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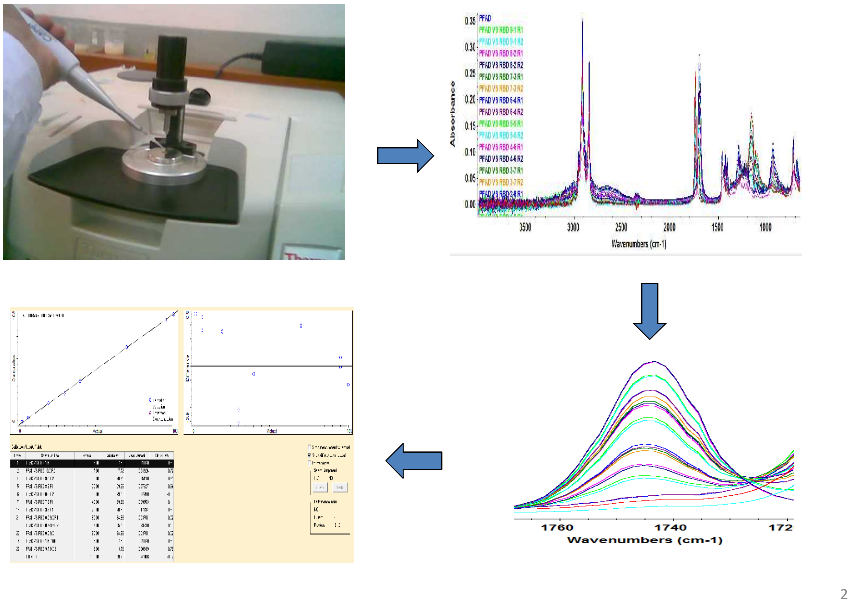
Graphical Abstract



Green, reliable, rapid and novel SB-ATR FT-IR method has been reported in which only one drop of sample is required, and less than 2 min are required to take results for the quantitative assessment of neutral oil present in PFAD.

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Abstract

This study developed a green, reliable, rapid and economical analytical method for the determination of neutral oil present in deodorizer distillates (DD) by Single Bounce Attenuated Total Reflectance (SB-ATR) Fourier-Transform Infrared Spectroscopy (FT-IR). Beer's Law was applied to develop calibration model based on the band height at 1744 cm^{-1} with two point baseline between 1800 to 1600 cm^{-1} of the carbonyl group (C=O) related to triglycerides. It was found to be linear for neutral oil over wide concentration range of 0 - 100% with correlation coefficients greater than 0.999. No solvent or costly standards were used in the proposed method. The accuracy of the method was assessed by comparison to a conventional standard American Oil Chemists' Society (AOCS) titrimetric procedures, which provided good correlation (> 0.999). Therefore, proposed method could be used as an environmentally friendly alternative to the AOCS titrimetric method for the quantitative determination of neutral oil in Palm Fatty Acid Distillates (PFAD) for the routine analysis in quality control laboratories of oil processing industries.

Keywords. PFAD; Quantitative Assessment; Neutral Oil; Beer's law; SB-ATR; FT-IR

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1. Introduction

Palm oil is totally different from other vegetable oils due to its unique fatty acids profile and tocols composition. Tocols composition of palm oil include γ tocotrienol (46%), α tocotrienol (22%), α tocopherol (20%) and δ tocotrienol (12%).[1,2] Due to their activity as antioxidant, hypocholesterolaemic, antithrombotic, anti inflammatory, anti atherogenic, hepatoprotector and immunomodulator, tocotrienols are significant components of many pharmaceutical and food supplements.[3,4] Palm oil is rich in phytonutrients such as vitamin E (600-1000 ppm), carotenoid (500-700 ppm), phytosterols (300-620 ppm), squalene (250-540 ppm) and phospholipids (20-100 ppm).[5] Generally for palm oil refining, degumming (to remove gum), bleaching (to reduce color) and deodorization (to remove odorous components) processes are carried out. Palm fatty acid distillate (PFAD) is by product of deodorization and generally quantity of PFAD is about 4% of crude palm oil (CPO).[6,7] Basically deodorization is the last but very essential refining process. It is the process where neutralized and bleached oil are stripped under high vacuum and high temperature steam. The temperature of deodorizer is maintained in between 180–260°C through high pressure steam boiler by heat exchange system. High vacuum atmosphere of about 2–8 mmHg is created in the deodorizer to avert the oil from oxidative damage. [8,9] Unwanted volatile compounds such as free fatty acids (FFAs), peroxides, aldehyde, ketones, alcohols, and carbohydrates are removed during deodorization. The volatile odoriferous compounds are stripped off by direct injection of high temperature steam. The volatile fraction is then collected in a condensed form of deodorizer distillate (DD).[10,11] Due to vigorous conditions in deodorizer equipment, some fractions of valuable components such as tocopherols, sterols, squalene and neutral oil as well are also distilled off and become part of the deodorizer distillate.[12] Mostly PFAD is utilized in non food industries as a sources of fatty acids particularly in oleochemical industries. Deodorizer distillates are also used for biodiesel, soap and feed manufacturing.[13-16]

Fatty acids and triglycerides are major components (~96.%) and minor bioactive compounds such as squalene (0.76%), tocopherols and tocotrienols (0.48%), phytosterols (0.37%), and other hydrocarbons (0.71%) are also the part of PFAD [17]. But still unluckily, PFAD has not been explored as it deserves source of bioactives. AOCS standard method is commonly used in the industries to assess neutral oil in DD which is very time consuming and laborious method. Furthermore, it involves lot of chemicals and glassware. Usually it takes more than five hours to produce result. During this time if neutral oil is going to DD results in great economical loss to industry. Therefore, quick result is desired to control the loss. FT-IR Spectroscopy has proved itself as a green technique in comparison to other methods which generate a lot of wastes and leads to environmental pollution. To the best of our knowledge no instrumental method has been reported yet to check neutral oil in PFAD. Therefore, a green method for the quantitative analysis of neutral oil in palm fatty acid distillates by single bounce attenuated total reflectance (SB-ATR) Fourier-transform infrared spectroscopy (FT-IR) was developed for quick information to technical staff of industry to avoid great loss.

2. Materials and Methods

2.1. Apparatus, Reagents and Samples

In present study, for FT-IR analyses no chemical or solvent or standard was used. For conventional standard method chromatographic column, Soxhlet flasks, separating funnel, wash bottle, funnels, stirring rods and forced draft oven were used. Furthermore, ether, hexane, Aluminum oxide (grade F-20) were used and purchased from Merck (Darmstadt, Germany). Two different edible oil industries were selected from where samples of PFAD as well as pure freshly produced refined, bleached and deodorized (oil) palm oil were collected. Eight samples were collected from each physical refinery on each continuous day.

2.2. Sample and standard preparation

Laboratory sample containers from which samples were collected were shakened vigorously and the samples were mixed thoroughly to achieve uniform distribution of sample's components. A series of standards ranging from 0 to 100% neutral oil were prepared by gravimetrically mixing of refined, bleached and deodorized (RBD) palm oil in PFAD which was free of neutral oil at 50 °C.

2.3. Neutral oil analysis

Initially standard AOCS Official Method (Ca 9f-57)[18] was used to check the neutral oil in prepared standards. Values achieved were used as a standard values for FT-IR Method.

2.4. FT-IR Instrumentation

Thermo Nicolet 5700 FT-IR spectrometer equipped with a pyroelectric deuterated triglycine sulfate (DTGS) detector and extended KBr optics. Instrument was controlled by OMNIC software version 7.2 (Thermo Nicolet Analytical Instruments, Madison, WI). SB-ATR accessory (Spectra-Tech, Shelton, CT) with a removable ZnSe crystal was mounted in the sample compartment of the spectrometer. The FT-IR spectra were recorded from 4000-650 cm^{-1} by co-addition of 32 scans at a resolution of 4 cm^{-1}. A fresh background spectrum of bare ATR crystal was taken before recording the spectrum of each standard and sample. ATR crystal was carefully cleaned with propanol to remove any lipo- or hydrophilic residues of the previous sample, and residual solvent was then evaporated using a stream of nitrogen gas.

2.5. FT-IR Analysis

Turbo Quant (TQ) analyst 7.2 software package from Nicolet (Thermo Electron Corp) was applied as chemometric tool for the development of FT-IR method for the determination of neutral oil in PFAD. The grouped spectra of standards (prepared by gravimetrically mixing of RBD palm oil in PFAD which was free of neutral oil) from OMNIC program was opened in TQ Analyst program. Values of neutral oil for standard obtained from standard AOACS method were put into the software to develop Beer’s Law calibration model.

2.6. FT-IR calibrations for neutral oil analysis

One sample of PFAD containing about 5.26% neutral oil was heated to 150 ^{o}C to convert neutral oil present in PFAD to free fatty acids. After continuously 2 hour heating, no any neutral oil was observed which was confirmed by standard AOCS method.[18] Therefore for clear and good calibration model, a wide range between 0-100% neutral oil for Beer law calibration model was selected. A set of 11 standards were prepared in concentration range of 0 to 100% neutral oil. Calibration was carried out by focusing on carbonyl band at 1744 cm^{-1} of triglycerides. Initially calibration was tried on v/v bases but no good results were obtained. As palm fatty acid distillate (PFAD) is solid at room temperature therefore both PFAD and refined, bleached and deodorized (RBD) palm oil were melted before mixing (v/v) to develop PLS calibration model. To avoid any error in calibration, measured volumes were weighed and for Beer law calibration model on the bases of mass/mass (w/w). Concentration of each prepared standard was determined by standards AOCS method.[18] Software and data treatment Nicolet Turbo Quant (TQ) Analyst chemometric software was used to construct Beer's law calibration model. To assess the performance of Bear law model, the correlation coefficient between predicted and reference values were measured as per reported procedures.[19-20]

2.7. Efficiency of FT-IR Method

Beer's law was applied on peak height as well as peak area by selecting different regions and baselines. But calibration model at band height 1744 cm^{-1} of the carbonyl group (C=O) related to neutral oil with baseline between 1800 to 1600 cm^{-1} provided outstanding results. Efficiency of FT-IR method was evaluated by the determination of intraday and interday precision, roughness, recovery, LOD and LOQ as per reported method. [21]

2.8. Intraday and interday precision

The intra as well as interday precisions were checked by analysis of the same PFAD samples on the same day (n = 3) and on three different days (n =3), respectively.

2.9. Roughness of methods

The robustness of the method was evaluated by assay of the sample, by two different analysts on the same instrument on two different days (n =3).

2.10. Efficiency of FT-IR Method

The efficiency was evaluated by the recovery through standard addition method. Different amount of the PFAD with known concentration of neutral oil was added to the RBD palm oil and then the proposed FT-IR method was applied to check the neutral oil concentration in PFAD. The recovery was calculated by following equation.

$$R\ (\%) = (C.B/A) \times 100$$

Where R is concentration of neutral oil recovered, C is total concentration after addition, B is the concentration of sample taken before addition and A is concentration of standard added.

2.11. Limit of detection and quantification

Free fatty acids and triglycerides are major components (~96.%) while squalene (0.76%), tocopherols, tocotrienols (0.48%), phytosterols (0.37%) and other hydrocarbons (0.71%) are minor components in PFAD.[17] Therefore no any matrix of PFAD affects on absorption of selected carbonyl band (1744 cm^{-1}) for calibration model. Even carbonyl band at 1710 cm^{-1} due to free fatty acids has no any interference in selected region. Limit of detection (LOD) and limit of quantification (LOQ) for the FT-IR method were determined by calculating the height of the IR bands at 1744 cm^{-1} for neutral oil at a low concentration. FT-IR analysis was repeated eleven times on lowest amount at which signals were differentiated from signal to noise ratio to calculate LOD and LOQ using the following equations.[21]

$$LOD = 3 \times SD \times C/ M$$

$$LOQ = 10 \times SD \times C/ M$$

Where SD is the standard deviation; C is the concentration of analyte and M is the mean band height. In this study, AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals were used to calculate the coefficient of variation of the data set and used as relative standard deviation (RSD, %).[22]

3. Results and discussion

3.1. FT-IR Spectroscopic Method Development

FT-IR Spectroscopy is a common tool used for qualitative analyses since long time but recently gained much attention for quantitative analyses due to its advantages as a green technique.[23-25] Figure 1 represents stacked FT-IR spectra of the neat refined, bleached and deodorized (RBD) palm oil (Fig. 1A) and palm fatty acid distillate (PFAD) (Fig.1B) which were recorded in the mid infrared region with one drop of sample at 50 °C. Carbonyl band of RBD palm at 1744 $\mathrm{cm^{-1}}$ due to triglycerides band is clearly differentiated from the carbonyl band at 1711 $\mathrm{cm^{-1}}$ due to free fatty acids of PFAD.

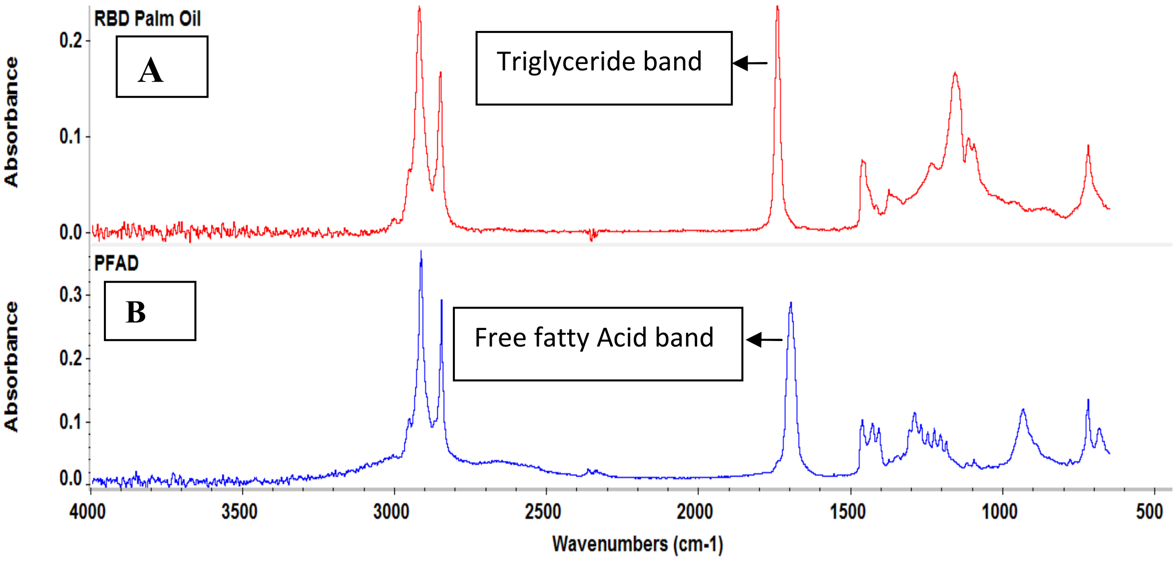


Fig.1. FT-IR spectra of (RBD) palm oil (Fig 1A) and palm fatty acid distillate (PFAD) (Fig. 1B).

3.2. Analytical Performance of FT-IR Method

Figure 2 represents complete group spectra of neutral oil mixed in PFAD (2A) with different ratio and expanded group spectra of selective carbonyl band (1744 cm^{-1}) in between 1765 -1720 cm^{-1} used in calibration model. Absorbance of free fatty acid band occurs at ~1710 cm^{-1} which has no interference with carbonyl band of neutral oil at 1744 cm^{-1}. Both signals are clearly separated from each other in very low as well as large amount which is obvious from Figure 2A. Key plan of achieving green method was completely accomplished as pre-treatment or extraction or pre-treatment steps were totally avoided. No solvent or chemical or even water was used for the development of the proposed method which is very clear from the spectra of neat RBD palm oil and pure PFAD. A simple Beer's law model using band peak height at 1744 cm^{-1} was applied for the quantitative determination of neutral oil in PFAD. A linear calibration curve with regression coefficient 0.999 was achieved shown in Figure 3 which showed good linearity in the range 0 to 100% of neutral oil. The ANOVA was calculated for the calibration developed for the evaluation of neutral oil in PFAD. The data obtained indicate that statistically ($P<0.05$) there is no linearity deviation. Following regression equation achieved from calibration of standards was used for the estimation of amount of neutral oil in the real PFAD samples.

$$Y = 0.00250x + 0.00$$

Where Y is calculated valve of neutral oil by Beer Law calibration model and x is the actual value obtained by standard AOCS method.

The % difference plot between the actual and calculated values for the calibration standards was also calculated and shown in the Figure 3. Linear calibration with excellent regression coefficient clearly revealed that FT-IR spectroscopic method can be used for accurate determination of neutral oil in PFAD.

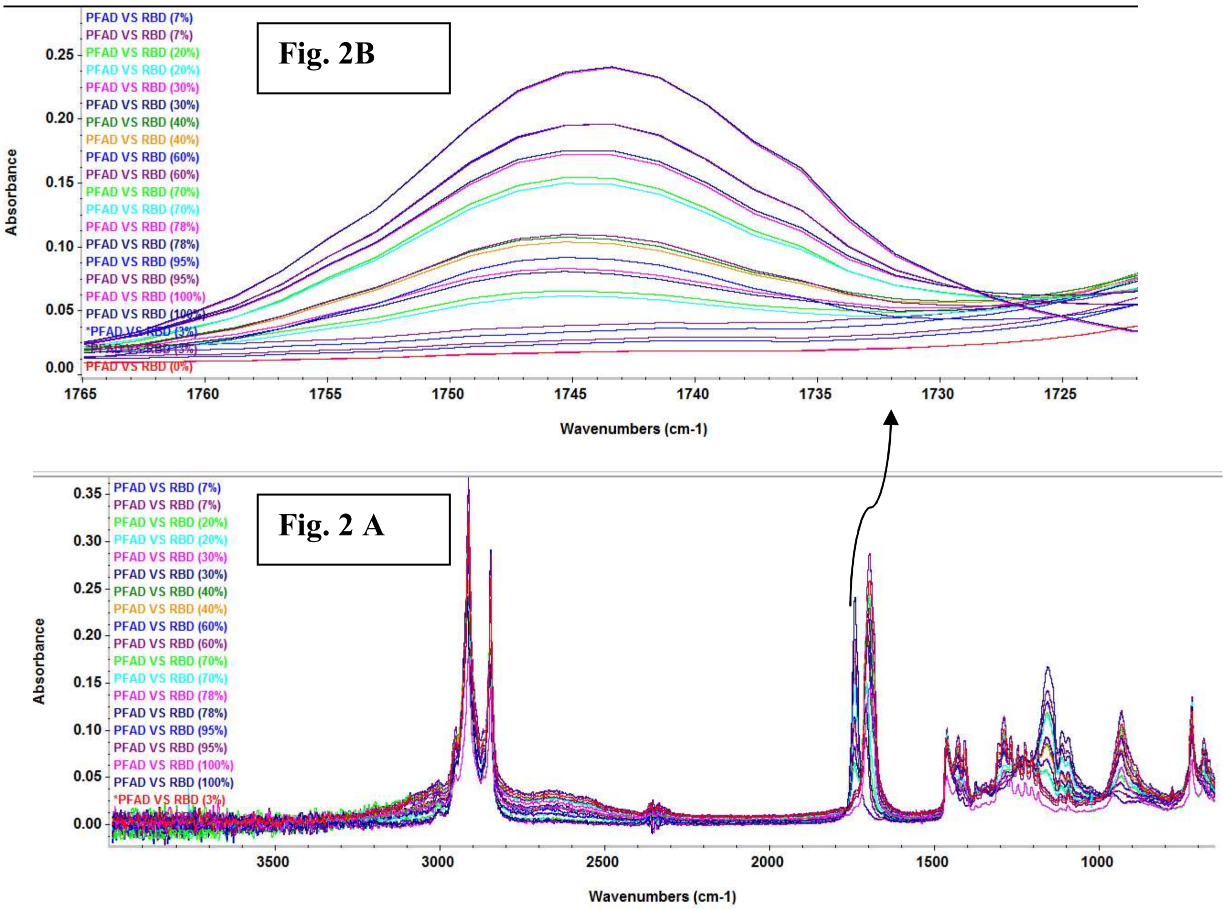
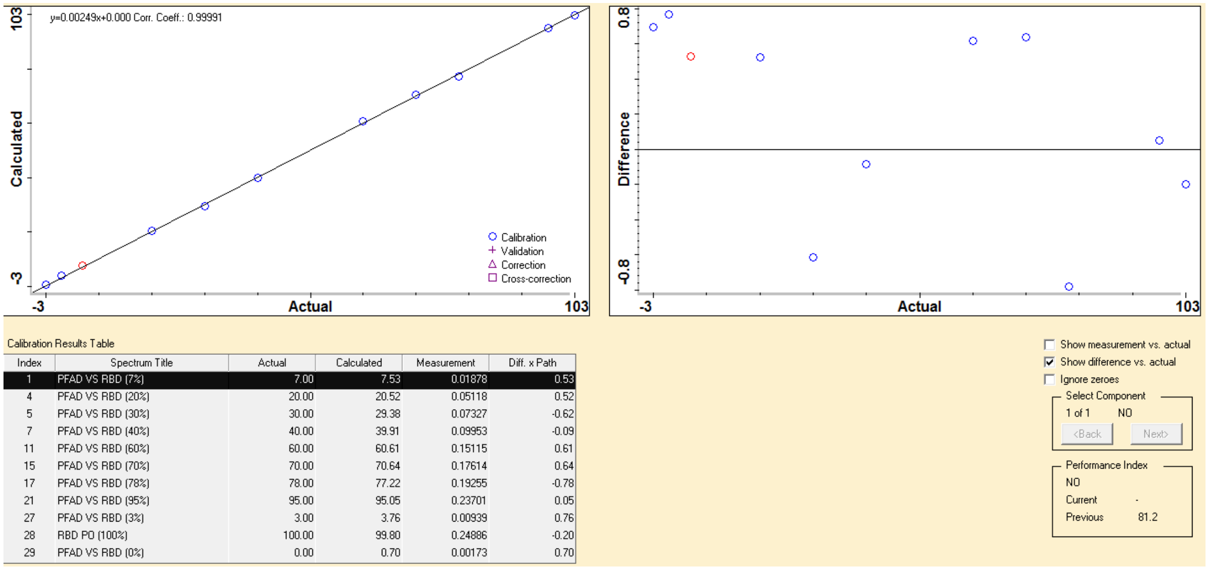


Fig. 2 Complete group spectra of neutral oil mixed in PFAD (2A) and expanded group spectra of selective carbonyl band (1744 cm^{-1}) used in calibration model.



y=0.00249x+0.000 Corr. Coeff.: 0.99991

Calibration Results Table

Index	Spectrum Title	Actual	Calculated	Measurement	Diff. x Path
1	PFAD VS RBD (7%)	7.00	7.53	0.01878	0.53
4	PFAD VS RBD (20%)	20.00	20.52	0.05118	0.52
5	PFAD VS RBD (30%)	30.00	29.38	0.07327	-0.62
7	PFAD VS RBD (40%)	40.00	39.91	0.09953	-0.09
11	PFAD VS RBD (60%)	60.00	60.61	0.15115	0.61
15	PFAD VS RBD (70%)	70.00	70.64	0.17614	0.64
17	PFAD VS RBD (78%)	78.00	77.22	0.19255	-0.78
21	PFAD VS RBD (95%)	95.00	95.05	0.23701	0.05
27	PFAD VS RBD (3%)	3.00	3.76	0.00939	0.76
28	RBD PO (100%)	100.00	99.80	0.24886	-0.20
29	PFAD VS RBD (0%)	0.00	0.70	0.00173	0.70

☐ Show measurement vs. actual
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Fig. 3. Calibration of 0 to 100% of neutral oil standards using Beer law in TQ Analyst program

Method validation was performed according to AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals.[22] Recovery test on the selected sample of PFAD was carried out by the addition of known amount of RBD palm oil. The statistics of recovery test of the proposed method (Table 1) demonstrated high recovery performance i.e. 100.17, 100.37 and 99.3 % with excellent RSD values of 1.64, 1.82, and 1.93 % at levels of 50.38, 30.08 and 5.16 % neutral oil, respectively; whereas acceptable recovery is 90–108%.[22] The % recovery results revealed that there is no significant interference effect from any other component present in PFAD. Therefore, it could be confidently claimed that the proposed method is feasible without using any solvent or chemical neutral oil could be rapidly checked by FT-IR spectroscopy with a green approach.

Tab. 1. Recovery test of neutral oil by Standard Addition Method by FT-IR

***A (g)**	****B (g)**	**Ratio of A & B**	*****C (%)**	**Amount found by FT-IR (%)**	**Recovery (%) with S.D (±)**	**RSD (%)**	**Acceptable recovery[29] (%)**
10.0	0.0	100:0	0.0	0.27±0.00	---	---	
5.0	5.0	50:50	50.0	50.38±0.83	100.76	1.64	90 – 108 %
7.0	3.0	70: 30	30.0	30.08±0.55	100.17	1.82	
9.5	0.5	95:05	5.0	5.16±0.10	103.20	1.93	

***A- PFAD with no neutral oil**

****B- Amount of RBD palm oil added**

*****C- Actual amount of neutral oil by Standard AOCS Method**

Statistical Figures of merit provided in Table 2 clearly indicates that SB-ATR FT-IR method could be used as alternative to standard AOCS method.

Tab. 2. Statistical Figures of merit for FT-IR method validation

Parameters	Beer's Law model
Mean Recovery (%)	101.38
Relative Standard Deviation (RSD)	1.80
Correlation Coefficient	0.999
Limit of Detection (LOD) (%)	0.48
Limit of Quantification (LOQ) (%)	0.85

The intra-day precision (Table 2) provided RSD value of approximately 1.9%. The data show a good precision of the method, since mean RSD value is less than 2%. Precision and roughness results of FT-IR method are shown in Table 3 and 4, respectively. The recoveries, standard deviation and coefficient of variance were found to be excellent and workable. As RSD in all cases was less than 2% and the recoveries were in between 100-103% indicating high degree for the accuracy of FT-IR method. Results of robustness of the method are given in Table 4. As RSD values are smaller than 2% and furthermore, robustness was confirmed by F test homogeneity of variance, P = 5%.

The results of present study accomplished significant benefits in terms of speed, simplicity, accuracy as a green method for determination of neutral oil present in PFAD using FT-IR spectroscopy.

Tab. 3. Assay of neutral oil in selected PFAD sample

Day	Concentration of Neutral oil (%)	RSD (%)
1	5.16±0.10	1.93
2	4.93±0.09	1.82
3	5.12±0.10	1.95
Mean Value	5.07±0.10	1.90

Tab.4. Data of ruggedness studies

FT-IR Results		RSD
Analyst 1	5.16±0.10	1.93
Analyst 2	5.08±0.10	1.97
Mean Value	5.12±0.10	1.95

3.3. Application of FT-IR Method

Results of neutral oil in PFAD samples by AOCS Standard and FT-IR methods in two selected industries are presented in Table 5. Results of both methods were found to be comparable. However, great variations were found among the samples of both industries. The amount of neutral oil by FT-IR method in the samples of Industry 1 was observed in the range between 9.52-22.91% whereas the amount of neutral oil in samples of Industry 2 was detected in the range between 5.72-9.96% with the mean value 15.87% and 5.38%, respectively. Reason behind the enormous variation between the results of two industries is not clearly known that either it is efficiency of designing of deodorizer or skilled manpower or check and control system that all PFAD samples of Industry 2 contained less amount of neutral oil than Industry 1.

Tab. 5. Neutral oil (%) in PFAD samples by AOCS Standard and FT-IR methods in two selected industries. Each value is mean of triplicate analysis and RSD was less than 5%.

PFAD Samples Number	**Industry 1**		**Industry 2**	
	AOCS Method (%)	**FT-IR Result (%)**	**AOCS Method (%)**	**FT-IR Result (%)**
1	12.70	12.82	8.63	8.73
2	9.47	9.52	ND	ND*
3	22.72	22.91	5.26	5.72
4	10.13	10.46	5.63	5.88
5	19.32	19.76	6.15	6.82
6	18.74	18.91	ND	ND
7	13.45	13.95	9.64	9.96
8	18.27	18.67	5.91	5.97
AV**	**15.6**	**15.87**	**5.15**	**5.38**

***ND, Not detected**
****AV, Average value of all eight samples**
All other values in table are mean of triplicate analysis and RSD was less than 5%.

4. Conclusions

Results of present study clearly indicated that for the determination of neutral oil, FT-IR spectroscopic method using Beer's law provided remarkable advantages in terms of speed and cost over the AOCS standard method which is very laborious and needs large amounts of toxic chemicals and solvents. For preparation of standards, only gravimetrically mixing of PFAD and RBD palm oil is involved to generate a calibration model. The proposed FT-IR method is a green method in which no extraction, pre-concentration, purification or any other pretreatment was used. Therefore, use of any solvent, chemical or costly standard was avoided which leads to economical and environmental friendly method. Environmental issue and cost for the disposal of used solvents is not associated with the proposed FT-IR method. Besides, only one drop of sample is required, and less than 2 min are required to take results of neutral oil present in deodorizer distillates from FT-IR spectrum to increase sample throughput. By using proposed method great loss of neutral oil from deodorizer to PFAD could be timely controlled by getting quick information from the FT-IR method. Also from food security point of view it is very important to control the neutral oil which is going to be waste in deodorizer distillate in addition to economical loss of industry.

Conflict of interest

The authors declare that they don't have any conflict of interest.

Acknowledgements

The authors thank the Universiti Teknologi Malaysia (UTM), Johor Bahru, Johor, Malaysia and National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan for the financial support of this work.

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