

## FTIR spectroscopy combined with multivariate calibration for analysis of cod liver oil in binary mixture with corn oil

<sup>1,2</sup>Rohman, A., <sup>1\*</sup>Che Man, Y. B., <sup>1</sup>Ismail, A. and <sup>1</sup>Puziah, H.

<sup>1</sup>Laboratory of Analysis and Authentication, Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

<sup>2</sup>Laboratory of Analytical Chemistry, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia.

**Abstract:** FTIR spectroscopy in combination with multivariate calibrations, i.e. partial least square (PLS) and principle component regression (PCR) was developed for quantitative analysis of cod liver oil (CLO) in binary mixture with corn oil (CO). The spectra of CLO, CO and their blends with certain concentrations were scanned using horizontal attenuated total reflectance (HATR) accessory at mid infrared (MIR) region of 4,000 – 650  $\text{cm}^{-1}$ . The optimal spectral treatments selected for calibration models were based on its ability to provide the highest values of coefficient of determination ( $R^2$ ) and the lowest values of root mean error of calibration (RMSEC). PLS was slightly well suited for quantitative analysis of CLO compared to PCR. FTIR spectroscopy in combination with multivariate calibration offers rapid, no excessive chemical reagent, and easy in operational to be applied for determination of CLO in binary mixture with other oils.

**Keywords:** FTIR spectroscopy, cod liver oil, partial least square, principle component regression

### Introduction

Currently, cod liver oil (CLO) has attracted widespread interest in the scientific fields because of its beneficial effects to human health attributed to high levels of the omega-3 fatty acids, like *cis*-5,8,11,14,17-eicosapentaenoic (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic (DHA) present in the form of triglycerides (Zeng *et al.*, 2010). CLO is one of the oil components used in pharmaceutical preparations as a source of vitamins A and D, and is persistently being sold as medicines or functional food oils, either in capsule or suspension formulations (Aursand *et al.*, 2007). In addition, corn oil which is obtained from seeds of *Zea mays* is usually used as vehicle in certain pharmaceutical formulation such as suspension and emulsion in which CLO is one of the active components in such formulation (Alvarez and Rodríguez, 2000). For this reason, analysis of CLO in binary mixture with corn oil needs to be developed.

Edible oils are typically analyzed by determining certain components such as fatty acid and triglyceride compositions rather than analysis of oils as a whole matter (Rohman and Che Man, 2010<sup>a</sup>), therefore Fourier transform infrared (FTIR) spectroscopy is developed in order to overcome this problem. FTIR spectroscopy is a fast and non-destructive technique, sensitive, and simple in sample preparation. Currently, FTIR has been widely used for analysis of edible fats and oils due to its capability to serve as “fingerprint technique”, especially in combination

with chemometrics of multivariate calibration (Che Man *et al.*, 2010). Two multivariate calibrations commonly used are partial least square (PLS) and principal component regression (PCR). Both calibration methods are based on reduction of spectral data and inverse calibration (Paradkar *et al.*, 2002).

Our group has developed FTIR spectroscopy in combination with chemometrics of multivariate calibration for determination of virgin coconut oil (VCO) in binary mixtures with palm kernel oil (Manaf *et al.*, 2007) and palm oil (Rohman and Che Man, 2009<sup>a</sup>); quantification of lard in CLO (Rohman and Che Man, 2009<sup>b</sup>) and lard in binary mixtures with animal fats (Rohman and Che Man, 2010<sup>a</sup>) and with some vegetable oils (Rohman *et al.* 2010<sup>b</sup>), and for analysis of extra virgin olive oil mixed with palm oil (Rohman and Che Man, 2010<sup>b</sup>). In addition, we also developed FTIR spectroscopy for quantitative of selected edible oils in ternary mixtures (Rohman and Che Man, 2010<sup>c</sup>). In the present study, we developed FTIR spectroscopy combined with PLS and PCR for quantitative analysis of CLO in binary mixture with corn oil as a oil model.

### Material and Methods

#### Sample preparation

Cod liver oil (CLO) and corn oil (CO) were purchased from local retail market in Serdang, Selangor, Malaysia. In order to assure the purity

\*Corresponding author.  
Email: yaakobcm@gmail.com

criteria of both oils, fatty acid composition was determined using GLC as derivative of fatty acid methyl ester (FAME) according to Rohman and Che Man (2009<sup>a</sup>). The standard of 37 FAME (C4 – C24) was obtained from Sigma Aldrich Chemicals (Deisenhofen, Germany). All reagents and solvents used throughout this study were of pro analytical grade.

#### Calibration and prediction samples

For calibration samples, a series of 20 samples containing CLO at concentration ranges of 1.0 – 50.0 % (v/v) as neat form in binary mixture with CO is prepared. For prediction samples, 17 independent samples with known concentration were also made. To ensure the homogenization of mixtures, the samples were mixed using vortex at 2000 rpm for 2 min. All samples were subjected to FTIR analysis.

#### FTIR analysis

FTIR spectra of oil samples were scanned using FTIR spectrometer Nicolet 6700 (Thermo Nicolet Corp., Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS). The software of OMNIC (Version 7.0 Thermo Nicolet) was used to operate and treat FTIR spectra. Using Pasteur pipette, the oil samples were located in good direct contact with horizontal attenuated total reflectance (HATR) composed of zinc selenide crystal at 25°C. FTIR spectra were collected in region of 4000 – 650 cm<sup>-1</sup> by co-adding 32 scans and at resolution of 4 cm<sup>-1</sup>. All spectra were rationed against a background of air spectrum. For quantitative analysis, all FTIR spectra were recorded as absorbance modes.

#### Data treatment

The software of TQ Analyst<sup>TM</sup> (Thermo electron Corporation) was used to construct calibration and validation models using PLS and PCR. In order to overcome the over fitting problem, the cross-validation using “leave-one-out” procedure was used to verify the calibration models. The values of root mean error of calibration (RMSEC) and coefficient of determination (R<sup>2</sup>) were used as the validity criteria for the calibration. The RMSEC value can be used as an indicative of the uncertainty in calibration model; consequently, the smaller the RMSEC value, the smaller the uncertainty in the developed model. Furthermore, the R<sup>2</sup> value and root mean square error of prediction (RMSEP) were used as validity criteria in prediction samples (Gallardo-Velázquez *et al.*, 2008).

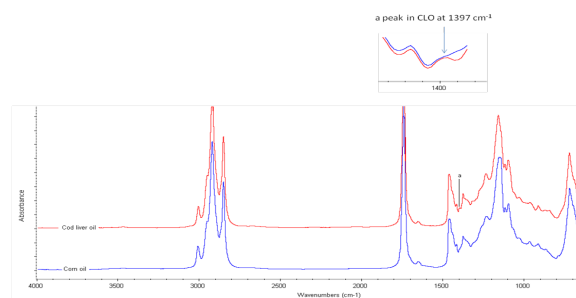
## Results and Discussion

The fatty acid compositions of CLO and CO were shown in Table 1. As cited in Standal *et al.* (2008), European Pharmacopea specified that CLO intended for medicinal use has the level of EPA and DHA in the range of 7-16 % and 6 – 18%, respectively. Both fatty acids contained in the used CLO (EPA of 15.70 ± 0.35%, DHA of 8.90 ± 0.22%) were in agreement in the specified monograph of European Pharmacopea. In addition, the used CO has the FA compositions which are in accordance with those ranged in Codex Alimentarius.

**Table 1.** Fatty acid composition of cod liver oil and corn oil

Fatty acids <sub>‡</sub>	Cod liver oil	Corn oil
Myristic (C14: 0)	4.15 ± 0.21 11.86	0.05 ± 0.00 12.99
Palmitic (C16: 0)	± 0.43 6.53	± 0.60 0.10
Palmitoleic (C16: 1)	± 0.23 2.3	± 0.01 2.01
Stearic (C18: 0)	± 0.09 21.18	± 0.06 27.48
Oleic (C18: 1)	± 0.30 1.42	± 0.18 53.13
Linoleic (C18: 2)	± 0.07 0.96	± 0.69 0.39
Linolenic (C18: 3)	± 0.05 0.11	± 0.00 0.72
Arachidic (C20: 0)	± 0.01 11.49	± 0.02 0.42
Eicosenoic (C20:1)	± 0.41 0.2	± 0.00 0.20
Behenic acid (C22:0)	± 0.00	± 0.00
Eicosapentaenoic acid (C20:5 n3)	16.80 ± 0.35	nq
Docosahexaenoic acid (C22:6 n3)	8.90 ± 0.22	nq

<sup>‡</sup>obtained from three replications. Standard deviation (SD) values of means are after ±. nq = not quantified



**Figure 1.** FTIR spectra of cod liver oil and corn oil scanned at 4,000 - 650 cm<sup>-1</sup>

#### Spectral analysis

Figure 1 reveals FTIR spectra of cod liver oil and corn oil at mid infrared region (4,000 – 650 cm<sup>-1</sup>). Two spectra show very closely and make difficult to be differentiated using naked eye, because approximately of 95% of the edible oil triglyceride, a glycerol esterified with fatty acids. However, due to its capability as fingerprint technique (meaning that there is no two oils having the same spectra in term of exact frequency positions and band intensities), FTIR spectroscopy can be used as tool to make a

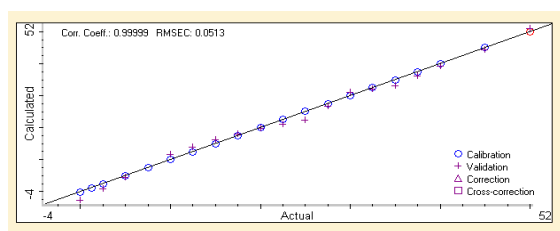
first differentiation, especially in combination with chemometrics. Using a closer scrutiny, there is a peak at  $1397\text{ cm}^{-1}$  (attributed to C-O stretching vibration) in CLO as assigned with “a”, which is absent in corn oil. For this reason, frequency regions at  $1480 - 1375\text{ cm}^{-1}$  were selected for quantitative analysis of CLO in binary mixtures with CO. Dufour (2009) as well as Guillen and Cabo (1997) have described the functional groups which absorb IR radiation, together with the mode of vibration.

### Quantitative analysis

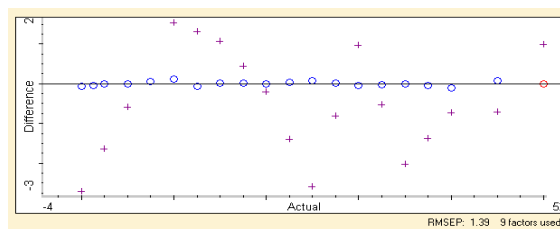
Quantitative analysis of CLO in binary mixture with CO was performed using multivariate calibrations of PLS and PCR, either using normal spectra or its derivatives (first and second). In the PLS and PCR calibration models, the method linearity was evaluated to show the relationship between responses (FTIR spectra) versus concentrations of CLO in the binary mixture with CO spanning the working concentration range. The values of  $R^2$  value and RMSEC from the PLS and PCR calibrations were used to choose the best treatments of spectra (normal or derivatives) in the spectral ranges of  $1480 - 1375\text{ cm}^{-1}$ . Table 2 exhibits the results obtained from the PLS and PCR calibrations in terms of  $R^2$  and RMSEC values.

**Table 2.** Calibration statistics for analysis of CLO content in CO using PLS and PCR techniques at frequencies of  $1480 - 1375\text{ cm}^{-1}$

Spectral ranges ( $\text{cm}^{-1}$ )	PLS		PCR	
	$R^2$	RMSEC (% v/v)	$R^2$	RMSEC (% v/v)
normal	0.999	0.051	0.996	0.874
First derivative	0.994	1.12	0.998	0.614
Second derivative	1.000	0.063	0.9991	0.063



A



B

**Figure 2.** Calibration model for relationship between actual value of CLO and FTIR calculated value (A), and the difference of both values in validation model (B)

The multivariate calibration model was subsequently used to predict CLO levels in prediction samples, which are prepared independently with known concentrations. The  $R^2$  and RMSEP values were computed for the relationship between actual CLO value and FTIR calculated value at  $1480 - 1375\text{ cm}^{-1}$ . The  $R^2$  and RMSEP values were employed to evaluate the analytical figure of merits of prediction samples. These statistical parameters are compiled in Table 3.

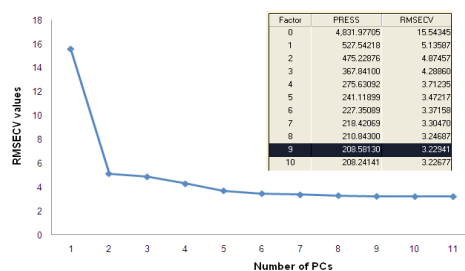
**Table 3.** The validation performance for analysis of CLO content in CO using PLS and PCR techniques at frequencies of  $1480 - 1375\text{ cm}^{-1}$

Spectral ranges ( $\text{cm}^{-1}$ )	PLS		PCR	
	$R^2$	RMSEP (% v/v)	$R^2$	RMSEP (% v/v)
normal	0.992	1.39	0.988	1.83
First derivative	0.982	2.16	0.982	2.03
Second derivative	0.883	5.48	0.879	5.67

Based on the highest values of  $R^2$  and the lowest values of RMSEC and RMSEP (as shown in Table 2 and 3), it can be concluded that normal spectra was well suited for determination of CLO in the mixture with CO using PLS calibration. Both high value of  $R^2$  and the low value of RMSEP indicated the success of the PLS calibration model in normal spectra for such analysis. Figure 2A exhibits the scatter plot for the relationship between actual value of CLO (x-axis) and FTIR calculated value (y-axis) using PLS in calibration model using the software of TQ Analyst, meanwhile the difference between actual and FTIR calculated values of CLO in validation model is represented in Figure 2B.

The proposed PLS model was further cross validated using ‘leave one out’ technique in order to test whether over-fitting happens during PLS calibration. Cross-validation evaluates the data by leaving one of calibration samples from the regression model and then performing a new model for the remaining calibration samples. The model is further evaluated using the samples leaved from the model and followed by calculating the error values for the predicted observations (Miller and Miller, 2005). After predicting all the samples using cross-validation technique, the root mean square error of cross validation (RMSECV) value was computed. The low RMSECV value obtained is 3.22% v/v. The number of principal components used in this study is 9, because this number offers the low value of the predicted residual error sum of square (PRESS). Figure 3 showed the RMSECV values obtained using different number of PCs. From the results, it can be declared that FTIR spectra with PLS regression is a promising method for analysis of CLO in the mixture with other oils. Besides, the developed method can

be extended to be applied in the analysis of CLO adulteration with the lower price oils like corn oil.



**Figure 3.** The correlation between the number of PCs or factor with RMSECV values in PLS calibration model for quantification of CLO in binary mixture with CO

## Conclusions

It can be concluded that Fourier transform Infrared (FTIR) spectroscopy combined with chemometrics of multivariate calibrations of PLS and PCR at frequency regions of 1480 – 1375  $\text{cm}^{-1}$  can be used to quantitatively analyze CLO contents blended with CO. The developed method was fast; with a total analysis time at about 3 min for each measurement, and it is environmentally friendly. Furthermore, the tedious time and chemical consuming reagents and solvents can be avoided.

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