

## ANALYSIS OF GLYCEROL IN NIPA (*Nypa fruticans* Wurmb.) KERNEL EXTRACT (NKE) USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Mario Dario L. Berdolaga Jr.  
Philippine Science High School - MIMAROPA Region Campus

<https://doi.org/10.5109/5909110>

---

出版情報 : Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 8, pp.317-322, 2022-10-20. Interdisciplinary Graduate School of Engineering Sciences, Kyushu University

バージョン :

権利関係 : Copyright © 2022 IEICES/Kyushu University. All rights reserved.

## ANALYSIS OF GLYCEROL IN NIPA (*Nypa fruticans* Wurmb.) KERNEL EXTRACT (NKE) USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Mario Dario L. Berdolaga Jr. <sup>1</sup>

<sup>1</sup>Philippine Science High School – MIMAROPA Region Campus  
Rizal, Odiongan, Romblon 5505, Philippines  
Corresponding Author: [mdberdolaga@mrc.pshs.edu.ph](mailto:mdberdolaga@mrc.pshs.edu.ph)

**Abstract:** *The quantification of glycerol from Nipa Kernel Extract (NKE) using HPLC method was undertaken and resulted 4.06% concentration using hydrolysis reaction with methanol and KOH catalyst. The pure and hydrolyzed NKE were monitored with Hydrophilic-Interaction Chromatography (HPLC-HILIC) using isocratic elution. The chromatograms extracted from the LC were analyzed and validated and showed good performance in terms of linearity as implicated ( $R^2 = 0.9928$ ), repeatability (%RSD range from 2.6758% and 16.6130%), intermediate precision ( $p$  – value ( $0.00119$ )  $< \alpha$ ), limit of detection 0.0001458% (w/w), limit of quantification 0.0004182% (w/w), and accuracy (76.7105% to 82.8505%) of hydrolyzed NKE. The method executed, determines the concentration of glycerol in pure and hydrolyzed NKE sample.*

**Keywords:** concentration, glycerol, hydrolysis, NKE

### 1. INTRODUCTION

Nipa fruit (*Nypa fruticans* Wurmb.) is a good source of biofuel due to its sugar-rich sap that can be transformed to alcohol upon fermentation. [22] A tropical mangrove plant, blooming throughout the year, nuts grow in clusters, and contain edible meat from its kernel. The edible meat is soft, sweet translucent with oily tannin-like [6].

Quantification of glycerol yields to application in research and industries possibilities for biodiesel sources. Petroleum and fossil fuels are approaching extinction despite these energy sources were non-renewable.

However, the goal to create alternatives for fossil fuels urge to develop renewable fuels from biomass and biofuels which supports green energy and production of its own by-products [9][5]. This potential breakthrough pushes researchers to assess and evaluate sources of biofuels by means of different indicators [24].

Glycerol is a result of transesterification process of plant oils. This polyhydric alcohol can be applied to pharmaceutical, cosmetics, food, and construction industries [8]. Its water-coating effect and usually applies as solvents in food additives [25]. Glycerol can also be used as anti-inflammatory agent in cosmetic products [14].

Glycerol can be applied in syrups, cream-based products, lubricants, and ointments. It also serves as an antimicrobial agent and solvents for hand sanitizers, plasticizers, sweeteners, and tonics [27].

Transesterification as a process of converting glycerol using methanol and with the aid of homogenous catalysts such as NaOH and KOH. Unlike acid catalysts, basic solutions minimize excess alcohol and lowers temperature for longer periods [1] [15]. In transesterification, glycerol sinks at the bottom in separated mixture. In addition, biodiesel which separates on the top of the glycerol is an important by product that is essential for cosmetic processes [11] [12].

In year 2015, the rise of biodiesel production creates an avenue for glycerol production for more than 300, 000 cubic meters globally. Glycerol is a renewable chemical used in chemical industry as solvents and beverages [28].

In the study of Alang et al (2022) [2], through the process of transesterification process yield of about 10% w/w glycerol as product together with biodiesel of palm kernel oil sample. In the tests conducted using IR spectrum reports absorption peaks that falls on the carbonyl compound, alcohols and esters which validates the occurrence of glycerol on the sample [5] [21]. The presence of triglyceride in oil sample can produce glycerol derivatives and biodiesel in transesterification.

According to the study of Muniz et al (2018) [20] wherein two proposed analytical methods were used to determine the glycerol content from biodiesel using HPLC. The data collected were compared based on linearity, limit of quantification, limit of detection, repeatability, and precision.

In the run tests made by Harabi et al (2019) [29], the content glycerol was about 30.40% w/w from waste frying oil which compared from the result of Kongjiao et al (2010) [31] yield of about 28.56%.

In the study of Belasin-Prieto, et al (2013) [4] employed analysis of glycerol oxidative products using HPLC method. To validate the analytical method, precision, accuracy, sensitivity, detection, and quantification limits are used.

Interesterification process used by Santoro, et al (2018) [26] to analyze the physico-chemical properties of fatty-acids using silver ion HPLC combined with mass spectrometry (MS) and another constituent triglyceride. In the analysis, triacylglyceride (TAG's) constituent were measured in % composition of different oil samples. Testing and analysis of potential sources of glycerol is an indicator of major product together with biodiesel. HPLC is an analytical method for analyzing single and attached molecules. There are studies that focused in analyzing fatty acid and glycerol profile of oil samples [13] [19] [16]. However, no study has validated or developed to analyze the glycerol profile of nipa kernel oil. The study's aim to establish novel, calibrated, simple, accurate analysis of the glycerol content using High Performance Liquid Chromatography (HPLC) method.

### 2. METHODOLOGY

#### 2.1 Materials

The nipa kernel meat is obtained from Rizal, Odiongan, Romblon Tablas Island mechanical pressed for oil extraction. The soxhlet extractor and rotary evaporator is used for yield oil extraction from nipa kernel. Glycerol is used as standard solution to measure the concentration of glycerol in nipa kernel oil extract. The AR grade chemicals that will be used in the study are glycerol (99.9% purity), potassium hydroxide pellets, methanol (99.9%) and are obtained from Vivanto Chemical Company, Terran Alchemy and Mt. Zion Chemicals [2].



Fig. 1. Site of nipa fruit collection

The procedures used in this study are ranged from oil extraction, purification of oil, hydrolysis of oil extract, instrumental analytical methods for the characterization of glycerol content.

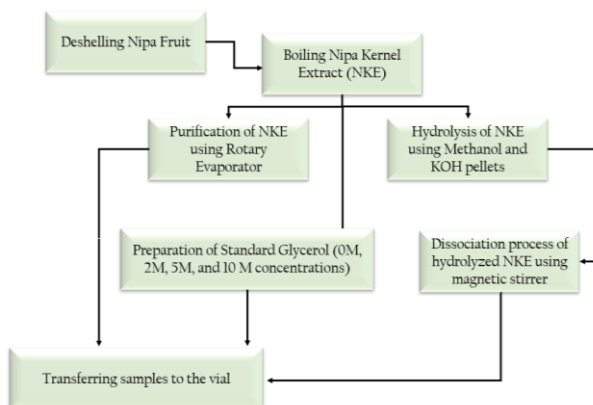


Fig. 2. Preparation of Standard and NKE Samples

## 2.2 Extraction of Nipa Kernel

Nipa kernel deshelled from the original fruit and subjected for boiling. To achieve the extract, 1360 grams of nipa kernel with 1360 mL distilled water is boiled for 2 hours in the first phase. The extract was transferred and

settled overnight for 5.2 – 5.4°C. It was then boiled for the same parameters for the second phase that lasted for 60 minutes. Using a soxhlet apparatus and rotary evaporator, to separate the solvent on the extract. The Nipa Kernel Extract (NKE) is purified by warming it to 100 °C-65°C (100 rpm) for 45 minutes and allowing it to stand for 2 hours for impurities to settle [30].

## 2.3 Hydrolysis of Nipa Kernel Extract

Glycerol will be synthesized from NKE using hydrolysis/transesterification following the stoichiometric equation of the reaction of triglyceride and methanol.

There are about 70 mL 20% methanol transferred into a beaker afterwards 2.3 g KOH catalyst are added on the NKE. The mixture is subjected to the magnetic stirrer for about 70°C for the methanol and KOH to dissolve properly. The NKE was transferred to a 1000-mL round bottom distillation flask to separate the water for the extract.

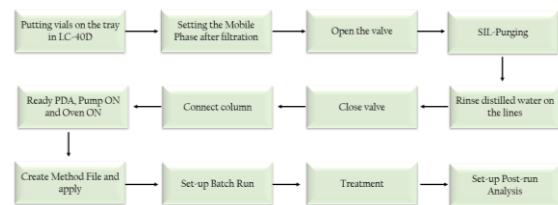


Fig. 3. LC Process

Table 1. Treatment formulation of standard glycerol

Treatment	Formulation of glycerol
Std0	0
Std1	2 M
Std2	5 M
Std3	10 M

## 2.4 Analysis of the samples by High Performance Liquid Chromatography (HPLC)

Glycerol with 99.9% purity is used as the standard reagent for High Performance Liquid Chromatography - Hydrophilic-Interaction Chromatography (HPLC-HILIC) Shimadzu Nexera Lite with quaternary low-pressure gradient pump (LC-40D), SPD-M40 (wavelength range of 190 to 800 nm UV detector), the Autosampler SIL-40C X3 with 0.01-50  $\mu$ L loop. The column measuring 300mm for the concentration of glycerol in NKE. The mobile phase was 100% prefiltered distilled 65% water (line A-C-D) and methanol 35% (line B-R) solution using isocratic elution. The total time of the analysis in each sample is 5 minutes with constant flow rate of 1 mL  $min^{-1}$  with injection volume of 10  $\mu$ L. Pump A Pressure is set in minimum of 2MPa and Pump A Degassing Line of -93kPa. Each sample was injected in triplicate to evaluate the repeatability precision for the calibration curve.

Peaks in the chromatogram measuring the maximum intensity of glycerol at (205 nm at  $\alpha=0.99$  UV Absorbance) and expressed in mAU (milli absorbance-unit).

## 2.5 Validation of the analytical method

The purpose of validating the proposed analytical method is to verify the reliability of the results. This validation is partial in a way that it must conform with the requirements and standards. The partial validation of the analytical method will be performed in this study. The parameters are linearity, precision (intermediate and repeatability precision), accuracy (recovery), limit of detection (LOD), limit of quantification (LOQ), [23].

### 2.5.1 Linearity

The linearity validation is performed through triplicate injections of standard solution that corresponds to every point of the analytical curve. The coefficient of determination ( $R^2$ ) is used to check the linearity. The range of values to make it linear falls above 0.99, and the angular coefficient of the line is constant [17].

### 2.5.2 Precision (Repeatability and Intermediate Precision)

The precision was assessed in terms of repeatability and intermediate precision. The repeatability was verified for four standard treatment formulations in triplicate for the analytical curve. The repeatability precision is also called as coefficient of variation and can also be expressed as the relative standard deviation (%RSD) and calculated using the equation.

$$\%RSD = \frac{\sigma}{\bar{X}} \times 100$$

Where:  $\sigma$  is standard deviation of the data sample and  $\bar{X}$  is the mean data sample. The intermediate precision was

evaluated using the analysis of variance (ANOVA) with different analytical curves.

### 2.5.3 Accuracy (Recovery)

The accuracy was measured as recovery indicated by (%R) and verified using three different concentrations. The accuracy was calculated using the formula.

$$\%R = \frac{C_m}{C_e} \times 100$$

Where:  $C_m$  is the measured concentration and  $C_e$  is the expected concentration.

### 2.5.4 Limit of Detection and Limit of Quantification

The limit of detection (LOD) was calculated using the standard deviation and slope of the analytical curve using the equation.

$$LOD = \frac{3s}{m}$$

Where:  $s$  is the standard deviation of the analytical curve and  $m$  is the slope of the analytical curve. The limit of quantification (LOQ) was calculated by multiplying 3.3 to the value of LOD (Muniz et al, 2018).

## 3. RESULTS AND DISCUSSION

### 3.1 Determination of concentration using Analytical Curve

Table 2. Linear Regression Table of Standard Glycerol and Absorbance in LC Method

Concentration (M)		Absorbance (mAU)	Predicted Y	Residual
0		0	24.3795	-24.3795
2		151.89	141.6109	10.2794
5		349.77	317.458	32.312
10		592.32	610.5365	-18.2119
$\beta_1$	$\beta_0$	$R^2$	Correlation coefficient	F
54.39	56.47	0.9928	0.9964	138.2272
				P-value
				0.054

$$\hat{Y} = 54.3866X + 56.4709$$

The ( $R^2$ ) is equal to 0.9928 which means that 99.28% of the variability of absorbance is explained by the concentration of the standard glycerol. The correlation coefficient ( $R$ ) represented by 0.9964 can be gleaned that there is a very strong direct relationship between absorbance and concentration.

The overall regression is right-tailed, where the  $F(1,2)$  which is equal to 138.2272, with a p-value of 0.0540. Since p-value is greater than  $\alpha$  (0.05), we reject the null hypothesis, hence that there is a significant relationship between concentration and absorbance of glycerol in the standard solution. The linear regression model,  $\hat{Y} = 56.4709 + 54.3866X$  is created, provides a better fit than the model without the independent variable. This model is used to compute for the concentration glycerol in the unknown pure and hydrolyzed sample of NKE. The  $\hat{Y}$  is the absorbance of the sample in mAU while  $X$  is the concentration of the sample.

Table 3. Table for the compared Percent Concentration of Glycerol from Pure and Hydrolyzed NKE

	Pure NKE	Hydrolyzed NKE
Absorbance (mAU)	309.628	1585.961
Concentration (ppm)	6.556	40.704
Measured Volume (L)	0.232 L	0.273 L
Mass of Glycerol (g)	0.00152	0.0111
% (w/v)	0.655%	4.06%

The concentration of glycerol from pure NKE is 6.556 ppm while in the hydrolyzed NKE, the concentration is equal to 40.704. The concentration of glycerol from hydrolyzed NKE is higher in yield than pure NKE as manifested by the % concentration in (g/L) of 4.06% in every 0.273 L sample.

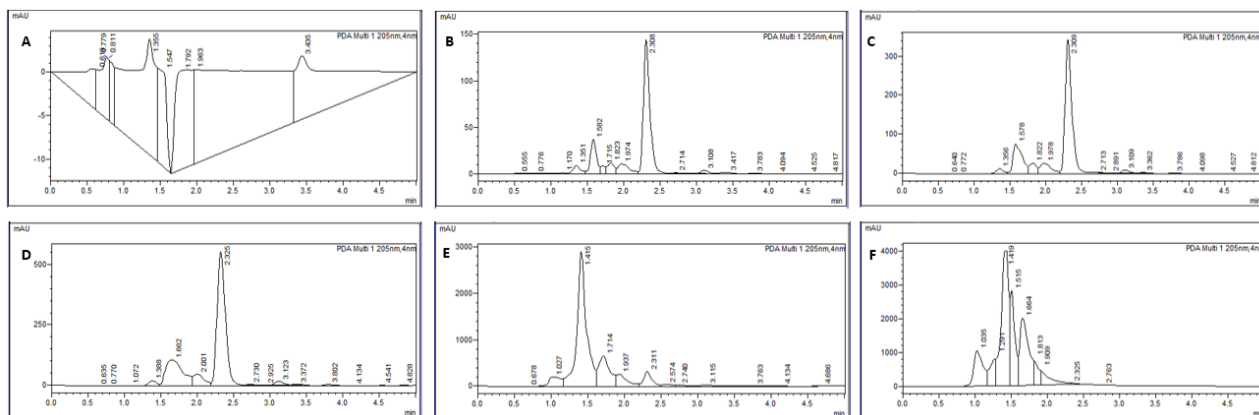


Fig. 4. Chromatogram of the Standard Glycerol (A) 0M; (B) 2M; (C) 5M; (D) 10M; and NKE Sample (E) Pure; (F) Hydrolyzed undertaken from the Post-Run Analysis of HPLC

Table 4. Comparison of %RSD obtained from Standard and NKE Samples

	R1	R2	R3	$\bar{X}$	$\sigma$	%RSD
2M	60.906	61.491	36.787	53.0613	14.0970	2.0526
5M	61.639	62.705	38.485	54.2763	13.6861	2.0067
10M	45.997	61.998	39.392	49.1290	11.6239	2.2169
Pure NKE	5.858	5.999	7.811	6.5560	1.0891	16.6130
Hydrolyzed NKE	45.714	35.708	40.689	40.7037	5.0030	2.6758

Legend: Acceptance criteria for %RSD = < 20%

### 3.2.1 Linearity Test

Figure 1 presents the analytical curve obtained from the glycerol standards in 1.5 mL ranging from 0M, 2M, 5M, and 10 M. The determination coefficient ( $R^2 = 0.9928$ ) shows a linear relationship, the model  $\hat{Y} = 56.4709 + 54.3866X$  is used to quantify the concentrations of glycerol from NKE. The determination coefficient of ( $R^2 = 0.9928$ ) is greater than the acceptance criteria of 0.990 from international standards for validating analytical methods. This indicates good linearity from the determined concentration ranges.

### 3.2.2 Repeatability and Intermediate Precision

The %RSD describes the repeatability precision undertaken from the triplicate injections done from pure and hydrolyzed NKE from post-run analysis made. According to UN Validation of Analytical Methodology (2009) [10], for HPLC methods the acceptable criteria for repeatability should fall below 20% and the RSD values from pure and hydrolyzed NKE along the range of 16.6130% and 2.6758% respectively. This shows that the concentrations measured by the chromatogram shows good precision.

For intermediate precision of the chromatogram from the standard glycerol and NKE samples and verified using one-way ANOVA. The p-value (0.00119) is less than  $\alpha$  which is 0.05, thus the null hypothesis is rejected which states that the concentration taken from the method is statistically significant. This shows that the concentration of glycerol from the hydrolyzed sample are evident in the standard sample.

### 3.2.3 Accuracy (Recovery)

Table 5 shows the %R (accuracy) of the measured concentration from the NKE samples compared to the concentrations of the standard glycerol at 2.30 min. retention time. The recovery values range from 76.7105% to 82.8505% shows strong agreement of experimental and expected concentrations of glycerol. The accuracy results fall within the acceptance criteria of international standards of analytical validation (70-120%) [20].

### 4.2.4 Limit of Detection and Quantification

The limit of detection (LOD) is defined as the 10% portion of %RSD. The requirement to detect the analyte at minimum concentration. In the calibration curve the standard deviation is equal to 26.44 and the slope is 54.39. The LOD and LOQ computed is 0.0001458% (w/w), and 0.0004182% (w/w), respectively. The accepted criteria according to the UN Validation of Analytical Methodology (2009) conforms on the range below the minimum concentration of the previous method undertaken from a laboratory. According to the study of Muniz et al (2018), the computed LOD is lower than the value obtained from the spectrophotometric detection of 0.000941% glycerol.

Table 5. Table for Accuracy Validation of the Concentration obtained the Chromatogram

Concentration (ppm)	%R	
	Pure NKE	Hydrolyzed NKE
53.061	12.3555	76.7105
54.276	12.0789	74.9933
49.129	13.3444	82.8505

#### 4. CONCLUSION

Nipa kernel extract attributed by HPLC method to quantify the concentration of glycerol were compared to standard glycerol from p-value (0.00119) hence imposed the values to be statistically significant due to distance of pure and hydrolyzed NKE concentration. This was strengthened by the concentration of glycerol in standard and hydrolyzed NKE to be comparable.

The method reflects a detection limit of 0.0001458% (w/w) and much lower based on literatures in quantifying glycerol. The precision (16.6130% and 2.6758%) and accuracy (76.7105% to 82.8505%) were within the ranges of acceptable criteria for validating analytical methodology. The results obtained from the method used are acceptable for the determination of glycerol concentration and can be an alternative procedure for chemical analysis. Further studies on the impurities and free fatty acids attached along glycerol that also absorbed similar absorbance units. Quantification of concentration of other unknown compounds in the matrix is expected to be analyzed and identified.

#### 5. REFERENCES

- [1] J. E. Andrade, A. Perez, P. J. Sebastian, D. Eapen, Retracted: A review of bio-diesel production processes, *Biomass Bioenergy*. 35 (2011) 1008–1020.
- [2] M. B. Alang, M. K. Ndikontar, Y. M. Sani, P. T. Ndifon, Synthesis and Characterisation of a Biolubricant from Cameroon Palm Kernel Oil Using a Locally Produced Base Catalyst from Plantain Peelings, *Green and Sustainable Chemistry*. 8 (2018) 275-287.
- [3] ASEAN Guidelines for Validation of Analytical Procedures. (2012). website:<https://asean.org/wp-content/uploads/2012/10/Asean-Analytical-Validation-gl.pdf>
- [4] J. C. Beltran-Prieto, J. Pecha, V. Kasparikova, K. Kolomaznik, Development of an HPLC method for the determination of glycerol oxidation products, *Journal of Liquid Chromatography & Related Technologies*. 2013.
- [5] G. Bagnato, A. Iulianelli, A. Sanna, A. Basile, Glycerol Production and Transformation: A Critical Review with Particular Emphasis on Glycerol Reforming Reaction for Producing Hydrogen in Conventional and Membrane Reactors, *Membranes*. 7 (2017).
- [6] C. A. Tabinas, Characterization and Glyceride Composition Approximation Analysis of Oil Extracted from Matured Meat of *Nypa fruticans* (Nipa) Nuts, *American Journal of Chemistry and Applications*. 4 (2017) 1-5.
- [7] R. Christoph, B. Schmidt, U. Steinberner, W. Dilla, R. Karinen, Glycerol, In *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2000.
- [8] J. Corach, E. F. Galván, P. A. Sorichetti, S. D. Romano, Estimation of the Composition of Soybean Biodiesel/Soybean Oil Blends from Permittivity Measurements, *Fuel*. 235 (2019) 1309-1315.
- [9] S. S. Dara S.S., A Textbook of Engineering Chemistry. First Edition, S. Chand and Company Ltd., Ram Nagar, 2008, 633-643. An ISO 9001: 2000 Comp.
- [10] Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens. (2009). United Nations, Vienna Austria. website: [https://www.unodc.org/documents/scientific/validation\\_E.pdf](https://www.unodc.org/documents/scientific/validation_E.pdf)
- [11] H. Hindarso, Production of Fatty Acid Methyl Ester from Microalgae Using Microwave: Kinetic of Transesterification Reaction Using CaO Catalyst, *Am. J. Chem. Eng.* 6 (2018) 54.
- [12] D. T. Johnson, K. A. Taconi, The glycerin glut: Options for the value-added conversion of crude glycerol resulting from biodiesel production, *Environ. Prog.* 26 (2007) 338–348.
- [13] D. K. Jain, N. Jain, R. Charde, The RP-HPLC method for simultaneous estimation of esomeprazole and naproxen in binary combination, *Pharm. Methods*. 2 (2011) 167–172.
- [14] A. Khan, A. Bhide, R. Gadre, Mannitol Production from Glycerol by Resting Cells of *Candida magnolia*, *Bioresource Technology*. 100 (2009) 4911-4913.
- [15] M. Y. Koh, T. I. M. Ghazi, A review of biodiesel production from *Jatropha curcas*, L. oil. *Renew. Sustain. Energy Rev.* 15 (2011) 2240–2251.
- [16] R. S. Kumar, U. G. Sree, N. B. Malleswara Babu, A RP-HPLC Method Development and Its Validation for the Simultaneous Estimation of Naproxen and Pantoprazole Sodium in Capsule Dosage Form, *Asian J. Res. Chem.* 6 (2013) 155–168.
- [17] P. A. Lopes, Internal publication of method validation course, Rede de Tecnologia, Rio de Janeiro, Brazil, p. 1, 2008.
- [18] D. W. Mayo, F. A. Miller, R. W. Hannah, Spectra of Carbonyl Compounds of All Kinds (Factors Affecting Carbonyl Group Frequencies), In: Mayo, D.W., Miller, F.A. and Hannah, R.W., Eds., *Course Notes on the Interpretation of Infrared and Raman Spectra*, Chapter 7, John Wiley & Sons, Inc., Hoboken, 179-204, 2003.
- [19] S. Mohideen, M. Shivakanth, P. Sureshkumar, S. N. Krishnan, Y. Surendranath, T. Satyanarayana, Development and validation of analytical method for naproxen and pantoprazole in capsule dosage form, *Int. J. PharmTech Res.* 3 (2011) 1169–1173.
- [20] R. O. Muniz, S. B. Martins, G. G. Hon'orio, J. N. da Cunha, C. G. de Souza, D. F. de Andrade, R. N. C. Pradelle, F. Turkovics, R. S. Neto, L. A. d'Avila, E. D'Elia, Total glycerol analysis in biodiesel samples using solid phase extraction coupled with enzymatic spectrophotometric determination, 2018.
- [21] M. R. Nanda, Y. Zhang, Z. Yuan, W. Qin, H. S. Ghaziaskar, C. C. Xu, Catalytic Conversion of Glycerol for Sustainable Production of Solketal as a Fuel Additive: A Review, *Renewable and Sustainable Energy Reviews*. 56 (2016) 1022-1031.
- [22] E. R. Jr, R. E. Raga, R. G. Junio, Morphological and sap yield variation in *Nypa fruticans* Wurbm.), 2011. website: <https://www.researchgate>.

- net/publication/259675674\_Morphological\_and\_sap\_yield\_variation\_in\_Nipa\_Nypa\_fruticans\_Wurmb
- [23] E. C. P. Rego, Internal publication of method validation course, Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO), Rio de Janeiro, Brazil, 2013.
- [24] C. A. G. Quispe, C. J. R. Coronado, J. A. Carvalho Jr., Glycerol: Production, Consumption, Prices, Characterization and New Trends in Combustion, Renewable and Sustainable Energy Reviews. 27(2013) 475-493.
- [25] D. Samul, K. Leja, W. Grajek, Impurities of Crude Glycerol and Their Effect on Metabolite Production, Annals of Microbiology, 64 (2014) 891-898.
- [26] V. Santoro, F. D. Bello, R. Aigotti, D. Gastaldi, F. Romaniello, E. Forte, M. Magni, C. Baiocchi, C. Medana, Characterization and Determination of Interesterification Markers (Triacylglycerol Regioisomers) in Confectionery Oils by Liquid Chromatography-Mass Spectrometry, Foods. 7 (2018) 23.
- [27] P. J. Sheskey, W. G. Cook, C. G. Cable, Handbook of Pharmaceutical Excipients. American Pharmacists Association, London, 2000.
- [28] A. Leoneti, V. Aragão-Leoneti, S. Oliveira, Glycerol as a by-product of biodiesel production in Brazil: Alternatives for the use of unrefined glycerol, Renewable Energy. 45 (2012) 138–145.
- [29] M. Harabi, S. Bouguerra, F. Marrakchi, L. Chrysikou, S. Bezergianni, M. Bouaziz, Biodiesel and Crude Glycerol from Waste Frying Oil: Production, Characterization and Evaluation of Biodiesel Oxidative Stability with Diesel Blends, Sustainability. 11 (2019).
- [30] M. Saifuddin, M. Khandaker, A. S. Hossain, M. Jahan, N. Mat, B. A. Nasrulhaq, Bioethanol Production from Mango Waste (*Mangifera indica* L. cv chokanan): Biomass as Renewable Energy, Australian Journal of Basic and Applied Sciences. 8 (2014).
- [31] S. Kongjao, S. Damronglerd, M. Hunsom, Purification of crude glycerol derived from waste used-oil methyl ester plant, Korean Journal of Chemical Engineering, 27 (2010).
- [32] L. Yongfu, K. Ryosuke, I. Yoshio, Correlation of vapor-Liquid Equilibria of Supercritical Methanol + Glycerol system, Journal of Novel Carbon Resource Sciences. 5 (2012) 19-22.