Table II—Potencies Computed by FDA and Lilly Procedures from the Best Straight Lines

Sample	True Concentration, μg/ml	Five-Point Line, Including Reference Point		Four-Point Line Excluding Reference Point, Lilly
		FDA	Lilly	
1	3.00	3.69	3.15	3.02
2	6.00	7.36	6.27	6.02
3	4.00	4.99	4.26	4.09
4	2.75	3.40	2.90	2.79
Reference, mm		16.79	16.12	16.12
Mean computational error, %		+24	+5	0

Standard responses were corrected to the observed mean reference concentration response ("3.75" μg/ml) on all standard plates. Sample plate responses were corrected to the theoretical reference response, the 3.75-μg/ml point on the best straight line through the five standards as required by regulations (1). These results are given in the "FDA" column of Table II.

A second set of potencies was obtained from the same data by correcting sample zones to the observed reference as was done for standards and interpolating from the same straight line. These results are given in the "Lilly" column of Table II. A third set of potencies was obtained by correcting to the observed reference and by rejecting the obviously erroneous reference response when computing the best straight line. These results were taken as the true sample assay.

The computational error of +24% in the FDA procedure was caused by the computed reference diameter required by the CFR (1). The error caused by including the erroneous reference point in the calculation of the best straight line was about 5%.

The plates for Sample 4 were incubated at a temperature slightly different from that of the standard plates. Since each response was corrected to the reference concentration, this difference in temperature and the resultant reduction in zone diameters had no effect on the calculated sample potency.

The correction procedure of Ref. 1 is without error only when the observed mean reference diameter is identical with that interpolated from the best straight line. Since this is an unlikely occurrence, a different correction scheme should be used. Correcting both standard and sample responses to the response observed for the reference and then calculating estimated potencies from the regression equation will avoid the errors of the CFR (1).

Proper plate correction is of more than academic interest because computational errors as great as 40% have been reported from other laboratories[2]. Such an error was caused by using a reference about three-fourths as concentrated as it was thought to be. Large errors seem to be rare, but smaller ones are not. In one series of 40 response lines from 16 laboratories, seven of the reference zones, including the one just mentioned, were obviously in error and nine other standard zones had errors. Except for the reference, errors were evenly distributed among the other four standards. Three calibration lines had two obvious errors in them. All of these lines were used in the laboratories reporting the work.

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ACKNOWLEDGMENTS

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[2] Dr. H. S. Ragheb, Department of Biochemistry, Purdue University Agricultural Experiment Section, West Lafayette, IN 47907, personal communication.

High-Pressure Liquid Chromatographic Feprazone Determination in Pharmaceutical Formulations

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Received September 12, 1978, from the *Istituto di Farmacologia, Università di Ferrara, 44100 Ferrara, Italy.* Accepted for publication November 30, 1978.

Abstract □ A high-pressure liquid chromatographic method was developed for the quantitative determination of feprazone, a nonsteroidal anti-inflammatory agent, in different pharmaceutical formulations. The results agree with those obtained with GLC and UV spectrophotometric assays.

Keyphrases □ High-pressure liquid chromatography—analysis, feprazone in various pharmaceutical formulations □ Feprazone—analysis, high-pressure liquid chromatography, various pharmaceutical formulations □ Analgesics—feprazone, analysis in various pharmaceutical formulations, high-pressure liquid chromatography

Feprazone (4-prenyl-1,2-diphenyl-3,5-pyrazolidinedione) is a nonsteroidal analgesic, antipyretic, and anti-inflammatory agent with a low ulcerogenic potential. Feprazone has been determined in pharmaceutical formulations and in body fluids by potentiometric titration (1), UV spectrophotometry (2), TLC (3), GLC (4–6), and radioisotopic techniques (7).

This paper describes the quantitative determination of feprazone in capsule, suppository, and cream formulations by a simple and rapid high-pressure liquid chromatographic (HPLC) procedure.

The proposed HPLC method was compared to GLC (5) and UV spectrophotometric (2) assays.

EXPERIMENTAL[1]

HPLC—*Equipment and Operating Conditions*—A liquid chromatograph[2], equipped with a UV–visible detector[3] and with a septum injector[2], was used. The column[4] was 50 cm × 2.2 mm i.d., and the microcell[5] volume was 8 μl. The flow rate was 0.49 ml/min under a constant pressure

[1] All solvents were BDH AnalaR grade and were used without further purification.

[2] Series 4000, Varian Aerograph, Palo Alto, Calif.

[3] Beckman DU, Beckman Instruments, Fullerton, Calif.

[4] Micropak SI 10, Varian Aerograph, Palo Alto, Calif.

[5] Circus, Milan, Italy.

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Table I—Comparison of HPLC, GLC, and UV Methods for Feprazone Determination in Capsule Formulations [a]

Sample	HPLC	GLC	UV
1	201.4	203.6	203.5
2	203.7	199.7	201.5
3	198.3	201.2	197.7
4	201.1	203.8	204.6
5	198.5	201.4	198.0
6	196.7	198.3	200.3
7	200.1	204.2	201.4
8	198.5	201.5	199.1
Average	199.8	201.7	200.8
SD	2.2	2.1	2.5

[a] All results are reported as milligrams per capsule based on a pool of five capsules.

Table II—Comparison of HPLC, GLC, and UV Methods for Feprazone Determination in Suppository Formulations [a]

Sample	HPLC	GLC	UV
1	302.1	302.8	301.7
2	296.8	296.1	298.0
3	296.4	297.2	298.1
4	301.5	302.3	304.3
5	300.5	298.3	295.6
6	301.2	301.4	301.8
7	296.9	296.2	298.7
8	296.2	297.6	299.5
Average	298.9	299.0	298.1
SD	2.6	2.8	2.8

[a] All results are reported as milligrams per suppository based on a pool of five suppositories.

Table III—Comparison of HPLC, GLC, and UV Methods for Feprazone Determination in Cream Formulations [a]

Sample	HPLC	GLC	UV
1	4.92	5.01	5.00
2	5.00	5.02	4.90
3	4.94	5.02	4.92
4	5.10	4.83	5.03
5	4.82	4.82	4.82
6	5.10	4.92	5.11
7	5.00	4.81	4.86
8	4.87	4.98	5.00
Average	4.97	4.93	4.96
SD	0.10	0.09	0.10

[a] All results are reported as milligrams percent based on a pool of five cream samples.

of 1000 psi (70.3 kg/cm^2). Detection was by UV absorbance at 265 nm.

The optimum wavelength was determined from the UV spectrum of feprazone obtained by a stop-flow technique (8). The flow was interrupted by means of a two-stem valve[6] placed between the constant pressure pump and the septum injector when an absorbance, previously determined by comparison with the solvent absorbance (and, therefore, belonging to the substance under examination), was detected. The complete sample spectrum was then recorded in the 320–220-nm range.

Reagents—As a reference standard, pure feprazone[7] was used. Promethazine chlorhydrate[8] was the internal standard. The mobile phase was methanol–32% ammonia (150:2 v/v).

Standard Solutions—Standard feprazone solutions were prepared by dissolving accurately weighed aliquots of the pure substance in the mobile phase to produce final concentrations of 0.1–3.2 mg/ml. All standard solutions contained 2.5 mg of promethazine/ml.

Capsules—Pools of five capsules[7] were crushed in a porcelain mortar to a fine powder. Then aliquots of 25 mg of powder plus 50 mg of the internal standard were dissolved in 25 ml of chloroform. After filtration, the solution was evaporated at 40° and the residue was dissolved in 25 ml of mobile phase.

Suppositories—An aliquot of a suppository[7] mixture, one-third of the weight of a suppository, and 200 mg of the internal standard were dissolved in 50 ml of carbon disulfide. After filtration and evaporation, the residue was dissolved in the mobile phase.

Cream—Cream (0.50 g) and 50 mg of the internal standard were dissolved in a water bath in 25 ml of ethanol. Then 1 ml of this solution was filtered, evaporated, and redissolved in 1 ml of mobile phase.

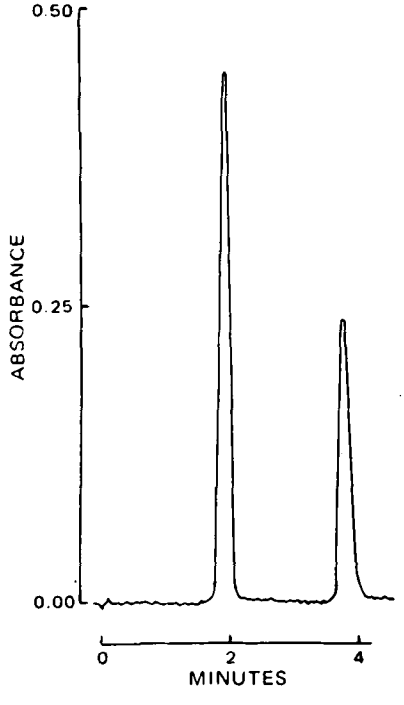

Figure 1—*Typical liquid chromatogram of feprazone (left) and internal standard (right) from capsules.*

GLC—A gas chromatograph[9], fitted with a dual-flame ionization detector and coiled glass columns[9] (1500 × 40 mm) packed with 2.5% SE-30 on 100–120-mesh Gas Chrom Q[9], was used. All working conditions were as described previously (5).

UV Spectrophotometry—A UV-visible spectrophotometer[3] was used, and the absorbance of feprazone was determined at 263 nm in 0.1 *N* NaOH. All working conditions were as described previously (2).

RESULTS AND DISCUSSION

Standard Curve—Linearity was shown by injecting into the column various amounts of feprazone over the concentration range 0.1–3.2 mg/ml with a constant amount of the internal standard (2.5 mg/ml). The mobile phase was methanol–32% ammonia (150:2 v/v). The standard curve was calculated by regression analysis as $y = 0.02 + 0.43x$ ($r = 1.0$), where y is the ratio of the feprazone peak area to the internal standard peak area, calculated at a sensitivity of 0.5 aufs deflection, and x is milligrams of feprazone per milliliter. To estimate the peak area standard deviations, five determinations were carried out for each concentration. Standard deviations were 3.1%.

HPLC—Eight samples of each pharmaceutical formulation were injected into the column using the stop-flow technique. Figure 1 shows a typical chromatogram of feprazone and the internal standard obtained from the capsule extraction. The retention times were 1.52 min for feprazone and 3.46 min for the internal standard. All measurements were carried out at room temperature (25°).

Recovery—Feprazone recovery from the pharmaceutical formulations was estimated. Formulations to which known feprazone amounts were added prior to extraction had an average percent recovery of 99.9, 99.9, and 99.8 with 95% confidence limits of 99.5–100.3, 99.4–100.4, and 99.2–100.4, respectively, for capsules, suppositories, and cream.

Peak Identification—Feprazone identification was accomplished by comparison with the pure standard retention times.

Suitable quantities of the liquid chromatograph cell effluent were collected corresponding to the drug chromatographic peak and were tested for homogeneity by qualitative monodimensional TLC using silica gel precoated glass plates (20 × 20 cm, 0.25 mm thick). Three solvents

[6] Whitey Co., Oakland, Calif.
[7] De Angeli Institute, Milan, Italy.
[8] Farmitalia Pharmaceuticals, Milan, Italy.
[9] Carlo Erba, Milan, Italy.

were used: (*a*) chloroform–acetone–ethanol (70:30:3 v/v); (*b*) cyclohexane–ethyl acetate–methanol–water (100:100:60:8 v/v); and (*c*) benzene–acetic acid (95:5 v/v). After each development, the plates were dried at 110° and spots were visualized in iodine vapors. The chromatograms corresponded perfectly in color (brown) and R_f values to those obtained with the reference compounds.

Comparison of HPLC, GLC, and UV Methods—The proposed HPLC method was compared to GLC (5) and UV spectrophotometric (2) assays. The results (Tables I–III) show that the proposed method is in good agreement with the GLC and UV assays.

The HPLC procedure described here provides a rapid, sensitive (the lower sensitivity limit is about 10 ng on the column), and precise assay of feprazone in pharmaceutical formulations.

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GLC–Mass Spectrometry of *Teucrium polium* Oil

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Received June 6, 1978, from the *Faculty of Pharmacy, University of Riyad, Riyad, Saudi Arabia.* Accepted for publication December 14, 1978.

Abstract □ The essential oil of *Teucrium polium*, growing in Saudi Arabia, was thoroughly investigated for its constituents by GLC–mass spectrometry, TLC, and spectrophotometric methods. This investigation revealed the presence of 10 terpenoidal compounds including the hydrocarbons β-pinene, limonene, α-phellandrene, and γ- and δ-cadinenes and the alcohols linalool, terpine-4-ol, cedrol, cedrenol, and guaiol. The oil was rich in alcohols and devoid of esters. Preliminary pharmacological screening showed that the oil possesses powerful antispasmodic activity.

Keyphrases □ GLC–mass spectrometry—analysis, *Teucrium polium* oil, antispasmodics, folk medicine □ *Teucrium polium*—oil, GLC–mass spectrometry □ Antispasmodics—analysis, *Teucrium polium* oil, GLC–mass spectrometry, folk medicine □ Folk medicine—antispasmodics, *Teucrium polium* oil, GLC–mass spectrometry

Teucrium polium (Family Labiatae) is one of the most fragrant plants in Saudi Arabia and is fairly distributed throughout the country (1). In folk medicine, it is used as an antispasmodic, antirheumatic, carminative, and flavoring agent. In an admixture with other powdered herbs, it is claimed to be therapeutic for peptic ulcer.

The aroma of this plant is due to its essential oil content (2). A literature review revealed that the oil constituents had not been investigated thoroughly (4). The present work was carried out to investigate the composition of this potentially therapeutic plant oil.

EXPERIMENTAL

Material—The plant was collected in the spring at the flowering stage from Sudair (Central Zone), 180 km from Riyad. Its identity as *T. polium* was confirmed[1]. It was dried and powdered.

Successive quantities (1 kg each) were subjected to steam distillation, which produced a volatile oil (average yield, 1.55 g).

The physical properties of the oil are presented in Table I.

TLC—TLC was done on precoated silica gel thin-layer plates[2] developed in chloroform–benzene (1:1) and chloroform–benzene (4:1) solvent systems (3). After development, the spots were visualized by spraying with anisaldehyde reagent (3).

Table I—Comparative Physicochemical Data

Property	Isolated Oil	Sardinian Oil
Color	Light yellow, darkens upon standing	Blue
Odor	Fragrant, characteristic	Agreeable, pungent
Refractive index[a]	n_D^{24}, 1.4850	n_D^{20}, 1.475 and 1.488
Optical rotation[b]	$[\alpha]_D^{24}$ +4.7° (10% in ethanol)	$[\alpha]_D^{20}$ +12.75°
Acetyl value[c]	117	47.45–47.59
IR[d]	1633, 1655, 1700, 2900, and 3400 cm^{-1}	—
NMR[e]	Broad singlets at 332, 306, 280, and 268 Hz; broad singlet at 217 Hz; singlet at 98 Hz; doublet at 73 Hz; two overlapping doublets centered at 58 Hz	—

[a] Zeiss Abbe refractometer. [b] Bellingham and Stanley polarimeter. [c] According to the BP method (5). [d] As film on Perkin-Elmer 567 grating IR spectrophotometer. [e] In deuterochloroform on Varian T 60A with tetramethylsilane as the internal standard.

GLC–Mass Spectrometry—Mass spectra of the volatile oil constituents were obtained using a gas chromatograph–mass spectrometer[3]. The chromatographic column (1.8 m long) was packed with 3% OV-17. The column was conditioned isothermally at 100° for 10 min, and then the temperature was programmed at 5 min until 260°. The injection port temperature was 185°, the separation temperature was 252°, and the ion source temperature was 290°. Mass spectra were obtained by operating at 60 MHz and with an ionization energy of 70 ev.

RESULTS AND DISCUSSION

The isolated oil was compared with Saridinian *T. polium* oil (4) (Table I). The IR and NMR data revealed free alcohols (3400 cm^{-1}; broad singlet at 217 Hz, disappeared on deuteration), alkene and highly conjugated

[1] The plant material was identified as *Teucrium polium* Linneus (Labiatae) by Dr. A. M. Migahid, Department of Botany, Faculty of Science, University of Riyad, Riyad, Saudi Arabia. A voucher specimen is available in the herbarium of Riyad University.

[2] GF plants, Anachem.

[3] Model 9000, LKB-Produkter, Brommo, Sweden.

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