Determinations of Tiodazosin and Levulinic Acid from Tablets by High-Performance Liquid Chromatography

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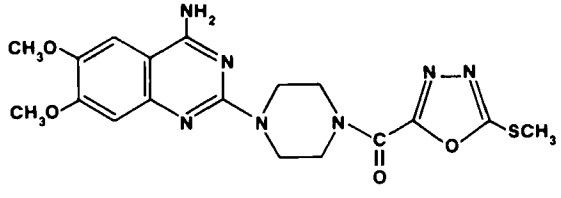
Received July 15, 1982, from the *Analytical Research and Development Department, Bristol Laboratories—Division of Bristol-Myers Co., Syracuse, NY 13201.* Accepted for publication September 7, 1982.

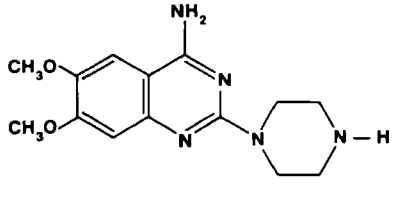
Abstract □ Specific assays of tiodazosin and levulinic acid from tablet preparations were developed using high-performance liquid chromatography. Tiodazosin was determined with acetonitrile 50% (v/v) in pH 5.7 acetate buffer on a 300 × 3.9-mm i.d. microparticulate C-18 column using 254 nm detection with butylparaben as the internal standard. Levulinic acid was determined at 280 nm with 1 *N* acetic acid on a 300 × 3.9-mm i.d. microparticulate phenyl column using an external standard. The assays were run sequentially. The relative standard deviations of sample variability (2σ, $n = 6$) and recoveries from synthetic preparation were ±1.6 and ~95%, respectively, for both assays.

Keyphrases □ Tiodazosin—tablets, high-performance liquid chromatographic assay, sequential with levulinic acid □ Levulinic acid—in tiodazosin tablets, sequential high-performance liquid chromatographic assay □ High-performance liquid chromatography—tiodazosin and levulinic acid from tablets, sequential assay

Tiodasozin, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl) -4- [[5-(methylthio) -1,3,4- oxadiazol-2-yl]carbonyl]-piperazine (I), is an orally active hypertensive agent currently undergoing clinical trials. It is formulated in tablets with levulinic acid (4-oxopentanoic acid). A reported high-performance liquid chromatographic (HPLC) assay method for tiodazosin (1) is limited to biological samples and involves work-up procedures and a fluorescence detection system that would not be suitable for the dosage form.



I



II

Levulinic acid has been quantitatively assayed as the methyl ester by GC (2). This method was found to be unsuitable, however, for the acid in the presence of the tablet excipients due to the incompleteness of the derivatization reaction. A simplified analytical method that does not employ derivatization is described in this study. The method utilizes the relatively weak UV absorptivity of the acid in methanol (3), which is ~20 liters/mole·cm at the 270-nm UV maxima and 15 liters/mole·cm at 280 nm.

Two simple HPLC procedures for the quantitative assay of tiodazosin and levulinic acid are described in the present report. The methods are accurate and specific in the presence of the tablet matrix ingredients.

EXPERIMENTAL

Apparatus—A modular HPLC system consisting of a pump[1], a septumless loop injector[2], and a fixed-wavelength detector[3] was used. Peak elution times and areas were obtained with a reporting integrator system[4].

Reagents and Materials—Tiodazosin[5] and levulinic acid[6] were used without further purification. Sodium acetate[7] and glacial acetic acid[7] were ACS reagent grade. Butylparaben NF[8] was used as received. Acetonitrile[9] was obtained as distilled-in-glass chromatographic grade, and water was distilled and deionized.

Sample Preparation—Ten tablets of tiodazosin were accurately weighed and then ground and blended to a uniform fine powder. A portion of the powdered blend was accurately weighed for each assay.

Tiodazosin Assay—*Chromatographic Conditions*—A stainless steel

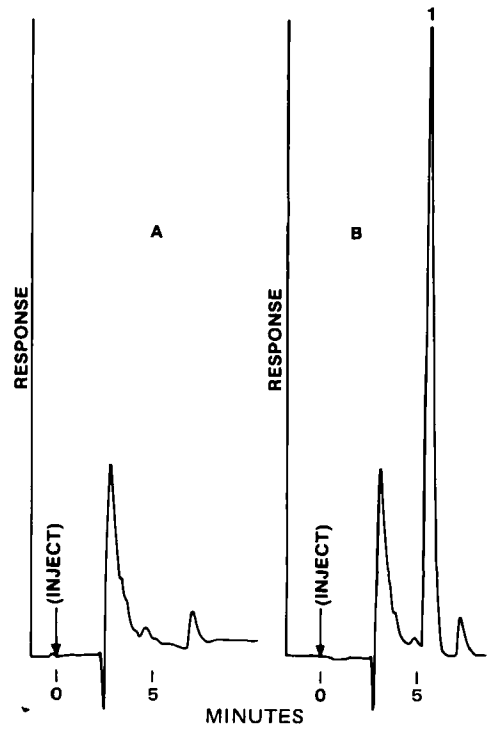


Figure 1—*Representative chromatograms from the levulinic acid assay. Key: (A) placebo with no levulinic acid present; (B) typical tablet sample with levulinic acid (1).*

[1] Model 6000A; Waters Associates, Milford, Mass.
[2] Model 7120; Rheodyne, Berkeley, Calif.
[3] Model 440; Waters Associates, Milford, Mass.
[4] HP-3354 Laboratory Automation System; Hewlett-Packard, Avondale, Pa.
[5] Bristol Reference Standard; Bristol Laboratories, Syracuse, N.Y.
[6] Sigma Chemical Co., Saint Louis, Mo.
[7] Fisher Scientific, Fair Lawn, N.J.
[8] Pfaultz and Bauer, Inc., Stamford, Conn.
[9] Burdick and Jackson, Muskegon, Mich.

0022-3549/83/1000-1168$01.00/0
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HPLC column (30 cm × 3.9-mm i.d.) prepacked with bonded octadecylsilane on silica[10] (10% loading by weight, 10-μm mean particle size) was used with a mobile phase of 50% by volume acetonitrile in 0.1 *M* sodium acetate, previously adjusted to pH 5.7 with glacial acetic acid. A flow rate of 1.5 ml/min and a loop injection volume of 20 μl were employed with the detector set at 254 nm.

Standard Solution—A solution of butylparaben in acetonitrile was prepared at a concentration of 100 μg/ml for use as an internal standard. A stock solution of tiodazosin reference material was prepared at a concentration of 50 μg/ml in mobile phase, and a 5-ml aliquot of this stock solution together with 5 ml of the internal standard solution were transferred to a 50-ml volumetric flask and diluted to volume with mobile phase for use as a standard.

Analytical Procedure—The powdered tablet sample equivalent to a theoretical level of ~5 mg of tiodazosin was transferred to a 100-ml volumetric flask. A 50-ml portion of mobile phase was added, the solution sonicated[11] for 5 min, and diluted to volume with mobile phase. A portion of this solution was filtered[12], discarding the first few milliliters, and a 5-ml portion of the filtrate was transferred to a 50-ml volumetric flask. The internal standard (5 ml) was added, and the solution was diluted to volume with mobile phase. Successive injections of the standard and sample solutions were made onto the chromatographic system at room temperature. The peak areas generated were applied to the calculation of milligrams tiodazosin per average tablet weight (ATW) using the formulas:

$$\text{Factor (F) for Tiodazosin} = \frac{\text{Peak Area Internal Standard} \times \text{mg/ml Tiodazosin Standard} \times \text{Purity of Standard}}{\text{Peak Area Tiodazosin Standard} \times \text{mg/ml Internal Standard}}$$

$$\text{mg Tiodazosin per ATW} = \frac{\text{F} \times 1000 \times \text{mg/ml Internal Standard} \times \text{Peak Area Tiodazosin} \times \text{mg per ATW}}{\text{Peak Area Internal Standard} \times \text{mg Sample}}$$

Levulinic Acid Assay—*Chromatographic Conditions*—A prepacked bonded phenyl on silica[13] (10% loading by weight, 10-μm mean particle size) HPLC column (30 cm × 3.9-mm i.d. stainless steel) was employed with a mobile phase of 1.0 *N* acetic acid. The mobile phase was filtered through a 0.45-μm membrane filter[14] prior to use. A flow rate of 1.0 ml/min and a loop injection volume of 200 μl were used with the detector set at 280 nm.

Standard Solution—A standard levulinic acid solution was prepared containing 0.26–0.30 mg of the acid per milliliter of mobile phase. To ensure noninterference, a 500-mg portion of placebo tablet granulation (containing all the tiodazosin tablet excipients) was mixed with 10 ml of the mobile phase. The resulting suspension was filtered[15], and an aliquot was injected into the chromatographic system.

Analytical Procedure—The powdered tablet sample, equivalent to one average tablet weight, was transferred to a 20-ml vial, 10 ml of mobile phase was added, the vial capped, and the suspension was mixed by shaking mechanically for 15 min in a reciprocating shaker. The contents of the vial was filtered[15] prior to chromatographic injection. Successive injections of the standard and sample were made at room temperature. The levulinic acid peak areas were used for the calculation of milligrams levulinic acid per ATW using the formulas:

$$\text{Factor (F) for Levulinic Acid} = \frac{\text{mg/ml Levulinic Acid Standard} \times 10 \times \text{Purity of Standard}}{\text{Peak Area Levulinic Acid}}$$

$$\text{mg Levulinic Acid per ATW} = \frac{\text{F} \times \text{Peak Area Levulinic Acid} \times \text{mg per ATW}}{\text{mg Sample}}$$

RESULTS AND DISCUSSION

The described HPLC method elutes levulinic acid in ~5.8 min and employs the relatively weak UV absorption of the acid at ~280 nm for determinations at concentration levels of $\geq 3 \times 10^{-4}$ *M*. The relatively larger injection volume of 200 μl was required for detectability of levulinic acid below the millimolar level. Figure 1 shows representative chromatograms for a placebo with no levulinic acid and a typical tablet sample.

The tiodazosin content can be determined sequentially from the same powdered tablet sample by employing the described, independent assay procedure. Figure 2 shows a typical chromatogram for this assay, with tiodazosin eluting at ~3.2 min and the internal standard at ~5.8 min. The total analysis time for a typical tablet sample is ~7 min.

Standard linearity was checked over the range 1.00–10.00 mg/ml for tiodazosin and 0.03–1.5 mg/ml for levulinic acid. Both compounds responded linearly over the stated concentration ranges, with least-squares regression analysis of the peak area responses producing correlation coefficients >0.999 in both cases. The difference in the slopes of the regression lines for levulinic acid in the presence of a placebo granulation compared with that in the absence of the placebo was <1% of the mean slope value. This implies a levulinic acid recovery of ~99% from the placebo matrix.

Chromatographic variability was determined by six identical injections of the standard preparations of tablet samples with a label claim of either 10 mg of tiodazosin or 7 mg of levulinic acid. Relative standard deviations (2*s*%) for chromatographic variability and sample variability are ±0.50

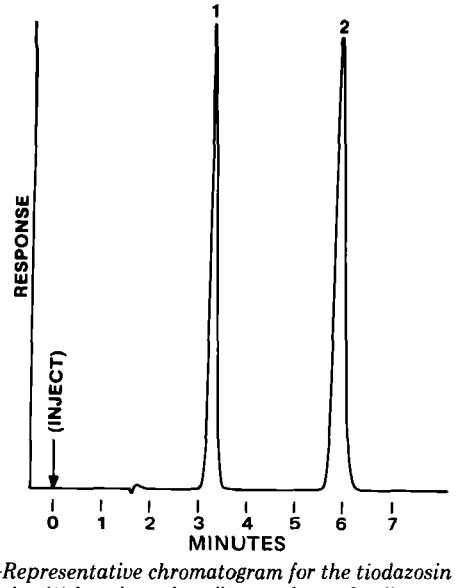

Figure 2—*Representative chromatogram for the tiodazosin assay. Key: (1) tiodazosin; (2) butylparaben (internal standard).*

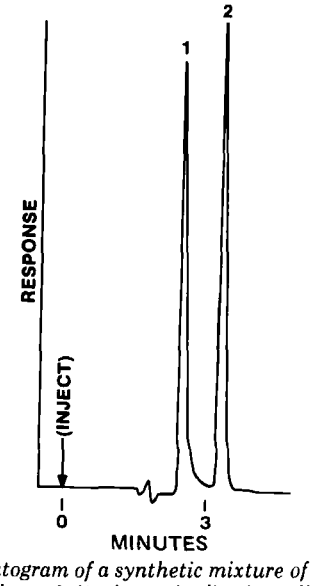

Figure 3—*Chromatogram of a synthetic mixture of tiodazosin (2) and 4-amino-6,7-dimethoxy-2-(1-piperazinyl)quinazoline (1).*

[10] μ-Bondapak C_{18}; Waters Associates, Milford, Mass.
[11] Model B52H; Ultrasonic Bath, Branson Co., Shelton, Conn.
[12] No. 6 Filter Paper; Whatman, Clifton, N.J.
[13] μ-Bondapak Phenyl; Waters Associates, Milford, Mass.
[14] Type HA; Millipore Corp., Bedford, Mass.
[15] No. 576 Filter Paper; Schleicher and Schuell, Keene, N.H.

and ±1.6% for the tiodazosin assay or ±1.8 and ±1.6% for the levulinic acid assay.

Accuracy of the assays was determined by recoveries of individual tiodazosin additions to spiked placebo formulation equivalent to 1, 2, 5, 10, and 20 mg of tiodazosin per tablet. Recoveries for the five spiked samples ranged from 95.7 to 99.3% with a mean recovery of 96.7% (s = ±1.8%), calculated from the regression line generated from the linearity study. The spiking of levulinic acid over the concentration range from 0.5 to 1.5 mg per tablet into a placebo matrix produced recovery results, calculated using the regression line generated in the absence of the placebo, of 87.5% at the 0.5-mg level, 95.6% at the 5-mg level, and 99.6% at the 15-mg level.

The forced degradation of 1-mg tiodazosin tablets under conditions of heat (100° for 20 hr), light (~15 W at 300 nm for 1 week), acid (1 N HCl for 20 hr), and base (1 N NaOH for 20 hr) was performed to test the specificity of the tiodazosin assay method. Absorbance ratios were calculated from chromatograms generated at 220, 254, or 340 nm from both undegraded and degraded samples. No apparent interference was observed from any of the degraded samples, and the relative standard deviations of the 254/220-nm and 254/340-nm absorbance ratios was ±1.2 and ±0.7%, respectively. For the levulinic acid assay, no apparent interferences were observed for degraded samples, where the degradation conditions were adjusted to produce a maximum acid degradation of 10%.

Specificity of the tiodazosin method was also demonstrated by the resolution of the principal known decomposition product, 4-amino-6,7-dimethoxy-2-(1-piperazinyl)quinazoline (II), from the active drug under the assay conditions. A chromatographic tracing of a mixture of I and II is shown in Fig. 3. The results of typical tablet blend analyses gave mean recoveries of 103% for tiodazosin and 99.7% for levulinic acid based on the label claims for a nominal 10 mg of tiodazosin per tablet level. In summary, the reverse-phase HPLC methods described in this paper provide simple, rapid, and quantitative methods for the determination of tiodazosin and levulinic acid from the tablet matrix.

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General Definition of Valence Delta-Values for Molecular Connectivity

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Received March 31, 1982 from the **Department of Pharmaceutical Chemistry, Virginia Commonwealth University, Richmond, VA 23298* and the ‡*Department of Chemistry, Eastern Nazarene College, Quincy, MA 02170.* Accepted for publication August 26, 1982.

Abstract □ The molecular connectivity valence delta-values have been defined in terms of the count of nonhydrogen valence electrons on a valence-state atom as screened from the nucleus by the core electrons. The core is defined as the nonvalence electrons minus 1. This general definition expresses the valence delta-values for second and third quantum level atoms and halogens. Valence delta-values have been derived for higher oxidation states of sulfur and phosphorus. The internal consistency of these delta-values is tested by their ability to closely correlate molar refraction values with $^1\chi^v$. It is found that a second variable, the count of the number of α hydrogen atoms, greatly increases the quality of the correlation. Some biological SAR applications reveal the general utility of these findings.

Keyphrases □ Molecular connectivity—valence delta-values, definition, applications to structure–activity relationships, comparison with molecular refractivity □ Structure–activity relationships—applications of molecular connectivity valence delta-values, comparison with molecular refractivity □ Molecular refractivity—comparison with molecular connectivity valence delta-values, structure–activity relationships

A major advance in the general applicability of molecular connectivity arose from the introduction of valence molecular connectivity indexes by Kier and Hall (1, 2). This innovation made it possible to encode information about heteroatoms and unsaturated features and to correlate this with the relative differences in properties among such molecules. The subsequent use of valence molecular connectivity has been summarized (3).

BACKGROUND

Early Estimates of δ^v—In the initial description of the valence connectivity delta a bonded atom was described by a count of the valence electrons other than those bonding hydrogen (1). This value, designated δ_i^v for atom i was incorporated into the valence connectivity index, $^1\chi^v$, using the same algorithm as for the simple connectivity index, $^1\chi^v = \Sigma(\delta_i^v\delta_j^v)^{-1/2}$. The structural information encoded in the simple δ, a count of σ bonds (electrons) other than those bonding hydrogen, and the δ^v-value was evident from an inspection of a matrix of the two values (3). The sum of the delta-values ($\delta^v + \delta$) for a hybrid atom relates closely to estimates of the hybrid atom volume. It was also shown that the difference between the delta-values ($\delta^v - \delta$) for a hybrid atom is a count of π and lone-pair electrons, referred to as "exterjacent" to describe their relationship to internuclear axes. A count of exterjacent electrons on an atom in its hybrid state, within a quantum level, bears a close relationship to the Mulliken–Jaffe electronegativity (3).

In the earliest consideration of heteroatoms (1), the δ^v-values for second quantum level atoms were verified by relating the calculated $^1\chi^v$ values to molar refraction (MR) data for substituted benzenes (4). The correlation was good, however the experimental value of fluorobenzene presented a problem. The fact that it has a value of 0.4 less than benzene led to the rejection of the logical $\delta_F^v = 7$ and to the derivation of an empirical value of −20. Thus the $(\delta_i^v\delta_F^v)^{-1/2}$ would have a negative value. This subtraction of the modest value of this term in the calculation of $^1\chi^v$ permitted a fairly accurate reproduction of the effect of fluorine in molecules as far as certain physical property values. Using the same approach, empirical values were derived for the other halogens (1) and sulfur (2). These are shown in Table I, column 1.

The Quantum Level Effect on δ^v—Following these initial empirical assignments of higher level atom δ^v-values, a fundamental significance for these numbers was sought. This led to the realization that the empirical δ^v-values for Cl, Br, I, and S were close to the numbers derived by a general expression:

$$\delta^v = \frac{Z^v - h}{Z - Z^v} \qquad \text{(Eq. 1)}$$

where h is the count of hydrogen atoms, Z^v is the count of valence electrons, and Z is the count of all electrons (5). The value $Z - Z^v$ is the count of all nonvalence electrons on the atom; thus, it constitutes information on the principal quantum number of the atom. The denominator in Eq. 1 may be viewed as simulating a radial dimension or a valence orbital screening factor associated with the particular quantum level. Indeed this influence on the valence electrons has been approximated by the use

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