CHAPTER 5
CONCLUSION

The work proposes a new method for the separation and quantification of five water-soluble vitamins (vitamin C, vitamin B_1, vitamin B_2, vitamin B_3 and vitamin B_6) and two fat-soluble vitamins (α-tocopherol and β-carotene). Solid-phase extraction proved to be an effective tool for performing adequate separation of the two groups of vitamins while HPLC provided a fast, accurate and reliable method for their determinations.

The method has been applied to the determination of water-soluble vitamins by coupling the solid phase extraction to isocratic chromatographic separation without using an inorganic buffer for the simultaneous determination of vitamins in *M. citrifolia* and *P. emblica* fruits and their fermented juices. We found that the Sep-Pak C_8 cartridge was useful for pre-concentration because it minimized loss of vitamins during separation process. The HPLC method offers high accuracy, precision, a relative short analysis time, unambiguous identification of vitamin C and vitamins B. The calibration curves are linear over the ranges of 5.0-800.0 and 100.0-1000.0 $\mu g.mL^{-1}$ for L-ascorbic acid (vitamin C), 10.0-100.0 $\mu g.mL^{-1}$ for thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2) and nicotinamide (vitamin B_3) and 0.50-50.0 $\mu g.mL^{-1}$ for pyridoxine hydrochloride (vitamin B_6), respectively. The limit of detections (S/N=3) are 0.50 $\mu g.mL^{-1}$ for L-ascorbic acid (vitamin C) and thiamine hydrochloride (vitamin B_1), 0.10 $\mu g.mL^{-1}$ for riboflavin (vitamin B_2), 2.00 $\mu g.mL^{-1}$ for nicotinamide (vitamin B_3) and 0.05 $\mu g.mL^{-1}$ for pyridoxine hydrochloride (vitamin B_6), respectively (Table 4.12). The precision of the LC method for determining vitamins was confirmed by analyzing each sample (n=5), using the proposed HPLC method. All the relative standard deviation for vitamin contents in both samples was less than 2%. The accuracy of the method for vitamin C and vitamin B group assay were studied by spike-placebo in aqueous extracts of *M. citrifolia* and *P. emblica*. Results are presented in Table 4.14–Table 4.23. The percentage recoveries of vitamin C in *M. citrifolia* and *P. emblica* extracts were found 100.37 and 99.32, respectively.

For vitamins B, the percentage recoveries were found to be 100.08, 100.07, 99.71 and 99.87 for vitamin B_1, vitamin B_2, vitamin B_3 and vitamin B_6 in *M. citrifolia* extract and 99.75, 100.03, 100.61 and 99.18 for vitamin B_1, vitamin B_2, vitamin B_3 and vitamin B_6 in *P. emblica* extract, respectively. The proposed method has been applied to the determination of vitamins in fermented juices containing *M. citrifolia* and *P. emblica*. The pH of fermented juices products were in the ranges of 3.25-4.45 and 2.91-3.94 for products containing *M. citrifolia* and *P. emblica*, respectively (Table 4.45 and Table 4.46). The amounts of vitamins C and vitamins B in fermented juices containing *M. citrifolia* and *P. emblica* decreased with increasing time (Table 4.47-Table 4.50). Statistical comparison of vitamin C and vitamins B between formulas within day in fermented juices of *M. citrifolia* and *P. emblica* showed significant at the level 0.05 ($P<0.05$) of all formula. The stability of vitamins in fermented juices product was also investigated. It was found that the amounts of vitamin C and vitamins B decreased with increasing time (Fig. 4.45-Fig. 4.49). Because vitamin C is easily destroyed during processing and storage through the action of metals such as copper and iron. Exposure to oxygen and prolonged heating in the presence of oxygen destroyed ascorbic acid. Thus, the stability of vitamin C in fortified foods depends on the product, processing method, and type of packaging used. Vitamin B_1 is one of the most unstable B vitamins. Baking, pasteurization, or boiling of products fortified with thiamine can reduce its content by up to 50 percent. Vitamin B_2 is susceptible to degradation on exposure to light. Vitamin B_6 is susceptible to light induced degradation and exposure to water can cause leaching and consequent losses. Vitamin B_6 is sensitive to light, heat and alkalis [179, 180].

A reverse-phase high performance liquid chromatographic (RP-HPLC) procedure was used for the determination of fat-soluble vitamins in *M. citrifolia* and *P. emblica* fruits. The method was validated and showed good linearity, precision, accuracy and recovery. The calibration curves are linear over the ranges of 1.0-10.0 $\mu g.mL^{-1}$ for α-tocopherol (vitamin E) and 1.0-10.0 $\mu g.mL^{-1}$ for β-carotene, respectively with $r^2>0.999$. The limit of detections (S/N = 3) are 0.01 $\mu g.mL^{-1}$ for α-tocopherol (vitamin E) and β-carotene (Table 4.12). The precision of the LC method for determining vitamins was confirmed by analyzing each sample (n=5), using the proposed HPLC method. All the relative standard deviation for vitamins content in

both samples was less than 2%. The accuracy of the method for vitamin E and β-carotene assay were studied by spike-placebo in organic extracts of *M. citrifolia* and *P. emblica*. Results are presented in Table 4.14 – Table. 4.23. The percentage recoveries were found to be 100.20 and 100.65 for vitamin E and β-carotene in *M. citrifolia* extract and 100.15 and 99.75 for vitamin E and β-carotene in *P. emblica* extract, respectively. The proposed method has been applied to the determination of vitamin E and β-carotene in fermented juices of *M. citrifolia* and *P. emblica*. Vitamin E and β-carotene were not present, because they are not soluble in fermented juices containing *M. citrifolia* and *P. emblica*.

The proposed RP-HPLC method was rapid, simple, very accurate and precise. The proposed method could be applied to the determination of multi-vitamin product from other medicinal plants.