
An Improved Method for Separation of Diltiazem and Related Substances by HPLC

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A simple, sensitive method has been developed for separation and estimation of cis-isomers (Des Acetyl Diltiazem and Des Acetyl Diltiazem-5H) present in Diltiazem HCl as related compounds. The present work involves the separation of these isomers from Diltiazem HCl which are normally accompanied with the bulk during the synthesis. The composition of the solvent system and the flow rate are optimized using Hypersil-ODS, 5 micron reverse phase column to achieve conditions for better and effective separation. The method is found to be rapid and reliable for routine quality control analysis of bulk and dosage form preparation of diltiazem. The results of the recovery experiments are also incorporated as a part of process validation. When compared with the USP method, the proposed method is found to be more sensitive and provides a better peak shape for each component of the chromatogram.

DILTIAZEM Hydrochloride is a calcium channel blocker (1). The synthetic process of Diltiazem HCl is normally associated with two related compounds which are less bioactive than the Diltiazem molecule (1). These related compounds are known as Desacetyl diltiazem - 5H and Desacetyl Diltiazem (dimethylamino derivative) and normally are quantitated (Fig.1) (2-7).

Earlier researchers reported the method of Diltiazem metabolites determination in human plasma or urine samples, using gas chromatography and HPLC method. (2-8)*.

EXPERIMENTAL

(a) **Chromatography conditions** :- The HPLC system consisted of a Hewlett-Packard 1050 model (USA), and a diodearray detector operated at 237 nm. The detector output was quantified by the integrator HP 3396A. Chromatographic experiments

*Preliminary results of this HPLC method, were communicated to 41st Indian Pharmaceutical Congress held at Bombay, 1989 (Ref. B-7 in Pharmaceutical analysis section prior to the publication of USP method).

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were carried out on Hypersil ODS, 5 micron column having dimensions (200 mm x 4.6 mm) (column source-Hewlett-Packard, Germany).

The mobile phase, prepared freshly, consisted of a mixture of (55:45) of acetonitrile (E. Merck, India) and 0.01 M ammonium phosphate (S.D. Fine Chemicals, India) buffer containing 0.06% triethylamine (S.D. Fine Chemicals, India) with pH adjusted to 3.75. All separations were performed isocratically at a flow rate of 1.0 ml/min at ambient temperature with a pressure of 1200 psi.

(b) **Reagents and Materials**:- Diltiazem Hydrochloride, desacetyl Diltiazem and Desacetyl Diltiazem - 5H were supplied by M/s Profarmaco, Italy and Central Drugs Laboratory, Calcutta, India. The identity of Diltiazem and the two related substances have been confirmed at our end by ¹H NMR (JEOL, FX-100, FT-NMR, Japan). Acetonitrile used was a HPLC grade (E. Merck, India). All other chemicals were analytical grade. All aqueous solutions were prepared using double distilled water.

(c) Standard solutions:- A stock solution of 1.0 mg/ml Diltiazem Hydrochloride was prepared with mobile phase. Concentration of 20 µg/ml of solution was made from the stock solution by suitable dilution with mobile phase.

(d) Preparation of sample:- The sample solutions and the solutions of related compounds were prepared exactly in the same manner as above method. 20 µl (loop capacity) of each sample, standard and related compounds were injected into the column for HPLC separation.

Extraction procedure from dosage form preparation (Tablets)

Twenty tablets (of 30 mg each) equivalent to 600 mg of Diltiazem were weighed accurately and finely powdered and transferred into a 500 ml volumetric flask. A stock solution (1 mg/ml) was made with the mobile phase after sonication for a period of 1 h. and cooled to room temperature. From the stock solution, 20 µg/ml solution was prepared with suitable dilution and used for HPLC separation.

Recovery experiment

To determine the accuracy, reproducibility and validity of the above method, recovery experiments were carried out. A fixed amount of the pre-analysed sample was taken and standard drug was added to it at 3 different concentrations (50%, 100% and 150%). Each of the concentration was repeated five times.

RESULTS AND DISCUSSION

Chromatography conditions were optimised for both bulk and dosage forms of Diltiazem to achieve excellent and rapid separation of Diltiazem and its related substances under isocratic condition. Since Diltiazem and the cis-isomers (as related compounds) are weak basic amines, we have monitored rigidly 3 specific parameter, viz. (a) ionic strength, (b) organic co-solvent (acetonitrile) and (c) the pH

of the mobile phase during separation. The use of high resolution columns (Hypersil-ODS) was mandatory to achieve better separation efficiency. The inclusion of short chain tertiary amine modifier (Triethyl amine) in mobile phase improved the peak symmetry and also reduced broadening due to interaction with amine groups of Diltiazem and other related compounds with the stationary phase. Concentration of triethylamine (TEA) was also optimized as TEA gives rise to loss of resolution and inactivation of silica matrix of the column at higher concentration. The inclusion of 0.06% TEA in the mobile phase consisting of acetonitrile and ammonium phosphate buffer resulted in an efficient separation of all compounds with reproducible peaks within 12 min. Under identical conditions both bulk and dosage form preparation of diltiazem gave reproducible results. The optimum conditions of separation of Diltiazem and its isomers as per the present method have been illustrated **Fig.2**.

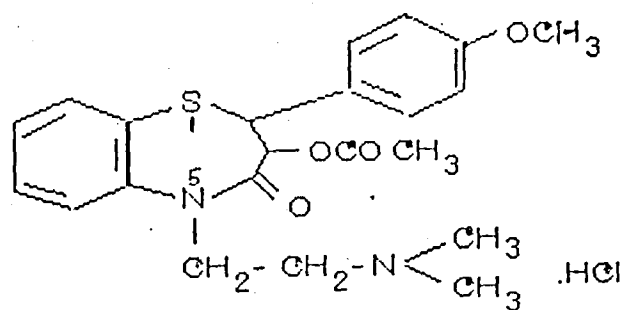
The advantages of the present method over the USP method are as follows:

- (1) total time required for separation of all peaks in the present method is less than half, as compared to USP method. (**Fig.3**).
- (2) reagents used are inexpensive and easily available.
- (3) The flow rate (1.0 ml/min) is lower as compared to (1.6 ml/min) USP method (9).
- (4) The peaks are more symmetrical.
- (5) Sensitivity is found to be significantly higher as analysis of Diltiazem was carried out in the present method was at a lower concentration than the USP method.

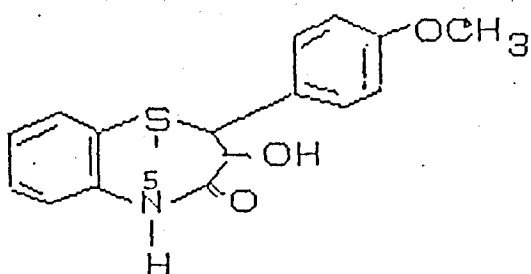
Standard chromatographic parameters have been calculated from the HPLC chromatogram taken using the same column and the same instrument

for the system suitability checks and are mentioned in Table-1.

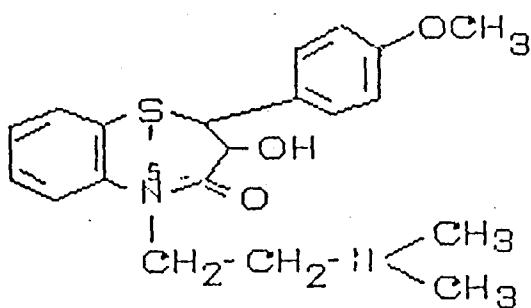
Recovery experiments were carried out at three different concentration levels and the results of recovery of 50% standard addition, 100% standard addition and 150% standard addition were found to be 95.62, 98.99 and 101.48% respectively.



DILTIAZEM HYDROCHLORIDE



DESACETYL DILTIAZEM-5H



DESACETYL DILTIAZEM
(DIMETHYLAMINO DERIVATIVE)

Fig. 1. Structure of Diltiazem Hydrochloride and its isomers.

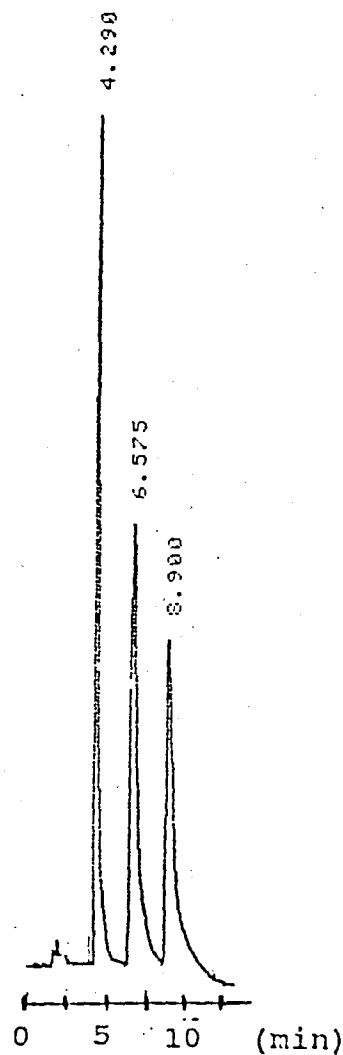


Fig. 2. Optimum conditions of separation of Diltiazem and its isomers by the present method. Chromatograms of
a) Desacetyl Diltiazem-5H-4.29 min.
b) Desacetyl diltiazem (Dimethyl amino derivative) 6.57 min.
c) Diltiazem Hydrochloride - 8.9 min.

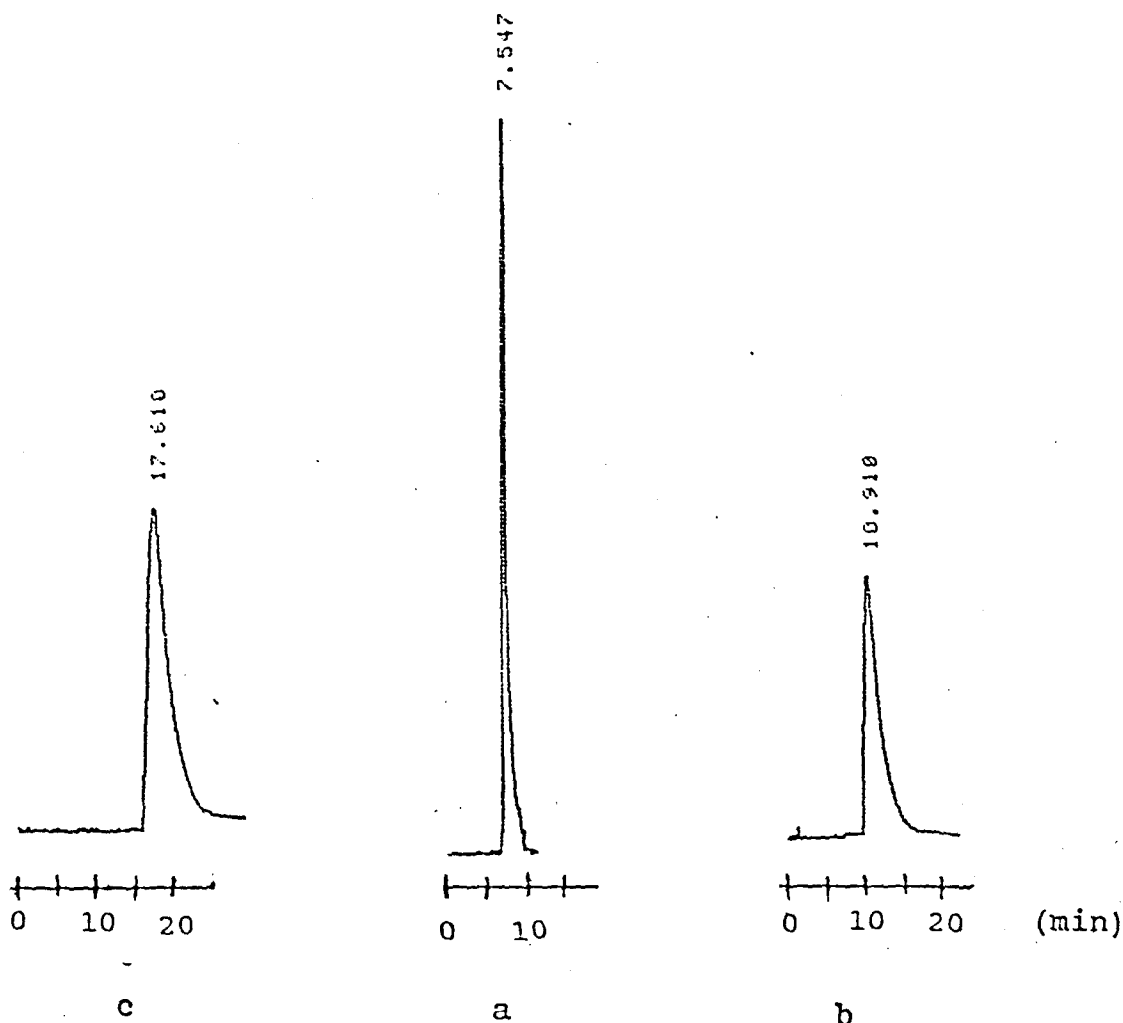


Fig. 3. Chromatograms of (a) Desacetyl Diltiazem - 5H
(b) Dimethylamino derivative and
(c) Diltiazem Hydrochloride by USP method.

The present method has low tailing factor, shorter retention time and low relative standard deviation indicating its suitability for routine analysis.

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Table 1: System suitability tests (SST) on DILTIAZEM HYDROCHLORIDE

	No. of Theoretical Plates(n)	Tailing Factor	Separation Factor (R)	Relative Retention Time(min)	Relative Std. Deviation (% RSD)
Proposed Method	628	1.125	4.323	0.730	1.100
USP Method	1213	1.625	4.999	0.619	2.140

(The SST parameters were checked six times.)

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