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Journal of Pharmaceutical and Biomedical Analysis 33 (2003) 33–38
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Assay method for quality control and stability studies of a new CVS disorder agent (compound 93/478)☆

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Received 11 June 2002; accepted 15 January 2003

Abstract

1-[4-(4-Fluorophenyl)-piperazine-1-yl]-3-(2-oxopyrrolidin-1-yl)-propane hydrochloride, (**I**), (CDRI code No. 93/478) is a new potent anti-ischemic and anti-hypertensive agent, being developed at the Central Drug Research Institute (CDRI), Lucknow, India. A sensitive high performance liquid chromatographic assay method has been developed and validated for in process quality control and for stability studies. HPLC separation was achieved on a C_{18} Purospher (Merck) column using a gradient of 0.02% tetra-methyl ammonium hydroxide (pH 7.5) and acetonitrile as mobile phase. The eluents were monitored by diode array detector at 240 and 290 nm. The lower limit of detection of **I** was 0.62 μg/ml, while the lower limit of quantitation was set to be 1.5 μg/ml. The calibration curves were linear in the range 1.5–62 μg/ml. Reproducibility of the method was determined by inter and intra assay variation, which were $<10\%$.

Keywords: 1-[4-(4-Fluorophenyl)-piperazine-1-yl]-3-(2-oxopyrrolidin-1-yl)-propane hydrochloride; HPLC; Anti-ischemic; Anti-hypertensive and in process quality control

1. Introduction

Among the 1-[4-piperazine-1-yl]-3-[2-oxo-pyrolidin-1-yl] propanes designed and synthesized for treatment of cardiovascular system (CVS) disorders, including hypertension, ischemia and dyslipidemia, mediated through α-adrenergic receptors, particularly α_{1a}, the compound 1-[4-(4-fluorophenyl)-piperazine-1-yl]-3-(2-oxopyrrolidin-1-yl)-propane hydrochloride (CDRI compound No. 93/478) (**I**, Fig. 1) has shown a good profile for development as a drug. This compound has now been patented [1–3] and taken up for further development at our organization. The present study was undertaken to develop a sensitive and reproducible high performance liquid chromatography assay method, for this compound, for in process quality control and stability studies.

☆ CDRI Communication No. 6154.

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0731-7085/03/$ - see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/S0731-7085(03)00350-9

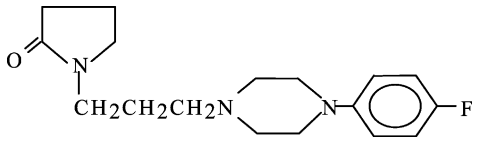

Fig. 1. Structure of CDRI compound No. 93/478 **(I)**.

2. Experimental

2.1. Reagents and standards

Standard compound 93/478 (**I**), starting materials and intermediates were supplied by the Medicinal Chemistry Division of this Institute. HPLC grade acetonitrile, methanol, tetra-methyl ammonium hydroxide (analytical grade) was obtained from E. Merck (India) Ltd. (Mumbai, India). Triply distilled water, from an all glass apparatus, was used in analysis. Other reagents used were of analytical grade.

2.2. Apparatus and chromatographic conditions

The HPLC system consisted of a binary gradient pump (model 250, Perkin-Elmer), a Rheodyne model 7125 injector with a 20 μl loop and diode array detector (model 235, Perkin-Elmer). HPLC separation was achieved on a C_{18} Purospher (Merck) column (250×4 mm I.D., 5 μm particle size). Column effluent was monitored at 240 and 290 nm. Data was acquired and processed using an IndTech HPLC interface and software. The mobile phase was a gradient of 0.02% tetra-methyl ammonium hydroxide (adjusted to pH 7.6 with orthophosphoric acid) and acetonitrile. The gradient was 10–90% acetonitrile in 20 min followed by 90% acetonitrile for 10 min. Both the solutions were filtered and degassed before use. Chromatography was performed at 27 ± 3 °C at a flow rate of 1.5 ml/min.

2.3. Preparation of stock and working standard solutions

Stock standard solutions containing 200 μg/ml of **I** as hydrochloride were freshly prepared in water. Working standard solutions were prepared in water in the range of 0.62–62 μg/ml by serial dilution. Quantitation was based on a six-point calibration curve.

2.4. Preparation of sample solution

Samples (5 mg) from various batches of the compound were dissolved in 25 ml of water. The solution was filtered and 1 ml further diluted to 10 ml with water to obtain a sample solution of ≈ 20 μg/ml.

2.5. Accuracy and precision

Known amounts of standard **I** were added to weighed amounts of bulk preparation samples. The content of **I** in the above mixture was determined by interpolation on the corresponding calibration graphs. The accuracy of the method was calculated on the basis of the difference between the mean calculated and expected concentrations. Precision was obtained by calculating the inter-day relative standard deviations (R.S.D.s) [5–8].

2.6. Effect of pH

Acid-base degradation studies were carried out to obtain the pH for maximum stability. This information is required for the preparation of the final formulations. Effect of pH on this compound was checked by the following method. Some 1 ml each of stock solution of **I** was taken in 10 ml volumetric flasks and the volumes were made up with buffers of pH 3–9. Samples were withdrawn at different time intervals and 20 μl were injected on to the HPLC column to analyze as described above. The reaction rate constants were calculated by LINREG program [4].

3. Results and discussion

3.1. Chromatography

Initial experiments with columns, such as C_{18} endcapped (250 mm, 4 mm, 5 μm, E. Merck) or CN (250 mm, 4 mm, 5 μm, E. Merck), using

several solvent systems including buffers were tried. The C_{18} endcapped (250 mm, 4 mm, 5 μm, E. Merck), column with 0.05 M phosphate buffer (pH 3):acetonitrile gave a single peak in the chromatogram (Fig. 2). The UV spectrum of this peak was, however, found to differ at peak onset, maximum and decline (Fig. 3). The peak purity index also indicated that peak was impure. HPLC of individual starting materials (1-(3-bromopropyl)-4-(4-fluorophenyl)-piperazine, 1-(3-chloropropyl)-2-pyrrolidone and 2-pyrrolidone) under the same conditions did not show any overlap with the elution of **I** (data not shown). When the NMR spectrum of the sample was analysed by a protocol of deletion of peaks corresponding to **I** and stating materials, it was found that the peaks remaining in the processed spectrum corresponded to 1-(3-hydroxy-propyl)-4-(4-fluoro-phenyl)-piperazine, a side product formed during preparation of **I**.

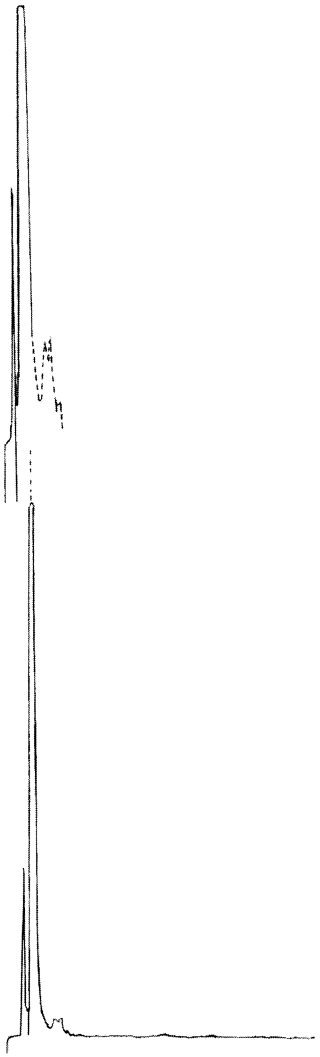

Fig. 2. Peak of the compound **I** in 0.05 M phosphate buffer (pH 3): acetonitrile.

The use of a C_{18} Purospher column with a gradient of acetonitrile in water containing 0.02% TMAH (pH 7.6) resulted in good separation of **I** from contaminants arising from starting material as well as possible degradation products (Fig. 4). Base line separation of **I** and the process impurity 1-(3-hydroxy-propyl)-4-(4-fluoro-phenyl)-piperazine was obtained (Fig. 5). Under the chromatographic conditions, other constituents did not interfere, since they eluted either before or after the peak of interest.

3.2. Selectivity and specificity

The retention times of **I,** 1-(3-hydroxy-propyl)-4-(4-fluoro-phenyl)-piperazine, 1-(3-bromo-propyl)-4-(4-fluoro-phenyl)-piperazine, 1-(3-chloropropyl)-2-pyrrolidone and 2-pyrrolidone were ≈ 13, 12, 18.5, 9 and 2.5 min, respectively. It would be recalled that 1-(3-bromopropyl)-4-(4-fluorophenyl)-piperazine, 1-(3-chloropropyl)-2-pyrrolidone and 2-pyrrolidone are the starting materials, while 1-(3-hydroxypropyl)-4-(4-fluorophenyl)-piperazine is the process impurity in the preparation of the final compound. None of the above interfered with the peak corresponding to **I** [6–8].

3.3. Linearity and reproducibility

External standardization by peak area was used for the quantitative determination of **I**. Based on a signal to noise ratio of 3, the detection limit was 0.62 μg/ml. The limits of quantitation were derived from extremes of concentration in which the interpolating polynomial was of the first order, i.e. linear. Thus, a straight line with a regression coefficient of 0.999 could be fit to data in the range

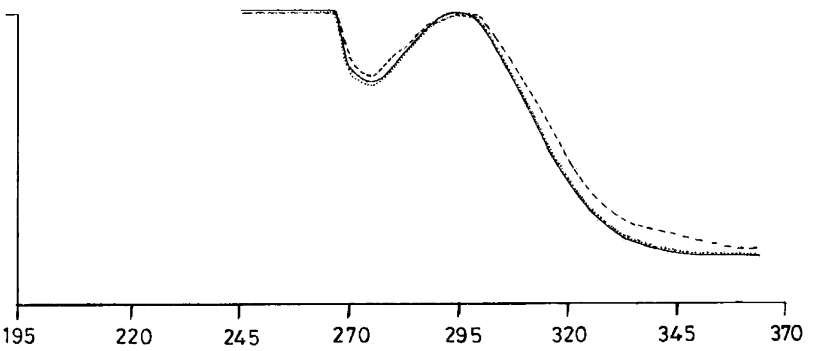

Fig. 3. UV spectra of the peak at peak onset, maximum and decline.

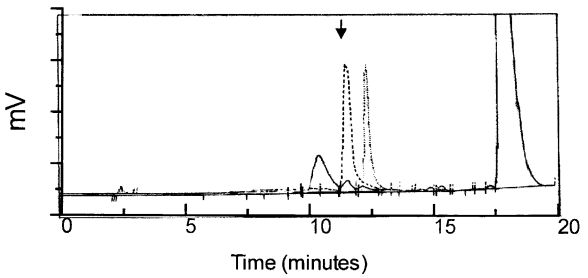

Fig. 4. The HPLC separation of **I** from the other impurities.

1.5–62 μg/ml (Fig. 6). Below 1.5 or above 62 μg/ml, significant deviation from linearity was observed. A typical standard curve ($r = 0.99966$) could be described by the equation

$$\text{Unknown conc. } (\mu g/ml) = [(4.8012 \times 10^{-7}) * \text{peak area}] - 0.09215.$$

The method showed adequate sensitivity for the determination of **I** in bulk preparations and checking pH stability of the compound.

The reproducibility and accuracy of the method were determined by intra and inter assay variation (Table 1). This data also illustrates that our choice of the lower limit of quantitation (1.56 μg/ml) is correct. At a concentration level of 0.62 μg/ml, the standard curve back-calculated a value showing $>29\%$ deviation from the actual (DFA), indicating that such low concentrations cannot be accurately determined by the standard curve.

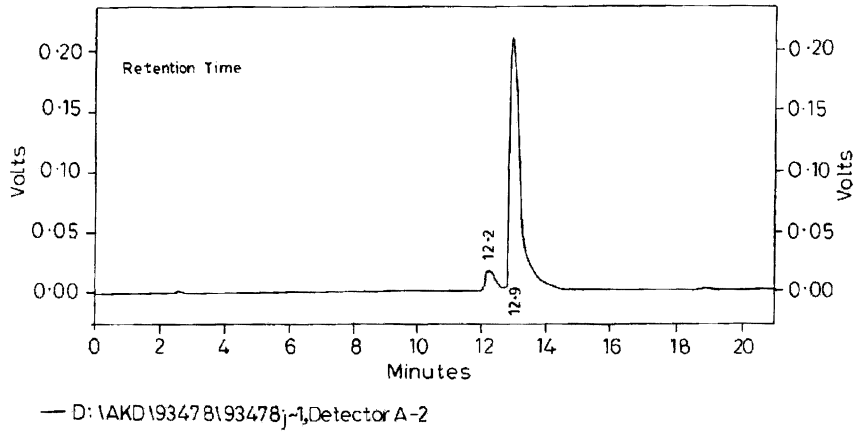

Fig. 5. Separation of **I** and 1-(3-hydroxy-propyl)-4-(4-fluoro-phenyl)-piperazine.

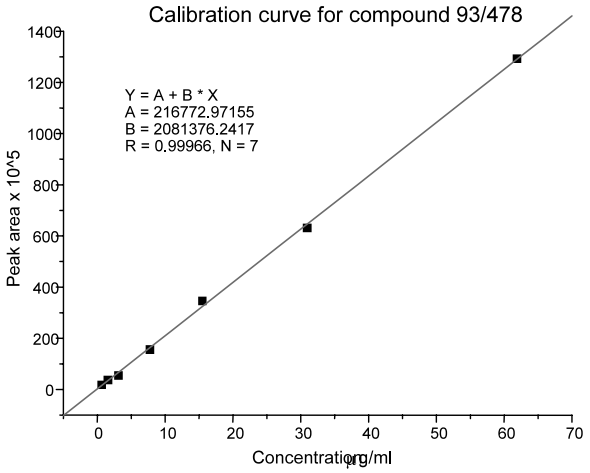

Fig. 6. Calibration graph of compound 93/478.

The % C.V. and % DFA were calculated on the basis of replicate injections of the calibration standards on the same day (intra day) and on different days (inter assay) and to check the variation in the analysis. All these were found to be within acceptable limits [5–8]. As is commonly observed, the % C.V. was always higher at lower concentrations.

3.4. Application of method in pharmaceutical analysis

Several samples of bulk preparation, required for pharmacological and toxicological studies were analyzed by the reported method. The method developed is simple and is being routinely used for quality control of **I** in bulk preparations, preformulation studies and for ongoing stability studies under stress conditions.

3.5. Effect of pH

The results of stability studies at different pH (at 30 ± 2 °C) indicated that the compound was most stable at about pH 4. A first-order degradation profile was observed. The reaction rate constants and half lives were calculated by LINREG program and are reported in Table 2. Therefore, any liquid formulation of this compound should be made around its most stable pH and this should be

Table 2
First order degradation parameters of the compound No. 93/478 at different pH

pH	Rate constant*	Half life (h)*
3	0.061	10.43
4	0.030	21.41
5	0.038	17.00
6	0.068	09.40
7	0.086	07.46
8	0.042	15.24
9	0.083	07.73

n, No. of experiments; rate constant, first order degradation rate constant of the compound 93/478; half life, first order degradation half life of the compound 93/478. pH of 1% solution of the compound in triple distilled water is ≈ 4.3.

* $n = 3$.

Table 1
Inter and intra assay variations

Conc. taken (µg/ml)	Intra assay variations			Inter assay variation		
	Conc. found* (µg/ml) mean ± S.D.	% C.V.	% D.F.A.	Conc. found* (µg/ml) mean ± S.D.	% C.V.	% D.F.A.
0.62	0.80 ± 0.011	1.37	29.03	0.78 ± 0.014	1.79	25.81
1.56	1.62 ± 0.016	0.988	3.85	1.53 ± 0.013	0.85	−1.92
3.12	3.16 ± 0.11	3.48	1.28	3.17 ± 0.15	4.73	1.60
7.8	8.10 ± 0.31	3.83	3.85	7.96 ± 0.10	1.26	2.05
15.6	15.37 ± 0.61	3.97	−1.47	15.72 ± 0.58	3.69	0.77
31.2	32.23 ± 0.79	2.45	3.30	31.46 ± 0.30	0.95	0.83
62.4	63.36 ± 1.24	1.96	1.54	62.52 ± 0.06	0.10	0.19

* $n = 3$.

kept in mind during designing of other drug delivery systems for this compound.

Acknowledgements

The authors thank CDRI for a research fellowship to D.S., Dr Nitya Nand for his encouragement and M. Chaudhry for technical assistance. Amit Misra's help in manuscript revision is also acknowledged.

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