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Short Communication

Solution stability study of terfenadine by high-performance liquid chromatography

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Introduction

Terfenadine (**I**, Fig. 1) is a new histamine H_1-receptor antagonist [1–5]. It is free from central nervous system side effects in pharmacological, toxicological and clinical studies [6–9]. During product development, it was necessary to determine its routes of degradation in order to evaluate the specificity of analytical methodology. This report describes a study of the stability of terfenadine in solution as a function of pH, light and oxygen.

Experimental

Materials

Terfenadine and various analogues (**II–VI**, Figs 1 and 4) were synthesised and characterised at the Merrell Dow Research Institute. The acetonitrile, chloroform and water used were of LC grade. Other reagents used were commercial reagent grade.

Sample preparation

Due to the low solubility of terfenadine in aqueous solution, all samples were prepared in 0.05 M aqueous buffer–acetonitrile (50:50, v/v) mixture at a concentration of about 0.6 mg ml^{-1}. The ionic strength of the aqueous buffers was adjusted to 0.13 M by adding a suitable amount of sodium chloride. The pH of the aqueous buffers and the apparent pH (indicated with *) of the aqueous buffer–acetonitrile mixtures used for sample preparation were measured by a Corning Model 130 pH meter (Table 1).

Helium-saturated samples were prepared by sparging the solutions with helium for about 40 min using a fritted glass cylinder dispersion tube prior to transferring into 10 ml ampoules in a glove bag under nitrogen. Each ampoule was corked, removed from the

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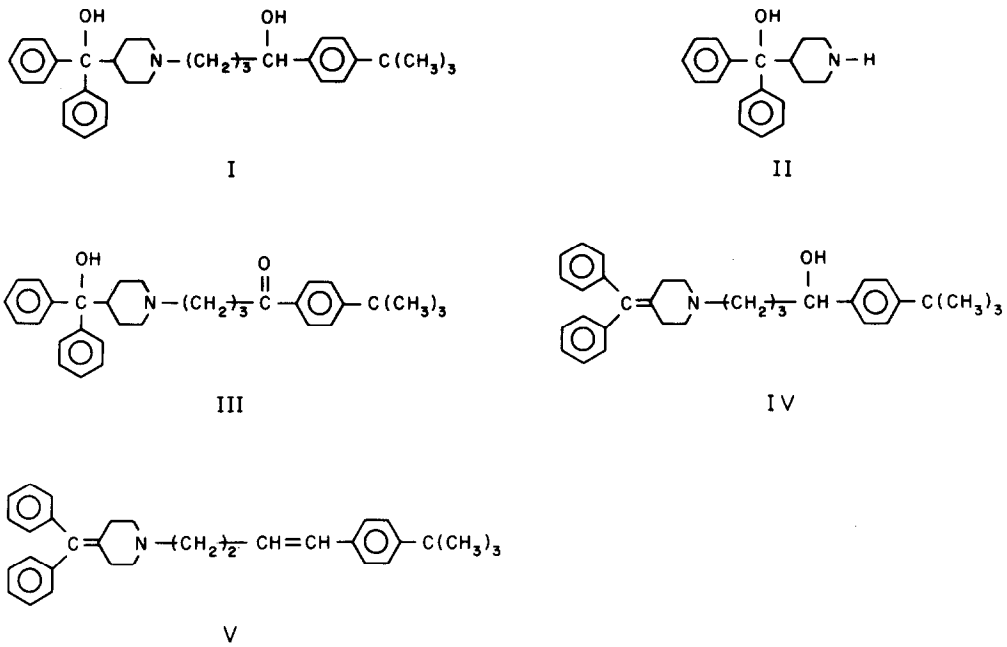


Figure 1
The structure of terfenadine and its analogues.

Table 1
Measured pH values of aqueous buffers and acetonitrile–aqueous buffers (50:50, v/v)

Buffer	Buffer pH	Mixture pH
Hydrochloric acid	1.5	1.5*
Acetate	3.9	5.0*
Phosphate	7.0	7.8*
Tris†	8.5	8.4*
Glycine	9.7	9.8*
Carbonate	10.4	11.4*

*Apparent pH.
†Tris = (hydroxymethyl)aminomethane.

glove bag and immediately sealed with a flame. These samples were then separately stored at 37°C in darkness, 27°C in darkness, and 27°C under a 500 foot-candle fluorescent light.

Oxygen-saturated samples were prepared by sparging the samples with oxygen for about 15 min and immediately sealing with a flame. The samples were then stored at 37°C in darkness.

Chromatographic conditions

The stability of terfenadine was studied by high-performance liquid chromatography using a system consisting of a Varian 5000 liquid chromatograph, a Varian 8055 autosampler, a Valco AH60 loop injector fitted with a 100-μl sample loop, and a Zorbax

C-8 column, 6 μm particle size, 15 cm × 4.6 mm i.d. (DuPont Instruments). The acetonitrile–0.1 M triethylammonium phosphate buffer (pH 7) (70:30, v/v) mobile phase was maintained at a flow rate of 1.5 ml min^{-1}. The column effluent was monitored by a Vari-Chrom UV-VIS detector (260 nm) and a Perkin–Elmer 650-10LC fluorescence detector (EX 260 nm, EM 305 nm) operated in tandem. The fluorescence detector was used to provide different sensitivity and qualitative information to complement the UV detector for the analysis of degradation products. Chromatograms were processed by a laboratory data system (Computer Inquiry Systems, Englewood, CA, USA). The above method was found to be suitable for the separation of terfenadine (**I**, Fig. 1) from the synthetic precursor (**II**), oxidation product (**III**) and dehydration products (**IV** and **V**) (k' = 4.7, 2.9, 6.3, 10.3 and 21.6, respectively). The method also showed linear UV (260 nm) response for terfenadine between 6 and 60 μg injected with a correlation coefficient of 0.99997. The relative standard deviation was 0.3% (N = 5) with a 30 μg injection.

Chromatographic procedure

The ampoules were allowed to cool to room temperature before opening and the contents (5 ml) were mixed well with an equal volume of mobile phase before transferring to septum-sealed vials for assay. External standard solutions of terfenadine were chromatographed every third injection. Averaged peak areas from the bracketing standards were used for the assay calculations.

Identification of degradation product

The fraction of the degradation product isolated by LC was adjusted to pH 12* with 5 M sodium hydroxide. The solution was evaporated almost to dryness (high vacuum rotovap, *ca* 40°C) extracted with 10 ml of chloroform and the chloroform layer was evaporated to dryness.

^{1}H-NMR spectroscopy

^{1}H-NMR spectra were obtained in $CDCl_3$ by means of a Varian FT-80A NMR spectrometer using a 1.7 mm capillary tube for the impurity and a 5 mm tube for the authentic sample of VI. Tetramethylsilane was used as the internal standard.

Mass spectrometry

Mass spectra were obtained by electron impact (solid probe) using a Finnigan Model 1015 mass spectrometer modified with the Model 3300 electronics.

Results and Discussion

Assay results from the samples stored at 37°C in darkness under oxygen as well as an inert atmosphere indicated that very little degradation of terfenadine had occurred over a 25 week period (assays were all 98% or higher). No degradation products were observed for storage periods of 25 weeks except for solutions at pH 1.5* where a degradation product (X) appeared with a retention of 8.1 min (Fig. 2). The retention time of X did not correspond to that of any of the known terfenadine derivatives, including **II**–**V**. The peak at 7.0 min, S, corresponded to the retention time of a biphenyl analog of terfenadine which was formed during synthesis and was observed in the sample before exposure to the storage conditions. Its relative response by either detector was much greater than that of terfenadine.

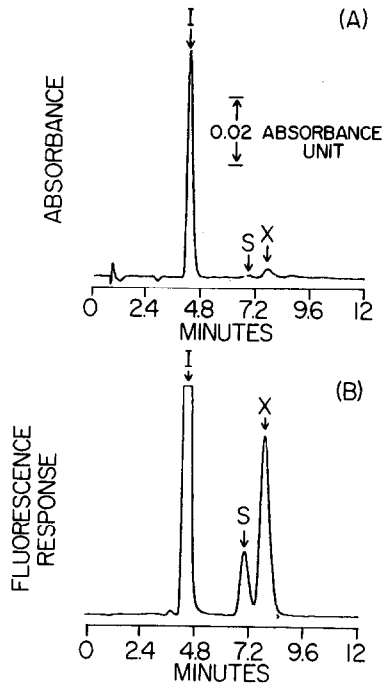


Figure 2
Chromatograms of terfenadine stored in hydrochloric acid–acetonitrile (50:50, v/v) solution under inert atmosphere, pH 1.5*, at 37°C in darkness for 25 weeks. A = UV 260 nm detection; B = fluorescence detection (λ_{ex} = 260 nm; λ_{em} = 305 nm).

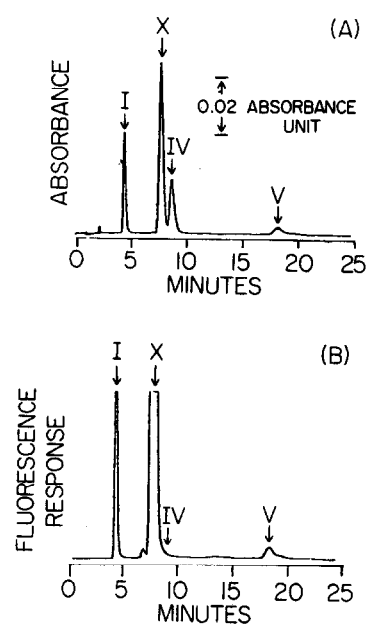
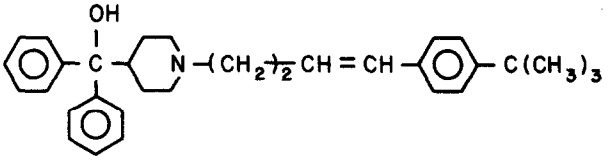


Figure 3
Chromatograms of terfenadine after overnight refluxing in hydrochloric acid–acetonitrile (50:50, v/v) solution, pH 1.5*. A = UV 260 nm detection; B = fluorescence detection (λ_{ex} = 260 nm; λ_{em} = 305 nm).

The amount of X in the pH 1.5* solution was increased greatly by refluxing the solution overnight (Fig. 3). The mass spectrum of X, after isolation from this solution, indicated a molecular ion at *m/z* 453, suggestive of a monodehydration product. Fragments at *m/z* 183 for $(C_6H_5)_2C{=}\overset{+}{O}H$ and at *m/z* 280 for α-cleavage at the nitrogen atom indicated dehydration at the asymmetric carbon. The ^{1}H-NMR spectrum had no signal near 4.6 ppm for the proton on the asymmetric carbon as seen with terfenadine, further indicating dehydration at this centre. In addition, a complex multiplet was observed at *ca* 6.0–6.5 ppm which was almost identical to that which had been observed previously for the bis-dehydration product (**V**). Structure **VI** (Fig. 4) was therefore indicated. Accordingly an authentic sample of this material was synthesized for comparison with X. The two samples gave the same LC retention time and the same family of peaks centered at *m/z* 453 for the molecular ion as well as fragments at *m/z* 434, 376, 320, 280 (base peak), 262, 183, etc. Both samples also gave proton NMR spectra having essentially the same pattern of aromatic (6.9–7.5 ppm) and olefinic (6.0–6.5 ppm) multiplets. Thus the structure of X was confirmed as **VI**.

The overnight refluxing produced not only **VI** (11.3%) but also **IV** (6.2%) and **V** (0.6%). The latter two degradation products were not studied extensively since they were not observed in the stability studies, and were identified on the basis of their retention times. The sensitivity of the detection of **IV** was less by fluorescence than by UV, in contrast to that of **V** and **VI** (Table 2).

Storage at 27°C in darkness under an inert atmosphere gave the same stability results as that at 37°C in darkness. However, samples stored at 27°C under intense fluorescent light began to show degradation after 8 weeks, as shown in Table 3. The degradation rate



VI

Figure 4
Product of the dehydration of terfenadine at the asymmetric carbon.

Table 2
UV and fluorescence detector response factors for compounds **I**, **IV**, **V** and **VI**

Compound	Response factor (area μg^{-1} injected)*	
	UV 260 nm†	Fluorescence (EX 260/EM 305 nm)‡
I	36	2600
IV	500	80
V	1440	74,150
VI	710	180,000

*Area: mV-s.
†Bandwidth = 8 nm; time constant = 1.0 s.
‡Sensitivity range = 10X; photomultiplier gain — low; response — normal; mode — normal.

Table 3
Stability of terfenadine in aqueous buffer solutions at 27°C under fluorescent light

Buffer	Initial concentration (ppm)	Percent terfenadine remaining after storage: Number of weeks 1	2	4	8	16	25
Hydrochloric acid	604	99	99	98	97	87	85
Acetate	605	100	99	100	100	98	98
Phosphate	605	100	99	99	100	96	85
Tris	605	99	99	99	99	95	90
Glycine	602	98	98	98	98	92	58
Carbonate	600	99	99	99	99	94	91

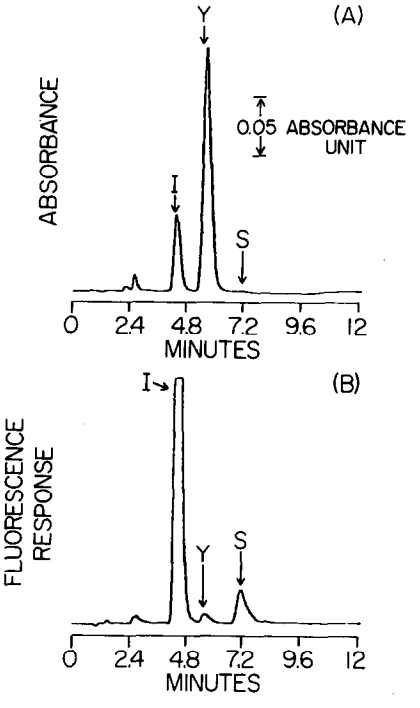


Figure 5
Chromatograms of terfenadine after storage in hydrochloric acid–acetonitrile (50:50, v/v) solution, pH 1.5*, at 27°C under fluorescent light for 25 weeks. A = UV 260 nm detection; B = fluorescence detection (λ_{ex} = 260 nm; λ_{em} = 305 nm).

was found to be independent of pH but highly dependent on buffer species. Thus acetate buffer provides protection from degradation whereas glycine buffer results in greater degradation than seen in the other buffer systems. Figure 5 shows the chromatograms of terfenadine solutions (pH 1.5*) stored for 25 weeks at 27°C under fluorescent light. The major degradation product (Y) was identified as the terfenadine oxidation product **III** on the basis of comparative retention times, UV/fluorescence response ratios, and UV absorbance ratios at 230 and 260 nm. The rate of formation of **III** at pH 1.5* in light is dependent upon the oxygen content of the solution, as shown in Fig. 6. However, the formation of **III** was found to be greatly reduced in the pH 5* solution and completely inhibited in basic solutions.

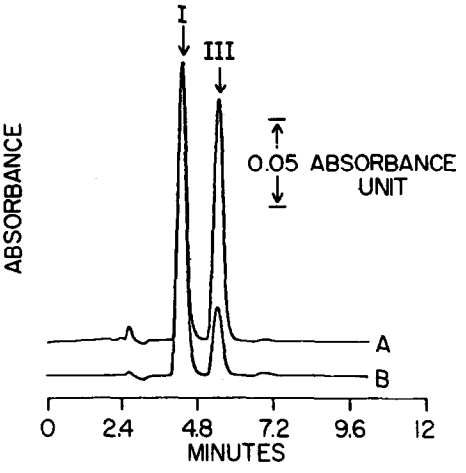


Figure 6
Chromatograms of terfenadine stored in hydrochloric acid–acetonitrile (50:50, v/v) solution, pH 1.5*, at 27°C under fluorescent light for 4 weeks with UV 260 nm detection. A = Oxygen atmosphere; B = inert atmosphere.

Conclusion

Terfenadine has excellent stability in pH 5–11* solutions that are protected from intense light. A low level of degradation is observed at pH 1.5* in darkness after 25 weeks storage. The stability of terfenadine is not affected by oxygen if the solutions are protected from light. Even under intense fluorescent light, terfenadine remains stable for up to 8 weeks storage at 27°C.

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