CHAPTER 2
REVIEW OF LITERATURES

2.1 Chromatographic Assay of Vitamins

The method of simultaneously analyzing vitamins A and E is based on the work of Egberg *et al*. [91] and De Vries *et al.* [92]. It has been used in the laboratory on a routine basis for over 4 years on a wide variety of samples. The HPLC method for niacin, nocotinamide, pyridoxine, thiamin, and riboflavin is based on an ion-pairing reverse-phase technique reported by Kirchmeier and Upton [93] and by Taillie *et al.* [94]. The reported methods were modified somewhat for routine use with automated HPLC equipment. The wavelength of the detector was adjusted so as to allow all of the vitamins to be analyzed using a single response range for most multivitamin premixes and preparation. The ion-pairing reagent was changed from hexanesulfonate to pentanesulfonate to improve the resolution between thiamin and riboflavin and thus substantially reduce the analysis time per sample. The modified method has been used successfully for several years on a variety of multivitamin matrices such as premixes, capsules, and tablets. The reproducibility and recovery obtained over this time period agree with the published data [93]. The method as described here applies primarily to vitamin concentrates. Methods of a similar nature have been applied to foods and urine [95-99].

2.2 Automated Vitamin Analysis

Ascorbic acid has been determined automatically by continuous flow using the reduction of 2,6-dichlorophenolindophenol [100-103], osazone formation with 2,4-dinitrophenyl hydrazine [104-106], quinoxaline formation with *o*-phenylenediamine [107-109], and reaction with diazotized 4-methoxy-2-nitronalin[110].

Riboflavin analysis has been automated with continuous flow by taking advantage of its fluorescence, removing impurities with permanganate oxidation, and obtaining a blank after reducing the riboflavin with hydrosulfite [111-113].

Oxidation of thiamin to thiochrome with potassium ferricyanide followed by extraction of the thiochrome into isobutanol with fluorescence measurement has been automated using continuous-flow automation [114-116]. A blank fluorescence is determined by omitting the ferricyanide oxidation step. The thiamin is extracted from the food matrix by acid hydrolysis followed by enzymatic hydrolysis with hydrolysis with a phosphatase such as Clarase or Takadiastase.

The Colorimetric analysis of niacin is based on the reaction of a cyanogenhalide, such as cyanogens bromide, with the pyridine nucleus of niacin giving rise to a chromophore upon further reaction with an aromatic amine. This chemistry has been subjected to continuous-flow automation [117]. A fluorometric method has been reported for foods but requires prior chromatographic separation [118]. Automated methods for vitamin B_6 in complex biological systems employ microbiological techniques [119]. Automated spectrophotometric methods [120] and automated radioassay [121] techniques have been used for fermentation broth and placental lactogen, respectively.

The effective use of chromatographic techniques for the determination of fat-soluble vitamins is perhaps the reason there exist few examples of the use of automation for these vitamins. Continuous-flow automation has been reported for the determination of vitamin A in milk [122], pharmaceuticals [123] and cattle feeds [124].

Column liquid chromatography determination of vitamins A and E in powdered milk and local flour: a validation procedure [125]. An isocratic LC method for the simultaneous determination of vitamins A, C, E and b-carotene [126]. Ultrasensitive determination of b-carotene in fish oil-based supplementary drugs by HPLC-TLS [127]. Determination of food constituents based on SFE: applications to vitamins A and E in meat and milk [128]. Determination of tocopherol acetate in emulsified nutritional supplements by solid-phase extraction and high-performance liquid chromatography with fluorescence detection [129]. Chromatographic determination of riboflavin and its derivatives in food [130]. Use of an amino acid in

the mobile phase for the determination of ascorbic acid in food by high-performance liquid chromatography with electrochemical detection [131]. Determination of vitamins A and E in milk powder using supercritical fluid extraction for sample clean-up [132]. A comparison of matrix resolution method, ratio spectra derivative spectrophotometry and HPLC method for the determination of thiamine HCl and pyridoxine HCl in pharmaceutical preparation [133]. Simultaneous determination of water-soluble vitamins excreted in human urine after eating an overdose of vitamin pills by a HPLC method coupled with a solid phase extraction [134]. Optimization of an Extraction Procedure for the Quantification of Vitamin E in Tomato and Broccoli using Response Surface Methodology [135]. Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables [136]. Determination of folic acid by ion-pair RP-HPLC in vitamin-fortified fruit juices after solid-phase extraction [137]. Voltammetric behavior of α-tocopherol and its determination using surfactant + ethanol + water and surfactant + acetonitrile + water mixed solvent systems [138]. Lipase-catalyzed reactions in organic and supercritical solvents: application to fat-soluble vitamin determination in milk powder and infant formula [139]. Supercritical fluid extraction and chromatography for fat-soluble vitamin analysis [140]. Liquid chromatographic method for the analysis of tocopherols in malt sprouts with supercritical fluid extraction [141]. Voltammetric determination of vitamins in a pharmaceutical formulation [142]. Electroanalysis at ultramicroelectrodes of oils and fats Application to the determination of vitamin E [143]. Simultaneous determination of carotenes and tocopherols in ATBC drinks by high-performance liquid chromatography [144]. Spectrofluorimetric method for the determination of piroxicam and pyridoxine [145]. Comparison of micellar and microemulsion electrokinetic chromatography for the analysis of water- and fat-soluble vitamins [145]. Optimizing separation conditions for riboflavin, flavin mononucleotide and flavin adenine dinucleotide in capillary zone electrophoresis with laser-induced fluorescence detection [147]. Flow injection spectrophotometric determination of L-ascorbic acid in biological matters [148]. Simultaneous determination of ingredients in a vitaminenriched drink by micellar electrokinetic chromatography [149]. Spectrophotometric determination of vitamin B_1 in a pharmaceutical formulation using triphenylmethane acid dyes [150].

Sequential injection redox or acid_/base titration for determination of ascorbic acid or acetic acid [151]. Simultaneous analysis of retinol, β-carotene and tocopherol levels in serum of Vietnams populations with different incomes [152]. Determination of total vitamin B6 in foods by isocratic HPLC: a comparison with microbiological analysis [153]. Reversed-phase liquid chromatography on an amide stationary phase for the determination of the B group vitamins in baby foods [154]. Sample preparation for routine high-performance liquid chromatographic determination of retinol palmitate in emulsified nutritional supplements by solid-phase extraction using monosodium L-glutamate as dissolving agent [155]. Automated determination of selected water-soluble vitamins in tablets using a bench-top robotic system coupled to reversedphase (RP-18) HPLC with UV detection [156]. A multicommuted flow system for sequential spectrophotometric determination of hydrosoluble vitamins in pharmaceutical preparations [157]. Stability of tocopherols in adapted milk-based infant formulas during storage [158]. Simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations by high-performance liquid chromatography coupled with diode array detection [159]. Flow injection renewable drops spectrofluorimetry for sequential determinations of Vitamins B_1, B_2 and B_6 [160]. Monomeric C18 chromatographic method for the liquid chromatographic determination of lipophilic antioxidants in plants [161]. Comparison of soluble manganese(IV) and acidic potassium permanganate chemiluminescence detection using flow injection and sequential injection analysis for the determination of ascorbic acid in Vitamin C tablets [162]. Comparison of two derivative spectrophotometric methods for the determination of α-tocopherol in pharmaceutical preparations [163]. The analysis of the zero-order and the second derivative spectra of retinol acetate, tocopherol acetate and coenzyme Q10 and estimation of their analytical usefulness for their simultaneous determination in synthetic mixtures and pharmaceuticals [164]. Development of a validated liquid chromatography method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction [165]. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii [166]. Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography [167]. Application of a liquid chromatography tandem mass spectrometry method to the analysis of water-soluble vitamins in Italian pasta [168].

Determination of carotene, tocopherols and tocotrienols in residue oil from palm pressed fiber using pressurized liquid extraction-normal phase liquid chromatography [169]. High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection [170]. Separation of thiamin and its phosphate esters by capillary zone electrophoresis and its application to the analysis of water-soluble vitamins [171]. Simultaneous determination of vitamins C, B6 and PP in pharmaceutics using differential pulse voltammetry with a glassy carbon electrode and multivariate calibration tools [172]. Spectrophotometric determination of ascorbic acid using copper(II)–neocuproine reagent in beverages and pharmaceuticals [173]. Method development and validation for monitoring in vivo oxidative stress: Evaluation of lipid peroxidation and fat-soluble vitamin status by HPLC in rat plasma [174]. High-throughput analysis of Vitamin C in human plasma with the use of HPLC with monolithic column and UV-detection [175]. Determination of vitamin C in tropical fruits: A comparative evaluation of methods [176]. A multicommuted fluorescence-based sensing system for simultaneous determination of Vitamins B2 and B6 [177]. HPLC-UV determination of total vitamin C in a wide range of fortified food products [178].

2.3 Stability of vitamins [179, 180]

Water-soluble vitamins

Ascorbic acid (vitamin C) is easily destroyed during processing and storage through the action of metals such as copper and iron. Both exposure to oxygen and prolonged heating in the presence of oxygen and alkalis destroy ascorbic acid.

Thiamine (vitamin B_1) is one of the most unstable B vitamins. Baking, pasteurization, or boiling of foods fortified with thiamine can reduce its content by up to 50 percent. The stability of thiamine during storage depends greatly on the moisture content of the food. Thiamine is highly sensitive to heat and alkalis.

Riboflavin (vitamin B_2) is very stable during thermal processing, storage and food preparation. Riboflavin, however, is susceptible to degradation on exposure to light. The use of light-proof packaging material prevents its deterioration.

Nicotinamide (vitamin B_3) is one of the most stable vitamins and the main loss occurs from leaching into cooking water.

Pyridoxine (vitamin B_6) losses depend on the type of thermal processing. For example, high losses of B_6 occur during sterilization of liquid infant formula; in contrast, B_6 in enriched flour and corn meal is resistant to baking temperatures. B_6 is susceptible to light induced degradation and exposure to water can cause leaching and consequent losses. Pyridoxine is sensitive to light, heat and alkalis.

Fat-soluble vitamins

α-tocopherol (vitamin E) occurring naturally in foods in the form of α-tocopherol, oxidizes slowly when exposed to air and sensitive to heat and alkalis. However, vitamin E added in the form of α-tocopheryl acetate shows excellent retention in wheat flour. Losses of vitamin E occur only during prolonged heating such as in boiling and frying.

β-carotene such as carotenoids, sometimes called pro-vitamin A, are water-soluble precursors which are made into vitamin A by the body. Beta-carotene was sensitive to sunlight and oxidizing agents.