ANALYSIS

METHOD VALIDATION AND STABILITY STUDIES OF DIFFERENTIAL ACTIVITY OF HYDROXYPROPYL- β -CYCLODEXTRIN AND HYDROPHILIC CARRIERS OF CARVEDILOL

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Abstract: Stability studies of the formulations of CAR solid dispersions were analyzed at 30°C/65% RH for a period of three months. A simple reverse phase HPLC was developed and validated for the quantification of CAR solid dispersions. Chromatographic separation was achieved on Waters Atlantis dC18 (4.6 × 150 mm) column with a mobile phase consisting of 0.033 M phosphate buffer and methanol (35 : 65). The mobile phase was filtered using an organic filter paper and sonicated for about 20 min. The flow rate was 1 mL/min and the wavelength used for detection was 242 nm. Forced degradation studies were conducted under three conditions namely: acidic, basic and hydroxide peroxide conditions. The HPLC linearity concentration was in the range of 5–80 µg/mL with a correlation coefficient (R^2) of 0.9995. There was no drug carrier interference. The suggested reverse phase HPLC methodology is simple, selective, linear, and robust in quantifying the amount of CAR in the various solid dispersion samples. In hydrogen peroxide, a degraded product was found on the chromatogram unlike that of the acidic and basic conditions. Degradation occurred more strongly in the acidic condition than in the basic condition. The binary systems were less stable than the ternary system solid dispersions due to the presence of HP- β -CD.

Keywords: Carvedilol, percentage degradation, solid dispersion, reverse-phase liquid chromatography, stress conditions, method validation

Carvedilol (CAR) is a 1-(9H-Carbazol-4-yloxy)-3-amino]-2-praonol (1) and it is basic in nature with a pKa of 7.8. CAR is both a beta and alpha receptor blocker that is used in the treatment or management of mild to moderate hypertension as

well as other cardiovascular ailments namely heart failure, cardiac arrhythmias, angina pectoris, and myocardial infarction (2). Fritz Wiedemann was the first to discover the drug and it is usually in the form of a white crystalline powder (3). It is highly soluble

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in organic solvents and poorly soluble in aqueous solution. Due to its poor water solubility, it is classified as a class II drug according to the biopharmaceutical classification system.

Cyclodextrins (CDs) are cyclic oligosaccharides made up of six to eight or more glucopyranose units and each unit is linked by α (1, 4) bond (4). In 1981 Villiers discovered this new compound from a starch digest of *Bacillus amylobacter* (5). Among the parent CDs, β -CD is commonly used because it is accessible and more economical as compared to the other two types (6). CDs form an inclusion complex with hydrophobic compounds in order to enhance their solubility, dissolution and stability profile of such compounds. Commonly used β -CDs are (2-hydroxypropyl- β - cyclodextrin (HP β CD) and sulfobutyl ether- β cyclodextrin (SB- β -CD)).

Solid dispersion is another technique by which formulation scientist use in enhancing solubility, dissolution and stability of drugs (7). It was first discovered by two scientists known as Sekiguchi and Obi (8). The solid dispersion concept deals with the metamorphoses of crystalline forms of drug into the amorphous state (9). Solid dispersions have the advantages of reducing particle size; improve wettability, higher porosity degree and amorphous structure for particles (10).

Many analytical techniques have been utilized for the determination of CAR in cardiac tissue (11), plasma (12), bulk and pharmaceutical dosage form (13, 14), serum (15) and urine (16). Methods such as HPLC-fluorimetry (17), differential pulse voltammetry (18), Chemiluminescence (19), spectrophotometric (20), fluorimetry (21, 22), tandem mass spectrometry (23), capillary electrophoresis (CE) (24) and high-performance liquid chromatography (HPLC) are employed for CAR analysis. Nevertheless, comparing these methods, HPLC is generally used for the determination of drugs in bulk and pharmaceutical dosage forms because of its high selectivity and sensitivity. Also, HPLC instruments are simple to use and require a simple sample formulation (25, 26). The experiment was intended to compare the stability of CAR when HP β CD, poloxamer (PLX), and polyvinyl pyrrolidone (PVP) carriers were used alone, as against when HP β CD was used in combination with either PLX or PVP.

MATERIALS AND METHODS

Materials

CAR was procured from Foster Pharmaceutical Company (Hangzhou, China). HP- β -CD (degree of substitution, 0.6, average Mw = 1541.5

Da), Poloxamer 188 and PVP K-30 were provided by Rick Chemicals Company (Kunshan, China), BASF SE Ludwigshafen, Germany and Colorcon Coating Technology Company (Shanghai, China). All other chemicals and solvents used in this research were of analytical grade.

Methods

Inclusion complex formulation for CAR- KD

CAR and HP β CD were weighed accurately using a (1 : 1) equimolar ratio. The two chemicals were uniformly mixed in a glass mortar and wetted using water and methanol (1 : 1 v/v). The resultant solution was kneaded for an hour, to form a paste and the paste was desiccated in an electric oven overnight. The desiccated mass gained, was crushed in a glass mortar and sifted using sieve number 80 and kept for further investigations.

Solid dispersion formulation of CAR-polymer and SD-polymers

CAR solid dispersions in PLX and PVP were formulated according to mass ratio of 1 : 1 (PVP1, PLX1), 1 : 3 (PVP2, PLX2) and 1 : 5 (PVP3, PLX3) using solvent evaporation method (27, 28). A known quantity of PLX and PVP were weighed and dissolved in ethanol/methylene chloride (1 : 1 v/v) and methanol. An equivalent amount of 200 mg of CAR was dissolved in all of the solutions and stirred for approximately one hour at 25°C with the help of a magnetic stirrer. The rotary evaporator was used to evaporate the remaining organic solvents present in the solutions at a temperature of 50°C. The solid mass was dried in an electric oven at 40°C, grounded and sifted through sieve number 80 and kept for further investigations.

Formulation of SD-polymers

The new solid dispersions SD-PLX (SD-PLX1, SD-PLX2, and SD-PLX3) and SD-PVP (SD-PVP1, SD-PVP2, and SD-PVP3) were prepared using the described method above. In this method, SD containing an equivalent of two hundred milligrams of CAR was weighed and added to the formulated polymer solutions.

Instrumentation and chromatographic conditions

The HPLC system comprises a model LC-10AT pump (Shimadzu, Kyoto, Japan), SPD-10A programmable photodiode array detector (Shimadzu, Kyoto, Japan), and Waters Atlantis dC18 (4.6 x 150 mm) were used in the analysis.

The mobile phase used was methanol and phosphate buffer (65 : 35 v/v). The flow rate for the

mobile phase was 1.0 mL/min with a column temperature of 30°C. The injection volume used was 20 µL. The CAR peak was measured at a wavelength of 242 nm. The retention time for each sample was 6 min.

Method validation

To ensure the validity of the developed reverse phase HPLC (RP-HPLC) method, validation parameters such as linearity, precision, accuracy, and robustness were performed in accordance with International Conference on Harmonization (ICH) guidelines (29).

Preparation of standard solutions and samples

The stock solution of CAR was freshly prepared and had a concentration of 1 mg/mL. Methanol was the solvent used in preparing the solution.

Specificity/Selectivity

CAR peak should not interfere with the polymers or other chemicals used in the formulation. Analytic interference was examined by injecting CAR organic solution to the HPLC.

Calibration solutions

A series of six standard solutions of CAR were prepared by dilution of the stock solution to obtain a concentration range of 5-80 µg/mL.

Precision

Four different concentrations (5, 20, 40 and 80 µg/mL) were measured. All the four samples were injected every 3 h within a day, continuously for 3 days. The calculation of both intra-day and inter-day precision was based on the standard curve.

Robustness

With this method, experimental conditions were deliberately changed and after each investigation alterations in the retention time of CAR were observed. The effect of flow rate on the retention time of CAR was analyzed by changing the flow rate to 0.9 mL/min and 1.1 mL/min respectively instead of 1 mL/min while other chromatographic conditions were held constant. The effect of temperature on CAR was also analyzed at 28°C and 32°C instead of 30°C while maintaining other conditions.

Sample preparation

The various samples (CAR, HPβCD, PVP, KD, and PVP1, PVP2, PVP3, SD PVP1, SD PVP2, and SD PVP3) were diluted with methanol to

achieve an equivalent concentration of 0.250 mg/mL.

Force degradation studies

Force degradation of CAR was carried out according to the ICH stress conditions such as acidic, basic, and hydrolysis using hydrogen peroxide (H₂O₂).

Acidic degradation

The acidic degradation studies were carried out by measuring 1 mg of CAR which was diluted in 1 mL of methanol and then transferred into a 50 mL volumetric flask containing 10 mL of H₂O and in different HCL concentrations (1 M and 2 M respectively). The solutions were refluxed and heated at a temperature of 80°C for 2 h and cooled. The resultant solution was injected into the HPLC system for further analysis. The use of both organic and inorganic solvents with high temperatures can affect the stability of the drug product (30).

Basic degradation

The basic degradation studies were carried out by measuring 1 mg of CAR which was diluted in 1 mL of methanol and then transferred into a 50 mL volumetric flask containing 10 mL of H₂O and in different NaOH concentrations (1 M and 2 M respectively). The solutions were refluxed and heated at a temperature of 80°C for 2 h and cooled and later injected into the HPLC system for further analysis.

Oxidation degradation

The hydrogen peroxide (H₂O₂) induced oxidative degradation studies were performed by measuring 1 mg of CAR and diluting it with methanol in a beaker. The content of the beaker was later transferred into a 25 mL volumetric containing 10 mL of 0.3% H₂O₂. The resultant solution was injected into the HPLC system for further analysis.

RESULTS AND DISCUSSION

Specificity

Only one specific peak of CAR was shown as a chromatograph. The retention time for CAR was 6 min. It is an indication that there was no major interference from the excipients used in the solid dispersion preparations and inclusion complex (31).

Linearity

Figure 1.

Precision

The relative standard deviation (RSD) of four varying concentrations of CAR was less than 2, suggesting a good precision method (Table 1).

Figure 1. The standard curve of CAR was $Y = 6331.6x + 7584.2$ ($R^2 = 0.9995$, $n = 3$ repetitions) with a linear range of 5-80 µg/mL.

Robustness

The relative standard deviation (RSD) of CAR at a specific concentration was less than 2, suggesting a good robust method (Table 2).

Force degradation

Acidic degradation

For acidic degradation of CAR using 1 M HCL and refluxed at a temperature of 80°C the percentage degradation of CAR was 87.15%. When 2 M HCL was refluxed at the same temperature the percentage degradation of CAR was found to be 56.86%. It was done in comparison with the pure drug (Figures 2, 3)

Basic degradation

For basic degradation of CAR using 1 M NaOH and refluxed at a temperature of 80°C the percentage of degradation of CAR was found to be 27.91%. For 2 M NaOH, the percentage degradation of CAR when it was refluxed at a temperature of 80°C gave a degradation of 19.02%. It was done in comparison with the pure drug (Figure 4, 5).

Table 1. Precision methodology with % RSD between 0.06-1.95%.

Concentration (µg/mL)	Intra-day		Inter-day	
	Mean (µg/mL)	RSD (%)	Mean (µg/mL)	RSD (%)
5	4.28	1.66	4.37	0.28
20	19.97	1.83	20.67	1.95
40	39.94	1.58	41.15	1.46
80	79.45	0.06	79.27	0.15

Table 2. Robustness methodology with % RSD between 0.003-0.98%.

Parameters	Concentration (100 µg/mL)		
	Normal condition	Retention time average	RSD (%)
Flow rate	1 mL/min	6.09	0.313
Column temp	30°C		
	Condition opted		
Flow rate	0.9 mL/min	6.64	0.217
Column temp	30°C		
Flow rate	1.1 mL/min	5.4	0.003
Column temp	30°C		
Flow rate	1 mL/min	6.1	0.98
Column temp	28°C		
Flow rate	1 mL/min	5.95	0.485
Column temp	32°C

Figure 2. HPLC chromatogram of CAR and 1 M HCL.

Figure 3. HPLC chromatogram of CAR and 2 M HCL.

Figure 4. HPLC chromatogram of CAR and 1 M NaOH.

Figure 5. HPLC chromatogram of CAR and 2 M NaOH.

Figure 6. HPLC chromatogram of CAR and H₂O₂.

Oxidation degradation

The oxidation degradation of CAR had other degradable compounds present on the chromatogram at different retention times and at a varying peak area. The other degradable compounds detected had a retention time of 1.173 min and 2.390 min. The percentage degradation of CAR was found to be 27.79% (Figure 6).

Sample degradation

After the three-month stability studies, there was no change in the physical appearance of the various formulations. The chromatogram of the first-month degradation profile of pure CAR was about 5.57%. With CAR-HP β CD/PLX/PVP, the range at which CAR degraded was in the range of 1.88-3.08% and that of the ternary system was in the range of 0.90-2.16%.

For the second month also, pure CAR degraded further to about 7.30%. The drug content in the binary system was in the range of 3.20% to 4.45% and that of SD-PLX/PVP was from 1.94-3.61% respectively. However, the third month led to an additional degradation of pure CAR to 11.22% and the drug content in the binary system degraded in the range of 4.41-5.39%. On the other hand, SD-carriers had drug content in the range of 2.83-4.12%.

Generally, pure CAR was the least stable and this result corroborates with other research results (32, 33). Again, the binary systems were less stable than the ternary system solid dispersions due to the presence of HP- β -CD. Generally, PVP polymer showered more stability than that of PLX carrier. It is because the glass transition temperature in PVP is higher than that in PLX thereby conferring stability on the formulations in a stability chamber of 30°C/65% RH (34-37) (Figure 7).

Figure 7. Stability studies of both binary systems and ternary systems for three months under 30°C/65% RH.

CONCLUSION

The HPLC method developed was specific, robust and linear over a concentration range of 5-80 µg/mL with a correlation coefficient (R^2) of 0.9995. During the force degradation experiment, there were degraded products found on the chromatogram when CAR was immersed in hydrogen peroxide. Comparatively, degradation occurred more strongly in the acidic condition than in the basic condition. The pharmaceutical excipients used in the formulation of the multicomponent systems enhanced the stability profile of CAR an indication that these drug excipients can be used to prolong the shelf life of active pharmaceutical ingredients.

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Competing interest

The authors declare that there are no competing interests.

REFERENCES

1. Brook C.S., Chen W., Dell'Orco P.C., Katrincic L.M., Louvet A.M., et al.: Google Patents, 2009.
2. Mancia G., Fagard R., Narkiewicz K., Redon J., Zanchetti A., et al.: Eur. Heart J. 34, 2159 (2013).
3. Zoghbi A., Geng T., Wang B.: AAPS PharmSciTech 18, 2927 (2017).
4. Lichtenthaler F.W., Immel S.: Tetrahedron Asymmetry 5, 2045 (1994).
5. Crini G.: Chem. Rev. 114 10940 (2014).
6. Rutkowska M., Płotka-Wasyłka J., Morrison C., Wiczorek P.P., Namieśnik J., et al.: Trends Anal. Chem. 102, 91 (2018).

7. Savjani K.T., Gajjar A.K., Savjani J.K.: *ISRN Pharm.* 2012, 10 pages (2012).
8. Sekiguchi T., Ikeura H., Tanaka K., Obi K., Ueno N., Honma K.: *J. Chem. Phys.* 102, 1422 (1995).
9. Sharma K., Thakkar S., Khurana S., Bansal A.K.: *Pharm. Excip.* pp. 97-144, Wiley, New York (2017).
10. Vasconcelos T., Sarmiento B., Costa P.: *Drug Discov. Today* 12, 1068 (2007).
11. Kaplan K., Gürkan Polat T., Duman O., Tunç S.: *J. Liq. Chromatogr. Related Technol.* 41, 532 (2018).
12. Zakrzewski-Jakubiak M., de Denus S., Leblanc M.-H., White M., Turgeon J.: *J. Pharm. Biomed. Anal.* 52, 636 (2010).
13. Raju V., Murthy K.: *Indian J. Pharm. Sci.* 73, 527 (2011).
14. Abdelwahab N.S.: *Arab. J. Chem.* 9, S355 (2016).
15. Gannu R., Yamsani V.V., Rao Y.M.: *J. Liq. Chromatogr. Related Technol.* 30, 1677 (2007).
16. Myung S.-W., Jo C.-H.: *J. Chromatogr. B Biomed. Sci.* 822, 70 (2005).
17. Rathod R., Prasad L.P.C., Rani S., Nivsarkar M., Padh H.: *J. Chromatogr. B Biomed. Sci.* 857, 219 (2007).
18. Behn F., Lärer S., Scholz H.: *J. Chromatogr. Sci.* 39, 121 (2001).
19. Pires C.K., Marques K.L., Santos J.L., Lapa R.A., Lima J.L., et al.: *Talanta* 68, 239 (2005).
20. Hameed E.A.A., Salam R.A.A., Hadad G.M.: *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 141, 278 (2015).
21. Xu L.X., Hui N., Ma L.Y., Wang H.Y.: *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 61, 855 (2005).
22. Zamani-Kalajahi M., Fazeli-Bakhtiyari R., Amiri M., Golmohammadi A., Afrasiabi A., et al.: *Bioanalysis* 5, 437 (2013).
23. Jeong D.W., Kim Y.H., Ji H.Y., Youn Y.S., Lee K.C., et al.: *J. Pharm. Biomed. Anal.* 44, 547 (2007).
24. Behn F., Michels S., Lärer S., Blaschke G.: *J. Chromatogr. B Biomed. Sci.* 755, 111 (2001).
25. Ho N.T., Desai D., Zaman M.H.: *Am. J. Trop. Med. Hyg.* 92, 24 (2015).
26. Pragst F., Herzler M., Erxleben B.-T.: *Clin. Chem. Lab. Med.* 42, 1325 (2004).
27. Sharma A., Jain C.: *Res. Pharm. Sci.* 5, 49 (2010).
28. Yuvaraja K., Khanam J.: *J. Pharm. Biomed. Anal.* 96, 10 (2014).
29. Grimm W.: *Drug Dev. Ind. Pharm.* 24, 313 (1998).
30. Davies O.R., Lewis A.L., Whitaker M.J., Tai H., Shakesheff K.M., et al.: *Adv. Drug Deliv. Rev.* 60, 373 (2008).
31. Tuchila C., Baconi D., Ciobanu A., Nitulescu G.: *Toxicol. Lett.* 258, S106 (2016).
32. Stojanović J., Vladimirov S., Marinković V., Velićović D., Sibinović P.: *J. Serb. Chem. Soc.* 72, 37 (2007).
33. Taymouri S., Varshosaz J.: *Adv. Biomed. Res.* 5, 48 (2016).
34. Sethia S., Squillante E.: *Int. J. Pharm.* 272, 1 (2004).
35. Weuts I., Kempen D., Decorte A., Verreck G., Peeters J., et al.: *Eur. J. Pharm. Sci.* 25, 313 (2005).
36. Paradkar A., Ambike A.A., Jadhav B.K., Mahadik K.: *Int. J. Pharm.* 271, 281 (2004).
37. Zhang X., Sun N., Wu B., Lu Y., Guan T., et al.: *Powder Technol.* 182, 480 (2008).