CHAPTER V
CONCLUSION

This study presents the development of spectrophotometry for simultaneous determination of CBR and CBF in vegetables. Spectrophotometric method of CBR and CBF is based on derivatization with appropriate reagents and measured the absorbance at visible region of the derivatives. Two reagents studied were DMA and AP. Conditions for derivatization were optimized and afterwards the method was validated. The optimum condition for derivatization of CBR with DMA was hydrolyzed CBR using NaOH 10.0 mmol L^{-1}. The obtained naphtholate anion was reacted with the diazonium ion which obtained from 0.6 mmol L^{-1}, 0.2 mmol L^{-1} and 2.0 mmol L^{-1} of DMA, $NaNO_2$ and HCl, respectively. The CBR-DMA derivative is orange-red color with the maximum absorption wavelength at 510 nm. DMA is selective for the detection of CBR under the above mentioned condition, only CBR give colored derivative.

Both CBR and CBF were derivatized with AP under the following conditions: 5.0 mmol L^{-1} AP, 9.6 mmol L^{-1} $K_3Fe(CN)_6$ and 5.0 mmol L^{-1} borate buffer pH 9.5. CBF has orange-red color with the maximum absorption wavelength at 510 nm, while red color with the maximum absorption wavelength at 480 nm was obtained for CBR. Therefore, AP was used as the derivatizing agent for the simultaneous analysis of CBF and CBR. Two techniques were investigated namely simultaneous equation and zero-crossing technique. For simultaneous equations technique, the amounts of reagents used were less than zero-crossing technique because the simultaneous equations technique did not require calibration curve. While, zero-crossing technique need one calibration curve for one point of the analysis. However, zero-crossing technique gave more accuracy than simultaneous equations technique.

Sample preparation studied in this thesis including extraction of the analytes from samples by QuEChERS and preconcentration of the analytes by CPE. The sample studied were cucumber, cabbage kale, yard long bean and mustard. In QuEChERS technique, 1.0% acetic acid in 99.0% acetonitrile was used as organic

solvent. Na_2SO_4, $MgSO_4$ and CH_3COONa were used for removing exceed water. The used amounts of salts depended on moisture of samples. Carbograph and PSA were used for removing chlorophyll, the used amounts of Carbograph and PSA were different for each sample. For CPE, TX-114 was used as the extractant, while NaCl was selected as dehydration reagent. The optimum condition for CPE was 1.5% w/v TX-114, 2.0% w/v NaCl, equilibration temperature at 55 °C, equilibration time for 20 min and centrifugation time for 10 min.

Analytical features of the proposed method were investigated in term of linearity, sensitivity, accuracy, precision and selectivity. The investigations were carried out using the optimum conditions of both sample preparation and analytical methods. Linearity was constructed using matrix match calibration with the 5 level concentrations of CBF and CBR in the range 0.5-7.0 mg L^{-1}. The results showed good linearity with R^2 higher than 0.99. Sensitivity is expressed as LOD and LOQ. LOD and LOQ obtained from spectrophotometry coupled to CPE were lower than those without CPE about 10 folds. LOD and LOQ for the determination of CBR using DMA as derivatizing agent were 0.10 mg L^{-1} and 0.50 mg L^{-1}, respectively. While, for the determination of CBF and CBR using AP as derivatizing agent were 0.20 mg L^{-1} and 0.50 mg L^{-1}, respectively. All the results indicated that the CPE-spectrophotometric method has capability for the detection at MRL. Accuracy and precision were investigated in terms of recovery and relative standard deviation of recovery, respectively. The recoveries of spiked samples (0.5, 1.0 and 3.0 mg kg^{-1}) were obtained in the range 72-102 % with the relative standard deviation less than 13%. The selectivity was investigated from the effect of interfering species which reported in term of tolerance limit by adding the other pesticides including carbamates and organophosphate pesticides into the mixture of 3.0 mg L^{-1} CBR and 1.0 mg L^{-1} CBF and analyzed using spectrophotometry. The results indicated that for the determination of CBR using DMA as the derivatizing agent is very selective, since all of the studied species did not interfere. While, simultaneous determination of CBR and CBF using AP as the derivatizing agent, some carbamate pesticides could interfere, especially IPC showing serious effect. However, most of the studied carbamates (BDC, MTM and MTC) and organophosphates (PTO) did not interfere when their concentrations were lower than 5.0 mg L^{-1}.

The accuracy for determination of CBR and CBF by the spectrophotometry was compared with HPLC via the recoveries. The results indicated that the obtained recoveries of two techniques were not significantly different (*t*- test, p=0.05). For the determination of CBR and CBF in vegetable samples, no contamination of CBR and CBF was observed in any samples studied.

In conclusion, the proposed spectrophotometric method coupled to CPE is a simple and reliable method for the simultaneous determination of CBF and CBR. CPE enhances sensitivity of the proposed method resulted in low LOD which provided the detection of CBF and CBR at MRL levels.