Research article Rapports De Pharmacie Vol.2 (2), 2016, 265-267
ISSN: 2455-0507

SIMPLE HPLC METHOD DEVELOPMENT FOR DILTIAZEM: APPLICATION TO BIOAVAILABILITY STUDIES

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ABSTRACT
Simple and effective high performance liquid chromatographic (HPLC) method was developed for estimation of diltiazem in drug free animal drug free blank plasma. The current method was used protein precipitating extraction of diltiazem from blank plasma. Separation was achieved on reversed-phase c_{18} column (250 mm × 4.6 mm, 5µ) and the detection was monitored by UV detector at 235 nm. The optimized mobile phase was used acetonitrile: 10mM potassium dihydrogen orthophosphate (ph 3.5), in the ratio of 70:30 % v/v at a flow rate of 1.0 ml/min. This linearity was achieved in this method range of 10.0 – 125.0 mcg/ml with regression coefficient range is 0.99. The present method is suitable in terms of precise, accurate and specific during the study. The present method was successfully applied for bioavailability studies studies.
Keywords: Simple Method; HPLC; diltiazem; Animal plasma; Bioanalytical study

INTRODUCTION
Diltiazem Hydrochloride (DTZ), 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1, 5-benzothiazepin-4(5H)-one monohydrochloride – a calcium channel blocker which inhibits influx of calcium (Ca^{2+}) ions – is used for treatment of several cardiovascular disorders [2], viz. essential hypertension [3] and supraventricular tachyarythmias [4]. Numerous analytical methods for the determination of DTZ in bulk drug as well as in formulations have been reported in literature viz. spectrophotometry [5] and [6], gas chromatography [7], HPTLC [8], HPLC [9], [10], [11], [12] and [13]. Recently, HPLC–MS and CE methods have been reported to characterize the DTZ metabolites [14], [15], [16] and [17]. A RP-HPLC method using monolithic silica support for separation of DTZ and its impurities has been published [18]. Two validated stability indicating HPLC methods have also been reported for DTZ in bulk drug [19] and in tablets [20-21]. These stability indicating analytical methods are validated for assay of DTZ, and not for analyzing the drug in the presence of its known impurities. To the best of our knowledge no reports were found for the validation of diltiazem in drug free animal plasma. The objective of this study was to develop and validate an assay for the estimation of diltiazem using HPLC.

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EXPERIMENTAL
Materials and reagents:
diltiazem was purchased from SunPharma India. Acetonitrile was purchased from Merck.
Equipment:
HPLC chromatographic separation was achieved on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). Princeton SPHER C_{18} (250mm x 4.6 mm i.d., 5µ) was used for the present analysis.
Preparation of the calibration standards and quality control (QC) samples:
The stock solution of diltiazem was prepared using water and acetonitrile mixture 1:1 at a concentration of 1.0 mg/mL each. diltiazem working solution was used to prepare the spiking stock solutions for construction of six-point calibration curve (10.0 - 125.0 mcg/mL) and QC samples at three different levels (10.0, 50.0, 125.0 mcg/mL). All the stock solutions were refrigerated (2-8° C) when not in use. Calibration standards and QC samples were prepared in bulk by spiking 25.0 µL of respective spiking stock solutions. These were stored at -70° C until analysis.
Sample preparation for analysis:
A calibration standard, validation QC samples was prepared by adding 0.5 ml plasma to 2.0 ml centrifuge tube and added 0.5 ml and 0.5 ml of precipitating agent (10% v/v perchloric acid) vortexed for 2 min. The resulting solution was centrifuged at 4000 rpm for 7 min. The supernatant

layer was separated and estimated by HPLC as shown in Figure 1

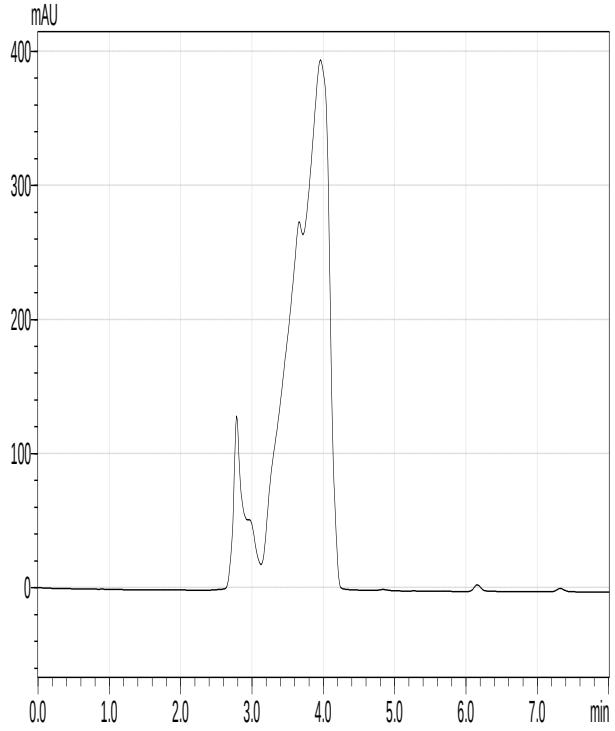

Figure 1: Typical chromatogram of blank plasma

Chromatographic conditions:

Standardization of diltiazem by RP-HPLC method was carried out using the optimized chromatographic conditions. The mobile phase used was acetonitrile: 10mM potassium dihydrogen orthophosphate (ph 3.5). Potassium dihydrogen ortho phosphate used was 10 mM solution in water with pH being adjusted to 3.5 with orthophosphoric acid solution. The injection volume was 20.0 µL. The UV-visible detector was set at 235 nm.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions are intended to take into account the various goals of method development and to weigh each goal accurately, according to the requirement of HPLC methods being used for the estimation of drugs in biological
fluids. Reverse phase HPLC method was chosen for diltiazem.

The standard solutions of diltiazem were scanned from 200–400 nm and the UV spectra obtained were recorded. From the UV spectra, the detection wavelength selected was 235 nm for diltiazem. The wavelength selected gave good peak response and the typical chromatogram of standard solution of diltiazem is shown in Figure 2.

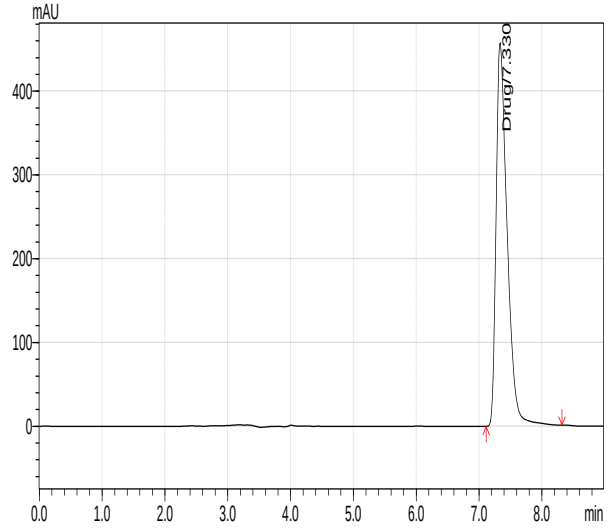

Figure 2: Typical chromatogram of standard drug

CONCLUSION

This method offers significant advantages over those previously reported, in terms of improved sensitivity and selectivity, faster run time (10 min) and lower sample requirements. Hence, this method is useful for single and multiple ascending dose studies in animal or human subjects.

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