Solid dispersion using Monoamino glycyrrhyzinate pentahydrate

4.1. Introduction

Glycyrrhizin, which is also known as glycyrrhizinic acid, is an oleanane-type triterpene glycoside whose use as sweeteners has been reviewed. This compound is extracted from the rhizomes and roots of licorice (*Glycyrrhiza glabra* L. Fabaceae) and other species in genus Glycyrrhiza (Annonymus, 1998; Kinghom and Compadre, 2001). In United states, ammoniated glycyrrhizin is included in the generally recognized as safe (GRAS) list of approved natural flavoring agent (Smith et al., 1996). Ammoniated glycyrrhizin has been rated as approximately 50 times the sweetness of sucrose (Hanrahan, 2001; Kinghom and Compadre, 2001). Monosodium glycyrrhyzinate together with flavors has been used to mask the bitter taste of guaifenesin (Fawzy et al., 1998) and extract containing pogostemi herba (Okudaira and Kakuta, 1997).

Many reported techniques mask the bitterness by controlling drug release at salivary pH (6.3-7.2). However it is a major challenge to develop palatable rapid disintegrating tablets (RDTs) with improved drug release. Thus in the present study an attempt has been made to formulate taste masked RDTs with improved dissolution so as to prepare a "patient-friendly dosage form" using mono amino glycerrhyzinate pentahydrate (GLY) as the hydrophilic carrier. Furthermore, the study undertakes to investigate solid-state characterization, and attempts to see the possible mechanism of taste masking and improved dissolution rate.

4.2. Artemether (ARM)

4.2.1. Experimental

4.2.1.1. Materials

Mono amino glycyrrhyzinate pentahydrate (GLY) was a gift from Sami labs, Bangalore. Methanol was purchased from Qualigens Fine Chemicals (Mumbai, India) and was used as received. Sodium hydroxide, hydrochloric acid, potassium chloride and potassium dihydrogen phosphate were purchased from S. D. Fine-Chem Ltd., (Mumbai, India) and were used as received.

4.2.1.2. Preparation of ARM-GLY solid dispersion

The solid dispersion of ARM and GLY in 1:0.5 and 1:1M was prepared using solvent evaporation method. Accurately weighed quantity of ARM (50 mg in 5 mL) was dissolved in methanol. Previously dissolved GLY (141 mg in 10 mL) in water was added with constant stirring on magnetic stirrer till solvent evaporates. The solid obtained after evaporation, was further dried to a constant weight in hot air oven at 70^0C for 48 hours. Dried powder was passed through sieve no. 44 and stored in desiccator until further evaluation.

The physical mixtures of ARM and GLY in 1:0.5 and 1:1M were prepared by mixing individual components geometrically that had previously been sieved through sieve no. 44, together with a spatula.

4.2.1.3. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

4.2.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

4.2.1.5. Tablet Formulation and Characterization

RDTs containing equivalent of 50 mg of ARM were compressed on an 8-station single rotary tabletting press (GMC, Mumbai, India) using an 9-mm round shaped flat punch with break line by direct compression technique (Mishra et al., 2006).

Two different superdisintegrants, croscarmellose sodium and crospovidone were tried to achieve rapid disintegration of tablets. Granular microcrystalline cellulose and lactose were used as diluents. Prepared RDTs were evaluated as mentioned in 3.2.1.6.

4.2.1.6. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

4.2.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

4.2.1.8. Mini-column Method

Mini-column method was carried out as mentioned in 3.2.1.9.

4.2.1.9. Stability studies

Stability studies were carried out as mentioned in 3.2.1.10.

4.2.2. Result and Discussion

4.2.2.1. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR studies were performed to detect the possible molecular interaction between ARM and GLY in the solid dispersion system. FTIR spectra of ARM, GLY, physical mixture and solid dispersion in 1:1M are presented in Figure 4.1. The characteristic peak of ARM at 2873 cm^{-1} is assigned to C-H stretching vibration in CH_3, CH_2. In addition the absorption peak at 2844 cm^{-1} can be assigned to C-H stretching vibration in C-O-CH_3. The peak at 1137cm^{-1} can be assigned to C-O stretching vibration in C-O-C.

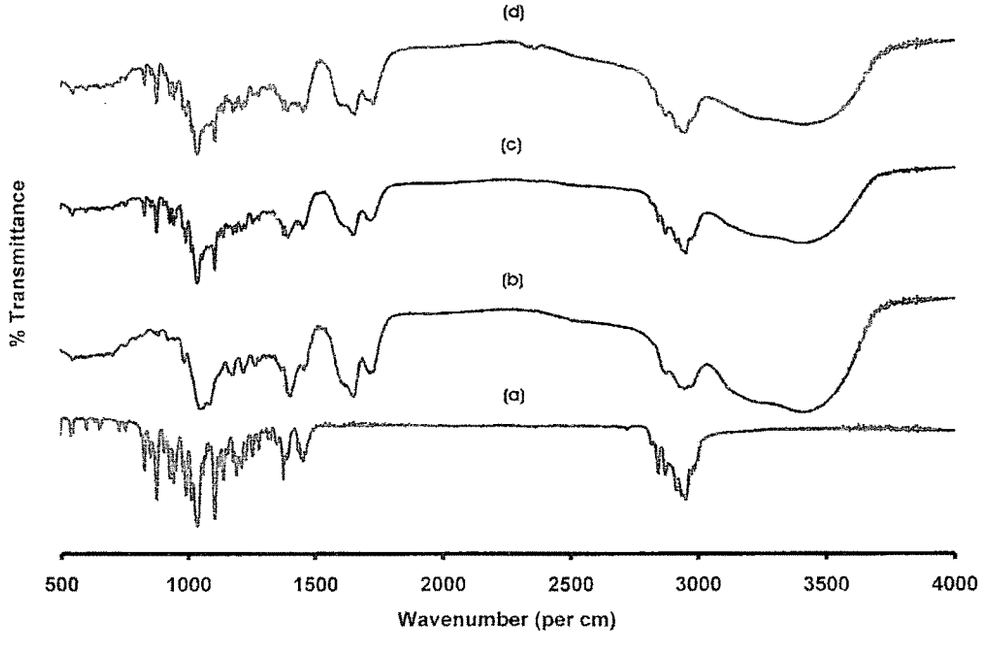

Figure 4.1. FT-IR spectra of (a) ARM, (b) GLY, (c) physical mixture and (d) solid dispersion

The peaks at 2953 and 2916 cm^{-1} are assigned to C-H stretching in $-CH_3$. All the above characteristic peaks appear in the spectra of binary systems at same wavenumber indicating no modification or interaction between the drug and carrier.

4.2.2.2. Differential Scanning Calorimetry (DSC)

Thermal behavior of ARM, GLY, physical mixture and solid dispersion in 1:1M are depicted in Figure 4.2. The pure ARM shows a sharp endothermic peak at 87.94^{0}C, followed by exothermic peak at 180.28^{0}C. The characteristic endothermic peak corresponding to melting peak of ARM was broaden and shifted towards lower temperature, with reduced intensity in both physical mixtures as well as solid dispersions. This could be attributed to higher GLY concentration and uniform distribution of drug in the crust of GLY, resulting in complete miscibility of molten drug in GLY.

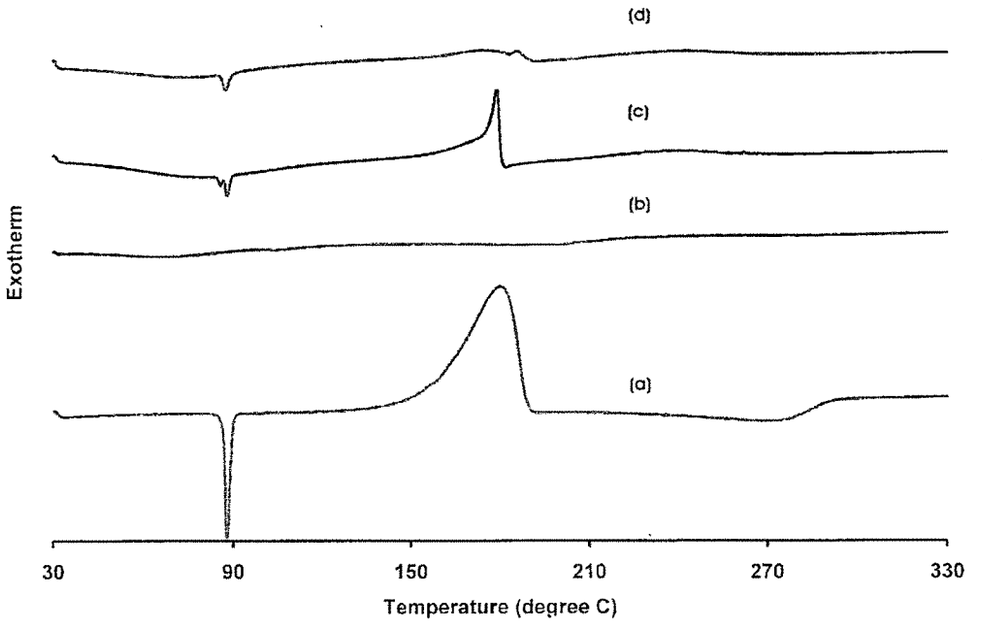

Figure 4.2. DSC curve of (a) ARM, (b) GLY, (c) physical mixture and (d) solid dispersion

4.2.2.3. Tablet Preparation and Characterization

To formulate RDTs of ARM, the 1:1M binary mixture was selected, based on its bitterness score. The formula of different tablets prepared is summarized in Table 4.1. Tablet characteristics of RDTs are summarized in Table 4.2.

Table 4.1 Formulation of RDTs

Drug/Excipients	RDT41	RDT42	RDT43	RDT44	RDT45	RDT46
ARM (mg)	-	-	-	-	50	50
GLY (mg)	-	-	-	-	-	141
Solid dispersion eq. to 50 mg ARM (mg)	191	191	191	191	-	-
Microcrystalline cellulose (Avicel PH 302) (mg)	185	-	185	-	326	185
Lactose (Lactopress) (mg)	-	185	-	185	-	-
Croscarmellose sodium (mg)	-	-	20	20	20	20
Crospovidone (mg)	20	20	-	-	-	-
Magnesium stearate (mg)	4	4	4	4	4	4

Table 4.2. Physical properties of RDTs

Parameters	RDT41	RDT42	RDT43	RDT44	RDT45	RDT46
Weight (mg) ± SD*	398.47 ± 1.78	399.12 ± 1.44	401.28 ± 1.29	398.86 ± 0.77	402.71 ± 1.41	400.63 ± 1.62
Disintegrating Time (sec)	56-63	42-49	28-32	53-58	32-35	37-42
Hardness (kg)	2.6-2.7	3.1-3.2	3.1-3.2	3.2-3.3	3.5-3.6	3.2-3.3
Friability (%) ± SD*	0.63 ± 0.14	0.59 ± 0.07	0.54 ± 0.09	0.56 ± 0.12	0.48 ± 0.08	0.51 ± 0.09

**Values represent the mean ± SD of 3 experiments.*

RDTs containing croscarmellose sodium and granular microcrystalline cellulose (RDT43) showed the fastest disintegration (28-32 seconds) with improved hardness and friability. The formula of optimized RDT was used to prepare RDT of pure ARM (RDT45) and physical mixture of ARM and GLY (RDT46).

4.2.2.4. *In vitro* drug release Studies

Drug release profiles of RDTs prepared from ARM, physical mixture and solid dispersion are presented in Figure 4.3. It is evident that the solid dispersion technique has improved the dissolution rate of ARM to a great extent. Table 4.3 summarizes % drug dissolved in 5 minutes (DP5), dissolution efficiency at 15 minutes (DE15), and dissolution efficiency at 60 minutes (DE60) for ARM and its binary systems with GLY. RDT43 showed excellent dissolution efficiency (DE60 = 97.83%) and rapid dissolution (DP5 = 91.77%) at pH 6.8. Similarly RDT43 showed improved dissolution efficiency (DE60 = 68.55%) and rapid dissolution (DP5 = 28.89%) at pH 1.2. This indicates increased availability of ARM in g.i.t. When compared with pure ARM formulation, tablets formulated with the binary mixture clearly perform better and a significant enhancement in dissolution characteristics was observed. RDT46 also showed improvement in dissolution to a significant extent when compared with drug alone.

Table 4.3. Percent dissolution and dissolution efficiency of ARM from binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
RDT45	8.50	12.00	10.32	14.91	25.10	34.89
RDT46	17.80	65.80	24.76	69.94	55.18	90.75
RDT43	28.89	91.77	35.49	91.48	68.55	97.83

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

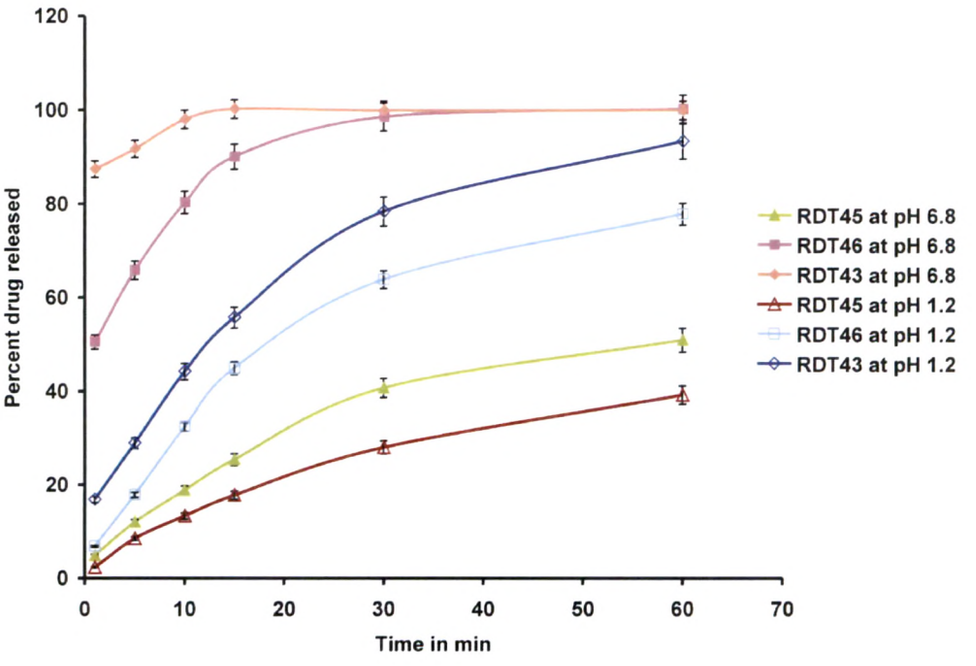

Figure 4.3. Dissolution of RDTs prepared from ARM, physical mixture and solid dispersion

This enhancement of dissolution of ARM from RDTs can be ascribed to several factors. It has been reported that GLY has structural similarity to triterpenes and show surfactant like action (Motlekar et al., 2006; Polyakov et al., 2005). Increased wettability and dispersibility are the main reasons for improvement of ARM dissolution (Ford, 1986).

RDTs prepared from physical mixing of ARM with GLY resulted in greater wetting and increased surface available for dissolution by reducing interfacial tension between hydrophobic drug and dissolution media. Furthermore, RDTs prepared from solid dispersion, results in uniform distribution of ARM in the GLY crust in a highly dispersed state. Thus, when such a system comes in contact with an aqueous dissolution medium, the hydrophilic carrier dissolves and results in precipitation of the embedded drug into fine particles, which increase the dissolution surface available (Modi and Tayade, 2006).

4.2.2.5. Gustatory sensation test

Bitterness evaluation results made by the consents of trained persons are listed in Table 4.4. No bitterness was imparted in solid dispersion with reference to pure drug. It has been reported that the bitter drug like ARM seem to bind G-protein coupled receptors, present on the apical taste cell membrane and produce bitterness (Yamamoto et al., 1998). GLY is astringent and hence might be directly interacting with G-proteins and paralyzing them, resulting in reduced taste transduction and thus reduced bitterness score. Further the sweet taste of GLY imparted additive effect.

ARM was uniformly distributed in the crust of GLY, which avoids contact of ARM with G-protein coupled receptors. Further the sweet taste of GLY imparted additive effect (Kinghom and Compadre, 2001). This may results in complete taste masking of ARM in GLY solid dispersion. Though the physical mixing of ARM with GLY brings the drug in close contact with carrier, ARM was not uniformly distributed in GLY as that of solid dispersion. This may not result in complete taste masking of ARM in GLY physical mixture.

Table 4.4. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Pure ARM						1	17	2
Physical Mixture		4	13	3				
Solid Dispersion	19	1						
RDT45						3	13	4
RDT46		2	16	2				
RDT43	20							

RDT45 was rated as moderate to strong bitter by 15% and strongly bitter by 65% and very strongly bitter by 20% of volunteers of panel. RDT43 was rated as tasteless by 100% of volunteers of panel.

4.2.2.6. Mini-column Method

The tapping frequency of column and flow rate of test solution were assumed to influence the mini-column method results. The tapping frequency was set at 30, 10 and 3 times. The effect of tapping frequency on the release rate is shown in Figure 4.4.

When the tapping frequency was 30 times, no release of ARM from compact powdered mass was observed. This might be because of the difficulty for the test solution to penetrate the compact powdered mass. The release rate increased, when the tapping frequency was 3 times. However the release rate decreased, when the tapping frequency was 10 times. The release rate decreased as the tapping frequency increased.

Next, the tapping frequency was set at 3 times and flow rates at 1, 1.3 and 1.5 mL/min. The results are shown in Figure 4.5. When the flow rate increased the release rate decreased. This was probably due to a delay in liquid penetration into the matrix, since the liquid flow rate on the matrix surface increased. The flow rate and tapping frequency were optimized, based on maximum drug release, at 1 mL/min and 3 times, respectively. Optimized flow rate and tapping frequency were further applied to RDT45 and RDT46.

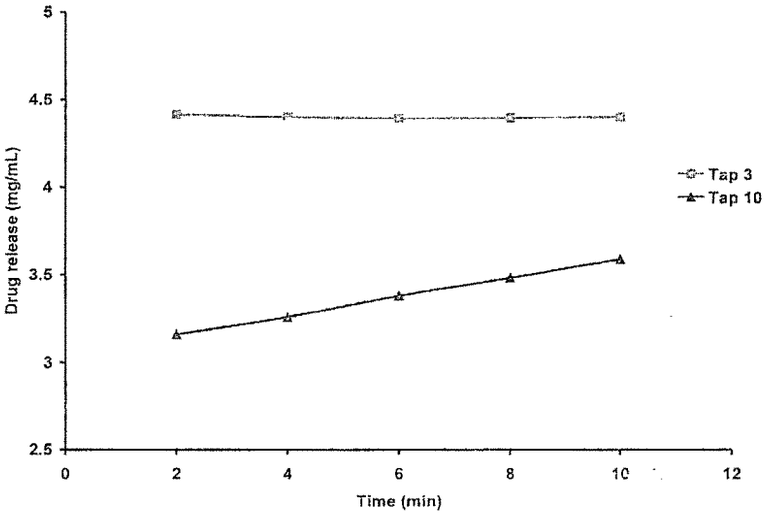

Figure 4.4. Effect of tapping frequency on the release of ARM from RDT43 with 1 mL/min flow rate

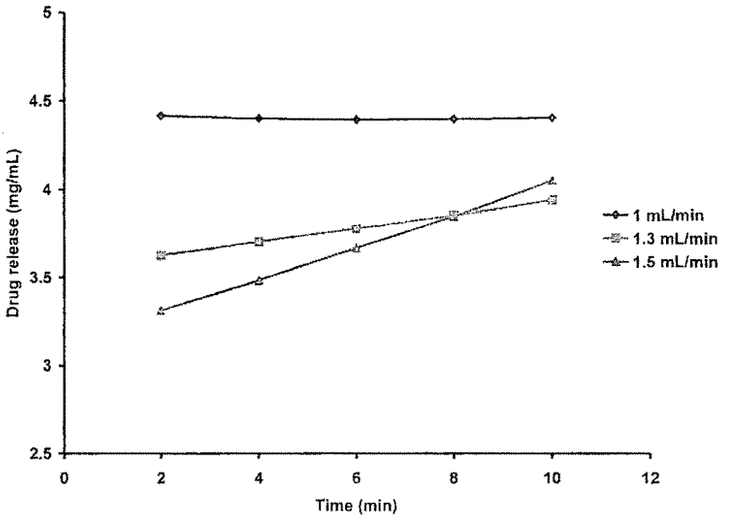

Figure 4.5. Effect of flow rate on the release of ARM from RDT43 with 3 times tapping frequency

Table 4.5 shows the results of the sensory tests and amount of ARM released after 2 min interval with the mini-column method. RDT43 sensed no bitterness with 4.42 mg/mL. The value (0.33 mg/mL) was about 14 times larger than that of pure ARM.

Table 4.5. Relationship between amount of release and results of sensory test of RDTs using mini-column method

Formulations	Time (min)				
	2	**4**	**6**	**8**	**10**
RDT45 (mg/mL) ± SD*	0.31 ± 0.07	0.33 ± 0.06	0.33 ± 0.09	0.32 ± 0.06	0.32 ± 0.08
Bitterness score	3	3	3	3	3
RDT46 (mg/mL) ± SD*	3.16 ± 0.08	3.14 ± 0.09	3.15 ± 0.04	3.12 ± 0.06	3.09 ± 0.05
Bitterness score	1	1	1	1	1
RDT43 (mg/mL) ± SD*	4.41 ± 0.06	4.40 ± 0.07	4.39 ± 0.04	4.39 ± 0.07	4.40 ± 0.07
Bitterness score	0	0	0	0	0

**Values represent the mean ± SD of 3 experiments.*

4.2.2.7. Stability studies

For the RDT43, the similarity factor (f2) was calculated by a comparison of the dissolution profiles at accelerated condition with the control at the initial condition. The f2 values ranged from 87 to 95 with a 2%-4% average difference at pH, 1.2 and 6.8. Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data showed little change over time, and so a shelf life up to 1327 days (44.23 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

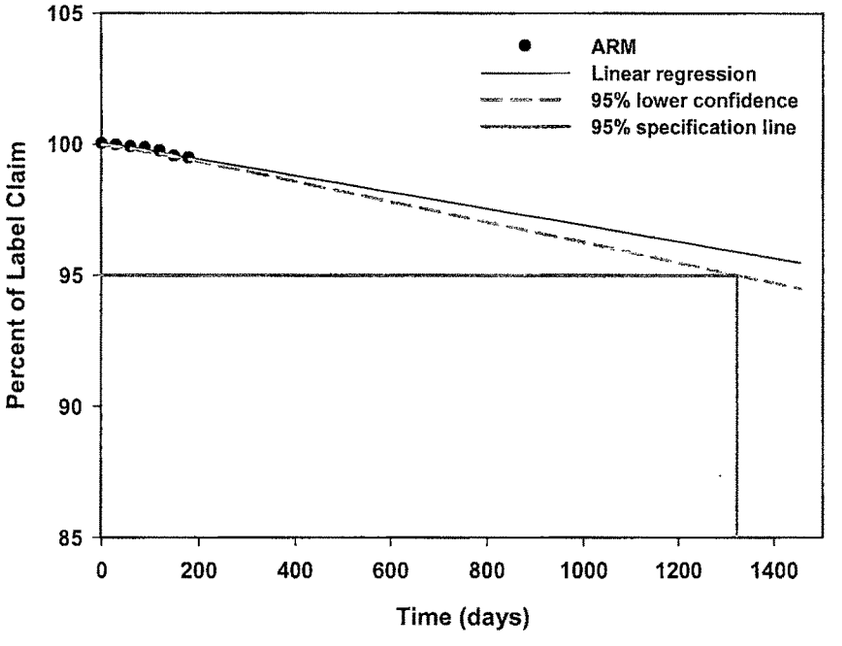

Figure 4.6. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated the complete taste masking of ARM with improved dissolution by solid dispersion technique. The FTIR and DSC studies indicated no interaction of ARM, at the molecular level, in GLY solid

dispersion. ARM-GLY solid dispersion along with use of superdisintegrant could be considered for formulation of a stable RDTs of ARM. The use of mini-column method, an *in vitro* model, could be applicable to evaluate bitterness score, which simulates the disintegration of tablet in oral cavity.

4.3. Mefloquine Hydrochloride (MFL)

4.3.1. Experimental

4.3.1.1. Materials

Materials used were as mentioned in 4.2.1.1.

4.3.1.2. Preparation of MFL-GLY solid dispersion

The solid dispersion of MFL and GLY in 1:0.5, 1:1, 1:1.5M was prepared using solvent evaporation method. Accurately weighed quantity of MFL (500 mg in 5 mL) was dissolved in methanol. Previously dissolved GLY (1518 mg in 10 mL) in water was added with constant stirring on magnetic stirrer till solvent evaporates. The solid obtained after evaporation, was further dried to a constant weight in hot air oven at 100^0C. Dried powder was passed through sieve no. 44 and stored in desiccator (Tarsons Products Pvt. Ltd, India) until further evaluation.

The physical mixtures of MFL and GLY in 1:0.5, 1:1, 1:1.5M were prepared by mixing individual components geometrically that had previously been sieved through sieve no. 44, together with a spatula.

4.3.1.3. Fourier transform infra-red spectroscopy (FTIR)

FT-IR study was carried out as mentioned in 3.2.1.3.

4.3.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

4.3.1.5. Tablet Formulation and Characterization

RDTs containing equivalent of 250 mg of MFL were compressed on an 8-station single rotary tabletting press (GMC, Mumbai, India) using a 16-mm x 8-mm caplet shaped punch by direct compression technique.

Two different superdisintegrants, croscarmellose sodium and crospovidone were tried to achieve rapid disintegration of tablets. Granular microcrystalline cellulose and lactose were used as diluents. Prepared RDTs were evaluated as mentioned in 3.2.1.6.

4.3.1.6. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

4.3.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

4.3.1.8. Mini-column Method

Mini-column method was studied as mentioned in 3.2.1.9.

4.3.1.9. Stability studies

Stability studies were carried out as mentioned in 3.2.1.10.

4.3.2. Results and discussion

4.3.2.1. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of MFL, GLY, physical mixture and solid dispersion in 1:1.5M are presented in Figure 4.7. The spectrum of MFL is dominated by N-H stretching vibration at 3226 cm^{-1}, quinine ring stretching vibration at 1603, 1363, 1111, and 1069 cm^{-1}, CF_3 stretching vibration at 1316 cm^{-1}. The peaks at 2875 and 2918 cm^{-1} can be assigned to C-H bridge and CH_2 respectively. The peak at 1555 cm^{-1} can be assigned to C=N/C=C. The peaks at 1288 and 1055 cm^{-1} can be assigned to C-N and piperidine ring, respectively. The peak at 1174 cm^{-1} may be due to the C-C/N-H stretching vibration. All above characteristic

peaks were appear in the spectra of physical mixture and solid dispersion at same wavenumber indicating no interaction between the drug and carrier.

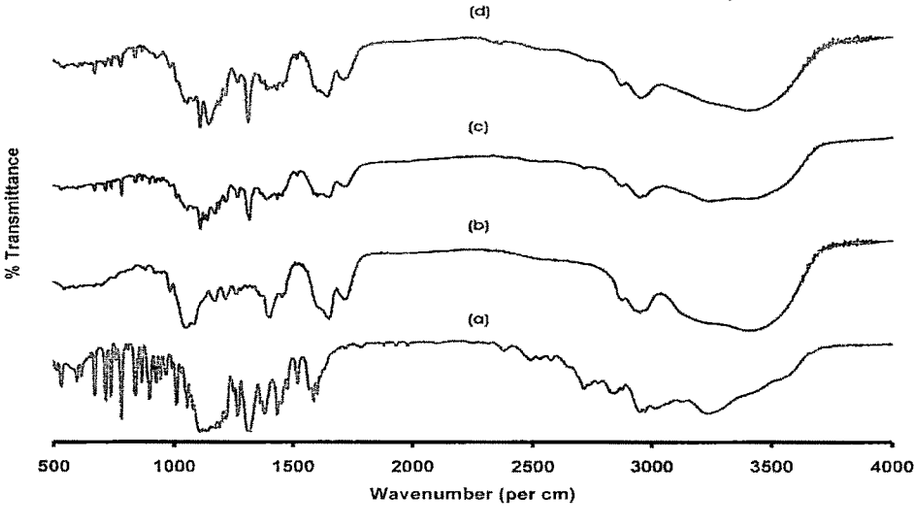

Figure 4.7. FTIR spectra of (a) MFL, (b) GLY, (c) physical mixture and (d) solid dispersion

4.3.2.2. Differential Scanning Calorimetry (DSC)

Figure 4.8 shows the DSC curve of MFL, GLY, physical mixture and solid dispersion in 1:1.5M.

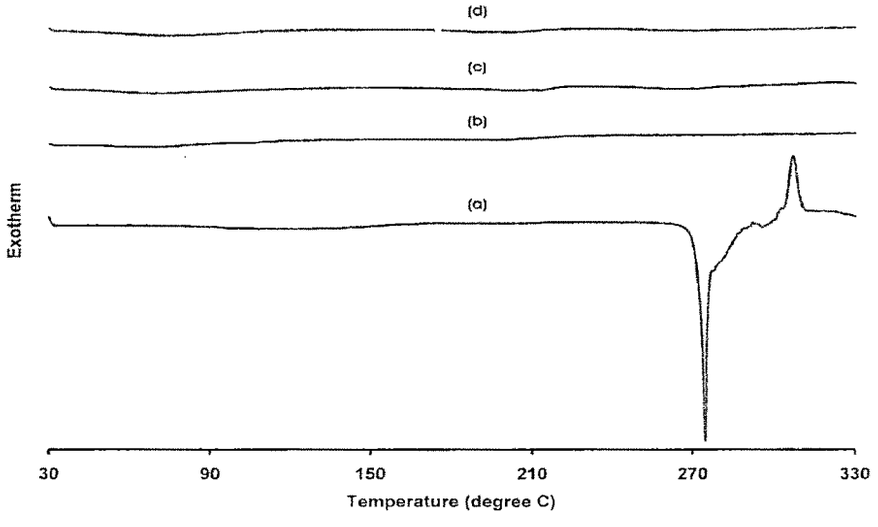

Figure 4.8. DSC curve of (a) MFL, (b) GLY, (c) physical mixture and (d) solid dispersion

The pure MFL showed an endothermic peak at 271.38^{0}C, followed by exothermic peak at 308.36^{0}C. The characteristic endothermic peak corresponding to melting peak of MFL was disappeared in physical mixtures as well as solid dispersions. This could be attributed to higher GLY concentration and uniform distribution of MFL in the crust of GLY, resulting in complete miscibility of molten drug in GLY.

4.3.2.3. Tablet Preparation and Characterization

To formulate RDTs of MFL, the 1:1.5M binary mixture was selected, based on its bitterness score. The formula of different tablets prepared is summarized in Table 4.6.

Table 4.6. Formulation of RDTs

Drug/Excipients	RDT51	RDT52	RDT53	RDT54	RDT55	RDT56
MFL (mg)	-	-	-	-	250	250
GLY (mg)	-	-	-	-	-	759.38
Solid dispersion eq. to 250 mg MFL (mg)	1009.38	1009.38	1009.38	1009.38	-	-
Microcrystalline cellulose (Avicel PH 302) (mg)	225.12	-	225.12	-	984.50	225.12
Lactose (Lactopress) (mg)	-	225.12	-	225.12	-	-
Croscarmellose sodium (mg)	-	-	65	65	65	65
Crospovidone (mg)	65	65	-	-	-	-
Magnesium stearate (mg)	6.5	6.5	6.5	6.5	6.5	6.5

RDTs containing croscarmellose sodium and granular microcrystalline cellulose (RDT53) showed the fastest disintegration (56-62 seconds) with improved hardness and friability. The formula of optimized RDT was used to prepare RDT of pure MFL (RDT55) and physical mixture of MFL and GLY (RDT56). Tablet characteristics of RDTs are summarized in Table 4.7.

Table 4.7. Physical properties of RDTs

Parameters	RDT51	RDT52	RDT53	RDT54	RDT55	RDT56
Weight (mg) ± SD*	1302.07 ± 2.31	1299.57 ± 2.48	1304.17 ± 2.37	1301.76 ± 2.19	1302.29 ± 2.14	1303.73 ± 1.97
Disintegrating Time (sec)	68-73	65-72	56-62	69-74	52-59	58-63
Hardness (kg)	5.7-5.8	5.8-5.9	5.7-5.8	5.8-5.9	5.5-5.6	5.6-5.7
Friability (%) ± SD*	0.62 ± 0.13	0.65 ± 0.11	0.56 ± 0.13	0.69 ± 0.16	0.52 ± 0.16	0.59 ± 0.09

**Values represent the mean ± SD of 3 experiments.*

4.3.2.4. *In vitro* drug release Studies

Drug release profiles of RDTs prepared from MFL, physical mixture and solid dispersion are presented in Figure 4.9. It is evident that the solid dispersion technique has improved the dissolution rate of MFL to a great extent. Table 4.8 summarizes % drug dissolved in 5 minutes (DP5), dissolution efficiency at 15 minutes (DE15), and dissolution efficiency at 60 minutes (DE60) for RDTs prepared from MFL and its binary systems with GLY. RDT53 showed excellent dissolution efficiency (DE60 = 95.38%) and rapid dissolution (DP5 = 83.30%) at pH 6.8. Similarly RDT53 showed improved dissolution efficiency (DE60 = 79.66%) and rapid dissolution (DP5 = 31.17%) at pH 1.2. This finding suggests increased availability of MFL in g.i.t. When compared with pure MFL formulation, tablets formulated with the binary mixture clearly perform better and a significant enhancement in dissolution characteristics was observed. RDT56 also showed improvement in dissolution to a significant extent when compared with drug alone.

This enhancement of dissolution of MFL from RDTs can be ascribed to several factors. It has been reported that GLY has structural similarity to triterpenes and show surfactant like action (Motlekar et al., 2006; Polyakov et al., 2005). Increased wettability and dispersibility are the main reasons for improvement of dissolution of MFL (Ford, 1986).

Table 4.8. Percent dissolution and dissolution efficiency of MFL from RDTs prepared using binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
RDT55	19.32	25.14	30.04	37.11	67.34	76.60
RDT56	21.18	56.00	32.85	60.20	72.03	85.52
RDT53	31.17	83.30	42.18	83.76	79.66	95.38

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

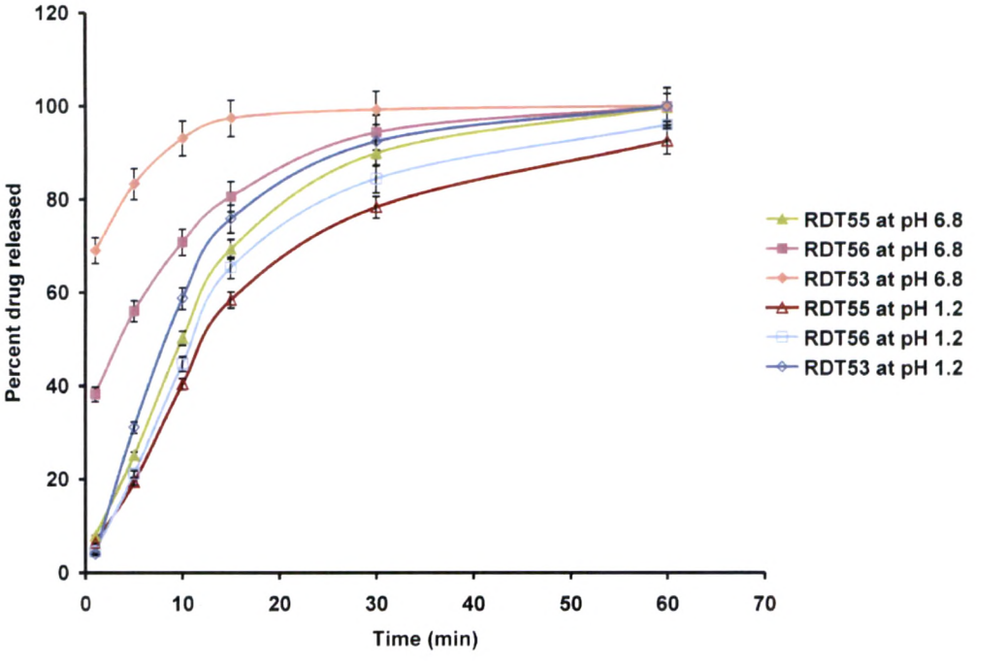

Figure 4.9. Dissolution of RDTs prepared from MFL, physical mixture and solid dispersion

RDTs prepared from physical mixing of MFL with GLY results in greater wetting and increased surface available for dissolution by reducing interfacial tension between hydrophobic drug and dissolution media. Furthermore, RDTs prepared from solid dispersion, results in uniform distribution of MFL in the

GLY crust in a highly dispersed state. Thus, when such a system comes in contact with an aqueous dissolution medium, the hydrophilic carrier dissolves and results in precipitation of the embedded drug into fine particles, which increase the dissolution surface available (Modi and Tayade, 2006).

4.3.2.5. Gustatory sensation test for RDTs

Bitterness evaluation results made by the consents of trained persons are listed in Table 4.9. No bitterness was reported in solid dispersion with reference to pure drug. It has been reported that MFL depolarize taste cells by closing K^+ channels and produce bitterness (Yamamoto et al., 1998). GLY is astringent and might be interacting with G-proteins and paralyzing them, resulting in reduced taste transduction and thus reduced bitterness score. Further the sweet taste of GLY imparted additive effect.

MFL was uniformly distributed in the crust of GLY, which avoids contact of MFL with G-protein coupled receptors. Further the sweet taste of GLY imparted additive effect (Kinghom and Compadre, 2001). This results in complete taste masking of MFL in GLY solid dispersion.

Table 4.9. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3+**
Pure MFL						2	15	3
Physical Mixture		4	15	1				
Solid Dispersion	20							
RDT55						2	17	1
RDT56		6	13	1				
RDT53	20							

Though the physical mixing of MFL with GLY brings the drug in close contact with carrier, MFL was not uniformly distributed in GLY as that of solid

dispersion. This might be the reason for not complete masking the bitter taste of MFL in GLY physical mixture.

RDT55 was rated as moderate to strong bitter by 20% and strongly bitter by 75% while RDT53 was rated as tasteless by 100% of volunteers of panel.

4.3.2.6. Mini-column Method

The tapping frequency of column and flow rate of test solution were assumed to influence the mini-column results. The tapping frequency was set at 30, 10 and 3 times. The effect of tapping frequency on the release rate is shown in Figure 4.10.

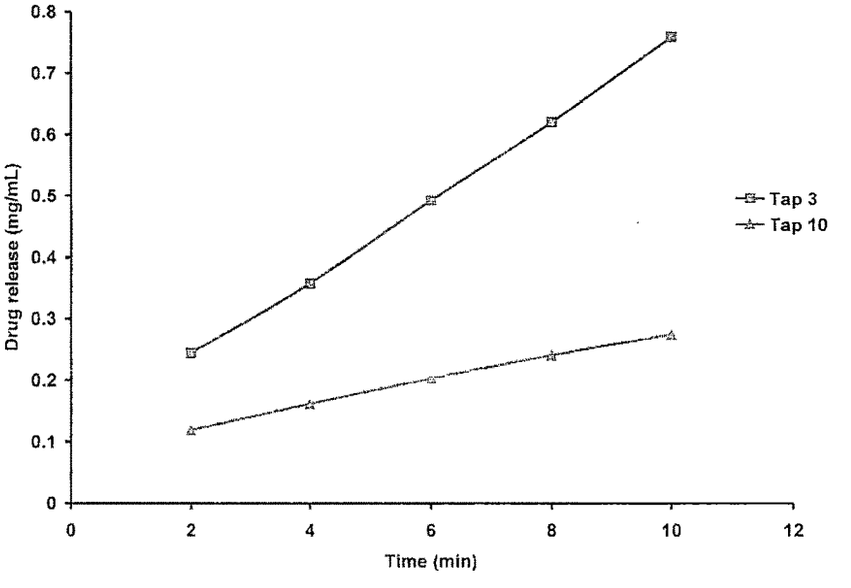

Figure 4.10. Effect of tapping frequency on the release of MFL from RDT14 with 1 mL/min flow rate

When the tapping frequency was 30 times, no release of PRM from compact powdered mass was observed. This might be because of the difficulty for the test solution to penetrate the compact powdered mass. The release rate increased, when the tapping frequency was 3 times. However the release rate decreased, when the tapping frequency was 10 times. The release rate decreased as the tapping frequency increased.

Next, the tapping frequency was set at 3 times and flow rates at 1, 1.3 and 1.5 mL/min. The results are shown in Figure 4.11. When the flow rate increased, the release rate decreased. This was probably due to a delay in liquid penetration into the matrix, since the liquid flow rate on the matrix surface increased. The flow rate and tapping frequency were optimized, based on maximum drug release, at 1 mL/min and 3 times, respectively. Optimized flow rate and tapping frequency were further applied to RDT55 and RDT56.

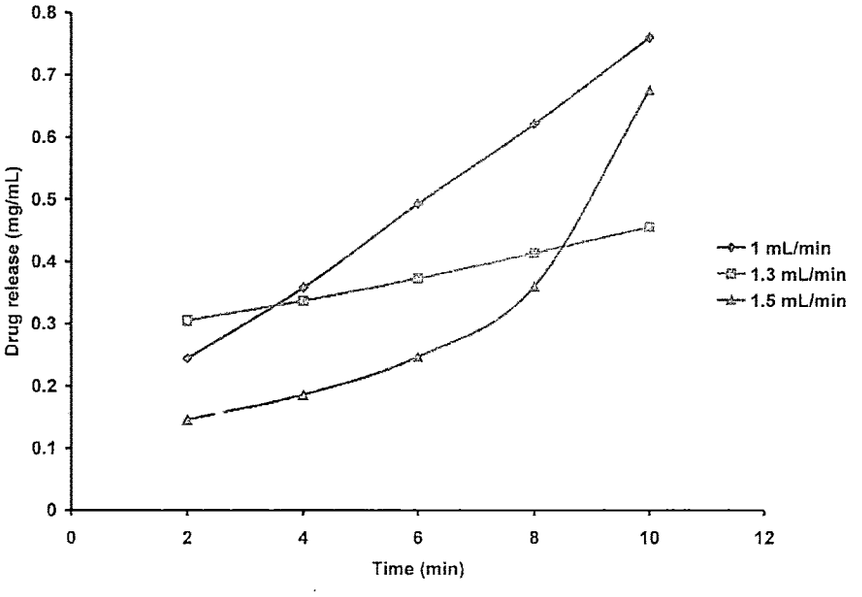

Figure 4.11. Effect of flow rate on the release of MFL from RDT53 with 3 times tapping frequency

Table 4.10 shows the results of the sensory tests and amount of MFL released after 2 min interval with the mini-column method. RDT55 showed increase in number of persons who sensed bitterness with increase in amount of MFL released. RDT53 sensed no bitterness with 0.31 mg/mL. The value (0.11 mg/mL) was about 3 times larger than that of pure MFL.

Table 4.10. Relationship between amount of release and results of sensory test of RDTs using mini-column method

Formulations	Time (min)				
	2	**4**	**6**	**8**	**10**
RDT55 (mg/mL) ± SD*	0.11 ± 0.08	0.18 ± 0.04	0.23 ± 0.06	0.36 ± 0.07	0.38 ± 0.04
Bitterness score	2.5	3	3+	3+	3+
RDT56 (mg/mL) ± SD*	0.16 ± 0.07	0.26 ± 0.09	0.31 ± 0.07	0.36 ± 0.08	0.41 ± 0.05
Bitterness score	0	1	1	1	1.5
RDT53 (mg/mL) ± SD*	0.31 ± 0.04	0.34 ± 0.09	0.37 ± 0.07	0.41 ± 0.05	0.46 ± 0.05
Bitterness score	0	0	0	0	0

**Values represent the mean ± SD of 3 experiments.*

4.3.2.7. Stability studies

For the RDT53, the similarity factor (f2) was calculated by a comparison of the dissolution profiles in each storage condition with the control at the initial condition.

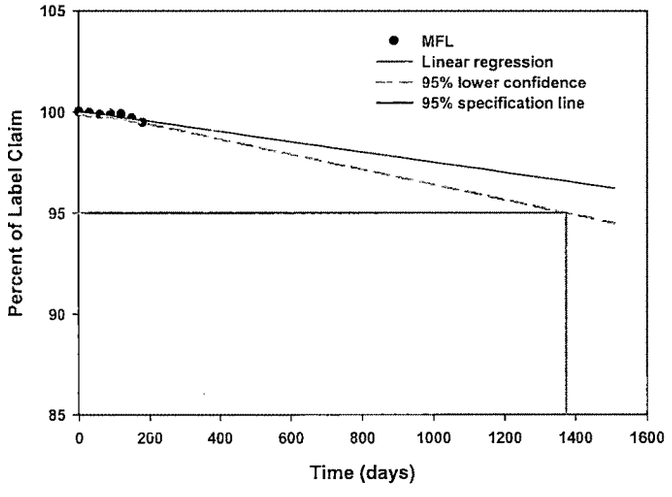

Figure 4.12. Extrapolation of accelerated stability data for shelf life calculation

The f2 values ranged from 78 to 91 with a 2%-3% average difference at pH, 1.2 and 6.8. Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data showed little change over time, and so a shelf life up to 1372.84 days (45.76 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

The study conclusively demonstrated the complete taste masking of MFL with improved dissolution by solid dispersion technique. The FTIR and DSC studies indicated no interaction of MFL in GLY solid dispersion. MFL-GLY solid dispersion along with use of croscarmellose sodium as disintegrant could be considered for formulation of a stable RDTs of MFL. The use of mini-column, an *in vitro* model, could be applicable to evaluate bitterness score, which simulates the disintegration of tablet in oral cavity.

4.4. Primaquine Phosphate (PRM)

4.4.1. Experimental

4.4.1.1. Materials

Materials used were as mentioned in 3.2.1.1.

4.4.1.2. Preparation of PRM-GLY solid dispersion

The solid dispersion of PRM and GLY in 1:0.5 and 1:1M was prepared using solvent evaporation method. Accurately weighed quantity of PRM (750 mg in 7 mL) was dissolved in water. Previously dissolved GLY (207.4 mg in 7 mL) in water was added with constant stirring on magnetic stirrer for 10 min. The water was removed by heating the solution under reduced pressure. The solid obtained was further dried to a constant weight in hot air oven at 100^0C. Dried powder was passed through sieve no. 44, and stored in desiccator (Tarsons Products Pvt. Ltd, India) until further evaluation.

The physical mixtures of PRM and GLY were prepared by mixing individual components geometrically that had previously been sieved through sieve no. 44, together with a spatula.

4.4.1.3. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

4.4.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

4.4.1.5. Tablet Formulation and Characterization

RDTs containing equivalent of 13.16 mg of PRM (equivalent to 7.5 mg primaquine base) were compressed on an 8-station single rotary tabletting press (GMC, Mumbai, India) using a 6-mm round shaped flat punch with break line by direct compression technique.

Two different superdisintegrants, croscarmellose sodium and crospovidone were tried to achieve rapid disintegration of tablets. Granular microcrystalline cellulose and dibasic calcium phosphate (DCP) were used as diluents. Prepared RDTs were evaluated as mentioned in 3.2.1.6.

4.4.1.6. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

4.4.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

4.4.1.8. Mini-column Method

Mini-column method was studied as mentioned in 3.2.1.9.

4.4.1.9. Stability studies

Stability studies were carried out as mentioned in 3.2.1.10.

4.4.2. Results and discussion

4.4.2.1. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of PRM, GLY, physical mixture and solid dispersion in 1:1M are presented in Figure 4.13. The characteristic peaks of PRM at 2968 and 2878 cm^{-1} are assigned to C-H stretching vibration in CH_3, CH_2. In addition, the absorption peak at 2844 cm^{-1} can be assigned to C-H stretching vibration in C-O-CH_3. The peak at 1119 cm^{-1} can be assigned to C-O stretching vibration in C-O-C. The peak at 3305 cm^{-1} can be assigned to N-H stretching in primary amines. All above characteristic peaks appear in the spectra of binary systems at same wavenumber indicating no modification or interaction between the drug and carrier.

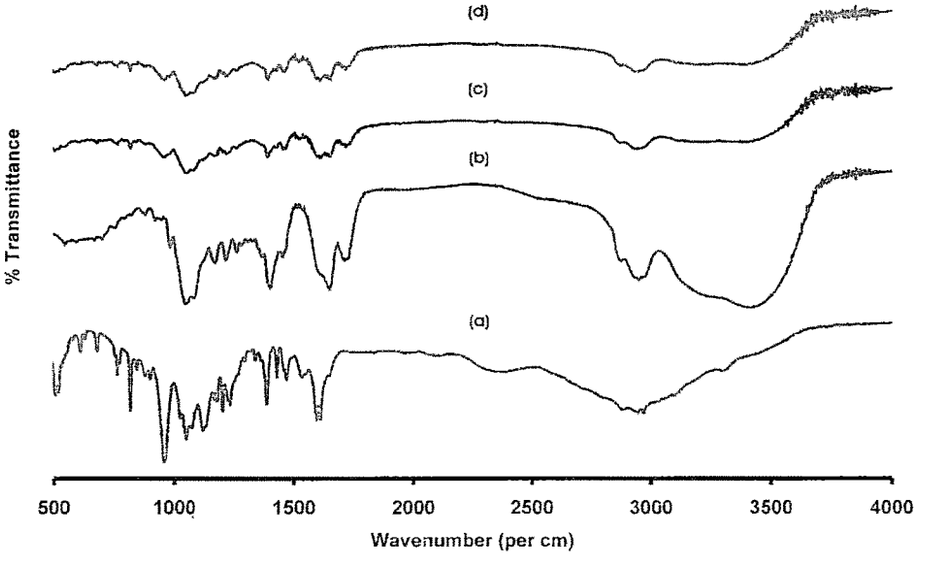

Figure 4.13. FT-IR spectra of (a) PRM, (b) GLY, (c) physical mixture and (d) solid dispersion

4.4.2.2. Differential Scanning Calorimetry (DSC)

Thermal behavior of PRM, GLY, physical mixture and solid dispersion 1:1M are depicted in Figure 4.14. The pure PRM shows a sharp endothermic peak at 202.68^{0}C. The characteristic endothermic peak corresponding to melting peak of PRM was broaden and shifted towards lower temperature (193.84^{0}C), with

reduced intensity in both physical mixtures as well as solid dispersions. This could be attributed to higher GLY concentration and uniform distribution of PRM in the crust of GLY, resulting in complete miscibility of molten PRM in GLY.

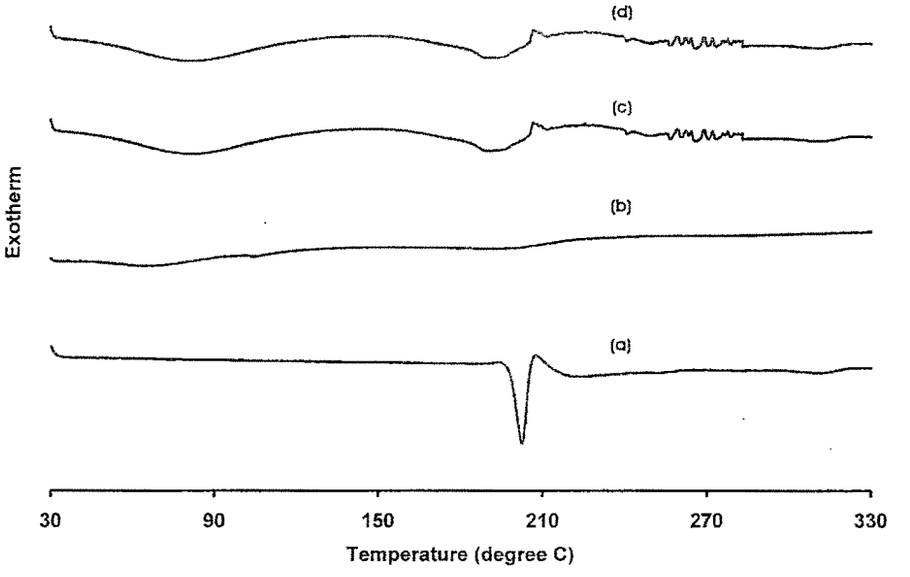

Figure 4.14. DSC curve of (a) PRM, (b) GLY, (c) physical mixture and (d) solid dispersion

4.4.2.3. Tablet Preparation and Characterization

To formulate a rapid disintegrating tablet (RDT) of PRM, the 1:1M binary mixture was selected, based on its bitterness score. The formula of different tablets prepared is summarized in Table 4.11.

RDTs containing croscarmellose sodium and granular microcrystalline cellulose (RDT63) showed the fastest disintegration (34-40 seconds) with improved hardness and friability. The formula of optimized RDT was used to prepare RDT of pure PRM (RDT65) and physical mixture of PRM and GLY (RDT66). Tablet characteristics of RDTs are summarized in Table 4.12.

Table 4.11. Formulation of RDTs

Drug/Excipients	RDT61	RDT62	RDT63	RDT64	RDT65	RDT66
PRM (mg)	-	-	-	-	13.16	13.16
GLY (mg)	-	-	-	-	-	24.26
Solid dispersion eq. to 13.16 mg PRM (mg)	37.42	37.42	37.42	37.42	-	-
Microcrystalline cellulose (Avicel PH 302) (mg)	29.88	-	29.88	-	54.14	29.88
Dibasic calcium phosphate (Emcompress) (mg)	-	29.88	-	29.88	-	-
Croscarmellose sodium (mg)	-	-	2.4	2.4	2.4	2.4
Crospovidone (mg)	2.4	2.4	-	-	-	-
Magnesium stearate (mg)	0.3	0.3	0.3	0.3	0.3	0.3

Table 4.12. Physical properties of RDTs

Parameters	RDT61	RDT62	RDT63	RDT64	RDT65	RDT66
Weight (mg) ± SD*	70.21 ± 1.28	69.89 ± 1.19	70.35 ± 0.87	69.75 ± 1.32	70.43 ± 0.69	70.28 ± 0.76
Disintegrating Time (sec)	43-49	47-52	34-40	44-51	36-41	36-42
Hardness (kg)	4.1-4.2	4.1-4.2	4.4-4.5	4.2-4.3	4.3-4.4	4.3-4.4
Friability (%) ± SD*	0.27 ± 0.09	0.29 ± 0.12	0.19 ± 0.10	0.31 ± 0.13	0.21 ± 0.09	0.21 ± 0.11

**Values represent the mean ± SD of 3 experiments.*

4.4.2.4. *In vitro* drug release Studies

Drug release profiles of RDTs prepared from PRM, physical mixture and solid dispersion are presented in Figure 4.15. It is evident that the solid dispersion technique has improved the dissolution rate of PRM to a great extent. Table 4.13 summarizes % drug dissolved in 5 minutes (DP5), dissolution efficiency at 15 minutes (DE15), and dissolution efficiency at 60 minutes (DE60) for PRM and its binary systems with GLY. RDT63 showed excellent dissolution efficiency (DE60 = 99.29%) and rapid dissolution (DP5 = 100.15%) at pH 6.8. Similarly RDT63 showed improved dissolution efficiency (DE60 = 92.30%) and rapid dissolution (DP5 = 84.14%) at pH 1.2. This finding suggests increased availability of PRM in stomach. When compared with pure PRM formulation, tablets formulated with the binary mixture clearly perform better and a significant enhancement in dissolution characteristics was observed. RDT66 also showed improvement in dissolution to a significant extent when compared with drug alone.

This enhancement of dissolution of PRM from RDTs can be ascribed to several factors. It has been reported that GLY has structural similarity to triterpenes and show surfactant like action (Motlekar et al., 2006; Polyakov et al., 2005). Increased wettability and dispersibility are the main reasons for improvement of dissolution of PRM (Ford, 1986).

Table 4.13. Percent dissolution and dissolution efficiency of PRM from binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
RDT65	70.30	99.87	68.39	95.65	73.09	99.03
RDT66	77.80	99.99	77.21	96.00	87.04	98.84
RDT63	84.14	100.15	82.87	96.56	92.30	99.29

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

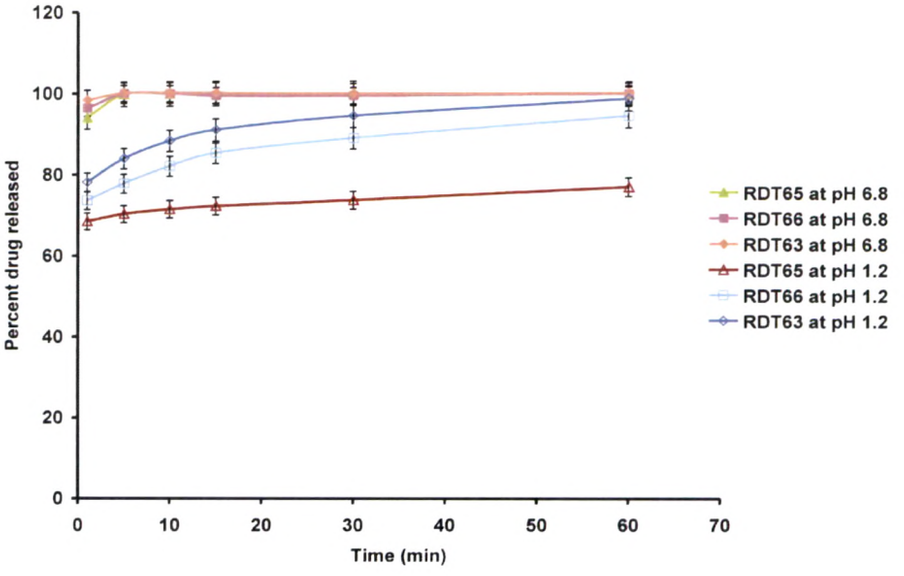

Figure 4.15 Dissolution of RDTs prepared from PRM, physical mixture and solid dispersion

RDTs prepared from physical mixing of PRM with GLY resulted in greater wetting and increased surface available for dissolution by reducing interfacial tension between hydrophobic drug and dissolution media. Furthermore, RDTs prepared from solid dispersion, results in uniform distribution of PRM in the GLY crust in a highly dispersed state. Thus, when such a system comes in contact with an aqueous dissolution medium, the hydrophilic carrier dissolves and results in precipitation of the embedded drug into fine particles, which increase the dissolution surface available (Modi and Tayade, 2006).

4.4.2.5. Gustatory sensation test for RDTs

Bitterness evaluation results made by the consents of trained persons are listed in Table 4.14. No bitterness was imparted in solid dispersion with reference to pure drug. It has been reported that PRM depolarize taste cells by closing K^+ channels and produce bitterness (Yamamoto et al., 1998). GLY is astringent and might be interacting with G-proteins and paralyzing them, resulting in reduced taste transduction and thus reduced bitterness score. Further the sweet taste of GLY imparted additive effect.

PRM was uniformly distributed in the crust of GLY, which avoids contact of PRM with G-protein coupled receptors. Further the sweet taste of GLY

imparted additive effect (Kinghom and Compadre, 2001). This results in complete taste masking of PRM in GLY solid dispersion. Though the physical mixing of PRM with GLY brings the drug in close contact with carrier, PRM was not uniformly distributed in GLY as that of solid dispersion. This might be the reason for not complete masking the bitter taste of PRM in GLY physical mixture.

RDT65 was rated as strongly bitter by 15% and very strongly bitter by 75% while RDT63 was rated as tasteless by 100% of volunteers of panel.

Table 4.14. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3+**
Pure PRM							2	18
Physical Mixture		3	15	2				
Solid Dispersion	19	1						
RDT65							3	17
RDT66		4	16					
RDT63	20							

4.4.2.6. Mini-column Method

The tapping frequency of column and flow rate of test solution were assumed to influence the mini-column method results. The tapping frequency was set at 30, 10 and 3 times. The effect of tapping frequency on the release rate is shown in Figure 4.16.

When the tapping frequency was 30 times, no release of PRM from compact powdered mass was observed. This might be because of the difficulty for the test solution to penetrate the compact powdered mass. The release rate increased, when the tapping frequency was 3 times. However the release rate

decreased, when the tapping frequency was 10 times. The release rate decreased as the tapping frequency increased.

Next, the tapping frequency was set at 3 times and flow rates at 1, 1.3 and 1.5 mL/min. The results are shown in Figure 4.17. When the flow rate increased, the release rate decreased. This was probably due to a delay in liquid penetration into the matrix, since the liquid flow rate on the matrix surface increased. The flow rate and tapping frequency were optimized, based on maximum drug release, at 1 mL/min and 3 times, respectively. Optimized flow rate and tapping frequency were further applied to RDT65 and RDT66.

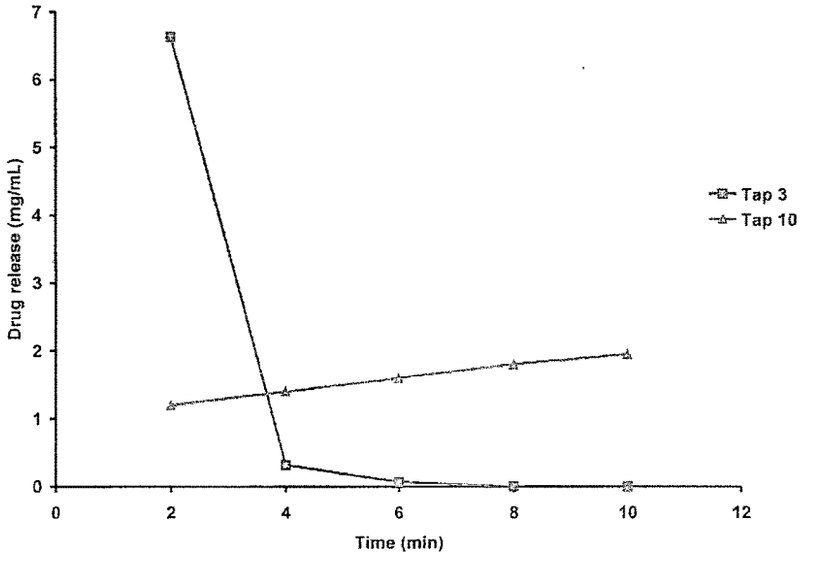

Figure 4.16. Effect of tapping frequency on the release of PRM from RDT63 with 1 mL/min flow rate

Table 4.15 shows the results of the sensory tests and amount of PRM released after 2 min interval with the mini-column method. Initial increased PRM concentration in eluate might be due to the higher solubility of PRM in pH 6.8. RDT63 sensed no bitterness with 6.63 mg/mL. The value (0.74 mg/mL) was about 9 times larger than that of pure PRM.

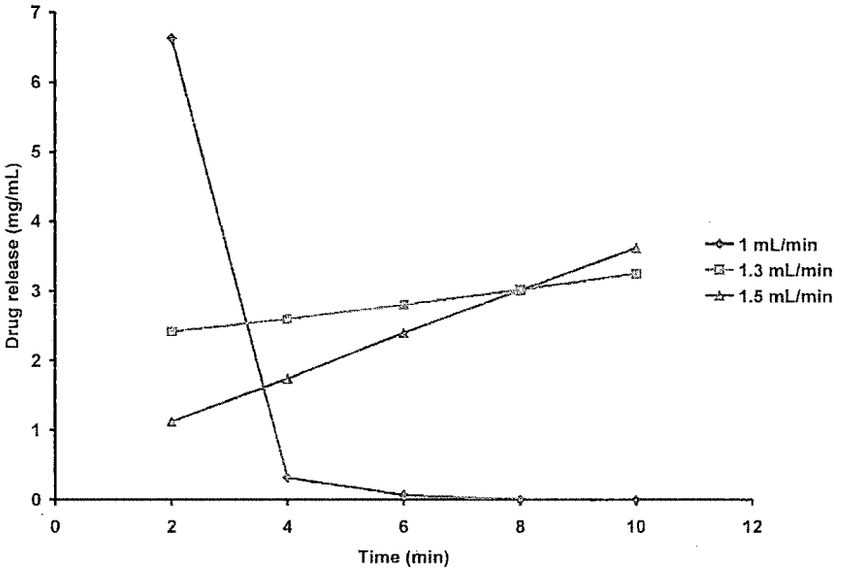

Figure 4.17. Effect of flow rate on the release of PRM from RDT63 with 3 times tapping frequency

Table 4.15. Relationship between amount of release and results of sensory test of RDTs using mini-column method

Formulations	Time (min)				
	2	4	6	8	10
RDT65 (mg/mL) ± SD*	6.37 ± 0.04	0.74 ± 0.07	0.38 ± 0.05	0.09 ± 0.03	0
Bitterness score	3+	3	2.5	2	0
RDT66 (mg/mL) ± SD*	6.46 ± 0.06	0.57 ± 0.08	0.24 ± 0.03	0	0
Bitterness score	1.5	1	1	0	0
RDT63 (mg/mL) ± SD*	6.63 ± 0.05	0.32 ± 0.04	0.07 ± 0.03	0	0
Bitterness score	0	0	0	0	0

**Values represent the mean ± SD of 3 experiments.*

4.4.2.7. Stability studies

For the RDT63, the similarity factor (f2) was calculated by a comparison of the dissolution profiles in each storage condition with the control at the initial condition. The f2 values ranged from 81 to 96 with a 2%-2.5% average

difference at pH, 1.2 and 6.8. Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data showed little change over time, and so a shelf life up to 1237.5 days (41.25 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

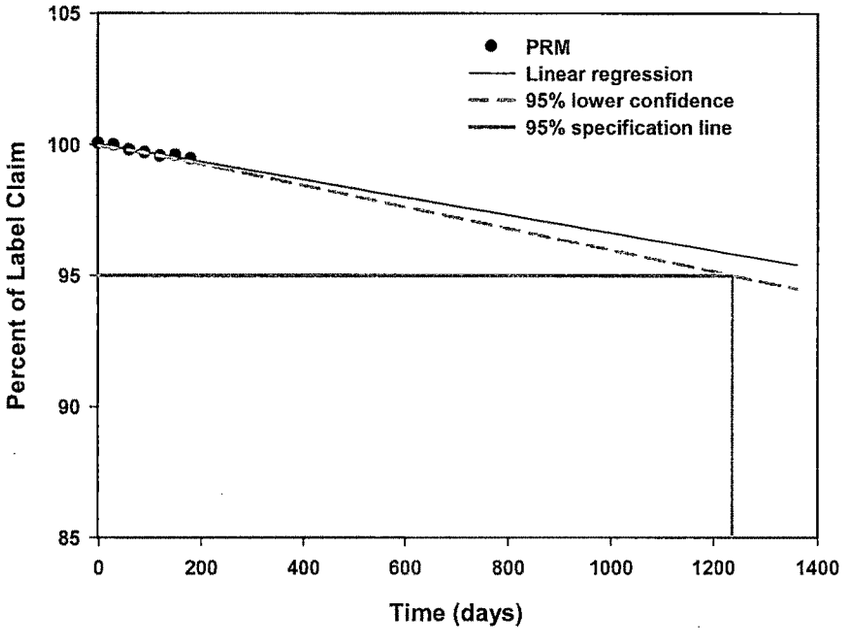

Figure 4.18. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated the complete taste masking of PRM by solid dispersion technique. FTIR and DSC studies showed no interaction of PRM with GLY in solid dispersion. PRM-GLY solid dispersion along with croscarmellose sodium could be considered for formulation of RDTs of PRM. The use of mini-column method, an in vitro model, could be applicable to evaluate bitterness score, which simulates the disintegration of tablet in oral cavity.

4.5. Summary

	ARM-GLY	MFL-GLY	PRM-GLY
GLY required per unit dose	50 mg ARM+ 141 mg GLY	250 mg MFL + 759.38 mg GLY	13.16 mg PRM + 24.26 mg GLY
Bitterness score (for solid dispersion)	0	0	0
Punch diameter	9 mm round shaped flat	16 mm x 8 mm caplet shaped	6 mm round shaped flat
Weight (mg)	400 mg	1300 mg	60 mg
Disintegration Time (sec)	28-32	56-62	34-40
DP5 at pH 1.2 (%)	28.89	31.17	84.14
DP5 at pH 6.8 (%)	91.77	83.30	100.15
DE60 at pH 1.2 (%)	97.83	79.66	92.30
DE60 at pH 6.8 (%)	68.55	95.38	99.29
Hardness (kg)	3.1-3.2	5.7-5.8	4.4-4.5
Friability (%) ± SD*	0.54 ± 0.09	0.56 ± 0.13	0.19 ± 0.10
Bitterness score (for RDTs)	0	0	0
Similarity factor (f2)	89 to 97	78 to 91	81 to 96
Shelf life (months)	44.23	45.76	41.25

**Values represent mean ± SD of 3 eperiments, DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min*

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