H_H0068: SAMPLE PREPARATION FOR ANALYSIS OF CHEMICAL COMPOSITION OF LICHEN BY ATR-FTIR SPECTROSCOPY

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Abstract: ATR-FTIR shorten sample preparation time for analyzing lichen secondary metabolite. This study examined sample preparation methods for quantitative analysis of lichen substances by ATR-FTIR spectroscopy. Lichens have complex chemical composition that made absorption bands of different functional groups complicated. Analysis and the data obtained depend on many factors. In quantitative analysis, proper sample preparation is necessary for high precision data. This experiment found that grounding lichen sample into size 500 μm was the most efficient method. Lichen samples which were collected from the different habitats had absorption bands at different intensity of ATR-FTIR spectra. The quantitative spectral analysis of chemical composition of lichen can be obtained by using optical density ratio (D_v/ D_{std}). In the future this technique will be very useful for analyzing chemical composition of lichen before and after transplant to polluted area to assess the impact of atmospheric pollution on metabolism of lichen products.

Introduction: Fourier transformed infrared (FTIR) spectroscopy has been used as a simple technique for obtaining rapid information on changes in composition of lichen due to air pollution[1]. Information on change in chemical compositionof lichens as a result of anthropogenic pollution is increasing used today to assess atmospheric quality. ATR (attenuated total reflection) is a sampling technique used in conjunction with infrared spectroscopy which samples could be examined directly and requires small sample size.Quantitative spectral analysis could be calculated from optical density of analyte band (D_v). According to the Bouguer-Lambert-Beer law, as follow:

$D=kcd$

where c is the concentration of absorbing by oscillator or vibration chemical groups; d is the sample thickness; k is the absorption coefficient for given oscillator.In order to eliminate the effect of sample thickness and other factors that influence the spectra used for calculation quantitatively, the optical density of the analyte absorption band (D_v) was divided by the optical density of standard band (D_{st}): D_v/ D_{st}. The standard band should be established from the band of spectra which undisturbed from environment. The ratio D_v/ D_{st} provided standard for the relative concentration of the studied chemical groups and its variation under anthropogenic exposure.

This paper examine sample preparation of lichen for analyzing by ATR-FTIR in order to get strongly intensity and high reproducibility of spectra band. The lichen *Parmotrema tinctorum* was used in this experiment.

Methodology: The FT-IR spectra of the lichen samples were record on Frontier FT-IR spectrometer (PerkinElmer, USA) using diamond/ZnSe UATR (universal attenuated total reflectance) over the wave number ranged 4000-700 cm^{-1}. The collected lichen samples were air-dry at room temperature, foreign materials on thalli were manually removed. Lichen sample were ground into powder with liquid nitrogen, and were then sieved through 500 μm sieve. The sample preparation methods were investigated as the following:

1) Weigh lichen sample 1.00 g extract with 10 ml acetone by soaking 2 hour. Filtered and evaporated to nearly dry, then used 3 pieces of filter paper dip into the solution.The filter papers were then evaporated to dryness, the lichen substances were fixed on filter paper and kept it in desiccators for analysis by ATR-FTIR.

2) Weigh lichen sample 0.100 g and extracted with acetone 1, 2, 3, 4 and 5 ml. by shaking on vortex mixer. Filtered and dropping the filtrate on 3 pieces of filter paper then evaporate filter paper to dryness, of which the lichen substances were fixed on filter paper. They were kept in desiccator until analysis by ATR-FTIR

3) Weigh lichen sample 0.0100 g and extracted with 10 ml acetone by soaking overnight. The solution/liquid was filtered and evaporated to nearly dry, and used 3 piece of filter paper dip into the solution. The filter papers were then evaporated to dryness, of which the lichen substances were fixed on filter papers. They were kept it in desiccator until analysis by ATR-FTIR.

4) Lichen sample were ground into powder with liquid nitrogen, and were then sieved through 180, 300 and 500 μm sieve. Using ground sample to analysis by ATR-FTIR.

5) Using intact lichen sample for ATR to record the spectrum.

Studied the precision of quantitative analysis was performed by using the appropriate sample preparation method to record the IR spectrum of lichen sample of 4 replicates.

Using the appropriate sample preparation method to record the IR spectrum of lichen samples collected from five sites at KhaoYai National park in Nakorn Rachasrima province and five sites at Queen Sirikit Botanic Garden in Chiang Mai province.

Results and Discussion: The sample preparation method 1) - 3) wereinvestigatedin order to separate and concentrated lichen substances which were suitable for FTIR analysis. It was found that the IR spectral band of method 1)had the high intensity, whereas method 2) and 3) had low intensity which the detail of spectral band were not enough for analysis. The FTIR spectrum of the intact lichen also gave low intensity band, which is not suitable for quantitative analysis. The results of method 4) showed the spectra of particle size 180, 300 and 500 μm no longer different. Although method 1) gave high intensity, but the pattern of spectrum was the same as ground sample.However it required large amount of lichen sample , which is relatively difficult to collect it for natural habitat.It can be concluded that grounding lichen was the appropriate technique for sample preparation. In this experiment choose 500 μm for analysis.Figure 1 is the FTIR spectrum of 4 replicatesanalysis of the lichen *Parmotrema tinctorum.* The quantitative results were calculated by using the ratio ofD_ν/ D_{std}.The band $\nu = 1615 \pm 2$ cm^{-1} was selected as the standard band, characterizing the bandc=c stretching of benzene to compare with $\nu = 2919 \pm 2$ cm^{-1}, characterizing the CH_2 group stretching vibration in the sample[1].These bands were structurally intensivewhich did not vary by the effect of environmental surrounding. Table 1 showed the value of D_ν/ D_{std} for 4 replicate records by using two standard band. The results showed the high precision which have %RSD lower than 5% of two D_{std} (ν = 2919 and 1615).It can be concluded that the value D_{std} can be selected from the value of both bands, 2919 cm^{-1} or 1615 cm^{-1}.

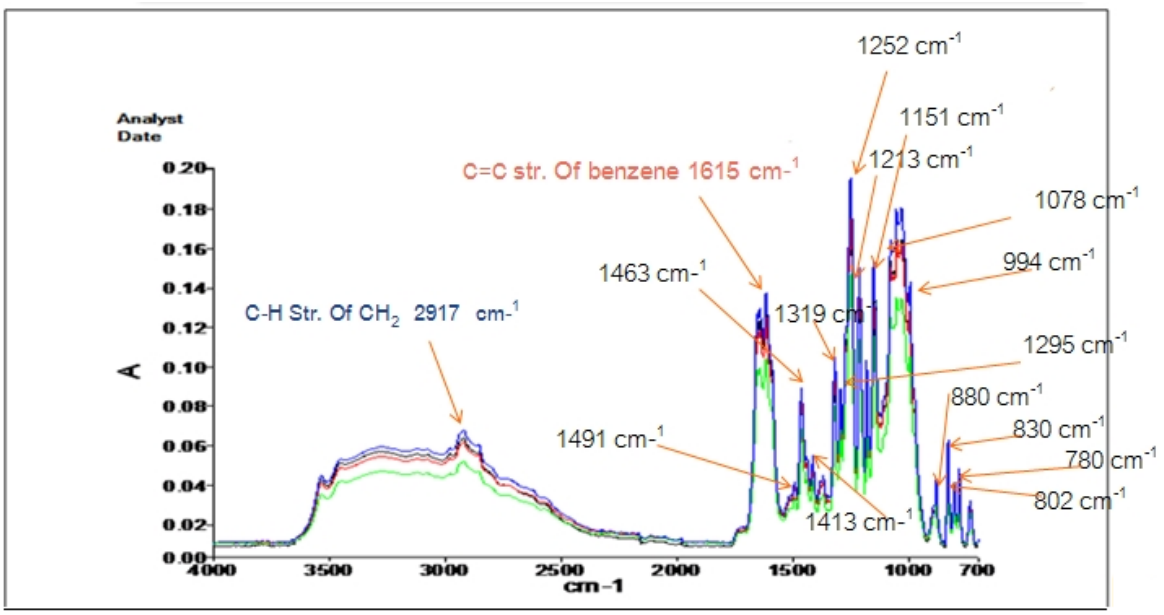

Figure 1. Absorption spectra of the lichen *Parmotrema tinctorum*in 4 replicates record of FTIR spectra

Table1. Comparison of%RSDbetween$D_v/D_{2919\pm2}$ and $D_v/D_{1615\pm2}$of the lichen *Parmotrema tinctorum*in 4 replicates record of FTIR spectra

$\nu(cm^{-1})$	Optical density (absorption) ratio $D_v/D_{2919\pm2}$		Optical density (absorption) ratio $D_v/D_{1615\pm2}$	
	$\bar{X} \pm SD$	%RSD	$\bar{X} \pm SD$	%RSD
2919±2	1.00±0.00	0.00	0.50±0.00	0.00
1615±2	2.02±0.02	0.99	1.00±0.00	0.00
1491±2	0.63±0.01	1.59	0.31±0.01	3.22
1463±2	1.32±0.01	0.76	0.66±0.01	1.52
1413±2	0.82±0.01	1.21	0.40±0.01	2.50
1368±2	0.67±0.01	1.49	0.33±0.01	3.03
1318±2	1.55±0.01	0.65	0.77±0.01	1.30
1294±2	1.33±0.02	1.50	0.66±0.01	1.51
1250±2	2.87±0.03	1.05	1.42±0.01	0.70
1212±2	2.22±0.02	0.90	1.10±0.01	0.91
1181±2	1.53±0.02	1.31	0.76±0.01	1.32
1150±2	2.25±0.02	0.89	1.11±0.01	0.90
1077±2	2.41±0.01	0.41	1.20±0.01	0.83
1052±2	2.62±0.03	1.15	1.30±0.01	0.77
993±2	2.05±0.05	2.44	1.01±0.02	1.98
880±2	0.63±0.02	3.17	0.31±0.01	3.22
830±2	0.94±0.01	1.06	0.47±0.01	2.12
802±2	0.61±0.02	3.28	0.30±0.01	3.33
780±2	0.73±0.02	2.74	0.36±0.01	0.28

The lichens from five sites at KhaoYai National Park and five sites from Queen Sirikit Botanic Garden had the ratios $D_v/D_{2919\pm2}$ and $D_v/D_{1615\pm2}$shown in Table 2 and 3. The resultsindicated that relative standard deviation (%RSD) of lichen samples collected from various sites gave higher value than repeatability analysis of lichen samples. Change of

chemical composition will affect the intensity of absorption band. The results showed that the sample collected from different sites which have different environmental surrounding give higher relative standard deviation (%RSD). Therefore it can be concluded that the chemical composition of lichens changes when the environmental surrounding change.

Table 2. The average value of ratio $D_\nu / D_{2919\pm2}$ and $D_\nu / D_{1615\pm2}$ of lichen samples collected from five sites at Khao Yai National Park in Nakorn Rachasrima Province and %RSD compared with replicated from table 1

$\nu(cm^{-1})$	Optical density (absorption) ratio $D_\nu/D_{2919\pm2}$			Optical density (absorption) ratio $D_\nu/D_{1615\pm2}$		
	$\bar{X}$ ± SD	%RSD	%RSDreplicated	$\bar{X}$ ± SD	%RSD	%RSDreplicated
2919±2	1.00±0.00	0.00	0.00	0.46±0.07	15.22	0.00
1615±2	2.20±0.35	15.91	0.99	1.00±0.00	0.00	0.00
1491±2	0.64±0.02	3.12	1.59	0.29±0.04	13.79	3.22
1463±2	1.40±0.06	4.29	0.76	0.65±0.08	12.31	1.52
1413±2	0.70±0.03	4.28	1.21	0.33±0.04	12.12	2.50
1368±2	0.63±0.05	7.94	1.49	0.29±0.02	6.90	3.03
1318±2	1.68±0.24	14.28	0.65	0.76±0.02	2.63	1.30
1294±2	1.49±0.12	8.05	1.50	0.68±0.05	7.35	1.51
1250±2	2.88±0.27	9.38	1.05	1.32±0.14	10.61	0.70
1212±2	2.35±0.19	8.09	0.90	1.08±0.13	12.04	0.91
1181±2	1.62±0.13	8.02	1.31	0.75±0.11	14.67	1.32
1150±2	2.18±0.18	8.26	0.89	1.01±0.13	12.87	0.90
1077±2	2.16±0.21	9.72	0.41	0.99±0.10	10.10	0.83
1052±2	2.36±0.25	10.59	1.15	1.08±0.11	10.18	0.77
993±2	1.76±0.15	8.52	2.44	0.81±0.09	11.11	1.98
880±2	0.61±0.03	4.91	3.17	0.28±0.03	10.71	3.22
830±2	0.96±0.07	7.29	1.06	0.45±0.07	15.56	2.12
802±2	0.54±0.05	9.26	3.28	0.25±0.05	20.00	3.33
780±2	0.87±0.27	31.03	2.74	0.39±0.06	15.38	0.28

Table 3. The average ratio of $D_{\bar{\nu}}/D_{2919\pm2}$ and $D_{\bar{\nu}}/D_{1615\pm2}$ of lichen samples collected from five sites at Queen Sirikit Botanic Garden in Chiangmai Mai province and %RSD compared with replicated from table 1

$\nu(cm^{-1})$	Optical density (absorption) ratio $D_{\nu}/D_{2919\pm2}$			Optical density (absorption) ratio $D_{\nu}/D_{1615\pm2}$		
	$\bar{X} \pm SD$	%RSD	%RSD replicated	$\bar{X} \pm SD$	%RSD	%RSD replicated
2919±2	1.00±0.00	0.00	0.00	0.43±0.07	16.27	0.00
1615±2	2.40±0.44	18.33	0.99	1.00±0.00	0.00	0.00
1491±2	0.69±0.08	11.59	1.59	0.29±0.04	13.79	3.22
1463±2	1.46±0.12	8.22	0.76	0.62±0.07	11.29	1.52
1413±2	0.74±0.04	5.41	1.21	0.32±0.04	12.50	2.50
1368±2	0.66±0.06	9.09	1.49	0.28±0.03	10.71	3.03
1318±2	1.79±0.31	17.32	0.65	0.75±0.03	4.00	1.30
1294±2	1.55±0.19	12.26	1.50	0.66±0.06	9.09	1.51
1250±2	2.90±0.33	11.38	1.05	1.23±0.16	13.01	0.70
1212±2	2.39±0.25	10.46	0.90	1.01±0.12	11.88	0.91
1181±2	1.69±0.18	10.65	1.31	0.71±0.09	12.68	1.32
1150±2	2.24±0.22	9.82	0.89	0.95±0.13	13.68	0.90
1077±2	2.16±0.17	7.87	0.41	0.92±0.14	15.22	0.83
1052±2	2.32±0.21	9.05	1.15	0.99±0.17	17.17	0.77
993±2	1.77±0.17	9.60	2.44	0.76±0.13	13.76	1.98
880±2	0.64±0.07	10.94	3.17	0.27±0.40	14.81	3.22
830±2	1.00±0.10	10.00	1.06	0.43±0.07	16.28	2.12
802±2	0.55±0.06	10.91	3.28	0.23±0.05	21.74	3.33
780±2	0.98±0.32	32.65	2.74	0.40±0.06	15.00	0.28

Conclusion: This experiment showed the ratio of D_{ν}/D_{std} from replicated analysis had high precision, and the lichen sample from different sites gave different amount of absorption ratio of D_{ν}/D_{std}. It revealed that ATR-FTIR can be used for quantitative analysis of chemical composition by comparing the ratio D_{ν}/D_{std} of absorption bands among sites.The further study is to quantitatively analysis lichens transplanted to polluted sites to assess the impact of atmospheric pollution on chemical composition of lichens.

References:

1. Meysurova AF, Khizhnyak SD, Pakhomov PM. J Appl Spectrosc. 2009;76(3):420-426.
2. Meysurova AF, Khizhnyak SD, Pakhomov PM. DOI:101134/S199542551102010X
3. Edwards HGM, Newton EM, Wynn-William DD, Lewis-Smith RI. Spectrochimicacta A. 2003;59:2301-2309.
4. Huneck and Yoshimura. SPINGER. 1996;493p

Acknowledgements: This work was supported by the National Research Council of Thailand (NRCT). We would like to thank Associate Professor Dr.Thitima Rukachaisirikul for her valuable suggestion.