DIETARY SUPPLEMENTS

Quantitative Analysis of Forskolin in *Coleus forskohlii* (Lamiaceae) by Reversed-Phase Liquid Chromatography

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A rapid method was developed for the evaluation of forskolin in *Coleus forskohlii* Briq. (Lamiaceae). Forskolin was quantitated in the root and stem of dried *C. forskohlii* and in 17 market products by reversed-phase liquid chromatography (LC) with a photodiode array detector at 210 nm. The temperature was held constant at 30°C, and the retention time of forskolin was approximately 6.8 min. The samples were extracted with acetonitrile by sonication. The precision of the method was confirmed by a standard deviation <5.0% (n = 3), and forskolin recovery was 99.1%. Limit of detection was 1.5 μg/mL, and the response was linear through zero from 6.3 to 630 μg/mL with a correlation coefficient (R^2) of 0.9998. Identity of the marker compound was confirmed by an LC/mass spectrometry experiment. The method was successful in the qualitative and quantitative evaluation of the marker compound in *C. forskohlii* plant material and in market products claiming to contain *C. forskohlii*.

Coleus forskohlii Briq. (Lamiaceae) grows wild in arid and semi-arid regions of India and Thailand; the roots have long been used in Ayurvedic medicine (1). A member of the mint family, it has been traditionally used to treat heart and lung disease, intestinal spasms, insomnia, and convulsions (1). Forskolin (Figure 1), a labdane diterpenoid, is considered the active secondary metabolite because of its ability to activate the enzyme adenylate cyclase (2). Recent research has shown that forskolin has positive effects against a wide range of conditions such as asthma, glaucoma, hypertension, hair loss, cancer, and obesity (3–9). It also inhibits the platelet-activating factor (PAF; 10).

In 1984, Inamdar et al. (11) reported a comparison of thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and liquid chromatography (LC) for the quantitative estimation of forskolin (11). Of the methods studied, normal-phase LC was determined to be the best procedure for the analysis of the benzene extract of *C. forskohlii*. The present study improved the extraction procedure and LC method.

To date, a maximum safe dose of forskolin has not been determined and no adverse effects have been reported from its use. This is evident by the information on the label of the market products, some of which have been standardized for forskolin at a low of 1%, while others list it at 20%. Because the plant has become increasingly popular in the Western world as a weight loss dietary supplement, an improved analytical method for quantitative and qualitative assessment is needed. This newly developed method was used to analyze the root, stem, and leaf of *C. forskohlii* and 17 products currently on the market containing *C. forskohlii* plant material or extract. A comparison of label statements with the obtained data is discussed.

Experimental

Apparatus

(**a**) *LC-photodiode array (PDA) system*.—Waters 2695 Alliance Separations Module equipped with a 996 PDA detector with an XTerra RP_{18} column, 150 × 4.6 mm, 5 μm particle size (Waters, Milford, MA), and maintained at 30°C. The mobile phase consisted of water (A) and acetonitrile (B). At a flow rate of 1 mL/min, the gradient elution was 50A/50B to 43A/57B in 10 min. Each run was followed by a 5 min wash with methanol and an equilibration period of 10 min. The detection wavelength was 210 nm, and the injection volume was 10 μL. The forskolin peak in a product was assigned by a comparison of the retention time and the UV spectra of the peak given in the standard compound chromatogram (Figure 2). Data were collected and analyzed by Waters Millennium[32] software and tabulated by Microsoft Excel.

(**b**) *LC/mass spectrometry (MS) system*.—Finnigan AQA mass spectrometer and Finnigan LC with AS3000 autosampler, P4000 pump, and UV6000LP detector (San Jose, CA), using an XTerra RP_{18} column, 150 × 4.6 mm, 5 μm particle size (Waters) at ambient temperature. The mobile phase consisted of water (A) and acetonitrile (B). At a flow

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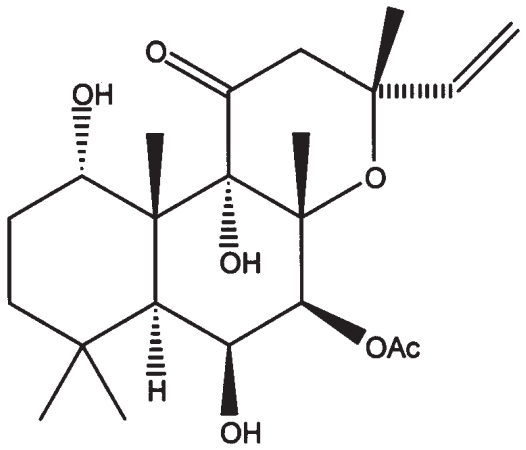

Figure 1. Structure of forskolin.

rate of 0.5 mL/min, the gradient elution was 50A/50B to 43A/57B in 10 min, hold at 43A/57B for 10 min. Each run was followed by a 5 min wash with methanol and an equilibration period of 10 min. The detection wavelength was 210 nm, and the injection volume was 5 μL. Best results were obtained in positive electrospray ionization (ESI) mode, with ionization voltage set to 25 V, source voltage to 3.0 kV, and probe temperature to 350°C.

(**c**) *Ultrasonic cleaner*.—Model FS20H (Fisher Scientific, Fair Lawn, NJ).

(**d**) *Centrifuge*.—Model 67377 Centricone, 1550 rpm (Chicago, IL).

(**e**) *Rotavapor*.—Buchi R-114 and water bath B-480 (Buchi, New Castle, DE).

Reagents

(**a**) *Acetonitrile and methanol*.—LC grade (Fisher Scientific).

(**b**) *Centrifuge tubes*.—Falcon BLUE MAX™ Jr, 15 mL polystyrene conical tubes (Becton Dickinson, Franklin Lakes, NJ).

(**c**) *Filters*.—Nylon membrane filters, 13 mm, 0.45 μm (Phenomenex, Torrance, CA).

(**d**) *Products*.—Purchased from various on-line vendors.

(**e**) *Coleus forskohlii*.—Obtained from the herbal garden of James Duke (Columbia, MD). A voucher specimen is maintained at the University of Mississippi herbarium.

(**f**) *Standard stock solution*.—Prepared by dissolving 6.3 mg forskolin (ChromaDex, Irvine, CA) in 10 mL acetonitrile. Further calibration levels (630.0–6.3 μg/mL) were prepared by diluting the stock solution with methanol. The 4-point calibration data (n = 3) were obtained: regression equation linear through zero ($y = 1.52 \times 10^3 x$, $R^2 = 0.9998$, and limit of detection = 1.5 μg/mL).

Extraction

The powder from one capsule or ground material from one tablet (range 200–1500 mg) of a product was weighed and then transferred to the respective centrifuge tube, followed by 3 mL acetonitrile. The samples were sonicated for 15 min. After centrifugation of the sonicated sample, the supernatant was pipetted into a 10 or 25 mL volumetric flask. This procedure was repeated twice (a total of 3 extractions per sample), and the samples were then diluted to the final volume with acetonitrile. Before injection, each sample was filtered through a 0.45 μm nylon membrane filter. A dried sample of *C. forskohlii* was separated into 3 parts (root, stem, and leaf). After grinding, ca 500 mg of each was extracted by the same method used for the purchased products.

Method Validation

The precision of the LC method was confirmed by injecting each sample in triplicate, and a standard deviation (SD) <5.00% was achieved. Reproducibility of the extraction method was verified by extracting one sample (n = 3) inter- and intraday. Accuracy of extraction was also determined by 2 methods. After sample CF-1 was exhaustively extracted until forskolin was no longer detectable by LC monitoring, CF-1 was spiked with standard solution (63 μg/mL). After the sample had dried, extraction then took place as described above and the sample was analyzed by LC. The recovery of forskolin was 99.2%. Accuracy was also tested by spiking sample CF-1 with a known amount of forskolin. Once the sample had dried, it was extracted and analyzed. Recovery of forskolin was 99.1% compared with the theoretical amount.

Results and Discussion

Method Development

The original quantitative estimation work used a benzene extract of *C. forskohlii*, which involved a total of 6 h of extracting at elevated temperatures followed by concentration in vacuo (11). Because of the environmental safety involved with benzene, a safer extraction solvent was used. Acetonitrile was chosen because of its low co-extractive properties and had proven efficient for the extraction of forskolin. Total extraction time was improved to about 1.5 h with sonication, and samples were then ready for analysis by LC after filtration.

Instead of a silica stationary phase, a reversed-phase C_{18} column was chosen (11). This allowed the use of water in the mobile phase, thereby eliminating the environmental safety issue with a halogenated solvent, such as methylene chloride, which was used in the reported method. Water was also more

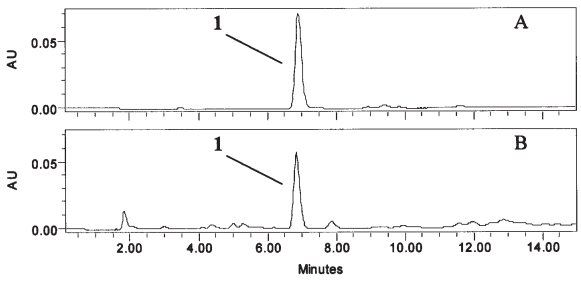

Figure 2. Liquid chromatogram of forskolin obtained (A) in the standard stock solution and (B) in a product claimed to contain forskolin, CF-4.

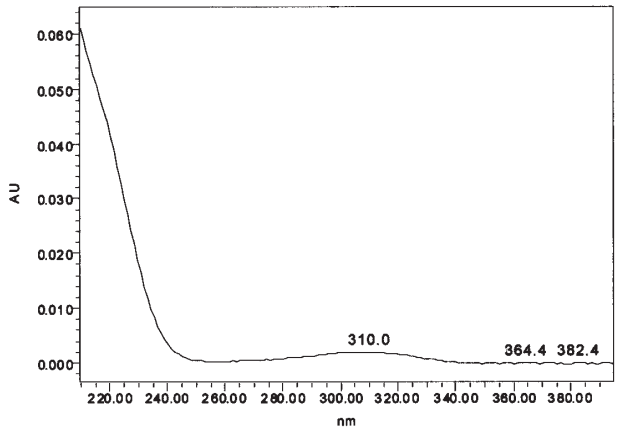

Figure 3. The UV spectrum of forskolin.

advantageous as a waste product for disposal. A gradient method using water and acetonitrile proved sufficient for the quantitation of forskolin. Chromatograms of the standard and a sample (CF-4) are shown in Figure 2. Forskolin was chosen as the marker compound for standardization because of its positive attributes described in the literature. The products tested were also standardized for the presence of forskolin according to their labels. Peak symmetry was good while retention time was retained at approximately 6.8 min. Peak purity and identity were verified by studying the PDA and MS data, as well as by spiking samples with reference compounds. No indications of impurities were found.

The LC/MS experiment was performed to confirm the identity of the peak of interest. The flow rate was modified to 0.5 mL/min to allow better detection of the compound. Although the retention time increased to about 12.3 min, the UV spectra (Figure 3) were a match for the peak of interest in the LC and LC/MS methods. In positive ESI mode, the spectrum of forskolin showed signals at *m/z* 428.2 $[M+NH_4]^+$ and 411.1 $[M+H]^+$ (Figure 4).

The efficiency of the extraction procedure was verified by repeatedly extracting sample CF-1 with 3 mL acetonitrile. Each extract was analyzed individually. Forskolin was no longer detectable after the fourth repetition. Forskolin was already 99.2% extracted after the third repetition, which confirmed that the extraction procedure was exhaustive.

Sample Analysis

Forskolin was detected in the dried root and stem extracts, 0.3 and 0.03%, respectively, but not in the leaf extract. Seventeen market products, consisting of tablets and capsules, were analyzed (Table 1). Products CF-1 to CF-8 contained only *C. forskohlii* extracts, and products CF-9 to CF-17 contained *C. forskohlii* extracts as part of a proprietary blend. Four products (CF-10and 15–17) did not specify the plant part extracted, and 2 products (CF-7 and 8), manufactured by the same company, contained leaf extract of *C. forskohlii*. All remaining products were made with the root or root extract of *C. forskohlii*. The labels reported percent standardization of forskolin as low as 1% in some products and as high as 20% in others. Only CF-15 did not list a percentage of forskolin on the label. Twelve of the products met or exceeded the percentage listed on the label. Products CF-4, CF-5, and CF-8 had a forskolin percentage <0.7% the label claimed value. Product CF-13 was the least desirable qualitatively, standardized for 16% forskolin, but only 10.5% was determined.

A much more effective comparison was made between the daily dose of forskolin stated on the label of the product and the actual amount determined by this method (Table 1). As stated earlier, a maximum safe dose of forskolin had not been determined and a wide range of dosage was shown by its standardization in milligrams reported on the product labels. One product stated a low of 2.5 mg forskolin per daily serving, while another stated a high of 26.6 mg per daily serving. The label claims on 14 of the products were relatively accurate, ± 3.6 mg (CF-1–12, 16, and 17).

Product CF-13 was of poor quality in terms of its low forskolin content compared with the label statement. Quality was also an issue for CF-14, in which the forskolin content was much greater than the label claim. Two tablets were to be taken twice a day. This was a daily consumption of 26.0 mg forskolin a day according to the label claim, but analysis showed the individual was ingesting almost 5 times that value, 124.8 mg. Because no maximum daily dosage of forskolin had been determined, a statement of risk was not issued about the consumption of CF-14.

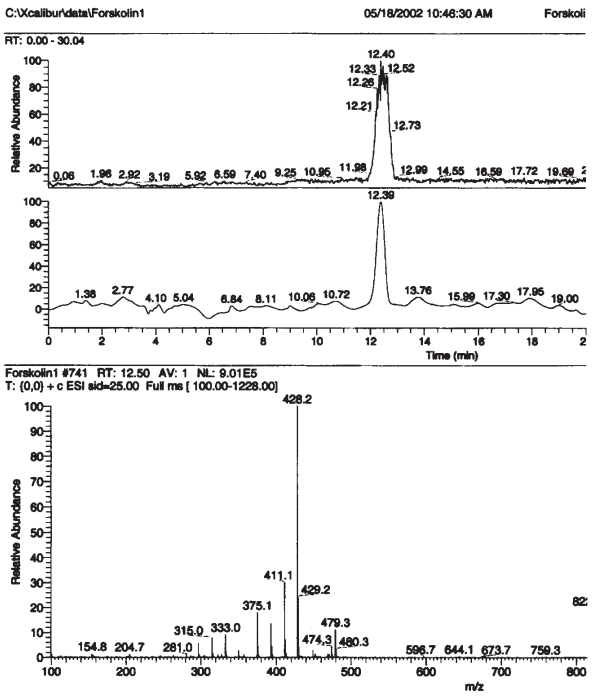

Figure 4. LC/MS chromatogram and mass spectrum of forskolin in standard stock solution and product extracts.

Table 1. Comparisons of percentage of forskolin by label claim and determined percentage in capsule or tablet, and of quantity (mg) in daily dose reported on label to quantitated values

Product code	Label claim, %	Found, %[a]	Label claim, mg	Found, mg
CF-1	18.0	20.1 (1.1)	9.0	10.0
CF-2	1.0	1.6 (0.2)	2.5	4.0
CF-3	1.0	1.3 (4.1)	2.5	6.1
CF-4	18.0	17.8 (2.9)	9.0	8.9
CF-5	10.0	9.3 (2.9)	10.0	9.3
CF-6	20.0	21.7 (3.6)	25.0	27.1
CF-7	10.0	12.9 (0.2)	10.0	12.9
CF-8	1.0	0.9 (0.2)	3.8	3.5
CF-9	10.0	10.6 (2.6)	16.7	17.6
CF-10	20.0	20.8 (2.7)	20.0	20.8
CF-11	20.0	22.2 (2.8)	20.0	22.2
CF-12	10.0	10.2 (0.7)	15.0	15.3
CF-13	16.0	10.5 (1.5)	26.6	17.5
CF-14	10.0	48.0 (1.4)	13.0	62.4
CF-15	[b]	11.2 (3.7)	[b]	33.6
CF-16	20.0	20.1 (0.1)	15.0	15.1
CF-17	10.0	13.8 (1.0)	5.0	6.9

[a] SD % is shown in parentheses.
[b] 300 mg *Coleus forskohlii* extract in 4 capsules.

In another interesting comparison, forskolin was not detected earlier by LC or LC/MS in the dried leaf extract of *C. forskohlii*, and previous research did not confirm its presence in the leaf of *C. forskohlii*. Yet, 2 of the products, CF-7 and 8, contained the leaf extract standardized for 20% forskolin. Its presence was verified by this method in which 20.8% forskolin was detected in CF-7 and 22.8% in CF-8. The reason for the high amount in the 2 products containing leaf extract was interesting because the root was the part of *C. forskohlii* used in medicinal preparations. Cultivation of *C. forskohlii* was focused on the quality of the root, not the leaf (12). Only one population of *C. forskohlii* was analyzed in this study, which means the chemical ecology of forskolin was not definitively addressed.

Conclusions

The method described here is rapid and accurate. In the ever-increasing market of dietary supplement and herbal products, the results obtained in this study strengthen the need for published analytical methods for their independent analysis. Even if a method was previously published, it is important to improve upon that method to ensure the safety and quality of products being consumed and to improve method safety.

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