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DETERMINATION OF CARVEDILOL IN HUMAN PLASMA BY RP-HPLC METHOD WITH ULTRAVIOLET DETECTION

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ABSTRACT

Carvedilol is an antagonist of α_1 , β_1 and β_2 membrane adrenoceptors and also a modulate of cardiac electro physiological properties. It is widely prescribed for treatment of cardio vascular diseases. A simple, selective and sensitive high performance liquid chromatography method for the determination for the carvedilol in human plasma was developed. The method utilizes the liquid-liquid extraction with n-hexane ethyl acetate (3:1 v/v) as the sample preparation technique .The samples were then analyzed using Phenominex Gemini c 18 column with UV detection at 241 nm. The calibration curve was linear through the range of 10-150 ng/ml. The lower limit of quantification was found to be 0.2 $\mu\text{g/ml}$ % RSD less than 3% for intra day and inter day precision. The mean recovery was found to be 93.09% for carvedilol. The method showed acceptable values for accuracy, precision, recovery, sensitivity and stability. The method is suited for routine analysis of carvedilol in human plasma and can further be extended for pharmacokinetic studies.

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Key Words

Carvedilol, Human Plasma, High Performance Liquid Chromatography (HPLC)

stored at 4°C. Quality control (QC) samples for carvedilol were prepared at concentration level of 1, 6 and 8 µg/ml, with 0.1, 0.6, 0.8 ml of working solutions of IS. The contents were evaporated to dryness on water bath at 10°C. The drug residue was then reconstituted with drug free human plasma and the samples were vortex mixed for 10 min, the analytes were further extracted from plasma with 4ml of n-hexane ethyl acetate (3:1 v/v) by vortexing for 3 min followed by centrifugation for 5 minutes at 2500 rpm. After centrifugation organic layer was separated from aqueous layer by freezing the aqueous layer at -5°C and organic layer was evaporated to dryness on water bath at 70°C. The residue was reconstituted with 1.0 ml of mobile phase the last three concentration levels of the Q.C sample were stored at -5°C and were used for validation of the method. The calibration curve for carvedilol was obtained by using eight calibration standard levels of (10, 20, 50, 75, 100, 125, 150 ng/ml) sample preparation technique for calibration standard level was same as that for Q.C samples. 20 µl aliquots of these solutions were injected on to HPLC system. For all calibration curves, a linear regression was used. Considering the ratio for the area of analyte to internal standard A correlation of more than 0.99 was desirable for each calibration curve.

Accuracy, Precision and lower limit of quantification:

The accuracy and precision of the method were evaluated on the Q.C samples. Intraday, accuracy and precision was measured by consecutively analyzing Q.C samples in one single day. The procedure was repeated for three different days to test the inter day accuracy and precision. Accuracy was calculated a percentage error whereas precision was measure in terms of relative standard precision (%RSD) of each calculated. Lower limit of quantification was set at the lowest calibration standard value 0.2µg/ml.

Recovery: Recovery for carvedilol at three concentration levels corresponding to three routine Q.C samples (1, 2,

6 and 8 µg/ml) analyzed in triplicate. Recovery was determined by comparing the ratio of the area of carvedilol with IS obtained after the injection of the processed plasma calibration samples with those achieved by direct injection of the same amount of drug and IS in the mobile phase.

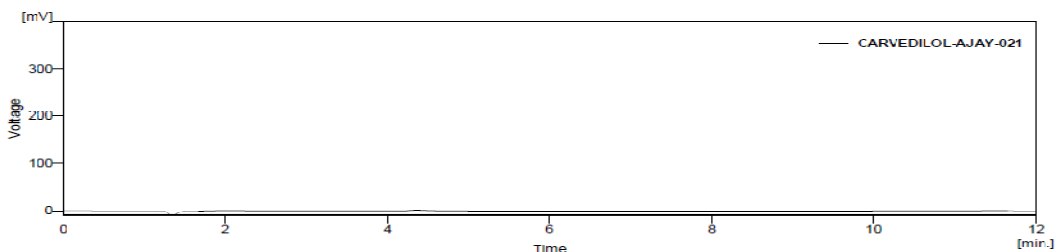
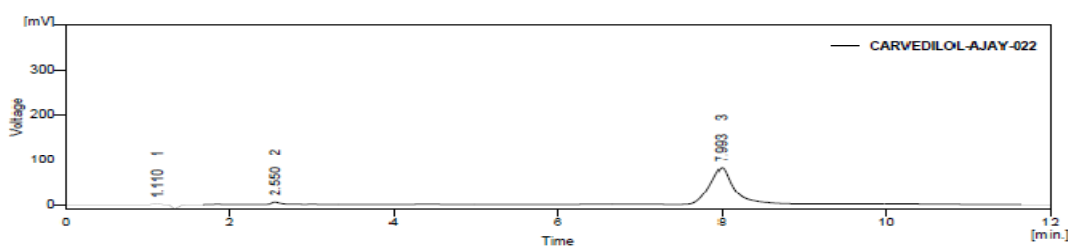
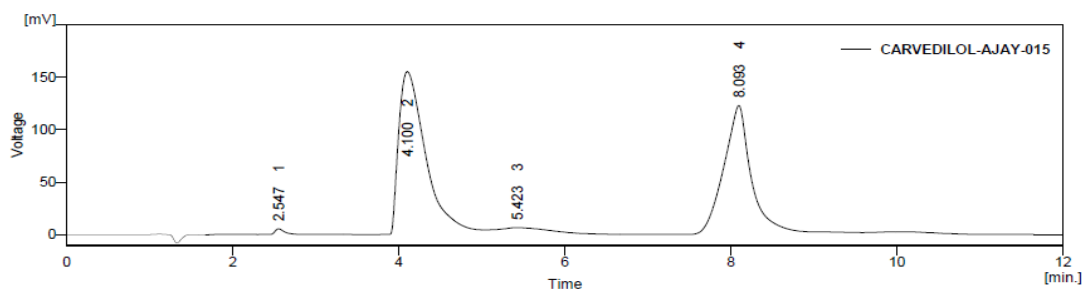
Stability:

Freeze-thaw stability of carvedilol was determined by assigning low and high Q.C samples [1 and 8 (µg/ml)] in triplicate over three freeze thawing cycles. First freeze-thaw cycle consisted of 24 hr frozen at -5°C followed by a complete thaw at a room temperature. The next two freeze-thaw cycles were of 12 hr each frozen at -5°C followed by a complete thaw at a room temperature short term stability consisted of two Q.C samples stored for 6 hr at room temperature long term stability involved storage of two Q.C samples for 5 days at 4°C and stock solution stability, where stock solutions were stored at room temperature for 6 hr. three Q.C samples were then evaluated in triplicate and the results were compared with the freshly prepared samples of same concentration.

RESULTS AND DISCUSSION**Carvedilol procedures****Chromatography**

Retention times for IS and carvedilol were 8.093 and 4.10 mins respectively. Representative chromatographs of blank human plasma, blank plasma spike with IS 100 µg/ml with carvedilol 100µg/ml are shown in figure 1, 2, and 3 respectively. The standard curves were linear over the concentration range of 10-150 ng/ml. the average correlation coefficient was $R^2 = 0.999$ with $y = 30604x$ regression equation.

Figures

Fig. 2:Chromatogram of blank plasma**Fig. 3:**Chromatogram of blank + IS**Fig. 4:**Chromatogram of IS and Carvedilol**Accuracy, precision and Lower Limit of Quantification:**

The method showed good accuracy & precision in plasma samples Table 1 show the results for intra-day and inter-day precision and accuracy for carvedilol in plasma samples. Inter-day and inter-day (%RSD) precision gave mean (S.D) of 2.11 ± 0.29 and 1.37 ± 0.26 respectively. Inter and inter- day accuracies were 98.82 ± 7.78 and 97.69 ± 7.21 respectively.

Table 1 - Intra-day and inter-day precision and accuracy of carvedilol in human plasma QC sample

Theoretical ($\mu\text{g/ml}$)	Observed (mean $\mu\text{g/ml} \pm \text{S.D}$)	Precision (%RSD)	Accuracy
Intra-day 1	0.8985 ± 0.0199	2.22	89.85
Intra-day 6	6.2260 ± 0.1449	2.33	103.77
Intra-day 8	8.2268 ± 0.1470	1.78	102.84
Inter-day 1	0.8941 ± 0.0149	1.67	89.41
Inter-day 6	6.0603 ± 0.0719	1.18	101.01
Inter-day 8	8.2116 ± 0.1023	1.25	102.64

Recovery:

Table 2 shows the results of the recovery test for the three Q.C levels tested (1, 2, 6 and 8 µg/ml). The extraction recovery in plasma samples ranged from 91.08 to 94.51 % for carvedilol at three concentration levels. The mean recovery for carvedilol was to be 93.09%.

Table 2 - Recovery of carvedilol in human plasma Q.C sample

Added (µg/ml)	Found (mean µg/ml±S.D)	(%RSD)	Recovery (%)
0.9715	0.8849± 0.0336	3.76	91.08
6.5477	6.1339±0.0876	1.43	93.68

Table 3 – Stability of carvedilol in human plasma Q.C sample

Stability	Added (µg/ml)	Found (mean µg/ml±S.D)	(%RSD)
Freeze thaw stability (three cycles)	0.8909	0.8849± 0.0336	3.76
	8.3154	8.3116±0.1035	1.25
Short term stability (for 6 hr at RT)	0.9065	8.991±0.0278	3.09
	8.1484	8.2078±0.2715	3.31
Long term stability (for 5 days at 4 C)	0.8981	0.8849±0.0336	3.81
	8.1484	8.1705±0.0658	0.81
Stock solutions stability (for 6 hrs at RT)	0.8981	0.9715±0.0148	1.52
	8.1484	8.3116±0.1035	1.25

A simple bio analytical method was developed to quantify carvedilol in human plasma. The validated method covers the wide range of linearity over 0.2-12 µg/ml and is therefore suitable for the determination of carvedilol in human plasma at different therapeutic dose levels. As compared to LC-MS/MS methods, the present method is economic and simple. Also it utilizes liquid-liquid extraction as the sample preparation; technique, which eliminates the drawbacks of ion suppression due to protein precipitation or the use of solid phase extraction cartridges which is not economic. The mobile phase used is buffer: methanol: ACN which is simple as compared to the reported methods. The mean recovery (±SD) was found to be 93.09% ±1.79.

CONCLUSIONS

A simple rapid and selective reverse phase HPLC method has been developed for assay of carvedilol in human plasma over the range of 0.2 to 12 µg/ml. The proposed

8.6882	8.2116±0.1023	1.25	94.51
All	0.8941±0.0149	2.16	93.09±1.79

Stability:

Plasma Q.C at two concentrations (1 & 8 µg/ml) was used for stability studies. It was performed to evaluate the influence of storage conditions from the sample collection to analysis. Table 3 represents the results of stability studies. Results indicated that carvedilol is stable in human plasma for the given stability conditions. The deviation of the mean test responses to the freshly prepared solution was less than 5% at any of the stability conditions.

method can be applied to monitor plasma concentration of carvedilol in pharmacokinetic studies. It can also be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis.

REFERENCES

1. Harish Padh, Rajeshwari Rathod, L Poorna Chandra Prasad, Shubha Rani and Manish Nivsarkar, J. Chrom. B., 857, 219(2007).
2. Li Xiao Xu, N Hui, L Y Ma and H Y Wang, Spectrochim Acta A Mol Biomol Spectrosc, 61(5), 855 (2005).
3. Maha Sultan, Asian journal of chemistry, 20 (3) 2283 (2008).
4. Ramesh Gannu, Vamshi Vishnu Yamsani and Yamsani Madhusudan Rao, J. Liq. Chrom. Rel. Tech., 30, 1677 (2007).

5. Wolfgang Lindnerh, Jana Oravcova, Dagmar Sojkova, *Journal of chromatography B.*, 682, 349 (1996).
6. P Ptacek, J Macek and J Klima, *Journal of Chromatography B*, 789, 405 (2003).
7. B. Yilmaz and Z. Erol, *Asian journal of chemistry*, 23 (1) 49-53.
8. A Zarghi, SM Foroutan, A Shafaati and A Khoddam, *Journal of Pharmaceutical and Biomedical Analysis*, 44, 250 (2007).
9. Bappaditya Chatterjee, Ayan Das and Tapan K. Pal, *Asian journal of chemistry*, 23 (3) 1334-1336.
10. Somaieh Soltani and Abolghasem Jouyban, *Asian journal of chemistry*, 23(4), 1728-1734.
11. Fibebe Analine Lanzanova, Debora Argenta, Marcela Zart Arend, Liberato Brum Junior and Simone Goncalves Cardoso, *Journal of Liquid Chromatography & Related Technologies*, 32(4), 526 (2009).
12. L.D. Srinivas, K.V.S. Prasad Rao and B.S. Sastry, *Asian journal of chemistry*, 17 (4), 2809(2005).
13. N Hokama, N Hobara, H Kameya, S Ohshiro, M Sakanashi, *J. Chromatogr B Biomed Sci Appl.*, 732(1), 233 (1999).
14. A Radi and T Elmogy, *IL FARMACO*, 60, 43 (2005).
15. Gottfried Blaschke, Frederike Behn, Stephan Michels and Stephanie Laer, *Journal of Chromatography B.*, 755, 111 (2001).
16. guidance for industry; Bioanalytical method validation, U.S Department of Health and Human Services Food and drugs administration center for drug evaluation & research (CDER) center for veterinary medicine (CVM) may 2001 BP.
