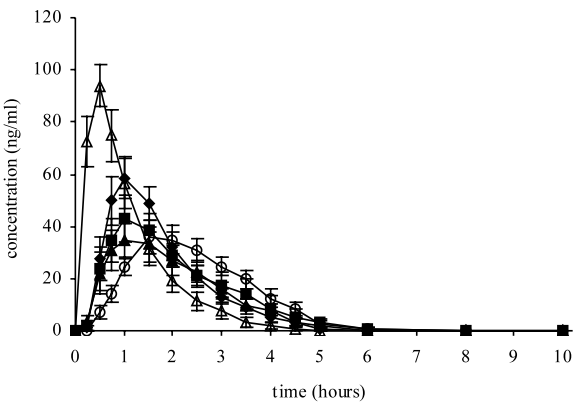


Fig. 3. Bioavailability of levosimendan from oral solution (E) and modified-release capsules (F–I): △ = E, ◆ = F, ■ = G, ▲ = H and ○ = I. Means ± S.E.M., $n = 9$.

Table 4
Pharmacokinetic parameters of oral solution (E) and modified-release capsules of levosimendan after a single dose of 2 mg, (means ± S.D., $n = 9$)

| Parameter | Formulation | | | | |
|---|---|---|---|---|---|
| | E | F | G | H | I |
| $C_{max}$ (ng/ml) | 95.9 ± 25.9 | 64.4 ± 26.3 | 55.5 ± 23.0 | 45.3 ± 20.6 | 36.9 ± 18.6 |
| $T_{max}$ (h) | 0.5 ± 0.2 | 1.0 ± 0.3 | 1.4 ± 0.9 | 1.3 ± 0.7 | 2.0 ± 0.4 |
| $AUC_{0-12\,h}$ (ng h/ml) | 118 ± 58 | 107 ± 49 | 99 ± 47 | 86 ± 39 | 95 ± 47 |
| MRT (h) | 1.0 ± 0.2 | 1.6 ± 0.3 | 1.8 ± 0.6 | 1.9 ± 0.7 | 2.4 ± 0.3 |

Table 5
ANOVA-test for pharmacokinetic parameters of modified-release levosimendan capsule formulations F, G, H and I

| | F vs. G | F vs. H | F vs. I | G vs. H | G vs. I | H vs. I |
|---|---|---|---|---|---|---|
| $C_{max}$ | n.s. | $P<0.05$ | $P<0.05$ | n.s. | $P<0.05$ | n.s. |
| $T_{max}$ | n.s. | n.s. | $P<0.05$ | n.s. | $P<0.05$ | $P<0.05$ |
| AUC | n.s. | $P<0.05$ | n.s. | n.s. | n.s. | n.s. |
| MRT | n.s. | n.s. | $P<0.05$ | n.s. | $P<0.05$ | $P<0.05$ |

ing amounts of these ingredients decreased the absorption rates as seen as longer $T_{max}$ and MRT but did not affect significantly the extent of levosimendan absorbed. The amounts of metabolities were negligible in most of the subjects so no formulation effect on metabolite formation could be evaluated after a single dose of levosimendan.

Levosimendan as a drug substance seems to be class I in the BCS. According to this and its modifed-release dissolution properties, it should be possible to determine the in vitro–in vivo correlation for modified-release levosimendan formulations.

The target to find out a predictive in vitro dissolution method was reached gradually. The first step was taken by observing which in vitro dissolution method predicted best similarities and differences in bioavailability: pH 5.8 and a rotation speed of 100 rpm seemed to be the best. The second step was to compare level C and B correlation coefficients: pH 5.8 with a rotation speed of 50 rpm appeared to be slightly better than 100 rpm. Cutler et al. (1997) suggested level C and B correlations for the first step in relating in vitro and in vivo performance of dosage forms. Comparison of the differences in the pharmacokinetic parameters with the differences in the in vitro dissolution curves seemed important: the dissolution method should discriminate only bioinequivalent batches. Based on this, pH 5.8 and a rotation speed of 100 rpm was selected for the evaluation of level A correlation for modified-release levosimendan formulation H. A high degree of level A in vitro–in vivo correlation was found.

Level A correlation for formulation H is formulation-specific. The dissolution rate of levosimendan was dependent on the dissolution conditions which indicated that level A correlation has to be investigated product by product. Level A correla-

Table 6
Level $C$ and $B$ correlation coefficients ($r$) for formulations F, G and H

| pH | Rotation speed (rpm) | Level C | Level B |
|---|---|---|---|
| 5.8 | 50 | 1* | 0.93 |
| 5.8 | 100 | 0.85 | 0.98* |
| 7.4 | 50 | 0.52 | 0.74 |
| 7.4 | 100 | 0.71 | 0.94 |

Formulation I was included only in the pH 5.8 and 100 rpm results. Level C IVIVC was represented for $C_{max}$ vs. $T_{80\%}$ and level B for $MDT_{in\ vitro}$ vs. $MRT_{in\ vivo}$.

* $P<0.05$

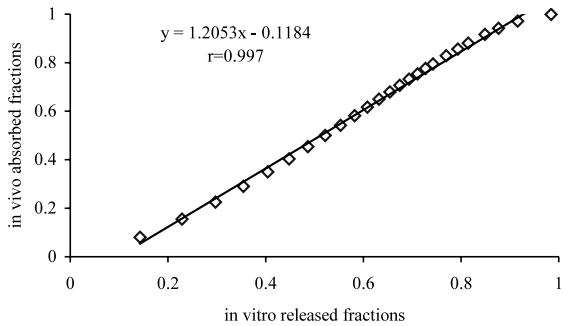


Fig. 4. In vitro released and in vivo absorbed fractions for modified-release levosimendan formulation H. In vitro conditions pH 5.8 and rotation speed 100 rpm.

tion was also found for formulations F, G and I, but with different mathematical transformations, i.e. with different lag times and time scale factors. In a number of studies it has been observed that the level A correlation is product-specific not drug substance-specific (Leeson, 1995; Munday and Fassihi, 1995; Yu et al., 1996; Hernández et al., 1996).

## 5. Conclusion

Level A correlation ($r = 0.997$) was found for formulation H at pH 5.8 with a rotation speed of 100 rpm. This dissolution method predicted also the best absorption rate for the modified-release levosimendan capsules studied. After internal or external validation of in vitro dissolution method it might be used as a surrogate for human bioequivalence studies. An in vitro dissolution test can replace absorption studies during the pre-approval process, to develop a desirable formulation, and to ensure batch-to-batch bioequivalence. It could also be extremely useful in performing possible post-approval changes in the formulation, scale-up or changes in the drug substance or excipient supplier.

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