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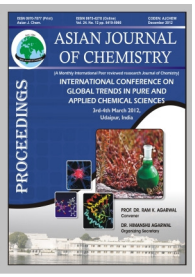


# High Performance Thin Layer Chromatography Method for Determination of Biotin in Edible Bird's Nest†

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In the present study, we detected biotin in the edible bird's nest for the first time and determined the optimal extraction and biotin. The biotin was extracted using *N,N*-dimethylformamide. Separation of biotin was performed by using a mobile phase consisting of chloroform: acetone:methanol:glacial acetic acid (2.5:2.5:1:0.01, v:v:v:v) on precoated silica gel 60 $F_{254}$ HPTLC plates. Densitometric evaluation of the spots was carried out in the absorbance wavelength at 400 nm. The spotting of the extract and standard solution is 0.47 of $R_f$ value. The method was validated for precision, intra and interday and recovery. The relative standard deviation (RSD) precision was 1.22 % in 0, 2, 4 and 6 h. Intra and interday variation studies gave 0.56, 1.17, 0.78, 1.02, 1.66, 0.55, 0.46 and 1.03 RSD (≤5 %) for the different concentration and time. Average recovery of 99.12 % was recorded and total content of biotin in edible bird's nest was 30.3 µg/g. Detection of biotin may also be an effective method to determine adulteration in edible bird's nest.

**Key Words: Biotin, *N,N*-Dimethyl formamide, High-performance thin layer chromatographic, Edible bird's nest.**

## INTRODUCTION

Edible bird's nest is an expensive traditional Chinese medicine dating as far back as the Tang (618-907 AD) and Sung (960-1279 AD) dynasties[1-3]. People attach many importance to edible bird's nest for their nutritional and medicinal value[3]. Based on the previous reports, edible bird's nest contains of many chemical compounds including lipid, ash, carbohydrate and protein[4]. It can be used in treating many diseases, such as consumption disease, stomach ulcers, haematemesis general debility and asthenia[5]. In this case, some adulterants for example karaya gum and tremella fungus have been discovered to be routinely added into the edible bird's nests during commercial processing prior to final sale[4]. The detection of biotin may be also a method to detect whether the nests are adulterated or not. Malaysia and Indonesia are the leading producer nation of edible bird's nest.

Biotin (hexahydro-2-oxo-1*H*-thieno[3,4-d]imidazole-4-pentanoic acid), is a water-soluble vitamin and also called vitamin H, which is the essential co-factor for five biotin-dependent carboxylases, namely acetyl-CoA carboxylase-α, acetyl-CoA carboxylase-β, propionyl-CoA Carboxylase, pyruvate carboxylase and β -methylcrotonyl-CoA, which carboxylase in many metabolic pathways[6,7].

Analytical techniques for biotin determination include microbiological assays[8,9], spectrophotometric[10,11], capillary zone electrophoresis[12], high performance liquid chromatography[13,14], thin layer chromatographic[15] and binding assays[16,17]. Among those, microbiological assays and HPLC are the most widely used assay methods to detect biotin. However, these two methods are not suitable for simple and fast detection of biotin. Nowadays, high-performance thin layer chromatographic is a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean-up. To our knowledge, no article related to the stability indicating HPTLC determination of biotin in edible bird's nest has been reported in the current literature. Therefore, the aim of this study was to provide simple and rapid extraction, quantification and new chromogenic methods of biotin in the edible bird's nest.

## EXPERIMENTAL

Unprocessed white edible bird's nest was obtained from CINRA Food Industries SDN. BHD (Malaysia). The bird's nests were processed as described by Guo *et al.*,[18]. The grounded nest samples were used in the following experiments. A biotin standard (B4501, ≥ 99 % lyophilized powder) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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All chemicals including solvents were at analytical grade. Pre-coated silica gel 60 $F_{254}$ HPTLC plates (10 × 10 $cm^2$) were purchased from Haiyang Chemical Co. (Qingdao, China).

A Camag (Muttenz, Switzerland) thin layer chromatography scanner 3 with Wincats 1.4.1 software, a development chamber consists of a twin trough glass chamber, Buchi rotating evaporation instrument, air oven, Eppendrof high-speed centrifuge were used.

**Chromatographic conditions:** The conditions were as follows. Test plate: pre-coated silica gel 60 $F_{254}$ HPTLC plates. Format: 10 × 10 $cm^2$. Spotting volume of sample: 15 µL. Development chamber: twin trough glass chamber 10 × 12 × 5 $cm^3$. Mobile phase: chloroform: acetone: methanol: glacial acetic acid (2.5: 2.5: 1: 0.01, v: v: v: v). Chamber saturation time: 60 min (mobile phase). Room temperature: 23 ± 2 ºC. Relative humidity: 55 × 3 %. Migration distance: 60 mm. Migration time: 20 min. Scanning speed: 20 mm/s. Data resolution: 50 µm step. Reflectance-absorbance wavelength: 400 nm (deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm). Operating software: Wincats 1.4.1.

**Chromogenic method:** 0.2 g of potassium permanganate was dissolved in 400 mL of distilled water. HPTLC plates were dried in a current of air for 5 min. Then, solutions of potassium permanganate were sprayed on the HPTLC plates and dried in the air oven (110 ºC) for 10 min. Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance wavelength at 400 nm and operated by Wincats 1.4.1.

**Standard solution preparation:** The solution of standard was prepared with accurately weighed biotin dissolved in DMF. The concentration of biotin was 0.4, 0.99, 1.48, 2.51, 3.19 mg/mL, respectively and every concentration was spotted 1 µL on the HPTLC plate. Quantitative concentration of each area was plotted with linear regression by TLC scanner.

**Extraction method:** 0.98 g powdered sample and 30 mL DMF were added in the 50 mL of centrifuge tube and the tube was placed into a boiling water bath (90-100) for 120 min. During the extraction processes, the tubes were shaked for at least 5 times and before shaking performed, the tubes were sealed. After cooling, the sample of tube was centrifuged at 10,000 rpm for 10 min. Then, the supernatant was concentrated by rotating evaporation instrument to less than 1 mL.

**Precision:** Precision was determined by spotting 1 µL of a 1.48 mg/mL standard solution on the same plate to give four spots of the same amount (1.48 µg) and scanned on 0, 2, 4 and 6 h.

**Intra and interday variation:** To ensure precision of the method, intra and interday variation studies were carried out at 1.48 and 3.19 µg. The concentrations chosen were fallen in the linearity range including the highest and middle points of the calibration curve. Standard solutions were analyzed in each morning and evening for two days.

**Recovery studies:** The extraction of solution was spiked with extra 50, 100 and 150 % of the standard biotin and the mixtures were analyzed by the proposed method. Samples were analyzed in triplicate.

**Statistical analysis:** Statistical analysis was carried out with SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Analysis of variance was performed by ANOVA.

## RESULTS AND DISCUSSION

In this study, biotin can be identified in the edible bird's nest and best proof was given in Figs. 1-4. Four spots can be observed in the same horizontal line in the Fig. 1. In Fig. 2 and 3, the standard peak and sample peak was aligned and $R_f$ value of 0.47 was recorded. This result proved that the sample and standard was in the same molecular weight. The sample and standard of absorption wavelength curve were approaching each other in the spectrodensitometric analysis graph (Fig. 4). The maximum absorption of 200 nm and second absorption of 400 nm can be found in Fig. 4.

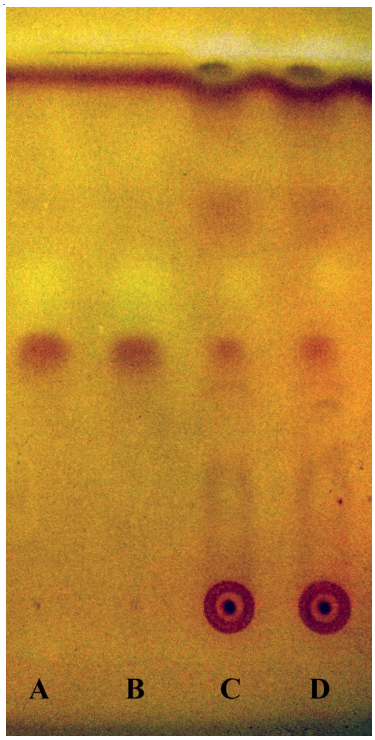


Fig. 1. HPTLC plates (A, B: standard solution and C, D: extraction of samples)

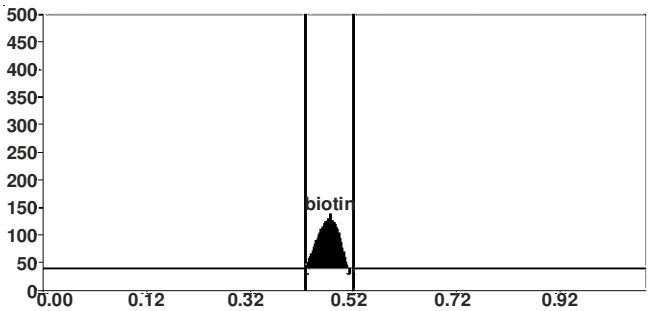


Fig. 2. Peak of standard solution

Table-1 presented the effects of the precision in different time (RSD ≤ 5 %) and there were no significant differences among the peak area value between 0, 2, 4 and 6 h. As shown in Table-2, the RSD of the intra and interday was 0.56, 1.17, 0.78, 1.02, 1.66, 0.55, 0.46 and 1.03, respectively, which could indicate that the robustness of this method was acceptable. Average recovery of biotin was 99.12 % as listed in Table-3. Through the above analysis of the data proved the repeatability and stability of this method.

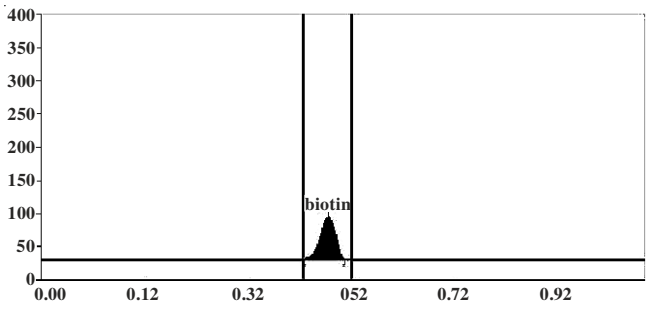


Fig. 3. Peak of extraction samples

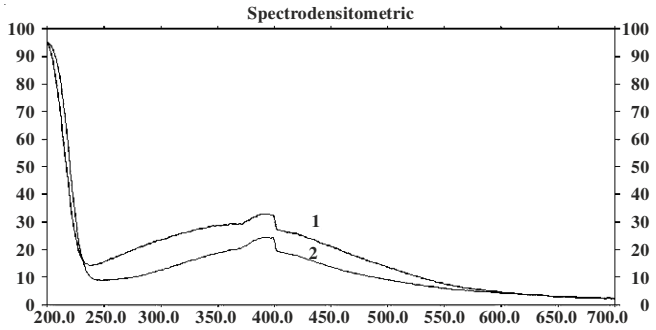


Fig. 4. Spectrodensitometric analysis graph of the biotin (1: standard solution, 2: extraction of samples)

TABLE-1
PRECISION

| Amount (µg/spot) | Time (h) | Peak area values | Mean ± SD | RSD (%) |
|---|---|---|---|---|
| 1.48 | 0 | 7092.25 | 7106.56 ± 86.49 | 1.22 |
| | 2 | 7110.75 | | |
| | 4 | 7099.75 | | |
| | 6 | 7123.50 | | |

TABLE-2
INTRA AND INTERDAY VARIATION

| | Amount (µg/spot) | Peak area values (mean) | SD | RSD (%) |
|---|---|---|---|---|
| Intraday precision | 1.48 µg | 7109 | 39.88 | 0.56 |
| | | 7170 | 84.02 | 1.17 |
| | 3.19 µg | 14491 | 112.69 | 0.78 |
| | | 14398.67 | 146.66 | 1.02 |
| Interday precision | 1.48 µg | 7134.67 | 118.09 | 1.66 |
| | | 7078.33 | 38.80 | 0.55 |
| | 3.19 µg | 14484.67 | 66.00 | 0.46 |
| | | 14491.33 | 149.37 | 1.03 |

TABLE-3
RECOVERY

| Level (%) | Observed (%) | Recovery (%) | Average recovery (%) |
|---|---|---|---|
| 50 | 49.2 | 98.4 | 99.12 |
| 100 | 99.7 | 99.7 | |
| 150 | 148.88 | 99.25 | |

Hot water extraction of biotin was carried out by the method of AOAC (1990). Through the pre-experiment, this extraction method was not ideal because impurities were found to be interfere with the determination of biotin. This experiment used DMF as extraction solvents could reduce the impurity effect on the detection of biotin.

The maximum absorbance wavelength of biotin in UV detection is 210 nm, but its silica gel plate has absorbed strongly at 190 to 210 nm. Thus, staining methods can be used to produce the absorption wavelength of 400 nm (Fig. 4). The major advantage of chromogenic method is the spotting can be shown as early and qualitatively and therefore the peak of biotin can be obtained.

Microbiological assays and HPLC are not necessarily applied to both qualitative and quantitative analysis of biotin in edible bird's nest. Microbiological assays are sensitive, but the sample's nutrients may promote certain microorganisms' growth and interfere with the assay results[19]. In addition, it is also rather tedious, time-consuming and expensive[20]. Whereby, UV detection in HPLC method is not suitable to the biotin detection because its maximum absorbance is at 210 nm, which is similar to that of most solvents. An analytical technique of HPTLC is widely used, which is mainly because of the environmental friendly concept and lowering the use of solvent amount purpose. Since several samples can be run simultaneously by using a small quantity of mobile phase, the analytical time and cost per analysis can be reduced. Statistical data analysis proved that this HPTLC method is repeatable, timesaving, accurate and dependable for the analysis of biotin in edible bird's nest.

Linear regression equation of biotin was Y = 397.891+ 4.499X (Y is the response while X is the amount of biotin) and r = 0.9995, RSD = 1.07 %. Total content of biotin was 30.3 µg/g in edible bird's nest.

## Conclusion

The proposed analytical HPTLC method is precise, specific, accurate and stable. This method may be applied in routine analysis of biotin in edible bird's nest and it may be a useful method to determine adulteration.

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