Determination of minor impurities in terazosin hydrochloride by high-performance liquid chromatography

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ABSTRACT

Minor impurities in the anti-hypertensive agent terazosin monohydrochloride dihydrate were determined using high-performance liquid chromatography. Manufacturing impurities and degradation products were separated using a reversed-phase system. Detector response was linear for the individual impurities to approximately 0.5 μg/ml which represents 0.05% of the drug concentration. The procedure provides quantitation of impurities to approximately the 0.05% level with precision (relative standard deviations) of ±0.1% to ±1.2% at a level of approximately 0.5%. A variety of reversed-phase columns were evaluated for the assay method with the optimum resolution achieved using a Zorbax Rx-C8 5-μm packing.

INTRODUCTION

Terazosin monohydrochloride dihydrate is an active antihypertensive agent commercially known as Hytrin (1).

Chemically the drug substance is 2-(4-[2-tetrahydrofuranylcarbonyl]-1-piperazinyl)-6,7-dimethoxy-4-quinazolinamine monohydrochloride dihydrate. The compound is prepared via a multistep synthesis and therefore the possibility of by-product formation exists. Several of these potential by-products have been identified in bulk terazosin and represent a range of size and polarity (Fig. 1).
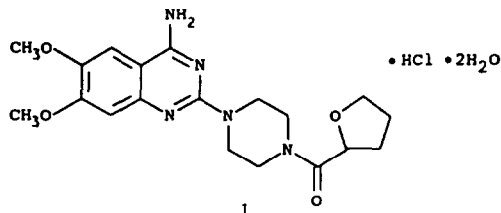
The effectiveness of terazosin as an antihypertensive agent is reported elsewhere in the litera-

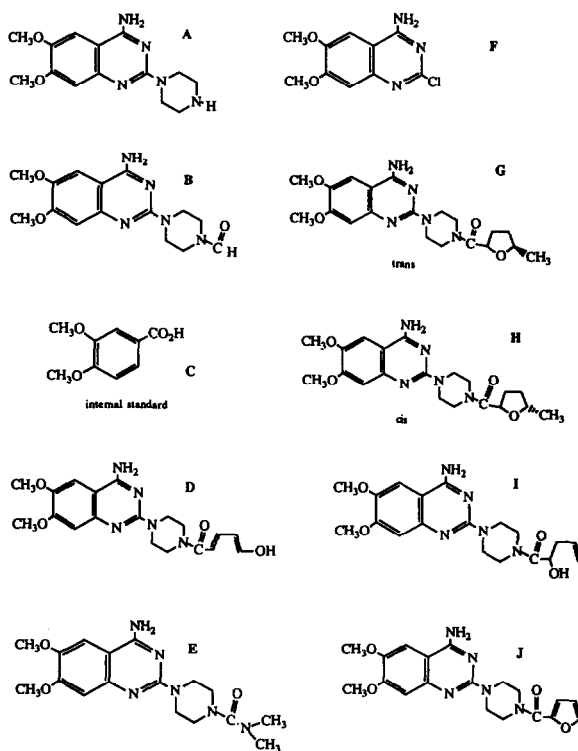Fig. 1. Potential by-products of terazosin synthesis.

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ture [1–6]. Various HPLC methods have been reported for the determination of terazosin and other quinazolinamines and their metabolites [7–9]. This paper describes the use of HPLC for the quantitation of minor impurities which can occur in terazosin bulk drug. The procedure is used to detect and quantitate potential manufacturing impurities and degradation products. The technique allows the direct determination of these materials without derivatization. In this work a reversed-phase chromatographic column and isocratic elution are used to achieve the required resolution of the drug substance and various impurities. This approach provides reliable quantitation of minor impurities which show marked differences in retention on different stationary phases.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Spectra-Physics isocratic pump (Spectra-Physics, Santa Clara, CA, USA), an autosampler (Waters, Milford, MA, USA), a variable wavelength ultraviolet detector Spectra 100 (Spectra-Physics) and a Model CR 4A data handling system (Shimadzu, Kyoto, Japan). Chromatographic separations described in the method were made using 5-μm Zorbax Rx C_8 columns measuring 15 cm $\times$ 4.6 mm I.D. (Mac Mod, Chadds Ford, PA, USA). Additional columns used included μ-Bondapak C_{18} (10 μm) measuring 30 cm $\times$ 3.9 mm I.D. (Waters), Nucleosil C_{18} (5 μm) measuring 15 cm $\times$ 4.6 mm I.D. (Macherey-Nagel, Düren, Germany). Bakerbond C_4 wide-pore (5 μm) measuring 25 cm $\times$ 4.6 mm I.D. (J.T. Baker, Philipsburg, NJ, USA), Versapak C_{18} (5 μm) measuring 30 cm $\times$ 4.0 mm I.D. (Alltech, Deerfield, IL, USA) and a Serva Techsphere C_8 (5 μm) measuring 25 cm $\times$ 4.6 mm (Serva Biochemicals, Paramus, NJ, USA).

Prior to use, the components of the eluent were filtered through 0.45-μm nylon membranes (Alltech).

Reagents

Acetonitrile and isopropanol were UV grade, distilled in glass, from Fisher Scientific (Fair Lawn, NJ, USA). 3,4-Dimethoxybenzoic acid, citric acid and sodium citrate dihydrate were reagent grade and were from Aldrich (Milwaukee, WI, USA). A 0.05 *M* solution of sodium citrate and citric acid prepared in deionized water was used in the HPLC eluent (pH = 4.4). All bulk drugs, reference standards and related impurity standards were synthesized at Abbott Laboratories (North Chicago, IL, USA). A 0.2 mg/ml solution of 3,4-dimethoxybenzoic acid in methanol was used as internal standard.

The eluent consisted of 175 ml of acetonitrile, 50 ml of isopropyl alcohol and 1775 ml of 0.05 *M* citrate buffer.

Instrument conditions

Instrument parameters were as follows: flow-rate, 2.0 ml/min; pressure, approximately 1500 p.s.i. (1 p.s.i. = 6894.76 Pa); detector, 254 nm at 0.10 a.u.f.s., attenuation at 16, ambient temperature (*ca.* 22°C) and an injection volume of 50 μl.

Analytical procedure

Terazosin bulk drug samples were prepared by dissolving approximately 100 mg of the drug substance in 100 ml of 90% citrate buffer and 10% acetonitrile containing 10 ml of internal standard solution. Impurity standards for impurities **A**, **F**, **I** and **J** were prepared at a concentration of 0.01 mg/ml (1% level in the same matrix). The amounts of these impurities were determined in the sample by comparing the corresponding peak area ratio (PAR) (*i.e.*, peak area/peak area of internal standard) in the sample and standard preparations. All other impurities were determined by comparing the peak area ratio for the impurity in the sample preparation to that of terazosin at the 1% level.

RESULTS AND DISCUSSION

The HPLC conditions described in the text were developed to resolve the drug substance, manufacturing impurities and the predominant degradation product (impurity A). Shown in Fig. 1 are the possible manufacturing impurities identified in the course of this work. Fig. 2 shows the degradation pathway which leads to additional
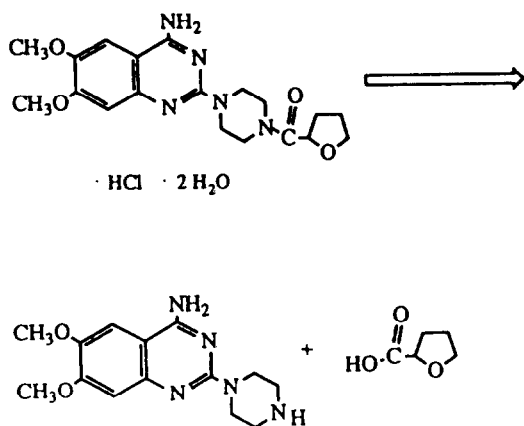
Fig. 2. Hydrolysis degradation pathway of terazosin.

impurity **A** when terazosin is hydrolyzed. These impurities were either isolated and identified by MS and NMR or identified by LC–MS techniques.

The presence of an amine moiety in both terazosin and its impurities required the use of ion-pairing to produce the most symmetrical peak shapes for each of the components. Although gradient elution may be used to resolve these components, a simple isocratic system with ion-pairing was developed that adequately resolves the drug from the early eluting impurities while still eluting the strongly retained impurities within a reasonable time. No single modifier provided optimum resolution of both early and late eluting impurities. Acceptable results were obtained using the ternary system described in the text where a small amount of acetonitrile aids in the resolution of the early eluting components and isopropanol helps to resolve the more strongly retained impurities while maintaining peak symmetry. Although the addition of isopropanol provided better peak shape, the majority of the packings investigated still showed poor resolution for the late eluting peaks (impurities **I** and **J**) which often co-eluted. The Rx-C_8 column, however, differs from the other reversed-phase columns used in that the silanes used are diisopropyl rather than dimethyl. These bulky groups provide steric protection of the siloxane bond and prevent substrate interaction with unprotected silanol groups resulting in a more uniform reversed-phase separation by minimizing interaction with the polar groups on the support. The Rx-C_8 column gave good resolution for all the known impurities including the late eluting components which are separated by six minutes. A detection wavelength of 254 nm provides a very similar response for the impurities and drug substance, providing the best estimate of unknowns quantitated *versus* the drug substance.

TABLE I

PRECISION DATA FOR IMPURITIES ANALYSIS IN BULK TERAZOSIN

Impurity	mg Found			
	Compound A	Compound F	Compound I	Compound J
Analyst I				
mg Added	6.45	9.97	9.25	11.23
Equivalent impurity level (%)	0.32	0.50	0.46	0.56
Mean	6.306	10.168	9.282	11.124
S.D.	0.053	0.051	0.011	0.138
R.S.D. (%)	0.84	0.50	0.12	1.24
Analyst II				
mg Added	9.5	12.9	11.3	10.3
Equivalent impurity level (%)	0.48	0.64	0.56	0.52
Mean	9.462	13.064	11.308	10.276
Std. Dev.	0.073	0.116	0.122	0.049
Rel. Std. Dev. %	0.77	0.89	1.08	0.47

Shown in Fig. 3 are typical chromatograms for a mixed standard preparation having individual concentrations for impurities of approximately 100 μg/ml and a typical lot of terazosin spiked with 0.05% of each impurity.

Detector response for terazosin and impurities was linear to approximately 0.5 μg/ml which represents 0.05% of the drug concentration (correlation coefficient > 0.9999). Linearity curves of concentration versus detector response essentially intersected the origin, allowing the use of one-point calibration for quantitation of known and unknown impurities. Since no lots of

terazosin were found which contained all the possible impurities, precision was performed on two artificial mixtures containing the four most frequently occurring impurities at approximately 0.5% each. The study was performed by two analysts on separate days. These data arc presented in Table I. As shown the assay precision (R.S.D.) ranged from ±0.12% to ±1.24% for impurities having mean values of 0.32% to 0.64%.

Several alternative reversed-phase packings were evaluated for the determination of terazosin impurities. For this evaluation the
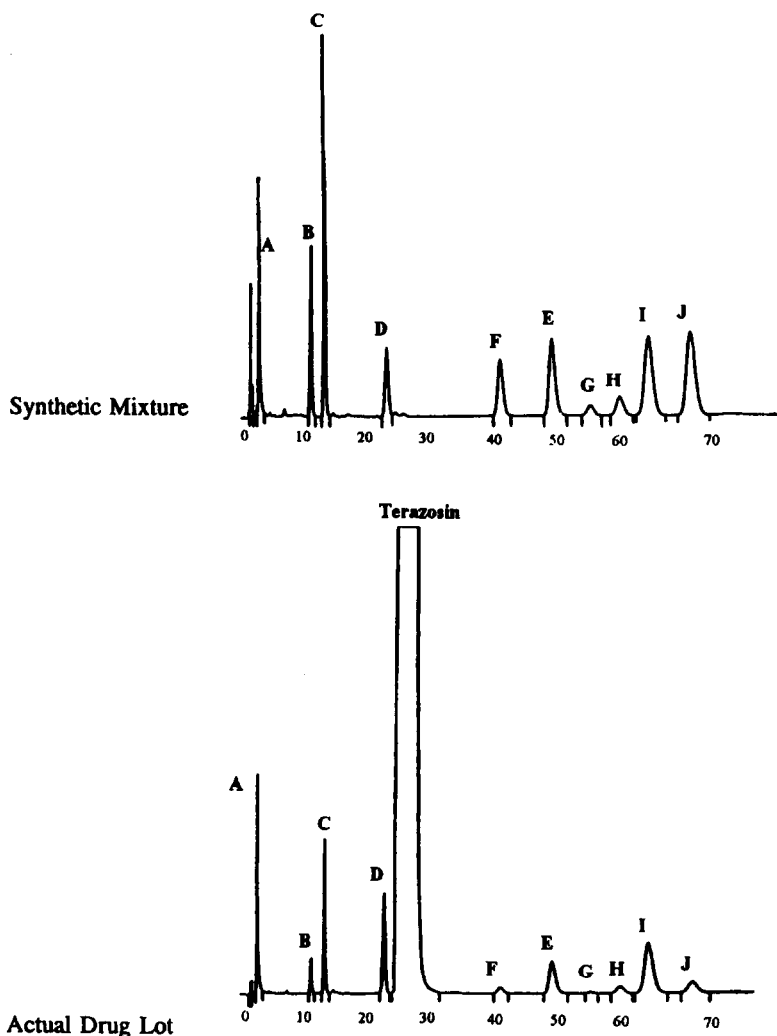
Fig. 3. Chromatograms of synthetic mixture and actual drug lot on Zorbax Rx C$_8$ column. Numbers indicate time (min).

same eluent was used as described in the experimental. The same synthetic standard mixture shown in Fig. 3 was used for this evaluation. The most discriminating difference in performance between the packings tested [Nucleosil C_{18} (Alltech and Macherey Nagel), Bakerbond C_4, Versapak C_{18}, μ-Bondapak C_{18}, Servo Techsphere C_8 and Zorbax Rx-C_8] was in the resolution of the late eluting peaks (impurities I and J). Several of the packings showed poor resolution for many of the impurities however, even when acceptable resolution was obtained for the majority of the compounds difficulty was encoun-

tered with impurities I and J. Depending on the column used, this pair of impurities could either co-elute or be resolved completely. Table II presents the corresponding resolution factor for this impurity pair for each packing tested. Fig. 3 and 4 show the HPLC traces.

To determine the reproducibility of the Rx-C_8 packing, a second lot of packing was obtained and evaluated. The separation obtained for terazosin and impurities was identical to the original lot.

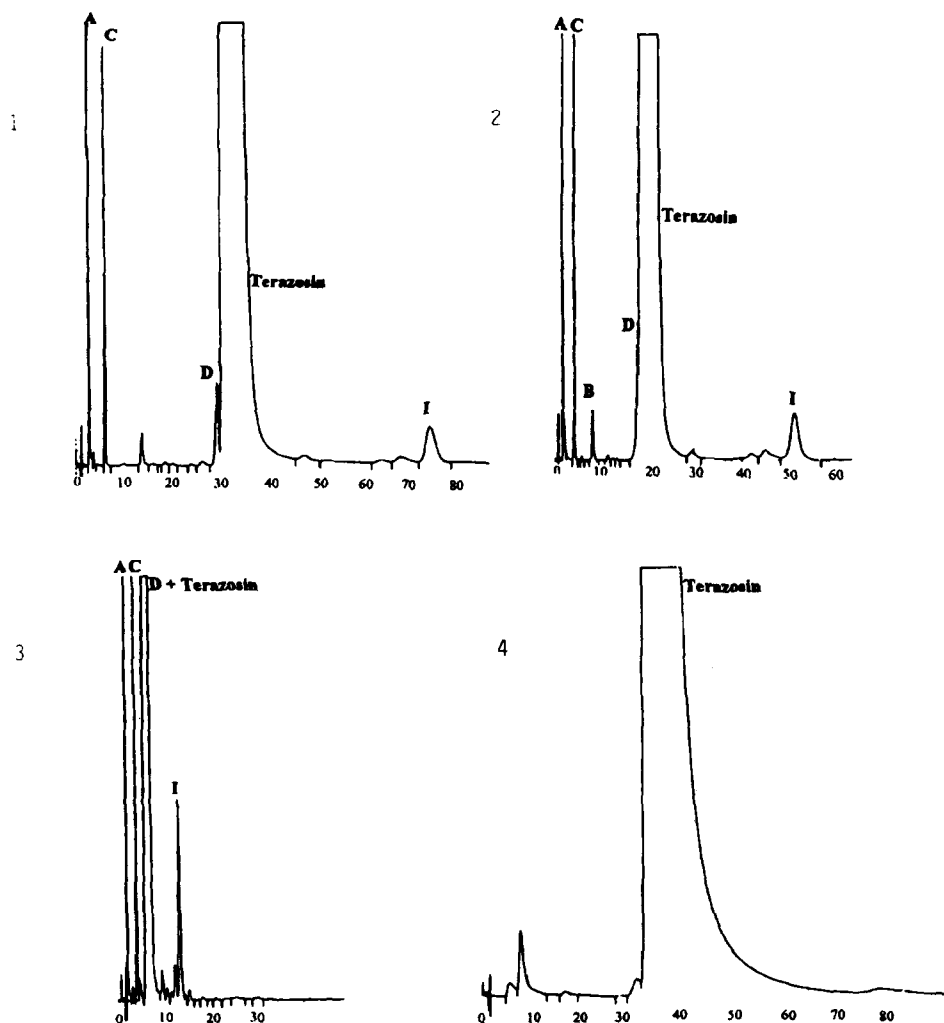The reproducibility of the separation and columns combined with their longevity and good
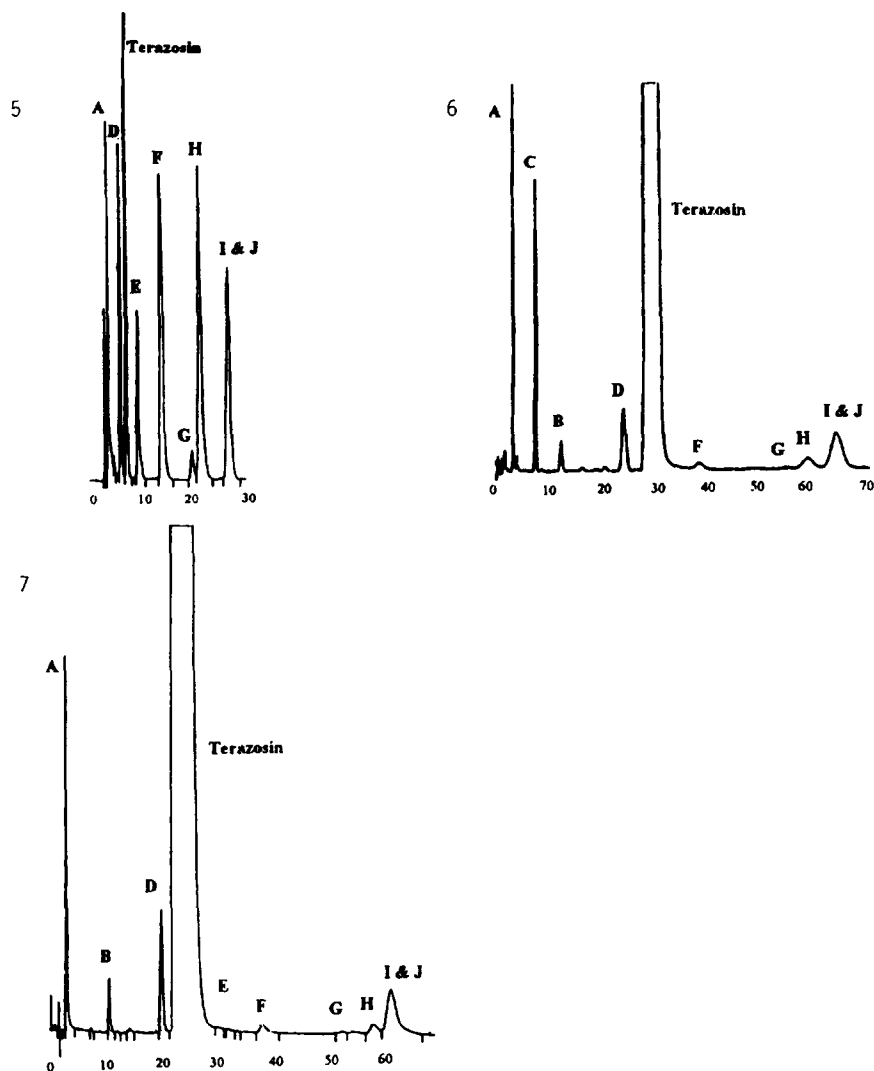

Fig. 4.

Fig. 4. Chromatograms of synthetic mixture on various packings. 1 = Alltech Nucleosil C_{18}; 2 = Macherey Nagel Nucleosil C_{18}; 3 = Bakerbond C_4; 4 = Versapak C_{18}; 5 = Bakerbond C_8; 6 = Waters μ-Bondapak C_{18}; 7 = Serva Techsphere C_8. Numbers indicate time (min).

TABLE II

PERFORMANCE OF VARIOUS REVERSED-PHASE PACKINGS FOR TERAZOSIN LATE ELUTING IMPURITY SEPARATION

Source	Packing	Column dimension	Resolution factor for impurities I and J
Alltech	Nucleosil C_{18} (5 μm)	15 cm × 4.6 mm	Co-elute
Macherey Nagel	Nucleosil C_{18} (5 μm)	12.5 cm × 4.0 mm	Co-elute
Waters	μ-Bondapak C_{18} (10 μm)	30 cm × 3.9 mm	1
Baker	Bakerbond C_4 (5 μm)	25 cm × 4.6 mm	0.9
Serva	Techsphere C_8 (5 μm)	25 cm × 4.6 mm	Co-elute
Zorbax	Rx C_8 (5 μm)	15 cm × 4.6 mm	2.4

solution stability of the drug make this a rugged determination which is well suited for automation.

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