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Application of FTIR Spectroscopy Combined with Multivariate Calibrations for Analysis of Chloramphenicol and Hydrocortisone Acetate in Cream Samples

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ABSTRACT

Analysis of chloramphenicol (CL) and hydrocortisone acetate (HCA) in cream samples using FTIR (Fourier Transform Infrared) spectroscopy. The objective of this study was to develop FTIR spectroscopy combined with multivariate calibrations for effective analysis of CL and HCA in cream formulation. High performance liquid chromatography (HPLC) method was applied for determining the actual value of CL and HCA. Cream samples containing CL and HCA were scanned using FTIR spectrophotometer employing attenuated total reflectance (ATR) technique. FTIR-ATR spectra were subjected to several optimization including wavenumbers selection and derivatization for quantitative analysis. The results showed that the optimum prediction model for correlation between actual values of CL and HCA as determined by HPLC and FTIR-predicted values was obtained using first derivative FTIR spectra at wavenumbers of 1500-1000 cm^{-1}. The R^2 value for calibration and internal validation for CL and HCA was > 0.9 with relatively small RMSEC (root mean square error of calibrations), RMSECV (root mean square error of cross validations) and PRESS (predicted residual error sum of squares) values. External validation of CL and HCA models produced R^2 > 0.98 with RMSEP values of 0.6501 and 1.0041, respectively. The results of CL and HCA assay using HPLC and FTIR spectroscopy were compared resulting non-significant results ($p > 0.05$) for these two methods. FTIR spectroscopy in combination with PLS can be used as a rapid and reliable analytical method for determination of CL and HCA in cream formulation.

Keywords: chloramphenicol; hydrocortisone acetate; FTIR spectroscopy; partial least square; cream

INTRODUCTION

Chloramphenicol (CL) is an antibiotic isolated from *Streptomyces venezuelae* and currently, this antibiotic has been produced synthetically. CL is a wide spectrum of antibiotics with bacteriostatic effects (Brayfield *et al*., 2014; Livingston *et al*., 2013). In addition, hydrocortisone acetate (HCA) is one of the synthetic steroid anti-inflammation drugs with very low solubility in water (Moffat *et al*., 2011; Altamimi *et al*., 2019).

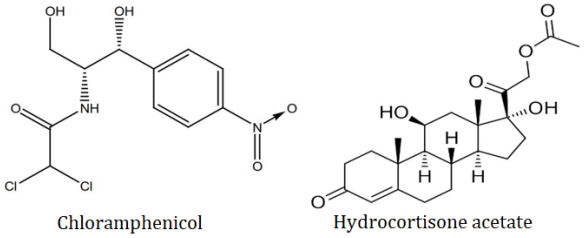

Figure 1. The chemical structures of chloramphenicol and hydrocortisone acetate.

The combination of chloramphenicol (CL) (Figure 1) and hydrocortisone acetate (HCA) (Figure 1) in cream preparation is intended to have an activity as anti-infection and to treat dermatitis, and this preparation is available in the market. In order to assure the quality of this cream preparation, fast and reliable methods must be developed.

Due to its capability to provide good separation, chromatographic-based methods are considered as method of choice for analysis of multicomponent active ingredients in pharmaceutical formulation. Thin layer chromatography (TLC) combined with densitometric measurement has been used for analysis of creams containing CL-HCA (Kristiningrum and Rakhmawati, 2021) while HPLC methods were successfully used for analysis of CL-prednison acetate in eye drops (Katakam, 2012), simultaneous analysis of CL-natrium dexametason in eye drops (Shadoul, *et al*., 2011), analysis of CL-dexametason-dexametason phosphate in suspension formulation intended to eyes (Iqbal *et al*., 2006), and analysis of CL-hydrocortisone (Xiaoyan, 1998). The other methods are ultraviolet spectroscopy for analysis of miconazole-HCA simultaneously (Abbas *et al*., 2017) and FTIR spectroscopy for analysis of CL (Karthikeyan, 2011). However, using literature review, there is no publication reporting the simultaneous analysis of CL-HCA using infrared spectroscopy method.

Analytical methods using FTIR spectroscopy and chemometric had proven effective for qualitative and quantitative analysis, fast, inexpensive, and non-destructive method (Roggo *et al*., 2007; Rohman *et al*., 2016; Guntarty *et al*., 2019). The results of the HPLC determination can be compared to the results of FTIR spectroscopy analysis using Partial Least Square (PLS) multivariate calibration. This research is expected to produce a new method that is simpler, fast, cheaper and more environmentally friendly in assay of CL and HCA by HPLC and FTIR spectroscopy combined with multivariate analysis.

MATERIAL AND METHODS

Materials

The reference standards of Chloramphenicol (CL) and Hydrocortisone Acetate (HCA) were obtained from *Badan Pengawas Obat dan Makanan* (The National Agency of Drug and Food Control), Republic of Indonesia. Methanol and acetonitrile for HPLC grade (*Lichrosolv)* were obtained from E. Merck (Darmstadt, Germany). Aqua pro injection was purchased from Ikapharmindo (Indonesia). The other solvents and reagents were of pro-analytical grade and bought from E. Merck (Darmstadt, Germany). The commercial samples were obtained from several pharmacy around Yogyakarta, Indonesia.

HPLC analysis

Analysis of CL and HCA was performed on Shimadzu LC20AD autosampler (SIL20A) using UV detector (SPD20) and photo-diode array PDA (SPD20MA) equipped with Labsolution software. Separation was carried out on a C-18 Waters X-Bridge (250x4.6 mm i.d, 5µm) column using mobile phase composed of acetonitrile-water (47:53 volume/volume). The mobile phase was delivered isocratically at flow rate of 1.0mL/min. For analysis of CL and HCA in cream samples, cream samples containing CL and HCA equivalent to 4mg CL and 5mg HCA were accurately weighed using analytical balance, added with 5mL methanol, sonicated for 10min and made to 10mL in volumetric flask. Amount of 4.0mL solution was taken and diluted with acetonitrile-water (1:1) and made into 20.0mL in calibrated volumetric flask. The solution was filtered using microfilter PTFE 0.45 µm and then injected into HPLC systems.

Analysis of CL-HCA in creams using FTIR spectroscopy

Samples (creams) were placed in direct contact with attenuated total reflectance (ATR) using FTIR spectrophotometer (Thermo Scientific Nicolet iS10) equipped with OMNIC and TQ Analyst softwares. FTIR spectra were obtained from 32 scanning at mid infrared region (4000-650cm^{-1}) using resolution of 16cm^{-1}. FTIR spectra acquisition were performed in absorbance values to facilitate quantitative analysis of analytes of interest. The air spectra were used as background spectra.

Data analysis

Two multivariate calibrations of partial least square (PLS) and principle component regression (PCR) were optimized for analysis of CF and HCA in creams. The selection of conditions used were analyzed statistically by calculating coefficient of determination (R^2 value), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) (Miller and Miller, 2010). All calculations were performed using software of TQ Analyst.

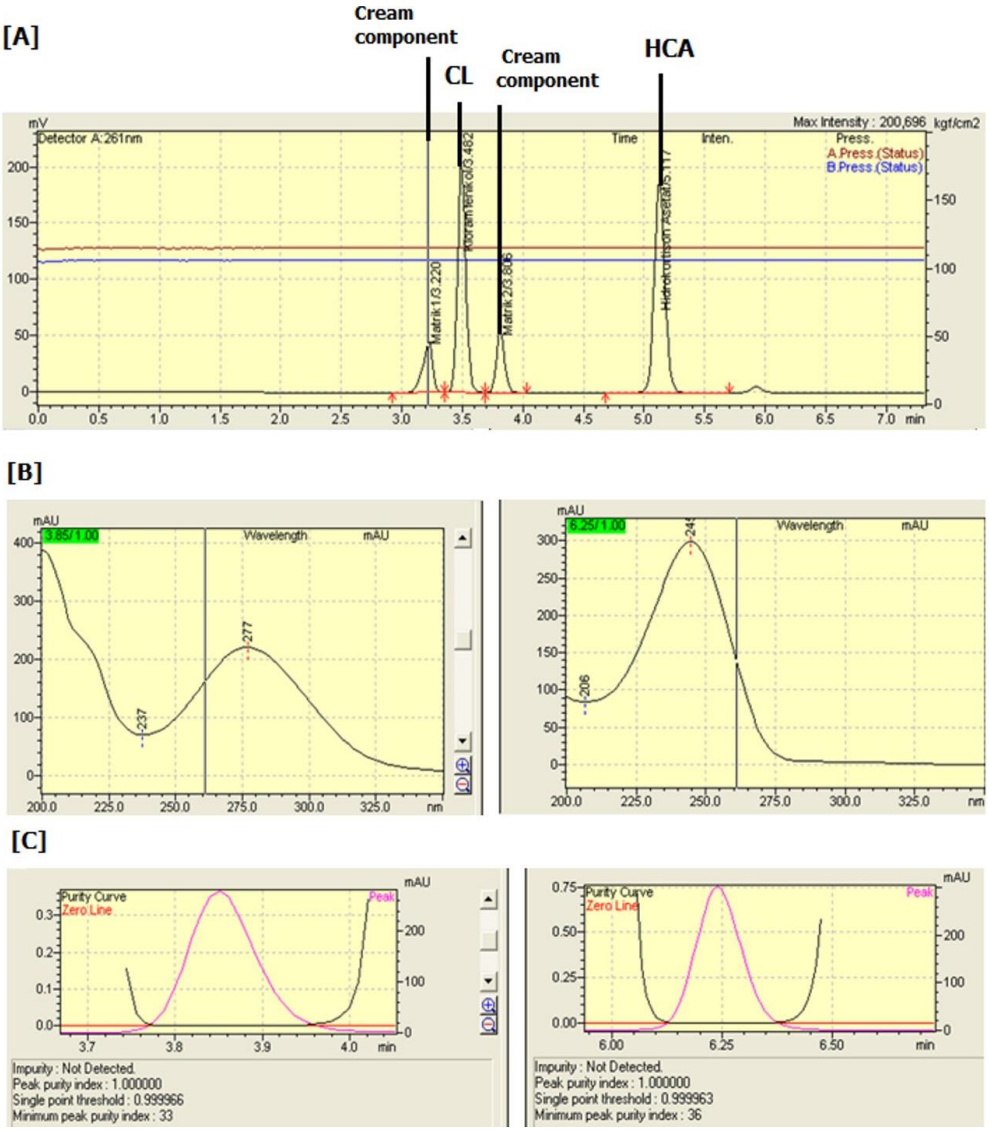

Figure 2. HPLC chromatogram for separation of chloramphenicol (CL) and hydrocortisone acetate with other matrix components in cream samples [A], along with UV spectra [B] and purity indexes [C] of CL (left) and HCA (right). See section methods for HPLC condition.

RESULTS AND DISCUSSION

Analysis of CL and HCA using FTIR spectroscopy was facilitated using multivariate calibration of partial least square (PLS) for modelling the actual values of CL and HCA as determined by HPLC and predicted values as determined using FTIR. Using the optimized condition based on experimental design approach, HPLC could separate analytes from other matrix with good separation with resolution (Rs) values of >2.00. The retention times (tR) of CL and HCA were of 3.482 and 5.117min, respectively (Figure 2A). In addition, the peaks in HPLC chromatogram corresponded with analytes (CL and HCA) as indicated by its UV spectra (Figure 2B) with high purity indexes (Figure 2C). The concentrations of CL and HCA in cream samples determined by HPLC were used as actual values and compiled (Table I).

The FTIR spectra of creams containing CL and HCA using attenuated total reflectance (ATR) technique (Figure 3). Quantitative analysis of CL and HCA was performed using FTIR spectroscopy and multivariate calibration assisted by software TQ Analyst®.

Table I. The actual values of chloramphenicol (CL) and hydrocortisone acetate (HCA) as determined with HPLC along with predicted values of CL and HCA using FTIR spectroscopy combined with multivariate calibration

Samples	Concentrations (mg/g)			
	Chlorampenicol		Hydrocortisone acetate	
	Actual concentrations as determined by HPLC	Predicted concentration as determined by FTIR-PLS	Actual concentrations as determined by HPLC	Predicted concentration as determined by FTIR-PLS
1	11.208	11.233	16.103	16.114
2	11.208	11.612	16.103	16.692
3	13.989	13.640	20.184	19.590
4	13.989	13.931	20.184	20.154
5	14.512	14.566	21.017	21.015
6	14.512	15.555	21.017	20.899
7	14.943	14.758	21.689	21.396
8	14.943	14.949	21.689	21.711
9	15.185	15.200	21.256	21.258
10	15.185	14.803	21.256	21.007
11	15.934	15.886	22.257	22.249
12	15.934	15.869	22.257	22.234
13	16.817	16.824	23.871	23.867
14	16.817	17.274	23.871	24.264
15	18.219	18.198	24.715	24.720
16	18.219	17.929	24.715	24.530
17	18.553	18.555	25.287	25.290
18	18.553	18.576	25.287	24.900
19	21.081	21.079	29.349	29.344
20	21.081	21.140	29.349	29.396
21	21.409	21.424	29.771	29.780
22	21.409	21.683	29.771	30.040
23	23.549	23.314	32.064	31.922
24	23.549	23.556	32.064	32.061
	Equation: $y = 0.9881 x + 0.2350$; $R^2 = 0.9930$; RMSEC = 0.3531		Equation: $y = 0.9985 x + 0.0064$; $R^2 = 0.9972$; RMSEC = 0.2941	

Some optimization steps were performed by evaluating the spectral treatment (normal versus derivatives) and selecting the best wavenumber region capable of reliable accuracy (indicated by R^2 value) and precision (low RMSEC and RMSEP values).

FTIR spectroscopy could be considered as selective method due to its properties as fingerprint analytical technique (Karoui *et al*., 2010). The wavenumbers range from 4000-650cm^{-1} are commonly used for sample measurement because it contains some important regions for differentiation (Rohman *et al.,* 2019). Peak at wavenumber of 3333 cm^{-1} corresponded to the stretching vibration of hydrogen bonding (-OH). The intense peak at 1639cm^{-1} associated with stretching vibration of carbonyl (C=O) group. The presence of functional group of N=O from ($R\text{-}NO_2$) was assigned from peak at wavenumber of 1356cm^{-1}, while the functional group of $–CH_2$- could be seen in wavenumber of 1468cm^{-1}. These functional groups confirmed the presence of CL and HCA in creams (Pavia *et al*., 2009).

The optimum results for simultaneous prediction of CL and HCA using FTIR spectroscopy were obtained using the first derivative FTIR spectra with 10 factors at wavenumbers of 1500-1000cm^{-1} providing R^2 of CL and HCA of > 0.9999 with RMSEC values of 0.0274 (CL) and 0.0358 (HCA), respectively (Table II). Figure 4 shows the calibration models which correlate between actual values of CL and HCA as determined by HPLC and FTIR predicted values.

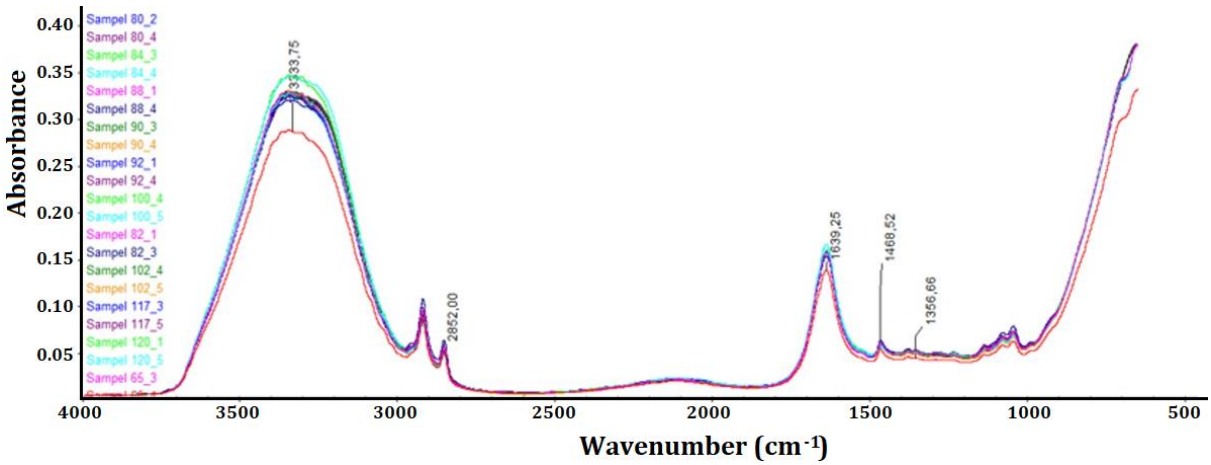

Figure 3. FTIR spectra of cream samples containing chloramphenicol and hydrocortisone acetate at wavenumbers of 4000-650cm^{-1}

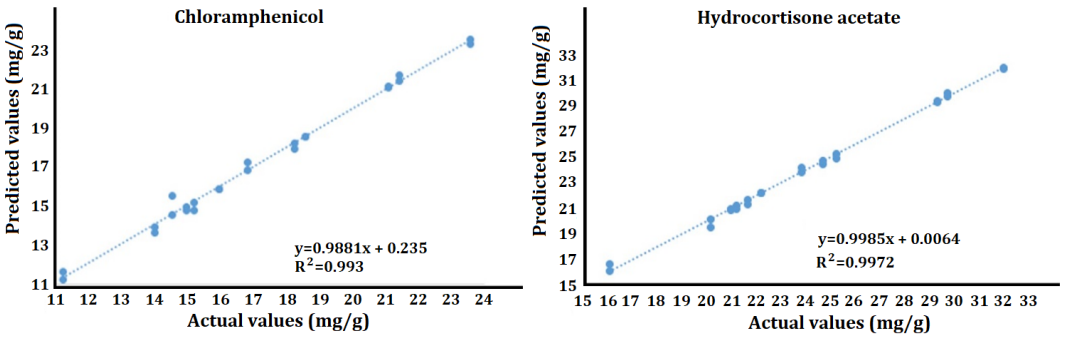

Figure 4. The correlation between actual values of chloramphenicol and hydrocortisone acetate as determined by HPLC and FTIR predicted values assisted with partial least square (PLS) calibration.

Table II. The performance of partial least square (PLS) for modelling the relationship between actual values of chloramphenicol and hydrocortisone acetate in creams samples.

Wavenumbers (cm^{-1})	Number of factors	Chloramphenicol					
		calibration		Validation		Cross validation	
		RMSEC	R^2	RMSEP	R^2	RMSECV	R^2
4000-650	8	0.0617	0.9988	1.4200	0.9352	1.7300	0.8627
4000-2500	5	0.4570	0.9910	1.8600	0.8741	2.8900	0.5742
1500-1000	10	0.0274	0.9999	0.2740	0.9968	1.0600	0.9544
1500-650	3	1.1900	0.9379	1.3700	0.9272	1.7900	0.8580
Wavenumbers (cm^{-1})	**Number of factors**	**Hydrocortisone**					
		Calibration		**Validation**		**Cross validation**	
		RMSEC	**R^2**	**RMSEP**	**R^2**	**RMSECV**	**R^2**
4000-650	8	0.0724	0.9999	1.9000	0.9188	2.2700	0.8552
4000-2500	5	0.5830	0.9911	2.3300	0.8763	3.5800	0.6072
1500-1000	10	0.0358	0.9998	0.3290	0.9973	1.2900	0.9614
1500-650	3	1.4200	0.9458	1.6900	0.9327	2.6400	0.8045

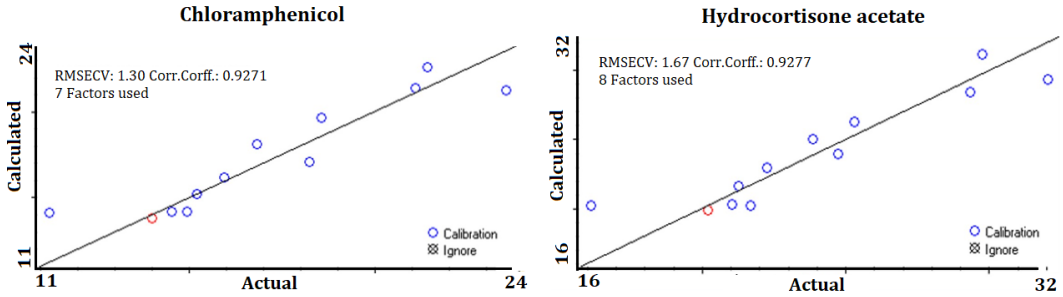

Figure 5. The correlation between actual values of chloramphenicol and hydrocortisone acetate and predicted values assisted with partial least square (PLS) calibration during cross validation

Table III. The results of chloramphenicol and hydrocortisone acetate determined by HPLC and FTIR spectroscopy combined with partial least square calibration.

Samples	Concentrations (mg/g)			
	Chloramphenicol		Hydrocortisone acetate	
	HPLC	FTIR-PLS	Aktual (KCKT)	FTIR-PLS
86.1	14.826	14.886	20.400	20.957
86.2	14.800	14.453	20.503	20.594
Average	14.813	14.670	20.452	20.776
RPD	0.176	2.952	0.504	1.747
94.1	15.910	15.464	22.623	22.521
94.2	15.930	15.580	22.448	22.695
Average	15.920	15.522	22.536	22.608
RPD	0.126	0.747	0.777	0.770
96.1	16.358	16.861	22.975	23.791
96.2	16.267	16.830	22.862	23.823
Average	16.313	16.846	22.919	23.807
RPD	0.558	0.184	0.493	0.134

RPD = relative percentage difference

The PLS calibration model was then cross validated using leave one out method. One of the calibration samples is taken out from PLS calibration model and the residual samples are used to build PLS model. Subsequently, the removed sample is calculated using the new PLS regression. This manner was repeated, leaving each sample out in turn and offering R^2 of 0.9271 and root mean square error of cross validation (RMSECV) of 1.30 (for CL) and R^2 of 0.9277 and RMSECV of 1.67 (HCA), as shown in Figure 5. Furthermore, the models were also validated using independent samples (external validation) and the correlation between actual values of CL and HCA determined using HPLC and FTIR predicted values were calculated and expressed by R^2-values. Besides, the difference between actual values and predicted values were expressed by root mean square error of prediction (RMSEP). During external validation, the obtained R^2 and RMSEP values were 0.9810 and 0.6501 for CL; 0.9859 and 1,0041 for HCA, respectively.

The optimized FTIR spectroscopy combined with PLS was then used for prediction of unknown samples and the results were compared with those determined by HPLC as presented in Table 3. PLS is one of multivariate calibrations commonly used to analyze analytes in complex samples (Khajehsharifi *et al.*, 2014). Both results were then statistically analyzed using independent t-test at significance level (P) of 0.05. Independent t-test resulted that both methods (HPLC and FTIR) are comparable for determination of CL and HCA as shown with P>0.05.

HPLC method using optimized condition showed good results for separation and quantification of CL and HCA. In this study, quantification result of CL and HCA using HPLC was used as an actual value when creating PLS model using FTIR spectra because HPLC is one of the confirmatory analytical methods which has become method of choice in some quantification studies. Result showed that combination of FTIR and PLS multivariate analysis demonstrated a good correlation with HPLC results. Correlation between FTIR and PLS toward HPLC analysis has also been studied for analysis of curcumin (Prabaningdyah *et al*., 2018). Therefore, FTIR is promising to be used for rapid quantification of CL and HCA in cream samples.

This study indicated that PLS multivariate calibration was successfully used for quantification of CL and HCA in cream samples. The variables used for creating PLS model were ordered systematically in matrix system and PLS look for latent variables to build high accuracy and precision model (Worley and Powers, 2013). Latent variables found the relationship between actual and predicted values in matrix. Evaluation of PLS model was performed using R^2, RMSEC, and RMSEP values. Model having high value of R^2 and low value of RMSEC and RMSEP was chosen as selected model. The high values of R^2 and low values of RMSEC and RMSEP indicated that FTIR spectroscopy using optimum conditions provided acceptable accuracy and precision for simultaneous analysis of CL and HCA (Pebriana *et al*., 2017). The difference between RMSEC and RMSEP values should be minimum to be considered as a valid model because it demonstrated the association between calibration and validation model. Model validation is important to be carried out to confirm the validity of the selected model to avoid overpredicting model that resulted bias data (Worley and Powers, 2013). Another validation method namely cross validation is an internal validation technique using different algorithm with external validation technique. Internal validation used the data originally from calibration model not from external samples. Low RMSECV value is preferred for model validity.

The selected PLS calibration model was applied to investigate the performance for quantification of samples with unknown concentration of CL and HCA. Results from several samples showed that combination of FTIR and PLS model could be used for quantification of CL and HCA in unknown samples. It suggested that combination of FTIR and PLS multivariate analysis is very potential and promising method to be used as rapid analytical technique for determination of CL and HCA in cream samples.

CONCLUSION

FTIR spectroscopy using variables of absorbance values of first derivative spectra at wavenumbers region of 1500-1000 cm^{-1} was successfully used for quantitative analysis of CL and HCA in cream samples. The analytical results of CL and HCA obtained using FTIR-partial least square were comparable to those by HPLC. The developed method is fast, simple in operation and environmentally friendly, and therefore can be proposed as alternative method to HPLC.

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