ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1371–1375

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short communication

Method for the control of known impurities in hydroxyzine hydrochloride[1]

D. Simpson[a,*], G.G. Skellern[a], J.H.McB. Miller[b]

[a]*Department of Pharmaceutical Sciences, University of Strathclyde, Royal College, Glasgow, G1 1XW, UK*
[b]*European Pharmacopoeia Laboratory, Council of Europe, 226 Avenue de Colmar, BP 907, F - 67029 Strasbourg Cedex, France*

Received for review 14 September 1995; revised manuscript received 14 December 1995

Keywords: Hydroxyzine hydrochloride; Related substances; Reversed phase HPLC

1. Introduction

Hydroxyzine hydrochloride or (*RS*)-2-(2{4-[(4-chlorophenyl) phenylmethyl] piperazine-1-yl}-ethoxy) ethanol dihydrochloride (**I**, Fig. 1) is a piperazine derivative which is a rapid acting anxiolytic used principally as an anti-emetic [1].

The main impurities from the synthesis of hydroxyzine hydrochloride are 1-[(4-chlorophenyl)phenylmethyl]-piperazine (**II**) and deschlorohydroxyzine (**III**, Fig. 1).

It has also been shown [2,3] using thin-layer chromatography (TLC) and spectrophotometry that the decomposition products after the exposure of hydroxyzine HCl to ultraviolet light were chlorobenzophenone, 4-chlorobenzaldehyde, 4-chlorobenzoic acid and 1-[(2-(2-hydroxyethoxy)-ethyl)]piperazine dihydrochloride.

Both gas chromatography [4] and high performance liquid chromatography (HPLC) [5] have been used to assay hydroxyzine in formulated preparations.

Although there is a TLC method described in the Belgian Pharmacopoeia [6] to limit related substances, it is known that deschlorohydroxyzine is not separated from hydroxyzine. A recently published monograph of the European Pharmacopoeia [7] describes a normal phase high performance liquid chromatographic method which separates the two known related impurities (**II** and **III**) from each other and from hydroxyzine. These official methods for related substances permit a mixture of 0.3% for each individual impurity and 1.5% for the sum of all impurities. However, this HPLC method suffers from a number of shortcomings which are: insufficient separation of analytes on some silica columns; an increase in the retention time of analytes with continued use caused by deactivation of the silica by the aqueous component of the mobile phase; and possible degradation or dissolution of silica due to the

* Corresponding author.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(95)01752-9

low pH of the eluent (pH 1.1). To overcome such problems, an assay method for the separation of impurities **II** and **III** from hydroxyzine utilising reverse phase HPLC was developed. This study reports on the validation of the method including linearity of response, limits of detection and quantitation, selectivity and ruggedness.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile, sodium methane sulphonate and sulphuric acid were obtained from Merck (Darmstadt, Germany); triethylamine (TEA) was supplied by Fluka (Buchs, Switzerland). Samples of hydroxyzine hydrochloride and impurity reference standards were supplied by UCB (Belgium).

2.2. Composition of mobile phase

The composition of mobile phase was a solution containing 9.8% v/v triethylamine and 0.35% m/v sodium methane sulphonate (pH adjusted to 2.85 with sulphuric acid: acetonitrile (70:30, v/v)).

2.3. Instrumentation and conditions

An HPLC system (Spectra Physics Analytical, Fremont, CA) comprising a P1000 Isocratic pump, a fixed volume loop injector (20 μl) and a CE 1200 High Performance Variable Wavelength Detector (Cecil Instruments, Cambridge, UK) was used. Chromatograms were recorded on a data jet integrator 3390 A (Hewlett Packard). The separation was carried out on 120 mm × 4.0 mm stainless-steel columns containing different octadecylsilyl silicas (ODSs) (Lichrosorb RP-18, Hypersil ODS (5 μm) and Nucleosil 100-5 C18 (5 μm)). The mobile phase, which was monitored at 230 nm, was delivered at a flow rate of 1 ml min^{-1}, resulting in a typical operating pressure of approximately 80 psi at ambient temperature.

A DU-6 spectrophotometer (Beckman Instruments, USA) using 1 cm quartz cells was employed for absorbance measurements.

2.4. Preparation of solutions for validation

2.4.1. Response factor

Hydroxyzine hydrochloride (5.0 mg) was dissolved in 100.0 ml of the mobile phase to give a concentration of 0.05 mg ml^{-1} (0.005% w/v). A portion (5.0 ml) of this solution was then further diluted to 100.0 ml with mobile phase. The same

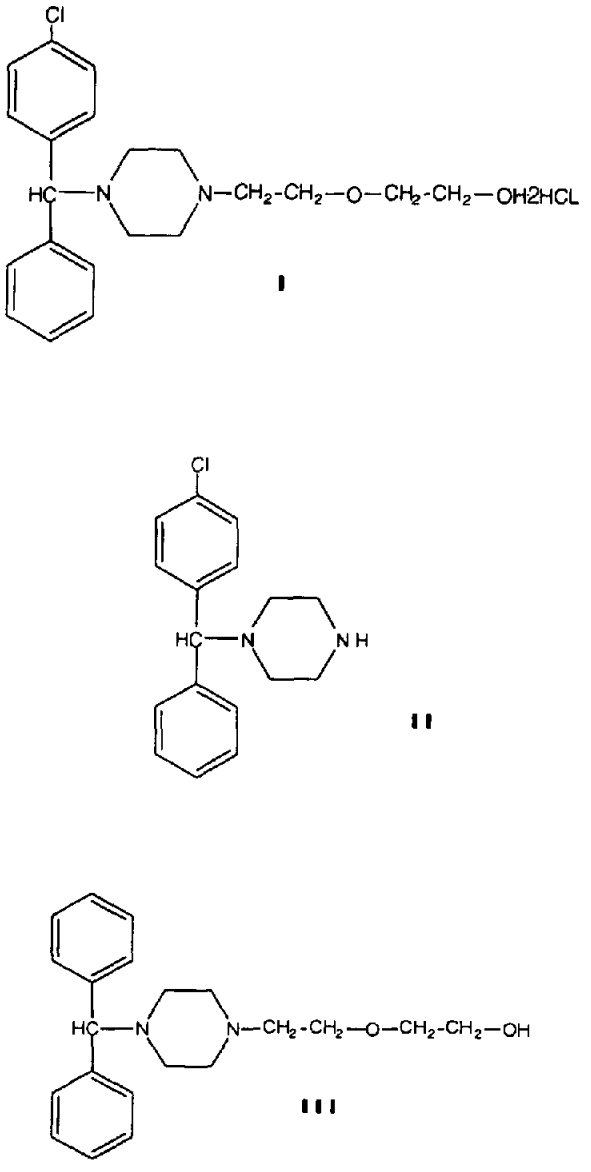

Fig. 1. Structural formulae of hydroxyzine hydrochloride and its impurities.

procedure was followed for the preparation of solutions (0.005% w/v) of the impurities **II** and **III**.

The absorbances of the impurities were measured at 230 nm using the DU-6 spectrophotometer and the response factors of the impurities were calculated relative to the absorbance of hydroxyzine.

2.4.2. Resolution

Hydroxyzine hydrochloride (10.0 mg) and impurity **II** (10 mg) were dissolved in 10.0 ml of the mobile phase. This solution was diluted to produce a final concentration for each of 3 μg ml^{-1}; aliquots (20 μl) of it were injected on to the different ODS columns. Resolution was calculated according to the method described in the European Pharmacopoeia [7].

2.4.3. Limits of detection and quantitation (LOD and LOQ)

The base-line noise of the system was determined. Sequential dilutions of the solution of impurity **II** (0.005% w/v) were prepared and injected on to the column to determine the LOD (peak height three times that of the base-line noise) and LOQ (peak height 10 times that of the base-line noise).

2.4.4. Linearity

Hydroxyzine hydrochloride (10.0 mg) was diluted to 100.0 ml with the mobile phase to give a concentration of 0.001 mg ml^{-1} (stock solution). Stock solutions of impurities **II** and **III** were prepared in the same manner.

Calibration standard solutions of 1.0, 2.0, 3.0, 4.0 and 5.0 μg ml^{-1} (33%–166% of the limiting concentration 0.3%) were prepared by pipetting 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the three stock solutions into 50.0 ml volumetric flasks and diluting to volume with the mobile phase.

20 μl of each calibration standard was injected on to the column. Peak areas were recorded and plotted against concentrations for each analyte.

2.4.5. Repeatability

A solution containing hydroxyzine hydrochloride (1.0 mg ml^{-1}) and impurities **II** and **III** at their limiting concentrations (3 μg ml^{-1}) was prepared. Another solution was also prepared of hydroxyzine hydrochloride at the limiting concentration (3 μg ml^{-1}).

A series of ten replicate injections of these solutions was made for the determination of the relative standard deviations for the individual analytes.

2.4.6. Impurity profile of various batches of hydroxyzine hydrochloride

A solution was prepared by transferring 10.0 mg of the test substance into a 10.0 ml volumetric flask and dissolving it in the mobile phase.

A reference solution (external standard) was prepared by diluting 3.0 ml of this solution to 200.0 ml with mobile phase. Then 5.0 ml of this solution was diluted to 25.0 ml with the mobile phase (3 μg ml^{-1}).

3. Results and discussion

3.1. Method optimisation

Initially, mixtures of acetonitrile and water were investigated for their suitability in separating hydroxyzine hydrochloride from its impurities on an ODS column. Although a separation was achieved, peak tailing occurred which was considered to be unsatisfactory. This phenomenon was attributed to the free silanol groups of the stationary phases employed and the basic nature of the analytes. However, by including the amine (triethylamine) in the mobile phase there was an improvement in peak shape.

Subsequently the system was optimised for separation of the impurities **II** and **III** from hydroxyzine by studying the effects of pH and addition of anionic counter ions. As well as effecting the retardation of analytes the presence of anionic counter ions also improved peak shape.

A mobile phase of triethylamine:acetonitrile:water (14:30:386, v/v/v) containing sodium methane sulphonate (0.5 g) with the pH of the aqueous phase adjusted to 2.85 with sulphuric acid was considered to be satisfactory for the

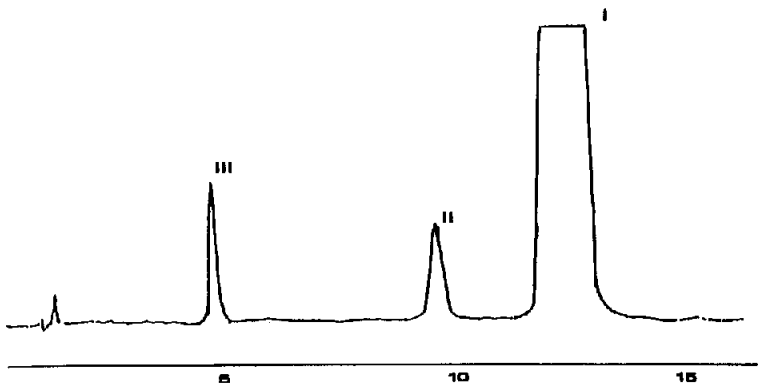

Fig. 2. HPLC chromatogram of a solution of hydroxyzine hydrochloride (I) and impurities II and III. Retention times (min): I = 12.5, II = 10.3, III = 4.84. Mobile phase: solution of 9.8% v/v triethylamine and 0.35% m/v sodium methane sulphonate (pH adjusted to 2.85): acetonitrile (70:30, v/v).

separation of the known synthetic impurities of hydroxyzine from hydroxyzine.

A typical chromatogram (Fig. 2) for the separation of impurities **III** ($t_R = 4.7$ min), **II** ($t_R = 9.7$ min) and hydroxyzine hydrochloride, **I** ($t_R = 12.3$ min) shows base-line separation. The resolution factor determined using the method in the European Pharmacopoeia [7] between **II** and hydroxyzine (**I**) was 3.0.

The method was then validated using the Linchrosorb RP-18 column.

3.2. Method validation

The response factors at 230 nm for 1-[(4-chlorophenyl) phenylmethyl]-piperazine (**II**) and deschlorohydroxyzine (**III**) relative to hydroxyzine were 0.6 and 1.0 respectively.

Impurity **II** was selected to determine the limits of detection and quantitation (0.18 μg ml^{-1} and 0.6 μg ml^{-1} respectively) because it eluted between hydroxyzine and **III** and had a lower response factor than **III**.

Little variation was observed (Table 1) between retention times with a maximum relative standard deviation of 2.3% for replicate injections ($n = 10$) for a mixture of **I, II** and **III**. Peak area repeatabilities for the three analytes were less than 5%, with impurities **II** and **III** giving RSD values of $\pm 3.6\%$ and $\pm 3.4\%$ respectively at the limiting concentration of 0.3% w/v.

The linearity of response for each impurity and for hydroxyzine was evaluated. Detector response (peak area) versus concentration of analyte was shown to be rectilinear from 1.00 to 5.5 μg ml^{-1} which corresponds to the range 33–166% of the impurity limit (0.3%) and the correlation coefficients were 0.998, 0.997 and 0.979 for **I, II** and **III** respectively.

Although the majority of the validation work was performed with the Lichrosorb RP 18 column, two other octadecylsilyl columns were also tested to evaluate the ruggedness and selectivity of the LC method. All three columns were efficient for separating hydroxyzine from the impurities **II** and **III**. Based on these observations it was considered that a minimum resolution of 2.0 is required for satisfactory chromatography.

In the several batches of hydroxyzine hydrochloride, which were examined using the chromatographic system described, no impurities were detected.

4. Conclusion

The separation of impurities **II** and **III** from hydroxyzine hydrochloride can be performed by a rapid and selective, reversed phase liquid chromatographic method. Deschlorohydroxyzine and 1-[(4-chlorophenyl)phenylmethyl]-piperazine have

Table 1
Comparison of three different reversed-phased columns for their ability to separate all three components

Column	Retention time (min)			Resolution factor between hydroxyzine and II
	Impurity III	Impurity II	Hydroxyzine	
Lichrosorb RP-18	4.7	9.8	12.3	3.0
Hypersil ODS	3.9	8.4	10.4	2.6
Nucleosil 100-5C18	4.0	7.7	9.9	2.4

relative retention times (to hydroxyzine) of 0.38 and 0.79 respectively.

The method was linear over the range corresponding to 33–166% of the limiting concentration of impurities (3.0 $\mu g\ ml^{-1}$). The limits of detection and quantitation were shown to be 0.18 $\mu g\ ml^{-1}$ and 0.6 $\mu g\ ml^{-1}$ respectively.

The method described is considered by the authors to be an improvement on the HPLC method described in the European Pharmacopoeia. This new proposed method has been demonstrated to be suitable for the control of impurities in hydroxyzine hydrochloride.

References

[1] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, 30th edn., 1993, pp. 1313–1314.
[2] J. Pawlaczyk, Ann. Pharm. (Poznan), 7 (1969) 127–128 (Chem. Abstr. No. 72: 103676r, 1970).
[3] J. Pawlaczyk, Poznan. Tow. Przyj. Nauk, Wyd Lek. Pr. Kom. Farm., 5 (1966) 117–120 (Chem. Abstr. No. 67: 5679w, 1967).
[4] C. Cardini, V. Quercia and A. Cavo, J. Chromatogr., 37 (1968) 190–193.
[5] S.E. Roberts and M.F. Delaney, J. Chromatogr., 242 (1981) 364–368.
[6] Belgian Pharmacopoeia, 1984.
[7] European Pharmacopoeia, 2nd edn., Maisonneuve, St. Ruffine, 1986.