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Simultaneous Determination of Hydrocortisone and Benzyl Alcohol in Pharmaceutical Formulations by Reversed-Phase High-Pressure Liquid Chromatography

ALBERT REGO ˣ **and BRETT NELSON**

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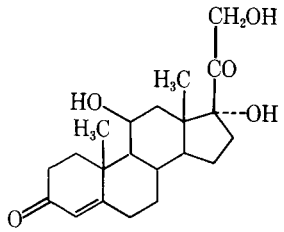
Abstract □ An accurate, reproducible, and specific reversed-phase high-pressure liquid chromatographic (HPLC) procedure that simultaneously determines hydrocortisone and benzyl alcohol in a variety of pharmaceutical formulations is presented. Cream, gel, ointment, and solution formulations containing varying hydrocortisone and benzyl alcohol concentrations can be analyzed with only minor modifications in sample preparation. To provide optimum accuracy and reproducibility, phenethyl alcohol is used as an internal standard. The specificity of the procedure allows for the quantitative determination of hydrocortisone and benzyl alcohol in the presence of their degradation products and without interference from the phenethyl alcohol. The determinations can be performed with an analysis time of 10–13 min/sample.

Keyphrases □ Hydrocortisone—simultaneous determination with benzyl alcohol, pharmaceutical formulations, reversed-phase high-pressure liquid chromatography □ Benzyl alcohol—simultaneous determination with hydrocortisone, pharmaceutical formulations, reversed-phase high-pressure liquid chromatography □ High-pressure liquid chromatography, reversed-phase—hydrocortisone, simultaneous determination with benzyl alcohol, pharmaceutical formulations

Hydrocortisone (I) has gained wide acceptance as a topical agent for the relief of inflammatory manifestations of corticosteroid-responsive dermatoses (1). Benzyl alcohol (II) is commonly used as an antimicrobial agent in a variety of topical formulations (2).

Various methods have been used for the determination of hydrocortisone, including TLC, polarography, colorimetry, GC, and high-pressure liquid chromatography (HPLC). TLC, though somewhat specific, lacks precision and short analysis time (1, 3–6). Polarographic determinations can be erroneous due to interferences, and they can



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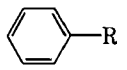

II: R = CH_2OH
III: R = CH_2CH_2OH

be nonspecific in that a variety of similar steroids can elicit the same reduction potential for the same functional groups (7–10). Colorimetric methods include reaction with aldehyde–sulfuric acid (11), ammonium molybdate (12), and 4,5-dimethyl-*o*-phenylenediamine (13). These methods lack specificity, because degradation products may provide a colorimetric response not indicative of actual steroid concentration. Numerous GC methods are available (14–18); however, most steroids cannot be directly analyzed but must be initially derivatized. The complexity of steroids precludes the direct formation of a single derivative (19). The USP methods for hydrocortisone formulations were recently changed from a colorimetric reaction with tetrazolium blue (1, 20–23) to HPLC procedures (1). Recently, literature on hydrocortisone analysis *via* HPLC has been published (19, 24–29). While these methods may be specific, the sample preparation and analysis time can be prohibitive to a rapid assay. Benzyl alcohol can be determined by spectrophotometry and GC. Spectrophotometric methods have the inherent problems of nonspecificity and formulation base interferences. GC methods, including the USP compendial method, are specific and efficient (2). However, these methods may have interferences due to longer retained compounds from the formulation base[1].

The described procedure allows for the simultaneous determination of hydrocortisone and benzyl alcohol in cream, gel, ointment, and solution formulations with a general sample preparation. The procedure incorporates phenethyl alcohol (III) as an internal standard to achieve optimum accuracy and reproducibility with an analysis

[1] Unpublished data.

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Table I—Recovery Studies[a]

Formulation	Hydrocortisone			Benzyl Alcohol		
	Spike, %	N	Recovery, %	Spike, %	N	Recovery, %
A	0.460	6	99.60 ± 0.46	0.730	3	99.85 ± 0.34
B	1.02	3	100.17 ± 0.05	1.06	4	100.27 ± 1.15
C	2.52	3	99.14 ± 0.05	0.99	4	99.81 ± 1.07
D	0.553	3	100.10 ± 0.20	1.16	3	100.54 ± 0.03
E	1.00	3	100.08 ± 0.23	2.00	4	99.87 ± 0.68
F	1.05	5	100.08 ± 0.17	—[b]	—	—
G	0.500	3	100.67 ± 1.53	—	—	—
H	2.36	3	100.20 ± 0.82	—	—	—

[a] Mean ± %*RSD* for *N* samples. [b] The dash denotes formulations not containing benzyl alcohol.

time of 10–13 min/sample. Phenethyl alcohol cannot be used as an internal standard for those formulations containing methylparaben, as these compounds elute at the same time. Benzyl alcohol could be used as an internal standard for those formulations containing methylparaben and propylparaben. The procedure is shown to be specific for hydrocortisone and benzyl alcohol in the presence of their degradation products in these formulations.

EXPERIMENTAL

Materials—Hydrocortisone[2], benzyl alcohol[3], methylparaben[4], propylparaben[4], and phenethyl alcohol[5] were used as standards. HPLC grade acetonitrile[6] and distilled, deionized water were used as reagents.

The HPLC system consisted of a pump[7], an automatic injector[8], a 254-nm detector[9], a radial compression module[10], and a reversed-phase octadecylsilane cartridge[11] fitted with a 2-μm precolumn filter and a recorder[12]. All peaks were electronically integrated with the laboratory data system[13].

The system was operated at ambient temperature, and the detector sensitivity was 0.1 or 0.2 aufs, depending on the hydrocortisone concentration. The chromatographic parameters include a 10-μl loop, an analysis time of 10 min, and a chart speed of 0.25 cm/min. After the baseline had stabilized, replicate standards were run to ensure reproducibility.

Mobile Phase—The mobile phase was 22.5% (v/v) acetonitrile in distilled, deionized water. Prior to use, the mobile phase was filtered through a 3.0-μm filter[14] and degassed with a water aspirator. A flow rate of 6.0 ml/min was used with a cartridge pressure of 1500–2000 psi.

Internal Standard Preparation—The stock phenethyl alcohol internal standard solution was prepared as 1.0% (w/v) in acetonitrile.

Standard Preparations—The hydrocortisone reference standard and benzyl alcohol reference standard were diluted to give the required working standard concentration levels. To provide accuracy and reproducibility, 25.0 ml of the phenethyl alcohol internal standard solution

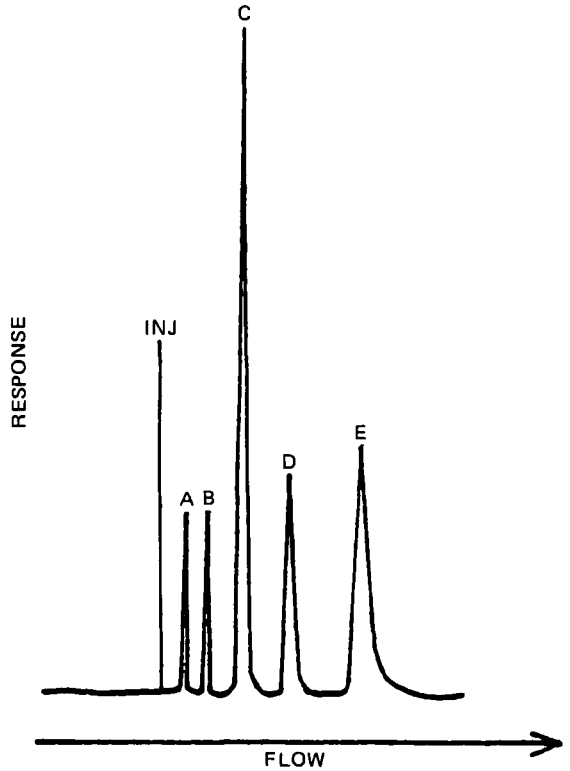

Figure 1—*A chromatogram showing the separation of components from benzyl alcohol degradation products. Key: (A) benzoic acid; (B) benzyl alcohol; (C) phenethyl alcohol (internal standard); (D) benzaldehyde; (E) hydrocortisone.*

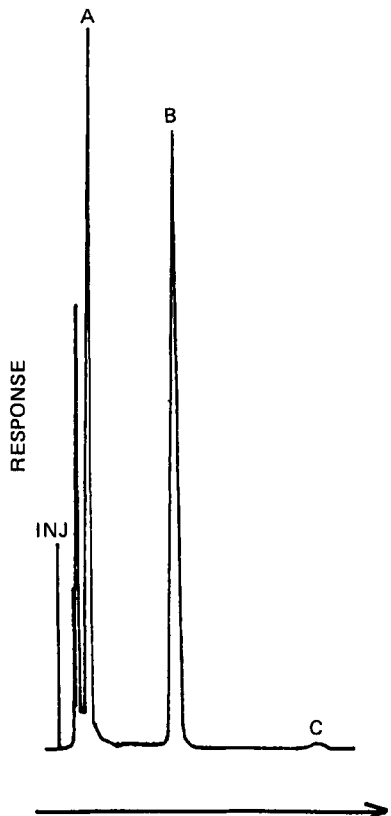

Figure 2—*A chromatogram showing the separation of components from hydrocortisone degradation products. Key: (A) polar degradation products of hydrocortisone; (B) hydrocortisone; (C) nonpolar degradation product of hydrocortisone.*

[2] The Upjohn Co., Kalamazoo, Mich.
[3] Stauffer Chemicals, Westport, Conn.
[4] Quad Chemicals, Long Beach, Calif.
[5] Matheson, Coleman, and Bell, Los Angeles, Calif.
[6] J. T. Baker, Phillipsburg, N.J.
[7] Model 6000A, Waters Associates, Milford, Mass.
[8] Model 725, Micromeritics Instrument Corp., Norcross, Ga.
[9] Model 440, Waters Associates, Milford, Mass.
[10] RCM-100 Radial Compression Module, Waters Associates, Milford, Mass.
[11] Radial Pak A, 10-cm × 8-mm cartridge (10-μm particles), Waters Associates, Milford, Mass.
[12] Omniscribe, Houston Instruments, Austin, Tex.
[13] Model 3352B, Hewlett-Packard, Fullerton, Calif.
[14] Millipore Type FS, Millipore, Corp., Bedford, Mass.

Table II—Reproducibility Studies[a]

Formulation	Hydrocortisone				Benzyl Alcohol			
	Day-to-Day	*N*	Operator-to-Operator	*N*	Day-to-Day	*N*	Operator-to-Operator	*N*
A	0.545 ± 1.91	19	0.550 ± 1.15	19	1.06 ± 0.83	19	1.06 ± 0.90	19
B	1.04 ± 1.30	20	1.05 ± 0.64	20	1.05 ± 0.58	19	1.05 ± 0.58	19
C	2.70 ± 0.72	19	2.71 ± 0.50	20	1.05 ± 0.85	19	1.05 ± 1.36	20
D	0.559 ± 0.89	20	0.557 ± 0.73	20	1.09 ± 0.81	20	1.08 ± 1.11	20
E	1.13 ± 0.94	19	1.13 ± 1.02	19	2.23 ± 0.56	19	2.22 ± 0.57	19
F	1.05 ± 0.85	18	1.04 ± 1.05	19	—[b]	—	—[b]	—
G	0.484 ± 0.97	18	0.484 ± 1.05	19	—	—	—	—
H	2.54 ± 0.74	18	2.54 ± 9.90	18	—	—	—	—

[a] Mean ± %*RSD* for *N* samples. [b] The dash denotes formulations not containing benzyl alcohol.

was added before the diluted standards were brought to 100 ml final volume with acetonitrile. The diluted working standard concentrations included 0.125 mg/ml, 0.250 mg/ml, and 0.625 mg/ml of hydrocortisone (equivalent to 0.5, 1.0, and 2.5% hydrocortisone on a product basis) and 0.250 and 0.500 mg/ml of benzyl alcohol (equivalent to 1.0 and 2.0% benzyl alcohol on a product basis).

Sample Preparation for Hydrocortisone and Benzyl Alcohol Determinations—*Cream and Gel Formulations*—Five-hundred milligrams of the sample was weighed into a 30-ml screw-capped culture tube. Acetonitrile (15.0 ml) and 5.0 ml of phenethyl alcohol internal standard solution, were accurately added and tightly capped. The tube was vigorously shaken by hand to disperse the sample and shaken for an additional 30 min on a mechanical shaker. The sample was centrifuged for 20 min, and the injector vial was filled with the clear supernatant. An aliquot (10 μl) was injected onto the liquid chromatograph.

Ointment Formulations—Five-hundred milligrams of the sample was accurately weighed into a 30-ml screw-capped culture tube. HPLC mobile phase (15.0 ml) and 5.0 ml of the phenethyl alcohol internal standard solution were accurately added, then tightly capped. The sample was then placed into a constant temperature (60–65°) water bath for 30 min and shaken by hand at 5-min intervals. The solution was then cooled to room temperature and centrifuged for 20 min. An aliquot was injected onto the liquid chromatograph following the procedure used for cream and gel formulations.

Solution Formulations—Five-hundred milligrams of the sample was accurately weighed into a 30-ml screw-capped culture tube. Acetonitrile (15.0 ml) and 5.0 ml of the phenethyl alcohol internal standard solution were accurately added and tightly capped. The sample was then shaken for 20 min on a mechanical shaker. The solution was injected as described in the previous two preparations.

RESULTS AND DISCUSSION

Linearity—To determine the linearity of the detector response, separate calibration curves for hydrocortisone (0.5, 1.0, and 2.5% product level) and benzyl alcohol (1.0 and 2.0% product level) were obtained. Each curve contained points equivalent to 50 and 150% of label claim and three intermediate points. In all cases, the component concentration was plotted *versus* the ratio of component peak area over internal standard peak area. The internal standard was held constant throughout the study. A composite hydrocortisone calibration curve passed through zero and was linear from 0.050 to 1.008 mg/ml with a correlation coefficient of 0.999. A composite benzyl alcohol calibration curve passed through zero and was linear from 0.129 to 0.854 mg/ml with a correlation coefficient of 0.999.

Recovery Studies—Each formulation was received with the appropriate placebo base. Each base was prepared and analyzed by HPLC to ensure that no UV absorbing peaks were present in the regions of benzyl alcohol, phenethyl alcohol, and hydrocortisone. To determine method accuracy, each placebo was spiked a minimum of three times with benzyl alcohol to the appropriate levels (1.0 and 2.0% on a product basis). A second placebo series was spiked a minimum of three times with hydrocortisone to the appropriate levels (0.5, 1.0, and 2.5% on a product basis). The samples were prepared and subjected to HPLC analysis. In all cases, satisfactory recoveries were obtained (Table I).

Reproducibility Studies—The reproducibility of the HPLC procedure was determined by the criteria of day-to-day reproducibility and operator-to-operator reproducibility. The following scheme was applied to each formulation: Day 1, Operator 1; Day 2, Operator 1; and Day 2, Operator 2 (Table II).

Limit of Detection—The limit of detection, defined here as an acceptable signal to noise level of >2:1 was performed by diluting the working standard 1–100 with acetonitrile and performing the analysis at 0.01 aufs. In all cases, benzyl alcohol, phenethyl alcohol, and hydrocortisone were easily detected and within acceptable limits.

Specificity Studies—It is known that benzyl alcohol degrades *via* oxidation to yield benzoic acid and benzaldehyde[15]. As confirmation of method specificity, a sample was spiked with benzoic acid and benzaldehyde and analyzed. The two peaks were noted and did not create any interference problems (Fig. 1).

The degradation of a steroid, such as hydrocortisone, is complex and involves hydrolysis, oxidation, and possible transesterification (19). Each formulation and formulation base placebo was artificially degraded by adjusting the sample to ~pH 12 with 50% NaOH and also by heating a second series of samples at 100° for 1 hr.

The four base formulation placebos, with or without benzyl alcohol depending on the formulation, were analyzed by the HPLC procedure. In all cases, no interfering peaks were noted in the area of benzyl alcohol, phenethyl alcohol, or hydrocortisone.

Further work demonstrated that degradation products of hydrocor-

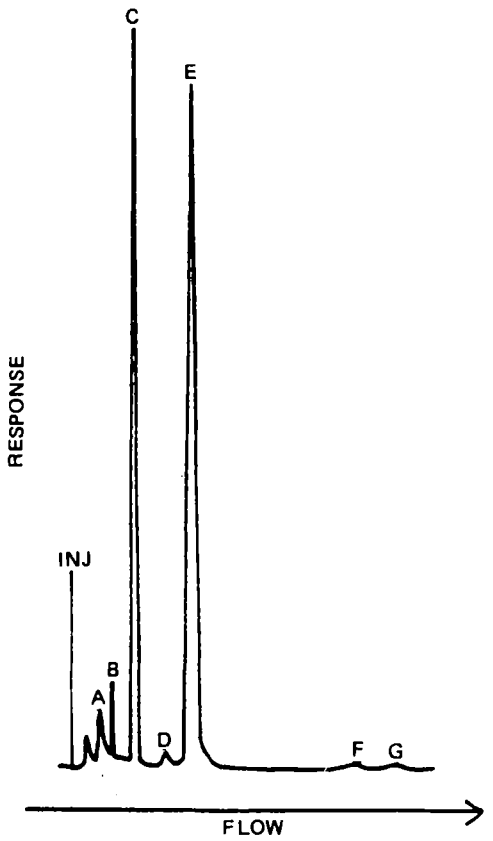

Figure 3—*A chromatogram showing the separation of components from degradation products of an aged sample. Key: (A) benzoic acid and polar degradation products of hydrocortisone; (B) benzyl alcohol; (C) phenethyl alcohol; (D) benzaldehyde; (E) intact hydrocortisone; (F, G) nonpolar degradation products of hydrocortisone.*

[15] The degradation products benzaldehyde and benzoic acid were verified by GC using 1.83-m × 0.318-cm stainless steel Porapak P column (Waters Associates, Milford, Mass.). Chromatographic parameters include gas flow of 45 ml/min, nitrogen, 220° oven temperature, and a flame ionization detector.

Table III—Stability Study[a]

Age, Days	Percent Hydrocortisone Found[b] Lot A	Lot B	Lot C	Percent Benzyl Alcohol Found[b] Lot A	Lot B	Lot C
0	0.517	0.527	0.528	1.06	1.06	1.05
36	0.517	0.519	0.518	1.02	1.04	1.04
49	0.472	0.476	0.480	1.05	1.07	1.06
86	0.467	0.470	0.464	1.06	1.07	1.04
119	0.391	0.390	0.393	1.00	0.99	1.00

[a] Claim was 0.525% hydrocortisone and 1.0% benzyl alcohol. [b] Each result represents the mean of four values.

tisone do not interfere with benzyl alcohol or the internal standard phenethyl alcohol. An artificially degraded gel formulation, not containing benzyl alcohol or phenethyl alcohol, gave a similar pattern of degradation peaks as noted in Fig. 2 with no benzyl alcohol or phenethyl alcohol interferences.

As a final test, a tube of a sample stored at 45° for >300 days was analyzed. The hydrocortisone level was 72% of label claim, substantially below the allowed lower specification limit. The chromatogram revealed a variety of peaks, none of which interfere with the benzyl alcohol, phenethyl alcohol, or hydrocortisone (Fig. 3). To ensure that there were no extraneous peaks under the hydrocortisone peaks, the sample was injected onto the HPLC coupled with a UV-visible spectrophotometer[16].

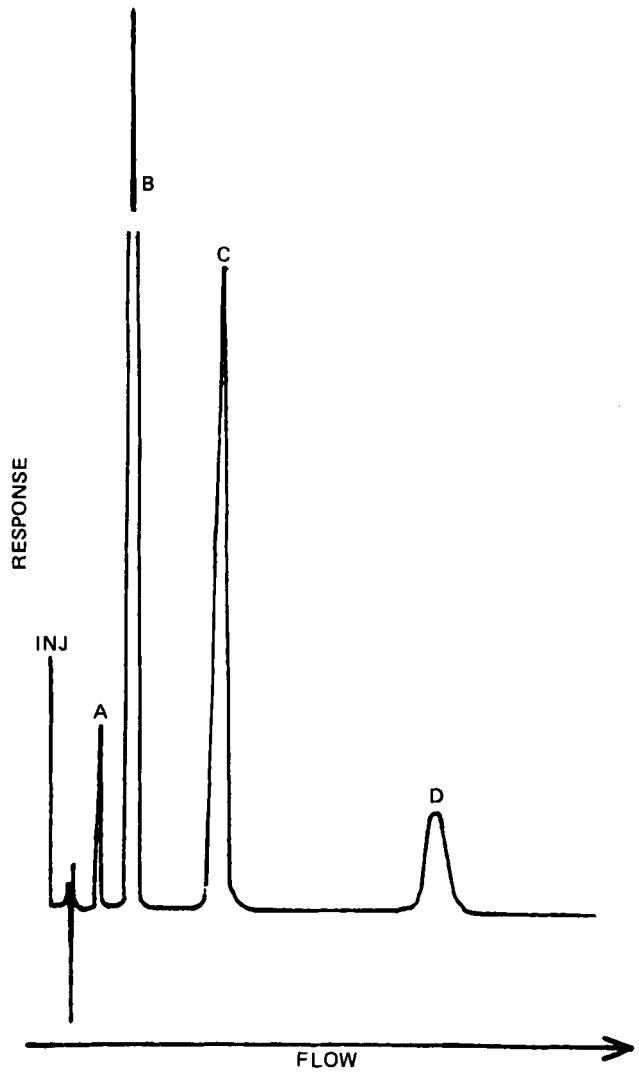

Figure 4—*A chromatogram showing the separation of preservatives and hydrocortisone. Key: (A) benzyl alcohol; (B) methylparaben; (C) intact hydrocortisone; (D) propylparaben.*

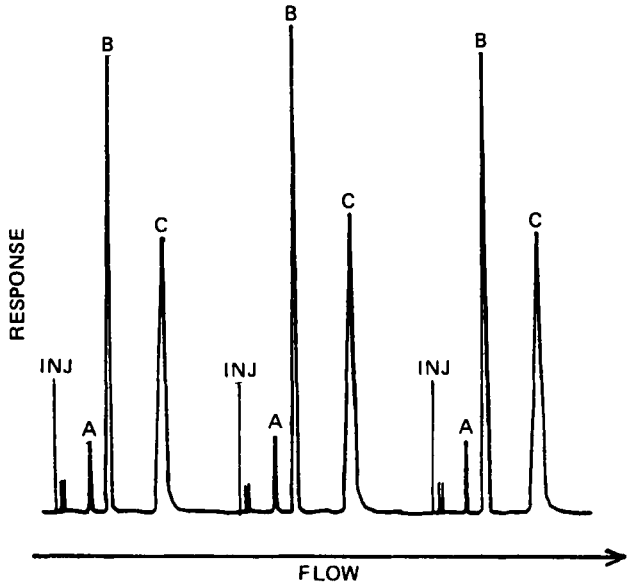

Figure 5—*A typical chromatogram of an experimental formulation. Key: (A) benzyl alcohol; (B) phenethyl alcohol; (C) hydrocortisone.*

As the hydrocortisone peak eluted, a UV scan was taken every 3 sec. These 14 scans were compared to a standard hydrocortisone UV scan. No differences were noted during the elution of the peak. As a further check, the first derivatives of the peak segments were subtracted from the first derivative of the hydrocortisone standard. In all cases, the predicted waveform was noted, indicating there are no UV absorbing interferences at the location of hydrocortisone. No attempt was made to identify the peaks associated with the degradation of hydrocortisone.

Phenethyl alcohol was used as a chromatographically pure reagent for all studies and was not subjected to a simulated degradation.

Stability Study—Actual data were generated with this procedure (Table III). The samples were a 0.5% hydrocortisone experimental formulation stored at 45° for accelerated stability studies for 120 days. The data for hydrocortisone revealed the expected downward trend and demonstrated the method could easily detect changes in hydrocortisone concentration. The data for benzyl alcohol revealed no significant loss throughout the study. Note that specificity was previously established to confirm the validity of the data.

Paraben Study—The methylparaben reference standard and propylparaben reference standard were diluted to 0.005 and 0.016 mg/ml, respectively. The hydrocortisone reference standard was added equivalent to 0.125 mg/ml to the diluted paraben standard. The three components are completely resolved. The methylparaben would interfere with the phenethyl alcohol. A second injection showed benzyl alcohol does not interfere with the parabens (Fig. 4). In formulations containing parabens the benzyl alcohol could be utilized as the internal standard in place of phenethyl alcohol.

The principal goal of any analytical separation procedure is to achieve maximum resolution in minimum time. Initial studies were conducted with a reversed-phase stainless steel column[17] and a 25% (v/v) acetonitrile solution in distilled, deionized water mobile phase pumped at 2.0 ml/min. Under these conditions, 15–25 min were required to achieve baseline to baseline resolution of the benzyl alcohol, phenethyl alcohol, and hydrocortisone, as well as the total elution of the degradation products. In comparison, the radial compression system with the modified mobile phase pumped at 6.0 ml/min achieved the same analysis in 10–13 min. The time factor was of obvious importance in the choice of this approach, allowing for the analysis of a large number of samples in a short period of time (Fig. 5).

An efficient feature of this procedure is the general sample preparation scheme and analysis parameters. The sample weights, sample dilution volumes, and internal standard concentration are constant. There are only two minor variations in sample preparation. The first is the centrifugation step for the cream, gel, and ointment formulations (relative to the solution) prior to injection. The second is the heating of the ointment formulation in the HPLC solvent prior to centrifugation. With this

[16] Model 8450A UV/VIS Spectrophotometer, Hewlett-Packard, Palo Alto, Calif.

[17] μ Bondapak C-18, 30 cm × 3.9-mm i.d. (10-μm particles), Waters Associates, Milford, Mass.

general scheme, the analysis parameters do not require solvent modification, variable injection volumes, or a change in analysis time. The only chromatographic variation is an attenuation change from 0.1 to 0.2 aufs for the 2.5% hydrocortisone formulations.

A variety of hydrocortisone formulations may contain methylparaben and propylparaben as preservatives. Though methylparaben interferes with phenethyl alcohol, benzyl alcohol could be substituted as the internal standard and yield satisfactory chromatographic separation.

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Synthesis and Evaluation of an ^{111}In-labeled Porphyrin for Lymph Node Imaging

RICHARD VAUM *, NED D. HEINDEL *‡ˣ, H. DONALD BURNS *, JACQUELINE EMRICH*, and NATALIE FOSTER‡

Received October 20, 1981, from the **Department of Radiation Therapy and Nuclear Medicine, Hahnemann Medical College and Hospital, Philadelphia, PA 19102* and the ‡*Center for Health Sciences, Lehigh University, Bethlehem, PA 18015.* Accepted for publication January 18, 1982.

Abstract □ ^{111}In-labeled tetra(4-*N*-methylpyridyl)porphyrin was investigated as a possible lymph node imaging agent. A clinically feasible method for the preparation of this radioactive pharmaceutical was developed from experiments with the synthesis and characterization of the unlabeled complex. The *in vivo* distribution of the compound in Wistar rats was determined as a function of time. Favorable lymph node–blood, lymph node–muscle, and specific lymph node–surrounding tissue ratios were obtained.

Keyphrases □ Porphyrin—^{111}In-labeled, synthesis and evaluation, biodistribution, lymph node imaging, rats □ Imaging, lymph node—^{111}In-labeled porphyrin, synthesis and evaluation, biodistribution, rats □ Biodistribution—^{111}In-labeled porphyrin, synthesis, lymph node imaging, evaluation, rats

The diagnostic imaging methods now being used for evaluating disease in the lymphatic system, oil lymphography and technetium Tc 99m antimony trisulfide colloid lymphoscintigraphy, possess significant inherent limitations. These methods image only those nodal groups that drain the subcutaneous injection site, and thus, require patent lymphatic vessels between the injection site and the nodes to be imaged (1–3). An intravenously administered radiopharmaceutical agent that allows the visualization of all nodal groups with a single injection would be an improvement in the diagnosis of lymph node involvement in malignancy.

BACKGROUND

It has been known since the 1940s that many porphyrins and metalloporphyrins display an affinity for lymphatic and neoplastic tissues when injected intravenously (4, 5). These early studies, however, depended solely upon differential fluorescence to detect the presence of the porphyrin in the target tissue and, consequently, were of limited diagnostic utility. A method is available for introducing indium, an electronic isostere of iron, into porphyrins (6). Two nuclides of that element, indium 111 and indium 113m, would be clinically acceptable from the viewpoint of decay energies, gamma-emission, and half-life for use in *in vivo* diagnostic radiopharmaceuticals. Furthermore, it has been shown that several porphyrins can transport β-emitting nuclides (cobalt 58, zinc 65, and palladium 109) to nodal tissue where their ionizing radiation results in selective nodal ablation and diminished rejection rate for skin homographs in dogs dosed with these radio porphyrins (7–9). It has also been demonstrated that an intravenously administered cobalt 57 derivative of a water-soluble porphyrin developed a tumor–blood ratio of 44:1 at 5 days postdosing in the TCT-4904 rat bladder tumor (10). Sufficient precedent exists to indicate that a soluble indium-labeled porphyrin might be nodal specific. This study reports a facile synthesis for radioactive [^{111}In]tetra(4-*N*-methylpyridyl)porphyrin (I) whose biodistribution in rats illustrates its potential as a radiodiagnostic agent for major nodal systems.

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