Journal of Chromatography, 519 (1990) 125–136
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 700

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

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(First received March 20th, 1990; revised manuscript received July 17th, 1990)

ABSTRACT

Minor impurities in the antibacterial agent temafloxacin hydrochloride were determined using high-performance liquid chromatography. Manufacturing impurities and degradation products were separated using a reversed-phase system with gradient elution. Detector response was linear for the individual impurities to approximately 50 μg/ml which represents 2.5% of the drug concentration. The procedure provides quantitation of impurities to approximately the 0.05% level with precision (relative standard deviations) of 4.7% to 29% in typical bulk drug lots. A variety of reversed-phase columns were evaluated for the assay method with optimum resolution achieved using a 5-μm Nucleosil C_{18} packing.

INTRODUCTION

Temafloxacin hydrochloride is a synthetic broad-spectrum fluoroquinolone antibacterial (Fig. 1). Chemically the drug substance is 6-fluoro-1-(2,4-difluorophenyl)-7-(3-methyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid hydrochloride. As reviewed previously [1], this class of compounds shows activity against gram-positive and gram-negative organisms. This paper describes the use of high-performance liquid chromatography (HPLC) for the quantitation of minor impurities which can occur in temafloxacin hydrochloride bulk drug. Potential manufacturing impurities and degradation products are determinable by the procedure.

Various methods using HPLC have been reported for the determination of fluoroquinolones and their metabolites [2–7]. The HPLC technique allows the direct determination of these materials without the derivatization which is necessary in gas chromatographic procedures [8,9]. In this work, a reversed-phase chromatographic column and gradient 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 in reversed-phase systems.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model SP-8100 ternary pump and autosampler and a Model SP-4270 data handling system (Spectra-Physics, Santa Clara, CA,

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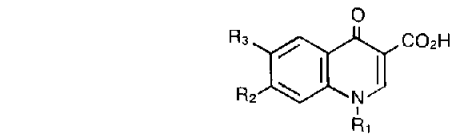

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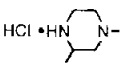
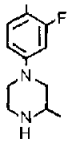
		F	Temafloxacin Hydrochloride
		F	Imp. A
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	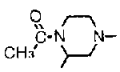	F	Imp. C
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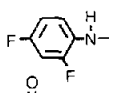
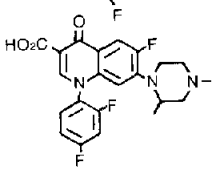
		F	Imp. H
		F	Imp. I

Fig. 1. Structures of temafloxacin hydrochloride and related manufacturing impurities.

U.S.A.). A Model SF-769 variable-wavelength UV detector (ABI Analytical Kratos Division, Ramsey, NJ, U.S.A.) was used. Chromatographic separations described in the method were made using Nucleosil C_{18} columns (5 μm) measuring 15 cm × 4.6 mm I.D. (Alltech, Deerfield, IL, U.S.A; Chromatographic Resolutions, San Jose, CA, U.S.A.; ES Industries, Marlton, NJ, U.S.A.; Keystone Scientific, Bellefonte, PA,

U.S.A.) or measuring 12.5 cm × 4.0 mm I.D. (Macherey-Nagel, Düren, F.R.G.). Additional columns used included μBondapak C_{18} (10 μm) measuring 30 cm × 3.9 mm I.D. (Waters Assoc., Milford, MA, U.S.A.), AQ-302 ODS (5 μm) measuring 15 cm × 4.6 mm I.D. (YMC, Morris Plains, NJ, U.S.A.), Chromegabond alkylphenyl (5 μm) and Chromegabond pentafluorophenyl (5 μm) measuring 15 cm × 4.6 mm I.D. (ES Industries, Marlton, NJ, U.S.A.), and Adsorbosphere HS C_{18} (5 μm) measuring 25 cm × 4.6 mm I.D. (Alltech). Prior to use, the components of the eluent were filtered through 0.45 μm nylon membranes (Alltech).

Reagents

Acetonitrile and tetrahydrofuran were UV grade, distilled in glass, from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Both monobasic potassium phosphate and orthophosphoric acid (85%) were ACS reagent grade and were from J. T. Baker (Phillipsburg, NJ, U.S.A.) and Fisher Scientific, respectively. A 0.02 *M* solution of monobasic potassium phosphate prepared in deionized water and adjusted to pH 2.4 using orthophosphoric acid was used in the HPLC eluent. All bulk drugs, reference standards and related impurity standards were synthesized at Abbott Labs. (North Chicago, IL, U.S.A.).

Chromatographic conditions

A linear gradient was used, mixed with the ternary pump as shown in Table I. Other conditions were: flow-rate, 1.0 ml/min; pressure, approximately 120 bar; detector, 325 nm at 0.10 a.u.f.s., attenuation at 16, and injection volume, 50 μl.

TABLE I

LINEAR GRADIENT FOR HPLC ELUENT

Time (min)	Phosphate buffer (%)	Acetonitrile (%)	Tetrahydrofuran (%)
0	90	5	5
50	35	5	60
60	35	5	60
65[a]	90	5	5
80[a]	90	5	5

[a] Used to re-equilibrate the column to the initial conditions.

Analytical procedure

Temafloxacin hydrochloride bulk drug samples were prepared by initially dissolving approximately 100 mg of the drug substance in 25 ml of acetonitrile–water (1:1) containing 1 ml of 1 *M* sodium hydroxide. The solution was mixed with 15 ml of 0.02 *M* phosphate buffer at pH 2.4 and diluted to 50 ml in acetonitrile -water (1:1) for a sample concentration of approximately 2 mg/ml. Impurity standards were prepared at concentrations of 10 μg/ml to 50 μg/ml (0.50 to 2.5% levels) in the same matrix. The amounts of known impurities were determined in the sample by comparing the corresponding peak areas in sample and standard preparations. Impurity content was calculated on the anhydrous basis by correcting the sample concentration for the

amount of water contained in the drug substance. The drug substance typically contains approximately 1–2% water measured by Karl Fischer titration. Unknowns were estimated by comparing the unknown peak areas in the sample preparation to that of temafloxacin hydrochloride at the 0.5% level.

RESULTS AND DISCUSSION

The HPLC conditions described in the text and Table I were developed to resolve the drug substance, manufacturing impurities and possible degradation products. Shown in Fig. 1 are possible manufacturing impurities identified in the course of this work. Shown in Fig. 2 are some of the degradation products formed when te-

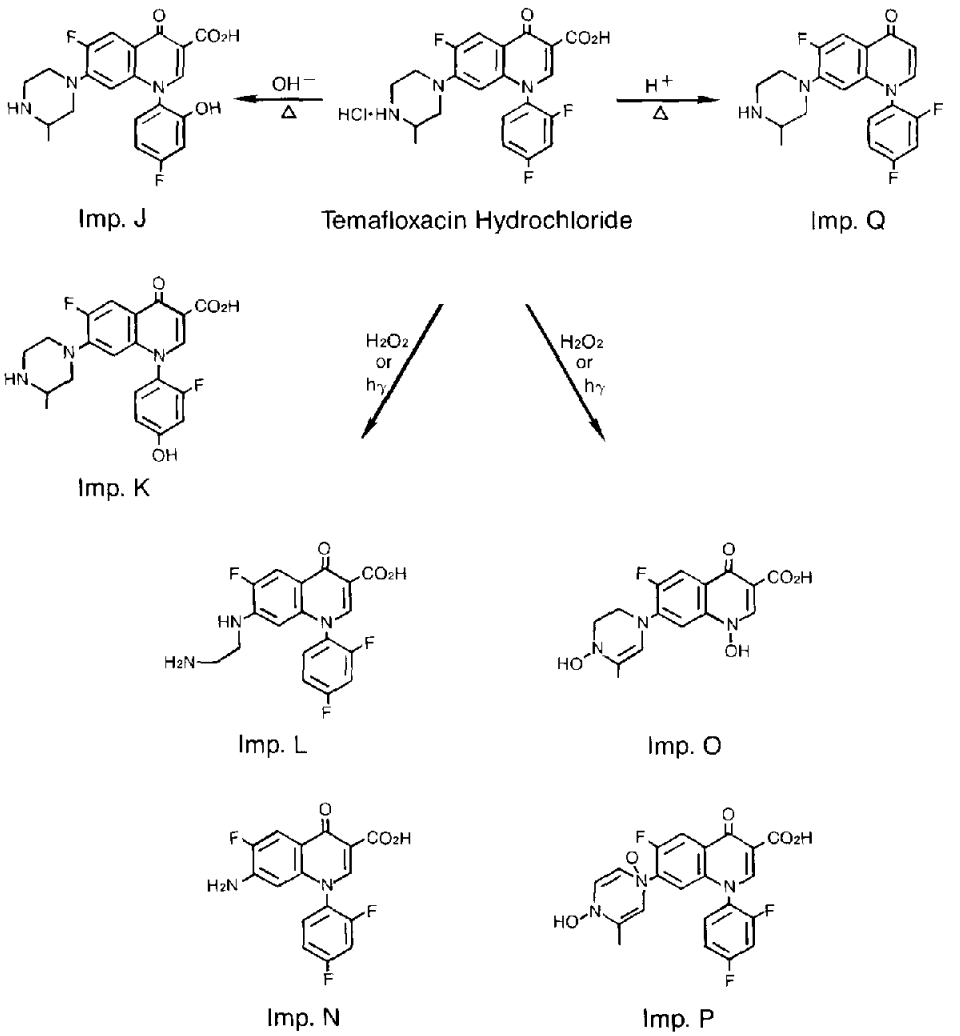


Fig. 2. Structures for possible degradation products of temafloxacin hydrochloride under various conditions.

mafloxacin hydrochloride is stressed under various conditions. The drug substance exhibits excellent thermal stability and only by vigorous chemical degradation was it possible to produce sufficient decomposition for the identification of the degradation products. To investigate the degradation chemistry in strong mineral acid, slurries of the drug substance were heated at 150°C in concentrated orthophosphoric acid or sulfuric acid for two days. For base degradation, 4 mg/ml solutions of the drug substance were refluxed in 0.5 *M* or 1 *M* sodium hydroxide for three hours. Degradation of temafloxacin hydrochloride in hydrogen peroxide was achieved by refluxing 2 mg/ml solutions of the drug substance in 0.5% or 1% hydrogen peroxide for one hour. In preparative experiments, saturated solutions of the drug substance in 30% hydrogen peroxide were refluxed for two hours. To investigate the degradation chemistry in light, a 1-mm layer of temafloxacin hydrochloride was stressed with a high intensity UV lamp for sixteen hours.

Under prolonged heating in concentrated mineral acid the drug can be decarboxylated. In refluxing aqueous base, temafloxacin hydrochloride undergoes nucleophilic substitution of OH^- for the F^- at positions 2 or 4 of the N-phenyl ring. These degradation products were isolated by semi-preparative HPLC. The structures of the isolates from acid and base degradation were confirmed by mass spectroscopy (MS) and NMR. In either UV light or in hydrogen peroxide at reflux temperature, two possible degradation pathways occur. The drug degrades under these conditions by ring opening followed by cleavage of the piperazine ring or by oxidation of the secondary and tertiary amines. These degradation products were either isolated and identified by MS and NMR or identified by LC–MS techniques. The identities of the degradation products formed by opening and cleavage of the piperazine ring were independently synthesized. The synthesized compounds were identical to the isolates by HPLC, NMR and MS.

Since temafloxacin has both acid and base functional groups, the chromatographic behavior on reversed-phase columns is dependent on pH as well as the organic modifier of the eluent. The most symmetrical peak shapes for the drug substance and impurities were obtained in eluents containing aqueous buffers at pH values of approximately 2 to 3. The amount of retention of the drug substance and impurities varied significantly in these systems. Single isocratic eluents with and without added ion-pairing reagents failed to adequately resolve the drug from early eluting impurities while still eluting the more strongly retained impurities within a reasonable time. For this reason, a gradient elution system was developed. Single organic modifiers of tetrahydrofuran methanol and acetonitrile were used in this approach. Again no single modifier provided optimum resolution of both early and late eluting impurities. Acceptable results were obtained using the ternary solvent system described in the text where a small amount of acetonitrile aids in the resolution of the front running components, while tetrahydrofuran is added to more efficiently resolve the more strongly retained impurities. A detection wavelength of 325 nm provides a very similar response for the impurities and drug substance, providing an accurate estimate of unknowns quantitated *versus* the drug substance.

Shown in Fig. 3 are typical chromatograms for a mixed standard preparation having individual concentrations for impurities of approximately 30 μg/ml to 50 μg/ml. Also included in this figure are chromatograms of a typical lot of temafloxacin hydrochloride bulk substance which was prepared as described and a lot of temaflox-

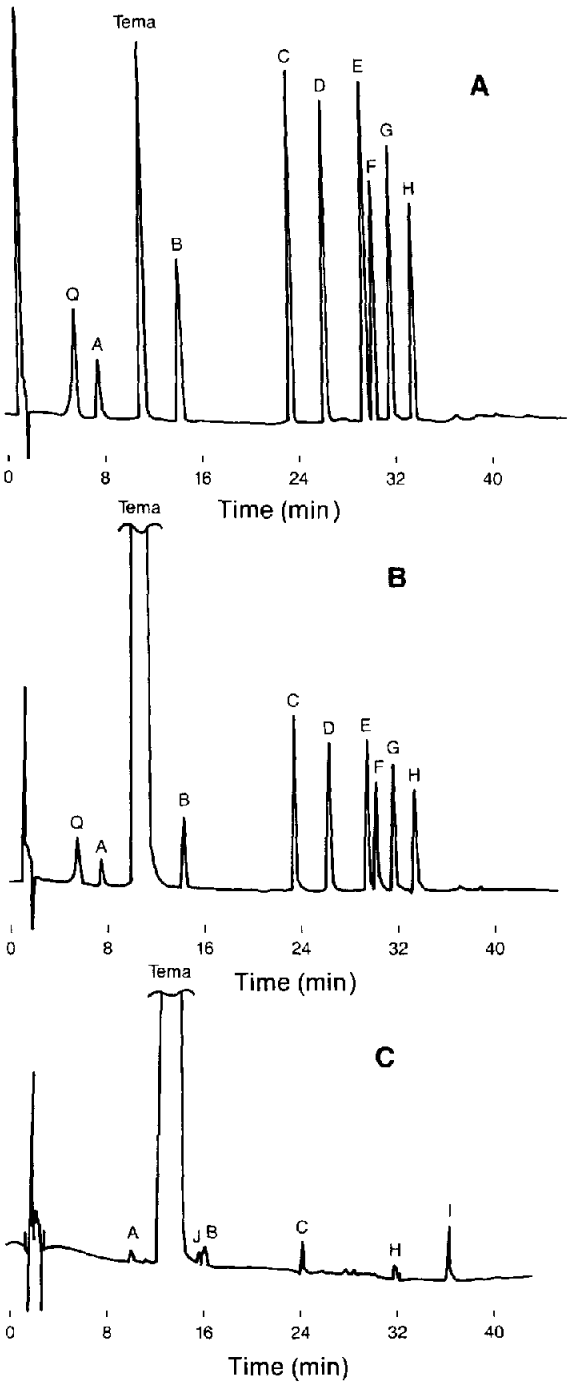


Fig. 3. Typical chromatograms for temafloxacin hydrochloride and impurities. Peak identities: Tema = temafloxacin, all other peak identities as shown in Figs. 1 and 2. (A) Mixed standard preparation; (B) temafloxacin hydrochloride (lot 1) spiked with known impurities at *ca.* 0.5% to 1.2%; (C) typical temafloxacin hydrochloride bulk.

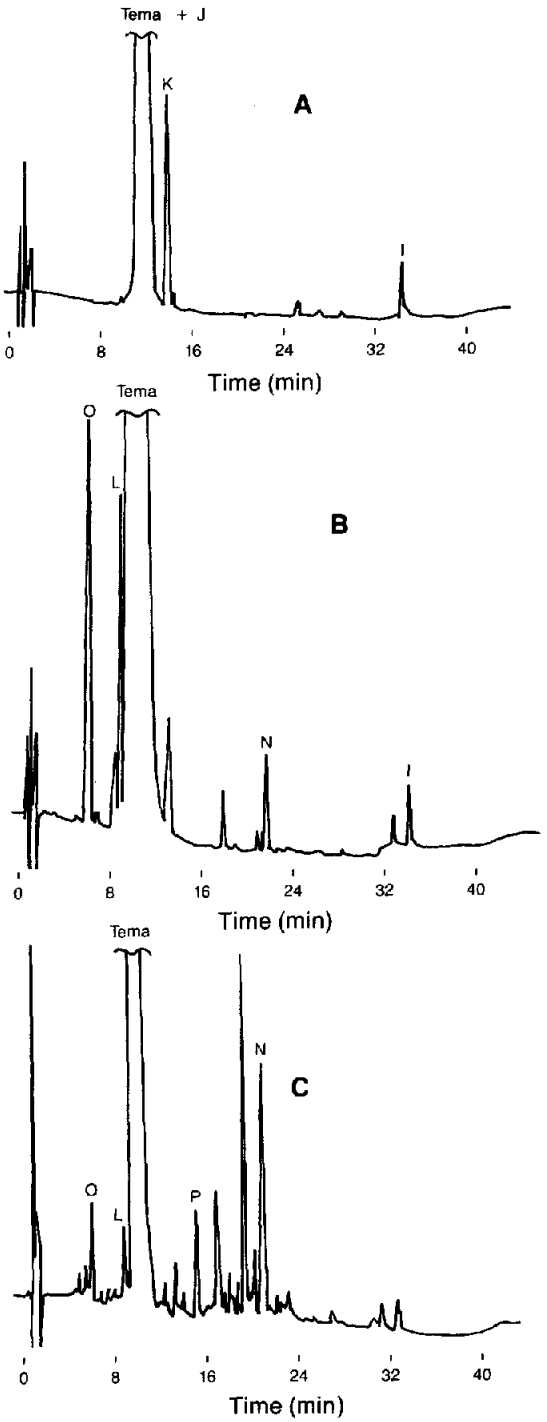


Fig. 4. Typical chromatograms for temafloxacin stressed under various conditions. Peak identities: Tema = temafloxacin, all other peak identities as shown in Figs. 1 and 2. (A) Refluxed in 0.5 *M* NaOH for 1 h; (B) refluxed in 1% H_2O_2 for 1 h; (C) high intensity UV light for 16 h.

acin hydrochloride spiked with the individidual impurities at 0.5% to 1.2%. By the conditions described in the assay procedure, impurities are determinable to approximately 0.05%. Presented in Fig. 4 are chromatograms of temafloxacin hydrochloride which were stressed by refluxing in aqueous sodium hydroxide or hydrogen peroxide and light stressed as a powder under high intensity UV light. The refluxed sample

TABLE II

PRECISION DATA FOR TEMAFLOXACIN HYDROCHLORIDE IMPURITIES

	Day	Analyst	% Impurity[a]				
			Imp J	Imp B	Unknown	Imp E	Imp I
Lot 1	1	1	0.045	0.46	0.10	0.042	0.035
	1	1	0.054	0.54	0.14	0.048	0.041
	2	2	0.050	0.44	0.087	0.035	0.038
	2	2	0.048	0.43	0.078	0.041	0.040
	2	2	0.032	0.41	0.089	0.042	0.036
	3	3	0.076	0.45	0.089	0.041	0.039
	3	3	0.036	0.42	0.085	0.048	0.034
		Mean	0.049	0.45	0.095	0.042	0.038
		Standard deviation	0.014	0.043	0.021	0.0045	0.0026
		Relative standard deviation	29%	9.6%	22%	11%	6.8%

	Day	Analyst	Imp B	Imp C	Imp I
Lot 2	1	1	0.041	0.078	0.13
	1	1	0.044	0.085	0.14
	2	2	0.041	0.063	0.13
	2	2	0.030	0.061	0.12
	2	2	0.027	0.060	0.12
	3	3	0.032	0.065	0.12
	3	3	0.046	0.067	0.12
		Mean	0.037	0.068	0.13
		Standard deviaiton	0.0075	0.0095	0.0079
		Relative standard deviation	20%	14%	6.1%

	Day	Analyst	Imp A	Imp C	Imp I
Lot 3	1	1	0.044	0.19	0.11
	1	1	0.045	0.19	0.11
	2	2	0.031	0.15	0.11
	2	2	0.036	0.16	–
	2	2	0.034	0.15	0.11
	3	3	0.030	0.16	0.12
	3	3	0.034	0.15	0.12
		Mean	0.036	0.16	0.11
		Standard deviation	0.0060	0.018	0.0052
		Relative standard deviation	17%	11%	4.7%

[a] Impurities were calulated on the anhydrous basis *versus* temafloxacin hydrochloride standard at 0.5%. Impurity identities are shown in Figs. 1 and 2.

solutions were neutralized then diluted in acetonitrile–water (1:1) containing about 30% of 0.02 *M* phosphate buffer. The light-stressed material was prepared as described in the assay procedure. Using the conditions stated in the text, one of the base degradation products coelutes with the drug substance. By increasing the amount of acetonitrile in the gradient profile the two major base degrates can be more adequately resolved from the parent drug.

Detector response for temafloxacin and impurities was linear to at least 50 μg/ml (correlation coefficients ≥ 0.999). 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. Assay precision was assessed by performing the procedure on three different lots of temafloxacin hydrochloride. Three analysts performed the determinations on separate days. These data are presented in Table II. As shown, the assay precision (relative standard deviation values) ranged from 4.7% to 29% for impurities having mean values of 0.036% to 0.45%.

Several alternate reversed-phase packings were evaluated for the determination of temafloxacin hydrochloride impurities. For this evaluation, similar gradient profiles were used as described in the text. However, the initial amount of organic solvents in the starting conditions was increased for the more retentative alkylphenyl and fluorophenyl packings. When necessary, conditions were modified to elute temafloxacin with approximately the same capacity factor (k') as achieved using the Nucleosil C_{18} packing. The same synthetic standard mixture shown in Fig. 3 was chromatographed on each column. Representative chromatograms from this evaluation are shown in Fig. 5. Of the columns tested (AQ-302 ODS, μBondapak C_{18}, Adsorbosphere HS C_{18}, Chromegabond alkylphenyl and Chromegabond pentafluorophenyl), none provided the combined peak symmetry and resolution for the impurites which was achieved using the Nucleosil C_{18} packing.

A final experiment was conducted to evaluate the performance of Nucleosil C_{18} (5 μm) columns used in the procedure which are packed and marketed by different manufacturers. For this study new columns were initially equilibrated in acetonitrile–water (60:40). Three gradient runs were made prior to chromatographing the same synthetic impurity standard used in Figs. 3 and 5. For each column the number of theoretical plates and tailing factors [10] were calculated for the drug peak. The relative retention times of impurities were also determined (RRT of temafloxacin = 1.00). The results of this experiment are presented in Table III. The primary difference between the columns tested was in the resolution of impurity peaks E and F (Fig. 1). Depending on the column used, this pair of impurities could either co-elute or be resolved almost completely. The amount of resolution is not directly reflected in the calculated plate count. The most ideal overall performance was achieved with the Macherey-Nagel column having a relatively modest plate count of less than 6000. Typical Nucleosil C_{18} columns used in the procedure provided reasonable column life (*e.g.* approximately 100–200 injections) with intermittent use. The reproducibility of the separation in the columns tested, combined with their longevity and the good solution stability of the drug make this a rugged determination which is well suited for automation.

TABLE III

PERFORMANCE OF NUCLEOSIL C_{18} (5 μm) COLUMNS FROM DIFFERENT MANUFACTURERS FOR TEMAFLOXACIN HYDROCHLORIDE IMPURITY DETERMINATION

Source	Column dimensions	N^a	$t_{0.05}{}^b$	Relative retention times for impurities (Temafloxacin RRT = 1.00)[c]								
				Q	A	B	C	D	E	F	G	H
Alltech	15 cm × 4.6 mm I.D.	9660	1.3	0.58	0.76	1.2	1.8	2.0	2.2	2.2 (co-elute)	2.4	2.4
Chromatographic Resolutions	15 cm × 4.6 mm I.D.	9634	1.4	0.54	0.74	1.2	1.8	2.0	2.3	2.3 (co-elute)	2.4	2.5
Keystone Scientific	15 cm × 4.6 mm I.D.	13095	1.4	0.69	0.75	1.2	1.8	2.0	2.3	2.3	2.4	2.5
ES Industries	15 cm × 4.6 mm I.D.	7694	1.5	0.52	0.74	1.2	1.8	2.1	2.4	2.4	2.5	2.6
Machery-Nagel	12.5 cm × 4.0 mm I.D.	5518	1.1	0.52	0.69	1.3	2.1	2.3	2.6	2.7	2.8	2.9

[a] $N = 16\left(\frac{t}{W}\right)^2$ for temafloxacin peak.

[b] $t_{0.05} = \frac{W_{0.05}}{2 \cdot f}$ for temafloxacin peak, where f is the distance from the peak maximum to the leading edge of the peak at 5% height.

[c] Peak identities as shown in Figs. 1 and 2.

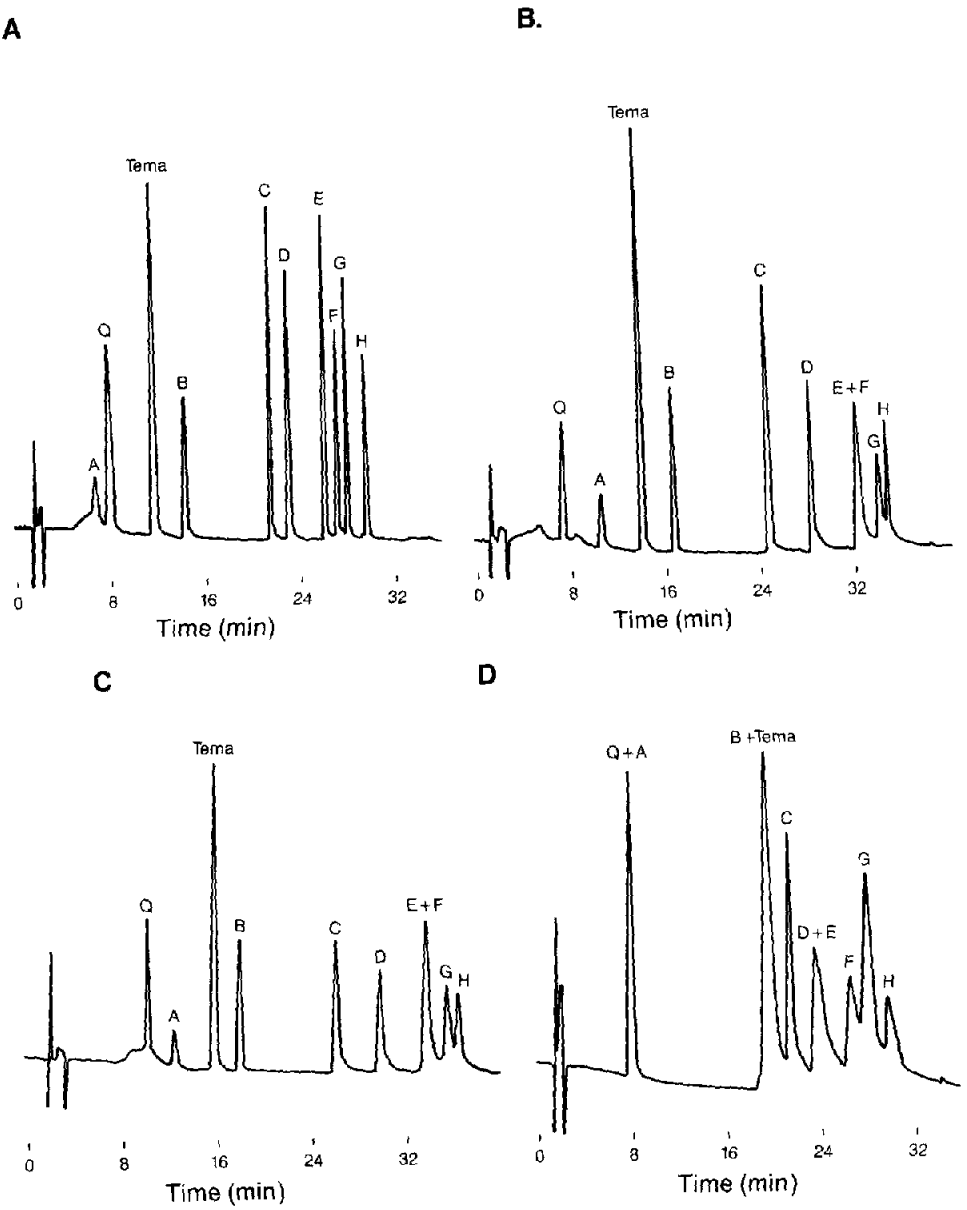


Fig. 5. Typical chromatograms for temafloxacin spiked with impurities using different reversed-phase packings. Peak identities: Tema = temafloxacin, all other peak identities as shown in Figs. 1 and 2. (A) Chromegabond alkylphenyl (15 cm × 4.6 mm I.D.); (B) Adsorbosphere HS C_{18} (25 cm × 4.6 mm I.D.); (C) μBondapak C_{18} (30 cm × 3.9 mm I.D.); (D) Chromegabond pentafluorophenyl (15 cm × 4.6 mm I.D.).

ACKNOWLEDGEMENTS

The authors thank Mr. Steve Cepa and Mr. Mike Cirovic for their assistance in the spectral identification of related minor impurities and Ms. Margie Bender for her assistance in preparation of the manuscript.

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