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Development of Salting-out Assisted Liquid-Liquid Extraction for the Determination of Orange I

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In this study, a fast and simple salting-out assisted liquid-liquid extraction (SALLE) followed by UV-Visible spectrophotometric method was developed and applied for the determination of Orange I in beverage. The sample treatment is based on the use of water-miscible solvent as the extractant and phase separation under high-salt conditions. Water-miscible extraction solvent was added to aqueous solution (10 mL) before the addition of $(NH_4)_2SO_4$. The mixture was vortexed until $(NH_4)_2SO_4$ dissolved. Then, the mixture was centrifuged. The target analytes in the sample were extracted into the organic phase. The aqueous phase was transferred to a vial for analysis. The absorbance of aqueous phase was measured by UV-Visible spectrophotometric method at the wavelength of 475 nm. The effects of parameters such as type of salt, amount of salt, pH, extraction solvent and contact time were evaluated. Under optimum conditions, a good linearity was obtained in the range of 5 – 20 ppm correlation coefficient of 0.9918. The limit of detection (LOD) and limit of quantification (LOQ) were 0.019 ppm and 0.057 ppm, respectively. The percentage of relative standard deviation (RSD%) of intra-day (n = 3) and inter-day (n = 9) were 2.54 and 8.45, respectively. The efficiency of the developed method was proven in the analysis of Orange I in commercial fruit juice sample with the recovery of 85.19%.

Key words: Orange I; salting-out liquid-liquid extraction; beverage sample

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Azo dyes are an important class of synthetic colourants. Aromatic azo compounds, in particular, are utilised as commercial colourants for clothes, polymers, cosmetics, and food and beverages. The dye biodegradation rate can be influenced by azo dye structure depends on the number and placement of azo linkages [1]. Oxidizing reagents, light and water will not disturb its chemical structure which make it more difficult to degrade in the environment. The azo dyes present in the environment are poisonous and may cause cancer. The development of efficient techniques to eliminate azo dyes is always challenging but crucial endeavour because they pose risks to both human health and the environment [2].

Several methods for the determination of azo dyes in various matrices have been reported, including liquid–liquid extraction (LLE) and solid phase extraction (SPE) [3,4]. LLE is also known as solvent extraction. The basic procedure is separation of chemicals based on their relative solubility in two distinct immiscible liquids, generally organic phase and water [4]. LLE is time-consuming, laborious, and low-sensitivity, and it frequently needs significant volumes of harmful organic solvents, which may be costly. SPE requires significantly lesser solvent than LLE, but costly. Salting-out assisted liquid-liquid extraction (SALLE) was developed as a sample preparation method as it provides shorter time in partition equilibrium, simplicity and ease of purification and condensation of analyte extracts. Salting out is a process that take places when the solubility of a nonelectrolyte substance in water diminishes when the concentration of a salt increases. As time passed, salting-out liquid-liquid extraction are widely used such as in the determination of insecticide [5,6], preservatives and artificial sweeteners [7], biogenic amines [8], phenol and chlorophenol [9].

Towards this end, of the plethora of micro-extraction techniques, the SALLE seemed to be the best candidate. Thus, this paper is dedicated to the development of a SALLE pre-treatment method to extract Orange I from fruit juice prior to the analysis using UV-Visible spectrophotometer. Several parameters such as type of salt, amount of salt, pH, type of extraction solvent and effect of contact time affecting the removal percentage of Orange I was evaluated. Finally, the method was employed for the determination of Orange I in fruit juice.

EXPERIMENTAL

Chemicals and Reagents

Standard of Orange I in powder form was purchased from Fluka (United States). HPLC grade tetrahydrofuran, acetonitrile (HPLC grade), methanol (HPLC grade), ammonium sulphate, magnesium sulphate and sodium chloride were purchased from QREC (New Zealand).

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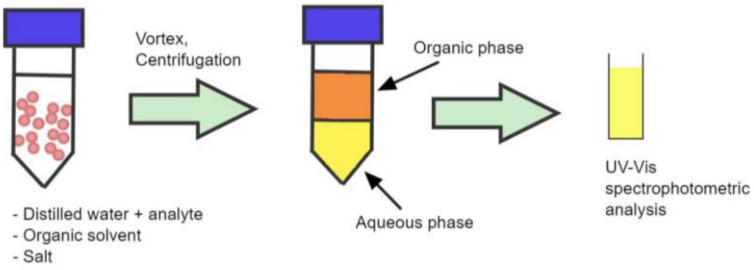

Figure 1. Salting-out assisted liquid-liquid extraction procedure.

Instrumentation

The instruments used were Shimadzu UV-2600 Double Beam UV-Vis Spectrophotometer (Japan) and pH meter was purchased from Eutech Instruments (USA).

SALLE Procedure

About 10 mL Orange I solution (10 ppm) was placed in a centrifuge tube. Then, 4.5 mL of water-miscible organic solvent was added. After that, 2.5 g of salt was added. The mixtures were then mixed well until the salt was completely dissolved before centrifugation for 10 mins. The mixture was separated into two layers. The upper layer was removed, and the bottom layer was transferred into small vials for UV-Vis spectrometry measurement. Figure 1 depicts the schematic diagram of SALLE procedure.

Several significant parameters were investigated with the aim of achieving the optimum results. The parameters involved were type of salt, amount of salt, pH, extraction solvent and contact time. All the optimization studies were carried out using UV-Visible spectrophotometer at 475 nm. The percent of removal were calculated using Equation 1.

$$\textbf{Removal percentage} = \frac{\textbf{initial absorbance} - \textbf{final absorbance}}{\textbf{initial absorbance}} \textbf{ x 100} \qquad \textbf{(1)}$$

Real Sample Analysis

The orange juice was purchased from local shop in Pulau Pinang. About 20 mL of orange juice was filtered before analysis and diluted in 100 mL volumetric flask. The diluted orange juice was spiked with Orange I dye.

RESULTS AND DISCUSSION

Optimization of SALLE Parameters

Effect of Type of Salts

The type of salt is a significant parameter in the SALLE procedure. The degree of phase separation was affected by the type and amount of salts used. In this study, the effect of Mg_2SO_4, NaCl and $(NH_4)_2SO_4$ were measured. The results showed that $(NH_4)_2SO_4$ provided more efficient of extraction with the highest percent removal (Figure 2). This might be related to a better salting-out ability and a higher solubility of $(NH_4)_2SO_4$ in water [10] as compared to other salts.

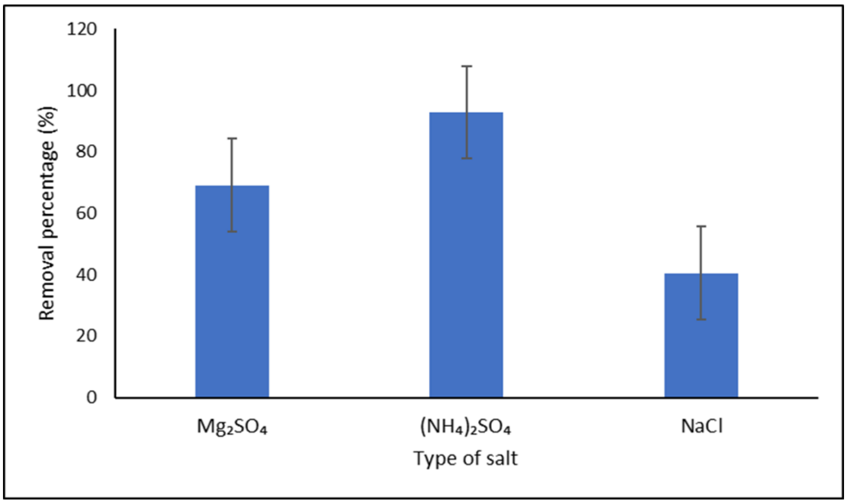

Figure 2. Effect of type of salting-out reagent on removal percentage. Conditions: Orange I concentration was 10 ppm; initial volume of aqueous solution and acetonitrile were 10 mL and 4.5 mL, respectively; 2 g of each of salts; vortex for 20 s; centrifuge for 2 mins at 3000 rpm.

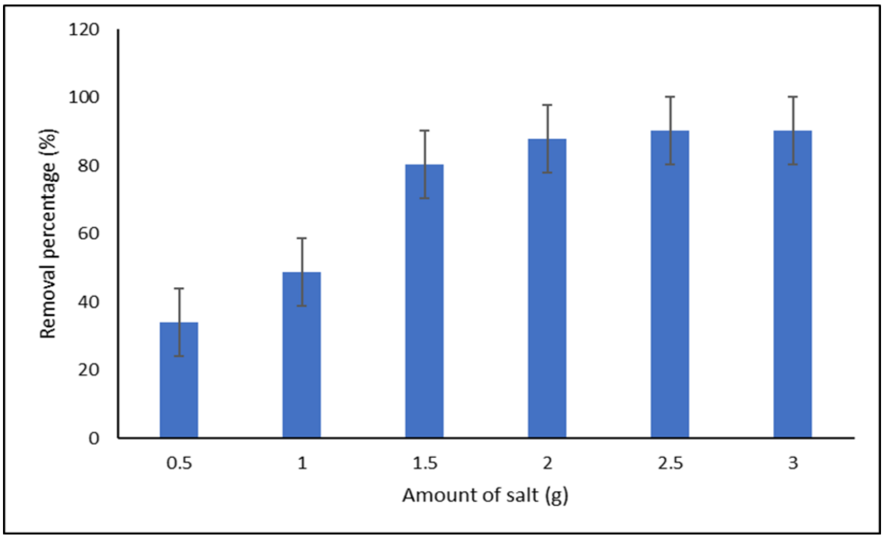

Figure 3. Effect of amount of salting-out reagent on the removal percentage. Conditions: Orange I concentration was 10 ppm; initial volume of aqueous solution and acetonitrile were 10 mL and 4.5 mL, respectively; 0.5 – 3.0 g of $(NH_4)_2SO_4$; vortex for 20 s; centrifuge for 2 mins at 3000 rpm.

The salting-out effect is usually applied to lower the phase solubility of hydrophilic and hence enhance the transition of analytes from the inorganic to the organic phase. Moreover, the importance of salting effect is enhanced by the fact that they are strongly related to Hofmeister series. Ammonium sulphate ($(NH_4)_2SO_4$) is frequently employed as a salting-out reagent because of its great solubility, which allows for the preparation of solutions with high ionic strength, ease of availability and cheap [11]. Thus, $(NH_4)_2SO_4$ was chosen for the study.

Effect of Amount of Salts

Different amount of $(NH_4)_2SO_4$ on the efficient of extraction was evaluated. The analytes solubility in the sample solution was also affected by ionic strength. The ionic strength of solution increases with the addition of salts [15]. The amount of $(NH_4)_2SO_4$ must be significant enough to clearly separate the two phases [3]. The amount was varied from 0.5 – 3.0 g. Based on Figure 3, the optimum amount of salt for extraction was 2.5 g. After 2.5 g, the removal percentage was constant. This can be explained by the fact that a substantial amount of precipitate generated at high concentrations absorbed the analyte [10]. The salting-out effect is frequently used to lower the solubility of hydrophilic compounds in the aqueous phase and therefore enhance the partition of analytes into the organic phase [3]. Hence, 2.5 g of $(NH_4)_2SO_4$ was selected for further analysis.

Effect of pH

pH value of sample solution influenced the extraction efficiency of a weak organic base or acid. To improve the extraction efficiency, the pH of sample should be modified to suppress the ionization of any acidic or basic analytes, considering the pK_a of the studied compounds. The pH was varied in the range of 5 – 9 using different concentrations of HCl and NaOH. The experimental results showed that increasing the pH up to 7 enhanced the signals, which then decreased when the pH was increased further. The degree of ionization of dye molecule was affected by the pH of aqueous solution [12]. The decrease in removal percentage after pH 7 can be described as the ionization of the phenol structure in Orange I dye and the absence of requirements for the production of an ion pair [13]. Thus, pH 7 was selected for further analysis.

Effect of Extraction Solvent

The type of extraction solvent is important parameter in SALLE method. The optimal extraction solvent should be water miscible, polar, and likely to cause phase separation when salt is added. Acetonitrile (ACN) is the most likely to use as organic solvent for previous SALLE method. In this study, acetonitrile (ACN), tetrahydrofuran (THF) and methanol (MeOH) were tested for the effect of different extraction solvent. The results showed that THF exhibited the highest extraction efficiency in comparison with the other solvents (Figure 5). Extraction ability of organic solvents can be explained by Gutmann donor number with the following sequences: acetonitrile (14.1) < methanol (19.0) < tetrahydrofuran (20.0) [14]. THF has the best aqueous phase separation due to lowest solubility in salt solutions and the lowest salt water residue in the separated organic layer. THF has lower polarity, compared to more polar solvents such as ACN, may aid in obtaining a cleaner extraction. Thus, THF was chosen for further analysis.

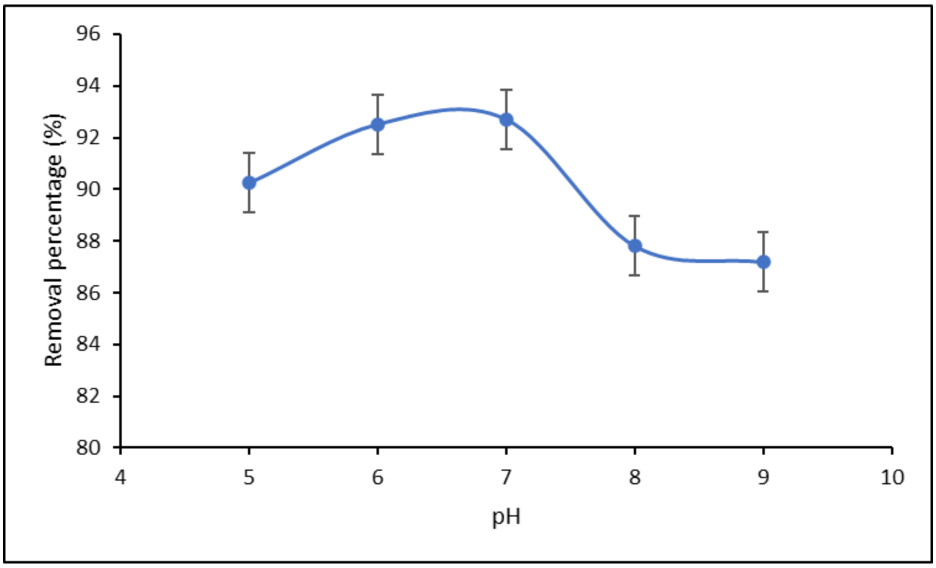

Figure 4. Effect of pH of aqueous solution on the removal percentage. Conditions: Orange I concentration was 10 ppm; pH 5 – 9; initial volumes of aqueous solution and acetonitrile were 10 and 4.5 mL, respectively; 2.5 g of $(NH_4)_2SO_4$; vortex for 20 s; centrifuge for 2 mins at 3000 rpm.

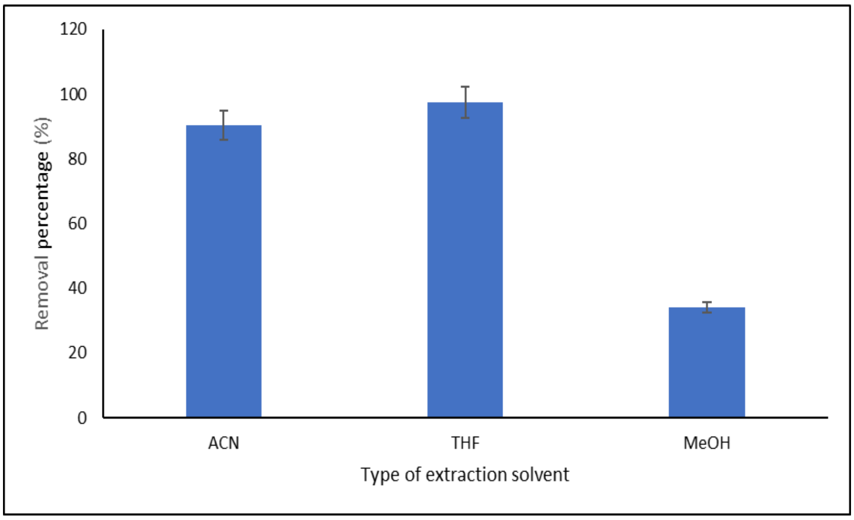

Figure 5. Effect of type of extraction solvent on the removal percentage. Conditions: Orange I concentration was 10 ppm; pH 7; initial volumes of aqueous solution and extraction solvents were 10 and 4.5 mL, respectively; 2.5 g of $(NH_4)_2SO_4$; vortex for 20 s; centrifuge for 2 mins at 3000 rpm.

Effect of Contact Time

The kinetics of the extraction are visibly influenced by centrifugation of the reaction mixture, which improves interaction between the organic solvent and the aqueous solution and so accelerates the development of the two-phase system [5]. The effect of contact time was varied from 5 – 25 mins with interval of 5 mins at 3000 rpm. 10 mins of centrifugation time exhibits the highest percentage of removal. Longer contact time resulted in decrease of analyte extraction. Therefore, 10 mins was chosen as centrifuge time.

Method Validation

Under the above optimum experimental conditions, the proposed SALLE method was validated by linearity, limits of detection, limits of quantification, repeatability and recovery. The calibration plots were found to be linear in the range 5 – 20 ppm (Figure 7). The calibration curve was plotted by concentration of Orange I against absorbance with correlation coefficient obtained is 0.9918.

For determination limit of detection (LOD) and limit of quantification (LOQ) for Orange I in this study,

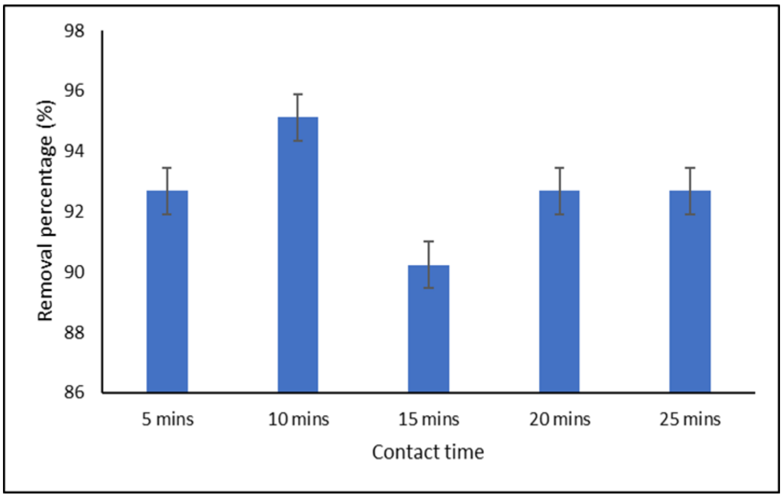

Figure 6. Effect of contact time on the removal percentage. Conditions: Orange I concentration was 10 ppm; pH 7; initial volumes of aqueous solution and tetrahydrofuran were 10 and 4.5 mL, respectively; 2.5 g of $(NH_4)_2SO_4$; vortex for 20 s; centrifuge 5 – 25 mins at 3000 rpm.

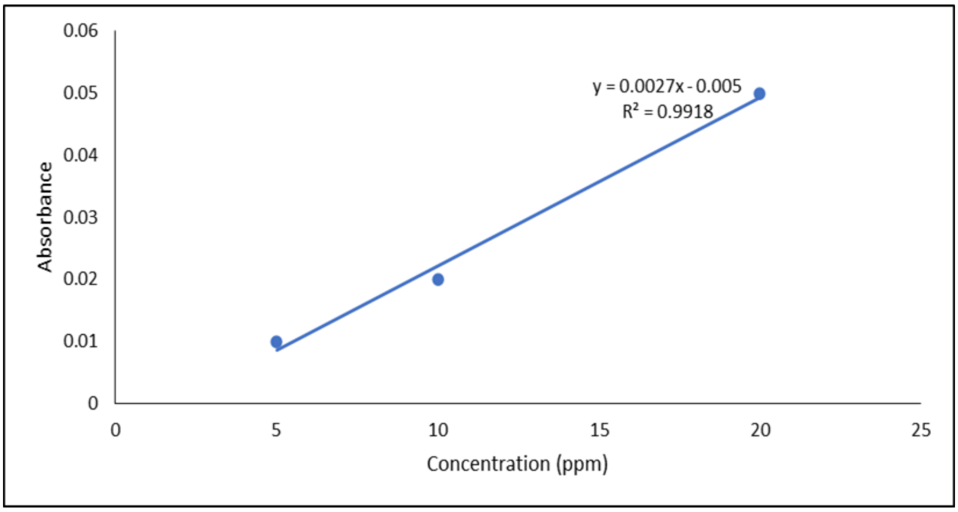

Figure 7. Calibration plots of Orange I over the range of 5 – 20 ppm.

the linear regression method was used. The LOD and LOQ are 0.019 ppm and 0.057 ppm, respectively.

The relative standard deviations (RSDs) of the analysis were evaluated by repeatability of sample. For intra-day (n=3) and inter-day (n=9), the RSD are 2.54% and 8.45%, respectively. Both RSD shows percentage less than 15.

The new SALLE technique together with UV-Vis was applied for the analysis of Orange I in orange juice (n=1) that was bought from local mart in Pulau Pinang. The percent of recovery for the real sample was 85.19%.

CONCLUSION

The relevance of the studies in salting-out method was able to extract Orange I dye from sample into small volume of organic solvent. The new sample preparation method based on SALLE method as sample preparation for the determination of Orange I in orange juice using UV-Visible Spectrophotometer was developed and applied. The advantages of this method were faster, simpler and less amount of solvent to be used as compared to liquid-liquid extraction. The results from validation indicate the proposed method can be used for the determination of Orange I in drinks sample.

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