# GLC Method for Assay of Forskolin, a Novel Positive Inotropic and Blood Pressure-Lowering Agent

**P. K. INAMDAR ˣ, H. DORNAUER, and N. J. de SOUZA**

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**Abstract** □ A GLC method for the quantitative determination of forskolin, a novel positive inotropic and blood pressure-lowering agent, is described. This method is simple, rapid, and sensitive and is suitable for the routine assay of plant materials and pharmaceutical preparations containing forskolin.

**Keyphrases** □ Forskolin—GLC analysis in plant extracts and pharmaceutical preparations □ GLC—analysis, forskolin in plant extracts and pharmaceutical preparations □ Antihypertensive agents—forskolin, GLC analysis in plant extracts and pharmaceutical preparations

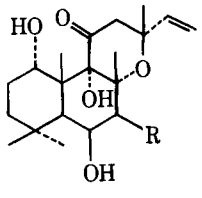
Forskolin (1α,6β,9α-trihydroxy-7β-acetoxy-8,13-epoxy-labd-14-en-11-one, Ia) is a novel positive inotropic and blood pressure-lowering agent isolated from the Indian plant *Coleus forskohlii* Briq. (Labiatae) (1, 2). Compound Ia currently is under preclinical development.

Different rapid, precise methods for the quantitative estimation of forskolin in plant materials and pharmaceutical preparations, as well as for application to pharmacokinetic studies, are under investigation in these laboratories. This report describes a GLC method for the assay of forskolin; it is applicable to forskolin in plant extracts and pharmaceutical preparations.

## EXPERIMENTAL

**Instrumentation**—The gas–liquid chromatograph[1] was equipped with a flame-ionization detector and a 180-cm × 5-mm glass, coil-shaped column packed with 3% OV-1[2] on 100–120-mesh Gas Chrom Q[3]. Nitrogen was the carrier gas at a flow rate of 20 ml/min, and the air and hydrogen flow rates were set to maximize the detector response. A potentiometric continuous-line recorder[4] (2.5 mv) with a speed of 300 mm/hr was used. The temperatures were: injector, 250°; column oven, 220°; and manifold, 270°. A microsyringe[5] was used in the GLC analysis.

**Reagents and Materials**—Samples of forskolin (Ia), 7-deacetylforskolin (Ib), plant materials, and tablets of forskolin were obtained[6]. The solvents were analytical grade.




Ia: R = $OCOCH_3$
Ib: R = OH

[1] Model 900, Bodenseewerk, Perkin-Elmer Co., Überlingen, West Germany.
[2] E. Merck, Darmstadt, West Germany.
[3] Supelco, Bellefonte, Pa.
[4] Kompensograph, 1288 × 288, type M-810-417, Siemens Aktiengesellschaft, Erlangen, West Germany.
[5] Hamilton 701N, Micromesure AG., 7402, Bonaduz, Switzerland.
[6] These samples were obtained from the research and development laboratories of Hoechst Pharmaceuticals Ltd. The plant materials were identified as *C. forskohlii* Briq. (No. HOE 141) and *C. spicatus* Benth. (No. HOE 142), both of the Labiatae family, by Miss Virbala Shah of Hoechst Pharmaceuticals Ltd. Voucher specimens are preserved at the Herbarium of the Research Centre, Hoechst Pharmaceuticals Ltd., Mulund, Bombay 400 080, India.

**Standard Solutions and Calibration Curves**—Forskolin (120 mg) was dissolved in chloroform (10 ml). The internal standard solution was prepared by dissolving Ib (80 mg) in chloroform (10 ml). Forskolin solutions were prepared in triplicate from these solutions to contain 1.2, 2.4, 3.6, 4.8, and 6 mg of forskolin/ml of chloroform, in which the concentration of the internal standard was 4 mg/ml.

Each solution (1 μl) was injected in triplicate onto the column. The peak area ratio of forskolin to the internal standard was plotted against the ratio of the weight of forskolin to the internal standard to yield a calibration curve.

**Preparation of Semipurified Extracts of Plant Materials**—The dried and finely divided plant material (15 g) was extracted with benzene (3 × 150 ml) at 40–50° for 5 hr, and the combined extracts were filtered and concentrated *in vacuo*. The residue was triturated with petroleum ether (bp 60–80°) (2 × 50 ml), and the petroleum ether layer was decanted. The petroleum ether-insoluble residue was treated further with methanol (2 × 50 ml), and the methanolic solution was separated and concentrated *in vacuo* to constant weight to provide the semipurified extract.

**Assay of Plant Materials**—The semipurified plant extract was dissolved, depending on its weight, in chloroform (0.125–3.0 ml). The solution always was adjusted to contain the internal standard at a concentration of 4 mg/ml. One microliter of the solution was injected onto the column. The mean peak area ratio of six injections was used for quantitation of the amount of forskolin in *C. forskohlii*.

**Assay of Forskolin Tablets (5 mg/Tablet)**—Ten tablets, each weighing an average of 125 mg, were powdered. A quantity equivalent to one tablet (125 mg) was transferred to a 25-ml centrifuge tube. Chloroform (10 ml) was added, and the suspension was centrifuged for 10 min; the procedure was repeated three times.

The pooled chloroform extracts left a residue upon evaporation of the chloroform *in vacuo*, and this residue was dissolved in chloroform (0.5 ml) and mixed with the internal standard solution (0.5 ml). One microliter of this solution was injected in quadruplicate onto the column.

Placebo tablets also were extracted in parallel with the sample. The placebo chromatogram was checked for interfering peaks at the retention times for forskolin and the internal standard.

## RESULTS AND DISCUSSION

The gas chromatogram of forskolin and the internal standard is presented in Fig. 1. The retention times were 21.6 and 18.0 min, respectively. The GLC responses for forskolin and Ib were linear over a range of at least 1.2–6.0 μg of the drug and the internal standard injected separately. The minimum detectable level was 30 ng of forskolin. A calibration curve indicated that a linear relation existed in the 1.2–6.0-μg range between the peak area ratio of forskolin to the internal standard and the ratio of the weight of forskolin to the internal standard.

Typical chromatograms for a semipurified extract of *C. forskohlii* and the semipurified extract of *C. forskohlii* together with the internal standard (4 μg) are presented in Fig. 2. A GLC–mass spectrometric analysis of the semipurified extract of *C. forskohlii* revealed that the peak with the retention time of that for forskolin gave a mass spectrum identical to that of a standard forskolin sample. By referring to the calibration curve, the amount of forskolin present in *C. forskohlii* was calculated. In the different samples assayed, forskolin occurred in amounts ranging from ~0.05 to 0.1% of the dry weight of the plant material, depending on the part of the plant that was assayed as well as on the time and location of collection of the plant material. The results agreed well with amounts of forskolin isolated from bulk quantities of plant material (1). Therefore, the method is a rapid and sensitive technique for evaluating and controlling the quality of *C. forskohlii*.

To find alternative and, possibly, richer sources of forskolin than *C. forskohlii*, the method was applied to semipurified extracts of other

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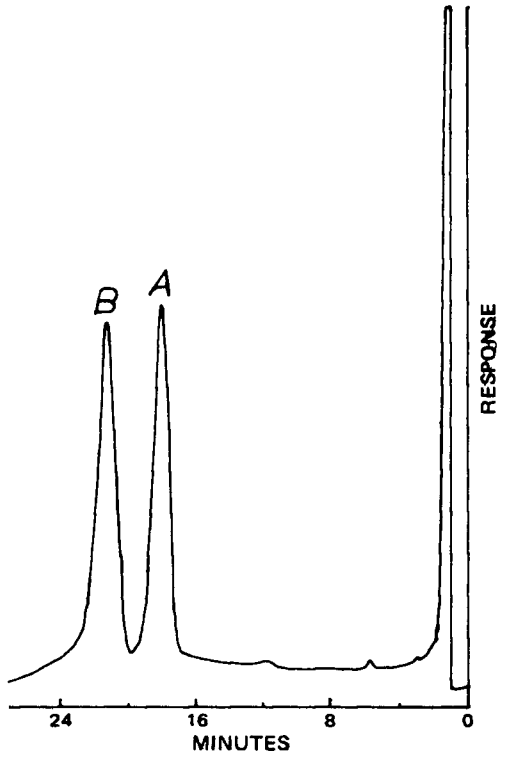


**Figure 1**—*GLC separation of forskolin (B) and the internal standard (A).*

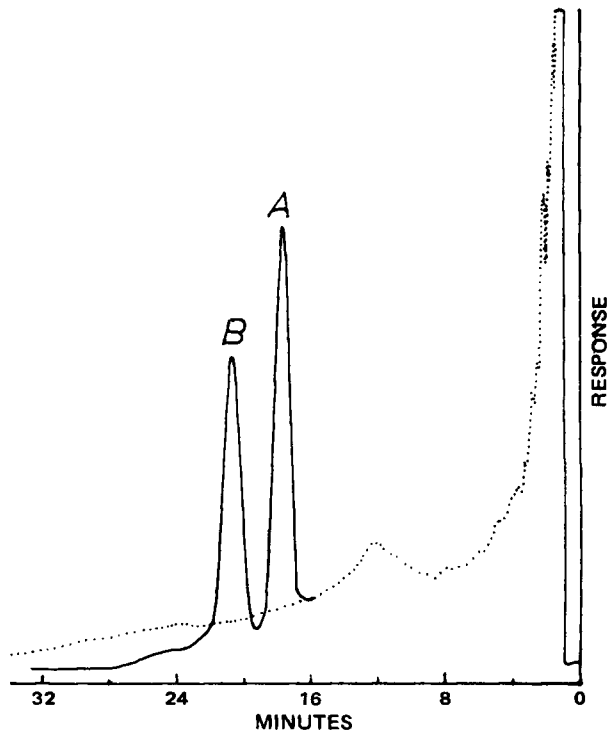


**Figure 3**—*Gas–liquid chromatogram of a semipurified extract of* C. spicatus (····) *and of the semipurified extract of* C. spicatus *spiked with forskolin (B) and the internal standard (A)* (—).

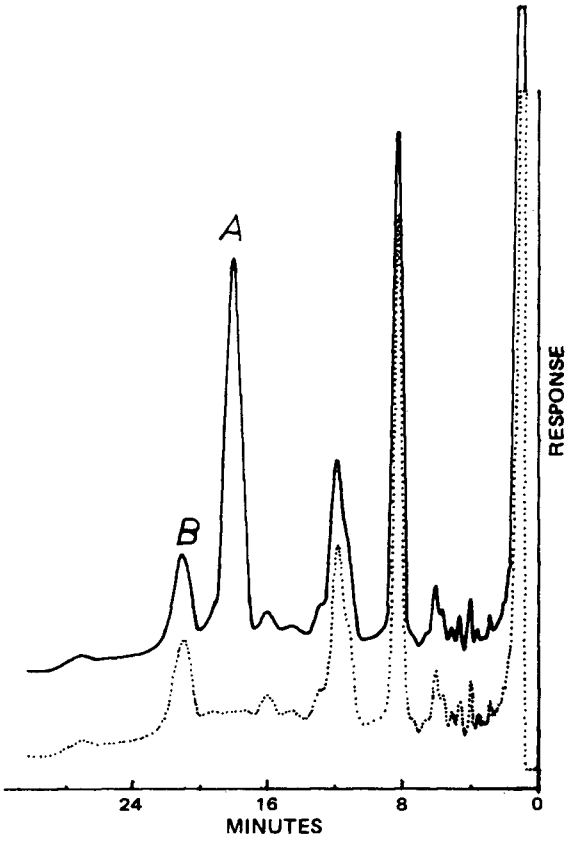


**Figure 2**—*Typical GLC separation of a semipurified extract of* C. forskolii (····) *and of a semipurified extract of* C. forskohlii *together with the internal standard* (—). *Key: A, internal standard; and B, forskolin.*

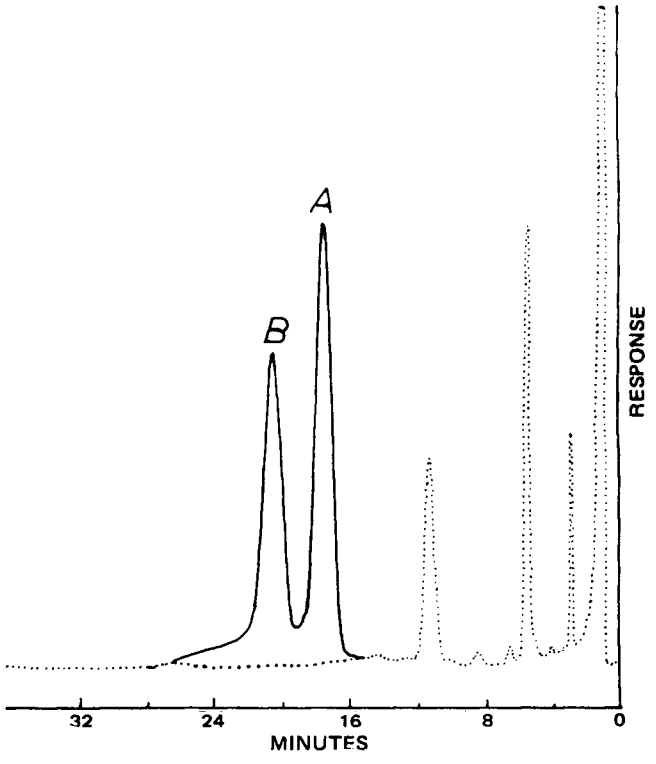


**Figure 4**—*Gas–liquid chromatogram of the placebo tablet* (····) *and of the tablets of forskolin with the internal standard* (—). *Key: A, internal standard; and B, forskolin.*

*Coleus* species and taxonomically related *Plectranthus* species (3). As typical examples, chromatograms for the semipurified extract of *C. spicatus* Benth. (Labiatae) and the semipurified extract spiked with forskolin and the internal standard are presented in Fig. 3. In none of the

six *Coleus* species other than *C. forskohlii* and the six *Plectranthus* species assayed was forskolin detected at levels as low as $\sim 1 \times 10^{-4}\%$ of the dry weight of the plant material (3).

Chromatograms from the assay of forskolin tablets are presented in Fig. 4. There was no interference due to peaks observed in the placebo samples. Assays were carried out in quadruplicate on five samples. The results indicated 5.12 mg of forskolin/tablet (label claim of 5.0 mg/tablet) with a range of 5.07–5.19 mg/tablet and a *RSD* of ±0.28%.

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# Anti-Inflammatory Activity of Diazomethyl Ketone and Chloromethyl Ketone Analogs Prepared from *N*-Tosyl Amino Acids


**I. H. HALL ˣ, J. E. HALL, Jr., M. MOHSENI, and Z. SAJADI**



Received January 7, 1980, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication June 9, 1980.



**Abstract** □ A series of diazomethyl ketone and chloromethyl ketone analogs prepared from *N*-tosyl amino acids was shown to have anti-inflammatory activity in mice at 20 mg/kg and in rats at 10 mg/kg. *N*-Tosyl-L-alanine and *N*-tosyl-β-alanine chloromethyl ketones demonstrated the most potent anti-inflammatory activity. The writhing reflex also was inhibited at 20 mg/kg in mice. In the tail flick test, *N*-tosyl-D,L-alanine and *N*-tosyl-D,L-isoleucine chloromethyl ketones demonstrated the highest increase in time. Toxicity studies indicated good therapeutic indexes for most of these agents.



**Keyphrases** □ Anti-inflammatory activity—diazomethyl ketone and chloromethyl ketone analogs prepared from *N*-tosyl amino acids, biological evaluation □ *N*-Tosyl amino acids—diazomethyl ketone and chloromethyl ketone analogs, evaluation for anti-inflammatory activity


A series of *N*-tosyl, *N*-benzoyl, *N*-acetyl, *N*-carbobenzyloxy, and *N*-cinnamyl cyanomethyl esters of amino acids was shown to have anti-inflammatory and immunosuppressive activities in mice (1). The standard proteolytic inhibitor tosylphenylalanyl chloromethyl ketone also was active as an anti-inflammatory agent. This report discusses a series of diazomethyl ketones and chloromethyl ketones of *N*-tosyl amino acids that demonstrated similar activity.

## EXPERIMENTAL

The synthesis and physical data of this series of compounds were reported previously (2). The synthetic methods were essentially those outlined by Schoellman and Shaw (3). Male $CF_1$ mice (~30 g) or male Sprague–Dawley rats (~160 g) were administered the test drugs at 20 or 10 mg/kg ip, respectively, in 0.05% polysorbate 80, 3.5 hr prior to the injection of 1% carrageenan in 0.9% saline into the plantar surface of the right hindfoot. Isotonic saline was injected into the left hindfoot to obtain baseline data. After 3 hr, both feet were excised at the tibiotarsal (ankle) joint according to a modification of the method of Winter *et al.* (4, 5).

As an analgesic screen, the tail flick (6) method was employed with male $CF_1$ mice (~30 g) who received the test drugs at 20 mg/kg ip 5 min prior to the analgesic test. An apparatus was designed and implemented in these laboratories to measure the tail flick response time of mice (Fig. 1). A control box housed all of the timing, logic, and sensing circuitry and the feedback controls. A secondary box provided the self-contained heater (55 ± 0.5°), the temperature-sensing elements, and special custom-made optical sensors to detect the flick of the tail. The sense signals were coupled to the control box to regulate the surface temperature and to freeze the digital clock (accuracy of 0.001%) display at the time of the first tail flick.

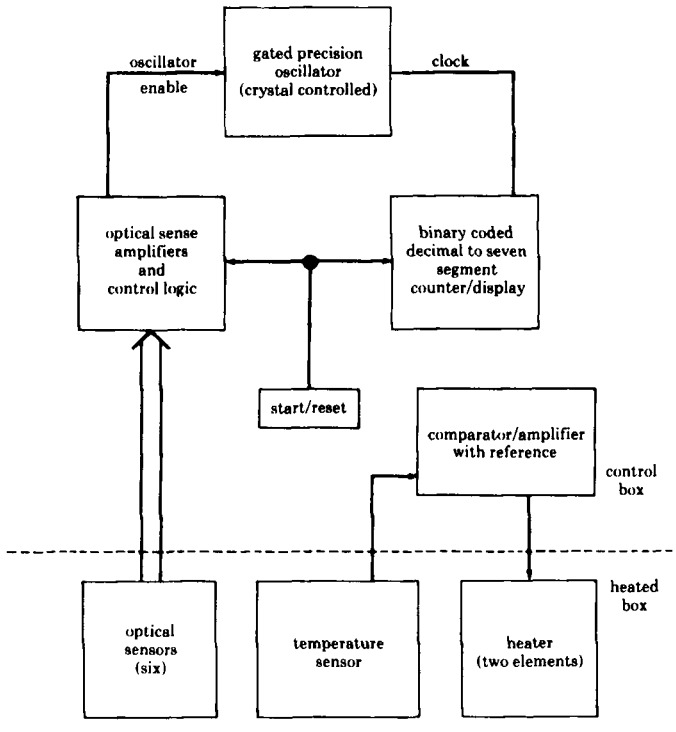


**Figure 1**—*Functional diagram of tail flick apparatus.*

The writhing reflex also was utilized as an analgesic test. Mice were administered the test drugs at 20 mg/kg ip 20 min prior to the administration of 0.5 ml of 0.6% acetic acid (7). After 5 min, the number of


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