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## Application of Experimental Design for Determination of Methanol and Ethanol in Transformer Industrial Oils Using Headspace Single-Drop Microextraction

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A simple, inexpensive, nearly solvent-free headspace single-drop microextraction (HS-SDME) followed by gas chromatography and flame ionization detection (GC-FID) was developed for determining methanol and ethanol in samples of transformer insulation oils. Five effective parameters in HS-SDME process, including extraction solvent volume, sample content, extraction time, extraction temperature, and agitation speed were screened and subsequently optimized using fractional factorial screening methodology (FFSM) and response surface methodology using Box-Behnken design (BBD). The extraction was carried out with dimethyl sulfoxide (DMSO) as micro drop, with 1  $\mu\text{l}$  of extraction phase injected into the GC-FID system. The calibration curve for the analytes was found to be linear within the range of 0.05-10.00  $\mu\text{g g}^{-1}$ , and the limits of detection for methanol and ethanol were 0.02  $\mu\text{g g}^{-1}$  and 0.03  $\mu\text{g g}^{-1}$ , respectively. The relative standard deviation obtained within 1.8-5.2% and a good recovery achieved within the range of 95.0-102.6% in the analysis of oil samples revealed that methanol and ethanol could be determined as a chemical marker of the transformer life using this method.

**Keywords:** Ethanol, Experimental design, Headspace single-drop microextraction, Methanol, Transformer oil

### INTRODUCTION

Transformers are the most expensive power grid equipment playing a very important role in transmission systems and distribution of electrical energy. Thus, any action to increase their useful life is a significant cost saving; hence, their quality control after production and monitoring their status during operation is of great importance. The life span of electrical equipment depends directly on the quality of their insulation. For over a century, cellulose, a natural polymer with mineral oil, has been used as a transformer insulation system [1]. The lifetime of transformers is evaluated by the solid insulation health checking [2], where the remaining life of solid insulation and subsequently the life of the transformer are

determined by the amount of insulation paper degradation [3]. In recent decades, methanol has been used as a marker for evaluating solid insulation situation in transformers [4]. As chemical markers of transformer diagnosis, methanol and ethanol are known as the third generation. In general, these alcohols in transformer oil are determined by headspace gas chromatography (HS-GC) with detectors such as mass spectrometry (MS) or flame ionization (FID) [5].

Due to the extremely low concentration of methanol and ethanol as well as matrix effects in the transformer oil samples, a separation-pre concentration step is usually required before analyzing the trace amounts of analytes [6]. HS-SDME is a rapid and cost-effective extraction technique where long-term stability of the droplet is possible during rapid stirring of the sample solution and better sensitivity is achievable due to reduced non-volatile matrix interferences

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[7]. In this method, a micro-syringe needle is held down into the septum of a vial and a hanging droplet of the extraction solvent is brought into contact with the headspace of the sample vial. The drop is retracted after a defined extraction time for analyte enrichment and injected into the GC injector for measurement [8].

However, the success of HS-SDME process is highly dependent on the selection of experimental conditions leading to a maximum analyte extraction [9]. The factors affecting the extraction process, including extraction solvent volume, volume of sample, time of extraction, extraction temperature, and stirring rate should be optimized.

The procedures for optimization are mostly based on one variable in time approaches, which facilitate the experimental results in interpretation, but the interplay between parameters is barely taken into consideration. As a result, a probable incorrect maximal leads to special conditions in which the composition of the variable does not produce the maximum analytical signal [10].

In the optimization process, design of experiments (DOE) is the prevalent approach to recognize and control the most effective parameters. In DOE, where statistical equations are used, major factors are identified and uncontrollable parameters are reduced. The DOE developed by Fisher and Yates in 1920, for the first time, was a DOE with controlled conditions for studying the effect of different variables on the response. The DOE approach seeks different purposes including identifying effective factors, estimating the effect of parameters on the defined response, identifying interactions of factors, as well as optimizing and modeling to define the mathematical relationship between the parameters and response. Considering that the parameters can also affect each other and that the best value for one parameter could be related to the other variables, DOE involves changing all parameters from one test to the next [11].

Application of statistical methods and particularly DOE has led to a considerable decrement in the number of required tests in an evaluation, thereby saving time and cost. As extraction methods are affected by the multiple parameters, utilization of experimental design leads to high efficiency [12].

Factorial design, as a useful method to investigate the main and interaction effects of the variables, is used to

evaluate the effects of two or more parameters on the operation outcome. As effective factors increase, the required number of experiments for full factorial design is increased. In this case, fractional factorial design (FFD) is selected for running only a fraction of the full factorial tests [13]. In this design, the number of experiments is calculated from the equation by  $Lk-p$ , where  $L$  is the parameter level,  $k$  denotes the factors number, and  $p$  defines the amount of the fraction of the full factorial used.

Recently, a combination of this form of screening, optimization, and extraction has found more applications in analytical procedures. For example, choosing variable before optimization for the dispersive liquid-liquid microextraction of melatonin from plasma samples has been used using the fractional factorial design [14]. The other example is examination of the maximum extraction of fragrance allergens from water samples [15]. Elsewhere, the same procedure has been reported for acquiring the optimum conditions for the heavy metal removal from environmental water samples [16,17].

In this work, for the first time, we applied HS-SDME method for extracting and determining methanol and ethanol as markers for degradation of insulation paper in transformer insulation oil using GC-FID available in Sarkhoon and Qeshm Gas Refining Company. The fractional factorial experimental design was performed for optimization of the variables affecting the procedure, including the volume of extraction solvent, sample volume, extraction time, agitation speed, and extraction temperature.

## EXPRIMENTAL

### Reagents and Solutions

All solvents and reactants, including methanol, ethanol, 2-propanol, dimethyl sulfoxide (DMSO) dioxane, chlorobenzene, ethylene glycol, diethanolamine, dimethylformamide, and glycerin, were obtained from Merck Company in Germany. Furthermore, the solvent used for all solutions was a new and unused transformer oil of Sarkhoon & Qeshm Gas Refinery.

### Instrumentation

For analyzing the volatile compounds extracted using a headspace-single drop microextraction technique, a gas

chromatographer model GC 3400 Varian equipped with a flame ionization detector (FID) was used. A capillary column (5.0 $\mu$ m CP- Sil Pona CB 50m 0.21 mm) was applied for chromatography separation which is appropriate for analyzing polar compounds in trace amount. A 5 $\mu$ l SGE syringe and vial, crimper, magnet, heater-stirrer and Helium carrier gas were employed to develop a headspace method.

### Preparation of Standard Solutions and Samples

Standard working solutions with ethanol, methanol, and 2-propanol were prepared daily through continuous diluting of stock ethanol, methanol and 2-propanol solution (1000 ppm) with a new transformer oil solvent. Once prepared, the stock solution should be kept at +4 °C so that the concentration of the analytes would not change. Glass containers were washed with n-hexane and acetone after usage. Due to the viscosity of the transformer oil sample, all the solutions were prepared by the weight-weight method. 2-propanol has been selected as an internal standard for measuring methanol and ethanol in oils, which has acceptable stability in oils and is not produced during the life of the paper or oil. It can adequately be separated from analyte compounds under chromatographic conditions [4].

### HS-SDME Procedure

Here, 10 ml of oil and stock solution was added to the vial with an appropriate volume; after inserting the magnet, it was sealed with a septum, and then placed in a paraffin bath for 20 min on a hotplate at 110 °C. At the top of the vial cap, a 5  $\mu$ l micro-syringe was placed. After 20 min of heating in the paraffin bath, the microsyringe needle was pushed into the headspace and microextraction was done with 2.5  $\mu$ l of extraction solvent (DMSO) which was suspended in the headspace until equilibrium was achieved and extraction was done for 5 min (Fig. 1).

The extraction solvent was then pulled up by the micro-syringe, and once the micro-syringe was removed from the vial, 1  $\mu$ l of residual extraction solvent was injected into the gas chromatography (Fig. 2). Table 1 reports the optimum instrumental conditions for gas chromatography analysis.

### Experimental Design

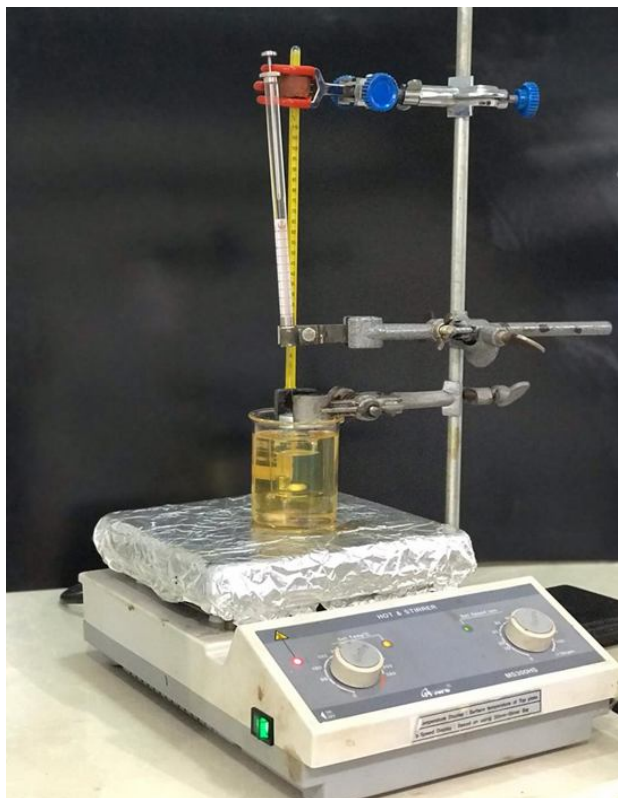
As mentioned, optimization through experimental design was carried out to investigate the factors affecting

the extraction process. MiniTab 16 software was used to create, analyze, and visualize the experiments designed in this study.

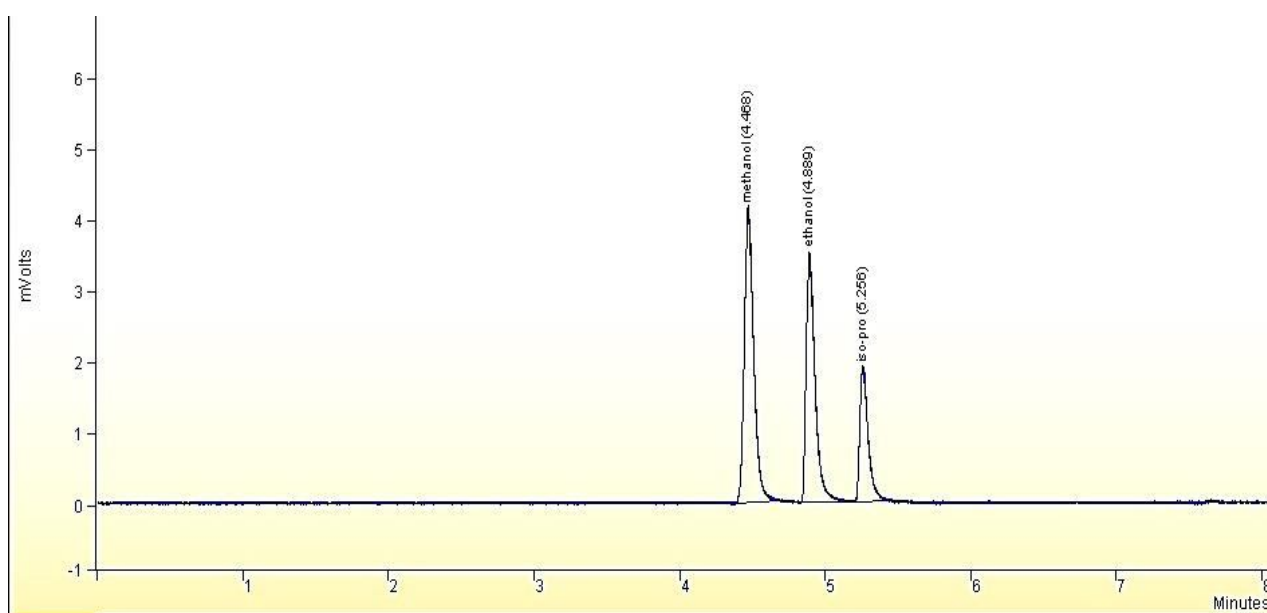
In order to achieve optimum conditions for the procedure, a fractional factorial DOE was chosen to investigate the primary importance of the parameters and their interactions. Extraction solvent volume, volume of sample, extraction temperature, extraction time, and agitation speed were the variables investigated. The variables were studied at two levels, low (-1) and high (+1). In the design, a three replicate center point (level 0) was included until the experimental variance was estimated and the linearity loss between the levels selected for each factor was checked [10]. Statistical analysis fitted a second-order polynomial regression model including the coefficient of linear, quadratic and interaction terms. Table 2 summarizes the levels for the experimental design. The analyte concentration was kept constant at 10 ppm during the experiments. Using Box-Behnken design, which is a response surface methodology based on a highly fractionalized three-level factorial design, and Pareto chart, which is obtained after multiple linear regression and analysis of variance, the significant variables were optimized. Correlation between the variables and the response was shown by the quadratic equation, and through this mathematical relationship, the critical points of this equation could be estimated. The necessary number of experiments, which is a very important factor in obtaining this second-order equation, is dependent to the parameters number, as stated by:  $n = 2k(k - 1) + C$ , where n is the number of tests, k is the parameters number, and C denotes the replicates number at the center point [10].

## RESULTS AND DISCUSSION

In this study, HS-SDME combined with GC-FID was developed for methanol and ethanol determination in transformer insulation oil samples. The parameters affecting the procedure such as the volume of extraction solvent, sample volume, extraction temperature, extraction time and agitation speed were examined, and optimal conditions were selected. The DOE approach was chosen to find the most significant factors, including extraction solvent volume plus sample volume extraction temperature. The peak area of



**Fig. 1.** HS-SDME sample preparation design for extraction.



**Fig. 2.** GC-FID chromatogram of analytes and internal standard after extraction of HS-SDME extraction conditions: concentration: 10 ppm, droplet volume: 2.5  $\mu$ l, sample volume: 10 ml.

**Table 1.** Optimized Instrumental Conditions for GC/FID

Condition	
Injection Temperature: 250 °C	$T_{in}$ : 40 °C for 3min
Split less mode	12 °C min <sup>-1</sup> to 300 °C
Detector temperature: 300 °C	300 °C hold for 5 min
Flow: 2 ml min <sup>-1</sup>	

**Table 2.** Variables and Levels Investigated Using the Fractional Factorial Experimental Design

Variables	-1	0	+1
Extraction solvent volume (μl)	1.5	2	2.5
Sample volume (ml)	5	7.5	10
Extraction time (min)	5	7.5	10
Extraction temperature (°C)	75	92.5	110
Agitation speed (rpm)	200	300	400

chromatographic analysis was used to evaluate the efficiency of extraction in experimental conditions.

### Extraction Solvent Selection

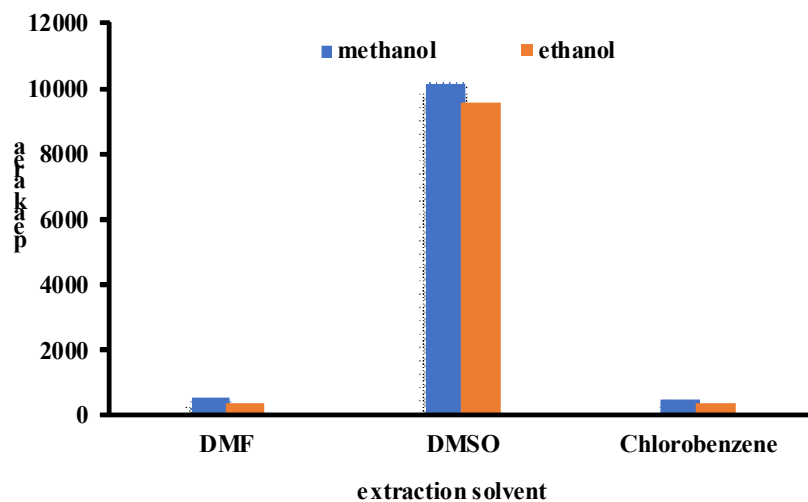
Choosing an appropriate solvent for HS-SDME method is very important as it affects the extraction efficiency. The extraction solvent must be able to meet the following conditions. Since the boiling temperature of analytes and internal standard of methanol is 64.7, ethanol is 78.3 and 2-propanol is 82.3, from among applicable and available solvents, solvents whose boiling temperature is higher than 100 °C are considered since droplet not being decomposed due to evaporation is important. Also, the amount of adsorbed analytes decreases as the solvent volume evaporates. Since it is very important to keep the extraction solvent suspended in the syringe needle, the selective solvent must be able to maintain its stability during

extraction.

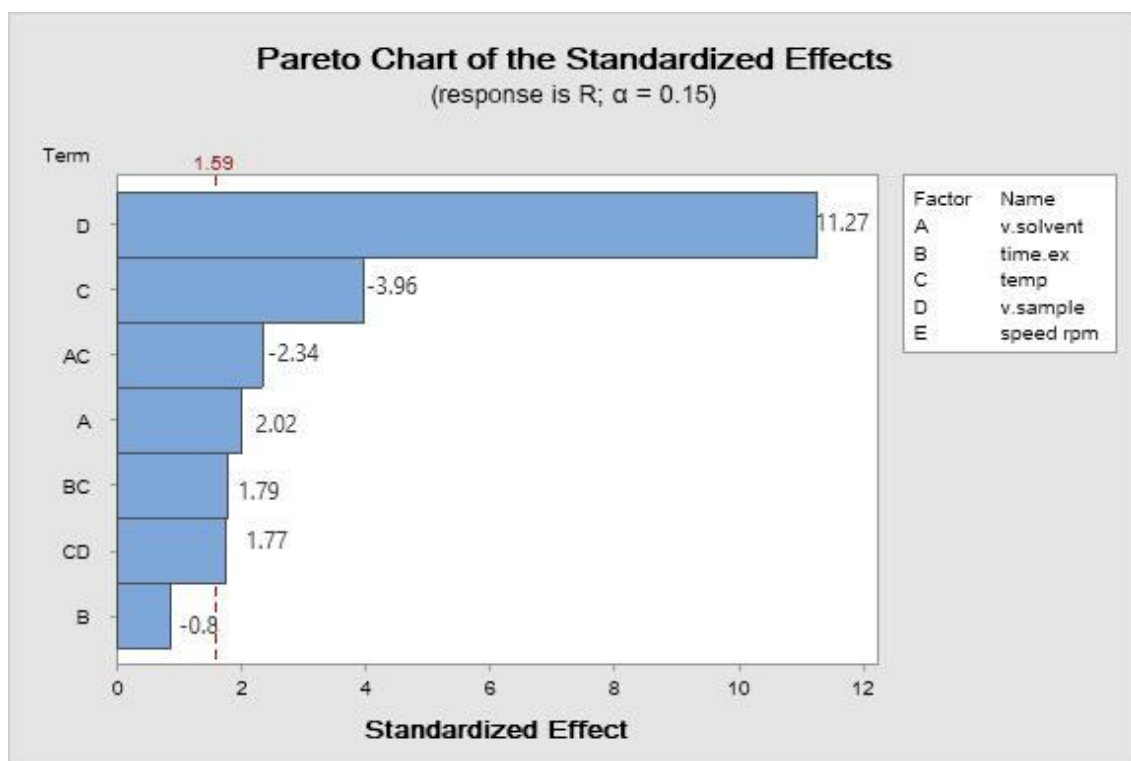
According to the limitations of solvent selection for this study included the formation of a stable droplet as well as the proper behavior of chromatography, in other words, the overlap in the chromatogram spectrum, dimethyl sulfoxide, chlorobenzene and dimethylformamide were investigated. According to the results obtained (Fig. 3), among these solvents, dimethyl sulfoxide was selected as the extraction solvent in the preliminary experiments.

### Design for Screening

In order to reduce the number of tests and to prevent loss of important data, the fractional factorial DOE was selected for screening the parameters. The peak areas obtained for methanol and ethanol as the responses for each test were calculated. The levels selected for effective factors



**Fig. 3.** Comparative graph of tested solvents after extraction of HS-SDME conditions: concentration: 10 ppm, temperature: 100 °C, droplet volume: 2.5  $\mu$ l, sample volume: 10 ml.



**Fig. 4.** Pareto chart obtained for the fractional factorial design for screening of the variables: (A) solvent volume, (B) time extraction, (C) temperature, (D) sample volume and their interactions.

were considered accordance between sample throughput and maximum evaporation of the solvent drop. By drawing Pareto and normal plots as well as residual plot, the data and model adequacy were analyzed. For data evaluation, analysis of variance (ANOVA) test was used, where significant effects at 5% level were explored [10]. A Pareto chart is shown in Fig. 4, in which the results have been visualized using bar charts. *Via* a linear regression and curvature check, the mathematical correlation between analytical response and the variables was performed. An increase in the response was found with the positive value for the estimated effect if the variable increases to its high level and a negative value indicates that a better response is obtained at low levels of the variable. A positive value for the interactions indicates that the response will increase if both variables change to the same level, low or high. The change of variables means that one variable drops to a low level and the other grows to a high level leading to a negative value, indicating an increase in the response [10]. According to these results, as can be shown in Fig. 4, the variables of sample volume and extraction temperature, and solvent volume were chosen for further study *via* Box-Behnken design (BBD) as a response surface method.

### Design for optimization

Optimizing the values of significant variables is the second step in achieving the best response. To explore the region of interest for the factors identified by the former part of the study, response surface method was applied, followed by the factorial experimental design. The three significant factors obtained from the screening test were evaluated about broader effects using Box-Behnken design. Figure 5 shows that three response surfaces were achieved from the outcome of the BBD. The quadratic equation correlating the three parameters and the analytical signal is:

$$R = 203245 - 11018(D) - 2568(C) - 54949(A) + 562.4(D^2) + 12.42(C^2) + 14072(A^2) + 51.42(D*C)$$

where A is the solvent volume ( $\mu\text{l}$ ), C is the temperature ( $^{\circ}\text{C}$ ), and D is the sample volume (ml).

In this step, we analyze the data and the adequacy of the model. Then, by drawing contour plots and surface plots, the relationship between responses and variables as well as factors is graphically shown. First, by drawing surface plots,

the effect of two other factors on the relation of responses can be checked by keeping one factor constant (Fig. 5).

Furthermore, by drawing the contour plot, it is possible to show the simultaneous investigation of the factors concerning the answers; as can be seen in Fig. 6, the contour plots, in fact, are from the top of the surface plot. After modeling and testing the accuracy and adequacy of the model as well as checking the plots, the optimization was done to find the final optimal point to achieve the maximum values. The optimal points were identified according to the optimization plots. The final optimal point for measuring the maximum amount of methanol and ethanol in the transformer oil sample was when the sample volume was 10 ml, droplet volume was 2.5  $\mu\text{l}$ , and extraction temperature was 110  $^{\circ}\text{C}$  (Fig. 7).

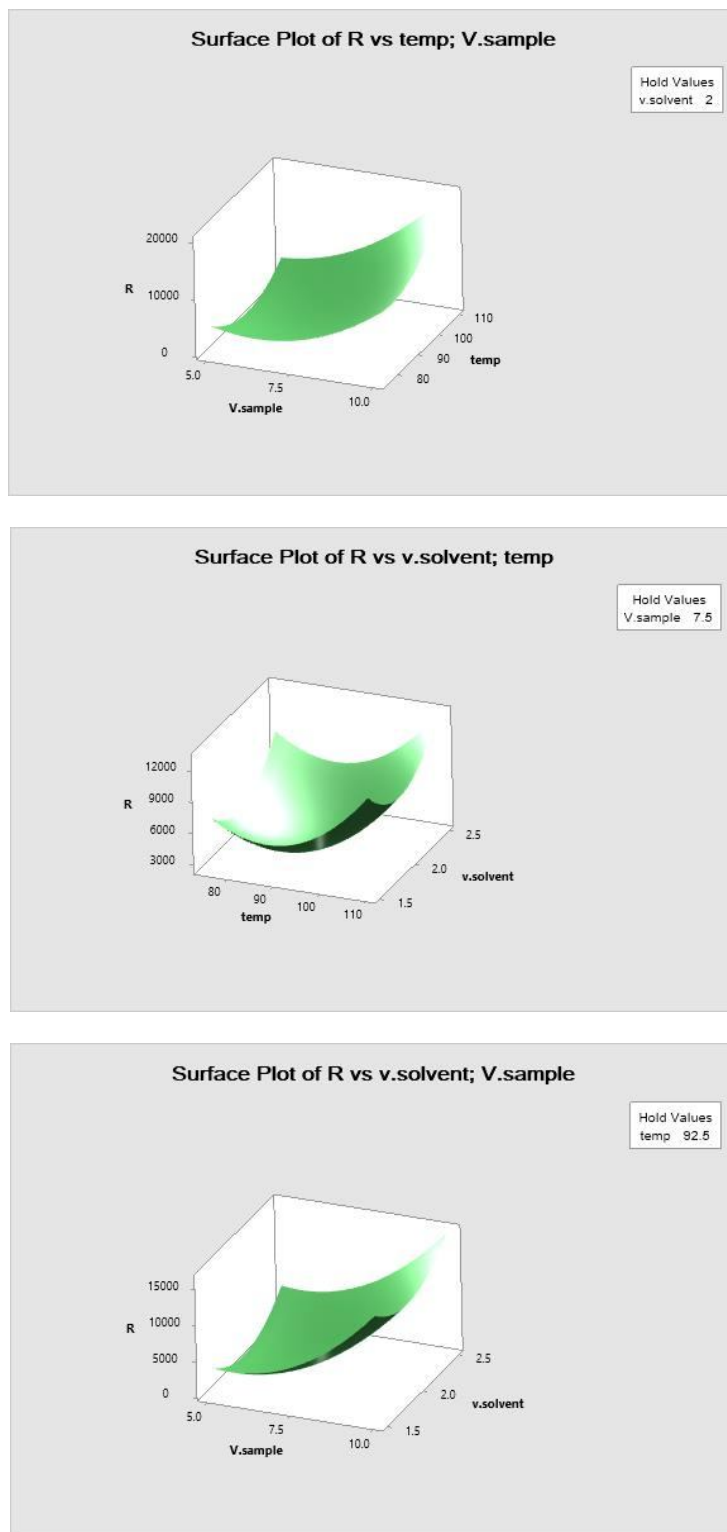
### Method Validation

Calibration curves were plotted under the optimum conditions, with the measurements of methanol and ethanol performed in the above-mentioned conditions. To draw the calibration curve, first, the standards with concentrations of 0.05-10.0  $\mu\text{g g}^{-1}$  were prepared. After performing the extraction steps, according to the final optimal points, they were injected to the gas chromatography, and the calibration curve was drawn. After drawing the curve, the transformer oil was extracted as the real sample in the final optimal conditions with the extracted phase injected into the gas chromatography. In this study, the detection limits of the proposed method for measuring methanol and ethanol were 0.02 and 0.03  $\mu\text{g g}^{-1}$ , respectively, as obtained through continuous injection of analytes until signal detection against the background noise [19-21].

### Analytical Performance

After drawing the calibration curves under optimum conditions, the real sample, which was the transformer oil selected from Sarkhoon and Qeshm Refining Company, was extracted. Once injected into the gas chromatograph and after interpretation of the resulting chromatogram, it was observed that methanol and ethanol were not detected in the real sample which was used transformer oil; thus, the cellulose insulation was not degraded, and accordingly the transformer was at optimum operating conditions.

To validate the method, we added standards 1, 3, 5 and



**Fig. 5.** Response surfaces from Box-Behnken design.



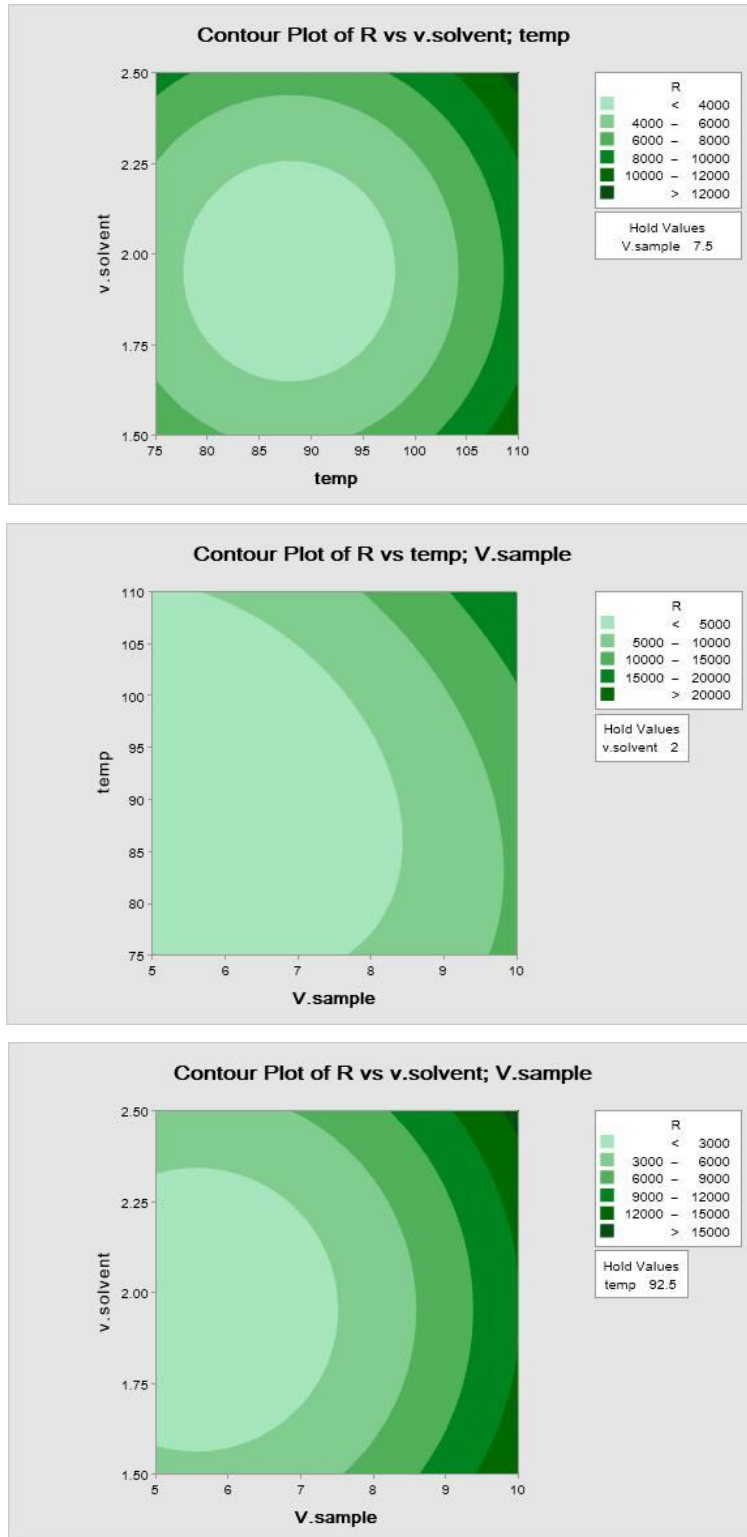
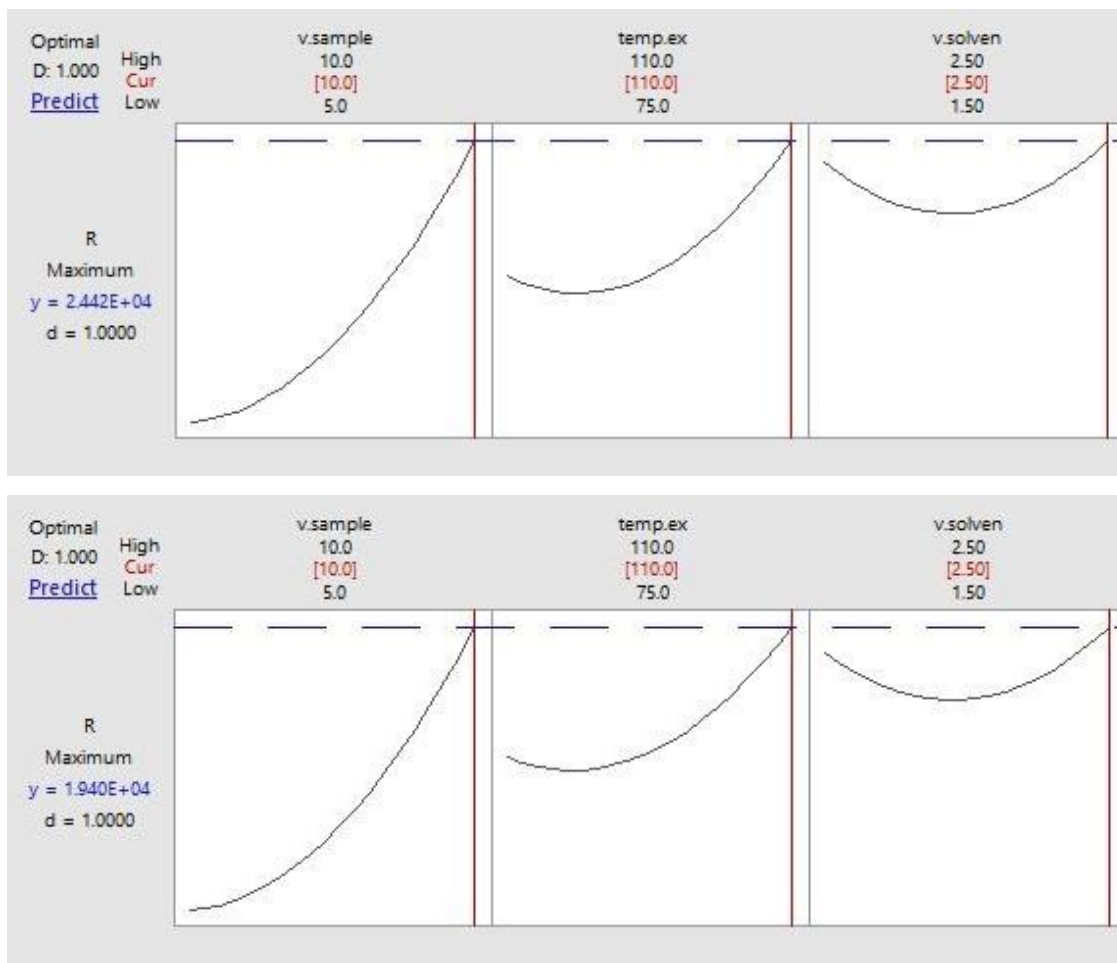


Fig. 6. Response contour from Box-Behnken design.



**Fig. 7.** The final optimal point for methanol (a) and ethanol (b).

**Table 3.** Results of Methanol and Ethanol Determination in the Spiked Oil Sample

Added ( $\mu\text{g g}^{-1}$ )	Found ( $\mu\text{g g}^{-1}$ )	RSD (%) <sup>a</sup>	R (%) <sup>b</sup>
Methanol			
1.0	0.95	5.2	95.0
3.0	3.05	5.0	101.6
5.0	4.93	1.8	98.6
7.0	6.95	2.1	99.2
Ethanol			
1.0	0.96	4.1	96.0
3.0	3.08	3.7	102.6
5.0	5.02	2.3	100.4
7.0	6.94	2.0	99.1

<sup>a</sup>Relative standard deviation. <sup>b</sup>Relative recovery.

**Table 4.** Comparison of the Limit of Detection and the Limit of Measurement for some of the Recent Studies

	Jalbert <i>et al.</i> 2007	Schaut <i>et al.</i> 2011	Jalbert <i>et al.</i> 2012	Bruzzoniti <i>et al.</i> 2014	Bruzzoniti <i>et al.</i> 2014	Matharage <i>et al.</i> 2014	Perrier <i>et al.</i> 2015	Molavi <i>et al.</i> 2017	Current work
Oil sample	10 ml	7 ml	10 ml	7 g	7 g	10 g	N.R	10 ml	10 ml
Instrumental setup	HS-GC- MS	HS-GC- MS	HS-GC- MS	HS-GC- FID	HS-GC- MS	HS-GC- MS	HS-GC- MS	HS-GC- MS	HS- SDME- GC-FID
HS equilibration time (min)	60	40	40	N.R	N.R	40	N.R	20	20
HS equilibration temperature (°C)	60	100	90	90	90	90	N.R	85	110
GC column	Stabil wax	Stabil wax	VF-624ms	Ultra 2, Rxi-624	Ultra 2, Rxi-624	VF-wax ms	N.R	VF-wax ms	CP Sil Pona
Internal standard	N.A.	N.A.	Ethanol d-6	N.A.	Ethanol d-6	Ethanol d-6	Ethanol d-6	2-ptopanol	2-ptopanol
LOD (ng g <sup>-1</sup> )	10	50	4	12	1.3	N.R.	4.8	144	20
Linearity (ng g <sup>-1</sup> )	N.R.	100-1000 (>0.99)	14-8000 (>0.999)	36-3500 (>0.9991)	3.9-2500 (>0.9993)	50-1890 (>0.99)	16-9750 (>0.995)	250-10000 (>0.996)	50-10000 (>0.996)
R <sup>2</sup>									

N.A: Not applicable. LOD: Limit of detection. N.R: Non-reported. R<sup>2</sup>: Regression coefficient.

7 µg g<sup>-1</sup> of methanol and ethanol to the standard oil sample three times each. Also, the extraction was done according to the HS-SDME extraction method, where it was injected into the device, and the obtained values were compared with the theoretical values. The results obtained from triplicate sample analysis in Table 3 indicate that there is a good agreement between the results of methods regarding methanol and ethanol. This suggests that the developed method is useful in analyzing these analytes as a marker in transformer oils.

### Comparison of the Proposed Method with Similar Procedures

As shown in Table 4, comparable results are obtained given the simplicity and cost of the method. One of the advantages of the proposed method is that it does not require applying an autosampler [1,2] or special syringe [18] which are used in similar methods to measure ethanol

and methanol causing higher costs. The relative standard deviation for methanol was obtained between 1.8 and 5.2% and recovery for selective concentrations was obtained between 95 and 101%. Also, the relative standard deviation for ethanol was obtained as 2.0 to 4.1% and recovery for selective concentrations ranged from 96-102%.

### CONCLUSIONS

This study examined a feasible method for extraction and determination of methanol and ethanol in transformer insulation oil samples, and optimization of the process variables using the design of experiments approach. For the first time, HS-SDME method was successfully applied for extracting and determining methanol and ethanol as markers for insulation paper degradation in transformer insulation oil samples using GC-FID. The method showed high extraction capacity of methanol and ethanol because of the

pre-concentration step. DOE was used to determine the most significant factors such as extraction solvent volume, sample droplet volume, extraction time, extraction temperature, and stirring rate on the extraction percentage of methanol and ethanol as the response. The proposed method has a lower and better detection range in comparison to other similar methods. It is a reasonably low-cost technique for measuring methanol and ethanol as indicators of solid insulation degradation in power transformers.

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