1 **Comparative evaluation of rivastigmine permeation from a transdermal system in the**
2 **Franz cell using synthetic membranes and pig ear skin with *in vivo-in vitro* correlation**
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ABSTRACT

In the present study, *in vitro* permeation experiments in a Franz diffusion cell were performed using different synthetic polymeric membranes and pig ear skin to evaluate a rivastigmine (RV) transdermal drug delivery system. *In vitro-in vivo* correlations (*IVIVC*) were examined to determine the best model membrane. *In vitro* permeation studies across different synthetic membranes and skin were performed for the Exelon® Patch (which contains RV), and the results were compared. Deconvolution of bioavailability data using the Wagner–Nelson method enabled the fraction of RV absorbed to be determined and a point-to-point *IVIVC* to be established. The synthetic membrane, Strat-M™, showed a RV permeation profile similar to that obtained with pig ear skin (R^2 = 0.920). Studies with Strat-M™ resulted in a good and linear *IVIVC* (R^2 = 0.991) when compared with other synthetic membranes that showed R^2 values less than 0.90. The R^2 for pig ear skin was 0.982. Strat-M™ membrane was the only synthetic membrane that adequately simulated skin barrier performance and therefore it can be considered to be a suitable alternative to human or animal skin in evaluating transdermal drug transport, potentially reducing the number of studies requiring human or animal samples.

Keywords: transdermal drug delivery system; permeation, synthetic membrane; Strat-M™; Franz diffusion cell; rivastigmine; point-to-point *IVIVC*.

58 **1. Introduction**

59

60 Transdermal drug delivery is an attractive alternative delivery route compared with
61 more conventional routes, such as oral drug delivery, as it avoids first pass metabolism, and
62 may overcome issues of poor patient compliance (Bartosova and Bajgar, 2012). A
63 transdermal drug delivery system (TDDS) is a formulation or device (e.g. transdermal patch)
64 which provides controlled and continuous drug delivery through the skin, maintaining the
65 drug concentration in the blood within the effective therapeutic window (Kalia and Guy,
66 2001, Wokovich *et al*., 2006). Over the past two decades, TDDS development has become
67 increasingly important in the pharmaceutical industry, leading to an increased number of
68 TDDS being approved by regulatory authorities for commercialization (Prausnitz *et al*., 2004;
69 Prausnitz and Langer, 2008; Wiedersberg and Guy, 2014). The rivastigmine Exelon® Patch is
70 an example of a commercially available TDDS which is used for the symptomatic treatment
71 of mild to moderately severe dementia in Alzheimer's disease (Williams *et al*., 2003).
72 Rivastigmine (RV) is a molecule with a partition coefficient (log $P_{octanol/water}$) of 2.1,
73 exhibiting good permeability and solubility and thus falling into Class I of the
74 Biopharmaceutics Classification System (Tannergren *et al.*, 2009).
75 The therapeutic efficiency of a TDDS, such as a patch, depends on its performance,
76 which involves two main steps: (1) drug release from the patch, and (2) permeation and
77 diffusion through the stratum corneum, via the epidermis and dermis until it reaches the
78 systemic circulation (Kalia and Guy, 2001; Wokovich *et al*., 2006). Thus, the efficacy of a
79 TDDS must be tested by means of reproducible and reliable *in vitro* performance tests that are
80 able to measure drug release and permeation for the finished dosage form (Ueda *et al*., 2009).
81 A permeation test coupled with an *in vitro-in vivo* correlation (*IVIVC)* can aid the
82 development of a new transdermal system by anticipating patch performance before clinical
83 trials, saving time and reducing development costs. Furthermore, this type of testing can be
84 applied to evaluate the performance of products that have undergone scale-up and post-
85 approval changes (e.g. drug supplier, formulation and manufacturing site changes) (FDA,
86 2014) relieving companies of the need to repeat extensive studies. However, few reports can
87 be found in literature on *IVIVC*s for TDDS (Shen and Burgess, 2015).
88 The flow-through or static diffusion cells are recommended by international guidelines
89 to measure drug release and permeation rate for topical and transdermal dosage forms (EMA,
90 2012; FDA, 1997a; Franz, 1975; Ueda *et al*., 2009), and for the development of *IVIVC*s (Shen
91 and Burgess, 2015). The most common diffusion cells are Franz-type (static) cells which

92 consist of two compartments - donor and receptor - which must be separated by a membrane.
93 Synthetic membranes are commonly used for drug release studies and natural (human or
94 animal) skin for permeation studies (Addicks *et al.*, 1987; Agyralides *et al*., 2004; Clement *et*
95 *al*., 2000; Davaran *et al*., 2005; Frum *et al*., 2007; Hai *et al*., 2008; Joshi *et al*., 2012;
96 Limpongsa and Umprayn, 2008; Ng *et al.*, 2010; Santoyo *et al*., 1996; Uchida *et al.*, 2015),
97 due to the different diffusion properties offered by membrane models.

98 The best *in vitro* model reported to date for the prediction of the *in vivo* permeation of
99 topical products is the use of excised human skin in the Franz Cell (Franz *et al*., 2009; Yang
100 *et al*., 2015), and is also strongly recommended by regulatory agencies (FDA, 1997a; EMA,
101 2012; OECD, 2004). Nonetheless, the use of excised animal skin is also recognized as
102 acceptable, and is frequently used as a replacement for human skin (EMA, 2012; OECD,
103 2004), since human skin use is subject to national and international ethical considerations and
104 is not always accessible (OECD, 2004). A number of studies have demonstrated that pig ear
105 skin exhibits similar structural and biochemical characteristics to human skin (Barbero and
106 Frasch, 2009; Dick and Scott, 1992; Simon and Maibach, 2000), making it well-suited for
107 permeation studies, providing comparable results to human skin (Dick and Scott, 1992; Godin
108 and Touitou, 2007). In addition, it is generally recognized that natural tissue samples can
109 demonstrate variability from one species to another and even within the same species (e.g.
110 based on age, sex, race, degree of hydration), potentially affecting drug permeability across
111 skin (WHO, 2006). The ready availability of skin sources is not always possible, hindering
112 the application of *in vitro* performance testing for TDDS in Franz Cells.

113 The lack of biological skin availability recurrently leads to the use of synthetic
114 membranes for *in vitro* performance testing; a fit for purpose synthetic membrane must be
115 inert, provide high permeability and not occlude the drug penetration (EMA, 2012; FDA,
116 1997a; Ueda *et al.*, 2009). The Food and Drug Administration (FDA, 1997a) has suggested
117 the application of simple polymeric membranes, which have often been used in *in vitro* drug
118 permeation studies, such as: polysulfone (Clement *et al.,* 2000, Ng *et al.,* 2010),
119 polyethersulfone (Joshi *et al.,* 2012; Ng *et al*., 2010; Uchida *et al.,* 2015), cellulose (Santoyo
120 *et al.,* 1996; Clement *et al*., 2000; Ng *et al.,* 2010; Borges *et al*., 2013), or
121 polydimethylsiloxane (Addicks *et al*., 1987; Clement *et al.,* 2000; Frum *et al.,* 2007; Ng *et al*.,
122 2010). However, depending on the physicochemical characteristics of the synthetic
123 membrane, the Franz Cell test may only reflect the drug release, and may not reflect the drug
124 permeation across the skin, providing limited information about the effectiveness of the
125 TDDS (Borges *et al.,* 2013).

126 Due to the difficulty in obtaining biological skin samples, sample to sample variability
127 and ethical issues, there is an increasing interest in establishing an inexpensive and
128 reproducible *in vitro* membrane model that simulates the skin barrier performance in terms of
129 release and permeation. In this context, the aim of the present study was to develop an *in vitro*
130 drug permeation test utilizing synthetic membranes that can potentially replace the use of
131 human or animal skin. The study was coupled with the development of an *IVIV*C method for
132 rivastigmine patch (Exelon® Patch) as the chosen model TDDS.
133
134
135 **2. Materials and methods**
136
137 2.1 Materials
138
139 All reagents used were of analytical grade. Sodium hydroxide (NaOH), ammonium
140 hydroxide (NH_4OH), ammonium monobasic phosphate ($NH_4H_2PO_4$), sodium phosphate
141 (Na_2HPO_4) sodium chloride (NaCl), potassium chloride (KCl) and potassium monobasic
142 phosphate (KH_2PO_4) were purchased from Vetec (Rio de Janeiro, Brazil). The high
143 performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased
144 from Tedia (Rio de Janeiro, Brazil). For all filtration procedures 0.45 µm polyvinylidene
145 fluoride filters were used (Millex Millipore, São Paulo, Brazil). Purified water was obtained
146 using a Milli-Q water purification system (Millipore, Bedford, MA, USA).
147 Rivastigmine USP Reference Standard (batch no. F0J302) was purchased from the
148 U.S. Pharmacopeia. The TDDS containing RV, which is commercially available as Exelon®
149 Patch10 (produced by Novartis (Brazil)), was purchased from a local pharmacy. Each
150 transdermal patch of 10 cm^2 contains 18 mg of RV and is reported to release 9.5 mg RV over
151 a period of 24 hours (Novartis, 2015).
152 Pig ear skin was obtained from young animals sacrificed at a local slaughterhouse
153 (Suibom Comércio Atacadista de Carne Ltda/ME, Minas Gerais/ Brazil). Synthetic
154 membranes with different characteristics were acquired from different suppliers and are listed
155 in Table 1.
156
157
158 2.2 Methods
159

160 2.2.1 High performance liquid chromatography (HPLC) analysis

161

162 RV quantification was performed using an Elite LaChrom HPLC system from Merck-
163 Hitachi, (Darmstadt, Germany) coupled to a photodiode array detector (DAD L-2130),
164 quaternary pump (L- 2455), column oven (L-2350), autosampler (L-2200), and Eze-Chrom
165 software. RV in solution was quantified using a modified U.S. Pharmacopeia method (USP,
166 2015a). Chromatographic separation was achieved isocratically at room temperature with a
167 Kromasil 100Å C8 column (4.6 x 150 mm; 5 µm). The mobile phase consisted of a mixture of
168 monobasic ammonium phosphate buffer (8.6 mg/mL; pH 7.0), acetonitrile and methanol in a
169 50:25:25 (v/v/v) ratio and, was run at a flow rate of 1.2 mL/min (Amaro *et al.,* 2015; Simon *et*
170 *al.,* 2016). The eluent was monitored for 6 min with ultraviolet-visible detection at 215 nm for
171 RV quantification; the retention time was approximately 4 min.

172

173 2.2.2 Validation of the quantification method

174

175 The chromatographic method was validated by testing the parameters of specificity,
176 linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ)
177 (ICH 2005; USP, 2014b). The specificity was assessed by comparing the spectral scans (200-
178 400 nm) of the receptor medium, RV standard solution and product sample. The linearity was
179 evaluated by linear regression analysis of three replicates obtained on different days, at 6
180 levels ranging from 20 to 300 µg/mL, which are equivalent to 10 – 150 % of the drug working
181 concentration (200 µg/mL). The accuracy of the method was determined by a recovery test, in
182 which product solutions of predetermined concentration were spiked with a known amount of
183 RV standard solution at four levels (40, 100, 200 and 230 µg/mL) equivalent to 20, 50, 100,
184 115 % of working concentration. The repeatability of the method was estimated by
185 calculating the relative standard deviation (RSD) of the quantification of six patch samples
186 (Shabir, 2003; USP, 2015b). Each patch was submerged in 50 mL of acetonitrile and receptor
187 medium (1:1, v/v) and placed in an ultrasonic bath for 1 hour. The solutions were diluted in
188 receptor medium resulting in samples with a concentration of ~200 µg/mL. The LOQ and
189 LOD were calculated based on the equations: $LOQ = SD \times 10/S$ and $LOD = SD \times 3/S$,
190 where SD is the standard deviation of the intercept with the y-axis of three calibration curves
191 and S is the average of angular coefficients of the respective curves (ICH, 2005). The stability
192 of prepared solutions in receptor medium containing RV standard (10, 75 and 200 µg/mL),

193 was tested by storage at room temperature and evaluation at 0, 12, 24 and 48 h. The
194 chromatographic system suitability was monitored by controlling the retention time,
195 theoretical plates and asymmetry of the RV chromatographic signal.
196
197 2.2.3 Drug solubility in receptor medium
198
199 RV solubility was determined in phosphate-buffered saline (PBS) pH 7.4 at 37 ± 0.5
200 °C. PBS contains 138 mM NaCl, 2.7 mM KCl, 1.43 mM KH_2PO_4 and 8.57 mM Na_2HPO_4.
201 The solubility studies were carried out by adding an excess of drug in a beaker containing 5
202 mL of receptor medium to obtain a saturated solution. The solutions were kept under constant
203 magnetic stirring at 500 rpm for 24 h. Thereafter, the solutions were centrifuged (Eppendorf
204 5430R, Germany) for 15 minutes at a speed of 5000 rpm. The supernatant was filtered
205 through a 0.45 µm Millipore membrane, and the filtrate was assayed by the HPLC method for
206 RV quantification, according to the methodology described in section 2.2.1. The solubility
207 test was carried out in triplicate.
208
209 2.2.4 Pig skin and synthetic membranes preparation
210
211 The young white porcine ears from different animals were initially cleaned with tap
212 water, followed by excision of the dorsal skin from the underlying cartilage using a scalpel.
213 The hairs and subcutaneous fat tissue were removed using surgical scissors to obtain a full-
214 thickness skin (~400 µm), which was washed with distilled water and visually inspected to
215 ensure its integrity (Junyaprasert *et al.*, 2007). When not used immediately, the skin was
216 wrapped in filter paper, moistened with saline solution and packed in aluminum foil for
217 storage at -4°C for up to 3 days (Pupe *et al.*, 2013). At point of use, the frozen skin samples
218 were rehydrated by incubation in saline solution 0.9 % (w/v) at room temperature for one
219 hour before being placed in the Franz Cell units.
220 No pre-treatment was performed on silicone membranes (Sil2, Sil5, Sil10) or
221 polyethersulfone membrane (StrM). Cellulose membranes (Cup and Cel) required hydration
222 in boiling distilled water before use.
223
224 2.2.5 *In vitro* permeation studies
225

226 The *in vitro* permeation studies were carried out in a Franz Cell system (Hanson
227 Research, Chatsworth, LA, USA) with a diffusion area of 1.767 cm^2 and capacity of 7 mL for
228 the receptor medium, PBS pH 7.4. The Franz Cell system was maintained at a constant
229 temperature of 37 ± 0.5 °C through thermostatic bath circulation, while the receptor medium
230 was stirred constantly at 350 rpm during the experiments. For the permeation studies,
231 synthetic membranes with different characteristics were used (Table 1), as well as the pig ear
232 dermis. For each membrane evaluated, an assay was performed with six diffusion cells. Each
233 membrane was carefully placed at the interface between the donor and receptor
234 compartments, with placement of the Exelon® Patch 10 over the membrane. Aliquots of 1 mL
235 were collected at time 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 22, 23 and 24 hours (since the
236 TDDS is intended for single daily application). Sink conditions were maintained with the
237 replacement of the same volume of receptor medium held at 37 ± 0.5 °C. All collected
238 samples were analyzed by HPLC and RV quantification was obtained by the regression
239 equation obtained from a standard curve prepared on the same day as the analysis.
240 The cumulative amount of RV permeated through the membrane was calculated taking
241 the patch area into account (mg/cm²), with results plotted as a function of time (hours). To
242 study the kinetics of RV permeated through different membranes between 1 and 24 hours,
243 data were treated according to zero-order (cumulative amount of drug permeated versus time),
244 first-order (log cumulative amount of drug remaining versus time), Higuchi (cumulative
245 amount of drug permeated versus square root of time), and Korsmeyer-Peppas (log
246 cumulative amount of drug permeated versus log time) models. The best-fit kinetics model
247 was selected based on the correlation coefficient values obtained by linear regression (Costa
248 and Lobo, 2001). The significance of the differences observed from the application of
249 different membranes was verified by a repeated measures test using one-way ANOVA
250 (GraphPad Prism® software, La Jolla, CA, USA) and considered significant if $p < 0.05$.
251
252 2.2.6 Methodology applied in the development of *IVIVC*
253
254 The RV *in vitro* permeation data was retrieved from studies described in section 2.2.5,
255 and the *in vivo* plasma concentration data (ng/mL) versus time (h) of the RV after TDDS
256 (Exelon® Patch 10) skin application was obtained from a previously reported pharmacokinetic
257 study (Lefèvre *et al.*, 2008). Mean plasma concentration data of RV as a function of time was
258 deconvoluted using the Wagner-Nelson mathematical equation (eq. 1) and thereby the RV

fraction absorbed (%Fa) at different times was calculated (Wagner and Nelson, 1964; Yang *et al.,* 2015).

$$\%Fa = \frac{C_t + k_{el} \cdot AUC_{0\to t}}{k_{el} \cdot AUC_{0\to\infty}} \times 100\% \quad \text{(eq. 1)}$$

Where, C_t is the plasma concentration of RV at time (*t*), k_{el} is the elimination rate constant, $AUC_{0\to t}$ is the area under the curve from time 0 to time *t,* and $AUC_{0\to\infty}$ is the area under the curve from time 0 to infinity (Wagner and Nelson, 1964; Yang *et al.,* 2015).

A point-to-point *IVIVC* for each membrane investigated was evaluated through assessment of the linear correlation between *in vitro* permeation and the *in vivo* input rate (FDA, 1997b). *In vitro* and *in vivo* fractions were plotted as independent (*x*) and dependent (*y*) variables, respectively, and a linear regression analysis established (Silva *et al*., 2015; Yang *et al*., 2015). The linear regression calculations were performed using Microsoft® Office Excel® 2007.

2.2.7 Statistical analysis

Microsoft® Office Excel® 2007 was used to determine *in vitro* permeation data. Difference between means was determined through statistical analysis of variance, ANOVA with Tukey's multiple comparison post-hoc test using GraphPad Prism® software (version 5.0), with a 95 % confidence level.

3. Results and discussion

3.1 HPLC method validation

Accurate and reliable analytical methods for drug assay in *in vitro* permeation studies are mandatory to evaluate the performance of transdermal systems (USP, 2015b). A modified U.S. Pharmacopeia method was used to quantify RV hydrogen tartrate raw material. The chromatographic method demonstrated specificity, since no chromatographic signal was detected on the injection of receptor medium alone. The RV chromatographic peak obtained from the standard and sample solutions injection had a retention time of 4.07 min with a peak purity of more than 0.999. The linearity was established with a correlation coefficient of 0.999

292 (Table 2). The repeatability of the method was adequate (RSD < 5%). The accuracy was
293 satisfactory, presenting recoveries between 99.2 and 100.0 %. The solutions containing RV at
294 concentrations of 10, 75 and 200 µg/mL remained stable after storage for 48 hours at room
295 temperature, with RSD of 0.52, 0.73 and 0.67 %, respectively. The chromatographic system
296 suitability to RV assay in the receptor medium exhibited satisfactory parameters, with
297 theoretical plates higher than 4300, peak asymmetry between 1.5-1.7 and, peak purity greater
298 than 0.99 for all determinations.
299
300 3.2 *In vitro* permeation studies
301
302 The use of a Franz Cell system requires the selection of a receptor medium which can
303 maintain sink conditions or at least the capacity to solubilize the drug content in the patch
304 (Clément *et al.,* 2000). In order to maintain sink conditions, it was considered that, if the
305 entire amount of RV contained in the patch was transferred to the receptor medium volume
306 (as migration of the drug can occur to the diffusion area), the solution concentration should be
307 no more than one third of the saturation solution concentration, as recommended by the U.S.
308 Pharmacopeia (USP, 2015c). The RV solubility in PBS pH 7.4 was established
309 experimentally to be 18.69 ± 0.27 mg/mL (n = 3). Considering that the receptor compartment
310 volume of each cell is 7 mL, and the declared maximum drug content in the patch is about 18
311 mg, the maximum concentration that may be attained in the receptor compartment is 2.57
312 mg/ml (which is 14% of the saturation solubility) and thus sink conditions are maintained.
313 The RV cumulative permeation profiles from TDDS are shown in Figure 1 where
314 panel A shows the permeated amount from synthetic membranes plotted against time and,
315 panel B shows the permeated amount from pig ear skin against time. The total drug amount
316 (mg ± SD) permeated over 24 hours through synthetic membranes Cel, Cup, Sil2, Sil5, Sil10
317 and StrM was 18.53 ± 0.21 mg, 18.81 ± 1.32 mg, 19.23 ± 0.91 mg, 19.22 ± 1.35 mg, 19.41 ±
318 1.14 mg and 14.89 ± 2.23 mg respectively, and through pig ear skin was 15.85 ± 1.62 mg. The
319 RV cumulative permeation profiles obtained over the 24 hours of experimentation were not
320 significantly different for cellulose (Cel, Cup) and silicone membranes (Sil2, Sil5, Sil10)
321 when evaluated by one-way ANOVA with Tukey's post-hoc test ($p > 0.05$). These permeation
322 profiles were found to be characteristic of anomalous kinetics (non-Fickian mechanism) by
323 fitting of the Korsmeyer-Peppas model, which resulted in R^2 values of 0.902, 0.837, 0.874,
324 0.881 and 0.899 for Cel, Cup, Sil2, Sil5 and Sil10 respectively, corroborating with previous
325 literature (Costa and Lobo, 2001). The Sil10 membrane, which is thicker (0.254 mm) than

326 other membranes, demonstrated lower drug permeation at initial time points in comparison
327 with the other synthetic membranes; however, after 2 hours the permeated amount was not
328 significantly different to other membranes, suggesting that the membrane also offers the least
329 possible diffusional resistance. Therefore, these results suggest that the cellulose and silicone
330 membranes do not present considerable resistance to the diffusion of RV from the patch.
331 Conversely, the synthetic membrane, StrM, when compared to all other synthetic membranes
332 evaluated in this study, exhibited a significantly different ($p < 0.001$) permeation profile, in
333 which the cumulative amount of RV diffused across the membrane showed similarity with the
334 permeation data for the pig ear skin (Skin). These permeation profiles were best characterised
335 by the Higuchi (square root of time) kinetic model for Skin ($R^2 = 0.987$) and StrM ($R^2 =$
336 0.997). This kinetic model is frequently used to describe the controlled release rate of a drug
337 from a dosage form by a constant diffusion process (Siepmann and Peppas, 2011). The
338 synthetic membrane StrM showed a RV permeation profile that was statistically similar to
339 that obtained with the Skin, with comparable permeation-time profiles ($p > 0.05$).
340 Considering the similarity between the RV permeation profiles obtained for StrtM and
341 Skin membranes shown in Figure 1, a linear relationship was investigated between
342 permeation across StrtM versus Skin. A significant and strong correlation ($p < 0.0001$) was
343 found with a correlation coefficient (r) of 0.962 between the RV diffusion through StrM and
344 Skin (Figure 2), suggesting that the synthetic membrane provides a diffusion barrier function
345 which is similar to that of pig ear skin.
346 Skin is constituted by a multilayer structure, and the outmost layer, the stratum
347 corneum, is an effective barrier against drug permeation across skin due to the presence of
348 lipid components (Prausnitz and Langer, 2008). Previously, Clément and co-workers
349 (Clément *et al.*, 2000) evaluated synthetic membranes impregnated with synthetic
350 hydrophobic material and demonstrated the importance of the lipophilic characteristics to act
351 as skin lipid-like materials, simulating the stratum corneum barrier. Uchida and co-workers
352 (Uchida *et al.*, 2015) observed, by microscopic analysis, the presence of synthetic lipids in
353 two of the three polymeric layers which form the StrM membrane, imparting additional
354 hydrophobic skin-like properties to this synthetic membrane. It was also reported that
355 polymeric layers create a porous structure with a total thickness of approximately 325 μm,
356 which is divided into three layers of different densities, providing a permeation gradient in
357 terms of pore size and diffusivity (Merck, 2012; Uchida *et al.,* 2015).
358 Consequently, this similar RV diffusion behaviour is thought to be related to the
359 hydrophobic characteristics and irregular polymeric structure of the StrM membrane, which

360 provided for the correlation between *in vitro* drug permeation profiles obtained using the
361 synthetic StrM membrane and pig ear skin. The results presented here indicate that the
362 synthetic membrane may be successfully used as a substitute for biological skin for *in vitro*
363 permeation studies, taking into consideration that pig ear skin exhibits structural and
364 biochemical characteristics similar to those of human skin (Barbero and Frasch, 2009; Dick
365 and Scott, 1992; Simon and Maibach, 2000).
366
367 3.3 *IVIVC* studies
368
369 The collected *in vitro* permeation data was mathematically correlated with the
370 bioavailability data of RV from TDDS, Exelon® Patch 10, as the same principles of *IVIVC*
371 utilized for extended release oral dosage forms (FDA, 1997b) can be applied to non-oral
372 dosage forms, such as parenteral depot formulations and new drug delivery systems (Nandy *et*
373 *al.*, 2011; Shen and Burgess, 2015).
374 Based on the RV plasma concentration data versus time obtained from the
375 bioavailability curve (Lefèvre *et al.*, 2008), the RV fraction absorbed (%Fa) as a function of
376 time was calculated using the Wagner-Nelson mathematical equation. RV pharmacokinetics
377 from a transdermal patch has been previously described by a one compartment model
378 (Mercier *et al.*, 2007; Lefevre *et al.*, 2008). Figure 3 presents the curve of the RV fraction
379 absorbed versus time after dermal application of the TDDS until C_{max} was reached.
380 According to Yang and co-workers, only Level A correlations are applicable to *IVIVC*
381 of TDDS, due to the complex mechanism of drug permeation (Yang *et al.,* 2015). Therefore,
382 the point-to-point *IVIVC* approach was used in this study for the RV patch (TDDS), i.e. a
383 point-to-point relationship between *in vitro* dissolution/permeation and the *in vivo* absorption
384 of drug from the dosage form (FDA, 1997b). The correlation was performed by plotting the
385 data obtained during the time interval from 0 to 8 hours after dermal application, to reflect the
386 time required for RV plasma concentration to reach a maximum (t_{max}) before beginning to
387 decrease as a function of elimination processes. The coefficient of correlation (R^2) and the
388 slope were obtained by plotting the *in vivo* fraction absorbed (%Fa) against the *in vitro*
389 fraction permeated, assuming the *in vivo* time was the same as *in vitro* time (point-to-point)
390 without any change in the time scale. *IVIVC* data is shown in Table 3.
391 The validity of the regression curves was represented by the $F_{cal} >> F_{tab}$, indicating that
392 the slope is significantly different from zero with p values no more than 0.0007 ($p < 0.05$).
393 The best-fit values ($R^2 > 0.90$) for linear correlation were found for Skin and StrM

membranes. For these membranes all points evaluated fitted close to the linear plot with minimum scatter (Figure 4), resulting in a low residual standard deviation (Sy.x). On the other hand, the Sil2, Sil5, Sil10, Cup and Cel membranes showed a high residual standard deviation, reflecting a less linear distribution, that is, the regressed data was less homogeneously distributed and more scattered around the regression line of the model, resulting in a poorer correlation, with $R^2 < 0.90$, and reduced predictability of the model.

It is interesting to note that the StrM synthetic membrane presented a strong and significant linear correlation with the *in vivo* data, with $R^2 = 0.991$, in the same way as the pig ear skin (Skin), with $R^2=0.982$. These good and similar correlations may be justified by the similar properties of the pig ear skin and StrM synthetic membrane and human skin. The good linear correlations obtained by *in vitro* RV permeation across StrM and Skin with *in vivo* RV absorption indicates that the methodology used in the *in vitro* permeation studies reflects the *in vivo* conditions, which is the correlation level which is most desirable for TDDS (Shen and Burgess, 2015; Yang *et al.,* 2015).

TDDS development is gaining increasing interest by the pharmaceutical industries, and also represents one of the most successful and innovative areas of research in drug delivery (Bartosova and Bajgar, 2012). However, during the development of new formulations, the number of animal and human studies should be kept to the minimum necessary to achieve the aims of the research, thus, the use of predictive *in vitro* studies of *in vivo* performance are increasingly needed. Despite the recognized importance of *IVIVC*s for oral dosage forms, the development of *IVIVC*s for TDDS has not yet been given proper importance, to date, and there are only a few literature reports on *IVIVCs* for TDDS (Chaturvedula *et al.,* 2005; Mateus *et al.,* 2014; Mohammed *et al.* 2014; Shen and Burgess, 2015, Yang *et al.,* 2015). In view of this, the data presented in this section suggests, once more, StrM synthetic membrane as a viable alternative to human and animal skin models for transdermal permeation studies, since the results showed an *in vitro* permeation test coupled with *IVIVC*, under controlled conditions, for prediction of *in vivo* RV transdermal absorption from a TDDS.

Furthermore, the permeation rate specifications for a transdermal dosage form are essential for the quality control analysis in order to assure a reproducible performance of TDDS, and consequently its therapeutic efficacy. If an *in vitro* permeation test can be related to the *in vivo* performance of a product, it is of significant interest and importance to the pharmaceutical industry, since a generic sponsor would have an efficient tool to evaluate different formulations and select the optimal formulation for use in the pivotal bioequivalence

428 study (Lionberger, 2008). The use of artificial membranes in *in vitro* methods with potential
429 to mimic the biological skin and predict product performance and drug permeability is a key
430 step in the development phase of a new transdermal product containing RV, as well as
431 screening of drug candidates for transdermal delivery, in order to ensure the drug permeation
432 rate and safety in clinical studies.
433

434 **4. Conclusion**

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436 In the current study, a point-to-point *IVIVC* was developed using the Franz Cell with a
437 synthetic membrane for evaluation of RV delivery from a rivastigmine TDDS. This *in vitro*
438 method may be used for quality control, stability verification, to test a product after post-
439 approval changes and/or for equivalence studies of a new generic TDDS. Permeation of RV
440 across the StrM synthetic membrane in Franz diffusion cells correlated well with results of
441 permeation studies using pig ear skin.
442 The results demonstrated that the use of the StrM synthetic membrane in Franz
443 diffusion cells could predict *in vivo* RV absorption, making this methodology promising to
444 assess other drugs. This synthetic membrane can be seen as an alternative to human or animal
445 skin when evaluating drug transdermal diffusion during the TDDS development, reducing
446 cost and time.
447

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449

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453

454 **Conflict of interest**

455

456 The author(s) confirm that this study content has no conflicts of interest.
457
458

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650 **LIST OF FIGURES**

651

652 Figure 1. *In vitro* permeation profile of RV from Exelon® Patch10 obtained from studies in
653 Franz Cell using different membranes. Panel (A) shows the cumulative RV permeation
654 profiles obtained for artificial membranes: dialysis tubing cellulose (Cel), Cuprophan® (Cup),
655 silicone of different thicknesses (Sil2, Sil5, Sil10) and Strat-M™ (StrM); and panel (B) shows
656 the cumulative RV permeation profile obtained with pig ear skin (Skin). Values represent the
657 mean ± standard deviation of six replicate cells.

658

659 Figure 2. Relationship between RV amount permeated (mg/cm²) through pig ear skin (Skin)
660 versus the RV amount permeated (mg/cm²) through synthetic membrane (StrM).

661

662 Figure 3. RV fraction absorbed *in vivo* from TDDS (Exelon®Patch 10) obtained by
663 deconvolution using the Wagner-Nelson model.

664

665 Figure 4. *In vivo-in vitro* correlation established for *in vitro* permeated data using synthetic
666 membranes (Sil2, Sil5, Sil10, Cel, Cup, StrM) and pig ear skin (Skin) as a function of the *in*
667 *vivo* fraction absorbed from TDDS containing RV (Exelon® Patch 10). Panel (A) shows the
668 linear regression obtained from Sil 2, Sil5, Sil10, Cup and Cel membranes; and Panel (B)
669 shows the linear regression obtained from StrtM membrane and pig ear skin (Skin).

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681 **LIST OF ABBREVIATIONS**

682

Cel	Cellulose dialysis tubing
Cup	Cellulose membrane - Cuprophan®
Fa	Fraction absorbed
F_{cal}	F calculated
F_{tab}	F tabulated
HPLC	High performance liquid chromatography
IVIVC	*In vitro-in vivo* correlation
LOD	Limit of detection
LOQ	Limit of quantification
PBS	Phosphate-buffered saline
RSD	Relative standard deviation
RV	Rivastigmine
SD	Standard deviation
Sil	Silicone membrane - Sil-Tec®
Skin	Pig ear skin
StrM	Polyethersulfone membrane - Strat-M™
TDDS	Transdermal drug delivery system

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