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RESEARCH ARTICLE

Application of central composite design and desirability function in the optimization of spectrophotometric method for quantification of fast green FCF using a green microextraction method

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ABSTRACT

The aim of this article is to develop a "switchable hydrophilicity solvent liquid phase microextraction" (SHS-LPME) for the effectual extraction of fast green FCF. Three "switchable hydrophilicity solvents" (SHSs) were practiced for the extraction of fast green FCF. The attained extract phase afterward phase separation was evaluated by UV-VIS spectrophotometry. The extraction parameters such as, SHS volume, HNO3 volume and NaOH volume, were enhanced using central composite design and desirability function. Under optimized conditions, the linear range 0.50-5.00 μg/ mL with R2 = 0.9886, limit of detection 0.341 μg/ mL, limit of quantitation 1.026 μg/ mL. The method showed a relative standard deviation (RSD) of 1.16% for 7 replicate measurements. Preconcentration and Enrichment factors were determined to be 20 and 35 respectively, indicating the method's efficiency in enhancing fast green detection. The proposed method was applied in real samples successfully.

Keywords: fast green (FCF); central composite design CCD); desirability functions witchable hydrophilicity solvent (SHS); liquid-liquid microextraction (LLME)

Abbreviations: SHS-LPME: switchable hydrophilicity solvent liquid phase microextraction, FG: Fast green FCF and df: degree of freedom

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1. Introduction

Food additives are frequently used to enhance the appearance of processed foods,, taste, flavor, color, preservation and nutritive value. Color is a fundamental aspect of food quality that is related to market acceptance, food colorants are chemical substances that are included into food matrices, to maintain the sensory characteristics of the food product, which may be lost during storage or processing, and to maintain the desired color appearance [1-3], dyes that can be frequently and commercially used in the food industries as a food colorants [4-10]. Food dyes are labeled into four groups: inorganic dyes, natural organic dyes, synthetic organic dyes, and caramel I through IV. Because of their higher stability and cheaper manufacturing costs, synthetic dyes are the most often used type in the food industry [11]. However, numerous studies indicate a direct correlation between synthetic dyes and a number of pathological disorders, including food allergies, cancer, and respiratory and gastrointestinal problems [12-17]. Furthermore, behavioral alterations in children are discussed, children with autism spectrum disorder (ASD) being among the most affected by these challenges [18] many types of these dyes are still in use today because of their low price, great stability, and high efficacy. Therefore, determining food dyes is crucial for control the amount of use permitted and quality control.

Fast green FCF (FG) is one of synthetic dye is extensively used in the coating of beverages, candy, ice cream, and skin care products [19]. FG's toxicity and allergic reactions have been published, Additionally, it could irritate the skin and eyes [20, 21], because of these concerns the monitoring of its content is essential. Several analytical methods have been used for this purpose, such as chromatographic (high performance liquid chromatography and thin layer chromatography [22, 23], spectrofluorimetric [24], Electrochemical [25] and spectrophotometric [26].

However, these methods often involve skilled operators and expensive equipment, making them less available to many laboratories. Also, because of the low levels of fast green in samples, the analysis using these instruments is often insufficient, needing an further step to enrich the sample. A variety of microextraction techniques, including magnetic solid-phase extraction (MSPE) [27, 28], liquid-liquid extraction (LLE), [29] and solid-phase extraction (SPE) [30]., have been reported for dye analysis. In recent years, there has been an increasing efforts on the employment of switchable hydrophilicity solvents (SHSs) as a novel green solvents in liquid-liquid microextraction methods. A switchable solvent is a solvent proficient of reversing its assets between a non-ionic liquid to an ionic liquid which is highly polar and viscous. These solvent reduce the need to add and remove numerous solvents because they are capable of switching properties and dissolving both the organic and inorganic components SHS-LLME offers several advantages over these conventional methods mentioned above... Unlike MSPE and SPE, SHS-LLME does not require solid sorbents, making it cost-effective and minimizing sample handling. Moreover, compared to LLE, SHS-LLME significantly reduces solvent consumption, aligns with green analytical chemistry principles, and enables easy phase separation by manipulating solvent polarity. These advantages make SHS-LLME an optimal choice for Fast Green FCF extraction[31-39]. The "response surface methodology (RSM)" is usually used to improve such extraction processes. RSM is a influential mathematical and statistical tool that permits researchers to discover the relationships between variables and responses and investigate their interactions. Through the use of analysis of variance (ANOVA), it develops a data-driven model to guide experimental, optimization and offers a mathematical model to evaluate the impacts of independent variables [40,41]. In this study, central composite design (CCD) was employed to optimize the "switchable-hydrophilicity solvent-based liquid-liquid microextraction (SHS-LLME)" method for the extraction and preconcentration of Fast green (FCF) dye before its spectrophotometric determination. This reversal of chemical properties by SHS minimizes the cost by removing the need for multiple solvents also provides easier separation of the product and decreases the overall environmental effect of the industrial process .

2. Materials and methods

2.1. Apparatus

A Shimadzu UV-1650 double-beam UV/Vis's spectrophotometer (Shimadzu, Japan) fitted with a quartz cell with a 1 cm path length (0.5 mL capacity) was utilized to measure absorbance at 620 nm. Additionally, a centrifuge EBA-20 (Hettich, Germany) and an ultrasonic bath LUC-410 (Labtech, Korea) were used in the experiment.

2.2. Standards and materials

A Fast Green FCF standard (90.0 %) and the solvents, including ethanol (EtOH, 99.9 %), N, N-Dimethylhexylamine (DMHA, 98 %) and N, N-dimethylcyclohexylamine (DMCHA, 99 %) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Additionally, N-methyl dipropylamine (MDPA, 98%) and other chemicals used in the study were supplied by Merck.

2.3. Standard microextraction procedure

2 mL of a Fast Green dye aqueous solution (2.0 µg/ mL) were placed into a 10-milliliter conical-bottom centrifuge tube. Next, the mixture of 0.5 mL of N, N- Dimethylhexylamine (DMHA) and 0.5 mL of 7.5 mol. L-1 HNO_3 was added and the tube was manually shaken for 20 seconds until a homogenous phase was seen. the tube was placed in a 40 °C water bath for 2 minutes and 0.6 mL of a 10 mol/ L NaOH solution was added, besides 40 seconds of ultrasonication were run. A cloudy solution developed at this stage. Centrifugation at 3500 rpm for 5 min was used to separate the fine droplets. A micro syringe was used to take the upper organic layer and diluted with 3 mL of ethanol, Lastly, a visible spectrophotometer set to 620 nm was used to measure the absorbance against the blank solution prepared in the same way without analyte [22].

2.4. Preparation of real samples

Two samples of soft drinks were purchased from local supermarkets in, Iraq. The preparation of drink sample was carried out based on Tülay and Emrah examination with a slight modification. The drink samples were diluted 10 times, with ultrapure water. After sonication for 20 min in an, ultrasonic bath, it was centrifuged at 3000 rpm for 10 min, an aliquot of 1 mL of sample was put in the centrifuge tube and the described SHS-LLME method was applied.

2.5. Optimization strategy

A "central composite design"(CCD) using "response surface methodology" (RSM) was used to optimize the key factors influencing the SHS-LLME procedure. After initial trials, it was found that three parameters DMHA volume, HNO_3 volume, and NaOH volume were the independent factors affecting the extraction of Fast Green. The dye's absorbance was chosen as the response for optimization. A three-factor, three-level CCD was created to optimize these parameters using Minitab version 22.0.1 (trial) for the analysis. In the CCD design, DMHA, HNO_3 and NaOH volumes were symbolized as A, B, and C, respectively. The codes, levels, and units of the enhanced factors can be found in **Table 1.** The three independent variables DMHA volume

(A), HNO_3 volume (B), and NaOH volume (C) were experienced at three different levels to understand their impact on the extraction effectiveness of Fast Green, maximizing the absorbance response was the goal.

Table 1. Levels, units, and codes of optimized factors.

Range and level	Factor	Range and level		
		-1	0	+1
Volume (mL) (DMHA)	A	0.3	0.5	0.7
Volume (mL) (HNO_3)	B	0.3	0.5	0.7
Volume (mL) (NaOH)	C	0.1	0.5	0.9

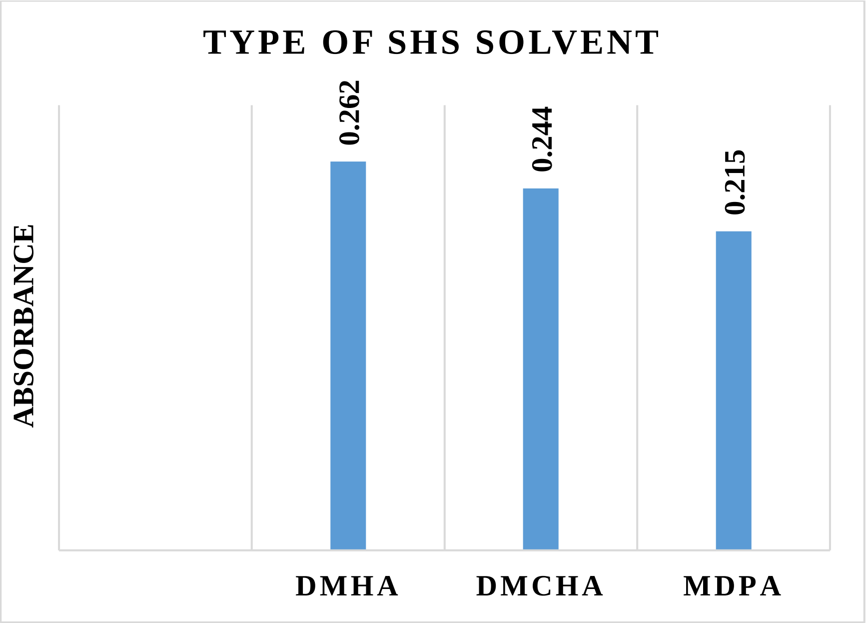
3. Results and discussion

3.1. Optimization of SHS-LLME factors

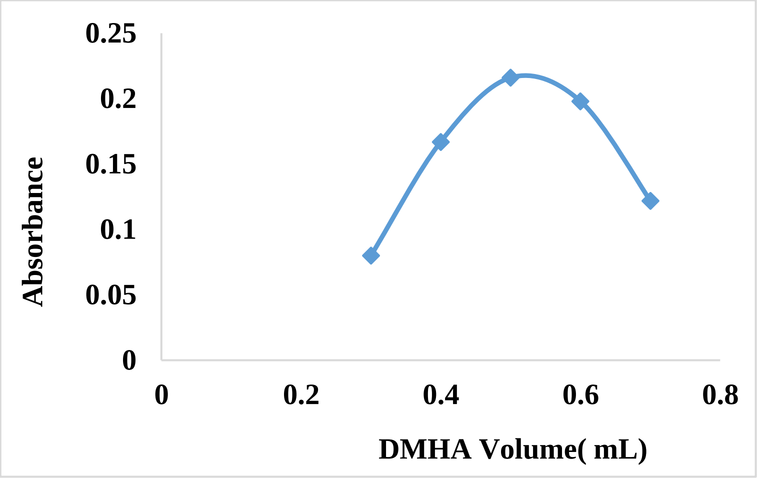
Single factor experiments were achieved to detect the key influencing factors on the extraction of fast green dye, and the central composite design (CCD) was used to examine the optimal effects of the above key influencing factors, this approach allowed it possible to systematically evaluate of each factor’s impact on the extraction efficiency, which helping to identify the optimal conditions for maximizing dye absorbance.

3.2. Optimization of type and volume of SHS

The selection of an appropriate switchable hydrophilicity solvent (SHS) is crucial that is have a strong extraction affinity for the analytes. Tertiary amines, are usually used as SHS solvents in -LLME due to their ability to make a large connection surface between the sample solution and the solvent in the experimental medium. Three different solvents were evaluated as SHS solvents then performed according to the extraction procedure above described. such as N-methyl dipropylamine (MDPA), N, N-dimethylhexylamine (DMHA) and N, N-dimethylcyclohexylamine (DMCHA). Among these, (DMHA) has been observed to yield the highest absorbance for the analyte (as shown in **Figure 1A**), likely due to its greater hydrophobicity. The volume of the SHS solvent can pointedly affect the volume of the consequential SHS phase and the recovery of the dye, thus the role of the volume of extraction solvent (0.3-0.7 mL) on the dye extraction was evaluated. As detected in **Figure 1B,** maximum recovery values were found for 0.5 mL of extraction solvent being constant up to 0.7 mL This volume allows a larger surface area for interaction between the dye and the solvent, which increases the efficiency of the dye transfer from the aqueous phase to the organic phase. Lower extraction solvent volumes (0.3 mL, corresponding to 0.1 mL of extraction solvent) produced in an impracticable phase separation, and then, unfortunate extraction recovery was detected. As a result, 0.5 mL of extractant phase was nominated for the consequent optimization experiments.



(A)



(B)

Figure 1. (A)- Effect of SHS Type and (B)- DMHA volume on absorbance of FG dye.

3.3. Optimization of acid volume

The effect of acid volume was another important factor to obtain the best extraction efficiency that can be changed between immiscible form and miscible form in the experimental environment., HNO_3 was selected as the acid, and its volume was analytically varied from 0.3 to 0.7 mL while using the extraction method (SHS-LLME)., while the volume of the DMHA was kept constant at the previously optimized level. As shown in **Figure 2,** the ideal HNO_3 volume for make the most of extraction efficiency was found to be 0.5 mLthis volume allows for sufficient conversion of the dye to its hydrophilic form, which facilitates the process of transferring the dye from the aqueous phase to the organic phase, because the larger acid volume enhances the protonation of the (SHS) molecules in a way that allows achieving the required balance between the phases to increase the extraction efficiency,

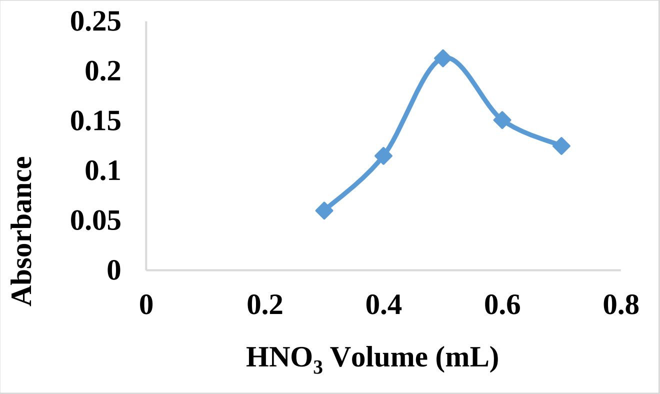


Figure 2. Effect of HNO_3 volume on absorbance of FG dye.

3.4. Optimization of NaOH volume as a switch-off trigger

NaOH assisted as a mediator for phase transitions for the extraction of FG from the polar solution to deprotonated, N, N-dimethylhexylamine (DMHA) form. A range of 10 M NaOH volumes, from 0.1 to 0.9 mL, were evaluated as revealed in **Figure 3.** It was detected that the absorbance was increased as the volume of 10M NaOH increased from 0.2 to 0.6 mL and stayed constant. Therefore, 0.6 mL was resolved to be the optimal volume for additional research. This volume of (NaOH) provides a basic medium capable of reversing the protonation process that occurred previously when using (HCl) and thus enhances the re-separation of the organic phase from the aqueous phase

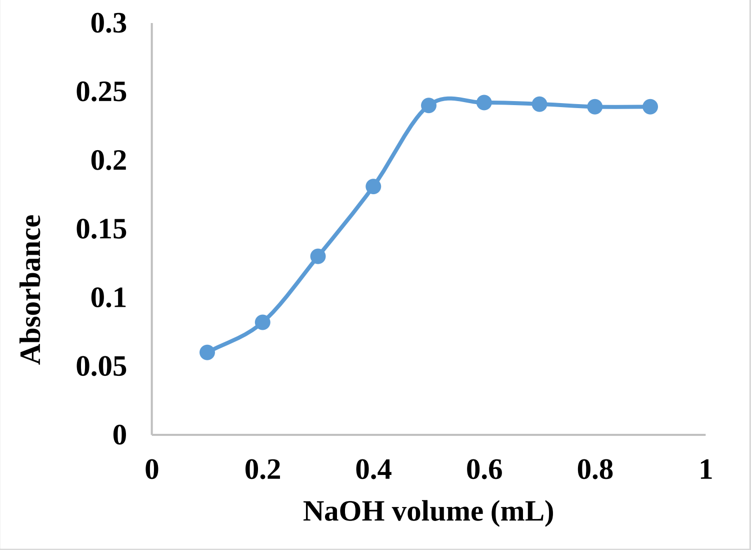


Figure 3. Effect of NaOH Volume on absorbance of FG dye.

3.5. Experimental design

In previous experiments, a One-Factor-At-Time (OFAT) approach was used to define the factor ranges for subsequent application of Response Surface Methodology (RSM). To determine the optimal conditions for the SHS-LPME method, a three-factor, three -level CCD was conducted The design included 8 factorial points, 6 axial points, and 6 center point replicates, for a total of 20 experiments (N) required for the CCD for three factors at three levels, this was achieved by adding 2k (for the factorial points, with k = 3, so 23 = 8) and 2k (giving 6 axial points) to the central point. The experimental design used for optimization and the conforming responses for the 20 experiments are offered in **Table 2.**

Table 2. The design matrix and the central composite design (CCD) responses.

Run order	Blocks	A	B	C	abs
1	1	0.500000	0.500000	0.50000	0.323
2	1	0.500000	0.500000	0.50000	0.321
3	1	0.300000	0.300000	0.90000	0.146
4	1	0.163641	0.500000	0.50000	0.219
5	1	0.500000	0.500000	1.17272	0.303
6	1	0.700000	0.300000	0.90000	0.193
7	1	0.836359	0.500000	0.50000	0.298
8	1	0.500000	0.500000	-0.17272	0.197
9	1	0.500000	0.500000	0.50000	0.313
10	1	0.500000	0.163641	0.50000	0.138
11	1	0.500000	0.500000	0.50000	0.326
12	1	0.300000	0.700000	0.10000	0.138
13	1	0.500000	0.836359	0.50000	0.244
14	1	0.700000	0.700000	0.10000	0.197
15	1	0.700000	0.300000	0.10000	0.112
16	1	0.500000	0.500000	0.50000	0.313
17	1	0.300000	0.300000	0.10000	0.110
18	1	0.300000	0.700000	0.90000	0.201
19	1	0.700000	0.700000	0.90000	0.285
20	1	0.500000	0.500000	0.50000	0.325

Quadratic regression modeling was used to the responses that corresponded to the coded values of the three different variables. The predicted absorbance (abs) for the fast green FCF was determined using the following equation:

$$abs=0.369 +0.753A +1.420 B+0.228 C- 0.836 A^{2} – 1.433 B^{2} -0.2278 C^{2} +0.294 A*B+0.109 A*C +0.053 B*C…. (1)$$

An ANOVA test was applied to the obtained experimental results. The results in **Table 3** showed that the model's p values were 0.000 (<0.05), confirming the significance of the model. equation and related terms were deemed meaningless if p-values were greater than 0.05 (p-value at 95 % confidence level), meaning that all binary interactions between variables were meaningless, while all linear variables and square variables were meaningful. Since the linear and squares of the linear variables were significant, they ought to be included in the experimental design; however, meaningless binary interactions were removed from the model.

Table 3." Analysis of variance (ANOVA) for the central composite design (CCD)" data.

Source	Sum of Squares	df	Mean Square	F Value	P-value
Model	0.108474	9	0.012053	12.12	0.000 significant
A-	0.772×10^{-3}	1	0.772×10^{-3}	7.77	0.019
B-	0.1406×10^{-3}	1	0.1406×10^{-3}	14.14	0.004
C-	0.145×10^{-3}	1	0.145×10^{-3}	14.66	0.003

AB	$0. 110 \times 10^{-4}$	1	$0. 110 \times 10^{-4}$	1.11	0.317
AC	$0. 61\times 10^{-5}$	1	$0. 61\times 10^{-5}$	0.62	0.451
BC	$0. 14 \times 10^{-5}$	1	$0. 14\times 10^{-5}$	0.15	0.711
A^2	0.161×10^{-3}	1	0.161×10^{-3}	16.20	0.002
B^2	0.473×10^{-3}	1	0.473×10^{-3}	47.58	0.000
C^2	0.191×10^{-3}	1	0.191×10^{-3}	19.24	0.001
Residual	0.99×10^{-2}	10			
Lack of Fit	0.97×10^{-2}	5	0.195×10^{-2}	57.91	0.000
Pure Error	0.16×10^{-3}	5	0.034×10^{-3}		
Total	0.118420	19			

The model’s determination coefficient (R^2) revealed that the independent variables explain 93.21 % of the variation in dye absorbance,The high value of the adjusted determination coefficient (91.60 %) further provisions the reliability and significance of the established model.Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots were generated to visualize the effects of interactions between variables on dye absorbance. **Figure 4**: Depicts the effect of DMHA and NaOH volumes on dye absorbance while keeping HNO_3 constant. **Figure 5**: Illustrates the impact of HNO_3 and DMHA volumes on absorbance with a fixed NaOH level. **Figure 6**: Shows the relationship between HNO_3 and NaOH with DMHA volume held at its center level. The results clearly indicate that absorbance depends on all the experimental variables studied. Additionally, the plots highlight significant interactions between variables.

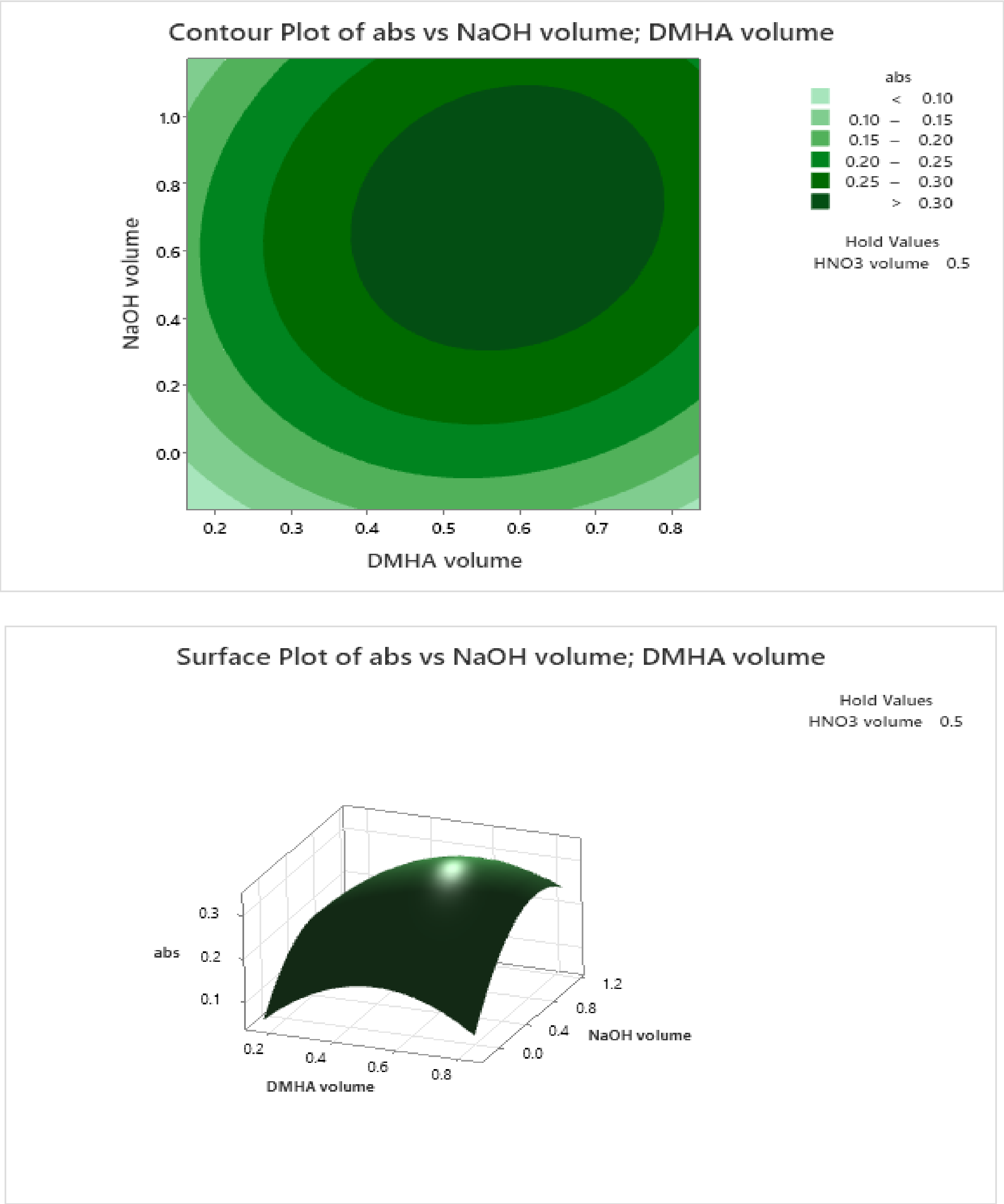


Figure 4. Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots showing the effect of DMHAvolume and NaOH volume on dye absorbance, with HNO_3volume held constant at the center level.

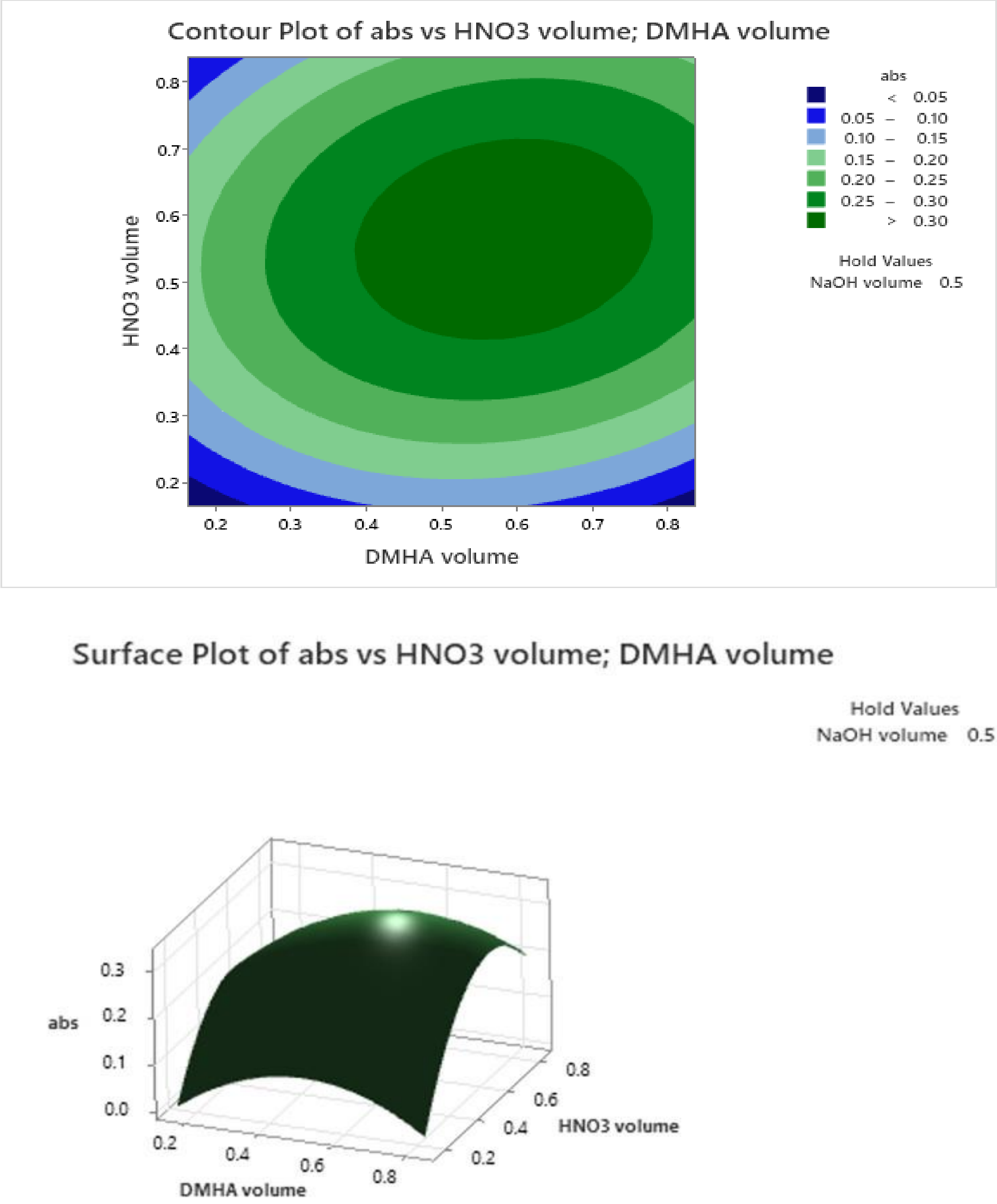


Figure 5. Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots showing the effect of HNO_3 volume and DMHA volume on dye absorbance, with NaOH volume held constant at the center level.

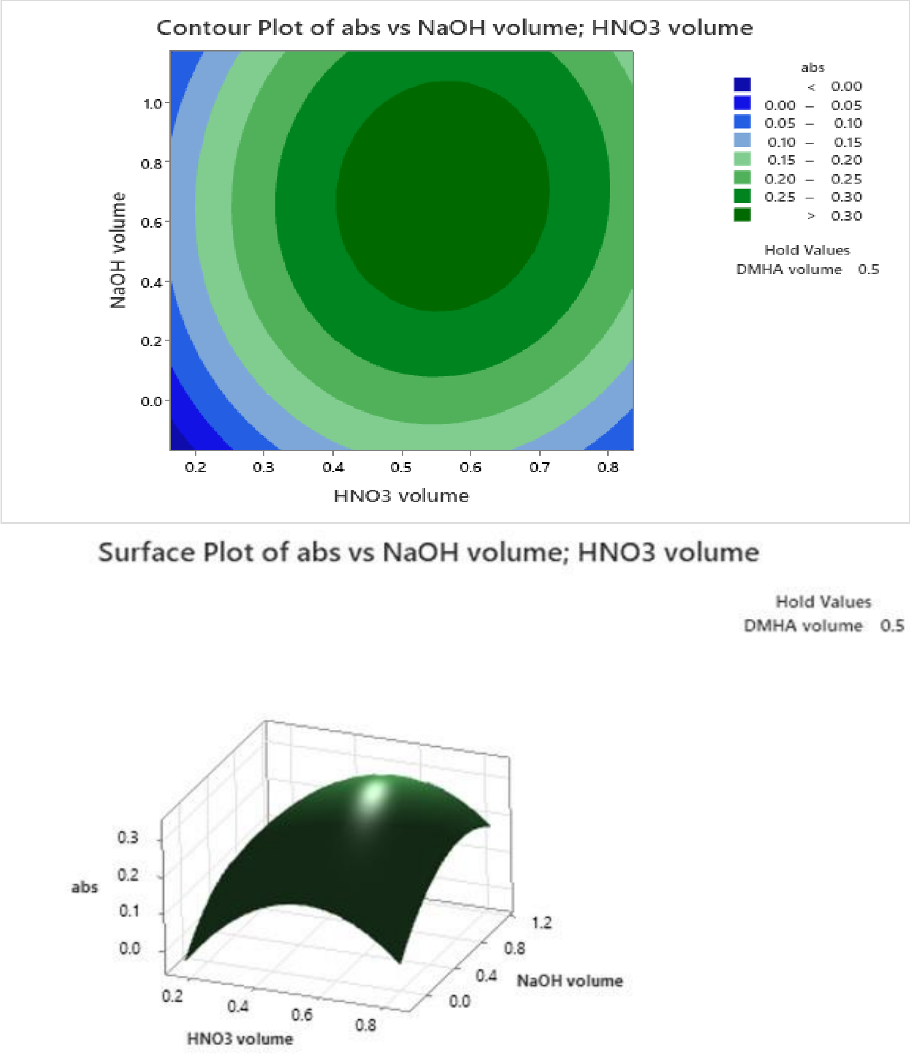


Figure 6. Three-dimensional (3D) surface plots and two-dimensional (2D) contour plots showing the effect of HNO_3volume and NaOH volume on dye absorbance, with DMHA volume held constant at the center level.

3.6. Desirability function

The performance of one or more responses is determined by the desirability function. In this procedure, each predicted response is altered to an individual desirability function (di) by the fitted model. For a fully undesirable response the value of di approaches to zero whereas di = 1 for a totally desirable response. **Figure 7** Presents the desirability function used to determine optimal extraction conditions

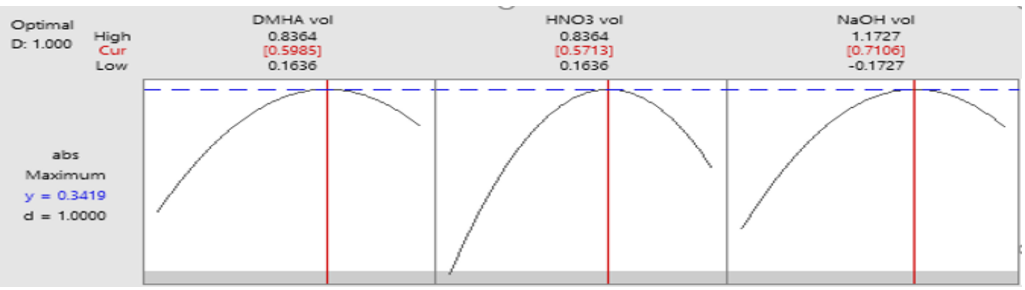


Figure 7. Desirability function (di).

The optimal condition, achieved by maximizing the equation 1, is 0.5985 mL of DMHA, 0.5713 mL of HNO_3 and 0.7106 mL of NaOH, it can be clearly seen that the global optimum condition is different from local optimum point obtained from OFAT. It is so because of considering interaction and square effects of the factors in the experimental design method in spite of OFAT.

3.7. Analytical performance of the enhanced technique

The method's analytical performance was evaluated by constructed the calibration graphs for FG dye at the established optimized conditions using combined (SHS-LLME) –Spectrophotometry by taken a series of standard FG solutions. The calibration graphs (**Figure 8**) were linear over the concentration range of 0.5–5 μg/mL, as indicated by the equation y = 0.128 x+0.0012, with a high correlation coefficient ($R^2 = 0.9886$). It was determined that the limit of detection (LOD) and limit of quantification (LOQ) to be 0.341 μg/ mL and 1.026 μg/ mL, respectively. These values were calculated using the formulas" LOD = $3.3Sb/m$ and LOQ = $10Sb/m$", where m is the calibration curve's slope and Sb is the standard deviation of seven replicate measurements of the blank solution. The relative standard deviation (RSD) for seven replicate measurements of a 1.0 μg/ mL solution was 1.16 %, indicating high precision. the enrichment factor which is the ratio of the calibration curve's slope with and without the SHS-LLME was found to be 35. The ratio of the sample volume 10 mL to the final volume 0.5 mL was used to calculate the preconcentration factor for the method and the result was 20.

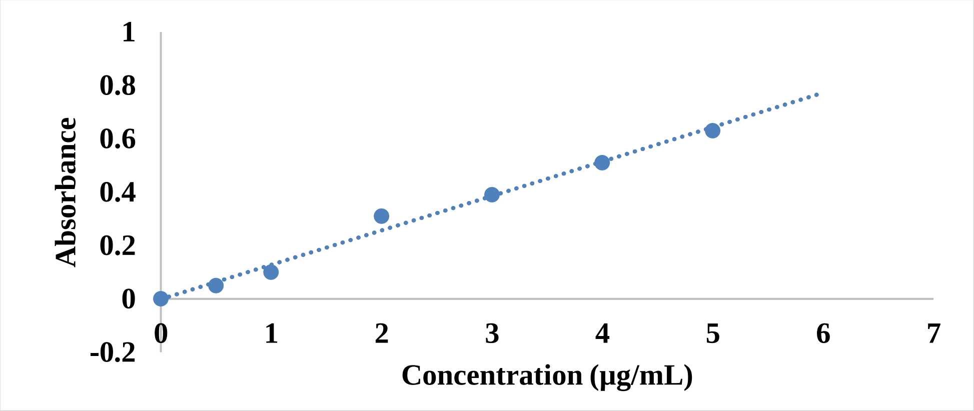


Figure 8. Calibration curve of FG dye after application (SHS-LLME) method.

3.8. Method selectivity

The selectivity of the suggested method was tested against some acids (ascorbic acid, citric acid and sucrose) which are usually confined in various drinks in large quantities and may effect on the extraction of dye. It was found that even at a 1000-fold in the system the percent relative error for ascorbic acid, citric acid and sucrose was 2.35 % ,6.18 %, 3.88 % respectively, indicating no serious effects on the separation and analyze of dye using (SHS-LLME) method [29].

3.9. Accuracy of the method and analysis of real samples

To investigate the accuracy and reliability of the described method, the analyses of the recovery experiments together with real samples were carried out. For this determination, dyes of known concentrations were additional to the real samples and analyzed after applying (SHS-LLME) method. As seen in **Table 4,** the recovery values of the dye in the samples were found to range from 93.0 % to 96.0 %.

Table 4. Accuracy of extraction method for determination of FG dye.

Sample. no	Added (μg. mL^{-1})	Found (μ g.mL^{-1})	Recovery %*
	0	0.412±0.831	-
1	1	0.16± 1.77	**94**
	2	0.6± 2.69	**93**
	0	0.1± 1.13	-
2	1	0.4±2.09	**96**
	2	0.1±2.99	**93**

** Calculated as* $(c_{found} - c^{0}_{found}) / c_{added} \times 100$ *%, where* c^{0}_{found} *and* c_{found} *are the found dye concentrations without and with an additive, respectively;* c_{added} *is the additional concentration.*

4. Conclusion

Fast green FCF was successfully separated and enriched using a switchable hydrophilicity solvent liquid-liquid microextraction (SHS-LLME) method that combined UV-Vis spectrophotometry. Several parameters were systematically examined, including the type and volume of the switchable hydrophilicity solvent (SHS), HNO_3 volume and NaOH volume. A Central Composite Design (CCD) was then used to optimize the most significant factors Response Surface Methodology (RSM) was used to analyze the CCD data in order to precisely identify the best extraction parameters for Fast green separation. RSM showed that in order to determine the exact relationship between extraction efficiency and this enrichment step careful experimental design is needed.

Author contributions

Authors contributed equally to the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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