

Aqueous injection of quercetin: an approach for confirmation of its direct in vivo cardiovascular effects

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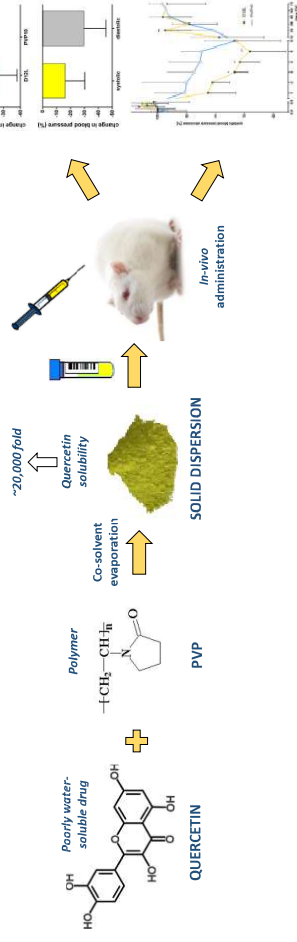
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**\*Graphical Abstract (for review)**



1 **Aqueous injection of quercetin: an approach for confirmation of its direct *in vivo* cardiovascular**  
2 **effects**

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47 **Authors contribution:**

48 Elena Piera Porcu, Massimo Cossu and Giovanna Rassu: carried out the experimental  
49 pharmaceutical work;

50 Paolo Giunchedi contributed to the interpretation of the results;

51 Guido Cerri performed the physical-chemical studies;

52 Jana Pourová, Iveta Najmanová and Thomas Migkos performed the *in vivo* study;

53 Veronika Pilařová and Lucie Nováková carried out pharmacokinetic analysis of blood samples;

54 Přemysl Mladěnka designed the *in vivo* experiments, critically analysed the data and wrote  
55 corresponding parts of the article;

56 Elisabetta Gavini provided the intellectual input and designs and approved the protocols to be  
57 followed in the study and wrote the article.

58 All authors discussed the results and contributed to the final manuscript.

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67 **ABSTRACT**

68 Potential positive effects of flavonol quercetin on humans were suggested by many studies. However, it  
69 is not clear if these effects are mediated by quercetin or its metabolites. The *in vivo* confirmation of  
70 quercetin effects is largely hindered by its low water solubility and thus impossibility to test directly its  
71 impact. Therefore, a solid dispersion of quercetin with polyvinylpyrrolidone (PVP) was developed to  
72 prepare an injectable formulation of water-soluble quercetin. The optimized formulation provided a  
73 20,000-fold increase in quercetin solubility. This formulation was tested on conventional and  
74 spontaneously hypertensive rats; it lowered their blood pressure in both short- and long-term basis.  
75 Pharmacokinetic data are also provided. [This study reports for the first time](#) an injectable water-soluble  
76 formulation of quercetin suitable for confirmation of its vascular effect *in vivo*.

77

78

79 *Keywords:* Solid dispersion; Cardiovascular effects; Quercetin; Polyvinylpyrrolidone; Solubility  
80 enhancement; Injectable aqueous solution.

## 81 1. INTRODUCTION

82 Flavonoids always attracted a great attention due to their presence in common diet and their possible  
83 positive effects on humans (Kumar and Pandey, 2013; Mladěnka et al., 2010). The major flavonol in  
84 the human diet is quercetin and indeed, most flavonoid studies have been performed with it. There is a  
85 huge number of studies showing its positive effects, and in particular, some are claiming lowering  
86 arterial blood pressure (Larson et al., 2010). However, notwithstanding such enormous quantity of  
87 articles, there are still no definite proofs if quercetin has clearly positive effects on human being. The  
88 major controversies arise from the facts that orally given quercetin has very low bioavailability (Li et  
89 al., 2009; Rothwell et al., 2016), and its *in vitro* effects were commonly observed in quite large  
90 concentrations which are hardly, if at all, achievable after oral intake. Another possible explanation is  
91 based on the fact that although quercetin is poorly absorbed, it is extensively metabolized by human  
92 microflora into a number of small phenolic compounds which can have significant biological effects in  
93 humans (Del Rio et al., 2013). For example, our group recently demonstrated that at least one of these  
94 metabolites lowers arterial blood pressure in rat (Najmanová et al., 2016). In order to confirm or refute  
95 the direct effect of quercetin on arterial blood pressure, a water-soluble formulation of quercetin is  
96 needed since only biologically friendly solvents are fully compatible with biological systems and can  
97 be applied intravenously without producing inadvertent additional effects.

98 In general, the use of quercetin in pharmaceutical area is still limited due to its poor solubility in water,  
99 which limits formulation strategies. Because of scarce hydrophilicity, quercetin shows low dissolution  
100 rate, and consequently minimal quercetin absorption occurs in the gastrointestinal tract (Li et al., 2009;  
101 Puerta et al., 2017). For this reason, several efforts have been made to improve the aqueous solubility  
102 and therapeutic effects of quercetin.

103 Various approaches such as polymeric nanoparticles (Wang et al., 2016), cocrystals (Smith et al.,  
104 2011), lipid nanoparticles (Bose et al., 2013; Li et al., 2009; Kumar et al., 2016), complexation (Jullian

105 et al., 2007; Sri et al., 2007), micelles (Gao et al., 2012), emulsions (Gao et al., 2009; Tran et al., 2014;  
106 Hädrich et al., 2016), liposomes (Caddeo et al., 2016) and solid dispersion have been developed to  
107 enhance the solubility, dissolution rate and bioavailability of quercetin. Solid dispersions refer to a  
108 solid product made up of at least two different components, generally a hydrophilic and inert matrix  
109 and one or more hydrophobic drugs. In these systems, the drug solubility and its dissolution profile can  
110 be improved through the amorphous solid state and by reducing the particle size of the drug.

111 Additionally, the use of hydrophilic carriers increases the wettability of hydrophobic drugs (Park et al.,  
112 2016). In particular, various polymers have been employed to increase quercetin solubility such as  
113 polyethylene glycols (PEGs) (Otto et al., 2013; Park et al., 2016), cellulose derivatives (Sansone et al.,  
114 2011; Li B. et al., 2013; Gilley et al., 2017) and polyvinylpyrrolidone (Povidone, PVP) (de Mello Costa  
115 et al., 2010; Kakran et al., 2011; Yan et al., 2014).

116 PVP is one of the most commonly used carriers for solid dispersions due to its amphiphilic properties,  
117 nontoxicity and biocompatibility. Indeed, PVP is known to form water-soluble complexes with several  
118 drugs including carbamazepine (Sethia and Squillante, 2004), lansoprazole (Zhang et al., 2008),  
119 efavirenz (Alves et al., 2014), atorvastatin (Jahangiri et al., 2015) and others. In all cases, the aqueous  
120 solubility of the product is largely improved.

121 PVP is available in different grades based on molecular weights (Kadajji and Betageri, 2011). The  
122 mean molecular weight of PVP is characterized by the K-value (e.g. Povidone K-12, Povidone K-17,  
123 Povidone K-25, Povidone K-30, Povidone K-90) (Foltmann and Quadir, 2008). PVPs with low K-  
124 value are suitable solubilizing agents particularly for injectables (e.g. rifampicin, sulfonamide,  
125 melphalan, metronidazole, trimethoprim formulations) due to their low viscosity.

126 The solid dispersions represent a profitable strategy because of its simplicity and efficacy for enhancing  
127 the solubility and bioavailability of poorly soluble drugs. This aspect has led to an increase in their  
128 applications in the area of cosmetics and pharmaceuticals. Solid dispersions can be prepared by

129 different techniques (Vasconcelos et al., 2007; Sareen et al., 2012). Solvent evaporation method is  
130 particularly suitable for PVPs due to their good solubility in most solvents. In addition, co-solvent  
131 technique is particularly important for parenteral dosage forms. Due to this method, it is possible to  
132 incorporate a large quantity of a drug in small volume of liquid, as required for injections (Soni et al.,  
133 2014).

134 Although there are many papers in the literature regarding the formulation improving delivery and  
135 solubility of quercetin in different delivery systems, very few studies have investigated the possibility  
136 of preparing quercetin formulations for injection (Yuan et al., 2006; Date et al., 2008; Sun et al., 2011);  
137 particularly scarce is the literature concerning the development of injectable aqueous solutions. These  
138 systems may be of significant utility not only for a possible therapeutic application but also for  
139 studying the real direct effects of quercetin *in vivo*, through a simple system enabling easy dose  
140 modification. For these reasons, the objective of the present work was to prepare an [aqueous](#)  
141 formulation of quercetin for [i.v.](#) injection to evaluate its antihypertensive effects *in vivo*. For this  
142 purpose, a solid dispersion powder of quercetin with PVP suitable for injections was developed by  
143 using the co-solvent method.

144

## 145 **2. MATERIALS AND METHODS**

146

### 147 **2.1 Materials**

148 Quercetin (purity  $\geq$  95%) and polyvinylpyrrolidone (PVP10, Mw=10000 g/mol, K-value 13-19) were  
149 purchased from Sigma Aldrich (Milan, Italy). Saline (0.9 % NaCl injectable solution) was supplied by  
150 Eurospital (Trieste, Italy). [Ethanol 96 % was purchased from Carlo Erba Reagents \(Italy\)](#). Analytical  
151 grade solvents were used.

152

153 **2.2 Preparation of solid dispersions**

154 Solid dispersions of quercetin with the different weight ratios of quercetin/PVP10 were obtained by  
155 using the co-solvent evaporation technique. Briefly, the solutions were prepared by dissolving 25 mg of  
156 quercetin in 12.5 ml of ethanol and variable amounts of PVP10 in 40 ml of deionized water (Table 1).

157

158 **Table 1.** Qualitative and quantitative composition of all formulations.

FORMULATION	QUERCETIN (mg)	PVP10 (mg)	Weight Ratio	Theoretical Drug Content
			QUERCETIN/ PVP10 (w/w)	QUERCETIN (%)
<b>D3L</b>	25	62.5	1/2.5	28.5
<b>D5L</b>	25	125	1/5	16.7
<b>D10L</b>	25	250	1/10	9.1
<b>D11L</b>	25	275	1/11	8.3
<b>D12L</b>	25	300	1/12	7.7
<b>D15L</b>	25	375	1/15	6.3
<b>D20L</b>	25	500	1/20	4.8
<b>D12E</b>	25	300	1/12	7.7

159 The code L indicates powders obtained by evaporation and freeze-drying, whereas the code E denotes  
160 the formulation obtained by evaporation until dryness.

161

162 The solutions were then mixed under magnetic stirring to produce a transparent yellow mixture. The  
163 solvent was evaporated until it reached 15-20 ml using a rotary evaporator under reduced pressure at 95  
164 °C. Finally, the resulting solutions were freeze-dried at  $-54.5^{\circ}\text{C}$  under vacuum (0.909 mbar) for  
165 approximately 8 h, without the addition of cryoprotectants, using a Lio 5P Cinquepascale (Trezzano sul  
166 Naviglio, Italy). The freeze-drying process was carried out for 8 h. The formulation having a weight  
167 ratio quercetin/PVP10 of 1:12 (D12L) was selected as the leader one.

168 Moreover, a solid dispersion with the same quercetin/PVP10 weight ratio was also prepared  
169 evaporating by rotary evaporator, under reduced pressure at 95 °C, until dryness. The crumbly powder  
170 obtained was denoted as D12E.

171

172

### 173 **2.3 Solubility studies**

174 Excess amounts of pure quercetin or quercetin solid dispersions were dispersed into 2 ml of deionized  
175 water and the samples were kept shaking in a water bath at 20 °C. Powder of solid dispersion was  
176 added to water solution until clouding solution was observed, in order to verify the maximum  
177 solubility. The suspensions were filtered through a 0.22 µm syringe filter and an aliquot of 600 µl was  
178 mixed with the same volume of 20 mM water solution of NaOH.

179 Due to the chemical changes of quercetin in basic medium over time (Yang et al., 2010), quantification  
180 of the drug was performed after 40 min to allow stabilization of solution. [According to Yang et al.](#)  
181 [\(2010\), the absorption bands of the reaction solution of quercetin and sodium hydroxide changed](#)  
182 [during the reaction due to the formation of intermediate and final products. After 40 min only the](#)  
183 [absorption band of the final product at 350 nm appeared.](#)

184 The concentration of quercetin was determined by measuring the absorbance using UV–VIS  
185 spectrophotometer (Thermo Spectronic, Helios Gamma, England) at 350 nm. The calibration curve was  
186 linear in the range of 1–20.0 mg/L ( $R^2 = 0.9999$ ).

187

### 188 **2.4 *In vitro* characterization of solid dispersions D12L and D12E**

189

#### 190 **2.4.1 Drug Content**

191 The amount of quercetin entrapped within the solid dispersions was determined spectrophotometrically  
192 by the same way as reported above. Briefly, 10 mg of each formulation were weighed accurately and  
193 dissolved in 100 ml of 20 mM solution of NaOH. Then, the absorbance was read after 40 min and the  
194 quercetin content was determined. The drug loading efficiency was then calculated as:

$$\text{Drug loading efficiency (\%)} = \frac{\text{Drug content (\% w/w)}}{\text{Theoretical drug content (\% w/w)}}$$

195

196

197

198

#### 199 **2.4.2 Particle size**

200 The particle size of D12L and D12E was determined using Coulter Laser Diffraction (Coulter LS 100  
201 Q Laser Sizer, Beckman Coulter, Miami, FLA, USA). D12L or D12E was suspended in silicon oil  
202 (Tegiloxan<sup>®</sup>). In both cases, sonication for 10 min was carried out. In addition, PVP10 (raw material)  
203 dispersed in vaseline oil was analysed. Three dispersions were prepared for each formulation and the  
204 values resulted from triplicate determinations of each dispersion (n=9). The average particle size was  
205 expressed as the mean volume-surface diameter,  $d_{vs}$  ( $\mu\text{m}$ ). The coefficient of uniformity (CU) was also  
206 calculated by applying the following formula:  $\text{CU} = d_{10}/d_{90}$  (Rassu et al., 2015). The values of  $d_{10}$   
207 and  $d_{90}$  indicate, respectively, the particle diameter for which 10% and 90% of the particles have a  
208 diameter less than the number indicated. A CU value approaching 1 indicates a dimensionally uniform  
209 sample.

210

#### 211 **2.4.3 Density and flow properties**

212 The true density ( $\rho_t$ ) of solid dispersions was calculated using a helium pycnometer AccuPyc II 1340  
213 (Micromeritics<sup>®</sup>, GA, USA). An exactly weighed amount of each sample was filled in the sample cup.

214  $\rho_t$  of the powder was determined for each batch as the ratio  $M/V_t$ , where M is the mass of the  
215 dispersion powder and  $V_t$  is the average volume occupied with powder calculated with pycnometer  
216 (Cerri et al., 2016).

217 The “bulk volume”  $V_{\text{bulk}}$  (i.e., volume of a given mass of powder filled into a graduated cylinder  
218 without any compacting) and the “tapped volume”  $V_{\text{tapped}}$  of the powder (i.e.,  $m/V_{50}$ , where  $V_{50}$  is the  
219 volume after 50 standardized taps) were also determined. The values of bulk density ( $\rho_{\text{bulk}}$ ) and tapped  
220 density ( $\rho_{\text{tapped}}$ ) were calculated. The results were reported as the mean of three replicates for D12L and  
221 D12E.

222 The Hausner’s ratio (HR) and Carr’s compressibility index (CI), used to predict powder flow  
223 characteristics, were calculated according to following equations:

$$\text{HR} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}}$$

$$\text{CI (\%)} = \frac{V_{\text{bulk}} - V_{\text{tapped}}}{V_{\text{bulk}}} \times 100$$

224 The results were compared with the scale of flowability given in the *Eur. Ph. 9<sup>th</sup> edition*.

225

226

#### 227 **2.4.4 X-ray powder diffraction**

228 X-ray powder diffraction (hereafter, XRPD) analyses of quercetin, PVP10, and solid formulations  
229 D12L and D12E were performed using a Bruker D2-Phaser diffractometer (Karlsruhe, Germany).

230 Instrumental parameters were:  $\text{CuK}\alpha$  radiation ( $\lambda = 0.15418$  nm), 30kV, 10 mA, LynxEye PSD  
231 detector set with an angular opening of  $5^\circ$ ,  $2\theta$  range  $5.8\text{--}70^\circ$ , step size  $0.020^\circ$ , time per step 1 s, spinner  
232 15 rpm. The alignment of the instrument was calibrated using the international standard NIST 1976b  
233 (Gaithersburg, MD, USA). The analysis were performed at  $26 \pm 2$  °C and  $50 \pm 2\%$  of relative humidity,  
234 conditions monitored with a Data Logger EBI20-TH1 (Ebro, Ingolstadt, Germany). A low-background  
235 silicon crystal specimen holder, manufactured by Bruker (Karlsruhe, Germany), was used. The XRD

236 patterns were evaluated using the software Bruker EVA 14.2 (DIFFRAC<sup>plus</sup> Package) coupled with the  
237 database PDF-2 (International Centre for Diffraction Data, Newtown Square, PA, USA).

238

#### 239 ***2.4.5 Thermal analyses***

240 Thermogravimetric and Differential Thermal Analyses (hereafter, TG and DTA) of quercetin, PVP10,  
241 and solid formulations D12L and D12E were performed using a Simultaneous Thermal Analyzer TA  
242 Instrument Q600 equipped with TA-Universal Analysis software (New Castle, DE, USA). About 7 mg  
243 of each sample was heated from room temperature to 500 °C under nitrogen flow (N<sub>2</sub> purity 99.999%)  
244 in an alumina crucible at the following operating conditions: 10 °C/min; gas flow 50 ml/min; an empty  
245 Al<sub>2</sub>O<sub>3</sub> crucible was employed as reference. The software TA-Universal Analysis was used to evaluate  
246 the results.

247

#### 248 ***2.4.6 Melting point determination***

249 All melting points of pure substances (quercetin and PVP10) and solid formulations (D12L, D12E)  
250 were determined with a Kofler melting point apparatus. A physical mixture of drug and polymer  
251 (D12M) with weight ratio of 1:12 was prepared for comparison.

252

#### 253 ***2.4.7 Fourier transform infrared spectroscopy (FT-IR)***

254 Fourier transform infrared (FT-IR) spectroscopy was performed using (Avatar 320 FT-IR, Thermo  
255 Nicolet, Madison, WI, USA). FT-IR spectra for pure quercetin, polymer PVP10 and solid dispersions  
256 D12L and D12E were obtained using the KBr disk method. A physical mixture of drug and polymer  
257 (D12M) was again analysed for comparison.

258

259 **2.5 Preparation and characterization of solutions**

260 D12L and D12E solutions were prepared by solubilizing 200 mg of each solid formulation (containing  
261 about 16 mg of quercetin) in 1 ml of deionized water. D12L was also dissolved in saline solution at the  
262 same concentration in order to obtain the solution at the highest concentration employed for *in vivo*  
263 experiments. The final solutions were characterized in terms of pH at 20°C, osmolality and viscosity.  
264 The latter was determined at 20 °C by using Oswald U Tube viscometer. Osmolality was measured  
265 using an Osmomat 030 (Gonotec GmbH, Berlin, Germany). In addition, the injectability of water  
266 solutions of D12E and D12L and saline solution of D12L was evaluated by using a device made from a  
267 syringe and a pan resting on the piston of the syringe (Schuetz et al., 2008) with some modifications  
268 (Porcu et al., 2017).

269

270 **2.5.1 Physical stability studies of water solutions**

271 The solutions obtained by dissolving solid dispersions were characterized in terms of particle diameter  
272 using a Coulter nanosizer N5 (Beckman-Coulter Inc. Miami, FL, USA). With the aim of preparing  
273 aqueous solutions containing quercetin suitable for the injection, the particle size of D12E and D12L in  
274 water solution was used as the indicator to evaluate the physical stability of the formulations over time,  
275 at room temperature, up to 4 weeks.

276

277 **2.5.2 Chemical stability studies of water solutions**

278 Chemical stability studies were carried out on the injection formulations D12L and D12E in water at  
279 room temperature for a period of 28 days. In order to calculate the drug content, the samples were  
280 analysed spectrophotometrically, as reported above, at specific time intervals. The residual portion (%)  
281 of quercetin for each solution was calculated.

282

### 283 **2.5.3 Sterilization**

284 On the basis of the results obtained, D12L dissolved in saline was selected for further *in vivo*  
285 investigation. It was sterilized by autoclaving at 121 °C for 30 min. After that, the resulting solution  
286 was characterized in order to verify the influence of sterilization process on formulation properties.  
287 Parameters such as quercetin content, viscosity and injectability were determined in the same way as  
288 mentioned above.

289

## 290 **2.6 In vivo studies**

291

### 292 **2.6.1 Animals and treatments**

293 The experiments were carried out on male both conventional (normotensive) and spontaneously  
294 hypertensive rats. The animals were housed in the animal house of the Faculty of Pharmacy, Charles  
295 University, Czech Republic at a constant temperature 23-25°C, under a 12-h dark/light cycle. They  
296 were provided with a standard pellet diet and tap water ad libitum. The study (reg. No. MSMT-  
297 7041/2014-10) was approved by the Experimental Animal Welfare Committee of Charles University,  
298 Faculty of Pharmacy in Hradec Králové and conformed to The Guide for the Care and Use of  
299 Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23,  
300 revised 1996).

301

### 302 **2.6.2 Pharmacodynamic study**

303 Six Wistar rats (Velaz, Czech Republic, average weight  $415 \pm 31$  g, blood pressure values under  
304 anesthesia:  $140 \pm 25 / 75 \pm 33$  mmHg) were anesthetized i.p. by urethane ( $1.2 \text{ g kg}^{-1}$ ) and the arterial  
305 blood pressure and heart rate were recorded via a pressure transducer MLT0380/D linked to the right  
306 common carotid artery using PowerLab equipped with the software LabChart 7 (AdInstruments,

307 Australia). The *vena saphena sinistra* was cannulated and after an acclimatization period of 15 minutes,  
308 D12L or pure polymer PVP10, both dissolved in saline, were administered as consequent bolus doses  
309 (100  $\mu$ l). In the case of D12L administration, the doses of pure quercetin were ranging from 0.05 mg  
310  $\text{kg}^{-1}$  to 5 mg  $\text{kg}^{-1}$  (corresponding to 0.65 to 65 mg  $\text{kg}^{-1}$  of D12L). The corresponding amount of PVP10  
311 polymer was calculated for each dose and administered in the same volume as control. The experiment  
312 was terminated by intravenous administration of 1 ml of 1 M KCl. The body temperature was  
313 maintained during the whole experiment at  $36.5 \pm 0.5$  °C.

314 Another fourteen, non-anesthetized, spontaneously hypertensive rats (Academy of Science, Czech  
315 Republic, average weight  $342 \pm 15$  g and initial systolic blood pressure value  $177 \pm 7$  mmHg) were  
316 treated intravenously with saline solution of D12L, via the tail vein (*vena caudalis*). The dose of  
317 quercetin was 5 mg  $\text{kg}^{-1}$ . Equivalent amount of pure polymer PVP10 was also dissolved in saline and  
318 served as the control. During the next 72 hours, the systolic blood pressure was non-invasively  
319 monitored using the tail-cuff method (PowerLab coupled to a NIBP System, with a pulse transducer  
320 and a tail occlusion cuff for rats, AdInstruments).

321

### 322 **2.6.2 Pharmacokinetic study**

323 In additional three rats (average weight  $359 \pm 5$  g) the plasma level of quercetin and its metabolites  
324 were measured. Male Wistar rats under urethane anesthesia (the same dose as above) received *via vena*  
325 *saphena sinistra* a dose of 5 mg/kg of D12L dissolved in saline. The blood samples in approximate  
326 volumes of 350  $\mu$ l were collected in subsequent time periods: 0; 1; 5; 10; 15; 30; 45; 60; 90; 120; 180  
327 and 240 min. The blood samples were immediately centrifuged (2,500 g, 10 min, MPW-52, MPW  
328 Med. Instruments, Poland) and the plasma samples were frozen at -80 °C for subsequent analysis.  
329 The determination of quercetin and its metabolites including quercetin-3-O-glucuronide, tamarixetin  
330 and isorhamnetin in these rat plasma was carried out using Acquity Ultra Performance LC <sup>TM</sup> (UPLC)

331 system (Waters, Milford, MA, USA) coupled with Micromass Quattro micro™ API benchtop triple  
332 quadrupole mass spectrometer (Waters, Milford, MA, USA). Analytical column BEH Shield RP C18  
333 (2.1 × 100 mm, 1.7 μm) kept at 40 °C was employed for the separation of all analytes from matrix  
334 components. Compounds were separated using gradient elution with methanol and 0.1% formic acid at  
335 flow rate 0.35 mL min<sup>-1</sup>. 5 μl of the extract were injected into the UHPLC-MS/MS system using the  
336 partial loop with needle overfill mode. All injected solutions were stored in the autosampler at 4 °C.  
337 The mass spectrometer parameters for electrospray ionization in positive mode were set up as follows:  
338 capillary voltage: 3.2 kV, RF lens voltage 0.5 V, extractor voltage 3.0 V, ion source temperature 130  
339 °C, desolvation temperature 450 °C. The nitrogen cone gas flow was at 100 L h<sup>-1</sup>; nitrogen desolvation  
340 gas flow at 800 L h<sup>-1</sup>. Argon was used as a collision gas. Selected reaction monitoring (SRM) was used  
341 for quantification using precursor [M+H]<sup>+</sup> and a corresponding product ion in specific transitions:  
342 303.0 > 153.0 for QCE (cone voltage 40.0 V and collision energy 30.0 eV), 479.2 > 303.1 for Q-3-Gl  
343 (cone voltage 25.0 V and collision energy 15.0 eV), and 317.2 > 302.0 for both TMX and IRN (cone  
344 voltage 40.0 V and collision energy 25.0 eV). The data were acquired using MassLynx 4.1 software  
345 (Waters) and processed by QuanLynx (Waters). The pretreatment of plasma samples consisted in  
346 simple protein precipitation, where acetonitrile acidified by 0.1% formic acid was added as  
347 precipitating agent rat plasma in 2:1 (v/v) ratio. Then, the sample was incubated for 10 min and  
348 consequently centrifuged for other 10 min. The supernatant was filtrated through 0.22 μm PTFE  
349 syringe filter and injected into the UHPLC system. The plasma samples containing quercetin and its  
350 metabolites were quantified using matrix calibration curve where the plasma samples were spiked by  
351 known amount of mentioned analytes.

352

353

## 354 **2.7 Statistical analysis**

355

356 Statistical analysis was performed with GraphPad Prism 6.02. Comparison between two groups was  
357 performed by Mann-Whitney test, while among more groups was carried out using Kruskal-Wallis test  
358 following Dunn's Multiple Comparison Test. The differences in blood pressure in relation to time (tail-  
359 cuff method) were compared by the use of two-way ANOVA. The significance level was set at  $p <$   
360  $0.05$ , if not state otherwise. Data are shown in both *in vitro* and *in vivo* experiments as mean  $\pm$  SD. For  
361 *in vitro* studies, at least triplicates were performed. The number of animals, used for *in vivo* studies, is  
362 mentioned in the corresponding parts of the article.

363

364

## 365 **3. RESULTS AND DISCUSSION**

366

### 367 **3.1 Solubility studies**

368 The co-solvent evaporation technique was employed for the preparation of solid dispersions in order to  
369 improve quercetin solubility in water. The solubility data indicated that the solubility of quercetin  
370 depends on the drug/polymer ratio employed. As shown in Fig. 1, formulations prepared with  
371 drug/polymer weight ratio of 1:12 were associated with maximal improvement in quercetin water  
372 solubility, regardless of preparation method used. In fact, D12L and D12E were prepared by co-solvent  
373 evaporation method, but in the case of D12L this step was followed by freeze-drying process.  
374 Nevertheless, in both formulations the solubility of quercetin increased almost 20,000 fold. Indeed, in  
375 the case of D12L/D12E solid dispersions, the water solubility quercetin increased to 41.6 mg/ml, which  
376 was much higher than that of anhydrous solubility (0.00215 g/L at 25 °C), as reported by Srinivas et al.  
377 (2010).

378 The use of larger amount of PVP in D15L and D20L compared to 1:12 weight ratio, resulted in  
379 solutions with very high viscosity; these systems were, therefore, not suitable for the preparation of  
380 parenteral injections. For this reason, D12L and D12E were chosen as leader formulations for the  
381 further studies.

382

383

### 384 **3.2 *In vitro* characterization of solid dispersions D12L and D12E**

385

#### 386 **3.2.1 *Drug content, drug loading efficiency and particle size***

387 Drug content studies showed that the real content of quercetin loaded in D12L and D12E was slightly  
388 lower than the theoretical values (Supplementary Table S2), but the mean values of drug loading  
389 efficiencies were above 90 % for both formulations.

390 The sizes of solid dispersion particles were compared. D12L particles tended to have a larger volume-  
391 surface diameter ( $d_{vs}$ ) than D12E particles (Supplementary Fig. S1) but no significant differences were  
392 observed ( $p > 0.05$ ). In both cases, the formation of solid dispersion led to a decrease in particle size of  
393 the polymer. Indeed preparation of solid dispersions leads to reduction of particle size to molecular  
394 level. This aspect has an important impact on solubility. When the soluble carrier dissolves, the  
395 insoluble drug is exposed to the dissolution medium in the form of very fine particles with increased  
396 contact area, allowing its quick dissolution and absorption (Sharma and Jain, 2010). Solid dispersions  
397 and PVP particles showed a wide size distribution, as indicated by low CU values ( $CU < 0.15$ ) reported  
398 in Supplementary Table S1, that decreased in the order  $D12E > D12L > PVP$ . Therefore, D12E was the  
399 most uniform preparation in size, with a CU value nearer to 1 in comparison to other samples. The  
400 particle distribution for all three samples is shown in Supplementary Fig. S1.

401

### 402 **3.2.2 Density and flow properties**

403 The values of density and flow properties are reported in Supplementary Table S2. The true density of  
404 D12L and D12E solid dispersions were similar ( $1.301 \pm 0.008$  vs  $1.230 \pm 0.002$  g/cm<sup>3</sup>) ( $p > 0.05$ ). The  
405 freeze-drying process increased markedly the bulk volume of D12L compared to D12E ( $p < 0.05$ ). The  
406 flowability of the solid dispersions was determined using Hausner's ratio and compressibility index. A  
407 Carr's CI of  $< 10$  or HR of  $< 1.11$  are considered 'excellent' flow whereas  $CI > 38$  or  $HR > 1.60$  are  
408 considered 'very very poor' flow according to *Eur. Ph.* D12L showed little inclination to flow  
409 (Supplementary Table S2). The HR and the CI were  $1.626 \pm 0.035$  and  $38.99 \pm 1.554$ , respectively,  
410 hence D12L exhibited "extremely poor" flowability. On the contrary, D12E showed good flowability  
411 as confirmed by good values of HR ( $1.092 \pm 0.037$ ) and CI ( $8.59 \pm 2.89$ ). Therefore, preparation  
412 technique affected the powder properties. In spite of high volume occupied by the powder, D12L  
413 shows good properties for a possible compression. This aspect confirms that the formulation can be  
414 proposed not only as solution, but also as powder to improve stability. On the contrary, D12E exhibited  
415 a lower bulk volume, favoring a practical package.

416

### 417 **3.2.3 X-ray powder diffraction**

418 The diffractograms of the samples are illustrated in Fig. 2. The XRPD pattern of quercetin showed a set  
419 of sharp peaks corresponding to three crystalline forms of quercetin, namely dihydrate (triclinic, space  
420 group P-1; PDF number 43-1695), anhydrous (monoclinic, space group  $P2_1/a$ ; Filip et al., 2013), and a  
421 second anhydrous polymorph with structure not yet identified (peaks at  $2\theta$  values of  $7.6^\circ$ ,  $9.4^\circ$ ,  $12.9^\circ$ ,  
422  $14.0^\circ$ ,  $22.1^\circ$ , and  $28.3^\circ$ ; Trendafilova et al., 2017). The coexistence of more forms of quercetin in raw  
423 materials supplied by pharmaceutical companies was already reported (Borghetti et al., 2012;  
424 Trendafilova et al., 2017).

425 PVP is an amorphous material, and its diffraction pattern presents two halos whose angular positions  
426 change with the adsorption of water (Teng et al., 2010). PVP10 showed two maximum at, respectively,  
427 11.7° and 20.4° 2 $\theta$  (Fig. 2), values in good agreement with those reported for a PVP of low molecular  
428 weight (PVP K-12) measured at ambient temperature and 53% of relative humidity (Teng et al., 2010).  
429 Basically, the XRPD patterns D12L and D12E resulted identical to PVP10, thus revealing the  
430 amorphous structure of the two formulations. Therefore, preparation technique did not influence XRPD  
431 patterns of solid dispersions.

432

### 433 **3.2.4 Thermal analyses**

434 The TG and DTA curves of the samples are reported in Fig. 3. The DTA path of quercetin exhibited a  
435 weak endothermic peak at 46.7 °C, linked to the evolution of adsorbed water (weight loss of 1.3%  
436 along the TG curve), followed by a more evident endothermic event at 108.9 °C, determined by the  
437 loss (3.1%) of H<sub>2</sub>O molecules from the crystal lattice of the hydrate form of quercetin. The drug  
438 exhibited a third endothermic reaction, marked by the sharp peak at 318.0 °C, related to the melting of  
439 the flavonoid, whereas the exothermic event at 343.8 °C, accompanied by a weight loss of about  
440 23.7%, is related to quercetin degradation process (Borghetti et al., 2012). Quercetin showed a residual  
441 mass of 57.1% at 500 °C. PVP10 displayed two main endothermic reactions: at 66.3 °C, associated to  
442 the loss of adsorbed water (10.8%), and at 430.9 °C, caused by the decomposition of the polymer  
443 whose residual mass at 500 °C was 2.1%. The TG-DTA curves of the formulations D12L and D12E  
444 turned out almost identical to each other, and very similar to those of PVP10. The first endothermic  
445 reaction occurred at 64.7 °C for D12L and at 66.9 °C for D12E, the second endothermic event between  
446 434.1 °C (D12L) and 434.5 °C (D12E). At 500 °C, D12L evidenced a residual mass of 8.0%, a bit  
447 higher than D12E (5.6%), a difference consistent with the drug content measured in the two  
448 formulations, slightly superior in D12L (see Supplementary Table S1).

### 449 **3.2.5 Melting point determination**

450 Both solid dispersions showed different melting points compared to single components (PVP10:  
451 165°C-170°C and quercetin: 305°C-307°C), confirming the formation of interactions between them.  
452 The physical mixture D12M containing quercetin and PVP10 displayed higher melting point (210°C-  
453 220°C) than D12L (180°C-185°C) and D12E (175°C-180°C).

454

### 455 **3.2.6 FT-IR**

456 The FT-IR spectra of quercetin, PVP, the solid dispersions D12L and D12E and physical mixture  
457 D12M were recorded in order to investigate the possible chemical interactions between the drug and  
458 the polymer in the solid dispersion formulation (Fig. 4). The spectra of quercetin exhibited a strong and  
459 broad stretching absorbance peak at 3405  $\text{cm}^{-1}$  due to hydroxyl groups, which might form inter-  
460 molecular hydrogen bonds with the phenolic –OH groups and aromatic ketone moiety. In addition, the  
461 stretching vibration peak of C=O aryl ketonic group was found at 1664  $\text{cm}^{-1}$  (Pralhad and  
462 Rajendrakumar, 2004). Other characteristic peaks in the quercetin molecule due to C=C aromatic ring  
463 stretching were detectable at 1610, 1560, and 1510  $\text{cm}^{-1}$  as reported previously in literature (Catauro  
464 2015, Borghetti et al., 2009). Band at 1319  $\text{cm}^{-1}$  was attributable to C–H bending in aromatic  
465 hydrocarbon, as well as peaks at 940, 825, 680 and 600  $\text{cm}^{-1}$ .

466 PVP exhibits a characteristic peak at the frequency 1655  $\text{cm}^{-1}$ , relative to the stretching of amide  
467 carbonyl group of the PVP units. Other peaks at 2958  $\text{cm}^{-1}$  as a result of  $\text{C}_{\text{sp}^3}\text{-H}$  stretching and at 3448  
468  $\text{cm}^{-1}$ , owing to N-H stretching were exhibited in FTIR spectra of polymer (De Mello Costa et al., 2010;  
469 Zhu et al., 2007). The peak at 1290  $\text{cm}^{-1}$  typical for C–N bond in PVP was also presented. The peak at  
470 1655  $\text{cm}^{-1}$  was characteristic for carbonyl group of PVP (Bryaskova et al., 2011).

471 FT-IR patterns of D12L and D12E were compared with pure quercetin and PVP spectra. Although  
472 D12L showed a peak of lower amplitude at 3448  $\text{cm}^{-1}$ , in both cases, solid dispersions spectra were

473 more similar to the polymer spectrum (peaks at 3448, 2955 and 1650-1670  $\text{cm}^{-1}$ ) (Fig. 4). Almost all  
474 peaks of quercetin shifted, decreased in intensity or totally disappeared, which suggests that hydrogen  
475 bonding occurs between quercetin and PVP (Li X.Y. et al. 2013; Yan et al., 2014).

476 The FTIR spectra of physical mixture D12M seemed to be only a summation of quercetin and PVP  
477 spectra. This aspect suggested that there was no interaction between drug and polymer in the physical  
478 mixture.

479

### 480 **3.3 Characterization of solutions**

481 D12L and D12E solubilized in water and D12L in saline solution were analyzed in terms of pH,  
482 osmolality and viscosity. As reported in Supplementary Table S3, the values of both formulations were  
483 very similar. The pH was acidic for both solutions (about 4) and therefore they are suitable for  
484 intravenous administration, which requires the pH in range of 4 to 8 (Tihanyi and Vastag, 2011). As  
485 confirmed by countless animal studies, a solution with a pH of 3-11 did not induce phlebotic changes,  
486 when drugs were administered over a few minutes (Roethlisberger et al., 2017). The viscosity of  
487 solutions was determined: solutions containing 200 mg/ml of solid dispersion showed values up to 3  
488 centipoises (Table S3 for Supplementary data). Nevertheless, diluted solutions with lower  
489 concentration of quercetin, used for *in vivo* experiments, exhibited low viscosities, comparable to the  
490 value of saline solution (1 cP) (Berteau et al., 2015).

491 The osmolality of solutions was measured (Supplementary Table S3), confirming the suitability of  
492 D12L in saline solution for injection ( $415.2 \pm 3.1$  mOsm/kg). Injectable of  $< 600$  mOsm/kg is  
493 considered to have a low-to-moderate risk of phlebitis; in particular, solutions with an osmolality less  
494 than 450 mOsm/kg are generally well tolerated by intravenous administration.

495 Nevertheless, very hypotonic solutions cause swelling of red blood cells and hemolysis, associated  
496 with pain and physiologic disturbances (Wang, 2015). Therefore, solid dispersions dissolved in water  
497 should not be administered (Supplementary Table S3).

498 The injectability of all solutions tested in *in vivo* experiments (200 mg/ml, 100 mg/ml, 40 mg/ml, 20  
499 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml) was evaluated by using the method reported by Schuetz et al.  
500 (2008) with some modifications. Injectability concept includes pressure or force required for injection,  
501 evenness of flow, and freedom from clogging (Cilurzo et al., 2011).

502 As expected, the injection speed rate was dependent on the concentration of samples; however, no  
503 solutions met any resistance. Obviously, injectability was related to product viscosity; indeed higher  
504 values of force required to push the solution through the syringe with more concentrated solutions  
505 compared to saline were obtained, by the virtue of PVP that increase the viscosity of solution (data not  
506 shown).

507

### 508 **3.3.1 Physical stability studies of water solutions**

509 Physical stability studies showed that aqueous solutions obtained from solid dispersions were quite  
510 stable over time. In fact, particle size of D12E increased from 20 nm to 100 nm after 28 days of storage  
511 at room temperature, as reported in Supplementary Fig. S2. Noteworthy, D12L was more stable than  
512 D12Es, showing a little increase in mean diameter until the 3<sup>th</sup> week, with no further changes during  
513 the last week of storage. Nevertheless, both solutions showed similar properties. Taking into account  
514 the data obtained, aggregation or precipitation of quercetin did not occur and thus a good stability of  
515 the drug in solution was supposed.

516

### 517 **3.3.2 Chemical stability studies of water solutions**

518 The drug contents of both solutions were determined spectrophotometrically over time in order to  
519 assess their chemical stability. During the 1<sup>st</sup> and 2<sup>nd</sup> weeks, the formulations were quite stable but after  
520 4 weeks, a rate of quercetin degradation corresponding to 30% was observed (data not shown). This  
521 behavior can negatively influence the therapeutic effect of the formulations. The product may be  
522 susceptible to physical and chemical degradation when stored as a ready-to-use solution. Therefore,  
523 considering the results obtained, the reconstitution of the powder prior to administration is highly  
524 recommended.

525

### 526 **3.4 Sterilization**

527 Sterilization process, required for injectable administration, did not modify the properties of D12L  
528 formulation dissolved in saline in terms of quercetin content, viscosity and injectability, confirming the  
529 feasibility of the process on the reconstituted powder (data not showed).

530

531

### 532 **3.5 *In vivo* studies**

533 By comparing the results from the *in vitro* characterization of the two solid dispersions, [there are no or](#)  
534 [minor differences between](#) D12E and D12L. Therefore, D12L was selected for *in vivo* studies because  
535 freeze-dried powders are more suitable and accepted for parenteral formulations.

536 Intravenous administration of D12L solid dispersion dissolved in saline led to decreases in both systolic  
537 and diastolic blood pressures in all anesthetized animals, however the dose of quercetin necessary for a  
538 blood pressure response was variable and ranged between 0.1 and 2.5 mg kg<sup>-1</sup> (Fig. Supplementary  
539 [Data S3A](#)). [Pure polymer](#) had apparently very little influence on the blood pressure [in this acute](#)  
540 [experiments](#). Comparison of maximal pressure drop between D12L and the control is shown in Fig. 5A.

541 Concerning the kinetics of blood pressure changes, the pressure started to decrease 2-3 minutes after  
542 the i.v. application of D12L and importantly the effect was gradual and persistent after one dose.  
543 Diastolic pressure tended to decrease more than systolic (Fig. 5B) in line with the assumption that  
544 quercetin acts [in this acute experiments](#) at the level of the vascular system. However, due to variable  
545 [responses among](#) animals, this change was not significant. The heart rate was not significantly  
546 influenced [after administration of neither](#) pure polymer solution nor quercetin dispersions (Fig.  
547 Supplementary Data S3B).

548 Pure quercetin is known to have vasodilatory effects on vascular smooth muscle cells *ex vivo* (Duarte et  
549 al., 1993); however its oral administration results in negligible concentrations of pure (non-  
550 metabolized) quercetin in plasma and its major conjugated metabolites had no direct vasodilatory  
551 effects neither *ex vivo* (quercetin-3-O-glucuronide, quercetin-3'-sulfate) (Lodi et al., 2009; Najmanová  
552 et al., 2016) nor *in vivo* (quercetin-3'-sulfate) (Galindo et al., 2012). In our experiments, a solid  
553 dispersion system carrying pure quercetin was used in order to demonstrate the *in vivo* direct  
554 vasodilatory activity of quercetin, administered in a biologically compatible solvent. Indeed, the arterial  
555 blood pressure decrease was observed few minutes after D12L intravenous administration. As  
556 mentioned, there was a marked difference between tested animals, for most of them the dose of 1 or 2.5  
557 mg kg<sup>-1</sup> of quercetin was needed to evoke a drop in arterial blood pressure. Because of these  
558 differences, we did not continue in dose-response studies, but the single dose of 5 mg kg<sup>-1</sup> of quercetin  
559 was selected for further confirmation of direct quercetin vasodilatory activity, in non-anesthetized,  
560 spontaneously hypertensive animals. Also in this case, the intravenous administration of D12L caused a  
561 decrease in systolic blood pressure (Fig. 6). The decrease started approximately 1 hour after  
562 administration and continued during the next 4 hours, reached an approximately 30% reduction of the  
563 initial blood pressure. After that, the systolic blood pressure slowly returned to the initial values.  
564 Interestingly, some decrease in blood pressure (10-20%) was observed also in the case of pure polymer,

565 but the effect of quercetin, given as D12L, was apparently stronger and reached significant difference  
566 vs. PVP10 at 3-5 hours after the administration. The explanation why the pure polymer caused also a  
567 decrease in arterial blood pressure is not known. Few papers described the PVP effect on the vascular  
568 system. Similarly to our study, PVP caused a profound decrease of blood pressure in dogs. Likely  
569 vasodilation and increased capillary permeability were responsible for the effect (Marshall and Hanna,  
570 1957). In another article, an increase in eNOS levels in aortic endothelial cells was reported after PVP  
571 administration to female rats, and the Rho-ROCK pathway was hypothesized to contribute to this  
572 vascular response. However, mostly non-consistent and non-significant tendencies in the contractile  
573 response of arteries were found *ex vivo*, and the results varied between pregnant and non-pregnant  
574 animals (Vidanapathirana, 2014). On the other hand, also results showing decreased ACh vasodilator  
575 response due to PVP are available (Mohamed, 2017).

576 As the last part, the analysis of pharmacokinetics of i.v. given D12L formulation was carried out. In  
577 addition to quercetin, also its metabolites appeared in plasma (Fig.7). Rapid decrease in plasma  
578 concentrations of quercetin with appearance of its known metabolites suggest that quercetin given in  
579 this formulation is rapidly dissolved in plasma similarly as it would be expected after administration of  
580 homogenous quercetin solution (e.g. by use of organic solvents). Concerning metabolites, isorhamnetin  
581 reached 10 times lower plasma concentrations than quercetin while its maximal concentration was  
582 more than 3 times higher than that of quercetin-3-O-glucuronide. Only very low concentrations of  
583 tamarixetin were observed.

584 There are no human studies, in which quercetin was given i.v. in a biologically compatible solvent.  
585 There is one clinical study in which large doses of quercetin dissolved in DMSO were given (Ferry et  
586 al., 1996). However, such administration cannot be used for tracking the effect of quercetin since  
587 DMSO can have negative effects.

588 Summarizing both pharmacodynamics experiments from this study, the arterial blood pressure started  
589 to decrease one hour after D12L i.v. administration, while similar blood-pressure lowering effects in  
590 anesthetized rats were observed within few minutes. This discrepancy can be related to both 1) the  
591 difference between the two types of experiments, with anesthetized and awake animals, and 2) the  
592 different mechanisms of action. For awake animals, the intravenous administration always represents a  
593 stress, even if it is performed in a gently way. Additionally, the blood pressure was measured by the  
594 occlusion cuff on the tail, which was also the place of application. Moreover, the repeated  
595 measurements at the beginning of the experiment in quick succession, could be another stress factor  
596 (even if the rats were previously trained for 2 weeks to adapt to this procedure). Despite these  
597 disadvantages, experiments on awake animals allow long-term monitoring of the studied effects that  
598 would not be realizable in animals under general anesthesia, and further confirmed the effect of  
599 quercetin. The acute and later effects of flavonoids on arterial blood pressure might be different.  
600 Acutely, flavonoids relax directly arteries while in a long-term base, they can have an effect on  
601 pathophysiology of hypertension. In terms of acute effects, there is no difference between quercetin and  
602 both its methylated metabolites isorhamnetin and tamarixetin while as mentioned above, quercetin-3-  
603 O-glucuronide is inactive (Najmanová et al., 2016). Regarding the later hypotensive effect, there are  
604 more suggested mechanisms. Quercetin is able to induce concentration-dependent phosphorylation of  
605 endothelial NO-synthase with subsequent improvement of vasodilation, both quercetin and  
606 isorhamnetin can decrease the expression of a NADPH-oxidase subunit, while isorhamnetin and  
607 quercetin-3-O-glucuronide, but not quercetin itself, are also blocking this enzyme (Chirumbolo, 2012;  
608 Mladěnka et al., 2010; Romero et al., 2009; Steffen et al., 2008). Hence the effect observed in this  
609 study in rats might be mediated not only by quercetin but also by its metabolites. In order to analyse the  
610 contribution of the metabolite(s), similar water-soluble preparation of isorhamnetin, in particular, will  
611 be needed, since isorhamnetin was apparently the major metabolite of quercetin given as D12L.

612 **4. CONCLUSION**

613 In this manuscript [the authors](#) described the preparation and characterization of solid dispersion  
614 enabling solubilization of quercetin in aqueous media for investigation of its direct *in vivo*  
615 cardiovascular effects. Low water solubility of quercetin represents a limit for testing and subsequent  
616 use of the drug. The *in vitro* characterization of the two solid dispersions prepared by using different  
617 techniques showed no [important](#) differences in both powder and solution forms. The solid dispersion  
618 [prepared](#) can be easily solubilized in aqueous media. However, considering the obtained results, the  
619 reconstitution of the formulation from freeze-dried powder before administration is highly  
620 recommended.

621 [This study gives also a direct proof of in vivo effects of i.v. given quercetin. Quercetin, administered in](#)  
622 [this i.v. way](#), reduced the arterial blood pressure in both awake and [anesthetized](#) rats. Nevertheless,  
623 rapid decrease of quercetin plasma concentration was observed after *in vivo* administration of solid  
624 dispersion. [Hence metabolites, in particularly isorhamnetin and possibly quercetin-3-O-glucoronide,](#)  
625 [could contribute to the vasodilatory effect of quercetin.](#)

626

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630

631 **Conflicts of interest**

632 The authors declare no conflicts of interest.

633

634

635

636 **REFERENCES**

- 637 Alves, L.D.S., Soares, M.F.D.L.R., de Albuquerque, C.T., da Silva, É.R., Vieira, A.C.C., Fontes,  
638 D.A.F., Figueirêdo, C.B.M., Sobrinho J.L.S., Neto, P.J.R., 2014. Solid dispersion of efavirenz in PVP  
639 K-30 by conventional solvent and kneading methods. *Carbohydr. Polym.* 104, 166-174.
- 640 Borghetti, G.S., Carini, J.P., Honorato, S.B., Ayala, A.P., Moreira, J.C.F., Bassani, V.L., 2012.  
641 Physicochemical properties and thermal stability of quercetin hydrates in the solid state. *Thermochim.*  
642 *Acta.* 539, 109-114.
- 643 Borghetti, G.S., Lula, I.S., Sinisterra, R.D., Bassani, V.L., 2009). Quercetin/ $\beta$ -Cyclodextrin solid  
644 complexes prepared in aqueous solution followed by spray-drying or by physical mixture. *AAPS*  
645 *PharmSciTech.* 10, 235-242.
- 646 Bose, S., Du, Y., Takhistov, P., Michniak-Kohn, B., 2013. Formulation optimization and topical  
647 delivery of quercetin from solid lipid based nanosystems. *Int. J. Pharm.* 441, 56-66.
- 648 Bryaskova, R., Pencheva, D., Nikolov, S., Kantardjiev, T., 2011. Synthesis and comparative study on  
649 the antimicrobial activity of hybrid materials based on silver nanoparticles (AgNps) stabilized by  
650 polyvinylpyrrolidone (PVP). *J. Chem. Biol.* 4, 185-191.
- 651 Caddeo, C., Nacher, A., Vassallo, A., Armentano, M.F., Pons, R., Fernández-Busquets, X., Carbone,  
652 C., Valentia, D., Fadda, A.M., Manconi, M., 2016. Effect of quercetin and resveratrol co-incorporated  
653 in liposomes against inflammatory/oxidative response associated with skin cancer. *Int. J. Pharm.* 513,  
654 153-163.
- 655 Catauro, M., Papale, F., Bollino, F., Piccolella, S., Marciano, S., Nocera, P., Pacifico, S., 2015.  
656 Silica/quercetin sol-gel hybrids as antioxidant dental implant materials. *Sci. Technol. Adv. Mater.* 16,  
657 035001.

658 Cerri, G., Farina, M., Brundu, A., Daković, A., Giunchedi, P., Gavini, E., Rassa, G., 2016. Natural  
659 zeolites for pharmaceutical formulations: Preparation and evaluation of a clinoptilolite-based material.  
660 *Microporous Mesoporous Mater.* 223, 58-67.

661 Cilurzo, F., Selmin, F., Minghetti, P., Adami, M., Bertoni, E., Lauria, S., Montanari, L., 2011.  
662 Injectability evaluation: an open issue. *AAPS Pharm. Sci. Tech.* 12, 604-609.

663 [Chirumbolo, S., 2012. Role of quercetin in vascular physiology. \*Can. J. Physiol. Pharmacol.\* 90, 1652-](#)  
664 [1657.](#)

665 Date, A.A., Nagarsenker, M.S., 2008. Parenteral microemulsions: an overview. *Int. J. Pharm.* 355, 19-  
666 30.

667 de Mello Costa, A.R., Marquiasavel, F.S., de Oliveira Lima Leite Vaz, M.M., Rocha, B.A., Pires  
668 Bueno, P.C., Amaral, P.L.M., da Silva Barud, H., Berreta-Silva, A. A., 2010. Quercetin-PVP K25  
669 solid dispersions: preparation, thermal characterization and antioxidant activity. *J. Therm. Anal.*  
670 *Calorim.* 104, 273-278.

671 Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P., Tognolini, M., Borges, G., Crozier, A., 2013. Dietary  
672 (poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against  
673 chronic diseases. *Antioxid. Redox Signal.* 18, 1818-1892.

674 Duarte, J., Vizcaíno, F.P., Utrilla, P., Jiménez, J., Tamargo, J., Zarzuelo, A., 1993. Vasodilatory effects  
675 of flavonoids in rat aortic smooth muscle. Structure-activity relationships. *Gen. Pharmacol.* 24, 857-  
676 862.

677 Eur Ph. Pharmaceutical Technical Procedures (Chapter 2.9) European Pharmacopoeia 8.0, 01/2014,  
678 Convention, Strasbourg, France (2014), pp. 285-370.

679 Ferry, D.R., Smith, A., Malkhandi, J., Fyfe, D.W., Anderson, D., Baker, J., Kerr, D.J., 1996. Phase I  
680 clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase  
681 inhibition. *Clin. Cancer Res.* 2, 659-668.

682 Filip, X., Grosu, I.G., Miclăuș, M., Filip, C., 2013. NMR crystallography methods to probe complex  
683 hydrogen bonding networks: application to structure elucidation of anhydrous quercetin. *Cryst. Eng.*  
684 *Comm.* 15, 4131-4142.

685 Foltmann, H., Quadir, A., 2008. Polyvinylpyrrolidone (PVP)-one of the most widely used excipients  
686 in pharmaceuticals: an overview. *Drug Deliv. Technol.* 8, 22-27.

687 Galindo, P., Rodriguez-Gómez, I., González-Manzano, S., Dueñas, M., Jiménez, R., Menéndez, C.,  
688 Vargas, F., Tamargo, J., Santos-Buelga, C., Pérez-Vizcaíno, F., Duarte, J., 2012. Glucuronidated  
689 quercetin lowers blood pressure in spontaneously hypertensive rats via deconjugation. *PloS one.* 7,  
690 e32673.

691 Gao, X., Wang, B., Wei, X., Men, K., Zheng, F., Zhou, Y., Zheng, Y., Gou, M., Huang, M., Guo, G.,  
692 Huang, N., Qian, Z., Wei, Y., 2012. Anticancer effect and mechanism of polymer micelle-encapsulated  
693 quercetin on ovarian cancer. *Nanoscale.* 4, 7021-7030.

694 Gao, Y., Wang, Y., Ma, Y., Yu, A., Cai, F., Shao, W., Zhai, G., 2009. Formulation optimization and in  
695 situ absorption in rat intestinal tract of quercetin-loaded microemulsion. *Colloids Surf. B Biointerfaces.*  
696 71, 306-314.

697 Gilley, A.D., Arca, H.C., Nichols, B.L., Mosquera-Giraldo, L.I., Taylor, L.S., Edgar, K.J., Neilson,  
698 A.P., 2017. Novel cellulose-based amorphous solid dispersions enhance quercetin solution  
699 concentrations in vitro. *Carbohydr. Polym.* 157, 86-93.

700 Hädrich, G., Vaz, G.R., Maidana, M., Kratz, J.M., Loch-Neckel, G., Favarin, D.C., de Paula Rogerio,  
701 A., Rodrigues, F.M., da Silva, F.M.R., Muccillo-Baisch, A.L., Dora, C.L., 2016. Anti-inflammatory  
702 effect and toxicology analysis of oral delivery quercetin nanosized emulsion in rats. *Pharm. Res.* 33,  
703 983-993.

704 Jahangiri, A., Barzegar-Jalali, M., Garjani, A., Javadzadeh, Y., Hamishehkar, H., Afroozian, A.,  
705 Adibkia, K., 2015. Pharmacological and histological examination of atorvastatin-PVP K30 solid  
706 dispersions. *Powder Technol.*, 286, 538-545.

707 Jullian, C., Moyano, L., Yanez, C., Olea-Azar, C., 2007. Complexation of quercetin with three kinds of  
708 cyclodextrins: an antioxidant study. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 67, 230-234.

709 Kadajji, V.G., Betageri, G.V., 2011. Water soluble polymers for pharmaceutical applications.  
710 *Polymers.* 3, 1972-2009.

711 Kakran, M., Sahoo, N.G., Li, L., 2011. Dissolution enhancement of quercetin through nanofabrication,  
712 complexation, and solid dispersion. *Colloids Surf. B Biointerfaces.* 88, 121-130.

713 Kumar, P., Sharma, G., Kumar, R., Singh, B., Malik, R., Katare, O.P., Raza, K., 2016. Promises of a  
714 biocompatible nanocarrier in improved brain delivery of quercetin: Biochemical, pharmacokinetic and  
715 biodistribution evidences. *Int. J. Pharm.* 515, 307-314.

716 Kumar, S., Pandey, A. K., 2013. Chemistry and biological activities of flavonoids: an overview. *Sci.*  
717 *World J.* 2013, doi:10.1155/2013/162750.

718 Larson, A.J., Symons, J.D., Jalili, T., 2010. Quercetin: a treatment for hypertension?—a review of  
719 efficacy and mechanisms. *Pharmaceuticals*, 3(1), 237-250.

720 Li, B., Konecke, S., Harich, K., Wegiel, L., Taylor, L.S., Edgar, K.J., 2013. Solid dispersion of  
721 quercetin in cellulose derivative matrices influences both solubility and stability. *Carbohydr. Polym.*  
722 92, 2033-2040.

723 Li, H., Zhao, X., Ma, Y., Zhai, G., Li, L., Lou, H., 2009. Enhancement of gastrointestinal absorption of  
724 quercetin by solid lipid nanoparticles. *J. Control. Release.* 133, 238-244.

725 Li, X.Y., Li, Y.C., Yu, D.G., Liao, Y.Z., Wang, X., 2013. Fast disintegrating quercetin-loaded drug  
726 delivery systems fabricated using coaxial electrospinning. *Int. J. Mol. Sci.* 14, 21647-21659.

727 Lodi, F., Jimenez, R., Moreno, L., Kroon, P.A., Needs, P.W., Hughes, D.A., Santos-Buelga, C.,  
728 Gonzalez-Paramas, A., Cogolludo, A., Lopez-Sepulveda, R., Duarte, J., Perez-Vizcaino, F., 2009.  
729 Glucuronidated and sulfated metabolites of the flavonoid quercetin prevent endothelial dysfunction but  
730 lack direct vasorelaxant effects in rat aorta. *Atherosclerosis.* 204, 34-39.

731 [Marshall, L.H., Hanna, C.H., 1957. Circulatory reaction of tolerant and nontolerant dogs to  
732 polyvinylpyrrolidone and of rats to dextran. \*Am. J. Physiol.\* 189, 209-213.](#)

733 Mladěnka, P., Zatloukalová, L., Filipický, T., Hrdina, R., 2010. Cardiovascular effects of flavonoids are  
734 not caused only by direct antioxidant activity. *Free Radic. Biol. Med.* 49, 963-975.

735 [Mohamed, T., Matou-Nasri, S., Farooq, A., Whitehead, D., Azzawi, M., 2017. Polyvinylpyrrolidone-  
736 coated gold nanoparticles inhibit endothelial cell viability, proliferation, and erK1/2 phosphorylation  
737 and reduce the magnitude of endothelial-independent dilator responses in isolated aortic vessels. \*Int. J.\*  
738 \*Nanomed.\* 12, 8813- 8830.](#)

739 Najmanová, I., Pourová, J., Vopršalová, M., Pilařová, V., Semecký, V., Nováková, L., Mladěnka, P.,  
740 2016. Flavonoid metabolite 3- (3- hydroxyphenyl) propionic acid formed by human microflora  
741 decreases arterial blood pressure in rats. *Mol. Nutr. Food Res.* 60, 981-991.

742 Otto, D.P., Otto, A., de Villiers, M.M., 2013. Experimental and mesoscale computational dynamics  
743 studies of the relationship between solubility and release of quercetin from PEG solid dispersions. *Int.*  
744 *J. Pharm.* 456, 282-292.

745 Park, S.H., Song, I.S., Choi, M.K., 2016. Preparation and Characterization of Quercetin-Loaded Solid  
746 Dispersion by Solvent Evaporation and Freeze-Drying Method. *Mass Spectrometry Letters.* 7, 79-83.

747 Pralhad, T., Rajendrakumar, K., 2004. Study of freeze-dried quercetin–cyclodextrin binary systems by  
748 DSC, FT-IR, X-ray diffraction and SEM analysis. *J. Pharm. Biomed. Anal.* 34, 333-339.

749 Porcu, E.P., Salis, A., Rassu, G., Maestri, M., Galafassi, J., Bruni, G., Giunchedi, P., Gavini, E., 2017.  
750 Engineered polymeric microspheres obtained by multi-step method as potential systems for  
751 transarterial embolization and intraoperative imaging of HCC: Preliminary evaluation. *Eur. J. Pharm.*  
752 *Biopharm.* 117, 160-167.

753 Puerta, E., Suárez-Santiago, J.E., Santos-Magalhães, N.S., Ramirez, M.J., Irache, J. M., 2017. Effect of  
754 the oral administration of nanoencapsulated quercetin on a mouse model of Alzheimer’s disease. *Int. J.*  
755 *Pharm.* 517, 50-57.

756 Rassu, G., Soddu, E., Cossu, M., Brundu, A., Cerri, G., Marchetti, N., Ferraro, L., Regan, R.F.,  
757 Giunchedi, P., Gavini, E., Dalpiaz, A., 2015. Solid microparticles based on chitosan or methyl- $\beta$ -  
758 cyclodextrin: A first formulative approach to increase the nose-to-brain transport of deferoxamine  
759 mesylate. *J. Control. Release.* 201, 68-77.

760 Roethlisberger, D., Mahler, H.C., Altenburger, U., Pappenberger, A., 2017. If euhydic and isotonic do  
761 not work, what are acceptable pH and osmolality for parenteral drug dosage forms? *J. Pharm. Sci.* 106,  
762 446-456.

763 Romero, M., Jiménez, R., Sánchez, M., López-Sepúlveda, R., Zarzuelo, M.J., O'Valle, F., Zarzuelo,  
764 A., Pérez-Vizcaíno, F., Duarte, J., 2009. Quercetin inhibits vascular superoxide production induced by  
765 endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. *Atherosclerosis*. 202, 58-67.

766 Sansone, F., Picerno, P., Mencherini, T., Villecco, F., D'ursi, A.M., Aquino, R.P., Lauro, M.R., 2011.  
767 Flavonoid microparticles by spray-drying: Influence of enhancers of the dissolution rate on properties  
768 and stability. *J. Food Eng.* 103, 188-196.

769 Sareen, S., Mathew, G., Joseph, L., 2012. Improvement in solubility of poor water-soluble drugs by  
770 solid dispersion. *Int. J. Pharm. Investig.* 2, 12-17.

771 Schuetz, Y.B, Gurny, R., Jordan, O., 2008. A novel thermoresponsive hydrogel based on chitosan, *Eur.*  
772 *J. Pharm. Biopharm.* 68, 19–25.

773 Sethia, S., Squillante, E., 2004. Solid dispersion of carbamazepine in PVP K30 by conventional solvent  
774 evaporation and supercritical methods. *Int. J. Pharm.* 272, 1-10.

775 Sharma, A., Jain, C.P., 2010. Preparation and characterization of solid dispersions of carvedilol with  
776 PVP K30. *Res. Pharm. Sci.* 5, 49-56.

777 Smith, A.J., Kavuru, P., Wojtas, L., Zaworotko, M.J., Shytle, R.D., 2011. Cocrystals of quercetin with  
778 improved solubility and oral bioavailability. *Mol. Pharm.* 8, 1867-1876.

779 Soni, L.K., Solanki, S.S., Maheshwari, R.K., 2014. Solubilization of poorly water soluble drug using  
780 mixed solvency approach for aqueous injection. *Br. J. Pharm. Res.* 4, 549-568.

781 Sri, K.V., Kondaiah, A., Ratna, J.V., Annapurna, A., 2007. Preparation and characterization of  
782 quercetin and rutin cyclodextrin inclusion complexes. *Drug Dev. Ind. Pharm.* 33, 245-253.

783 Srinivas, K., King, J.W., Howard, L. R., Monrad, J. K., 2010. Solubility and solution thermodynamic  
784 properties of quercetin and quercetin dihydrate in subcritical water. *J. Food Eng.* 100, 208-218.

785 Steffen, Y., Gruber, C., Schewe, T., Sies, H., 2008. Mono-O-methylated flavanols and other flavonoids  
786 as inhibitors of endothelial NADPH oxidase. *Arch. Biochem. Biophys.* 469, 209-219.

787 Sun, H., Xu, H., Yang, X., Li, N., Liu, Z., Pan, W., Yuan, Y., 2011. Formulation of a stable and high-  
788 loaded quercetin injectable emulsion. *Pharm. Dev. Technol.* 16, 609-615.

789 Teng, J., Bates, S., Engers, D.A., Leach, K., Schields, P., Yang, Y., 2010. Effect of water vapor  
790 sorption on local structure of Poly(vinylpyrrolidone). *J. Pharm. Sci.* 99, 3815-3825.

791 Tihanyi, K., Vastag, M., 2011. Solubility, delivery and ADME problems of drugs and drug-candidates,  
792 Bentham Science Publishers, Budapest.

793 Tran, T.H., Guo, Y., Song, D., Bruno, R.S., Lu, X., 2014. Quercetin- containing self- nanoemulsifying  
794 drug delivery system for improving oral bioavailability. *J. Pharm. Sci.* 103, 840-852.

795 Trendafilova, I., Szegedi, A., Mihály, J., Momekov, G., Lihareva, N., Popova, M., 2017. Preparation of  
796 efficient quercetin delivery system on Zn-modified mesoporous SBA-15 silica carrier. *Mat. Sci. Eng.*  
797 *C-Mater.* 73, 285-292.

798 Vasconcelos, T., Sarmiento, B., Costa, P., 2007. Solid dispersions as strategy to improve oral  
799 bioavailability of poor water soluble drugs. *Drug Discov. Today.* 12, 1068-1075.

800 Vidanapathirana, A.K., Thompson, L.C., Mann, E.E., Odom, J.T., Holland, N.A., Sumner, S. J., Han,  
801 L., Lewin, A.H., Fennell, T.R., Brown, J.M., Wingard, C. J., 2014. PVP formulated fullerene (C60)  
802 increases Rho-kinase dependent vascular tissue contractility in pregnant Sprague Dawley rats. *Reprod.*  
803 *Toxicol.* 49, 86-100.

804 Wang, W., 2015. Tolerability of hypertonic injectables. *Int. J. Pharm.* 490, 308-315.

805 Wang, W., Sun, C., Mao, L., Ma, P., Liu, F., Yang, J., Gao, Y., 2016. The biological activities,  
806 chemical stability, metabolism and delivery systems of quercetin: A review. *Trends Food. Sci. Technol.*  
807 56, 21-38.

808 Yan, J., Wu, Y.H., Yu, D.G., Williams, G.R., Huang, S.M., Tao, W., Sun, J.Y., 2014. Electrospun  
809 acid–base pair solid dispersions of quercetin. *RSC Adv.* 4, 58265-58271.

810 Yang, L., Li, P., Gao, Y., Wu, D., 2010. Qualitative observation of chemical change rate for quercetin  
811 in basic medium characterized by time resolved UV–VIS spectroscopy. *J. Mol. Liq.* 151, 134-137.

812 Yuan, Z.P., Chen, L.J., Fan, L.Y., Tang, M.H., Yang, G.L., Yang, H.S., Du, X.B., Wang, G.Q., Yao,  
813 W.X., Zhao, Q.M., Ye, B., Wang, R., Diao, P., Zhang, W., Wu, H.B., Zhao, X., Wei, Y.Q., 2006.  
814 Liposomal quercetin efficiently suppresses growth of solid tumors in murine models. *Clin. Cancer Res.*  
815 12, 3193-3199.

816 Zhang, X., Sun, N., Wu, B., Lu, Y., Guan, T., Wu, W., 2008. Physical characterization of  
817 lansoprazole/PVP solid dispersion prepared by fluid-bed coating technique. *Powder Technol.* 182, 480-  
818 485.

819

820

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## FIGURE CAPTIONS

**Figure 1. Water solubility of quercetin in solid dispersions compared to pure untreated quercetin.**

**Figure 2. XRPD patterns of quercetin (QCT), PVP10, and solid dispersions D12L and D12E.**

**Figure 3. TG and DTA curves of quercetin (QCT), PVP10, and solid dispersions D12L and D12E.**

**Figure 4. FT-IR spectra of quercetin, PVP10, D12M (a physical mixture of drug and polymer) and solid dispersions D12E and D12L.**

**Figure 5. Changes in arterial blood pressure. A:** maximal decrease in blood pressure between pure polymer PVP10 and D12L, **B:** differences in maximal decrease in systolic and diastolic blood pressure in D12L (quercetin) treated rats. [The experiments were performed on normotensive anesthetized rats.](#)

**Figure 6. Effect of D12L and PVP10 i.v. administration on arterial blood pressure in [non-anesthetized spontaneously hypertensive rats](#).** D12L was administered in the dose of  $65 \text{ mg.kg}^{-1}$  corresponding to the dose of  $5 \text{ mg.kg}^{-1}$  of pure quercetin. The dose of PVP10 was  $60 \text{ mg.kg}^{-1}$  (this dose corresponds to the amount of PVP10 administered during the D12L application). \*  $p < 0.05$ , \*\* $p < 0.01$

**Figure 7. Pharmacokinetics of D12L.** The [graphs are](#) showing plasma levels of quercetin and its metabolites after i.v. administration of D12L. [The administered dose of quercetin was  \$5 \text{ mg kg}^{-1}\$ .](#)



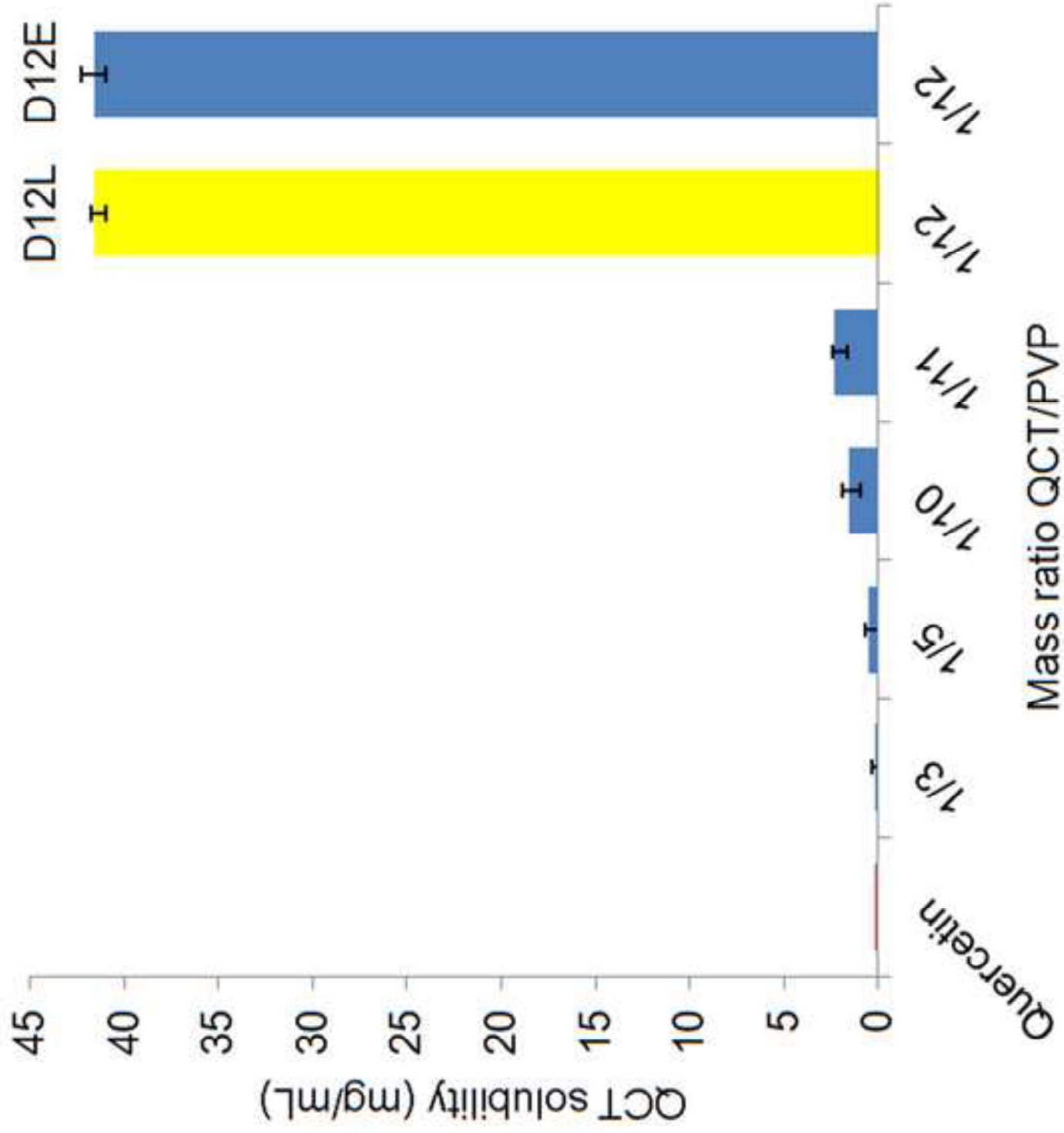


Figure1

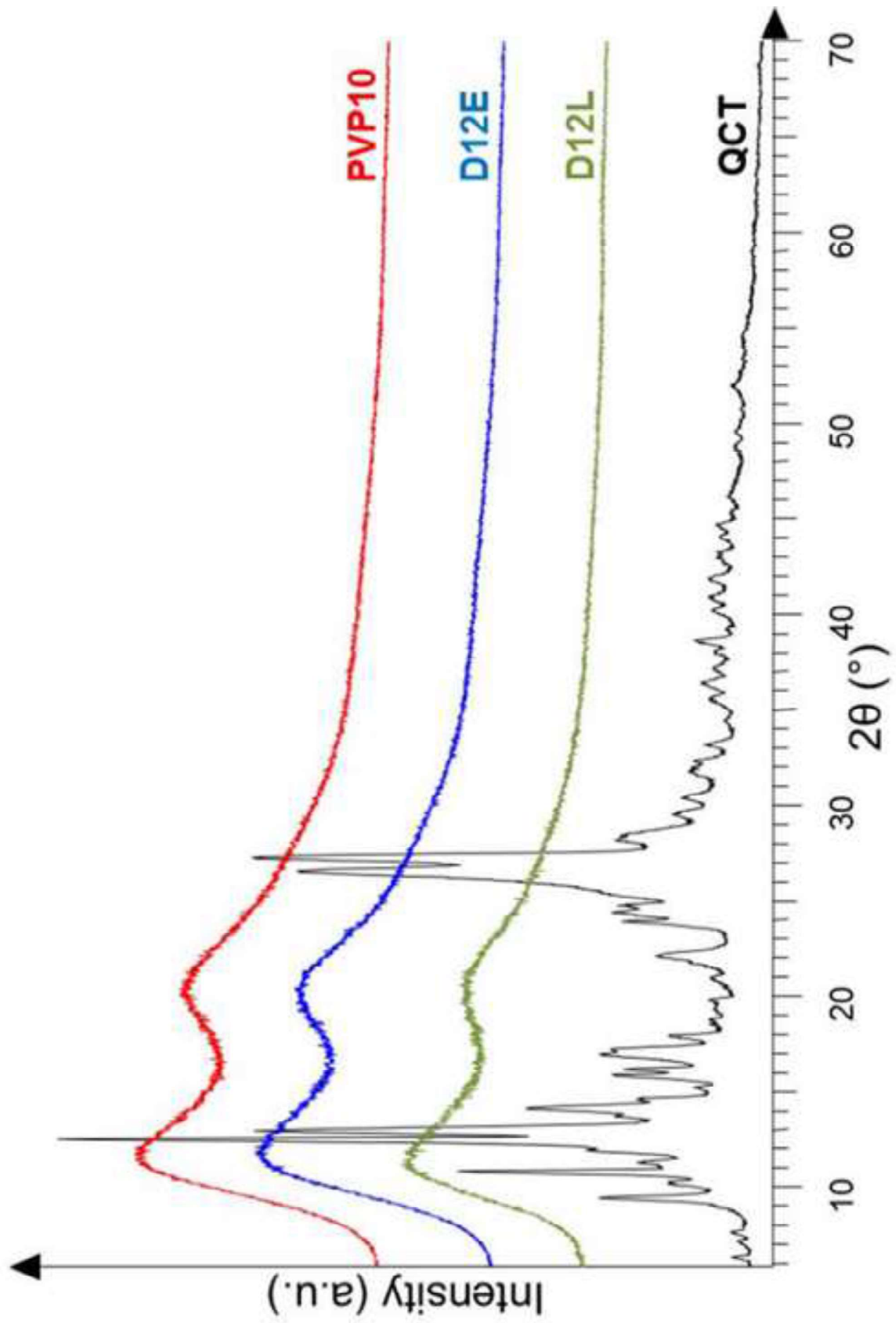


Figure2

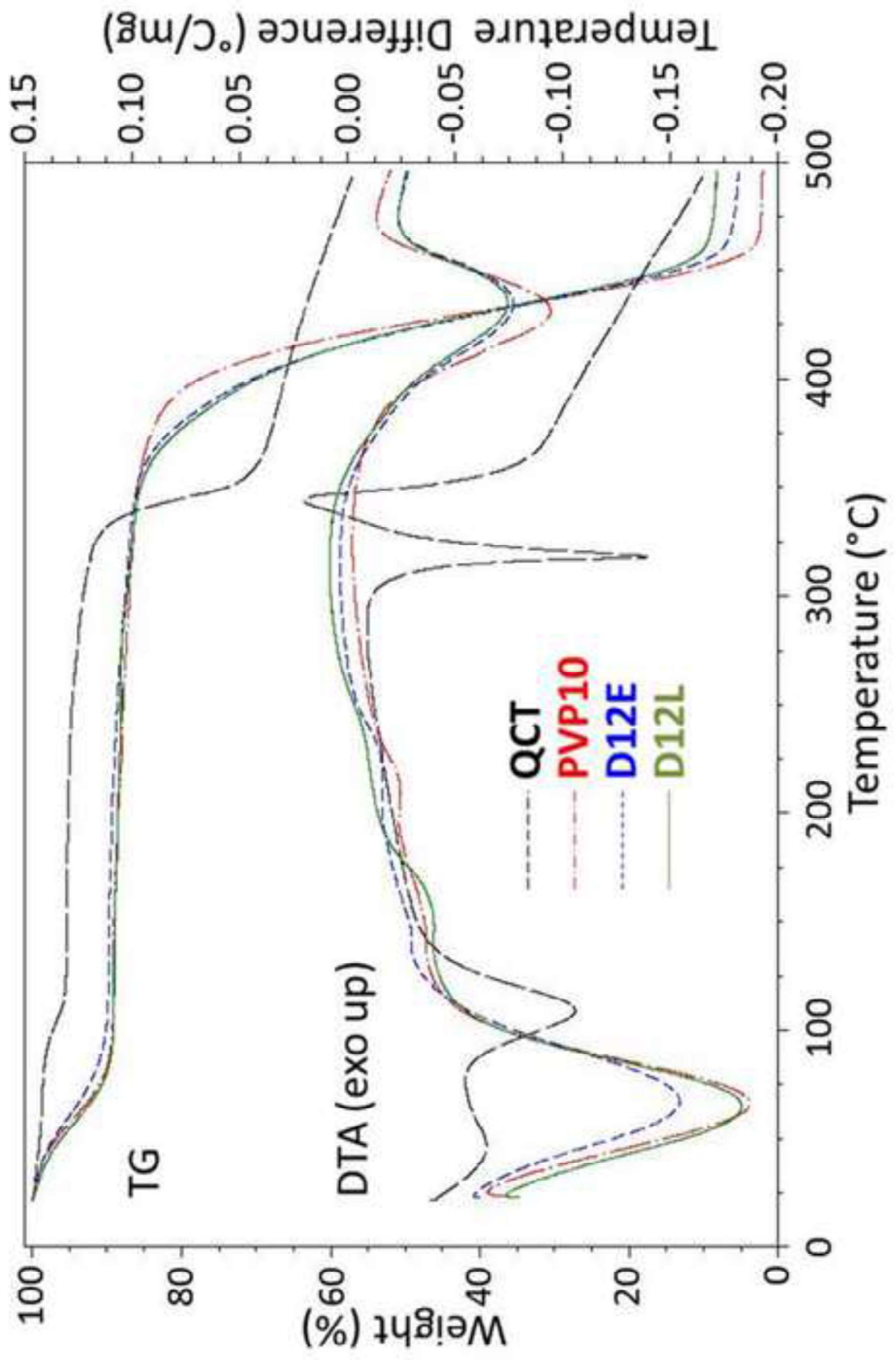


Figure3

Figure 4

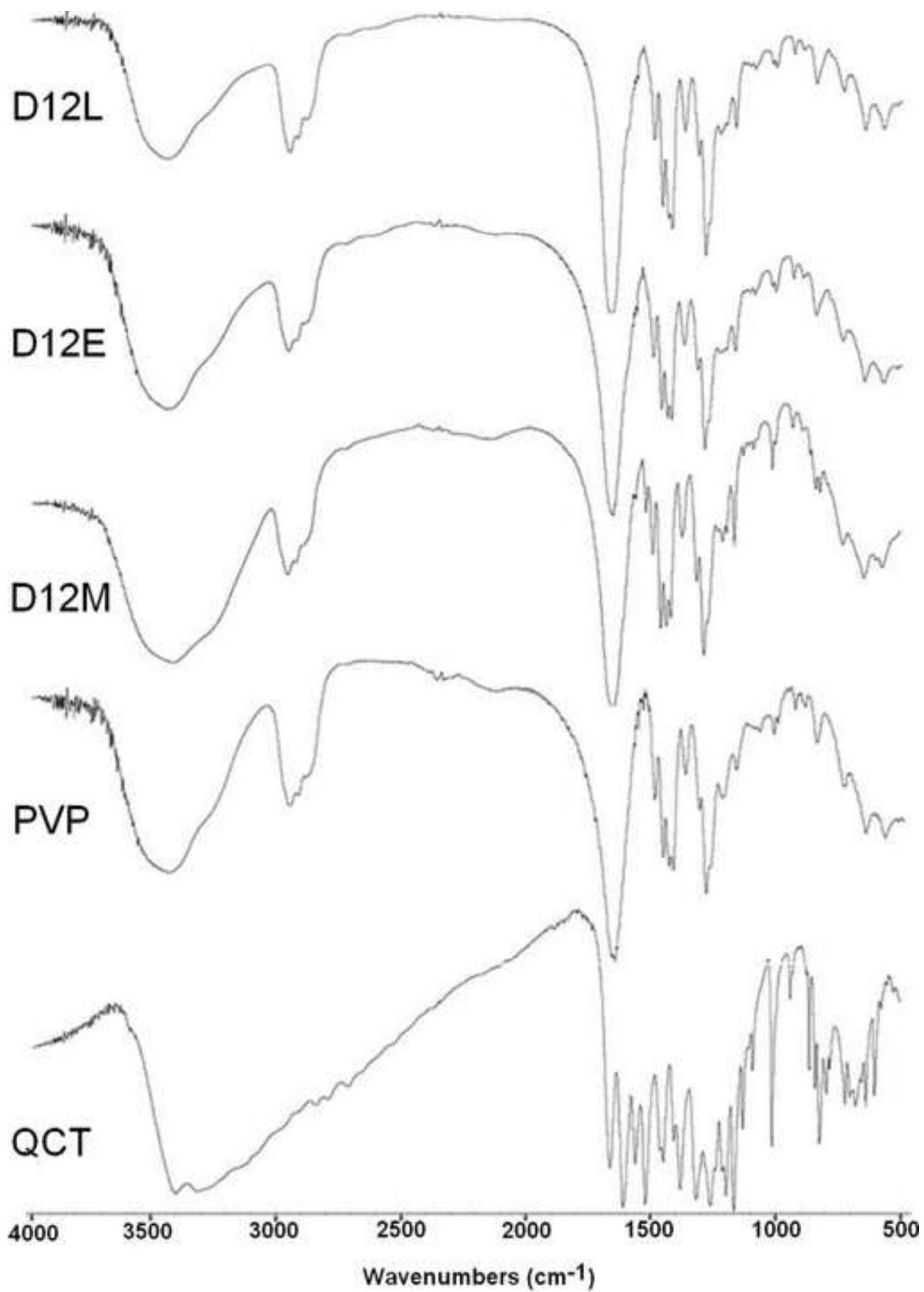


Figure5

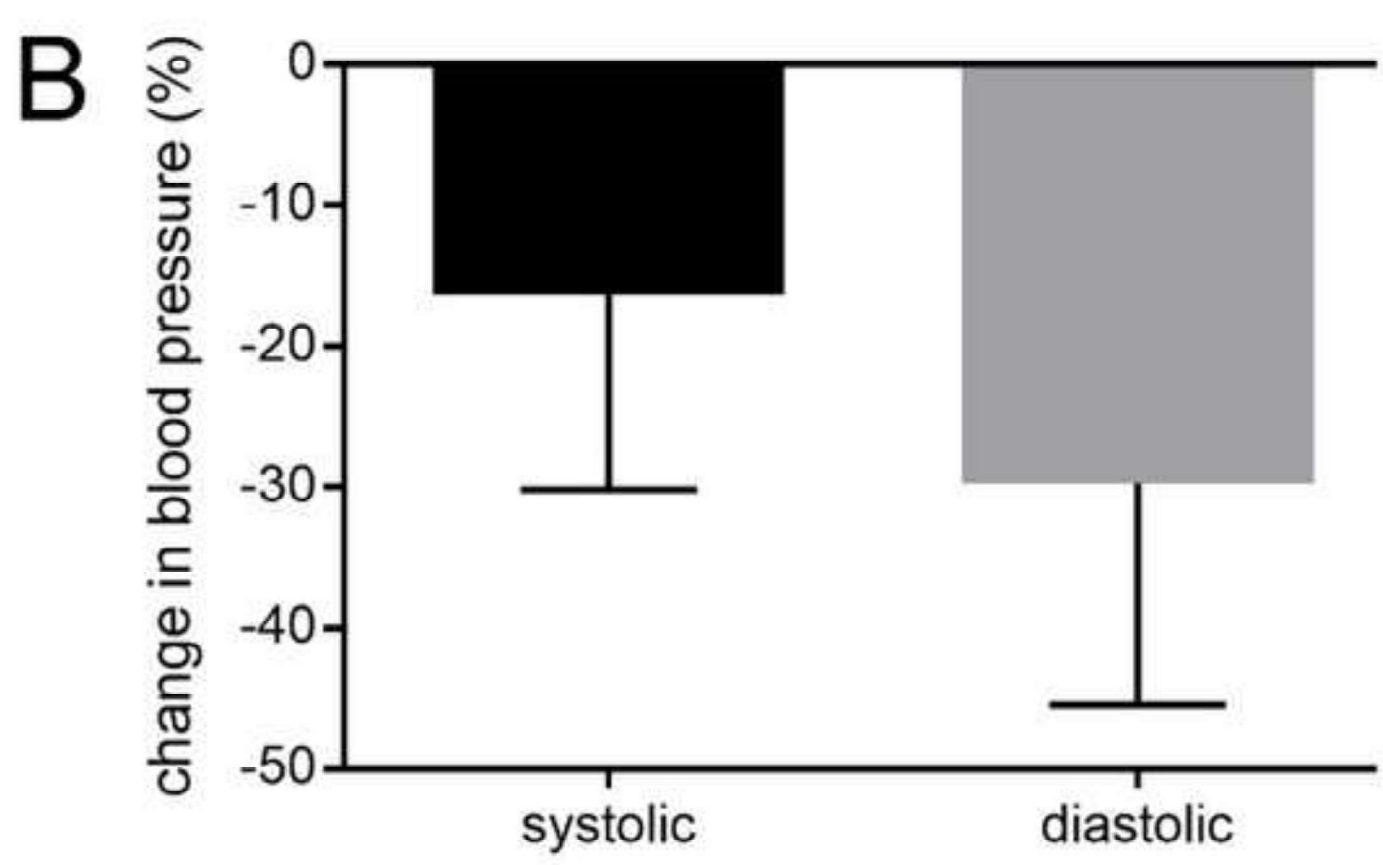
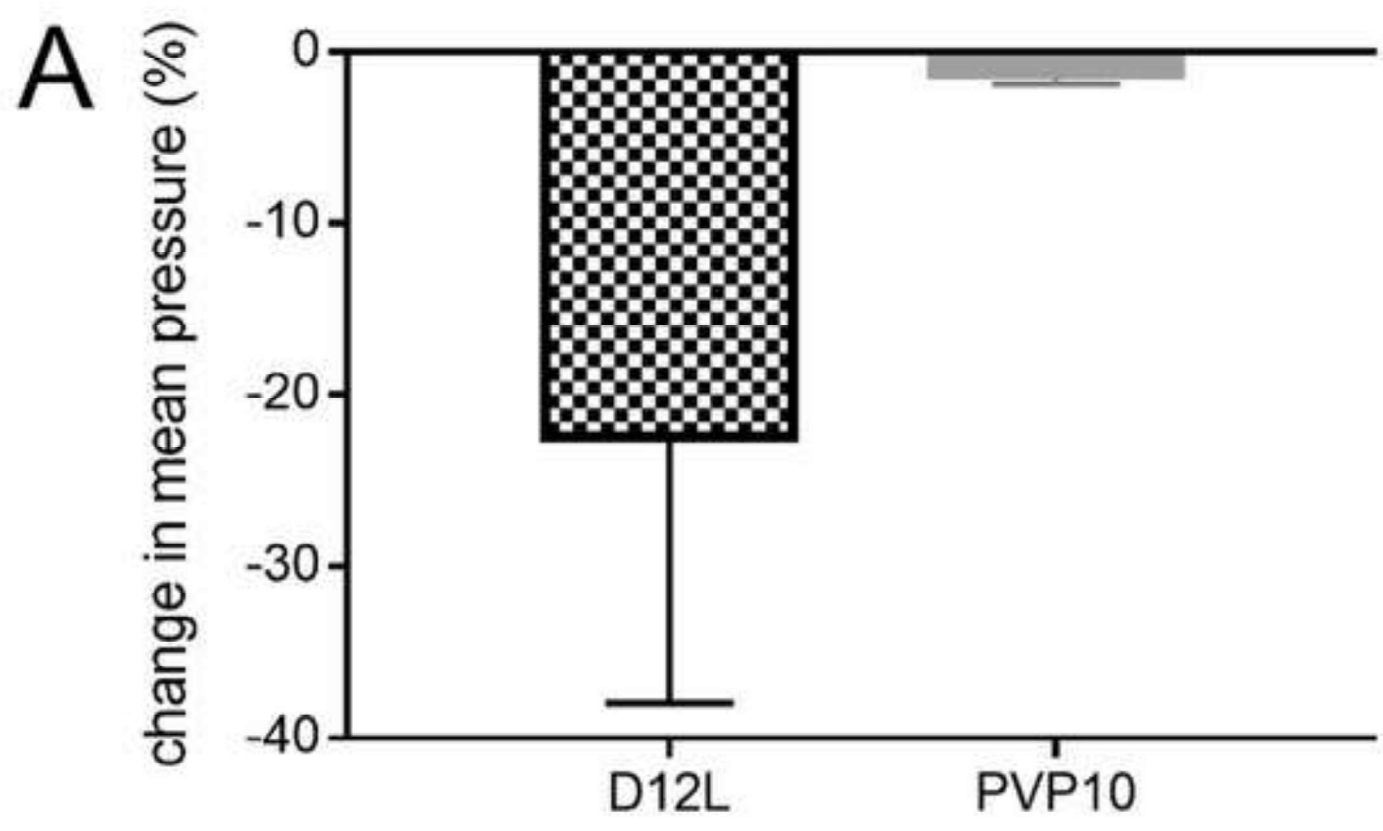


Figure 6

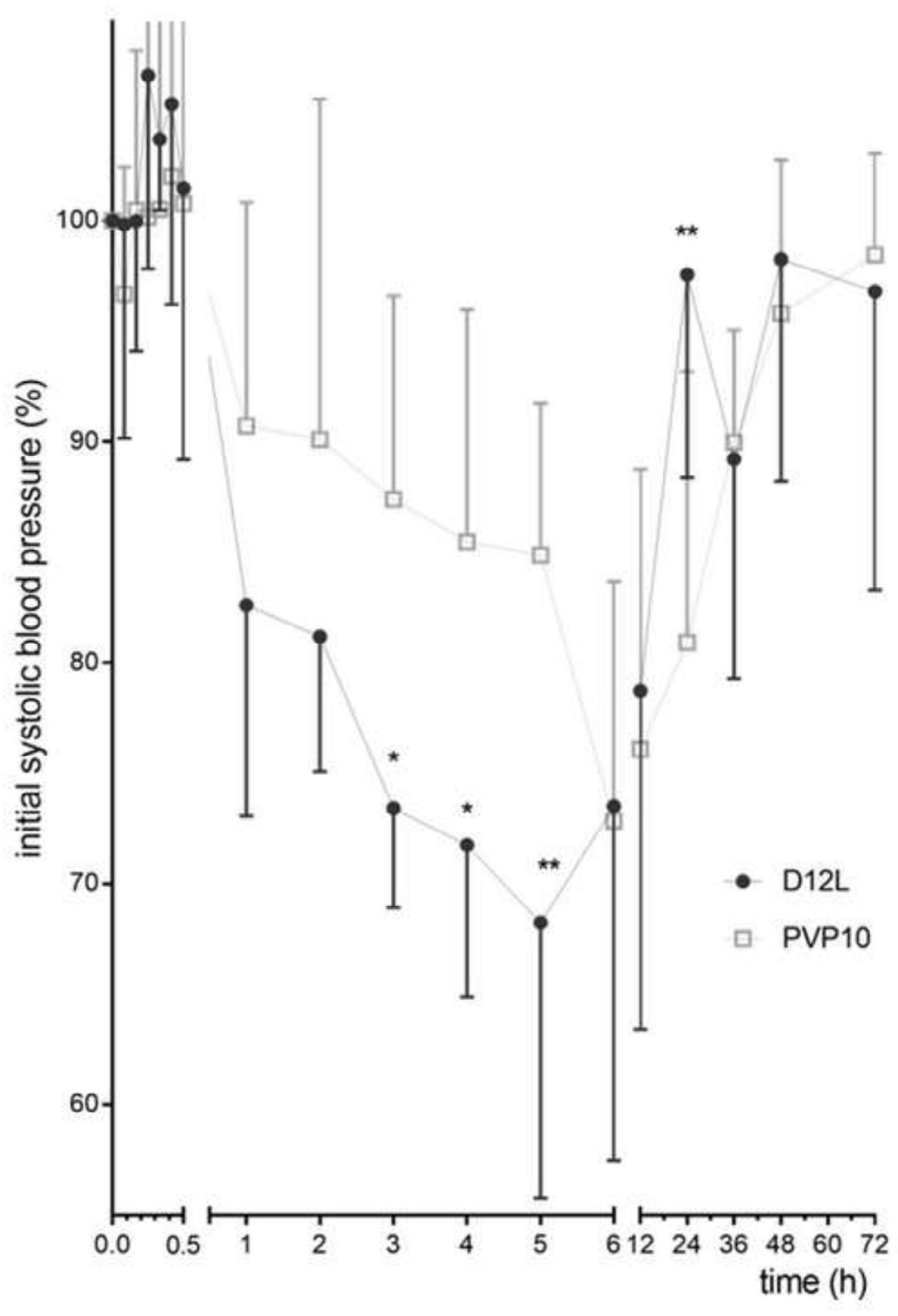


Figure 7

