Investigation of Cyclam-Containing Mobile Phases for the Liquid Chromatographic Analysis of Tenidap

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Abstract

Tenidap is a new oxindole-based cytokine-modulating agent used for the treatment of arthritis. Initial analyses of tenidap for tenidap content and process-related impurities using reversed-phase liquid chromatography were unsatisfactory because of severe peak tailing and batch-to-batch column variability. These difficulties are overcome by addition of cyclam, a nitrogen crown ether analog, to the mobile phase. The improved chromatographic performance after addition of cyclam to the mobile phase may be attributable to changes in the configuration of tenidap.

Introduction

Tenidap (1) (structures of both configurations are shown in Figure 1) is a new oxindole-based cytokine-modulating agent currently in Phase III of regulatory filing as an antiarthritic drug. The original reversed-phase chromatographic assay used to monitor the potency and purity of tenidap, tenidap sodium, and tenidap formulations employed a Waters Nova-Pak C_{18} column and a conventional mobile phase (water–acetonitrile–methanol–triethylamine–85% H_3PO_4, 800:150:50:10:3, v/v). Several years after the development of this chromatographic system, some newly purchased columns inadequately resolved tenidap from its 3-thenoyl isomer (see Figure 1), the prior eluting peak. On the inferior columns, tenidap and its 3-isomer tailed badly and merged together. Because these assays were to be transferred to many other locations, it was necessary to modify the chromatographic conditions to normalize column performance.

Tailing peaks that are observed for solutes containing amines or other functionalities capable of hydrogen bonding can be caused by interaction with residual silanols on the stationary phase surface. A common approach to minimize solute–silanol interaction is to add an amine to the mobile phase (2). Because the original mobile phase already contained triethylamine, a more aggressive tailing suppressor was needed. In the literature, cyclam (Figure 1) has been proposed for use as a silanophilic agent (3). Recently, triethylamine (TEA), cyclam, and *N,N*-dimethyloctylamine (DMOA) were compared as tailing suppressors (4). TEA is often the first choice to minimize peak tailing because short chain tertiary amine modifiers can efficiently penetrate to the support's residual silanol groups and block potential solute interactions (2). In some cases, longer chain modifiers such as DMOA have proved to be more effective. For reversed-phase ion-pair separations of antidepressive and neuroleptic amines, the favorable influence of long-chain compounds was attributed to the elimination of retention mechanisms other than liquid–liquid distribution (5).

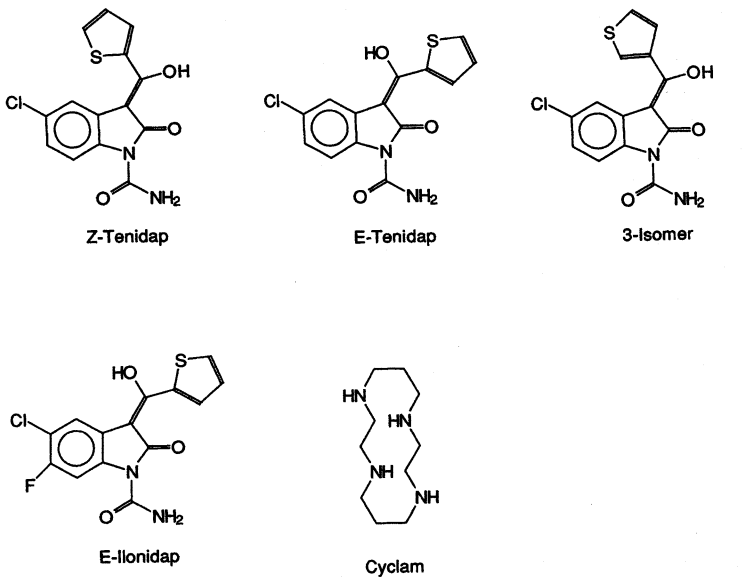

Figure 1. Chemical structures of tenidap and its 3-isomer, ilonidap, and cyclam.

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Cyclam and other cyclic tetraaza compounds have been evaluated as silanol blockers because these types of additives have a large number of nitrogens in a fixed ring and should generate intense silanol–amine interactions (3). Cyclam also has been used to form stable complexes with metal ions (6,7). Since DMOA is unpopular because of its stench, cyclam was evaluated as a tailing suppressor for tenidap.

This paper describes the effect of cyclam on the chromatographic performance of tenidap. Cyclam was included in the mobile phase for tenidap assays to improve peak shape, minimize column-to-column variability, and increase column life.

Experimental

Chemicals and solvents

Cyclam (1,4,8,11-tetraazacyclotetradecane) was purchased from Fluka Chemical (Ronkonkoma, NY), HPLC-grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ), and TEA was purchased from Eastman Kodak (Rochester, NY). Standards of tenidap sodium and its 3-isomer were prepared and characterized in-house at Pfizer (Groton, CT).

Apparatus

The HPLC system used for most of these studies was the Perkin-Elmer LC Analyst system (Norwalk, CT) with the associated LC analyst software. This system consisted of a PE Model 620 quaternary LC pump, a PE Model ISS 100-C autoinjector, a PE Model 235 diode-array detector, a Digital DECstation 316 SX, and a BAS Model LC-22A column heater. Chromatographic performance was evaluated using PE/Nelson Access*Chrom software. Waters Nova-Pak C_{18} columns (15 cm x 3.9-mm i.d.; 4-µm particles, P/N 86344) (Bedford, MA) were used for all separations. HPLC conditions were as follows: mobile phase, water–acetonitrile–triethylamine–85% H_3PO_4–cyclam, 800:200:10:3:variable, pH 7.0; flow rate, 2 mL/min; detection, UV (255 nm); injection, 10 µL; temperature, ambient; sample preparation, 1 mg tenidap sodium per milliliter.

Preparation of the cyclam salt of tenidap

Cyclam (0.12 g, 0.6 mmol) and tenidap (0.40 g, 1.2 mmol) were stirred in refluxing acetonitrile for 5 min. The resulting slurry was cooled to ambient temperature, filtered, and washed with acetonitrile to give 0.50 g (100% yield). The cyclam salt was characterized by proton nuclear magnetic resonance spectrometry and single crystal x-ray diffractometry as a 2:1 adduct of tenidap–cyclam. This 2:1 adduct was isolated even when 1:1 molar ratios of tenidap and cyclam were used. The product was a dull orange solid; the melting point was 179.5–180.7°C; the ^{1}H-NMR (solvent d-DMSO, 300 MHz) had the following characteristics: δ, 1.72 (s, 4H), 2.8 (s, 8H), 2.90 (s, 8H), 6.25 (broad s, 4H), 6.73 (d, 2H), 7.11 (d, 2H), 7.18 (s, 2H), 7.61 (d, 2H), 8.05 (d, 2H), 8.18 (s, 2H), 8.60 (d, 2H), 9.32 (s, 2H).

A representative crystal was surveyed and a 1 Å data set (maximum sin Θ/λ = 0.5) was collected on a Siemens R3RA/v diffractometer (Madison, WI). All crystallographic calculations were facilitated by the SHELXTL (8) system. The structure of the cyclam salt of tenidap, which was plotted using the SHELXTL plotting package, is shown in Figure 2. Two cyclam molecules are depicted in Figure 2; however, both molecules used a center of symmetry so that only one unique cyclam molecule was present in the structure. Both the E and Z configurations of tenidap are illustrated in Figure 2. Coordinates, distances, and angles will be submitted to the Cambridge Crystallographic Data Center, Cambridge, England.

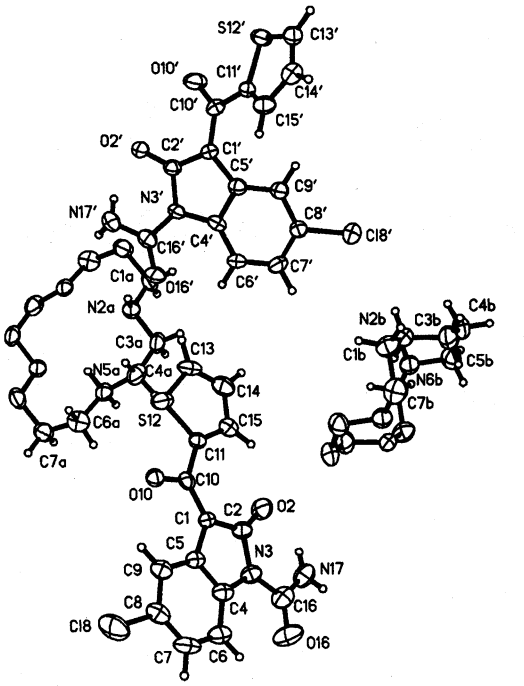

Figure 2. The structure of the cyclam salt of tenidap derived from single crystal x-ray diffractometry.

Results and Discussion

To study the effect of cyclam on the chromatography of tenidap and its 3-isomer, several mobile phases were evaluated. Two mobile phase reservoirs were prepared. The reservoirs were identical except that only one reservoir contained cyclam. The chromatographic system mixed the two reservoirs at predetermined ratios to obtain specific mobile phases. The column was equilibrated with each new mobile phase for 1 h before the sample was injected.

The effect of the system on the number of theoretical plates, retention, resolution, and tailing factor is summarized in Table I; chromatograms illustrating this effect are shown in Figure 3.

The data in Table I and in Figure 3 show that chromatographic performance improves with increasing concentration of cyclam. These data were generated on a new column whose performance was considered acceptable, even without the addition of cyclam. Other new columns have performed both better and worse than the column chosen for this illustration.

The chromatographic performance measured for the 3-isomer was consistently better than that measured for tenidap

Table I. Effects of Cyclam Concentration on the Analysis of Tenidap and its 3-Isomer

Cyclam (mg/L)	Retention time (min)		Tailing factor*			Plates (n)‡		Plates (n)§	
	3-Isomer	Tenidap	3-Isomer	Tenidap	Resolution†	3-Isomer	Tenidap	3-Isomer	Tenidap
0	12.6	16.0	1.79	2.54	3.1	3300	2300	1500	1100
250	12.2	15.8	1.59	2.08	3.5	4500	2200	2600	1200
500	11.7	15.3	1.55	2.07	3.5	4300	2200	2500	1200
750	11.4	14.7	1.44	2.05	3.6	5200	2200	2600	1200
1000	11.0	14.2	1.45	2.05	3.4	4200	2200	2900	1200
0‖	12.5	16.1	1.55	2.12	3.4	4300	2200	2400	1200
0#	12.5	16.1	1.49	2.10	3.5	4400	2100	2500	1200

* The tailing factor was calculated by the USP method, $T = w_h/2f$, *where* w_h is peak width at half height and f is the horizontal distance from point of ascent to a point coincident with maximum peak height.

† $R_S = \frac{2(t_{R2}-t_{R1})}{w_{h1}+w_{h2}}$ where t_{R1} and t_{R2} are the retention times of peaks 1 and 2, respectively, and w_{h1} and w_{h2} are the peak widths at half height for peaks 1 and 2, respectively.

‡ Theoretical plates were calculated using the tangential (USP) method: $n = 16(t_R/w_b)^2$, where t_R is retention time and w_b is peak width at base.

§ Theoretical plates were calculated using the Foley and Dorsey method (9).

‖ An injection was made into a mobile phase containing no cyclam after equilibration in the new mobile phase for 1 h. The previous mobile phase contained 1000 mg cyclam per liter.

An injection was made into a mobile phase containing no cyclam after equilibration in the new mobile phase for 2 h. The previous mobile phase contained 1000 mg cyclam per liter.

both with and without cyclam. This is because tenidap was present at roughly 100 times the 3-isomer concentration (approximately 0.93 mg/mL), which was required to allow concomitant quantitation of the parent drug (tenidap) and trace levels of process-related compounds and degradation products. The effect of cyclam is better measured using the 3-isomer peak because the improvement in column efficiency is not obscured by an overloaded analyte peak.

When cyclam was removed from a conditioned column, the peak shape started to deteriorate, but it remained better than in the untreated column (Table I). These data are consistent with the slow desorption kinetics of cyclam and with the theory that the stationary phase surface contributed, in part, to the improvement in peak shape. The effect of cyclam remained 2 h after its removal from the mobile phase. The slow desorption kinetics of cyclam is due to its high density of amine functions, a phenomenon that does not occur with straight chain analogs (3).

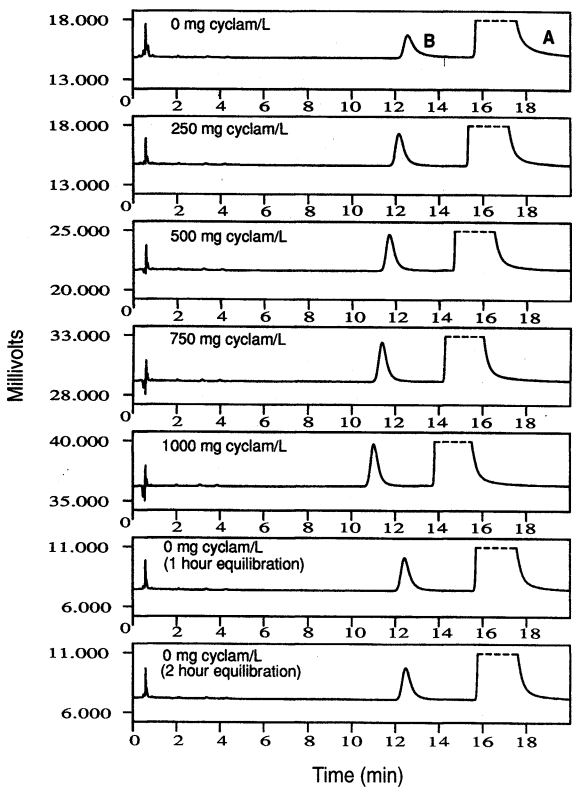

Figure 3. Illustration of the effect of cyclam concentration: A, tenidap; B, its 3-isomer.

Tenidap and 3-acyl substituted oxindoles contain an enolic system that can exist in an E or Z configuration (Figure 1). In the solid state, based on single crystal x-ray diffraction, tenidap and tenidap sodium hydrate exist in the Z configuration. In contrast, the crystalline cyclamate salt (a 2:1 adduct) contains both E and Z configurations of tenidap. This is also true of the analog, ilonidap (Figure 1) (M.P. Snyder and J. Bordner, Pfizer Central Research, unpublished data, 1992).

The pK_a measured for tenidap (2.8–3.3) varied slightly depending on its configuration and the environment of the measurement (E.M. Greer and E.F. Fiese, Pfizer Central Research, unpublished data, 1994). At pH 7.0, tenidap is significantly above its pK_a and can be considered completely ionized. In its E configuration, the enolic hydrogen cannot participate in intramolecular hydrogen bonding, and based on titration data, it is more acidic than tenidap in its Z configuration (E.M. Greer and E.F. Fiese, Pfizer Central Research, unpublished data, 1994). A stronger acid is a weaker proton acceptor and has weaker hydrogen bonding to silanols on the surface of the packing material, resulting in improved chromatographic performance. Because previous studies comparing tailing suppressors determined that cyclam was much less effective than TEA or DMOA (3), cyclam would have little impact on chromatographic performance if its only function was to mask surface silanols.

The unusual activity of cyclam may be due to perturbation of the equilibrium of tenidap tautomers to favor the E configuration, a form that has less interaction with silanols. Cyclam may have similar effects on other analytes amenable to isomerization to a less silanophilic (more column-friendly) form.

The chromatograms shown in Figure 4 also support the theory that tenidap and its 3-isomer perform better chro-

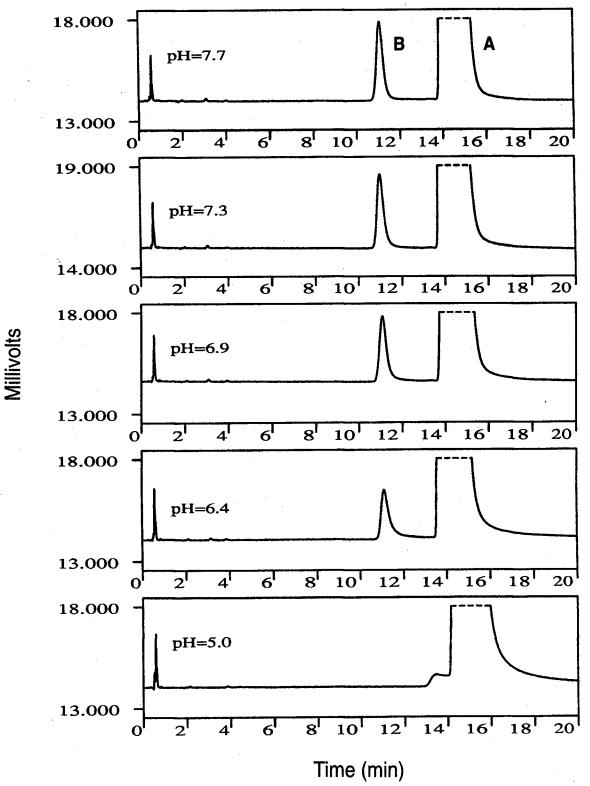

Figure 4. Illustration of the effect of mobile phase pH: A, tenidap; B, its 3-isomer.

Table II. Column-to-Column Reproducibilty (1 mobile phase)

Column i.d.	Plates (n)*	Comments
T90583 KO3	2400	Used for Development
T90591 R34	2300	New Column
T90591 S38	2300	New Column
T90452 KO2	2500	New Column
T70352 RO4	2300	Old Column

* Theoretical plates were calculated using the tangential (USP) method: $n = 16\,(t_R/w_b)^2$ where t_R is retention time and w_b is peak width at base.

matographically when they exists in their E configuration. These data were generated with the same two-reservoir approach used to generate the chromatograms in Figure 3. Cyclam concentration was held constant at 500 mg/L, and the apparent pH of the mobile phase was varied. The apparent pH values listed in Figure 4 were measured on the mobile phases (containing acetonitrile). An apparent pH of 7.7 corresponds to a buffer pH of 7.0. Decreasing the pH shifted the equilibrium of the E/Z conformers to the weaker acid (the Z conformer). This shift was accompanied by deterioration of the chromatographic performance. Although it is possible that deterioration of chromatographic performance was due to dissociation of the tenidap–cyclam ion-pair at low pH, the apparent pH of the mobile phases used to generate the chromatograms in Figure 4 ranged from 7.7 to 5.0. Under these conditions, tenidap can be considered completely ionized, and the concentration of the tenidap–cyclam ion-pair would not be affected by changes in mobile phase pH.

Probably the most valuable aspect of using cyclam-containing mobile phases for tenidap assays is that column-to-column variability is minimized and column life is increased. Before cyclam was used as an additive, failures of brand-new columns or failures within the first day of use were not unusual. With cyclam-containing mobile phases, columns routinely last for more than a year (> 600 injections). In addition, as is illustrated in Table II, column-to-column reproducibility is excellent. The data in Table II were generated on several different columns with different histories using a single batch of mobile phase. Assays developed to support pharmaceutical production must show this type of column-to-column reproducibility.

Conclusion

Cyclam has been identified as a potent tailing supressor for tenidap and tenidap analogs and appears appropriate for analytes susceptible to tautomerism. Column-to-column variation has been minimized, and column lifetimes have increased. Because the original mobile phases used for tenidap contained triethylamine, a strong silanophilic agent, and peak tailing remained a problem, it was concluded that cyclam was functioning as more than a silanophilic agent.

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