Determination of Water-and Fat-Soluble Vitamins in Gummies by Reversed-Phase Liquid Chromatography

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INTRODUCTION

Vitamins are essential substances for human health and growth. Storage, aging, and processing of foods may cause vitamin loss. There is a need to develop a fast and accurate analytical method for the determination of vitamins in foods. The determination of vitamins in sugar-based matrix is usually a challenging task due to the complexity of the matrix and instability of vitamins. Gummies are sugar-based candies which are fortified with selected vitamins. Until now, no method has been proposed for the determination of vitamins in gummies. In this work, the determination of six water-soluble vitamins and three fat-soluble vitamins is studied by reverse phase liquid chromatograph(RP-LC) with ultra-violet detector at different wavelengths, on a C-8 column using phosphate buffer and acetonitrile as mobile phase.

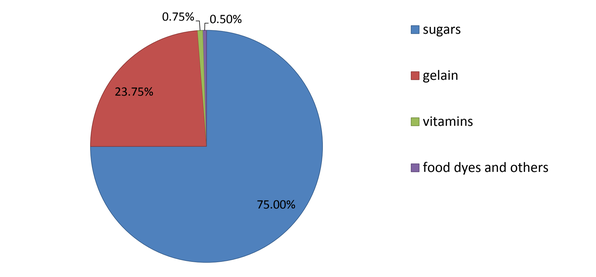


Fig. 1 Ingredients of Gummy

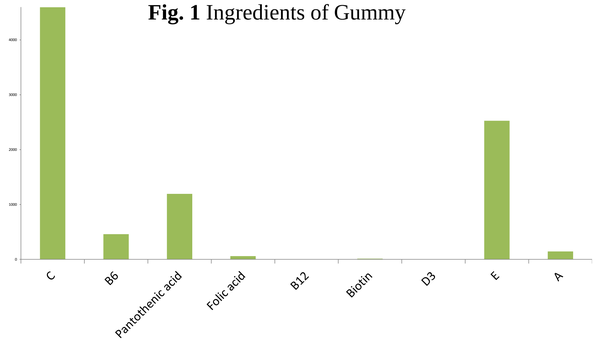


Fig. 2 Concentration range of vitamins in gummies (mg kg^{-1})

METHODOLOGY

Determination of six water-soluble vitamins [calcium pantothenate (B5), pyridoxine (B6), biotin (B8), folic acid (B9), cyanocobalamin (B12) and ascorbic acid (C)] and three fat-soluble vitamins [retinyl palmitate (A), cholecalciferol (D3) and alpha-tocopheryl acetate (E)] in gummies was studied by RP-LC with UV detector. Sample was prepared as follows:

- Gummy was dissolved in the mixture of water/methanol (v/v=1:1)
- Ethanol was used to precipitate the gelatin
- Nitrogen gas was used to evaporate the solvent
- The final sample was injected onto a C-8 column

Tab. 1 Elution program for the RP-HPLC determination of Water- and fat- soluble vitamins in Gummies

Time (min)	Solvent A (0.025 M Phosphate buffer, pH=3.5) %	Solvent B (Acetonitrile) %
0.0	100.0	0.0
3.5	100.0	0.0
4.0	87.0	13.0
12.0	70.0	30.0
12.5	0.0	100.0
53.0	0.0	100.0
54.0	100.0	0.0

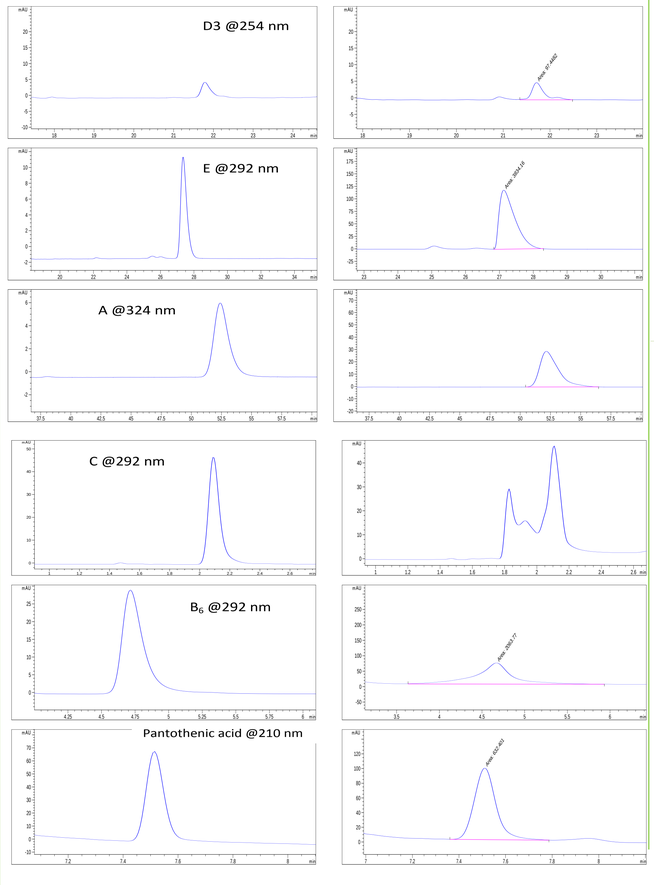
All chromatographic separations were performed under room temperature. The elution program is listed in table 1. The flow rate was 1.0 ml min^{-1}. The detection was achieved at five different wavelengths.

Tab. 2 Detection wavelengths for water- and fat-soluble vitamins

Vitamin	Detection wavelength(nm)
B_5, B_8	210
D_3	265
C, B_6, B_9, E	292
A	324
B_{12}	361

RESULTS

Vitamins were detected at different wavelengths. Figure 3 shows the chromatograms of standard and sample solution of nine fat- and water- soluble vitamins. Figure 4 presents recoveries of vitamins in standard and sample solutions.



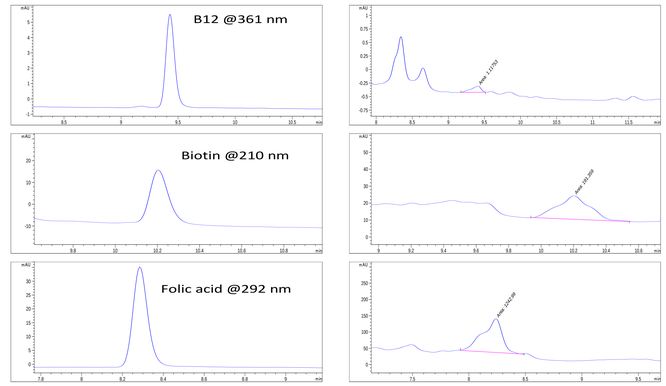


Fig. 3 Chromatograms of standard and samples of nine fat- and water-soluble vitamins

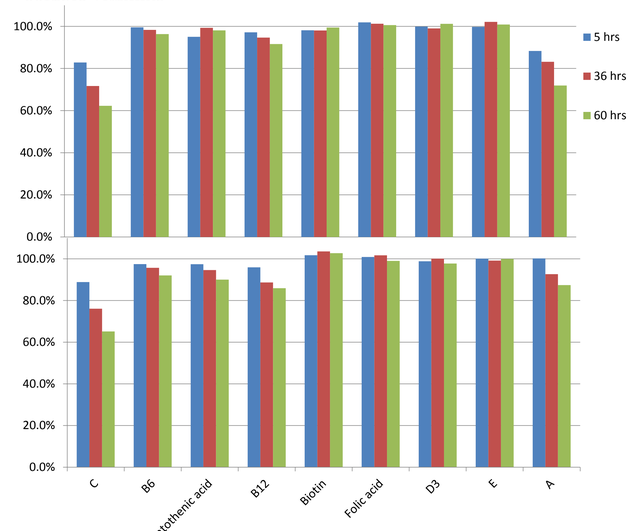


Fig. 4 recoveries of vitamins in standard and sample solutions

Tab. 3 Determination of water- and fat-soluble vitamins in gummies

Vitamins	Labeled (mg/kg)	Found (mg/kg)	SD	RSD (%)	Recovery (%)
C	4595.588	3919.469	73.52	1.88	85.29
B6	459.559	41.015	2.25	5.48	8.83
Pantothenic acid	1194.853	760.228	97.24	12.79	63.63
Folic acid	59.743	24.039	1.29	5.37	40.24
B12	1.379	0.119	0.02	17.50	8.66
Biotin	13.787	24.777	2.68	10.81	179.71
D3	2.298	2.391	0.02	0.70	104.05
E	2527.574	1450.034	7.62	0.53	57.37
A	144.761	251.286	15.85	6.31	173.59

CONCLUSIONS

Separation and determination of the vitamins was achieved by RP-LC. For most vitamins, quantitative results close to the label claim are determined. The stability of the vitamin extracts and potential interference by co-extracted sugar are being investigated to improve these results prior to validation of the final method.

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