

**COMPARATIVE EVALUATION OF CYCLODEXTRIN COMPLEXATION IN IMPROVING DISSOLUTION OF RITONAVIR**Bharani S. Sogali^{a*}, Sushant M. S^a, Ramana Murthy K. V^b^a Department of Pharmaceutics, Krupanidhi College of Pharmacy, ChikkaBellandur, Bangalore-560035, India.^b Department of Pharmaceutics, Andhra University, Visakhapatnam-530003, India.***Corresponding author e-mail: bssogali@gmail.com***Received on: 18-02-2016; Revised on: 29-03-2016; Accepted on: 01-04-2016***ABSTRACT**

The present study was focused on comparing different methods of preparations and the effect of hydroxy propyl beta cyclodextrin (HP β CD) and randomly methylated beta cyclodextrin (RM β CD) in enhancing dissolution and oral bioavailability of poorly soluble, antiretroviral protease inhibitor, ritonavir. Complexes of ritonavir-CD complexes were prepared at 1:1 ratio using physical mixing, co evaporation, spray drying and freeze drying methods. The prepared complexes were characterized using FTIR, differential scanning calorimetry (DSC), x-ray diffraction studies (XRD), nuclear magnetic resonance spectroscopy (NMR) and scanning electron microscopy. *In vitro* dissolution studies were performed in 0.1N HCl and the dissolution data were subjected to one way ANOVA. Phase solubility studies proved complexation of ritonavir and were of A_L type. All complexes showed enhanced dissolution rate compared to ritonavir but the complex prepared with RM β CD using freeze drying had shown complete and fast drug release. On the basis of above results, it was concluded that complex prepared with RM β CD by freeze drying was successful in improving solubility, dissolution rate and oral bioavailability of ritonavir.

Key words: Randomly methylated β cyclodextrin (RM β CD), Freeze drying, Ritonavir, Complex, Bioavailability.**INTRODUCTION**

Human immunodeficiency virus (HIV) infection, which leads to acquired immunodeficiency syndrome (AIDS), remains a serious worldwide health problem. HIV continues to be a major global public health issue, claiming more than 25 million lives over the past three decades. There were approximately 34 [31.4–35.9] million people living with HIV in 2011. In 2012, more than 9.7 million people living with HIV were receiving antiretroviral therapy (ART) in low and middle-income countries [1]. The discovery of HIV protease inhibitors introduced new and effective first-line therapies for HIV/AIDS. Helping to combat HIV-related diseases and prolong survival, protease inhibitors are commonly administered with reverse transcriptase inhibitors. However, poor patient compliance, noxious side effects, and viral

resistance have led to a recommendation to treat with different kinds of protease inhibitors.

Ritonavir, a widely prescribed antiretroviral protease inhibitor drug belongs to Class II under BCS and exhibits low and variable oral bioavailability due to its poor aqueous solubility. Its oral absorption is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Ritonavir, a CYP3A and P-gp inhibitor, helps to increase saquinavir oral bioavailability [2-4]. However, because HIV/ AIDS patients must take other drugs known to be metabolized by CYP3A or they are P-gp substrates, ritonavir has been shown to cause additional toxicity and safety issues [5]. Therefore, safe pharmaceutical formulations that enhance the bioavailability of ritonavir are needed.

Several approaches could be investigated to improve oral bioavailability of poorly soluble drugs, among them, complexation with cyclodextrins has drawn considerable attention, because of their low toxicity, low cost, biocompatibility, biodegradability and abundant availability. Drug-CD complexation and improvement in solubility and dissolution rate are influenced by both nature of the cyclodextrin (native or chemically modified, crystalline or amorphous) and the method of complexation, viz co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, spray drying, or freeze drying. The effectiveness of a method depends on nature of the drug and CD [6-8]. In many cases, spray drying and freeze drying were found to be more effective for drug complexation [9-13].

Therefore, in the present work, a series of binary systems of ritonavir with amorphous and highly soluble derivatives of cyclodextrins, HP β CD and RM β CD were prepared by using four different methods. The main objective of the study was to carry out comparative studies on effectiveness of the method of preparation and better cyclodextrin derivative in enhancing solubility and dissolution rate of ritonavir so that the drug dosing can be reduced for minimizing side effects and also the utilization of large amounts of cyclodextrins can be lowered.

MATERIALS AND METHODS

Materials: Ritonavir was kindly gifted from Hetero Chemicals, Hyderabad, India. HP β CD and RM β CD were gift samples received from RoquettePharma, Italy. All other chemicals used were of analytical grade.

Methods

Phase solubility studies: Phase solubility studies were performed as mentioned by (Higuchi and Connors, 1965) [14] in 0.1 N HCl to determine suitable cyclodextrin derivative in solubility enhancement. Solubility studies were carried out by adding excess amounts of drug separately in quantities exceeding its aqueous solubility to 50 mL of 0.1 N HCl, containing increasing concentrations of cyclodextrins (2-12 mM). The resulting suspensions were shaken at room temperature for a period of 72 hrs, until equilibrium was established. The samples were filtered through a 0.45 μ m membrane filter (Millex-HA filter units, Millipore) and suitably diluted with 0.1 N HCl before analysis. All studies were performed in triplicate.

Apparent 1:1 stability constants were calculated from the straight-line portion of the phase solubility diagrams, according to the **Equation 1**.

$$K_{a:b} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad \text{-Eq.1}$$

where S_0 is the solubility of the drug in examined buffer solution in the absence of ligand.

Preparation of drug-CD complexes

In the present work, drug-CD complexes were prepared using physical mixing, co evaporation, spray drying and freeze drying.

Physical mixture [15,16]: Drug and cyclodextrins at equimolar ratio (1:1) were weighed and mixed in a mortar by geometric dilution method for sufficient time (~5 min) to obtain a homogenous powder blend, passed through sieve no. 80 and stored in a sealed glass vials and kept in desiccator over fused calcium chloride until further use.

Co evaporation [16-18]: Required quantities of drug and cyclodextrin were dissolved in sufficient quantity of water-ethanol solvent mixture (1:1) and evaporated on a water bath at 50°C with stirring. Each solid product was sieved through #80 and stored in desiccator.

Spray drying: Spray drying was performed using Labultima LU22 spray dryer for the preparation of inclusion complexes of both drugs and cyclodextrins. Equimolar ratio of drug and cyclodextrins were dissolved in sufficient quantity of ethanol and water mixture (1:1). The solutions were atomized at a flow rate 2 mL/min using fixed values for compressed air (500 L/h) and aspirator 40-50 m³/hr with T_{in} value of 70°C, corresponding to a T_{out} of 48°C. After spray drying, each resulting powder was collected by cyclone separation and transferred to glass vials. After spray drying, all complexes were kept in an oven at 40°C for 24 hrs to remove traces of moisture present in the complexes.

Freeze drying/lyophilization [19,20]: Required quantity of cyclodextrin was dissolved in sufficient quantity of water and required quantity of drug was added and dispersed. The dispersion was kept under magnetic stirring at 200 rpm for 72 hrs in closed vials for complex formation which resulted in the formation of clear solution. The resulting solution was fast frozen at -20°C using liquid nitrogen and dried at -50°C and 0.0070 mbar pressure in a freeze dryer (Model MODUL YOD 230, Thermo Electron Corporation, India) for 48 hrs. The obtained freeze dried product in the glass vial was stored in desiccator.

In vitro release characteristics of complexes [20-23]: Dissolution experiments were carried out in triplicate (n = 3) with an USP XXIII paddle apparatus in 900 mL of 0.1 N HCl at 37°C using a rotation speed of 50 rpm. In each study drug-CD complex

equivalent to 100 mg of drug was used. 5 mL sample was withdrawn at intervals of 5, 15, 30, 45, 60 min using a syringe fitted with prefilter (0.45 μm). An equal amount of fresh dissolution medium maintained at the same temperature was replaced immediately after withdrawal of the test sample. Test samples were suitably diluted wherever necessary and the absorbance was measured as per the analytical procedures described. The mean percent of drug dissolved and the standard deviations were calculated.

Drug-excipient interaction studies

Drug excipient interaction studies were conducted for ritonavir, pure CDs, physical mixtures and freeze dried complexes to gain insight into interactions between drug and respective CDs in the complexed form.

Fourier transform infrared spectroscopy (FTIR): Fourier transform infrared spectra (FTIR) were used to identify the formation of complex. Samples were analyzed by potassium bromide pellet method in an IR spectrophotometer (Shimadzu, FTIR 1300) in the region between 400-4000 cm^{-1} . Complex formation was evaluated by comparing the IR spectra of the solid complex with drug. For comparison purposes, FTIR spectra were obtained for ritonavir, each βCD being studied as well as physical mixtures of both drugs with each cyclodextrin.

Differential scanning calorimetry (DSC): The thermal behavior of ritonavir and cyclodextrin inclusion complexes was studied using Differential scanning calorimetry in order to confirm the formation of solid complex. When guest molecules are incorporated in the cyclodextrin cavity or in the crystal lattice, their melting, boiling and sublimation points are usually shifted to a different temperature or disappear within the temperature range. The samples were heated from 0 to 350°C at a heating rate of 10°C/min under a nitrogen flow, flowing at a rate of 40 mL/min through the DSC cell.

Powder X-Ray diffraction study (XRD): X-Ray diffraction of inclusion complexes and the pure drug were performed to identify the interaction of the drug with cyclodextrins using a PW 1720 X-ray generator and a PW 1710 diffractometer control (Philips Electronic Instrument). The scanning range (2θ) was from 5° to 90°, and the scan step and scan speed were 0.04° and 0.02°/s, respectively.

Nuclear magnetic resonance (NMR) spectroscopy: $^1\text{H-NMR}$ studies were conducted to determine the electronic interactions between drug and cyclodextrins were obtained from a Bruker AM-400 spectrophotometer. Samples were prepared by dissolution of the drug in methanol as solvent for RTV. $^1\text{H-NMR}$ spectra of inclusion complexes and

pure components were recorded. Chemical shifts were reported in ppm (δ) downfield from tetra methyl silane (TMS) (internal reference).

Scanning electron microscopy (SEM): The morphological properties of the ritonavir, physical mixture, freeze dried powders of ritonavir and RM βCD complex were characterized by scanning electronic microscopy (Cambridge instrument: Stereoscan 360).

RESULTS AND DISCUSSION

Phase solubility studies: Ritonavir is practically insoluble in water. The phase solubility diagrams obtained were linear as shown in (**Figure 1**). Profiles obtained were according to Higuchi and Connors are of A_L type. Because these profiles were characterized by a slope of less than one, it was assumed that the solubility increase was due to the formation of a complex in 1:1 molar ratio. Stability constant values obtained for ritonavir cyclodextrin complexes were 119.01 M^{-1} for HP βCD and 192.96 M^{-1} for RM βCD (**Table 1**). 1.6 fold higher stability constant was obtained with RM βCD compared to HP βCD proved that RM βCD is having higher complexation efficiency.

Complexation is generally due to the hydrophobic interaction between poorly water soluble guest molecule ritonavir and the apolar cavity of cyclodextrin molecule. The hydrophobicity and geometry of the guest molecule as well as the cavity size and the derivative groups of the cyclodextrin are important for complex formation. In the present study, the enhancement of ritonavir solubility was highly dependent on the type of cyclodextrin used. RM βCD is more effective in solubilising ritonavir in 0.1N HCl than with HP βCD . The different complexation constants found for different cyclodextrin molecules indicated that the derivative groups on cyclodextrins appear to play an important role in the incorporation of ritonavir into cyclodextrin cavity, since much higher complexation constants were obtained with RM βCD for ritonavir.

Dissolution studies of RTV-CD complexes: The dissolution parameters of pure ritonavir and complexes prepared with HP βCD and RM βCD by physical mixing, co evaporation, spray drying and freeze drying are represented in **Table 2**. Dissolution profiles of RTV complexes prepared with both cyclodextrins by freeze drying are represented in **Figure 2**. 25.86% ritonavir dissolution was observed in 60 min. Physical mixtures with both CDs showed 57.89% and 62.45% drug release. Co evaporated and spray dried products exhibited 64.45-64.49% and

64.45-72% respectively. Complete drug release was obtained with freeze dried complex of RTV-RM β CD within 10 min and RTV- HP β CD complex released 98.87% RTV within 20 min. A marked enhancement in dissolution was observed in complexes prepared by freeze drying technique. The order of drug release based on method of preparation was as follows, FD>SD>COE>PM. RM β CD complexes of ritonavir gave higher dissolution compared to HP β CD. This may be due to RM β CD reduced the interfacial tension between the solid particles of RTV and the dissolution medium, leading to a greater rate of dissolution.

The higher drug release observed with inclusion complexes prepared by freeze drying method may be due to better interaction of drugs and cyclodextrins. Similar way, the dissolution efficiencies were 9 fold higher with RM β CD and 8.16 fold higher with HP β CD in DE₁₀ compared to RTV. 8.2 fold with HP β CD and 1.96 fold with RM β CD higher values in %DE₁₀ of freeze dried products corresponding physical mixture were observed. High dissolution efficiency and low T₅₀ values were obtained with freeze dried complexes of RTV-RM β CD. Both RTV-CD complexes prepared by co evaporation showed higher DE₆₀, DE₁₀, DP₃₀ and low T₅₀ values compared to spray dried products. Spray dried products showed very slow release but co evaporated products showed initial quick release followed by slowing down of release of drug. This may be due to long heating time in co evaporation could help in complex formation. Higher DE₆₀ and DE₁₀ and lower T₅₀ were observed with freeze dried complexes of RTV-RM β CD and HP β CD complexes.

Bar diagrams of MDT values for RTV-CD complexes prepared by freeze drying are shown in **Fig 3**. A drastic decrease in MDT values was observed with freeze dried complexes of ritonavir with both HP β CD and RM β CD. Ritonavir had shown higher MDT value of 200.6. The reduction in MDT to 3.0 was found with RTV-RM β CD complex indicated that effective complexation of RTV with RM β CD has been taken place. Freeze drying method proved in enhancing dissolution rate.

The enhancement with freeze dried complexes may be due to increased interaction and conversion of drug into highly amorphous state in freeze drying method. Increase in dissolution parameters of RTV complex with RM β CD has been attributed to the formation of an inclusion complex in the solid state and formation of readily soluble complexes in the dissolution medium with these cyclodextrins. One way ANOVA was performed for freeze dried

complexes with two CDS using Graph Pad Prism 5.03 (Graph Pad Software, Inc.CA, USA) software (trial version) at a level of significance of p<0.05 and the results are shown in **Table 3** and **4**.

Null hypothesis was rejected in the one way ANOVA test as p value is less than 0.05. Therefore it indicated statistically a significant difference in the dissolution profiles of RTV complexes. The results showed that there is significant difference among RTV-CD complexes prepared with four methods compared to ritonavir alone. But, no significant difference was observed between methods physical mixing and co evaporation. T₅₀ values showed no significant difference as all complexes showed quick release compared to pure RTV [24].

FTIR studies: FTIR patterns of ritonavir complexes with HP β CD and RM β CD are represented in **Figure 4** and **5** respectively. When FTIR was performed, the drug spectrum indicated characteristic peaks at 3344.88 cm⁻¹ (N-H stretching amide group), 2951 cm⁻¹ (hydrogen-bonded acid within the molecule), 1714 cm⁻¹ (ester linkage), 1627.97 and 1525.74 cm⁻¹ (-C=C- stretching aromatic carbons).

The FTIR spectra of ritonavir cyclodextrin complexes showed considerable difference compared to pure ritonavir and cyclodextrin spectra and showed decrease in frequency of a specific peak which is generally seen in complexation. Physical mixtures showed broadening of peak and shifting from 3344.68 cm⁻¹ to 3338.89 cm⁻¹ (NH stretching), 2952 cm⁻¹ to 2922 cm⁻¹ hydrogen bonded acid peak, 1734 cm⁻¹ (ester linkage) and 1639 cm⁻¹ C=C group with HP β CD. RM β CD physical mixture showed peaks with less intensity and broadening at 3452.39 cm⁻¹, 2939 cm⁻¹, 1716 and at 1631 cm⁻¹ regions. Freeze dried complexes showed similar pattern with widening. Freeze dried complexes with RM β CD showed one small additional peak at 2407.24 cm⁻¹. Intermolecular hydrogen bonding was observed in ritonavir cyclodextrin complexes prepared by freeze drying, due to broad absorption at 3389.04 cm⁻¹. The broadening and widening of peak at 1730 cm⁻¹ region may be due to the interaction between cyclodextrins at the aldehydic link. The results confirmed the formation of stable hydrogen bonds and interaction at the aldehydic group was responsible in complex formation, giving increase in solubility of the drug.

DSC studies: The thermograms of ritonavir, cyclodextrins and the complexes are presented in **Figure 6** and **7**. The DSC thermogram of ritonavir showed an endothermic peak at 124.55°C, which is corresponding to its melting point. Thermogram of

HP β CD showed a broad endothermic peak from 60-80°C. In case of RTV and HP β CD complex prepared by physical mixture showed endothermic peak at 118.99°C and freeze dried complex showed broad endothermic peak at 107.19°C that strongly indicates strong interaction between ritonavir and HP β CD and hence confirms complexation.

Thermogram of RM β CD showed a broad endotherm in the range of 80-100°C which can be attributed to desolvation. Physical mixture of RTV and RM β CD showed broadening of endothermic peak in the range of 100.41°C and freeze dried complex showed a broad endothermic peak at 100.63°C due to formation of inclusion complex.

Nuclear magnetic resonance spectroscopy (NMR): ¹H-NMR spectra of ritonavir, cyclodextrins, physical mixtures and its freeze dried complexes were carried out and are presented in **Figure 8** and **9**. All the proton signals in the ¹H-NMR spectrum of the pure ritonavir are essentially located in a narrow range 1.0 to 5.2 ppm including the aliphatic amine peak at 3.2 ppm and other prominent peaks at 7.0 and 7.3 ppm. ¹H-NMR signals reported for HP β CD was found at 1.12, 3.8, 4.7 and 5.19 ppm. ¹H-NMR spectrum of RM β CD showed proton signals at 3.48, 3.78, and 4.65 ppm.

In NMR spectra of ritonavir, NH and OH groups must have undergone hydrogen bonding and other molecular forces of attraction which can be shown by NMR data by disappearance of many OH, NH signals between 3.6-5.2 ppm and disappearance of few signals near 7.0-7.3 ppm that indicates strong interaction between ritonavir and cyclodextrins. Around 3.0 ppm, N-H in ritonavir has intense peak where as in freeze drying of ritonavir with HP β CD and RM β CD complex, peak was disappeared indicating complexation between ritonavir and cyclodextrins.

X-ray diffraction studies: XRD of ritonavir, cyclodextrins, physical mixture and freeze dried complexes were performed and are shown in **Figure 10** and **11**. In the X-Ray diffractogram of ritonavir, peaks at a diffraction angle (2 θ) of 17°, 18° and 22° were present but the peak intensity was less in pure ritonavir which suggested that the drug is not completely crystalline but it is a mixture of both crystalline and amorphous forms. In the physical

mixture complexes, prepared with HP β CD and RM β CD, the presence of drug was revealed by peaks of low intensity at 21°. Peaks were not observed in freeze dried complexes of ritonavir with HP β CD and RM β CD indicating that the drug is completely converted into amorphous form. A similar behavior was previously reported for ketoprofen and ibuprofen [25].

Scanning Electron Microscopy (SEM): The SEM images of RTV, RTV complexes prepared with HP β CD and RM β CD by freeze drying method are shown in **Figure 12**. Ritonavir has appeared as needle shaped crystals. Freeze dried samples appeared as elongated particles. The change of the particle's shape and disappearance of needle structure of pure RTV in freeze dried complexes confirmed single phase existence and loss of needle structure indicates loss of crystallinity, thus supporting with XRD studies. The present study clearly indicated the superiority of RM β CD in improving the solubility and dissolution of poorly soluble drug RTV. Among the methods of preparation freeze drying technique was found to be more useful compared to other methods.

CONCLUSION

For the first time, comparative studies with different CDs like HP β CD and RM β CD for improving bioavailability of BCS class II drug, ritonavir were carried out. Simultaneously, the effect of method of preparation and influence of CD on enhancement of solubility and dissolution were studied. With the help of phase solubility studies, quantities of CDs were minimized in the preparation of drug-CD complexes. Formation of inclusion complexes was established with the help of FTIR, DSC, ¹H-NMR, XRD and SEM studies. Freeze drying method was found to be useful in improving the solubility and dissolution of ritonavir and the *in vivo* studies confirmed the improvement in oral bioavailability of these drugs. Further studies on these complexes may lead to reduction of the dose of the drug due to improved bioavailability.

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Table 1: Stability constants of RTV with two CDs

Cyclodextrin	$K_{1:1}$ (M^{-1})	Phase diagram
HP β CD	119.01	A _L Type
RM β CD	192.96	A _L Type

Table 2: Dissolution parameters of RTV inclusion complexes

Type of complex	Method of preparation	DE ₆₀	DP ₃₀	DE ₁₀	T ₅₀ (min)	MDT
Pure Drug	---	17.97±0.95	16.80±0.55	8.02±0.35	---	200.46±4.5
RTV-HP β CD	PM	46.76±0.58	52.13±0.56	8.40±0.30	27.5±0.15	
	COE	55.83±0.78	58.73±0.69	36.13±0.55	7.50±0.25	
	SD	50.81±0.75	42.96±0.45	27.97±0.56	35.0±0.25	
	FD	93.80±0.65	84.69±0.52	69.08±0.45	2.50±0.15	3.67±5.2
RTV-RM β CD	PM	41.05±0.86	59.29±0.55	37.50±0.91	8.50±0.12	
	COE	56.86±0.75	59.25±0.45	36.69±0.95	8.01±0.32	
	SD	58.81±0.42	61.54±0.60	31.44±0.85	25.00±0.15	
	FD	94.76±0.35	92.86±0.42	72.40±0.75	2.00±0.15	3.00±4.9

Table 3: ANOVA for dissolution parameters of RTV- HP β CD complexes

Null hypothesis: H₀= there is no significant difference in dissolution parameters between products prepared by various methods

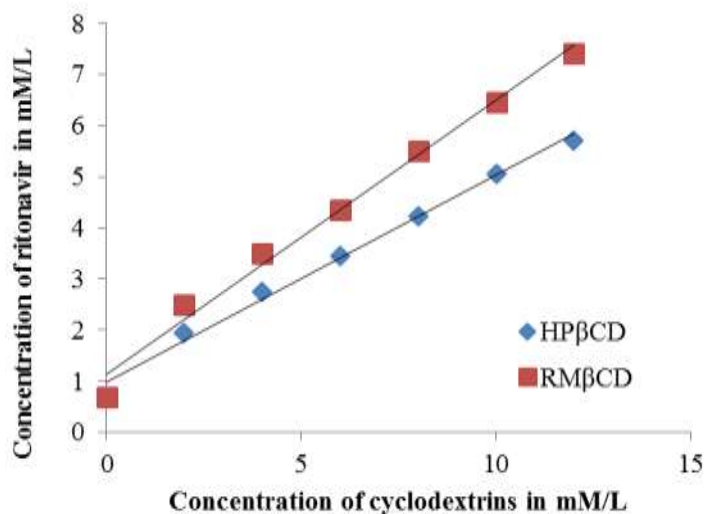
Parameter	Source of variation	df	SS	MS	F ratio	P
DE ₆₀	Between products	4	9466	2366	4743*	<0.0001
	Within products	10	4.989	0.4989		
	Total	14	9471			
DP ₃₀	Between products	4	9600	2400	4379*	<0.0001
	Within products	10	5.481	0.5481		
	Total	14	9605			
DE ₁₀	Between products	4	7572	1893	4181*	<0.0001
	Within products	10	4.527	0.4527		
	Total	14	7576			
T ₅₀	Between products	3	2189	729.7	21620*	
	Within products	8	0.270	0.0337		
	Total	11	2189			

*df: Degrees of freedom, SS: Sum of square, MS: Mean square
p: Significance level, *Significant difference (Null hypothesis rejected)*

Table 4: ANOVA for dissolution parameters of RTV- RM β CD complexes

Null hypothesis: H_0 = there is no significant difference in dissolution parameters between products prepared by various methods						
Parameter	Source of variation	df	SS	MS	F ratio	P
DE ₆₀	Between products	4	9483	2371	4960*	<0.0001
	Within products	10	4.780	0.4780		
	Total	14	9488			
DP ₃₀	Between products	4	11483	2871	6303*	<0.0001
	Within products	10	4.554	0.4554		
	Total	14	11488			
DE ₁₀	Between products	4	6372	1593	2552*	<0.0001
	Within products	10	6.242	0.6242		
	Total	14	6378			
T ₅₀	Between products	3	879.3	293.1	13027*	
	Within products	8	0.180	0.0225		
	Total	11	879.5			

*df: Degrees of freedom, SS: Sum of square, MS: Mean square
p: Significance level, *Significant difference (Null hypothesis rejected)*

**Figure 1: Phase solubility diagrams of RTV in two CDs**

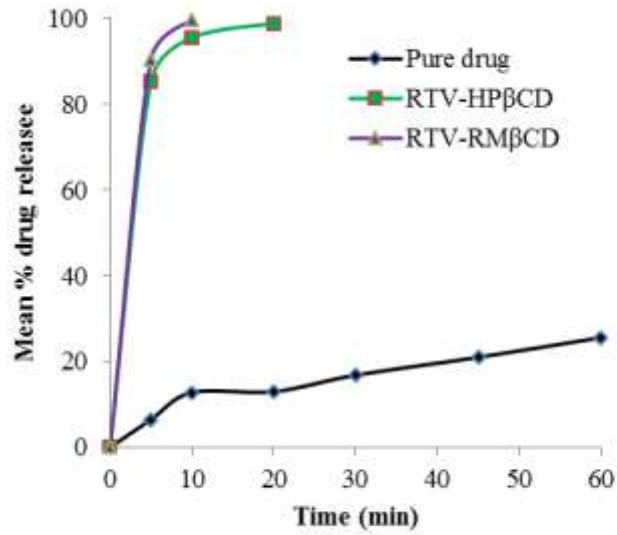


Figure 2: Comparative dissolution profiles of RTV inclusion complexes prepared by freeze drying

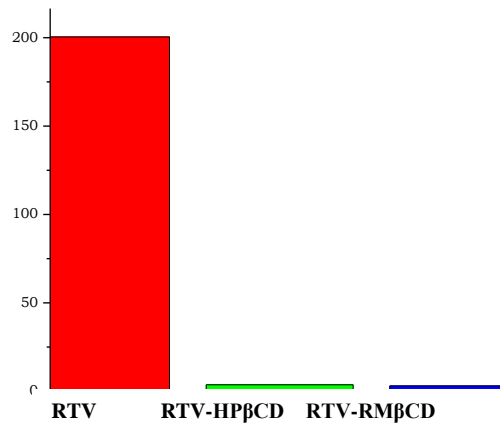


Figure 3: Bar diagram of MDT values for RTV-CD complexes prepared by freeze drying

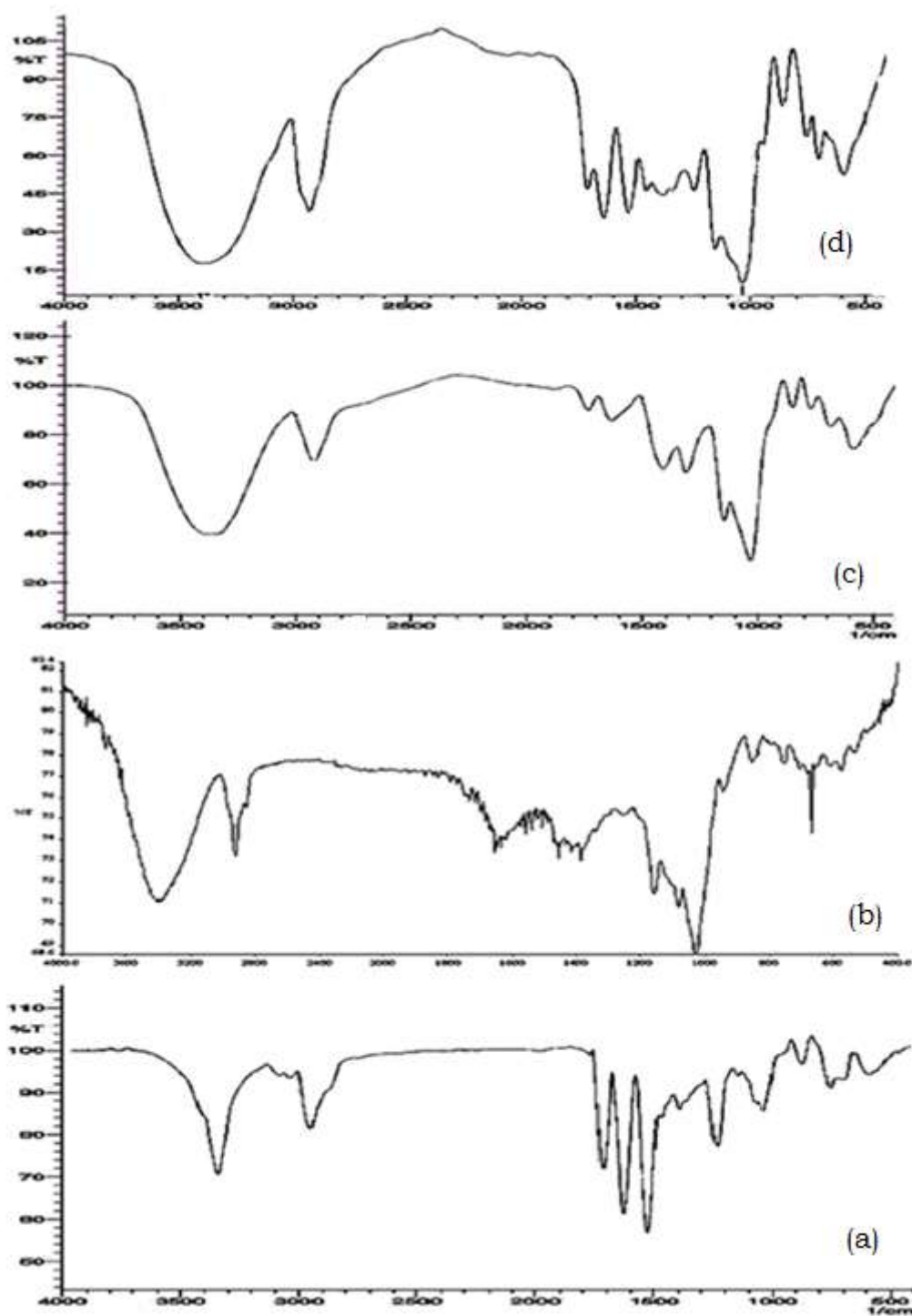


Figure 4: FTIR spectra of a) RTV b) HP β CD c) RTV-HP β CD physical mixture d) RTV-HP β CD complex prepared by freeze drying

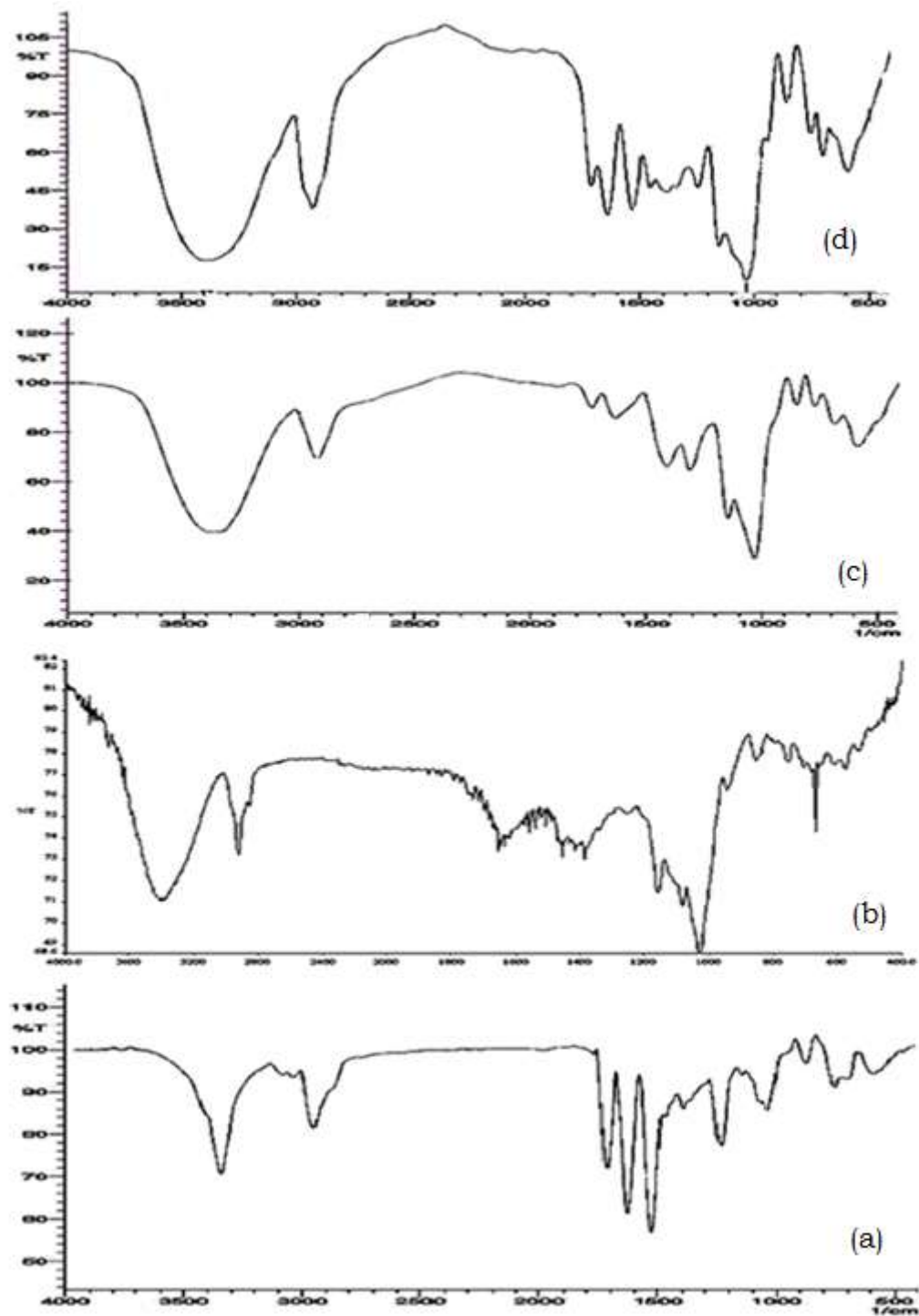


Figure 5: FTIR spectra of a) RTV b) RM β CD c) RTV-RM β CD physical mixture d) RTV- RM β CD complex prepared by freeze drying

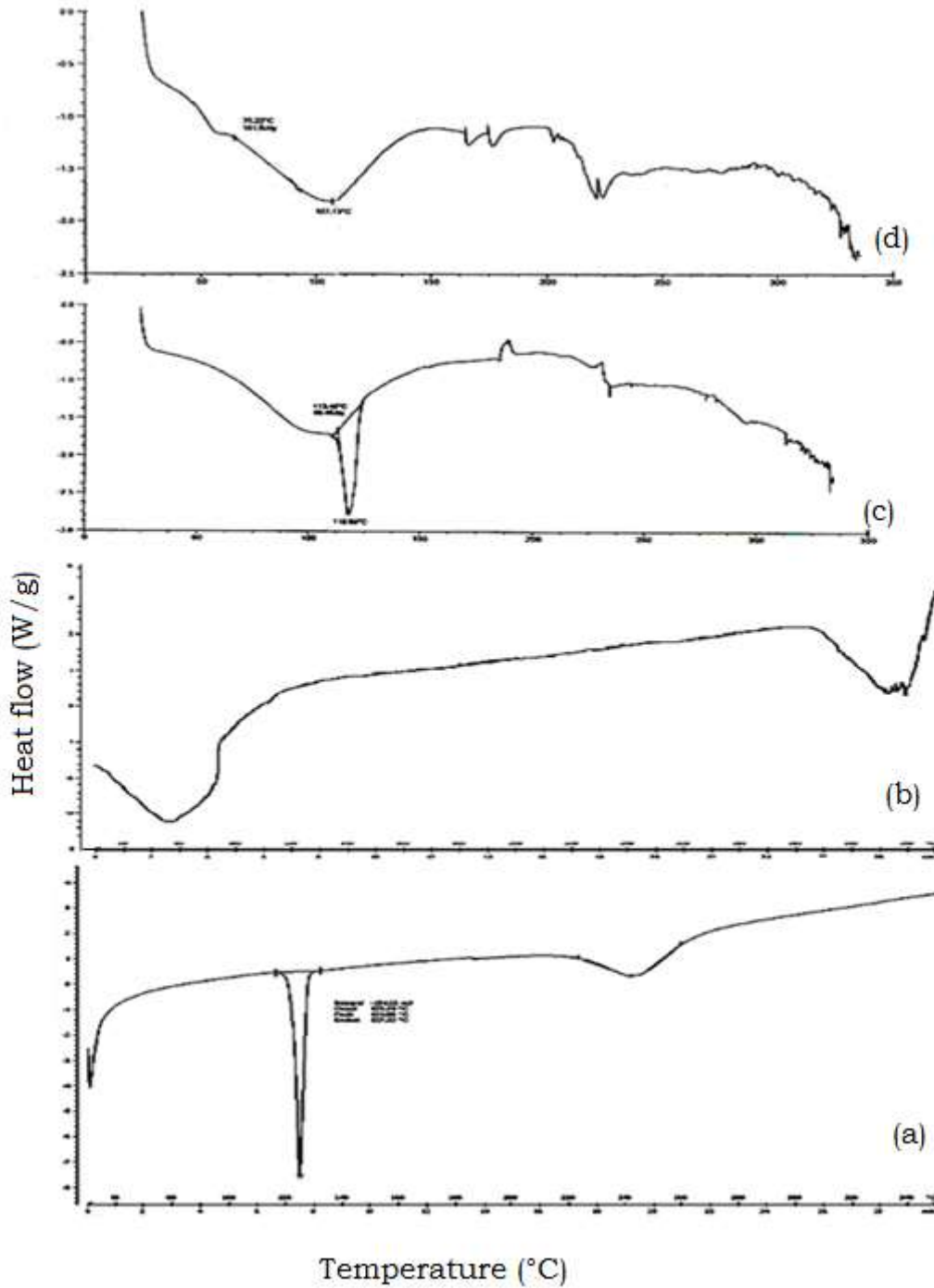


Figure6: DSC spectra of a) RTV b) HPβCD c) RTV- HPβCD physical mixture d) RTV- HPβCD complex prepared by freeze drying

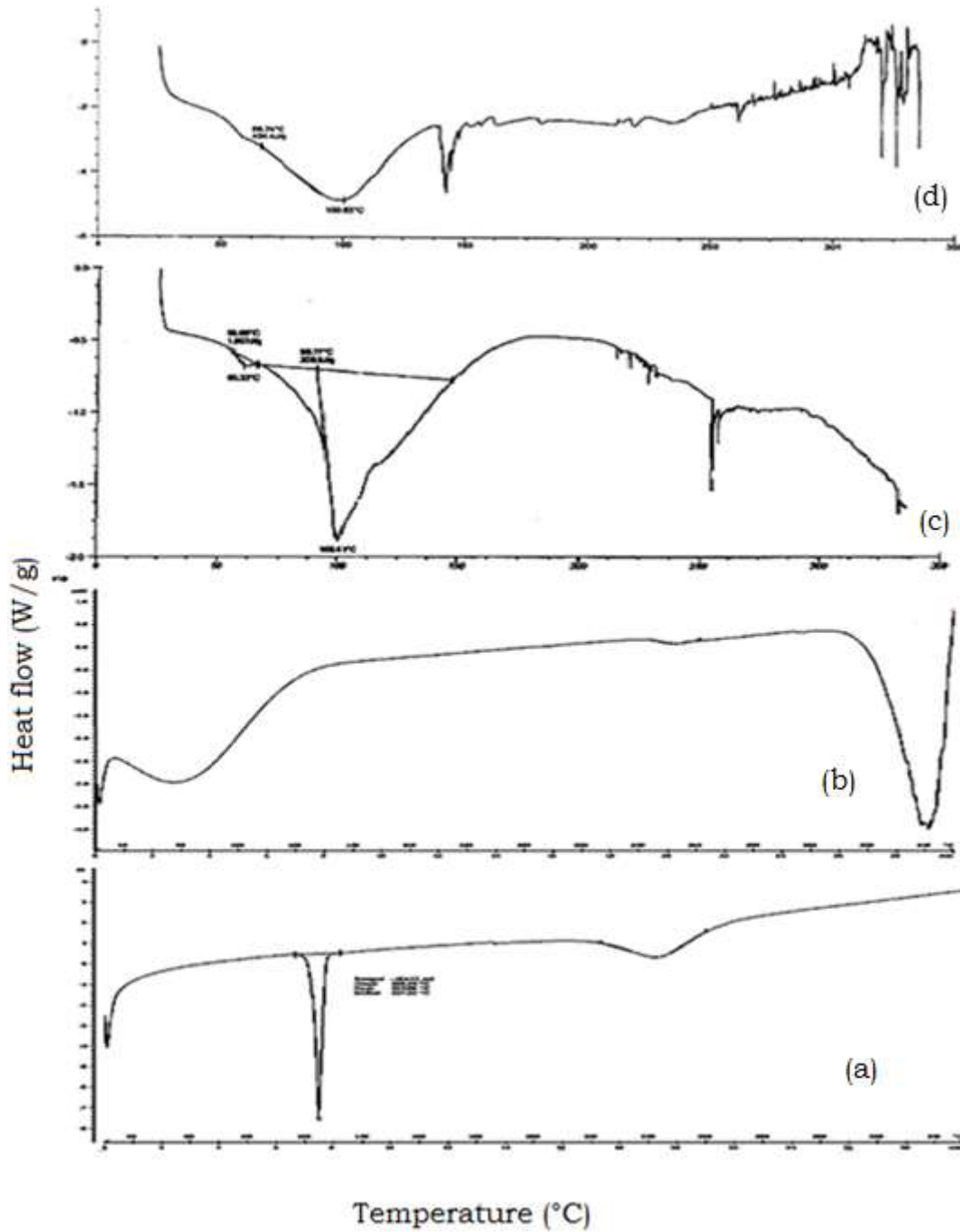


Figure 7: DSC spectra of a) RTV b) RM β CD c) RTV- RM β CD physical mixture d) RTV- RM β CD complex prepared by freeze drying

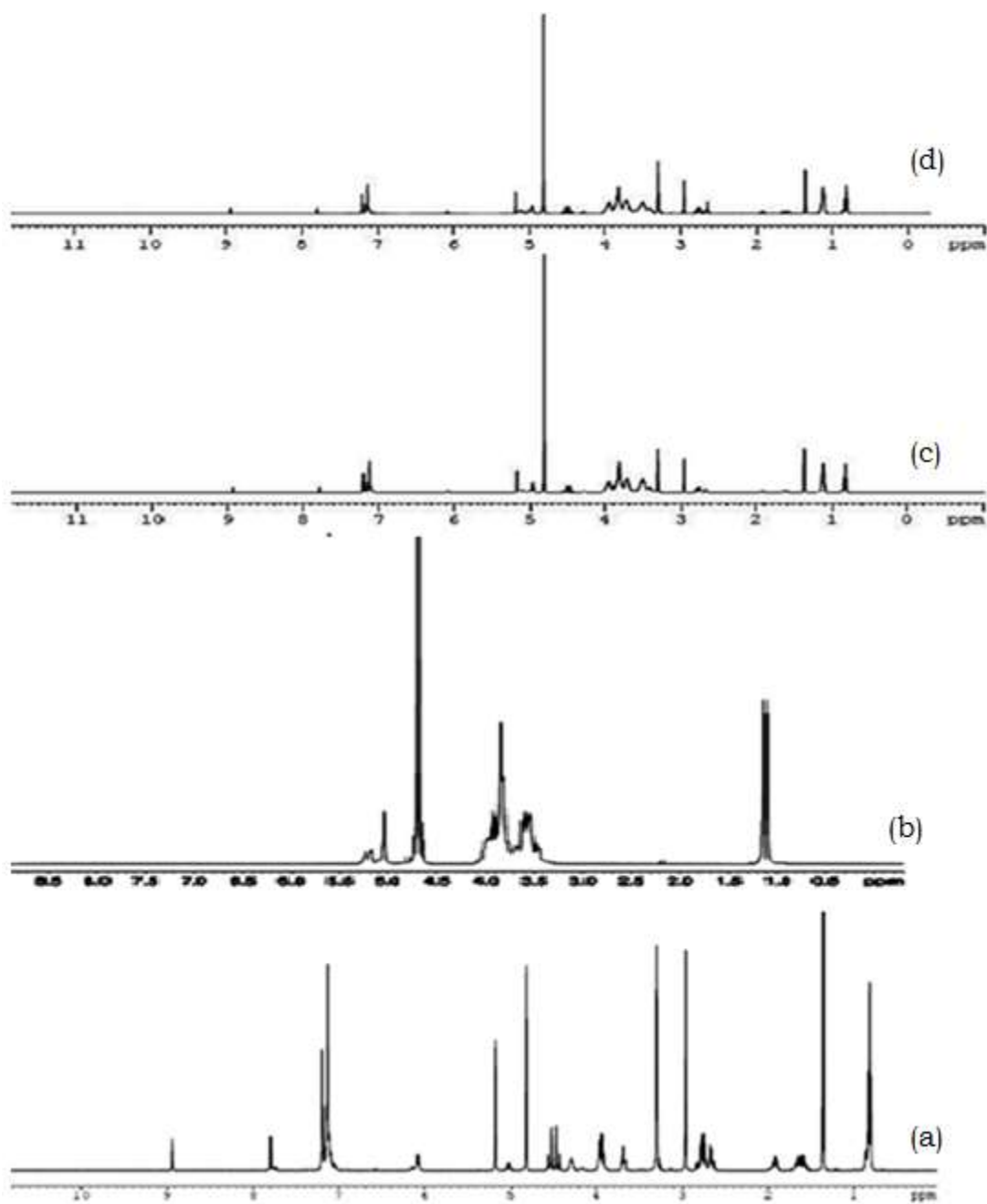


Figure 8: ¹H NMR spectra of a) RTV b) HPβCD c) RTV- HPβCD physical mixture d) RTV- HPβCD complex prepared by freeze drying

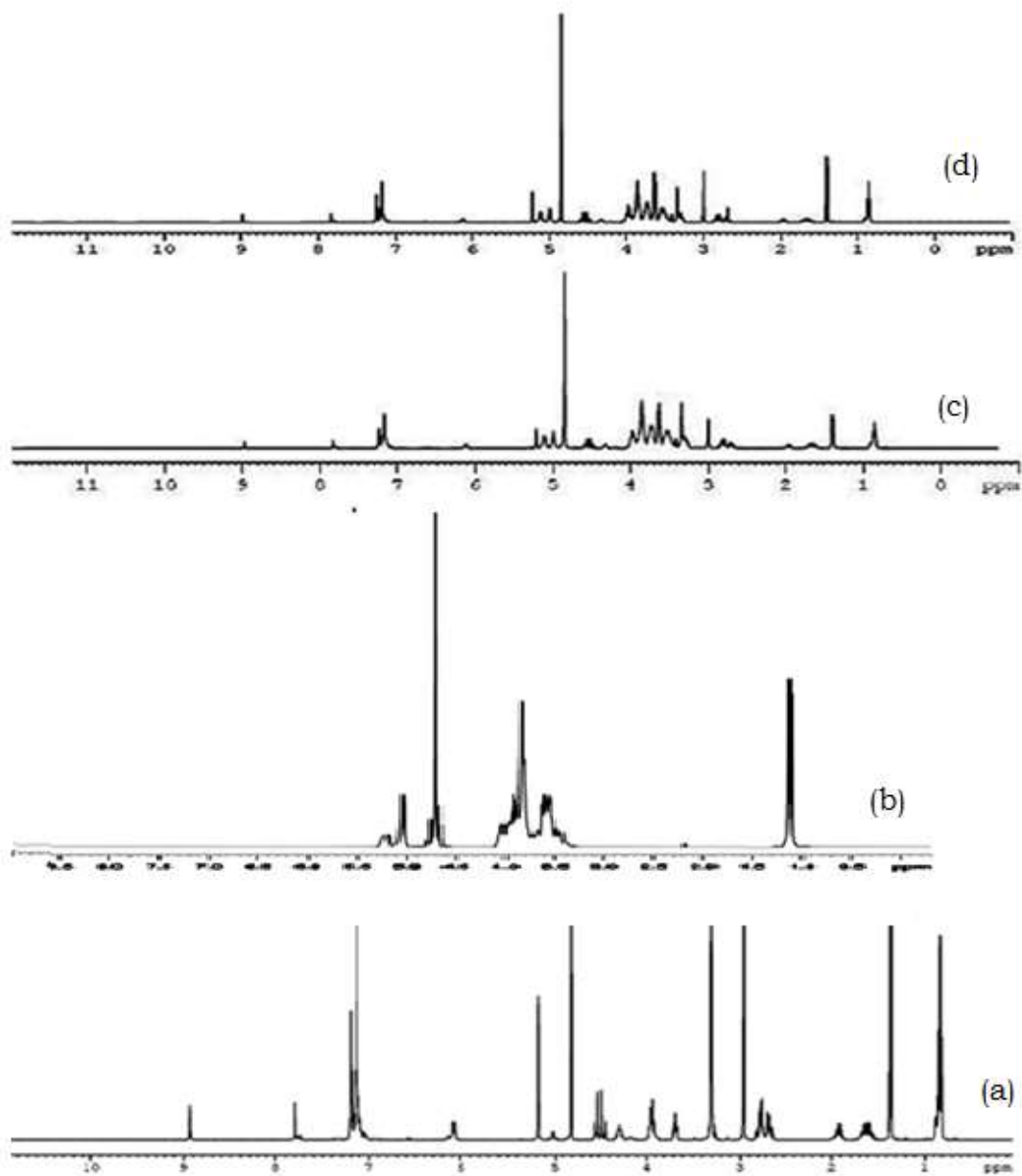


Figure 9: ¹H NMR spectra of a) RTV b) RMβCD c) RTV- RMβCD physical mixture d) RTV- RMβCD complex prepared by freeze drying

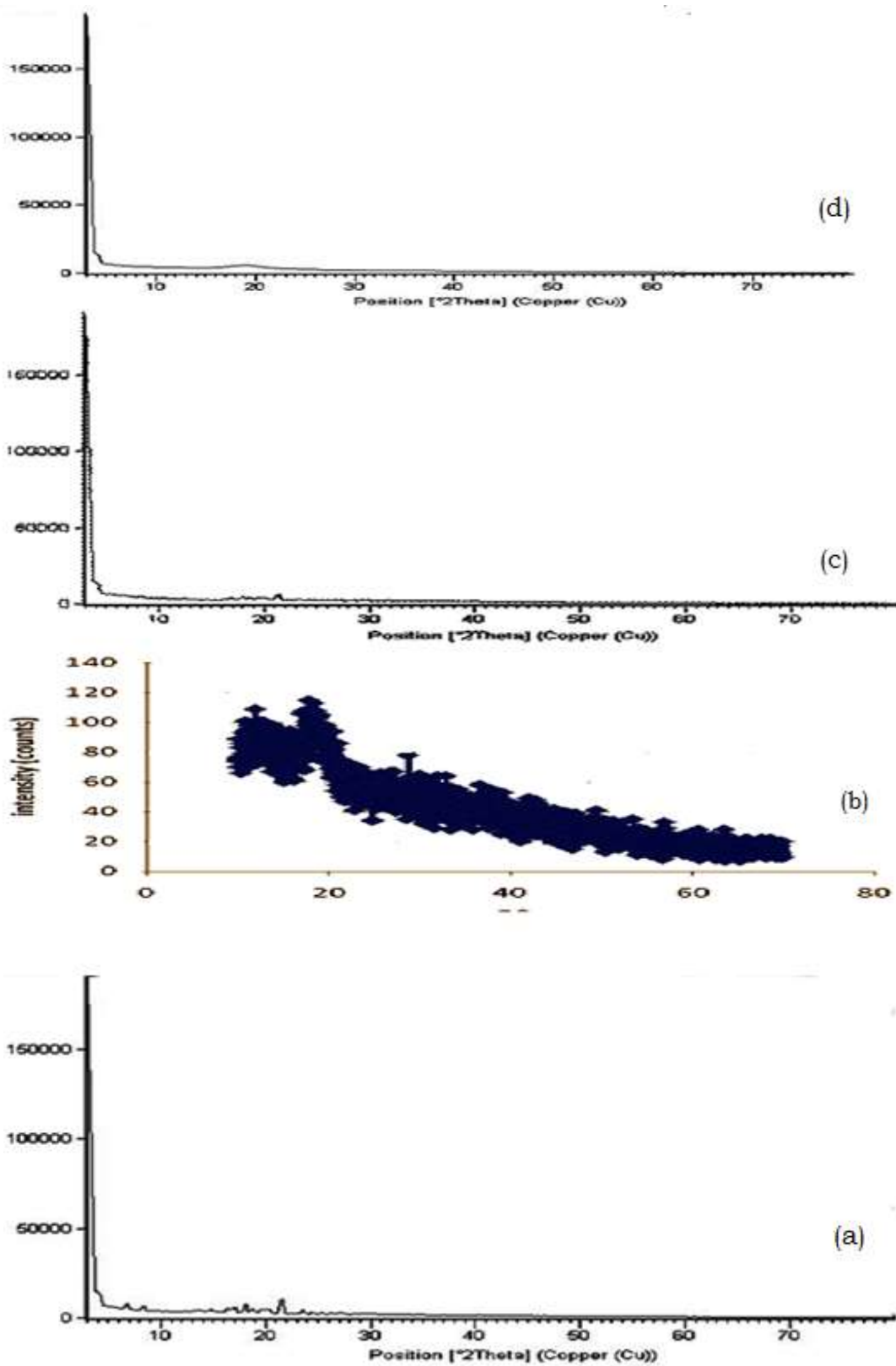


Figure 10: XRD patterns of a) RTV b) HPβCD c) RTV- HPβCD physical mixture d) RTV-HPβCD complex prepared by freeze drying

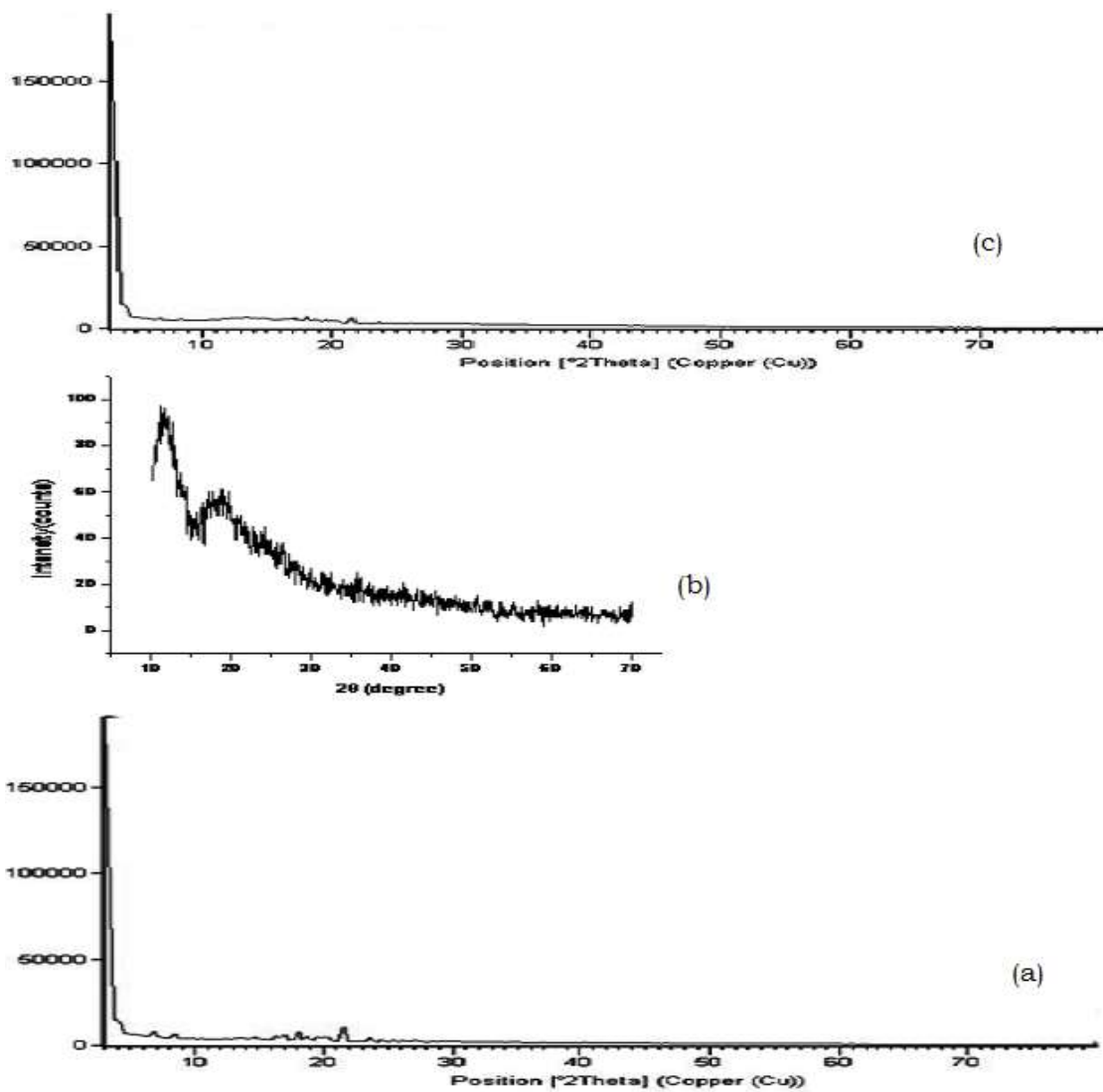


Figure11: XRD patterns of a) RTV b) RM β CD c) RTV- RM β CD physical mixture d) RTV- RM β CD complex prepared by freeze drying

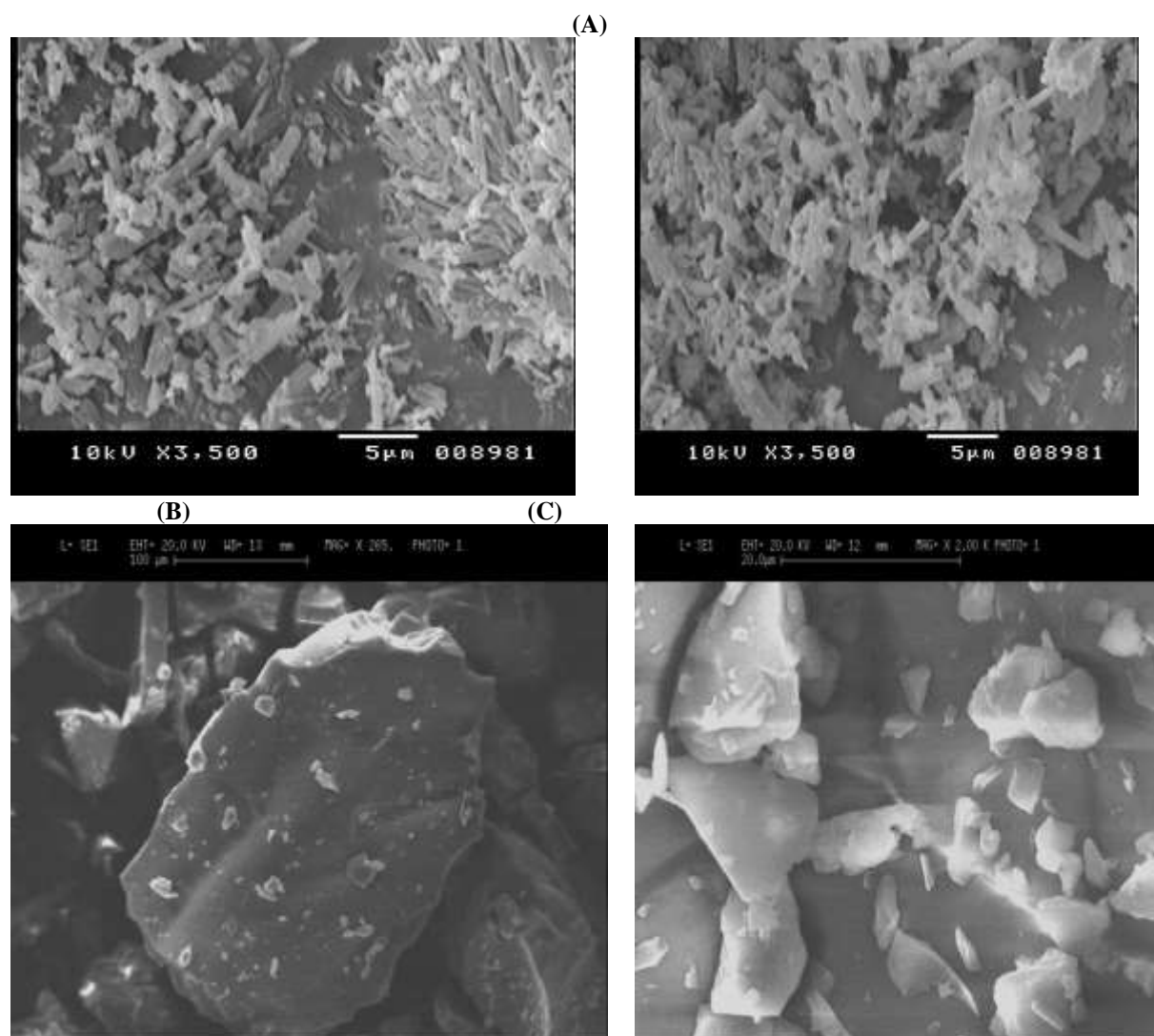


Figure 12: SEM images of A) RTV B) RTV- HP β CD complex C) RTV-RM β CD complex by freeze drying method

REFERENCES

1. Data Base on HIV/AIDS. Compilation prepared by World Health Organization (WHO)
2. Huisman MT, Smit JW, Wiltshire HR, Hoetelmans RMW, Beijnen JH, Schinkel AH. Mol Pharmacol, 2001; 59: 806–813.
3. Gao W, Kishida T, Kageyama M, Kimura K, Yoshikawa Y, Shibata N, Takada K. Antiviral Chem Chemother, 2002; 13(1): 17–26.
4. Farrar GAM, Mitchel H, Hooper FS, Malcolm SL. Br J Clin Pharmacol, 1994; 38: 162.
5. Pathak M, Musmade P, Dengle S, Karthik A, Bhat K, Udupa N. Eur J Pharm Sci, 2010; 41(3-4): 440-51.
6. Chowdary KPR, Nalluri BN. Drug Dev Ind Pharm, 2000; 26: 1217-1220.
7. Palmeiri GF, Angeli DG, Giovannucci G, Martelli S. Drug Dev Ind Pharm, 1997; 23: 27-37.
8. Palmieri GF, Wehrle P, Stamm A. Drug Dev Ind Pharm, 1993;19: 875–85.
9. Nagase Y, Hirata M, Wada K. Int J Pharm, 2001; 229: 163–72.
10. Moyano JR, Arias MJ, Gines JM, Perez JI, Rabasco AM. Drug Dev Ind Pharm, 1997; 23: 379–85.

11. Castillo JA, Canales JP, Garcia JJ, Lastres JL, Bolas F, Torrado JJ. Drug Dev Ind Pharm, 1999;25: 1241–48.
12. Diaz D, Escobar Lianos CM, Bernad MJB. Drug Dev Ind Pharm, 1999;25: 107–110.
13. Stella VJ, Rajewski R. United States Patent 5, 1992.
14. Higuchi T, Connors KA. Adv Anal Chem Instr, 1965; 4: 117-212.
15. Hirayama F, Uekama K. Methods of investigating and preparing inclusion compounds. In: Duchene D. (ed.) Cyclodextrins and their industrial uses. Paris, Editions de Sante :1987, pp. 131-172.
16. Hegdes AR. Chem Rev, 1998;98: 2035-2044.
17. Rajendrakumar K, Madhusudan S, Prahlad T. Eur J Pharm Biopharm, 2005; 60(1): 39-46.
18. Nagase Y, Hirata M, Wada K. Int J Pharm, 2001; 229: 163–72.
19. Miller LA, Carrier RL, Ahmed I. J Pharm Sci, 2007; 96: 1691-1707.
20. Salustio PJ, Feio G, Figueirinhas JL, Pinto JF, Cabral Marques HM. Eur J Pharm Biopharm, 2009 ;71(2): 377-86
21. Orienti I, Cerchiara T. Int J Pharm, 1999;190: 139-53.
22. Thoresteinn M, Brewster ME. J Pharm Sci, 1996; 85(10): 1017-26.
23. Shilpi S, Mushir A, Sanjula B, Alka A, Anil Kumar, Javed A. AAPS Pharm Sci Tech, 2010; 2(2): 518-527.
24. Bolton S and Bon C. Nonparametric methods. Pharmaceutical statistics: Practical and clinical applications. 5th ed, New York; Informa Healthcare: 2010, pp.390-424.
25. Mura P, Bettinetti GP, Manderioli A, Faucci MT, Bramanti G, Sorrenti M. Int J Pharm, 1998;166: 189–203.