INTRODUCTION

Vitamin B₂ can be found in nature as the free riboflavin (RF), but in most biological materials, it occurs predominantly in the form of two coenzymes, flavin mononucleotide (FMN) and flavin–adename dinucleotide (FAD), although there are other flavin derivatives present in nature (2). Most flavoprotein enzymes are involved in the complex respiratory processes that occur in the mitochondria of living cells, although some are involved in other aspects of metabolism. The Dietary Allowances Committee of the National Research Council recommends an intake of RF of 0.6 mg/1000 kcal, which is equivalent to 1.6 mg/day (3).

Several methods have been proposed for the determination of vitamin B₂ in food (4), usually involving the conversion of FMN and FAD to RF. The standard method for analysis of total RF in food is the AOAC fluorimetric method (5). There is growing interest in knowing not only total RF content but also flavin composition of food. Liquid chromatography (LC) using reversed phase columns has been applied to the determination of total RF (6–12) and also to the separation of the main flavins in foods, RF, FMN, and FAD (4, 13–22). RF has a strong inherent fluorescence and can be detected very specifically with high sensitivity at its maximum fluorescence intensity at pH 6–7. The main sources of RF in foods are milk, eggs, meat products, and yeasts. RF is one of the most stable vitamins, and the alkaline conditions in which it is unstable are rarely encountered in foodstuffs, although it is sensitive to light. Extraction with hot dilute acids can split vitamin B₂–protein compounds, but the phosphoric acid esters of RF can only be hydrolyzed completely by means of enzymes (23).

In this study, a liquid chromatographic reversed phase procedure for the separation of free RF, FMN, and FAD is optimized using fluorescence detection. Application of the ion-pairing technique was prevented by using an amide-based stationary phase endcapped with trimethylsilyl (24–26), which also produced a considerable decrease in the peak widths by avoiding the interaction of the vitamers with the silanol groups of the stationary phase. The procedure was applied to the determination of the B₂ vitamers in different types of food, such as milk and soy-based infant formulas, beer, fruit juices, and honey of different types. Most B₂ vitamin appeared as RF, while the coenzymes were present in lower amounts. The method was validated using two certified reference materials, and results within the certified range were obtained.

KEYWORDS: Liquid chromatography; fluorescence; riboflavin vitamers; foods