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Application of Hildebrand Solubility Parameter to Identify Ethanol-Free Co-Solvent for Pediatric Formulation

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ABSTRACT

Formulation of active pharmaceutical ingredients into liquid dosage form is frequently limited by solubility issues. Ethanol is commonly used as a cosolvent to improve the solubility of drugs. Due to the incomplete expression of ethanol metabolizing enzyme in children under 6 years, several drug authorities such as WHO, EMA, and FDA recommend avoiding the use of ethanol in pediatric formulation whenever possible. In addition, Muslim consumers are regulated by the halal practice where excessive use of ethanol in pharmaceutical products should be avoided. Thus, it is necessary to explore an alternative co-solvent to reduce the use of ethanol in pediatric formulation. This study is aimed to identify an alternative co-solvent to ethanol that is safe for the pediatric using Hildebrand Solubility Parameter approach. In this study, the solubility parameter of the model drug, MH2011, was determined using Hildebrand Solubility Parameter (HSP). The solubility parameter (δ) of MH2011 was determined using two approaches. The first method is by measuring the maximum solubility of the model drugs in the binary mixture of water and 1,4 dioxane. The second approach is by calculating solubility parameters based on Fedor's group substitution method. Using a binary solvent blend, the MH2011 solubility parameter was identified. MH2011 solubility parameter is comparable to those calculated using Fedor's Group substitution method. Data on the solubility parameter has then been used to identify the alternative solvent to ethanol. The studies also implied that propylene glycol 7% v/v gave similar solubility power as those of ethanol 7% (v/v). This study suggested that Hildebrand's Solubility Parameter can be applied to identify an alternate co-solvent to ethanol when an alcohol-free formulation is preferred; such as in halal pharmaceuticals and pediatric liquid formulations.

Keywords: Hildebrand Solubility Parameter, ethanol-free, pediatric, liquid dosage form

INTRODUCTION

Ethanol is a universal co-solvent that is commonly used to improve drug solubility in liquid formulation (Savjani *et al*., 2012; Vemula *et al*., 2010). This substance is the most studied organic solvent in terms of physicochemical properties, pharmacological action, pharmacokinetics, and safety profile. The fact that ethanol is affordable and accessible in large quantities leads this solvent to be the most commonly used technique to improve drug solubility in liquid dosage form (Lim *et al*., 2014).

Although the presence of ethanol in the liquid dosage form is considered safe for adults, the presence of ethanol in the pediatric drug product can raise potential toxicities. Children below five years old have an incomplete expression of ethanol-metabolizing enzymes such as aldehyde dehydrogenase and CYP2E1 (Bhatt *et al*., 2017; Zuccotti & Fabiano, 2011). Thus, Food Drug

Administration, World Health Organization, and European Medicine Agency recommend avoiding the use of ethanol in oral pediatric formulation whenever possible. If ethanol in the formulation can not be avoided, the FDA and WHO recommend the concentration of ethanol in the product should be below 0.5% v/v for children under 6 years old and below 5% for children under 12 years old. European Medicine Agency suggested that single administration of oral dosage form administered to children should not contain ethanol of more than 6 mg/kg. The restriction of ethanol in the pediatric formulation is also supported the by American Pediatrics Association which recommends that children's blood alcohol concentration (BAC) post single administration of ethanol-containing medication should be kept below 25 mg/dL (Zuccotti & Fabiano, 2011). These restrictions are aimed to prevent the accumulation of ethanol in the systemic circulation. Within blood vessels, ethanol crosses the blood-brain barrier via passive diffusion and acts as a depressant for the central nervous system (CNS). Ethanol altered blood-brain barrier permeability and changed transporter expression in brain endothelial cells (Laksitorini *et al*., 2021). The negative effect of ethanol on the blood-brain barrier can be observed in the BAC as low as 41 mg/dL (Cheng *et al*., 2021).

In addition toxicities issue, the presence of ethanol in pharmaceuticals is related to halal status. Most halal authorities in Moslem countries agree to not use ethanol in the pharmaceutical product unless there is no substitution available (Afifi *et al*., 2014; Annabi & Wada, 2016; Majelis Ulama Indonesia, 2018; MZ, 2019). The ethanol limit in the beverage is varied among the halal authority, ranging from 0.1-1% (Mansur *et al*., 2022). Indonesian Ulema Council as well as Malaysia Halal Authority suggest that the ethanol content in pharmaceutical preparation should be less than 0.5% v/v (Alzeer & Abou Hadeed, 2016). The increase in global halal awareness among consumers required the pharmaceutical industry to develop liquid formulations which did not include ethanol as a co-solvent.

Drug solubility enhancement has been an interest to many pharmaceutical scientists as nearly 40% of novel drug candidates are lipophilic (Markovic *et al*., 2020). Several strategies have been explored to improve drug solubility. This includes co-solvency, adding a surfactant, salt formation, and complex formation. A pediatric oral liquid formulation such as drop, syrup, and sterile preparation required a small volume and thus required solubility enhancement. Solubility enhancement using co-solvent addition has been a choice as this method is simple and affordable (European Medicines Agency, 2006).

To explore the prospectus co-solvent, data on compound polarity needs to be identified. This can be performed by identifying dielectric constant, dipole moments, or solubility parameters. The relationship between the solubility parameter and the number of drugs dissolved is explained by the extended Hildebrand solubility equation (Eq.1). X^2 is the solubility of the solute, ΔH_{fus} is the fusion enthalpy of the solute, T_{fus} is the melting point of the solute, R is the gas constant, T is the temperature of the solution, V_2 is the partial molar volume of the solute, $\phi 1$ is the volume fraction of the solvent. This equation suggested that the maximum solubility of a solute (X^2) can be achieved if the solvent's solubility parameter (δ_1) is similar to the solute's solubility parameter ($\delta 2$) (Rathi, 2010).

$$-\log X^2 = \frac{\Delta H_{fus}(T_{fus}-T)}{-2.303RT_{fus}T} + \frac{V2.\varphi^2}{2.303RT} + (\delta_1 - \delta_2)$$

.....(Eq. 1)

Although the extended Hildebrand solubility approach has been used to explore the appropriate solvent mixture to maximize drug solubility, this approach has not been used to facilitate the exploration of alternate solvents in the context of creating ethanol-free/halal pediatric formulations. The present study is aimed to explore alternate co-solvent to ethanol for improving the solubility of MH2011, a model drug that has solubility issues. The solubility of MH2011 is 0.117 mg/ml at room temperature (Muhammad, 2014; Suryarini, 2014). The result of the study will enrich the research area where the alcohol-free formulation is preferred, for example in the halal industry and pediatric liquid formulation.

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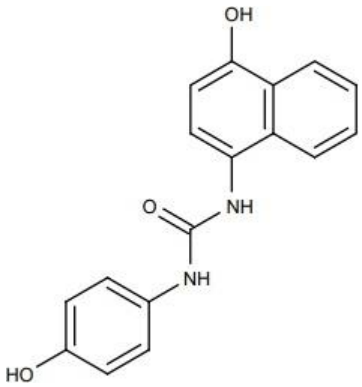

Figure 1: Molecular Structure of MH2011

Table I. Preparation of a binary mixture of water and 1.4 dioxane resulting solution with different solubility parameters

Solvent solubility parameter $(cal/cm^3)^{1/2}$	Dioxan volume (%v/v)	H_2O volume (% v/v)	Maximum Wavelength (nm)	Standard Curve	R-value
10	100	0	489	Y=1.56X-0.157*	0.9994
12	85	15	492	Y=1.146x+0.029*	0.992
14	70	30	504	Y=1.100x+0.014*	0.997
16	55	45	510	Y=0.928x+0.100*	0.997
18	40	60	504	Y=1.200x+0.024*	0.998

MATERIAL AND METHOD

MH2011 or (1-(4-hydroxynaphthalen-1-y1)-3-(4-hydroxyphenyl)urea) is a new molecular structure that was designed as analgesia (Figure 1). MH2011 was synthesized at the Department of Pharmacochemistry, Gadjah Mada University using urea, 4-amino-1-naphthol dan p-aminophenol as a starting material (Purnomo *et al*., 2016). Before the experiment, MH2011 was re-purified by a recrystallization process using ethanol. 1,4-Dioxane were from E. Merck, LTD. *Aqua destillata* was used for all the experimental purposes. Propylene glycol, buffer components were purchased from Sigma Aldrich. Double beam UV/Vis spectrophotometer, Genesis 10 model was used to measure the concentration of MH2011 in the solution. Thermostatic shaking water baths were used for the solubilization process.

Confirmation of MH2011 purity

To determine the purity of MH2011, the melting point of MH2011 and the starting material were evaluated using a hot-stage optical microscope. Briefly, the auto thermal controller was programmed with an increasing temperature of 5°C/minute. When the tested compound almost reached the melting point, the temperature programming was set at a 2°C increase per minute. The melting point is the temperature at which the compound starts to melt until all the samples were completely melted (Kumar *et al*., 2020).

In addition to melting point data, the determination of MH2011 purity was based on the compound separation method using thin-layer chromatography. MH2011 and its starting material, p-aminophenol. 4-amino-naphthol and urea were dissolved in methanol. One microliter of the solution was dropped into the stationary phase silica gel 60 F254. As the solution gets dried, the sample was eluted using chloroform: methanol (3:1) as a mobile phase. Detection of the compound was done with UV light 254 nm (Silver, 2020).

Determination of MH2011 solubility parameter

Solubility parameters were determined based on the maximum solubility of MH2011 in the binary mixture of 1,4 dioxane and water. These binary solvents were used as they are fully miscible in most of all proportions. These solvents provide two extreme solubility parameters (δ1) from 10 to 23.4 $(cal/cm^3)^{1/2}$ respectively. Thus, the combination of these solvents can create a mixture with a wide solubility parameter from 10-23.4 $(cal/cm^3)^{1/2}$. The experiment was done in triplicate at a temperature of 30±1°C; 14.5 Psi (Rathi, 2010).

To determine MH2011 solubility parameters, an excess quantity of MH2011 was introduced into a screw-capped vial. A six-milliliter binary mixture of water and 1.4 dioxane were added to the vial (Table I). The sample was placed on a rotary shaker thermostatic water bath for 6 hours at constant speed at 150 rpm, temperature 30 ±1°C. Samples were withdrawn every hour to determine the time required to reach the steady state. Each of the samples was filtered through Whatman paper No. 41. After dilution, MH2011 concentrations were determined using a UV spectrophotometer at λ_{max} of each binary solvent. Within this time frame, our preliminary studies suggested that two hours is required for the system to reach steady states. Similar experiments were conducted with different dioxane-water mixtures. All experiments were done in triplicate and expressed as mean ± SE (Rathi, 2010).

Exploration of non-ethanol co-solvent

Exploration of co-solvent to improve the solubility of MH2011 was determined based on MH2011 solubility parameter. Considering co-solvent toxicity data for pediatric subpopulation, propylene glycol was selected to be explored. The solubility of MH2011 in the solvent mixture was determined using the shake flask method. A mixture of propylene glycol and water (7-21%) was tested. Aqua distillates, phosphate buffer

saline 0.01 M, or ethanol 7% were used as a control. Briefly, an excess quantity of MH2011 was introduced into a screw-capped vial. Five milliliters of the cosolvent were added to the vial. To determine the time needed to reach equilibrium, all sample was placed in an ultrasonic water bath and sonicated for 5, 10, 15, 20, 25, 30, 35, 40, 45 dan 50 minutes. Samples were withdrawn at the end of the experiment and filtered through Whatman paper No. 41. Once the sample been diluted, MH2011 concentrations were determined using a UV spectrophotometer at λ_{max} of each solvent (Rathi, 2010). This experiment was performed to identify the time needed to reach a saturated solution. The solubilization process was terminated according to the time that was identified before as the time to reach saturation solution. Absorbance data were analyzed based on the standard curve made for each solvent mixture.

Data analysis

The data were calculated using Microsoft Excel. The determination of MH2011 solubility parameter and the effect of propylene glycol as a co-solvent were analyzed using GraphPad Prism 8.0. To compare different groups, one-way ANOVA was used followed by a Tukey post-comparison test. When there are only two groups, one tail t-tests were used. Data were expressed as mean±SE. When $p < 0.05$ was considered significantly different.

RESULT AND DISCUSSION

Determination of MH2011 purity

In this study, MH2011 was used as a model drug to explore an alternative co-solvent to ethanol. To confirm MH2011 purity, thin-layer chromatography was employed. Silica gel GF254 was used as a stationary phase and chloroform: methanol (3:1) was used as the mobile phase. The RF of MH2011 (0.33) is significantly different from its starting material which is p-amino phenol (0.27) and 4-amino-1- naphthol (0.16 and 0.22). On the other hand, urea migration can not be detected due to the absence of chromophore on its molecular structure (Figure 2).

Another approach to confirm the identity of MH2011 is the melting point. The melting point of MH2011 and its starting materials were tested using a hot-stage optical microscope. The melting point of MH2011 is distinct from its starting material. Para-aminophenol, 4-amino-1 naphthol, and urea showed melting points at 188-190°C, 94-96°C, and 133-135°C respectively. While MH2011 started melting at 246,5°C (Table II). Together with the result of thin-layer chromatography, it suggested that the compound meets the requirement for further examination.

Figure 2: Confirmation of MH2011 purity using thin-layer chromatography. Silica gel GF254 was used as a stationary phase and chloroform: methanol (3:1) were used as a mobile phase. From left to right are urea (A), 4-amino-1- naphthol, RF 0.16 and 0.22 (B), p-aminophenol; RF 0.27 (C), and MH2011; RF 0.33 (D), respectively.

Table II Melting point of MH2011 and its starting material

Compound	Melting Point (°C)
p-aminophenol	188-190
4-amino-1-naphthol	94-96
Urea	133-135
MH2011	>246.5

Determination of MH2011 solubility parameter.

Before the exploration of alternative co-solvent to ethanol, the solubility parameter of MH2011 was determined. MH2011 solubility parameter (δ2) was determined by two methods. Firstly, by measuring the solubility of the tested compound in the binary solvent of water and dioxane. Secondly, by calculating the solubility parameter based on Fedor's method.

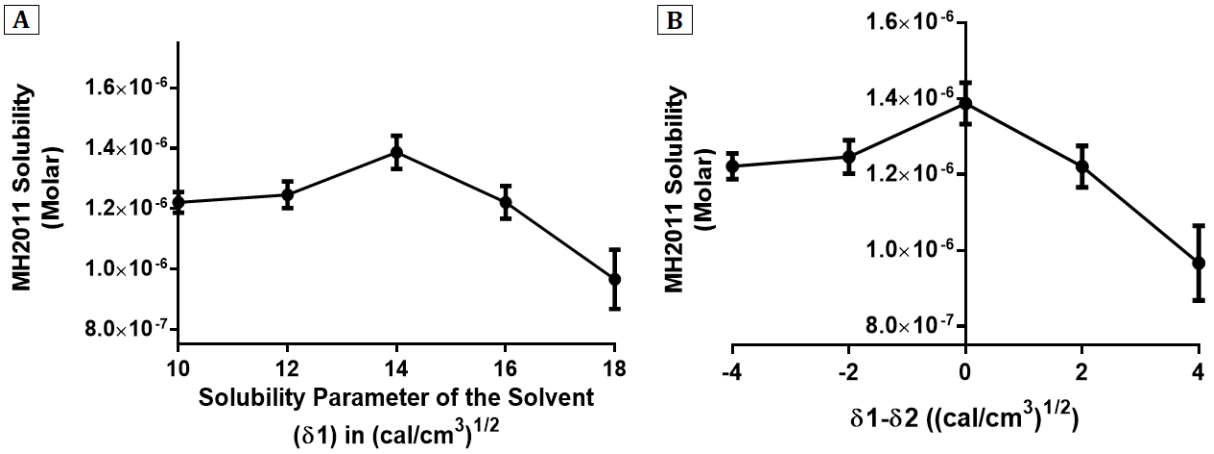

Figure 3. Determination of MH2011 solubility parameter (δ2). The solubility parameter was performed using the shake flask method with binary solvent dioxane and water at various proportions. MH2011 solubility parameters were determined based on the maximum solubility of MH2011 in the binary solvent

In the first method, the solubility parameter of MH2011 is assumed to be similar to the solubility parameter of the binary solvent (δ1) which produced MH2011 maximum solubility. The solubility of MH2011 was evaluated in binary solvent which contained water and dioxane. Some series of solvent blends were prepared to produce solvent with several solubility parameters (δ1) from 10-18 $(cal/cm^3)^{1/2}$. An excess of MH2011 was added to the vial. The solubility of MH2011 over different binary solvents showed a bell shape. MH2011 solubility peak (X2) of 7.6 X 10^{-5} M was observed in a binary solvent of 10% water and 90% dioxane which produce δ1 of 14 $(cal/cm^3)^{1/2}$ (Figure 3a). From the peak of the bell shape graph, MH2011 solubility parameter could be identified as 14 $(cal/cm^3)^{1/2}$. Theoretically, the solute's solubility parameter is the value of δ1 at which the solute, in this case MH2011, shows maximum solubility. As the maximum solubility of MH2011 was observed at δ1 14 $(cal/cm^3)^{½}$, it suggested that the solubility parameter of MH2011 is approximately 14 $(cal/cm^3)^{½}$. Figure 3a showed a bell-shaped curve suggesting that the solubility of MH2011 decreased in both at lower and higher values δ1 but reach the maximum in δ1 14 $(cal/cm^3)^{½}$. The confirmation of MH2011 solubility parameter (14 $(cal/cm^3)^{½}$) can be seen when Δδ is zero and it reaches the highest value of solubility (Figure 3b).

The second method employed to determine the MH2011 solubility parameter (δ2) was Fedor's group substitution approach. The method follows Equation 2.

$$\delta = 0.5\left(\frac{\Delta u}{\Delta V}\right) \text{ ..... (Eq.2)}$$

Δu is the values of cohesive energy per mole of the fragment while ΔV is the molar volume of the fragment. Briefly, MH2011 was divided into different fragments. The data on (Δu) and molar volume (ΔV) for each fragment were obtained from the original research paper done Fedors's group (Fedors, 1974). According to calculation using Eq.2 it suggested that the MH2011 solubility parameter was 14.31 $(cal/cm^3)^{½}$ (Table III). Based on the two different approaches, it suggests that the MH2011 solubility parameter was approximately 14 $(cal/cm^3)^{½}$ as the solubility parameter determined by the dioxane-water binary mixture agreed with that of Fedor's group substitution method.

Determination of alternative co-solvent to ethanol

Cosolvent is the most common technique to improve the solubility of poorly soluble drugs (Nayak & Panigrahi, 2012; Savjani *et al*., 2012; Solanki *et al*., 2013) It reduced the energy needed for the initial solubility process before the drug dissolved in water.

Table III. Calculation of MH2011 solubility parameter using Fedor's Substitution Method.

Drug Fragment	No. of Fragment	Cohesive Energy	Total Cohesive Energy	Molar Volume	Total Molar Volume
OH-	2	7122	14244	3.8	7.6
C=O	1	4150	4150	10.8	10.8
Phenylene (para)	2	7630	15260	52.4	104.8
NH	2	2000	4000	4.5	9
CH=	4	1030	4120	13.5	54
Ring closure	1	750	750	18	18
		Total Cohesive Energy	42524	Total Molar Volume	204.2
			208.2468168		
		Solubility Parameter	14.4307594		

Table IV. Solubility of MH2011 in different solvents and co-solvent.

Solvent (v/v)	Solubility parameter $(cal/cm^3)^{½}$	Solubility of MH2011 (mg/ml)	Solubility enhancement (fold-compared to water)
Propylene glycol 21%	21.59	1.77±0.04	14.75
Propylene glycol 14%	22.2	1.48±0.05	12.3
Ethanol 7%	22.67	1.01±0.01	8.41
Propylene glycol 7%	22.8	0.87±0.03	7.25
Water	23.4	0.12±0.05	baseline

*Solubility parameter of MH2011 is 14 $(cal/cm^3)^{½}$ experimentally and 14.43 $(cal/cm^3)^{½}$ based on Fendor's Methods.
*n.a: not performed

Some cosolvent is commonly used in pharmaceutical preparation among other ethanol, propylene glycol, and polyethylene glycol (Strickley, 2004). The current study focused to investigate an alternate cosolvent to ethanol. Based on the solubility parameter of the model drug, MH2011, which is 14 $(cal/cm^3)^{½}$, some co-solvent candidates were assessed. The solubilizing process was done with the aid of the sonication process.

The selection of an alternate solvent to ethanol was based on the solubility parameter of the model drug as well as the solubility parameter of ethanol. Pure ethanol and pure propylene glycol have a solubility parameter of 12 $(cal/cm^3)^{½}$ and 14.8 $(cal/cm^3)^{½}$, respectively. Another perspective co-solvent is PEG 400 and glycerin. This solvent has a solubility parameter of 11.3 $(cal/cm^3)^{½}$ and 17.7 $(cal/cm^3)^{½}$ respectively. A 7% v/v ethanol, the common syrup's common ethanol content, has a solubility parameter of approximately 22.67 $(cal/cm^3)^{½}$ (Table IV).

Even though pure ethanol or pure might enhance MH2011 solubility, excessive use of this solvent as a pediatric formulation is not feasible due to the toxicity issue and halal concern (Abrantes *et al*., 2016; Alserhan *et al*., 2020; Rouaz *et al*., 2021). Indonesian Ulema Council as well as Malaysia Ulema Council agrees that any product with ethanol concentration below 0.5% v/v is considered halal. An increase in halal awareness toward pharmaceutical product enforced pharmaceutical scientists to develop alcohol-free liquid dosage forms (Alserhan *et al*., 2020; Tushar Saha *et al*., 2019). In this study, propylene glycol was explored as a co-solvent due to its feature which is generally accepted as safe. The concentration of propylene glycol in liquid dosage form varied range is between 0-80% v/v (Lim *et al*., 2014). The European Medical Agency recommends the maximum propylene glycol content in the pediatric formulation should be less than 200 mg/kg body weight after a single intake (Lim *et al*., 2014). This corresponds to 2.0 g propylene glycol for a one-year-old infant which has an average weight of 10 kilograms.

In this study, we limit propylene glycol to 21% v/v to comply with European Medicine Agency. If a one-year-old infant (10kg) was administered with 5 ml syrup that contained propylene glycol 21% v/v, the intake is equal to 103 mg/kg which is much lower compared to the limit that was recommended by European Medicine Agency (200 mg/kg). As depicted in Table 4, propylene glycol 7-21 % could improve the

solubility of MH2011 from 7.25-14.75-fold which is significantly different compared to water ($p<0.001$). Interestingly, 7% propylene glycol can solubilize MH2011 which is not statistically significant compared to ethanol 7%, the common ethanol concentration that was added to syrup (Figure 4). This might be due to the similarity of ethanol 7% and propylene glycol solubility parameters, which are 22.68 and 22, 8 $(cal/cm^3)^{½}$, respectively. This study suggested that 7% ethanol can be substituted with propylene glycol 7% in the product development which required ethanol-free formulation.

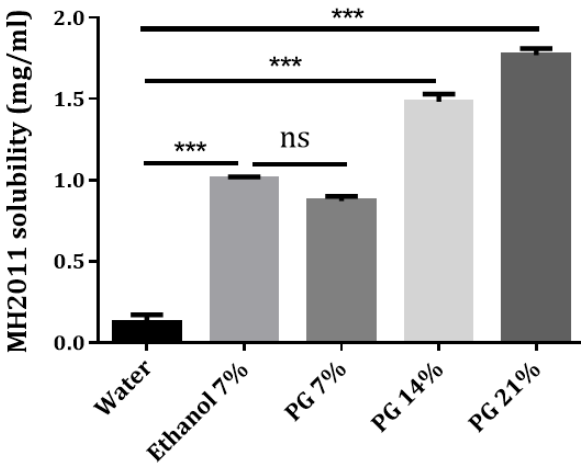

Figure 4: The solubility of MH2011 in several solvent mixtures. Each point represents mean±SEM. Each of the data represents 3 replications. The data were analyzed using One Way ANOVA followed by Tukey post comparison test. *: $p<0.05$ and ***: $p<0.001$.

Using Hildebrand Solubility Parameter (HSP), other potential co-solvent that will produce particular solubility parameters can be predicted (Martin *et al*., 1985; Sotomayor *et al*., 2013). In this study, other than propylene glycol 7% v/v, other potential co-solvent that can be used among other 6.2 part of glycerin and 93.8 part of water. A similar approach also has been used to explore an environmentally friendly solvent to extract several ingredients in dried herbs as a substitute for the common solvent such as dimethylformamide and chloroform (Sánchez-Camargo *et al*., 2019). This study highlights the importance of Hildebrand Solubility approach that can be considered for improving drug solubility in the pediatric liquid formulation which has some limitations in term of type and the number of co-solvent used.

CONCLUSION

In summary, the extended Hildebrand solubility parameter can be used to explore alternate co-solvent to ethanol concerning increased awareness of excipient safety and halal conformity. In the model drug used (MH2011), the proportion of cosolvent propylene glycol which can substitute ethanol 7% has been identified. These studies suggest Hildebrand Solubility Parameter can be applied to identify the alternate co-solvent when the ethanol-free formulation is preferred.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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