Author/s:
Li, W;Lu, HT;Doblin, MS;Bacic, A;Stevens, GW;Mumford, KA

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A novel efficient liquid-liquid solvent extraction process for cannabinoid mimic recovery

Wen Li [1], Hiep Thuan Lu [2,3], Monika S. Doblin [2,3], Antony Bacic [2,3], Geoffrey W. Stevens [1], Kathryn A. Mumford [1,2]

[1] Department of Chemical Engineering, Faculty of Engineering and Information Technology, The University of Melbourne, Parkville, VIC 3010 Australia

[2] Australian Research Council Medicinal Agriculture Hub, La Trobe University, AgriBio Building, Bundoora, VIC 3086 Australia

[3] La Trobe Institute for Agriculture and Food, Department of Animal, Plant and Soil Sciences, School of Agriculture, Biomedicine & Environment, La Trobe University, AgriBio Building, Bundoora, VIC 3086 Australia

Abstract:

Cannabinoids attract worldwide attention due to their well-known medicinal and psychoactive properties. Their efficient recovery from plant material is a significant challenge due to the presence of a complex mixture of secondary metabolites. In this work, 4-*tert*-amylphenol (4-TAP) was selected as a cannabinoid mimic from five mimic candidates based on an evaluation matrix that included several criteria – toxicity, market price, physiochemical properties, and extraction performance. The Conductor-like Screening Model (COSMO) was used to predict the physiochemical properties and extraction performance of the mimic candidates and cannabinoids, and assist in the mimic selection and mimic liquid-liquid extraction (LLE) process development. Three aqueous systems, three volatile organic compound (VOC) solvents, three biodegradable green solvents and one extractant were used to investigate the dissolution, extraction, and stripping of 4-TAP. A three stage LLE recovery process was developed for the selected cannabinoid mimic including leaching, extraction, and stripping. The COSMO model predictions show comparable trends to the experimental data and is shown to be useful for integrating fundamental modelling work into cannabinoid extraction process design.

Keywords: Neutral cannabinoids, Mimic, Solvent extraction, Liquid – Liquid Extraction (LLE), COnductor-like Screening MOdel (COSMO)

1. Introduction and background

Cannabis sativa L. (cannabis) is a remarkable plant that has been globally cultivated for centuries, and is a source of food, fuel, fiber, ritual and medicine [1-3]. Cannabis contains many valuable secondary metabolites, particularly cannabinoids, which have remedial potential against depression, inflammation, epilepsy, pain alleviation and have other effects of clinical relevance [4-8]. To date, 113 cannabinoids have been identified in *C. sativa* [9] in which the predominant cannabinoids are tetrahydrocannabinol acid (THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN) and cannabicyclol (CBL) [1, 2, 10]. The cannabinoids exist within plant tissues primarily in their acidic forms (e.g., tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), etc.), which are produced through a biosynthetic pathway from the precursors olivetolic acid and geranyl diphosphate [11]. Acidic cannabinoids are non-psychoactive [12] but under the effect of heat (e.g., drying and smoking processes), light and natural aging [10, 13], they are decarboxylated to form psychoactive neutral cannabinoids.

Several methods have been applied to extract cannabinoids from cannabis. The conventional cannabinoid extraction method is to macerate cannabis in either hexane, chloroform or other similar volatile organic compounds (VOCs) and then remove the solvent using temperature and pressure shifts to form the crude 'product' [10]. Due to the complexity of cannabis' phytochemistry, the crude 'product' contains a large amount and number of coextracted impurities which requires sophisticated downstream purification processes [10, 14]. Consequently, the utilisation of ethanol [15], deep eutectic solvents [16] and supercritical fluids [17-19] as a green cannabis extraction technology has been extensively studied in recent years. However, the high capital and operating costs, typical batch operation process, non-selective extraction as well as safety concerns, are some challenges that limit the application of those technologies at large scale [20, 21]. In comparison to other technologies, liquid-liquid extraction (LLE) provides the enrichment of active pharmaceutical compounds (APCs) in a four-step process (i.e., leaching – extraction – stripping – precipitation) which has the advantages of low cost, easy operation, small plant footprint when utilizing solvent extraction columns and can be environmentally friendly through the use of bio-degradable solvents [22, 23].

LLE has been applied to isolate cannabinoids from foods and biological samples for laboratory scale chromatographic analysis [7]. Due to the low solubility of cannabinoids in water, some small molecule organic modifiers such as ethanol, methanol and acetone were added into the aqueous solution to enhance the cannabinoid leaching efficiency [24]. LLE was also integrated into centrifugal partition chromatography (CPC), a liquid-liquid preparative chromatographic technique, to isolate cannabinoids from crude extracts after cannabis maceration and winterisation processes using hexane/methanol/water and hexane/acetone/acetonitrile liquid systems [8]. However, the high fouling potential when operating with turbid crude extracts and scalability were found as limitations to the LLE – chromatographic technique. Recently, Legrange et al. [25] patented a process to isolate cannabinoids from aqueous slurries and filtered aqueous solutions using ethyl acetate, heptane and hexane. However, while LLE processes have been widely applied at pilot- and industrial-scales for alkaloid manufacturing processes [22, 23, 26], most cannabis-related studies are limited to laboratory scale (i.e., experiments with milliliters to liters of cannabinoid-rich solution). In addition, these LLE processes predominantly relied on volatile and flammable VOCs (i.e., hexane and heptane), which are increasingly avoided in the cannabis manufacturing industry and related markets [21].

The biggest challenge when conducting research on cannabis and cannabinoids are regulatory barriers, that require significant effort and investment to overcome [27]. This issue is amplified when considering the scale of material required for pilot-scale studies, i.e., over 100 liters of cannabinoid-rich solution. As such, there are benefits to identifying a cannabinoid mimic that not only can simulate the leaching and extraction behavior of cannabinoids in the LLE process but also alleviate the costs and meet the requirements for large-scale extraction. The use of mimics in screening and optimising the operational conditions for LLE processes has been applied for metal [28, 29] and alkaloid extractions [22, 30], but there is not yet any study related to cannabinoid extraction.

In this study, the selection of a mimic for psychoactive neutral cannabinoids was based on using an evaluation matrix in which the selection criteria included toxicity, market price, physicochemical properties, and extraction performance. The COnductor-like Screening MOdel (COSMO), which was incorporated within the Amsterdam Modeling Suite software developed by Software for Chemistry & Materials (Amsterdam, The Netherlands) [31-33], was utilised to predict the physiochemical properties, solubility and distribution of cannabinoid mimic candidates and neutral cannabinoids in three aqueous systems (i.e., ethanol/water, methanol/water and variable pH water), three VOCs (i.e., xylene, toluene, *n*-heptane) and three green biodegradable organic solvents (i.e., *d*-limonene, α-pinene and *p*-cymene). The dissolution, extraction, and stripping performance of the selected neutral cannabinoid mimics in these solvent systems were investigated, forming the basis to develop an LLE process for neutral cannabinoids. In addition, the capability of phosphine-oxide, a well-known extractant for offering a high separation efficiency for various organic molecules [34], in mimic extraction and stripping was also studied.

2. Experimental methods

2.1 Materials

The chemicals utilised in this study were summarised in **Table 1**. The extractant CYTOP® 503 was kindly provided by Solvay (Cytec Canada). All the chemicals were used without further purification. Sulfuric acid and sodium hydroxide pellets were also used to prepare the diluted solutions for pH adjustment of the aqueous phase during experiments.

Table 1 Summary list of chemicals used in the experiments

Chemicals	**Purity**	**Supplier**
Ethanol	≥ 95%	ChemSupply (South Australia, Australia)
Methanol	≥ 99.9%	
n-Heptane	≥ 99%	
d-Limonene	≥ 97%	Sigma-Aldrich (Merck, Darmstadt, Germany)
p-Cymene	≥ 98.5%	
α-Pinene	≥ 97.5%	
4-*tert*-Amylphenol	≥ 98.5%	
Xylene (mixture of isomers)	≥ 98.5%	ChemSupply (South Australia, Australia)
CYTOP® 503	Not applicable	Solvay (Cytec Canada, Ontario, Canada)
Tributyl phosphate (TBP)	≥ 99%	Consolidated Chemical Company (Victoria, Australia)
Sulfuric acid	97.5 – 98.5%	ChemSupply (South Australia, Australia)
Sodium hydroxide	≥ 98%	

2.2 Solubility experiments

The solubility of the neutral cannabinoid mimic was measured in the mixtures of water and organic modifier (i.e., methanol and ethanol), the alkaline solution and the mixtures of ethanol and alkaline solution. The alcohol/water solutions were prepared by mixing the alcohol with reverse osmosis (RO) water using volumetric flasks at volume fractions of alcohol ranging from 0 – 80 v/v%. The pH of the solutions was measured and fine-tuned using either 0.01 mol/L H_2SO_4 or 0.02 mol/L NaOH. When making the mixed ethanol/alkaline solution, the pH of RO water was pre-adjusted close to the desired condition before mixing with ethanol. A 50 mL aliquot of each solution was transferred to a sealed conical flask and 4-TAP was weighed and dissolved into the solution to investigate the solubility at 22°C. The 4-TAP/solution was thoroughly mixed with a magnetic stirring system. Before reaching saturation, the dissolution of 4-TAP pellets completed within a short time (usually 5 mins) and the solution was considered saturated when solid residue (in white flocculent phase) was present 2 h after the last 4-TAP pellet was added. The saturated solution was filtered and diluted using acetonitrile prior to analysis.

The concentration of 4-TAP in solution was determined using an Agilent 1290 Infinity II Ultra-High-Performance Liquid Chromatography (UHPLC) instrument equipped with a diode array detector. The chromatographic separation of 4-TAP was carried out with a mobile phase of acetonitrile/water (60:40 v/v) at an isocratic flow rate of 0.5 mL/min using an Acquity UHPLC C_{18} column, 1.7 µm, 100 mm ×

2.1 mm (Waters Australia, NSW, Australia) at wavelength 225 nm. The 4-TAP standard samples were prepared using acetonitrile in six concentration points from 5 mg/L to 200 mg/L and all calibrations had a coefficient of determination (R^2) for linearity ≥ 0.999.

2.3 Equilibrium isotherm experiment

Isothermal shakeup experiments were conducted to measure the distribution of the cannabinoid mimic 4-TAP in the organic and aqueous phases at equilibrium. For experiments with 40 – 80 v/v% ethanol/water, a 20 g/L 4-TAP in ethanol/water aqueous solution was prepared by dissolving 4-TAP in pure ethanol and adding water to the mixture until the desired ethanol/water volume ratio was obtained. Due to the solubility limit of 4-TAP, a 0.9 g/L 4-TAP aqueous solution was prepared for experiment with 20 v/v% ethanol/water. The solutions were stored in well-sealed Schott bottles to prevent solvent evaporation. The aqueous to organic volume ratios (A/O ratios) were selected in the range of 0.1 to 20. The allocated volumes of the aqueous and organic solutions were added to separation funnels and shaken for 20 min until equilibrium was obtained. Extraction kinetics tests showed that approximately 0.5 min of mixing time was sufficient for the shakeup tests to reach equilibrium (see **Figure S1**). After phase separation, the aqueous solution was kept in well-sealed sample vials for further concentration analysis using the UHPLC as described in Section 2.2. The 4-TAP containing samples were diluted 100 – 2000 times using acetonitrile to ensure the diluted 4-TAP concentrations remained within the calibrated range. The concentration of 4-TAP in organic phase was calculated based on a mass balance.

The distribution of 4-TAP between the two immiscible liquids is characterized by the partition coefficient parameter (*P*), which is defined as the concentration ratio of 4-TAP in the organic phase to the aqueous phase at equilibrium (Equation *1*).

$$P_{4-TAP} = \frac{[4-TAP]_{org}}{[4-TAP]_{aq}}$$

Equation 1

where $[4-TAP]_{org}$ and $[4-TAP]_{aq}$ are the concentrations of the solute 4-TAP in the organic and aqueous phases, respectively, at equilibrium.

2.4 Conductor-like screening model (COSMO model)

The COSMO model was initially developed [35] using a spherical cavity Onsager model based on the quantitative study of tautomeric equilibria in gas-liquid phase [36]. It was originally implemented in 1993 in the quantum chemistry simulation program Molecular Orbital Package (MOPAC) [37]. Since its first publication, a number of modifications to the quantum chemical program packages have been implemented [38-42]. Compared to the more conventional chemical engineering tools, such as group contribution methods, the COSMO model broadens its application from simple compounds towards either more complicated or novel compounds (such as ionic liquids). Due to its ability to treat complex molecules in a wide range of solvent and solvent mixtures, the COSMO model has been successfully applied to the prediction of the partition coefficients of morphine and its mimic (i.e., phenol) in liquid-liquid systems [22, 43] and the solvent loss [23] in the extraction process of APCs.

Here, the COSMO model was used to test whether it can assist in the quick selection of a neutral cannabinoid mimic and in screening leach solutions and solvent systems for the LLE process. Treating the solvent as a dielectric polarizable continuum, the COSMO model can calculate the activity coefficient of a chemical species in either a single solvent or a mixture of solvents based on the σ-profile (the screening charge distribution of a chemical species). Therefore, the partition coefficients of the cannabinoids/cannabinoid mimic can be calculated by using Equation 2 to Equation 4:

$$P_C = \frac{[C]_{total,org}}{[C[_{total,aq}} \cdot \frac{\gamma_C^{aq,\infty}}{\gamma_C^{org,\infty}}$$

Equation 2

C represents the neutral cannabinoids (i.e., THC, CBD or CBN) or cannabinoid mimic candidates; $[C]_{total,org}$ and $[C]_{total,aq}$ are the total molar concentrations of organic phase and aqueous phase, respectively; $\gamma_C^{org.\infty}$ and $\gamma_C^{aq,\infty}$ are the activity coefficients of C in organic and aqueous phase, respectively, at infinite dilution.

$$ln\gamma_C^{aq,\infty} = \frac{\Delta G_C^{*sol,aq} - \Delta G_C^{*sol,C}}{RT} + ln\frac{[C]_{total,aq}}{[C]}$$

Equation 3

$$ln\gamma_C^{org.\infty} = \frac{\Delta G_C^{*sol,org} - \Delta G_C^{*sol,C}}{RT} + ln\frac{[C]_{total,org}}{[C]}$$

Equation 4

where $\Delta G_C^{*sol,aq}$, $\Delta G_C^{*sol,org}$ and $\Delta G_C^{*sol,C}$ are the solvation free energies in the system that are needed for cannabinoid/mimic molecules transferring from a fixed position in an ideal gas to a fixed position in the aqueous phase, organic phase and pure cannabinoid/mimic, respectively. $[C]$ are the molar concentrations of pure neutral cannabinoid/mimic.

The acid dissociation constants (pKa) of cannabinoids and cannabinoid mimic candidates can also be calculated based on the empirical correlations [44, 45]. The correlations are based on the solvation reaction of the compounds:

$$HA(aq) + H_2O(l) \rightarrow H_3O^+(aq) + A^-(aq)$$

Equation 5

$$HB^+(aq) + H_2O(l) \rightarrow H_3O^+ + B(aq)$$

Equation 6

In the solvation reactions, HA represents acid and HB^+ represents base. The COSMO model calculates the solvation free energies of HA, HB^+, A^-, B, H_2O and H_3O^+, so the free Gibbs energy of solvation can be calculated by the equations below:

$$\Delta G_{sol} = G(A^-) + G(H_3O^+) - G(HA) - G(H_2O)$$

Equation 7

$$\Delta G_{sol} = G(B) + G(H_3O^+) - G(HB^+) - G(H_2O)$$

Equation 8

A linear relationship [45] was developed by correlating the calculated free Gibbs energy of solvation based on the COSMO model with the corresponding experimental pKa data. Two empirical equations as Equation 9 and Equation 10 were concluded from the data sets including 94 acids and 75 bases.

$$For\ acids,\ pK_a = 0.62 \times \frac{\Delta G_{sol}}{RT \cdot ln10} + 2.10$$

Equation 9

$$For\ bases,\ pK_a = 0.67 \times \frac{\Delta G_{sol}}{RT \cdot ln10} - 2.00$$

Equation 10

Therefore the pKa values of cannabinoids and mimic candidates can be calculated based on the empirical equations above.

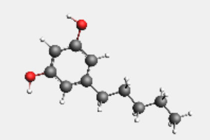
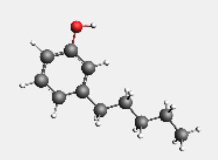
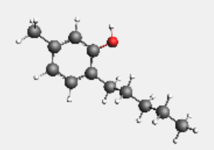
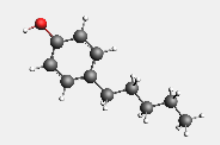
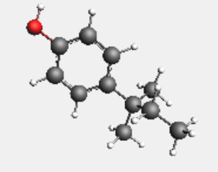
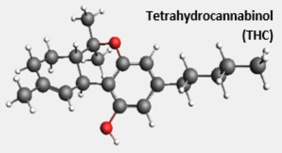
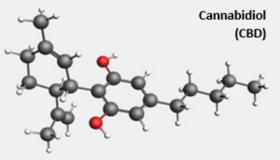
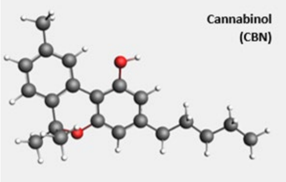
In this study, the partition coefficients and pKa of the neutral cannabinoids and cannabinoid mimic candidates in different liquid-liquid systems were predicted with the Amsterdam Modeling Suite, which was developed by Software for Chemistry & Materials (Amsterdam, The Netherlands) and incorporates the COSMO-SAC packages [32].

3. Results

3.1 Neutral cannabinoid mimic selection using COSMO

Five neutral cannabinoid mimic candidates which have similar but more simplified structures compared to the common neutral cannabinoids (e.g., THC, CBD and CBN) were initially selected. The COSMO predictions of the physical properties of the cannabinoid and mimic candidates are summarised in **Table 2**. The predicted density and flash point have good agreement with the experimental data obtained from literature sources. The deviation of the melting point is within ± 51°C for mimic compounds and in the range of 31 to 99°C for cannabinoids.

Table 2 Physical properties of neutral cannabinoid mimic candidates and three major neutral cannabinoids predicted by the COSMO model

Cannabinoid and mimic compounds	**Chemical structure** [a]	**COSMO prediction**			**Experiment**			
		Density (kg/L)	Melting point (ºC)	Flash point (ºC)	Density (kg/L)	Melting point (ºC)	Flash point (ºC)	Ref
Olivetol		0.978	99.60	177.91	-	46 – 48	> 110	[46]
3-pentylphenol		0.925	29.73	128.35	-	-	-	
5-methyl-2-N-pentylphenol		0.920	41.74	139.79	0.95	24	-	[47, 48]
4-pentylphenol		0.925	29.73	128.35	0.960	18-24	133	[48, 49]
4-*tert*-amylphenol (4-TAP)		0.920	49.81	113.41	0.962	92.7	111	[48, 50, 51]
Tetrahydrocannabinol (THC)		0.965	95.01	226.39	-	64.0	-	[52]
Cannabidiol (CBD)		0.967	167.20	244.96	1.04	67.5 – 88.5	-	[52, 53]
Cannabinol (CBN)		1.009	175.80	252.81	-	77	-	[54]

[a] Grey circles represent carbon atoms; red circles represent oxygen atoms and white circles represent hydrogen atoms

3.1.1 Selection criterion 1: Solubility in common cannabis leaching solutions such as alcohol/water mixtures

For the selection of the cannabinoid mimics, their solubility should be similar to or higher than the solubility of neutral cannabinoids in the same leaching solution. This ensures that the same concentration of the neutral cannabinoids can be achieved when using the mimic to simulate the cannabinoid leaching solution. Neutral cannabinoids have increasing solubility in ethanol/water with increasing ethanol content in the leaching solution and the rate of solubility increases significantly at ethanol contents ≥ 0.6 – 0.8 v/v (**Figure 1**). The solubilities of all neutral cannabinoid mimic candidates are higher than cannabinoids at the same ethanol content of the leaching solution. In some cases, such as olivetol at > 0.2 v/v ethanol/water, 5-methyl-2-N-pentylphenol and 4-pentylphenol at > 0.5 v/v ethanol water, the data points were not plotted on the figure as the mimic solubilities were over the calculation range of the COSMO model (i.e., ~ 900 g/L). Overall, all mimic candidates met this solubility criterion.

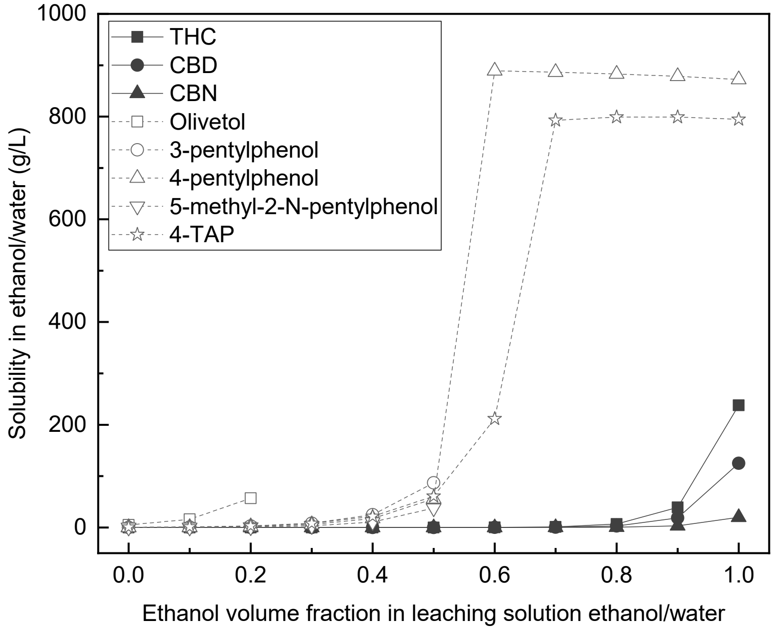


Figure 1 Solubility of major neutral cannabinoids (THC, CBD and CBN) and potential neutral cannabinoid mimic candidates predicted by COSMO (pH = 7)

3.1.2 Selection criterion 2: Partition coefficients in liquid-liquid solvent systems

The partition coefficient or distribution ratio of a solute is defined as the ratio of its concentration in the organic (hydrophobic) phase over the aqueous (hydrophilic) phase (Equation 2). In the cannabinoid extraction process, $P_{cannabinoid} > 1$ (or $\log P_{cannabinoid} > 0$) is an essential indicator of efficient extraction. To simulate the extraction behavior of the neutral cannabinoids, the mimic is expected to have a similar distribution and uniform mass transfer behavior from one phase to the other.

The COSMO model suggests that the ethanol content in the aqueous phase needs to be < 0.7 v/v to extract CBD effectively into toluene (**Figure 2**). The thresholds for THC and CBN are < 0.9 v/v.

Among the mimic candidates, 3-pentylphenol, 4-pentylphenol, 5-methyl-2-N-pentylphenol and 4-TAP crossed the x-axis at 0.65 – 0.80 v/v ethanol/water meaning that the preferred extraction condition for those mimics is < 0.65 v/v ethanol/water. This range is close to the extraction condition of neutral cannabinoids, so these mimics meet this partition coefficient criterion.

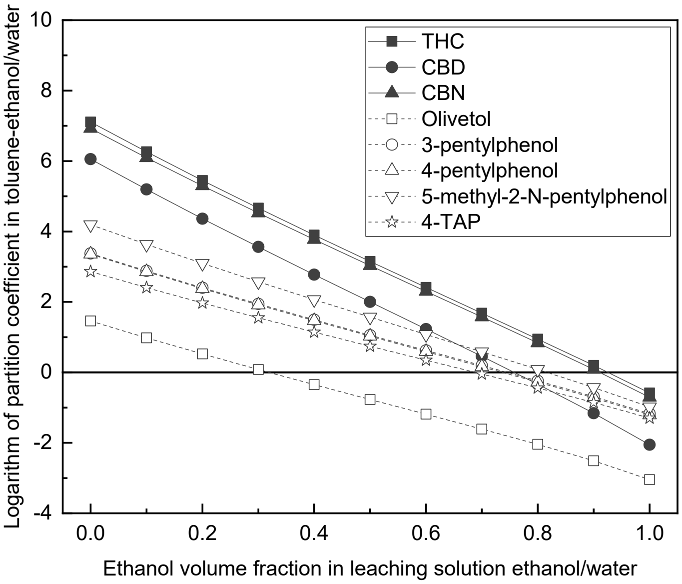


Figure 2 Partition coefficients of major neutral cannabinoids and potential neutral cannabinoid mimic candidates in ethanol/water – toluene liquid-liquid system predicted by COSMO

3.1.3 Selection criterion 3: Acid dissociation constant (pKa)

Neutral cannabinoids contain hydroxyl functional groups in their structures and therefore act as weak acids when dissolved into aqueous solution. pH adjustment of aqueous solutions will affect the concentration of a cannabinoid presenting in neutral and anionic forms and consequently its partition coefficients in the aqueous-organic liquid-liquid system. To determine the thermodynamic equilibrium and the acid strength of a cannabinoid, the acid dissociation constant (expressed as *pKa* value) is used. With *pKa* values close to neutral cannabinoids, the mimics are expected to have a response to pH changes similar to neutral cannabinoids during the LLE process.

The *pKa* of cannabinoids and mimic candidates in this study are predicted by the COSMO model and summarised in **Table 3**. The predicted values are comparable with data reported in the literature with 12.63% average absolute relative deviation. Comparing the *pKa* values of neutral cannabinoids and mimic candidates (**Table 3**), all mimic candidates show good agreement with the cannabinoids.

Table 3 pKa values of neutral cannabinoids and mimic candidates

Cannabinoid and mimic compounds	*pKa* predicted by COSMO	*pKa* from literature sources	Absolute relative deviation (%)
Olivetol	10.62	9.59 [55]	10.74%

3-pentylphenol	10.57	Not available	-
5-methyl-2-N-pentylphenol	11.88	10.55 [56]	14.34%
4-pentylphenol	10.55	Not available	-
4-TAP	10.26	10.43 [57]	1.63%
THC	11.83	10.60 [58]	11.60%
CBD	12.92	10.32 [59]	25.19%
CBN	11.24	9.73 [59]	15.52%

3.1.4 Evaluation of mimic candidates

Besides the physiochemical properties and extraction performance criteria discussed in Section 3.1.1 to Section 3.1.3, the toxicity and market price are also significant for cannabinoid mimic selection, particularly when the mimic is utilised to upscale the solvent extraction process. An evaluation matrix [22] for mimic candidates including a range of selection criteria and the weighting, with scoring from 1 (least suitable) to 5 (most suitable), is summarised in **Table 4**. The weighting for selection criteria (in brackets) is dependent on their impacts in the solvent extraction process with the highest score having the most significant impact. The overall score of each mimic candidate is the sum of weighting factors × scores.

Table 4 Evaluation of cannabinoid mimic candidates

Selection criteria		**Olivetol**	**3-pentylphenol**	**5-methyl-2-N-pentylphenol**	**4-pentylphenol**	**4-TAP**
Toxicity (4*)		3	3	3	2	3
Market price (3)		2	0 (Not available)	1	3	5
Physiochemical properties	**Water solubility (4)**	4	5	5	5	5
	Solubility in alcohol (4)	5	5	5	5	5
	***pKa* (4)**	4	4	5	4	5
Extraction performance	**partition coefficient (5)**	2	4	5	4	4
Overall score[#]		80	88	100	93	107

* Weighting factor

[#] Sum of selection criteria weighting x score

Four of these cannabinoid mimic candidates are currently available on the market and easy to source. Based on the material safety data sheets (MSDS) obtained from Merck [60], the toxicity of olivetol, 5-methyl-2-N-pentylphenol and 4-TAP are categorized as 4, practically non-toxic and not as an irritant. Only 4-pentylphenol falls in category 3 for toxicity (which is slightly toxic and slightly irritating), with the oral median lethal dose (LD_{50}, rat) being 231 mg/kg. Using Merck costings as an indirect market price guideline, the current selling price of mimic candidates are AUD 8780, AUD 1370, AUD 41600, and AUD 64 for each 100 grams of olivetol, 4-pentylphenol, 5-methyl-2-N-pentylphenol and 4-TAP, respectively.

Based on the evaluation matrix (**Table 4**), the cannabinoid mimic candidate with the highest score, 4-TAP was selected as the neutral cannabinoid mimic for dissociation, extraction, and stripping studies in this study.

3.2 Solubility of 4-TAP in leaching solutions

The solubility of 4-TAP in alcohol/water and pH water leaching solutions represents the maximum amount of 4-TAP that can be dissolved into the leaching solutions. This also represents the maximum leaching efficiency achievable under a specified leaching condition. The change of 4-TAP's solubility at different leaching conditions is expected to mirror the behavior of neutral cannabinoids in those conditions.

Having similar hydrophobicity as the major neutral cannabinoids with a C_5 alkyl chain (eg. THC/CBD) and a hydroxyl functional group attached to the benzene ring, 4-TAP rarely dissolves in pure water. Hence, the selected leaching solutions were alcohol/water solutions, which utilize organic alcohols to enhance 4-TAP solubility into water, and alkaline solutions, which enhance 4-TAP solubility based on the chemical dissolution of hydroxyl functional groups to release a proton.

3.2.1 Alcohol/water systems

The solubility of 4-TAP in a methanol/water mix increases slowly from 0.18 g/L to 3.96 g/L when the volume fraction of methanol increases to 0.4 v/v (**Figure 3A**). At this point, the solubility of 4-TAP dramatically increases up to 560.72 g/L at 0.7 v/v methanol/water before slowly levelling off at higher methanol content. A similar trend was observed for the ethanol/water system (**Figure 3B**). These data suggest that ≥ 0.4 v/v alcohol content is preferred to efficiently dissociate 4-TAP and neutral cannabinoids into leaching solutions.

The solubility of 4-TAP in alcohol/water was also predicted by a COSMO model and the predicted results are comparable with experimental results up to a 0.6 v/v alcohol content. At higher alcohol content, the COSMO model predicted a similar trend of 4-TAP solubility, but the predicted data are overestimated in both alcohol/water systems.

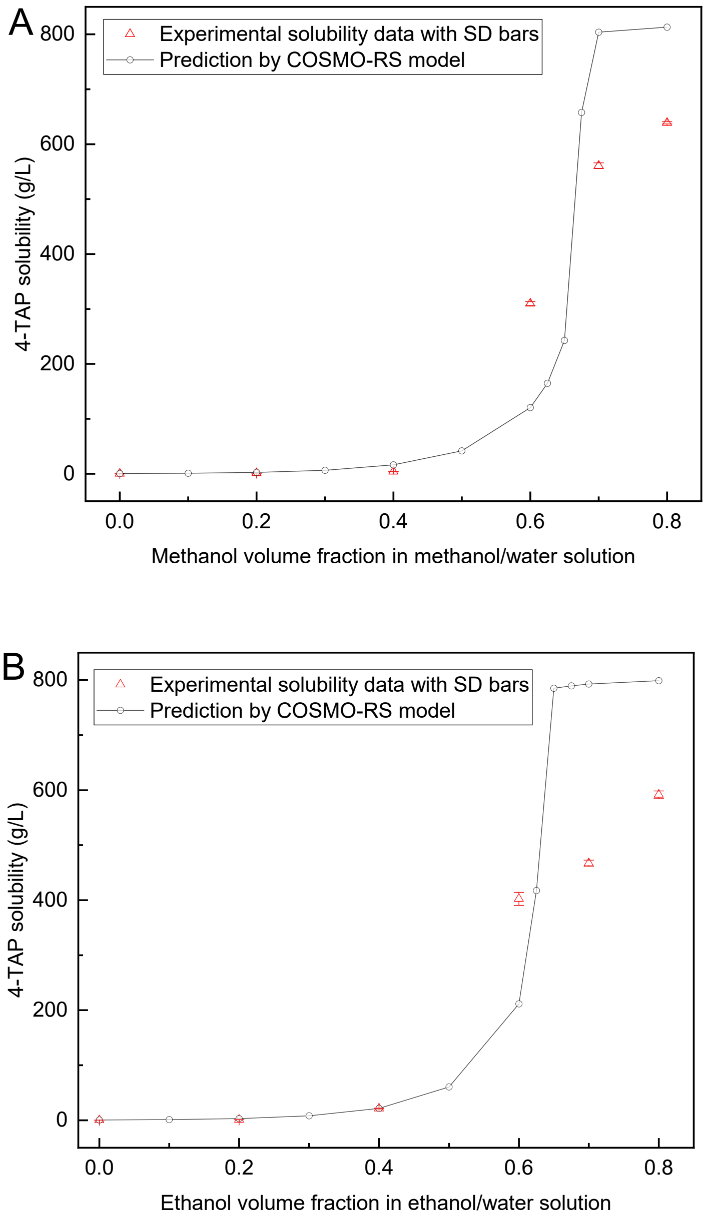


***Figure 3** Experimental and predicted solubility of 4-TAP in mixed leaching solution (pH = 7; A: methanol/water, B: ethanol/water)*

3.2.2 pH-controlled water-based system

Figure 4 shows the solubility of 4-TAP in pH-controlled water-based leaching solutions. The pH was adjusted by diluted either H_2SO_4 or NaOH solutions as required. The solubility of 4-TAP stabilised at 0.99 ± 0.09 g/L at pH ≤ 10. At higher alkaline conditions, the solubility enhanced significantly and reached a maximum solubility of 29.64 g/L at pH 12.5. The increase of mimic solubility in alkaline solutions is due to the ionization of the hydroxyl functional group. The limit of mimic solubility at pH > 12.5 is caused by the decrease of OH^- activity in concentrated alkaline solutions and the solubility limit of 4-TAP-sodium salt. Based on the comparable pKa of 4-TAP and neutral cannabinoids (**Table 3**), alkaline solutions with pH ≥ 10 are required to leach neutral cannabinoids from cannabis materials.

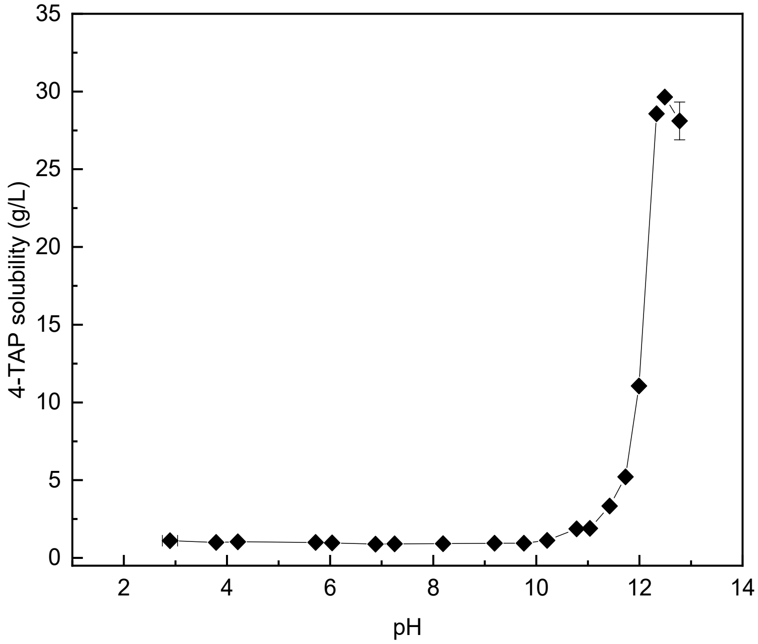


Figure 4 *Solubility of 4-TAP in pH-controlled water-based leaching solution*

3.2.3 <u>Combined alcohol/water and pH-controlled leaching system</u>

The high alcohol content enhances the solubility of 4-TAP but will reduce the extraction of 4-TAP and increase the solvent loss of ethanol into the organic phase. The integration of alkaline leaching and alcohol/water leaching therefore has the potential to enhance the solubility of 4-TAP while maintaining the low alcohol content in the leaching solution.

Solubilities of 4-TAP in 40 v/v% ethanol/water and pH 12.5 water solutions were only 21.34 g/l and 29.64 g/L, respectively (**Figure 5**). By adjusting the pH of 40 v/v% ethanol/water to pH 12.5, the solubility of 4-TAP was elevated 10-fold, reaching 264.53 g/L. This demonstrates the synergistic effect of physical dissolution and chemical dissociation.

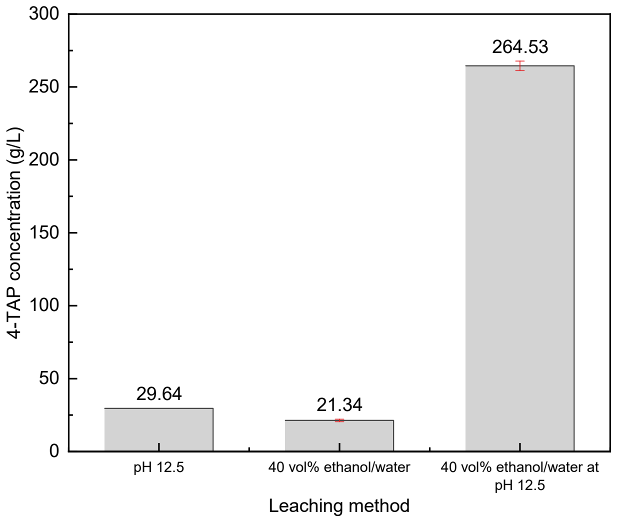


Figure 5 *Solubility of 4-TAP in three different leaching solutions (**left**: pH 12.5 alkaline solution; **middle**: 40 v/v% ethanol/water solution; **right**: pH 12.5 40 v/v% ethanol/water solution)*

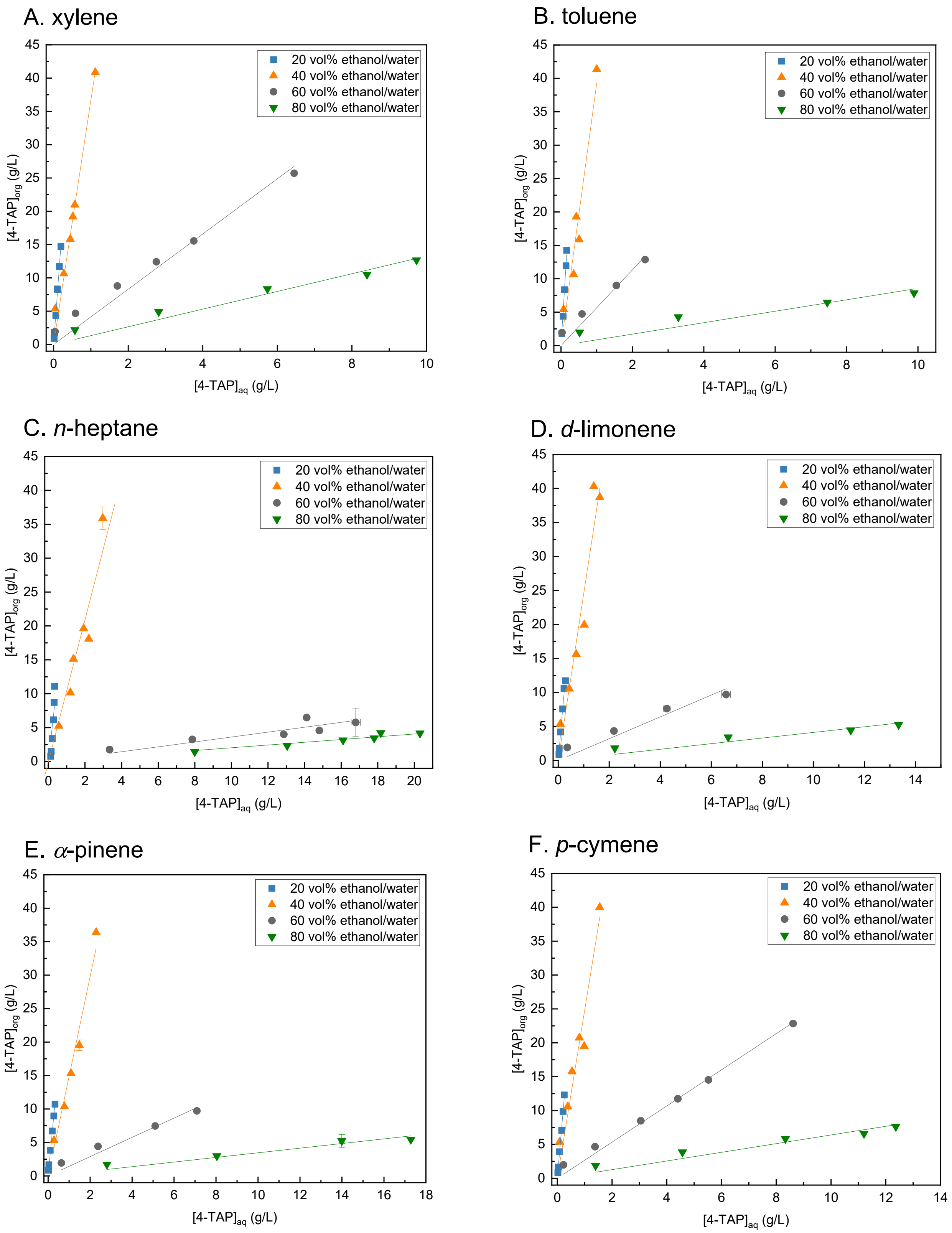
Overall, all three methods can efficiently leach 4-TAP into the aqueous phase. Alkaline leaching could maintain the high liquid-liquid immiscibility and minimize the solvent loss in the later LLE stage. However, a strong alkaline leaching can cause a large amount of caustic sludge in the waste stream and increases the acid consumption to drop the pH of aqueous solution during the LLE stage. Alcohol/water leaching is less corrosive than an alkaline solution, which can reduce the cost of the leaching process, but the presence of alcohol in the leaching solution might enhance the solubility of some hydrophobic "impurities" in cannabis extracts into the leaching solution and hence reduce the selectivity of the LLE process. Combining the above leaching methods has the advantage of enhancing leachability of cannabinoids but retains the disadvantages of both approaches.

3.3 Extraction and Stripping

The extraction and stripping isotherms of neutral cannabinoid mimic 4-TAP was also investigated. The driving force for extraction (i.e., 4-TAP transferred from aqueous to organic phases) and stripping (i.e., 4-TAP transferred from organic to aqueous phases) processes is via the mutual affinity of 4-TAP to aqueous and organic solutions in the liquid-liquid system. The distribution of 4-TAP between the two immiscible liquids is characterised by the partition coefficient, $P_{4-TAP} = \frac{[4-TAP]_{organic}}{[4-TAP]_{aqueous}}$. The modification of alcohol content in aqueous phase will alter the affinity of 4-TAP to this phase, which consequently controls the mass transfer direction of 4-TAP between phases. Similarly, the presence of effective extractant in the organic phase will increase the affinity and mass transfer of 4-TAP towards the organic phase.

3.3.1 Pure organic diluent systems

The extraction and stripping isotherms of cannabinoid mimic 4-TAP between 20% v/v – 80 v/v% ethanol/water aqueous phases and six pure organic diluents are plotted in **Figure 6**. The partition coefficients of 4-TAP represented by the slopes of isotherm lines using linear regression are summarized in **Table 5**.



A. xylene

B. toluene

C. *n*-heptane

D. *d*-limonene

E. *α*-pinene

F. *p*-cymene

***Figure 6** Experimental extraction and stripping isotherms of 4-TAP in 20 – 80 v/v% ethanol/water aqueous phases and six pure organic diluents (pH = 7)*

Table 5 Experimental partition coefficients for neutral cannabinoids mimic 4-TAP.

	20 v/v% Ethanol/H_2O	**40 v/v% Ethanol/H_2O**	**60 v/v% Ethanol/H_2O**	**80 v/v% Ethanol/H_2O**
xylene	75.29	36.71	4.15	1.33
toluene	85.59	39.28	5.67	0.86
***n*-heptane**	24.62	10.94	0.36	0.20
***d*-limonene**	42.97	24.52	1.60	0.41
α-pinene	33.14	14.54	1.44	0.35
***p*-cymene**	46.92	24.83	2.67	0.64

4-TAP is extracted efficiently when contacting 20 v/v% and 40 v/v% ethanol/water with pure organic diluents (**Figure 6** and **Table 5**). The extractability order of the six organic solvents for 4-TAP can be arranged from high to low as toluene ≥ xylene > *p*-cymene ≥ *d*-limonene > α-pinene > *n*-heptane, which suggests that the presence of a benzene ring in organic diluents enhances the extraction of 4-TAP. While the partition coefficients of 4-TAP in the green solvent systems (*p*-cymene, *d*-limonene and α-pinene) were 1.5 – 2.7 times lower than in the toluene and xylene systems, green solvents remain potential candidates for neutral cannabinoid mimic extraction with $P_{4\text{-}TAP} \geq 14$ at 20 – 40 v/v% ethanol/water. In contrast, at 80 v/v%, $P_{4\text{-}TAP}$ is < 1 in most liquid-liquid systems meaning that the mass transfer of 4-TAP is preferred from the organic phase towards aqueous phase. Therefore, 60 – 80 v/v% ethanol/water solutions are considered the optimal conditions for stripping neutral cannabinoid mimics.

The utilisation of a high ethanol content stripping solution will result in ethanol loss to the organic phase. The saturated concentrations of ethanol in the organic phase when contacting 60 – 80 v/v% ethanol/water with pure *n*-heptane and green solvents were in range of 5.37 – 27.49 g/L [23], which is < 0.05 volume fraction of the organic phase, thus the effect of solvent loss on extraction performance of the recycled solvent is expected to be minor. The ethanol losses in xylene and toluene systems are at a higher range (i.e., 22.76 – 86.06 g/L). It is worth emphasizing that all the stripping results at 60 – 80 v/v% ethanol/water take into account the impact of solvent loss. In the recycling of the organic solvent, the ethanol can be gradually accumulated and separated from the organic solvents (with the higher boiling points) by distillation. The LLE process with a modifier is schematically represented in **Figure 7**.

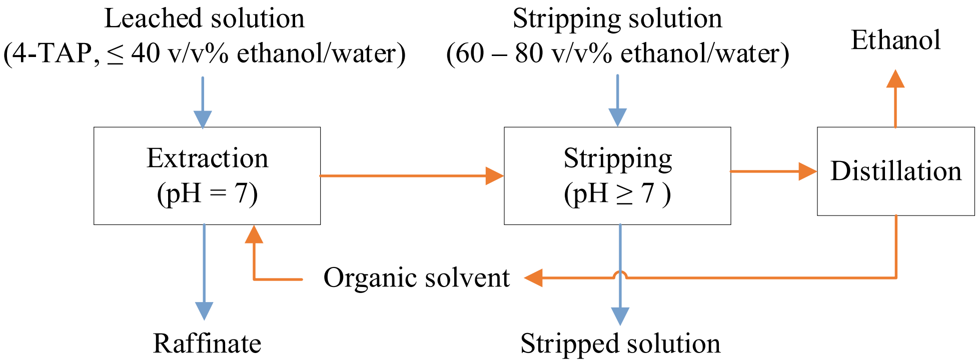


Figure 7 A flow diagram of cannabinoid mimic extraction and stripping

To test the veracity of these results, reproducibility tests were conducted in duplicate for isothermal shakeup experiments using toluene solvent with the partition coefficients data summarized in **Table 6**. The average relative standard deviation (RSD) of the isothermal shake-up experiment is 3.65% across the 20-80% ethanol/water mixes with a maximum of 5.01% observed in the 40% ethanol/water mix.

Table 6 Experimental partition coefficients of 4-TAP in reproducibility tests for isothermal shakeup experiments using toluene solvent.

	20 v/v% Ethanol/H_2O	**40 v/v% Ethanol/H_2O**	**60 v/v% Ethanol/H_2O**	**80 v/v% Ethanol/H_2O**
Toluene-1	85.59	38.54	5.59	0.86
Toluene-2	87.81	40.47	5.71	0.81
RSD%	2.60%	5.01%	2.02%	4.96%

The experimental results are comparable with the trends predicted by the COSMO model operated with the Klamt parameter (**Figure 8**). The absolute relative deviation of experimental and predicted log $P_{4\text{-}TAP}$ (**Table 7**) shows that COSMO model provided a good prediction of partition coefficients at systems with ≤ 40 v/v% ethanol content, whilst the model underestimated the partition coefficients in systems with higher ethanol content. This may be a consequence of the overestimation of the model in predicting 4-TAP solubility at high alcohol content solutions (**Figure 3**).

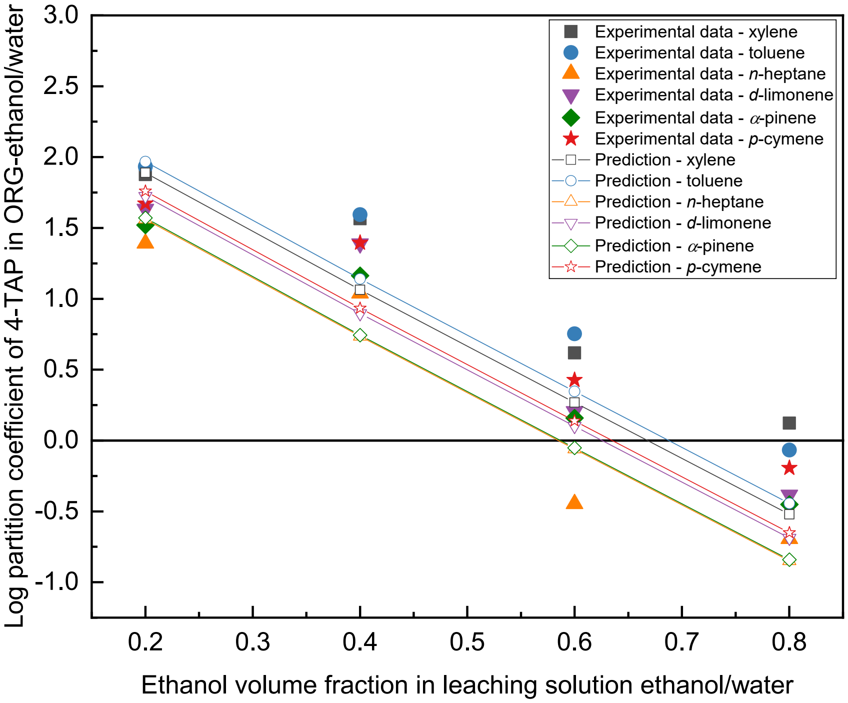


Figure 8 *Experimental and predicted partition coefficients of 4-TAP in between 20 – 80 v/v% ethanol/water aqueous solution and six pure organic diluents*

Table 7 The absolute relative deviations of experimental and predicted log $P_{4\text{-}TAP}$ in different liquid-liquid system (defined as $\left|\frac{\boldsymbol{log\ P_{pred} - log\ P_{exp}}}{\boldsymbol{log\ P_{exp}}}\right|$ x 100%)

	20 v/v% Ethanol/H₂O	**40 v/v% Ethanol/H₂O**	**60 v/v% Ethanol/H₂O**	**80 v/v% Ethanol/H₂O**
xylene	0.70%	32.08%	56.63%	523.51%
toluene	1.85%	28.44%	54.06%	559.31%
heptane	12.26%	29.30%	86.50%	22.73%
***d*-limonene**	5.52%	35.51%	50.63%	79.19%
***α*-pinene**	3.31%	36.05%	132.34%	86.29%
***p*-cymene**	5.31%	33.12%	67.60%	235.59%
Average	4.83%	32.42%	74.63%	251.10%

3.3.2 Extractant/organic diluent systems

To test whether the extraction step could be further optimised, a phosphine-oxide extractant, CYTOP®503 (Solvay, Canada), was added into toluene to investigate the partition coefficient of each mimic when contacting this extractant/diluent system with the 40 v/v% ethanol/water aqueous system. This phosphine-oxide extractant is known to offer a high separation efficiency for various organic molecules, good stability and fast extraction kinetics in both batch and continuous solvent extraction processes [34].

3.3.2.1 Effect of pH

The impact of pH in the isotherm experiment on 4-TAP extraction efficiency from 40 v/v% ethanol/water aqueous solution to 5 v/v% CYTOP®503/toluene organic solvent is illustrated in **Figure 9.** 4-TAP extraction efficiency was measured to be 97.3% - 98.4% under controlled acidic conditions. As pH increased to over 10, the extraction efficiency dropped and was only 86.0% at pH 13. These data suggest that the preferred pH conditions for mimic extraction are at 4 – 4.5 while pH > 12.5 has the potential to be used for the mimic stripping process.

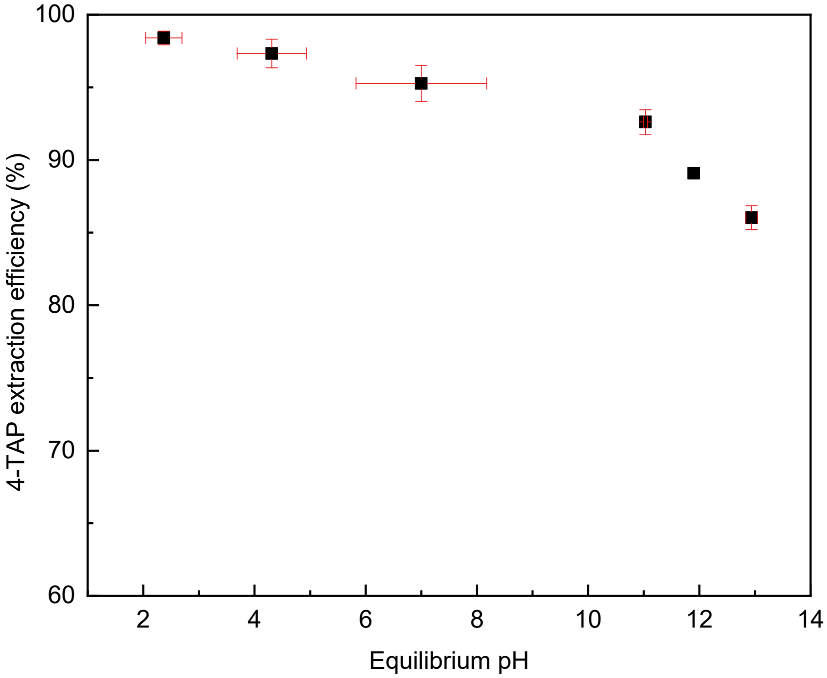


Figure 9 *Effect of pH on the 4-TAP extraction using 40 v/v% ethanol/water – 5 v/v% CYTOP®503/toluene system (A/O ratio = 1)*

3.3.2.2 Effect of extractant concentration

As CYTOP®503 extractant has high density and viscosity, the high concentration of CYTOP®503 in the organic solvent might reduce the activity of the solute (4-TAP), increase third phase formation potential and result in a longer equilibrium time. Therefore, the optimal concentration of extractant use is essential for designing the LLE process.

The extraction efficiency of 4-TAP using a 40 v/v% ethanol/water – CYTOP®503/toluene system at different pH and volume fractions of CYTOP®503 in toluene solution was investigated. The increasing concentration of phosphine-oxide extractant in toluene solvent up to 20 v/v% almost had negligible effect on 4-TAP extraction efficiency at pH 4 and pH 7 (**Figure 10**). However, when extractant content in the organic phase increased further, the extraction efficiency dropped due to the reduction in the activity of 4-TAP. Thus, pure toluene at pH 12.5 showed a poor extraction of 4-TAP but by adding CYTOP®503, the 4-TAP extraction efficiency increased and reached a peak of 60% when CYTOP®503 volume fraction in organic phase was at 50 v/v%. The results indicate that the phosphine-oxide based extractant has as similar affinity to 4-TAP as pure organic diluents when physical dissolution is used. When 4-TAP chemically dissociated into anion forms in caustic aqueous solution (e.g., pH 12.5), the extractant showed stronger affinity to the cannabinoid mimic than pure organic diluents.

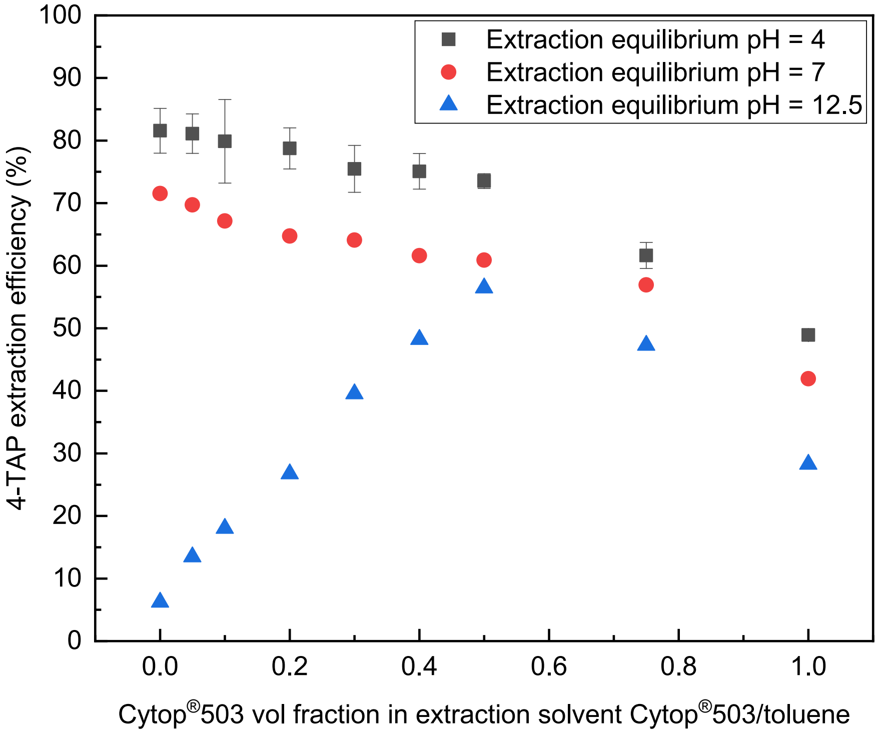


Figure 10 *Effect of pH and CYTOP®503 volume fraction on the 4-TAP extraction efficiency using a 40 v/v% ethanol/water – CYTOP®503/toluene system (A/O ratio = 1)*

The CYTOP®503 volume fraction also influences the overall stripping efficiency of 4-TAP from loaded organic phase using 80 v/v% ethanol/water at pH 7 and 12.5 (**Figure 11**). Clearly, pH 12.5 improved the extraction of neutral cannabinoid mimic 4-TAP from loaded organic solvent to aqueous stripping solution by around 20% in comparison to pH 7. When the extractant volume fraction increases, the 4-TAP stripping efficiency drops dramatically which suggests the addition of phosphine-oxide extractant will not benefit the mimic stripping process. Overall, the study found that pure organic diluents systems are sufficient for the extraction of the neutral cannabinoid mimic 4-TAP.

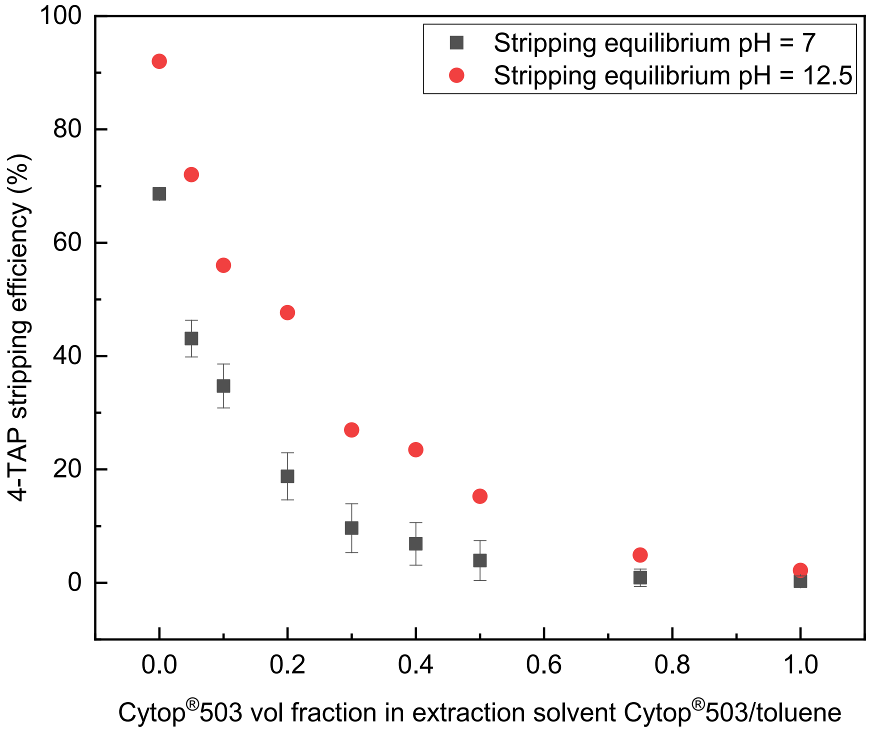


Figure 11 *Effect of pH and CYTOP®503 volume fraction on the 4-TAP stripping efficiency using 80 v/v% ethanol/water – CYTOP®503/toluene system (A/O ratio =1)*

4. Conclusions

This study has developed a proof-of-concept to utilize the COSMO model in selecting neutral cannabinoid mimics to simulate the performance of neutral cannabinoids in an LLE process. The dissolution, extraction, and stripping performance of 4-TAP as a neutral cannabinoid mimic in three aqueous systems and six organic solvents were investigated.

The conclusions from this work can be summarized as follows:

- The solubility of 4-TAP in aqueous solution was enhanced by introducing either ethanol or methanol as modifiers and increasing the pH of the solutions. This demonstrated that the combination of physical dissolution and chemical dissociation by increasing the pH of the alcohol/water solution had a synergistic effect in enhancing the solubility of 4-TAP.
- It is feasible to achieve efficient extraction and stripping of 4-TAP by controlling the volume fraction of modifier ethanol. To achieve high 4-TAP extraction efficiency, the preferred ethanol contents were < 60 v/v% whereas 80 v/v% offered a high mimic stripping efficiency.
- The extractability of six pure organic diluents was shown to be in the order toluene ≥ xylene > *p*-cymene ≥ *d*-limonene > α-pinene > *n*-heptane. The partition coefficients of 4-TAP in the green solvent systems were 1.5 – 2.7 times lower than in either the toluene or xylene systems but green solvents still provided high 4-TAP extractability.

- The experimental partition coefficient results were comparable with the trends predicted by the COSMO model. This supports the use of the COSMO model for proof-of-concept in pre-screening solvent systems and assisting in prediction of the key thermodynamic parameters.
- Considering the strong extractability of the phosphine-oxide based extractant, pure organic diluent systems are recommended for neutral cannabinoids and the mimic.

For further work, it is of great interest to validate the newly developed LLE process on cannabinoid-containing solutions or plant tissues which will provide a strong and solid base in scaling up this process.

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Supplementary Data

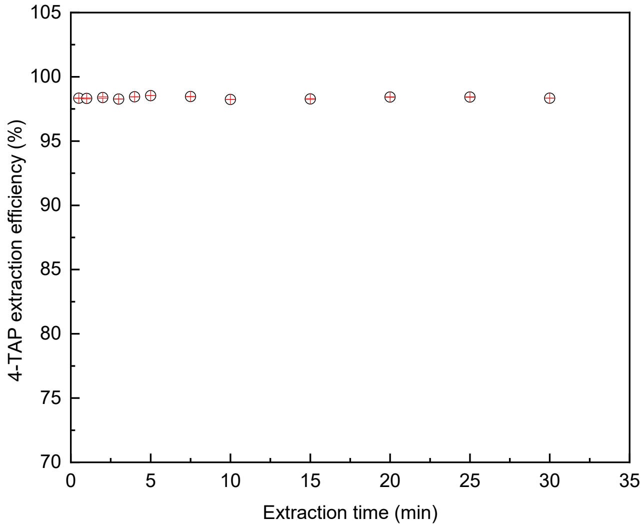

Figure S1 The rate of extraction of 4-TAP from 40% ethanol/water into toluene (pH = 7)