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Application of FTIR spectroscopy and multivariate calibration for analysis of curcuminoid in syrup formulation

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ABSTRACT

Curcuminoid, the main components in Curcuma species, is responsible for several biological activities including antioxidant and hepatoprotective effect, therefore fast and reliable analytical method is required for curcuminoid determination. The objective of this study was to develop Fourier transform infrared (FTIR) spectroscopy in combination with multivariate calibration of partial least square (PLS) and principle component regression (PCR) for quantitative analysis of curcumin (CUR) and desmethoxycurcumin (DMC) in syrup sample containing extract of *Curcuma xanthorriza* (temulawak). Syrup samples were directly scanned using FTIR spectrophotometer with attenuated total reflectance (ATR) sampling technique at wavenumbers 4000–650 cm^{-1} and its spectra were correlated with contents of CUR and DMC determined using high performance liquid chromatography. PLS offered better prediction model for the relationship between actual values of CUR and DMC and FTIR predicted values using absorbances at wavenumbers of 3004–974 cm^{-1} than that using PCR. PLS calibration model yielded R^2 of 0.9999 (calibration) and 0.9976 (for validation) for such correlation. The errors in calibration and validation expressed by root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were low, i.e. 0.0014 (RMSEC) and 0.0017 (RMSEP), respectively. FTIR spectroscopy in combination with PLS provide fast, accurate and precise method for determination of CUR and DMC in syrup samples.

INTRODUCTION

Curcumin (CUR) and desmethoxycurcumin (DMC) with chemical structures as in Figure 1 is main components of curcuminoid present in *Curcuma xanthooriza* (temulawak in Indonesia) (Lechtenberg *et al*., 2004). Both CUR and DMC was responsible for several biological activities including antioxidant, antibacterial, antiinflamation and anticancer activities (Duvoix *et al*., 2005; Shishodia, 2005). Both compounds were also used as chemical markers for quality assurance of pharmaceutical formulation using Curcuma species as main components (Li *et al*., 2011). Many traditional medicine and supplement containing curcuminoid had been produced in the form of tablet, syrup and also suspension.Therefore, reliable analytical method should be applied in order to assure product quality (Singh *et al*., 2014).

Several methods based on chromatographic methods such as thin layer chromatography (Péret-Almeida *et al*., 2005), high performance liquid chromatography using detectors of UV-vis (Korany *et al*., 2013), photodiode array (Korany *et al*., 2013), liquid chromatography-mass spectrometry (Jiang *et al*., 2006) and capillary electrophoresis (Anubala *et al*., 2016) were reported for analysis of CUR and DMC in different samples. However, this chromatographic method was time consuming and involving extensive laborious activities, thus, fast, simple and reliable analytical method for analysis of CUR and DMC in pharmaceutical formulation was highly needed (Tanaka *et al*., 2008). One of the potential method developed as alternative chromatographic technique was Fourier transform infafred (FTIR) spectroscopy (Rohman, 2012).

FTIR spectroscopy is fast and simple method for analysis of curcuminoid in any types of samples. FTIR spectroscopy

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at near region has been developed for analysis of CUR, DMC, bisdemethoxycurcumin and total curcuminoid, analysis curcuminoid in ethanolic extract of *Curcuma longa* (Tanaka *et al*., 2008), and for analysis of ethanolic extract of *Curcuma xanthorriza* in mid infrared region (Lestari *et al*., 2017). In our best knowledge, there is no publication reports regarding the application of FTIR spectroscopy for analysis of CUR and DMC in pharmaceutical formulation. The objective of this study was to develop FTIR spectroscopy in combination with multivariate calibration of PLS and PCR for assay of CUR and DMC in syrup samples.

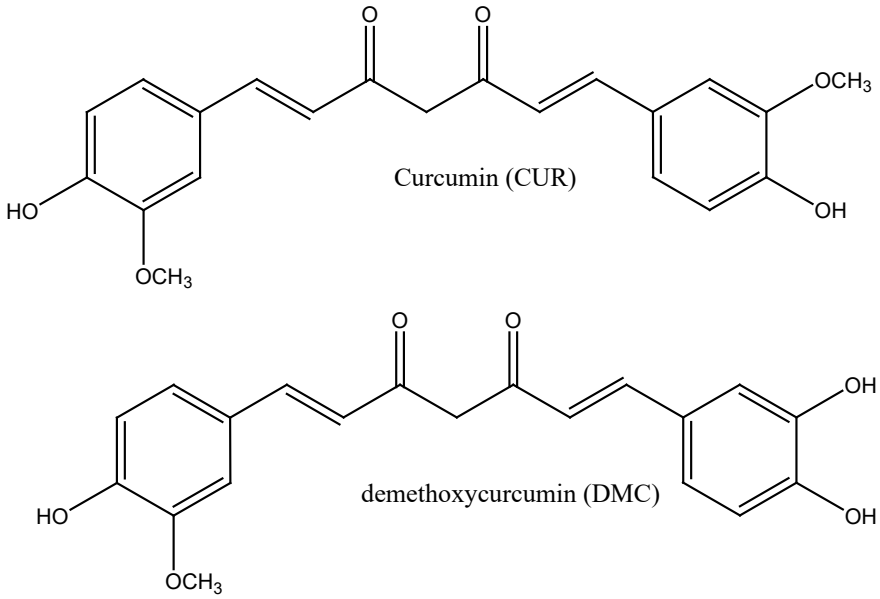


Fig. 1: The chemical structures of curcumin (CUR) and demethoxycurcumin (DMC).

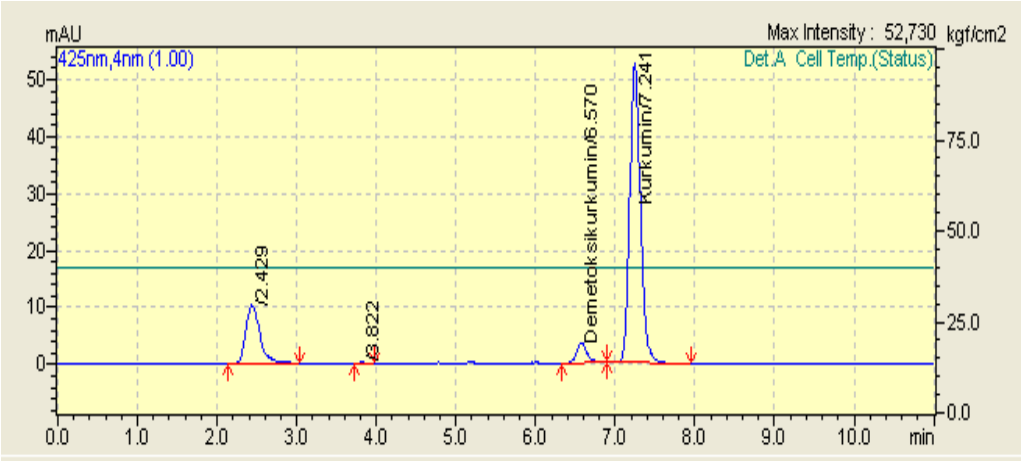


Fig. 2: Typical HPLC chromatogram of syrup samples containing Curcuma extract. CUR was stated as Kurkumin (RT. 7.241 min), DMC was stated as Demetoksikurkumin (RT. 6.570 min).

MATERIALS AND METHODS

Curcumin and demethoxycurcumin were isolated from curcuminoid purchased from E. Merck (Darmstadt, Germany). Isolation was performed using method as described in Péret-Almeida *et al*. (2005). To check the purity of both CUR and DMC, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were used. The samples of syrup formulations were purchased around Yogyakarta, Indonesia. The sample placebo was given by PT. SOHO Pharmaceutical Industry (Jakarta, Indonesia).

HPLC analysis

HPLC analysis was performed using Shimadzu HPLC instrument-LC-20AD (Japan) equipped with Rheodyne 7725i injection valve with a 20 µL loop volume and Binary gradient pump was used. The detector used was photodiode array (Shimadzu, SPD-M20A) operated at a wavelength of 425 nm. Data were acquired and processed by using LC-solution

software. Chromatographic separation was performed using RP 18 Waters® X-Bridge (250 mm × 4.6 mm i.d.; 5 µm) as reported by Prabaningdyah *et al*. (2017). The column temperature was set up at 40°C. The mobile phase composition was acetonitrile-acetic acid 4.08% (49: 51 v/v) delivered isocratically at flow rate 1.04 mL/min. For sample preparation, syrup samples (5.0 mL) were accurately taken and transferred into volumetric flask 100 ml. The sample was added with 50 mL mobile phase, shaken vigorously and made until volume with mobile phase. A-4.0 mL of this solution was taken and diluted until 20 mL, filtered using 0.45 µm filter, and injected into HPLC system.

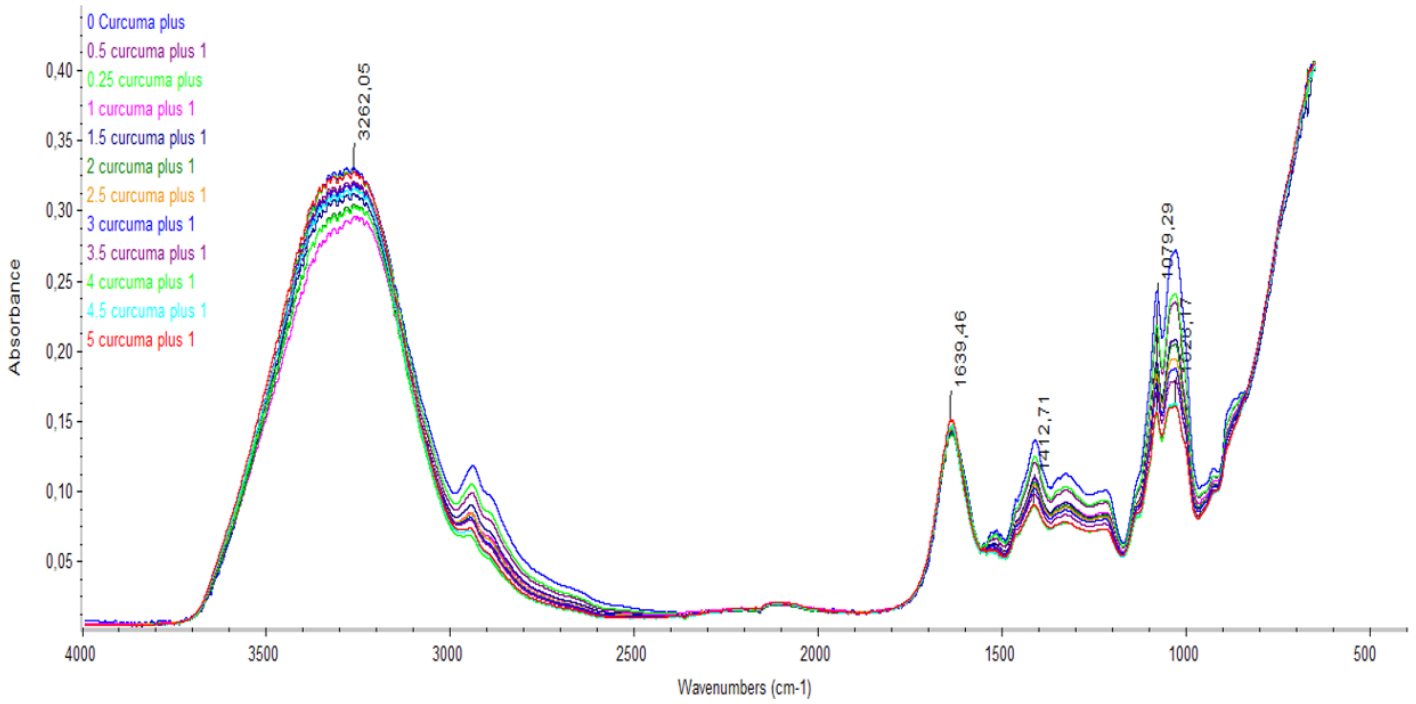


Fig. 3: The overlay of FTIR spectra of syrup samples containing curcuma extract scanned at mid infrared region (4000–650 cm^{-1}).

Table 1: The contents of CUR and DMC in syrup samples, analyzed using HPLC previously validated method.

Sample amount (g)	CUR (mg/g)	DMC (mg/g)
0.2693	0.0262	0.0018
0.5096	0.0327	0.0024
1.0584	0.0698	0.0050
1.5075	0.0930	0.0066
2.0106	0.0977	0.0071
2.5058	0.1414	0.0101
3.0022	0.1841	0.0128
3.5315	0.2364	0.0164
4.0311	0.2523	0.0175
4.5211	0.2677	0.0192
5.0521	0.3203	0.0226

Analysis using FTIR spectroscopy

The syrup samples were directly placed on Attenuated Total Reflectance (ATR) accessory composed of zinc selenide (ZnSe) crystal as sampling handling technique at controlled ambient temperature (25°C). All FTIR spectra were scanned using a FTIR spectrophotometer (Nicolet 6700 FTIR spectrometer, Thermo Nicolet Corp, Madison, WI), equipped with deuterated triglycinesulphate (DTGS) detector and beam splitter of potassium bromide (KBr)/Germanium. The instrument was connected to software of the OMNIC operating system (Version 7.0, Thermo Nicolet, Madison, WI). Spectra of FTIR were scanned in wavenumbers region of 4000–650 cm^{-1} with resolution of 4 cm^{-1} and number of scanning of 32. All spectra were calibrated using background of air spectrum as reference. After every scan, a new reference air background spectrum was taken. These spectra were recorded absorbance values at each data point in triplicate.

Table 2: The performance of partial least square (PLS) and principle component regression (PCR) for modelling the actual values of curcumin and demethoxycurcumin as determined with HPLC and FTIR predicted values.

Multivariat	Wave Number (cm^{-1})	Spectral treatment	Number of Factor	Curcumin				Demethoxycurcumin			
				Calibration		Validation		Calibration		Validation	
				R^2	RMSEC	R^2	RMSEP	R^2	RMSEC	R^2	RMSEP
PLS	1504–844	Normal	4	0.9975	0.00725	0.9854	0.00699	0.9999	0.000687	0.9920	0.000587
	1504–844	1st der.	8	0.9999	0.0073	0.9965	0.00835	0.9999	0.0000504	0.9962	0.000458
	3004–974	***Normal***	***10***	***0.9999***	***0.00110***	***0.9958***	***0.00444***	***0.9999***	***0.0000591***	***0.9980***	***0.000155***
	3004–974	1st der.	5	0.9998	0.00228	0.9676	0.0152	0.9998	0.000152	0.9725	0.001100
	3024–2497 and 1538–916	Normal	6	0.9986	0.00532	0.9761	0.00623	0.9991	0.000303	0.9796	0.000536
	3024–2497 and 1538–916	1st der.	6	0.9999	0.00107	0.9967	0.00947	0.9998	0.000139	0.9928	0.000597
	2344–844	1st der.	10	0.9983	0.00589	0.9761	0.0199	0.9985	0.000388	0.9786	0.0031
	2344–844	Normal	10	0.9999	0.00114	0.9976	0.00617	0.9999	0.0000628	0.9990	0.000246
PCR	2344–844	Normal	10	0.9978	0.00678	0.8838	0.00977	0.9978	0.00678	0.8838	0.00977
	2344–844	1st der.	10	0.9983	0.00595	0.9763	0.0202	0.9985	0.000393	0.9787	0.00133
	3024–2497 and 1538–916	1st der.	10	0.9990	0.00464	0.9841	0.0120	0.9992	0.000287	0.9845	0.000693
	3024–2497 and 1538–916	Normal	6	0.9975	0.00715	0.9784	0.9784	0.00715	0.9984	0.000402	0.0000554
	1504–844	1st der.	5	0.9974	0.00722	0.9908	0.0140	0.9981	0.000438	0.9908	0.000893
	1504–844	Normal	5	0.9974	0.00741	0.9590	0.00526	0.9981	0.000445	0.9740	0.000289
	3004–974	Normal	10	0.9980	0.00646	0.9307	0.00881	0.9986	0.000376	0.9575	0.000597
	3004–974	1st der.	10	0.9981	0.00627	0.9785	0.0194	0.9984	0.000403	0.9799	0.00127

1st der = first derivative spectra; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction; R^2 = coefficient of determination.

Chemometrics analysis

Multivariate analysis of PLS and PCR was established using TQ Analyst software version 7.0 (Thermo electron Corporation, Madison, WI) included in instrument of FTIR spectrophotometer. PLS and PCR calibrations were used for developing model which correlated between actual values of curcumin (CUR) and desmethoxycurcumin (DMC) obtained from HPLC determination and FTIR predicted values. Some statistical parameters namely coefficient determination (R^2), Root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Predicted (RMSEP) were computed using TQ Analyst.

RESULTS AND DISCUSSION

HPLC method is method of choice for quantitative analysis of curcuminoid due to its capability to make separation among curcuminoid components. In this study, FTIR spectroscopy combined with multivariate calibration was proposed as an alternative technique to HPLC method due to its simplicity in sample preparation. The analytical results obtained from HPLC measurement was used as actual values of CUR and DMC during analysis using FTIR spectroscopy. Two multivariate calibrations, namely partial least square (PLS) and principle component regression (PCR) were used for modelling the correlation between actual values of CUR and DMC in syrup samples containing Curcuma extract (*Curcuma longa* and or *Curcuma xanthorriza*) as determined by HPLC with predicted values using FTIR spectroscopy at optimized wavenumbers region. Table 1 compiled the contents of CUR and DMC in syrup samples, analyzed using HPLC previously validated by our laboratory (Prabaningdyah *et al*., 2017). Typical chromatogram of evaluated syrup samples was shown in Figure 2. It can be shown that peaks of CUR and DMC were well resolved from other components.

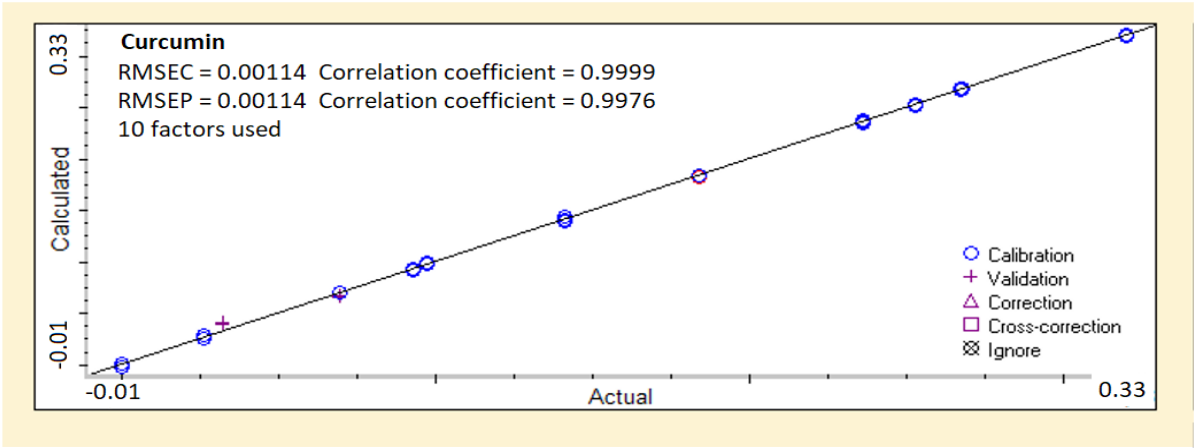
Figure 3 exhibited FTIR spectra of syrup samples containing Curcuma extract which showed typical absorption bands of curcuminoid. The interpretation of functional groups responsible for IR absorption in syrup samples could be correlated with functional groups in curcuminoid (CUR and DMC). Wide and intense absorption at 3265 cm^{-1} corresponded to the stretching vibration of the OH bond (–OH stretching) and was associated with the presence of hydrogen bond. The absorption peaks at 2960

and 2922 cm^{-1} were coming from CH_3- and CH_2- asymmetric stretching vibrations, respectively. The intense and characteristics peak at 1639 cm^{-1} corresponded to conjugated carbonyl (C=O) (Rohman *et al*., 2015). These absorption peaks proved that main components in syrup samples was curcuminoid.

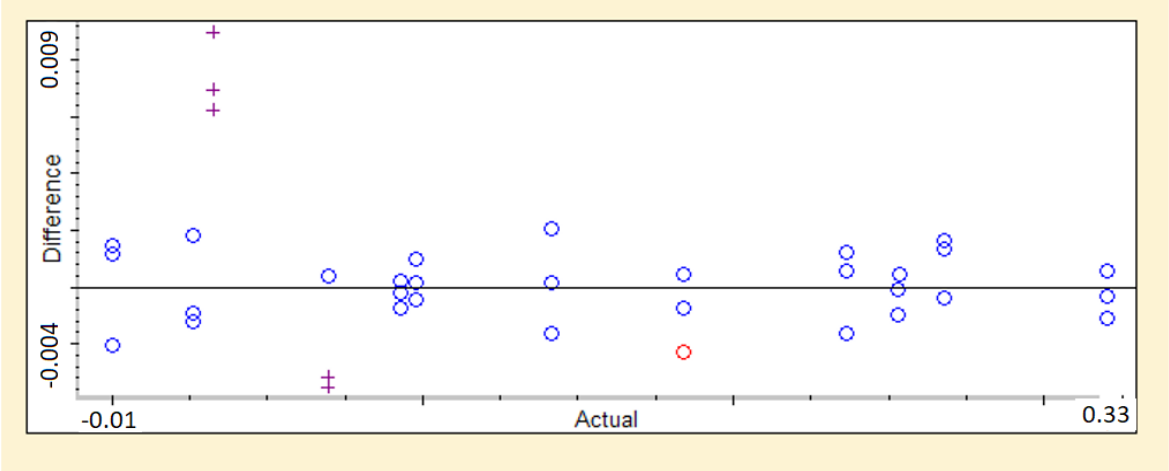
Table 3: Difference between actual and calculated curcuminoid content in syrup using PLS multivariat calibration assisted with TQ Analyst software.

Index	Curcumin			Demethoxycurcumin		
	Actual (mg/g)	Calculated (mg/g)	Diff. x Path	Actual (mg/g)	Calculated (mg/g)	Diff. x Path
1	0.1841	0.18281	−0.00129	0.01278	0.0127	−0.00008
2	0.2677	0.26643	−0.00127	0.0192	0.01911	−0.00009
3	0.3203	0.3194	−0.0009	0.0226	0.02254	−0.00006
4	0.1414	0.14379	0.00239	0.01005	0.0102	0.00014
5	0.2364	0.23739	0.00099	0.01644	0.0165	0.00006
6	0.2532	0.25315	−0.00005	0.01754	0.01754	0
7	0.1414	0.14017	−0.00123	0.01005	0.01001	−0.00004
8	0.23645	0.23471	−0.00174	0.01644	0.01635	−0.00009
9	0.18406	0.18344	−0.00062	0.01278	0.01274	−0.00004
10	0.2364	0.23728	0.00088	0.01644	0.0165	0.00006
11	0.09767	0.09797	0.0003	0.00706	0.00706	0
12	0.25323	0.25378	0.00056	0.01754	0.01759	0.00005
13	0.2532	0.25223	−0.00097	0.01754	0.01748	−0.00006
14	0.093	0.09201	−0.00099	0.00665	0.00661	−0.00004
15	0.14145	0.14096	−0.00049	0.01005	0.01004	−0.00001
16	0.093	0.09341	0.00041	0.00665	0.00667	0.00002
17	0.32031	0.32058	0.00027	0.0226	0.02261	0.00001
18	0.09303	0.09268	−0.00036	0.00665	0.00662	−0.00003
19	0.1841	0.18447	0.00037	0.01278	0.01283	0.00004
20	0.0977	0.09921	0.00151	0.00706	0.00713	0.00007
21	0.0977	0.09679	−0.00092	0.00706	0.00699	−0.00007
22	0.2677	0.27031	0.00261	0.0192	0.0193	0.00011
23	0.3203	0.32058	0.00028	0.0226	0.02265	0.00004
24	0.0698	0.06732	−0.00248	0.00503	0.0049	−0.00013
25	0.03272	0.03857	0.00585	0.00244	0.00252	0.00008
26	0.0327	0.03546	0.00276	0.00244	0.00237	−0.00007
27	0.0262	0.0252	−0.001	0.00177	0.0017	−0.00007
28	0.0698	0.06474	−0.00506	0.00503	0.00473	−0.0003
29	0.02618	0.02534	−0.00085	0.00177	0.00173	−0.00004
30	0.06982	0.07047	0.00065	0.00503	0.00508	0.00005
31	0	0.00108	0.00108	0	0.00005	0.00005
32	0.02618	0.02741	0.00123	0.00177	0.00183	0.00006
33	0	0.00089	0.00089	0	0.00003	0.00003
34	0.0327	0.03768	0.00498	0.00244	0.00239	−0.00006
35	0	−0.00162	−0.00162	0	−0.00006	−0.00006
36	0.26774	0.26758	−0.00016	0.0192	0.01919	−0.00001

For quantitative analysis, the selection of wavenumbers region was critical point during developing PLS and PCR models. The selection of wavenumbers region was based on its capability to provide the highest coefficient determination (R^2) and lowest errors expressed by RMSEC and RMSEP. To facilitate optimum model, FTIR spectra were also subjected to first derivative. Table 2 compiled the performance of PLS and PCR models using normal and first derivative spectra for the prediction of CUR and DMC in syrup samples along with statistical parameters, including the number of factors, R^2 in calibration and prediction (validation) models, RMSEC and RMSEP. Based on highest R^2 and lowest values, FTIR normal spectra at wavenumbers region of 3004–974 cm^{-1} using PLS calibration was selected for quantitative analysis of CUR and DMC in the syrup samples. Table 3 described difference between actual CUR and DMC from HPLC analysis and calculated CUR and DMC content in syrup using PLS multivariat calibration assisted with TQ Analyst software.



(A)



(B)

Fig. 4: The PLS calibration model for the relationship between actual values of curcumin and FTIR predicted value. (A) calibration model; (B) residual analysis.

Figure 4 exhibited the correlation between actual values of CUR determined by HPLC method and FTIR predicted values using optimized wavenumbers. The R^2 values obtained were 0.9999 (calibration) and 0.9976 (prediction). The errors in calibration (RMSEC) and validation (RMSEP) were relatively low, i.e. RMSEC of 0.00114% and RMSEP of 0.001167%. From residual analysis (Figure 4B), it is clear that systematic error did not occur because the residual value fall above and below zero value. Similarly, Figure 4 revealed correlation between the actual contents of DMC and predicted values using FTIR spectroscopy with high R^2 and low errors. Based on R^2, RMSEC and RMSEP, FTIR spectroscopy using PLS model was accurate and precise method for prediction of CUR and DMC in syrup samples. FTIR spectroscopy method is fast and reliable method for routine quality control of syrup formulation containing Curcuma extract.

CONCLUSION

FTIR spectroscopy using normal spectra at wavenumbers region of 3004–974 cm^{-1} coupled with PLS model was accurate and precise for determination of CUR and DMC in syrup formulation containing Curcuma extract. This method can be used as an alternative HPLC method suitable for routine monitoring of herbal medicine in which curcuminoid is main components. The developed method is fast, efficient and not involving chemical and reagents, therefore, FTIR spectroscopy in combination with PLS is considered as green analytical method.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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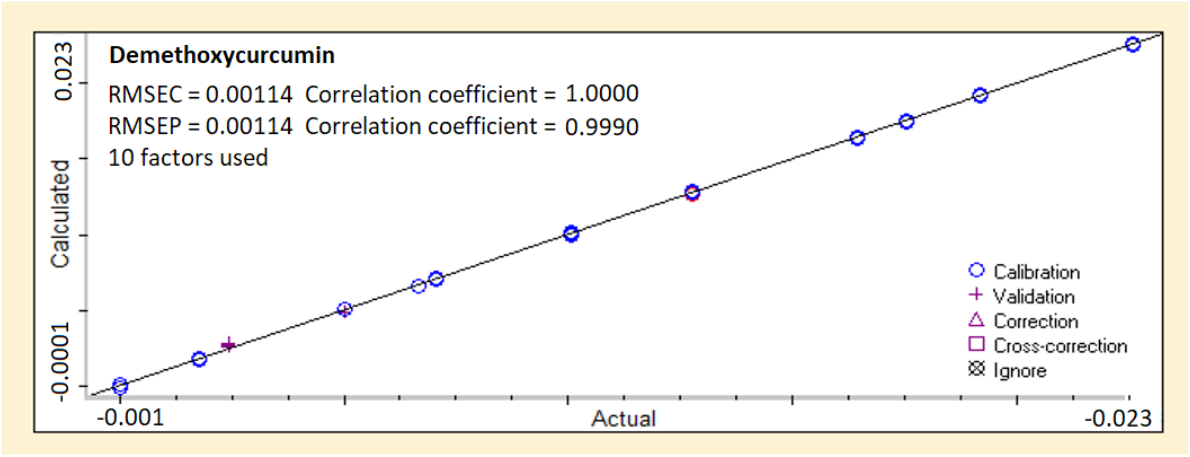
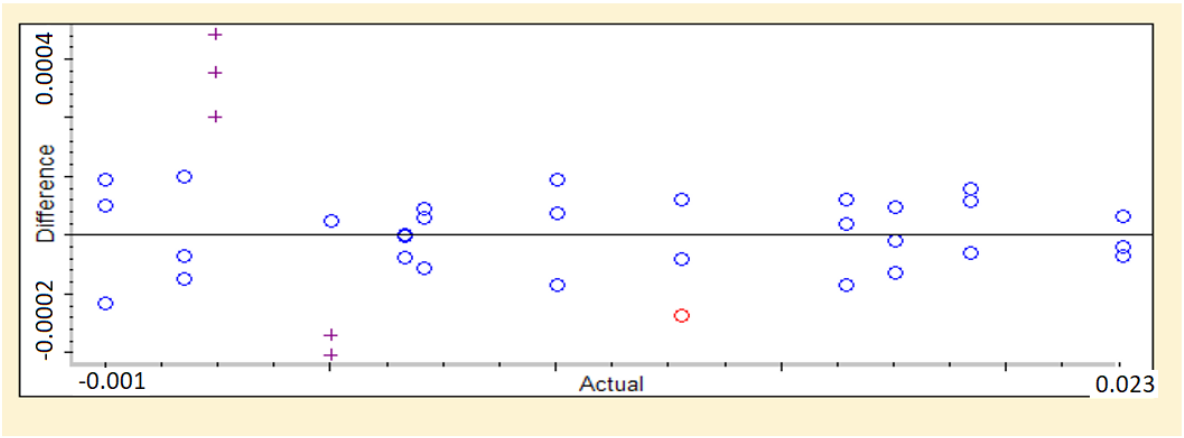


(A)



(B)

Fig. 5: The PLS calibration model for the relationship between actual values of demethoxycurcumin and FTIR predicted value. (A) calibration model; (B) residual analysis.

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