

In Vitro Evaluation of the Therapeutic Equivalence of Generic Sodium Polystyrene Sulfonate Formulations

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Received: (06/11/2016), Accepted: (09/03/2017)

ABSTRACT

Equilibrium and kinetic *in vitro* potassium ions (potassium chloride) binding studies were used to document bioequivalence between generic (T) and innovator (R) formulations of the cationic exchange resin, sodium polystyrene sulfonate (SPS) that is used in the treatment of hyperkalemia. The equilibrium binding studies were conducted under identical experimental conditions of constant time, and varying concentrations of potassium chloride in either simulated intestinal fluid (SIF) or water, with and without acid pretreatment of the drug product. The kinetic binding studies were conducted under constant concentrations of potassium chloride (0.3 and 3 mM) in either water or SIF with varying times of observation. Flame photometric technique was used to measure the concentration of non-bound potassium ions. Equilibrium binding studies of potassium ions to SPS in simulated intestinal fluid (SIF) did not follow Langmuir-type adsorption isotherm, otherwise, equilibrium binding studies in water have shown to obey Langmuir equation with correlation coefficient (R^2) of 0.972 for both formulations. The calculated affinity (k_1) and capacity (k_2) binding constants were found to be (1.41; 1.25) and (3.1; 3.55 mMole/g) for generic and innovator formulations, respectively. The T/R ratios for total potassium ions bound ranged from 0.85 to 1.01 at 0.1 – 30 mM concentrations. The T/R ratios for both affinity and capacity constants were 1.13 and 0.87 respectively. In kinetic binding studies, both drug products have exhibited the same rate of exchange for potassium ions. Therefore, based on both equilibrium and kinetic data the two drug products were comparable in terms of their *in vitro* binding characteristics.

Keywords: *In Vitro* Bioequivalence, Potassium Binding Resins, Sodium Polystyrene Sulfonate.

INTRODUCTION

Bioequivalence (BE) studies are required by regulatory authorities to ensure clinical safety and efficacy of drug products. However, this may be unsuitable for orally administered drugs intended for local action and not reaching the systemic circulation, documentation of bioavailability (BA) and bioequivalence (BE) can be achieved by suitably designed in-vitro studies (1).

Ion exchange materials are insoluble substances that contain loosely held ions exchangeable with other ions in solutions coming in contact with them. They can exchange either positively charged ions (cation exchangers) or negatively charged ones (anion exchangers). These exchanges take place without any physical alteration to the ion exchange material (2). Sodium

polystyrene sulfonate (SPS) is a cation-exchange resin prepared in the sodium form and has the structure shown in Figure (1)

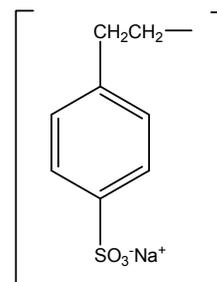


Figure (1): Chemical Structure of SPS.

SPS has been previously used to get rid of toxicity of different cations like lithium and iron (3) (4). Recently, SPS, either taken orally or by enema, has been used for the treatment of hyperkalemia, by exchanging its Na⁺ with K⁺ in the large intestine (5). In fact,

each gram of SPS exchanges usually not less than 110 mg and not more than 135 mg of K^+ , calculated on the anhydrous basis (6). As a requirement for registration, the Division of Bioequivalence at the Food and Drug Administration (FDA) has concluded that *in vivo* studies are not necessary to document the BE of a generic drug product in case of Cholestyramine resin formulations, since this drug shows low water solubility and intestinal permeability (7). Accordingly, *in vitro* BE studies including both equilibrium and kinetic studies were sufficient to confirm the BE between the generic and brand formulations (7). Despite SPS is rated "AA" in the FDA/CDER's Approved Drug Products with therapeutic Equivalence Evaluation where no known or specified bioequivalence issue is known or suspected, the information necessary to show bioequivalence between pharmaceutically equivalent products is presumed and considered self-evident based on acceptable *in vitro* studies that ensure the absence of effect for factors that can affect the exchange process in comparison with brand products (10) like the particle size. Following the same principles outlined above for the *in vitro* BE testing of Cholestyramine, *in vitro* testing of SPS can be used to in lieu of *in vivo* testing.

To the best of our knowledge, there is no published report on the *in vitro* approach to evaluate the *in vitro* equivalence between the generic SPS and the brand product. Accordingly, this work would be of great importance to the pharmaceutical industry and academic research that wish to develop new generic formulations and obtain preliminary prediction about the *in vivo* performance of the obtained formulation. In this study, both binding and equilibrium for SPS were carried out in order to compare the *In vitro* binding characteristics between the generic (Pharmacin-S[®]) and the innovator (Kayexalate[®]) formulations (*in vitro* BE studies).

MATERIALS AND METHODS

Materials

Pharmacin-S[®] powder (Jerusalem Pharmaceuticals, Ramallah, Palestine), Kayexalate[®] powder (Sanofi Aventis -

France), monobasic sodium phosphate (Merck-Germany), sodium hydroxide (Merck-Germany), KCl (Merck-Germany), sodium chloride (Merck-Germany), hydrochloric acid (Merck-Germany), sodium hydroxide (Merck-Germany).

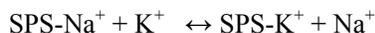
Methods

The simulated intestinal fluid (SIF) was prepared to contain 0.05 M sodium phosphate buffer solution with pH 6.8 without enzymes. Standard solutions of KCl with concentrations covering the range of 0.05 -1.5 mM were prepared. These concentrations were analyzed using flame photometer. To obtain standard curves, signal versus K^+ concentration plots were constructed. For equilibrium binding studies, 50 mg of SPS powder of either Pharmacin-S[®] or Kayexalate[®] were soaked in 10 mL of either SIF or water overnight at room temperature with or without acid pretreatment. The acid pretreated samples were previously soaked in 10 ml of 0.1 N HCl at 37°C for 1 hour. Then, the pH of the sample solutions was adjusted to 6.8 with 1 N NaOH then allowed to soak overnight at room temperature. In the day after, different concentrations of KCl (0.1 - 30 mM) solutions were prepared by adding the specified volumes of 100 mM KCl stock solution and complete the remaining volume with SIF or water. The samples were incubated at 37 °C for 24 hours. Then they were filtered, diluted and analyzed by means of flame photometer. All experiments were conducted as six replicates as per the FDA guidance on Cholestyramine (7).

For kinetic binding studies, 50 mg of SPS powder of either Pharmacin-S[®] or Kayexalate[®] were soaked in 10 ml of distilled water at room temperature overnight. Then, the volume of 100 mM KCl solution required to obtain a potassium concentration of 0.3 and 3 mM was added quickly and the volume was completed to 50 ml by water. The samples were incubated at 37 °C and samples were withdrawn at different time intervals (0.25, 0.5, 1, 2, 4, 8, 16, and 24 hours), filtered, diluted and analyzed by flame photometer. All experiments were conducted as six replicates.

Calculation of adsorption equilibrium and kinetic parameters:

For SPS, the ion exchange process is an equilibrium process that can be explained by the following reaction:



Usually the exchange depends on factors such as: (i) the concentration of both Na^+ and K^+ , (ii) the affinity of resin with these ions, and (iii) the number of reactive sites (i.e., the binding capacity) in the matrix. As proposed by Langmuir regarding the adsorption of gases on plane surfaces, the monomolecular adsorption of potassium ions from solution can be described by the following equation (8):

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{C_{eq}}{K_2}$$

Where C_{eq} represents the concentration of the adsorbate (K^+) remaining in the

solution at equilibrium, x is the amount of adsorbate bound to the adsorbent (SPS), m is the amount of adsorbent used, k_1 is the affinity constant, k_2 is capacity constant which is account for the maximum amount of adsorbate that can be adsorbed per unit weight of adsorbent.

Based on that, different types of adsorption isotherms have been proposed (8). In order for adsorption to follow Langmuir type, the adsorption isotherm should show saturable adsorption as the concentration of the adsorbate increases.

RESULTS

Standard curves of K^+ have shown linearity over the concentration range of 0.05-1.5 mM K^+ with correlation coefficient of 0.998 and 0.997 in SIF and water respectively. The standard curve of K^+ in water is shown in Figure 3.

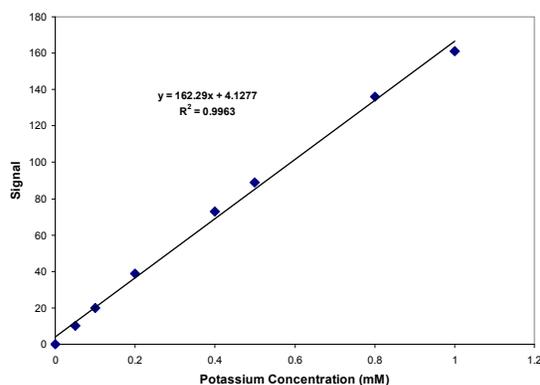


Figure (3): Standard curve of K^+ in water

Regarding the equilibrium binding studies in SIF, plots of micromoles K^+ bound /50 mg resin versus initial concentration of K^+ were constructed as in Figure 4. The plots do not follow Langmuir type isotherm, thus Langmuir equation was not applicable in this case. The same results were obtained with samples that were previously acid treated.

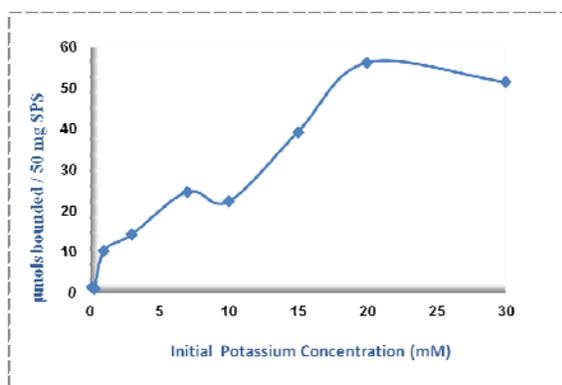


Figure (4): Micromoles potassium bound /50 mg SPS versus initial potassium concentration (mM) for Pharmacin-S in SIF without acid pretreatment.

Concerning the equilibrium binding studies in water, Figure 5 has shown to follow Langmuir equation with monolayer adsorption. In fact, the monolayer isotherms

obtained for both Pharmacin-S[®] and Kayexalate[®] in water without acid pretreatment can be observed.

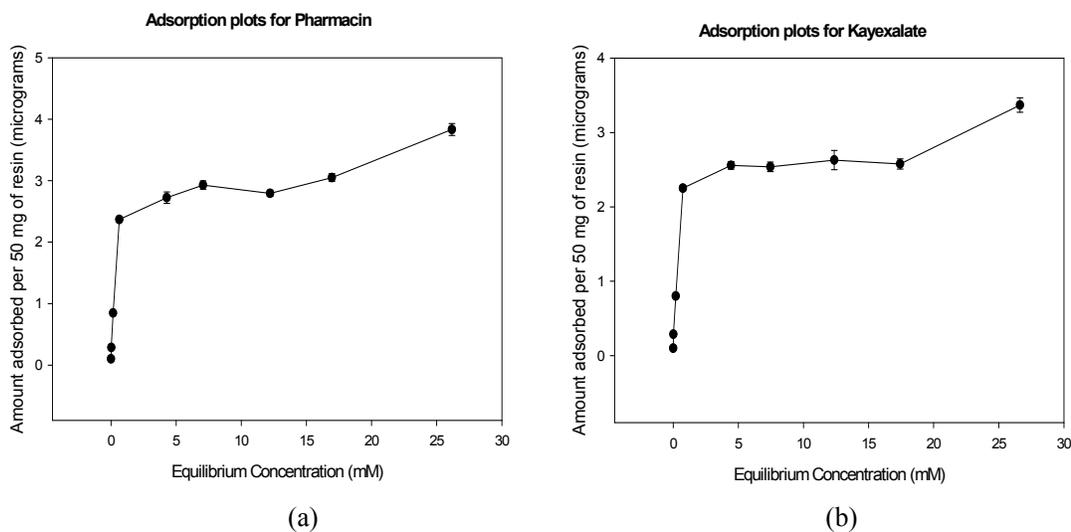


Figure (5): Micromoles bound of K^+ per 50 mg resin versus the initial potassium concentration (mM) for (a) Pharmacin-S[®] and (b) Kayexalate[®] in water without acid pretreatment. To apply Langmuir equation, the required parameters were calculated as in Table 1.

Table (1): Summary of calculations required to apply Langmuir equation for equilibrium studies in water without acid pretreatment for both formulations

Initial conc (mM)	Pharmacin -S®				Kayexalate®				Relative Binding
	Ceq(mM)	x/m (mmols/g resin)	Ceq/x/m	% Binding (T)	Ceq(mM)	x/m (mmols/g resin)	Ceq/x/m	% Binding (R)	T/R
0.1	0	0.1	0	100	0	0.1	0	100	1.00
0.3	0.009242	0.290758	0.031788	96.9	0.009242	0.290758	0.031788	96.92	1.00
1	0.202365	0.797635	0.253706	79.8	0.152527	0.847473	0.179978	84.75	0.94
3	1.151483	1.848517	0.622922	61.6	0.96459	2.03541	0.473905	67.84	0.91
5	2.542113	2.457887	1.034268	49.16	2.438284	2.561716	0.951817	51.23	0.96
7	4.421081	2.578919	1.714316	36.84	4.296486	2.703514	1.589223	38.62	0.95
10	7.535958	2.464042	3.058373	24.64	7.162173	2.837827	2.523823	28.38	0.87
15	12.51976	2.480239	5.047804	16.53	12.39517	2.604834	4.758524	17.37	0.95
20	17.62816	2.371842	7.432267	11.86	17.12978	2.870222	5.968103	14.4	0.83
30	26.86326	3.136743	8.56406	10.46	26.39603	3.603975	7.324143	12.01	0.87

Plots of $C_{eq}/x/m$ versus C_{eq} were constructed as shown in Figure 6 and Langmuir parameters were calculated for both formulations as shown in Table 2. Both

formulations have shown to follow Langmuir type adsorption with good correlation. It is obvious that deviation from Langmuir equation occurs at high concentrations.

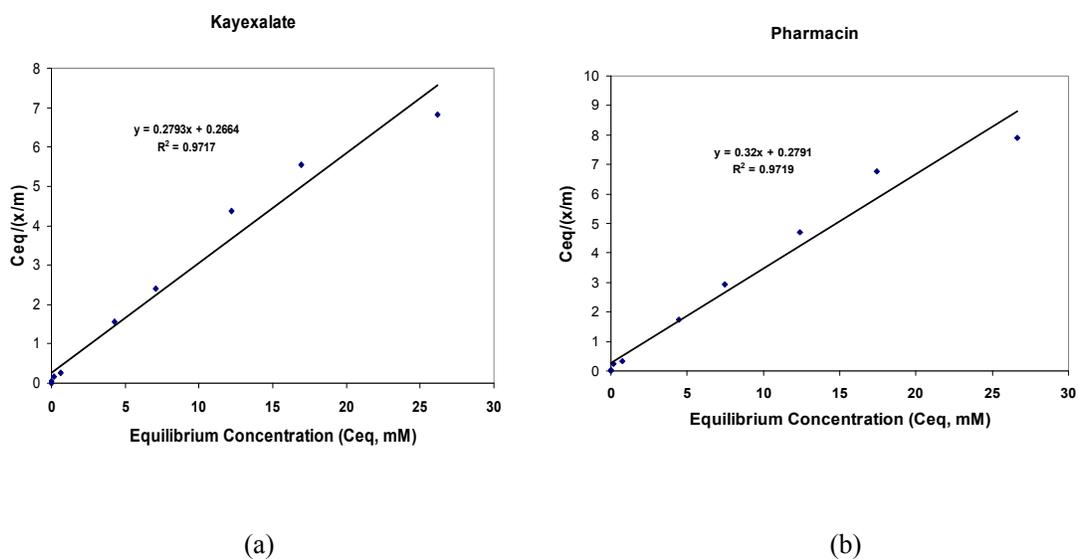
**Figure (6):** Langmuir Plots for both formulations (a) Kayexalate® (b) Pharmacin-S® for the equilibrium study in water without acid pretreatment.

Table 2 shows Langmuir parameters along with the results for the Statistical Analysis of the ratios using SPSS program.

Table (2): Langmuir Isotherm parameters with statistical analysis

Drug Product	Pharmasin S®	Kayexalate®	Reference/Test Ratio (R/T)	90% Confidence Interval
Affinity constant (k_1)	1.41	1.25	0.92	0.802-1.05
Capacity (k_2) [mmol/g]	3.1	3.55	1.14	1.12-1.17
Correlation Coefficient (r^2)	0.972	0.972	-----	-----

No difference was obtained in experiments done with acid pretreatment. The binding of K^+ to SPS at the two initial K^+ concentrations: 0.3 mM and 3.0 mM was

extremely rapid (equilibrium was achieved within 15 minutes) and identical for both drug products. The results for the kinetic studies are shown in Figure 7.

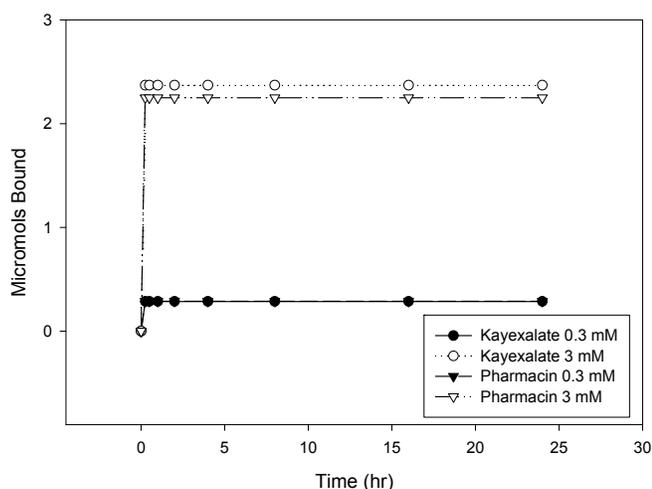


Figure (7): Kinetic studies for Pharmacin-S® and Kayexalate® for the equilibrium study in water without acid pretreatment.

DISCUSSION

A conventional human pharmacokinetic *in vivo* study is often considered the "gold standard" to determine bioequivalence of drug products. However, this BE approach is not always applicable to products not intended to be delivered into the systemic circulation. Examples of these drugs are the antihyperlipidemic resins Cholestyramine, phosphate binding drugs, potassium binding agents among others (9). In these situations, the FDA has proposed *in vitro* binding studies as an alternative approach for the *in vivo* studies. The FDA did not provide guidance for Kayexalate® specifically, but a guidance was given for Cholestyramine (7). The protocol suggested for Cholestyramine was used as the starting point to establish our own method of analysis to determine the equivalence between Kayexalate® and

Pharmacin®. The protocol suggested both equilibrium and kinetic experiments in order to assess both the extent and rate of release of the active ingredient from the dosage form in its intended site of action (i.e., the small intestines).

A validated analytical method for potassium based on flame photometric technique was established to assess K^+ concentrations upon incubation with the binding resin under different experimental conditions. The equilibrium studies in SIF have not obeyed type I isotherm but a Type II was obtained. Accordingly, Langmuir equation was not applicable. This may be due to the presence of high Na^+ concentrations in SIF that may compete for the adsorption of K^+ . This can be ensured by the adsorption isotherm obtained in water in which no Na^+ was found. These studies obeyed Langmuir

isotherm and the parameters needed to evaluate the BE between the reference and test products were calculated. Application of Langmuir equation on both drug products was satisfactory with good correlation. Statistical Analysis using SPSS program reveals that the Reference/Test ratios for affinity and capacity constants are acceptable with a 90% confidence interval that lies within the acceptance criteria stated by FDA guidelines (80% - 125%) regarding the bioequivalence.

CONCLUSIONS

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CONFLICT OF INTRESTS

The authors report no conflicts of interest in this manuscript.