

Formulation, Optimization & Evaluation of Fixed Dose Combination Moisture Barrier Film Coated Bilayer Tablet of Artesunate & Amodiaquine Hydrochloride

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Abstract: Fixed dose combination of artesunate & amodiaquine hydrochloride provided challenge in product development due to incompatibility of two drugs. So severe degradation of the drugs in presence of each other. The main issue was stability of artesunate in formulation. The aim of present investigation was to develop stable cost effective fixed dose combination moisture barrier film coated bilayer tablet of two incompatible drug artesunate and amodiaquine hydrochloride for improving patient adherence, compliance, convenience, reduce cost and improve stability of dosage form. Reduced pill burden, therapy period & effective treatment of multidrug resistance & falciparum malaria improved patient compliance, convenience. Results of preformulation study indicated that it was necessary to develop formulation in humidity & temperature controlled environment. Artesunate was very moisture sensitive drug so blend of artesunate layer was prepared by dry granulation method and blend of amodiaquine hydrochloride layer was prepared by wet granulation method. The formula of artesunate layer was optimized using 3^2 factorial designs & using calcium carbonate as basic stabilizing agent. The formula of amodiaquine hydrochloride was optimized using PVPK-30 as a binder. Coating parameters were optimized using 3^2 factorial designs. Instamoistshield moisture barrier coating material was ready to use mixture of polymers, plasticizers, pigments, opacifiers and other excipients which could be used with organic or hydro-alcoholic systems for protection against atmospheric moisture. Result of present study suggested that stable artesunate and amodiaquine hydrochloride moisture barrier film coated bilayer tablet could be successfully formulated using calcium carbonate as basic stabilizing agent & instamoistshield as moisture barrier coating material.

Key words: Artesunate, Amodiaquine hydrochloride, Bilayer tablet, Incompatible, Wet granulation, Dry granulation.

INTRODUCTION

Resistance to antimalarial drugs is caused by the ability of the parasite to survive or multiply in the presence of antimalarial drug concentrations that normally destroy the parasite or control their multiplication. Due to the high resistance of Plasmodium falciparum, there has been the urgent need for drug combination therapy. Artemisinin base

therapy is highly effective at treating Plasmodium falciparum in most places.⁽¹⁻⁵⁾ The fixed dose combination of drugs improve patient adherence, convenience, compliance, reduce cost, improve treatment efficacy & reduce resistance. This combination also available in market as co-packed kit. For that 4 tablets of artesunate & 4 tablets of amodiaquine required per day for complete cure of

malaria of adult patient (<14 years). Fixed dose combination reduces number of tablets administered & two tablets per day required for complete cure of malaria in adult patient. The basic aim of bi-layer tablet formulation is to separate physically or chemically incompatible ingredients and to produce repeat action or prolonged action tablet. Artesunate is degraded mainly due to water content (>1%), elevated temperature (80°C in dry condition) and possibly the 4-aminoquinoline moiety. Both drugs are hygroscopic so moisture barrier film coating is essential. (6-7) Artesunate is artemisinin derivative and active metabolite is dihydroartemisinin. The bioavailability, elimination half life and volume of distribution are 86.4%, 40-95 minute, 1.85lit/kg respectively. Amodiaquine hydrochloride is 4-aminoquinolon derivative and main active metabolite is desethylamodiaquine. The bioavailability, elimination half life and volume of distribution are 75%, 1 to 10days or more, 20-40 lit/kg respectively. (8-9) In the bilayer tablet blend of artesunate layer was prepared by dry granulation method & blend of amodiaquine layer was prepared by wet granulation method. In this research work the ingredients of formula were optimized via factorial design and trial and error method.

MATERIALS AND METHODS

Materials

Artesunate & Amodiaquine hydrochloride (IPCA Laboratories Limited, MP) MCC avicel, Calcium carbonate, Sodium hydroxide, Crosscarmellose sodium, Crosspovidone, Starch (Gujarat Microwax Pvt Ltd) Magnesium stearate, Talc, Aerosil (shital Chemicals Ltd.) Isopropyl alcohol, Methylene chloride (Ranchem Ltd.) Instamoistshield-MB (Ideal curve Pvt Ltd.)

Method

Preformulation study (10-12)

Angle of repose, bulk density, tapped density, compressibility index, hausner's ratio of pure drug artesunate & amodiaquine hydrochloride was determined. For drug-drug compatibility study physical compatibility study, FT-IRstudy⁽¹¹⁾, DSC study were performed.

Reverse phase HPLC analytical method for quantification of drugs (13)

Reverse phase HPLC method for quantification of artesunate in phosphate buffer 7.5

Chromatographic condition:

Mobile phase: Take 0.68 gm potassium dihydrogen orthophosphate in 500ml of water adjust PH=3.0 with

orthophosphoric acid, add 350ml acetonitrile to it. Sonicate for 10 minutes.

Diluent: 500ml acetonitrile: 500ml water

Column: HypersilBDS150*4.6mm, 5micr or equivalent

Wavelength: 216nm

Injected volume: 20 microlitre

Flow rate: 1.0 ml/min

Autosampler temperature: 20°C

Column temperature: ambient

Reverse phase HPLC method for quantification amodiaquine hydrochloride in water

Chromatographic condition:

Mobile phase: 750 ml of water:250 ml Acetonitrile:1.0 ml orthophosphoric acid

Diluent: Mobile phase as diluent

Column: water symmetryC18, 250*4.6mm, 5micr or equivalent

Wavelength: 300nm

Injected volume: 10 microlitre

Flow rate: 1.0ml/min

Formulation & optimization of artesunate layer

Artesunate tablets were prepared by dry granulation method. All ingredients were passed through 40 # sieve. Accurately weighed all the ingredients except magnesium stearate were mixed. After uniform mixing of ingredients, sieve passed half quantity of magnesium stearate as lubricant was mixed for 2 minutes. Slugs were prepared (6-8 Kg/cm² hardness tablets) using cadmach double rotary machine & passed with multimill 2mm screen. The granules were sieved through 20# sieves. The final granules were lubricated with remaining half quantity of magnesium stearate & compressed with MCC (avicel) as second layer using cadmach single rotary compression machine using 16/32 SC punch size. 3² full factorial designs for combination of concentration of disintegrate (Crosscarmellose sodium) & basic stabilizing agent (Calcium carbonate) was used. The concentration of crosscarmellose sodium(X₁) and concentration of calcium carbonate(X₂) were selected as independent variables & disintegration time & drug release at 30minute were selected as dependent variable. For validation of mathematical model check point batch was taken with coded value X₁= 0.5 & X₂=0.8.Coded value for factorial design & composition of formulations were shown in table 1 & 2.

Formulation& optimization of amodiaquine hydrochloride layer

Amodiaquine hydrochloride tablets were prepared by wet granulation method. All the ingredients were

passed through 40 mesh sieve. Accurately sifted amodiaquine hydrochloride, MCC were mixed thoroughly & granulated with binding solution (PVP K30 in isopropyl alcohol). Granules were dried in hot air oven at 60°C for 30 minutes & pass it 20 mesh sieve. These granules were mixed with crosscarmellose sodium, aerosil & talcum powder as disintegrate, antiadherent & glidant respectively. The blend was lubricated with magnesium stearate & compressed with MCC (avicel) as second layer using cadmach single rotary compression machine using 16/32 SC punch size. The compositions of formulations were shown in table 3.

Preparation of bilayer tablet

The blend of optimized formula of artesunate layer & amodiaquine hydrochloride layer was prepared & compressed using 35 station cadmach double rotary machine with 16-32 SC plain on both side punch size. Amodiaquine hydrochloride layer was first precompressed & final compression was given after addition of artesunate layer blend.

Preparation of Instamoistshield-MB (moisture barrier) film coating solution

Required quantity of isopropyl alcohol & instamoistshield material were taken in vessel & stirred with propeller stirrer to form vortex for 5 minute. Then methylene chloride was added & stirred for 40 minutes. Solution was passed through 80 mesh screen. The composition of coating solution was described in table 4.

Optimized film coating parameter⁽¹⁴⁻¹⁹⁾

Coating was performed at different spray rate of 8, 12, 16, 20 gm/minute, atomizing air pressure 1.5, 2, 2.5, 3 kg/cm², inlet air temperature 20, 40, 60, 80°C, rotating speed of pan 6, 12, 18, 24 rpm and % weight gain 3.0%w/w, 5.0%w/w, 7.0%w/w. From trial & error method coating parameters were optimized. 3² full factorial design was employed to study combine effect of independent variables spray rate (X1), inlet air temperature (X2) on dependent variables coating uniformity, coating process efficiency, and %LOD on the basis of the preliminary trials.⁽²⁰⁻²³⁾ The coded value was shown in table 5.

Evaluation parameters for coating^(14, 17, 19)

Coated tablets were evaluated for coating uniformity (CU), coating process efficiency (CPE) & %LOD

Evaluation parameter of uncoated & film coated tablet⁽²⁴⁻²⁹⁾

Two tablets from each formulation were randomly selected and organoleptic properties such as colour, odour, taste, and shape were evaluated. Thickness and diameter of ten uncoated & coated tablets were measured using vernier calipers. The prepared uncoated & coated tablets were evaluated for uniformity of weight using 20 tablets, hardness (Monsanto tester), friability using 10 tablets (Roche type friabilator), disintegration test using 6 tablets & water as medium (Disintegration apparatus).

Drug content (assay)

Chromatographic condition was same as described in calibration curve. For estimation of artesunate, standard solution & sample solution were prepared using 100mg artesunate in 100 ml diluent & 1 average weight (Eq. to 100mg) in 100 ml diluent respectively. For estimation of amodiaquine hydrochloride standard solution was prepared using 40mg amodiaquine in 100 ml with water & from 100ml, 10ml was taken & diluted with 100ml. Sample solution was prepared by dissolving 1 average weight tablet in 100 ml diluent & sonicated for 20 minute. Filter it through whatman filterpaper no1. From that 10 ml was taken & diluted with 100ml. From that 10ml was taken & diluted up to 100 ml with water.

In vitro Drug Release Study

Individual dissolution for each tablet was taken & injected immediately after completed dissolution. Delay in injecting the dissolution sample may cause the degradation of artesunate and the impurity generated. The in vitro dissolution study was carried out using dissolution medium consisted of 900ml phosphate buffer pH 7.5 for artesunate & water for amodiaquine hydrochloride using apparatus-2 USP (Paddle) at 75 RPM for 30 minutes. Temperature maintained at 37±1°C. Aliquots of 20ml were withdrawn at specified time and an equivalent amount of fresh dissolution fluid equilibrated at the same temperature was replaced. Aliquots withdrawn were diluted suitably, filtered and analyzed by reverse phase HPLC method with UV detection. Chromatographic condition for amodiaquine hydrochloride was same as described in calibration curve of amodiaquine hydrochloride. The chromatographic condition for artesunate was described following. Mobile phase & Diluents: same as in calibration curve.

Column: 150*4.6mm, 5micron (Thermohypersil BDS)

Wavelength: 210nm

Injected volume: 100 micro litre

Flow rate: 1.0 ml/min

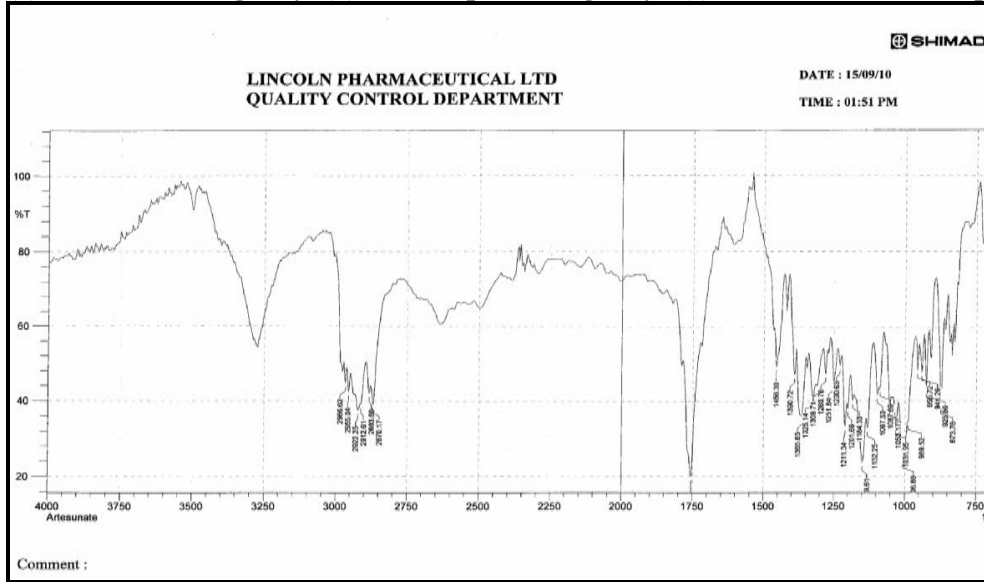
Accelerated stability study of best optimized batch⁽³⁰⁾

Stability study of tablets was carried out at critical room temperature, 40±20⁰C and 75%RH. Tablets were

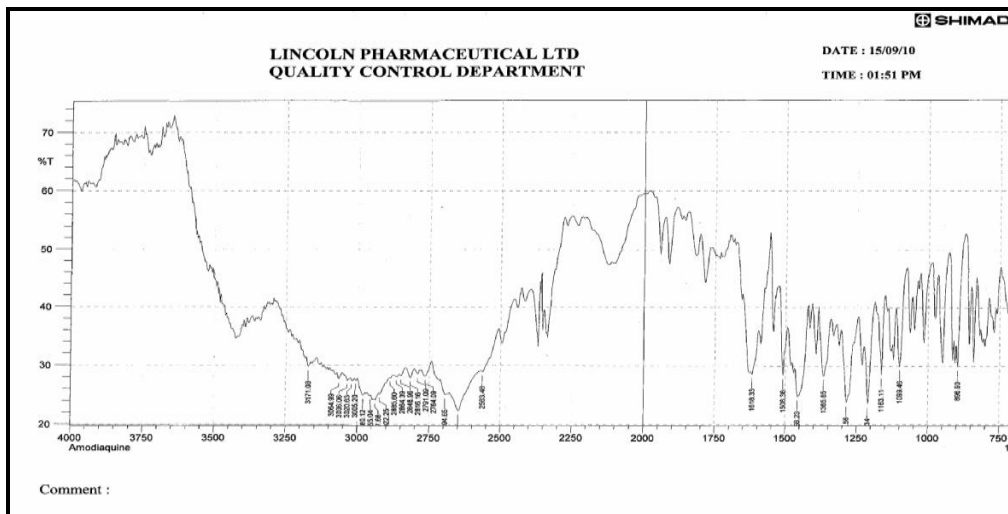
evaluated for all the parameter. It was packed in alu blister packing.

Figure1:FT-IR Spectra

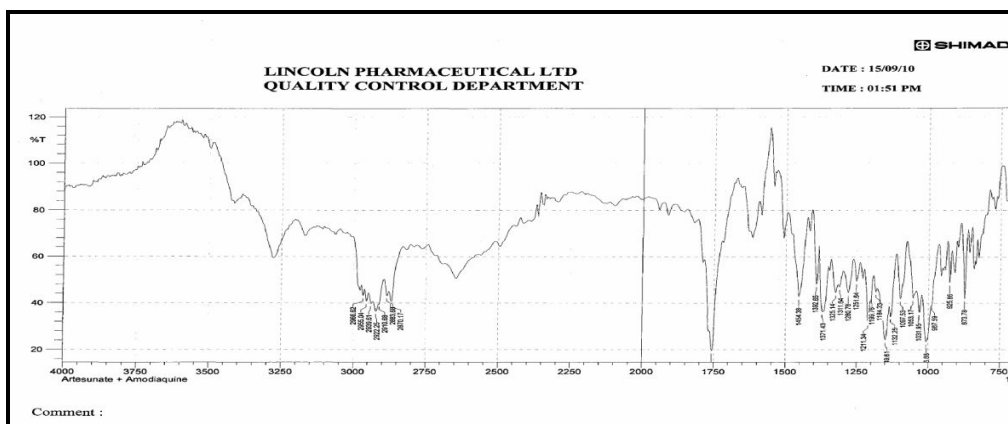
(a) artesunate drug only (b) amodiaquine drug only (c)Artesunate+Amodiaquine mixture



a



b



c

Figure 2: Drug scanning calorimetry spectra
 (a) artesunate drug only (b) amodiaquine drug only (c) Artesunate+Amodiaquine mixture.

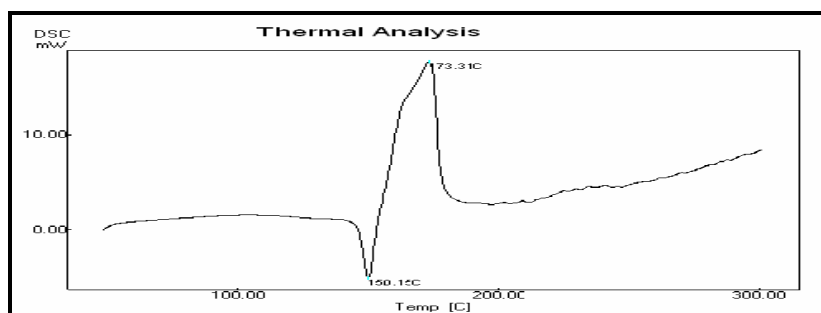
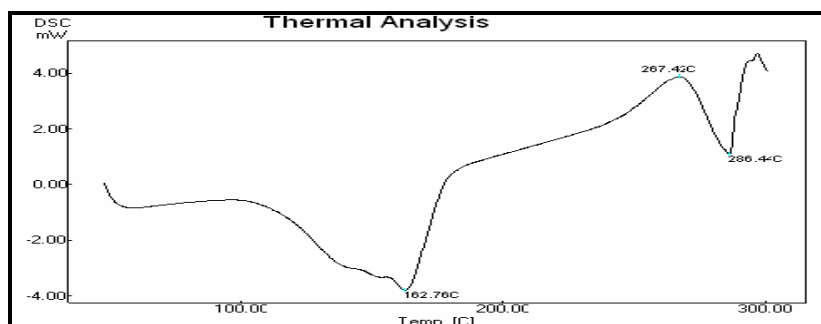
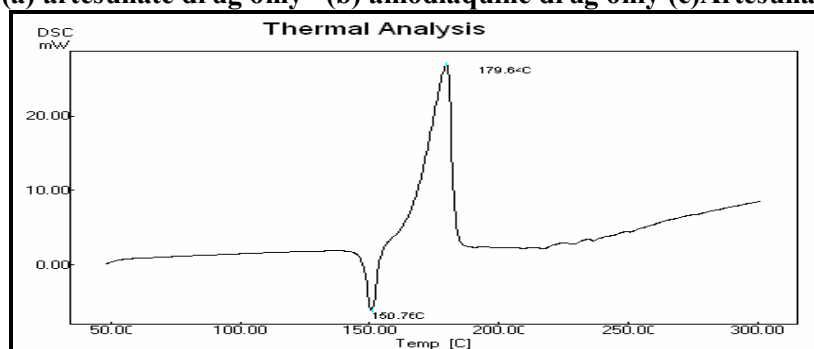


Table 1: Coded values for amount of ingredient

Coded value	Concentration of (disintegrate) Crosscarmellose sodium (X1)	Concentration of (basic stabilizing agent) Calcium carbonate (X2)
-1	1.5%	5%
0	2 %	15%
1	2.5 %	20%

Table 2: Factorial batches for artesunate layer

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Artesunate	100	100	100	100	100	100	100	100	100
MCC(Avicel)	66.33	46.83	37.08	65.35	45.85	36.1	64.38	44.88	35.13
Crosscarmellose sodium	2.92	2.92	2.92	3.9	3.9	3.9	4.87	4.87	4.87
Calcium carbonate	9.75	29.25	39	9.75	29.25	39	9.75	29.25	39
Talcum powder	3	3	3	3	3	3	3	3	3
Aerosil	10	10	10	10	10	10	10	10	10
Magnesium stearate	3	3	3	3	3	3	3	3	3
Total weight	195	195	195	195	195	195	195	195	195

All the weights are in mg.

Table 3: Formulation batches for amodiaquine hydrochloride layer

Ingredients	G1	G2	G3	G4	G5
Amodiaquine hydrochloride	400	400	400	400	400
MCC(Plain)	14.2	12.2	14.2	-	-
MCC(Avicel)	-	-	-	14.6	14.6
PVPK-30 (2%)	9	-	-	9	9
PVP K-90 (2.5)	-	11	-	-	-
Starch (2%)	-	-	9	-	-
Isopropyl alcohol	75	75	75	75	75
Crosscarmellose sodium (1%)	4.4	4.4	4.4	4.4	4.4
Talcum powder	4	4	4	4	4
Magnesium stearate	4	4	4	4	4
Aerosil	4.4	4.4	4.4	4	6.6
Total weight	440	440	440	440	440

All the weight are in mg

Table 4: Codes for variable in 3²factorial design

Variable	Low(-1)	Medium(0)	High(+1)
Spray rate (gm/min) X1	12	14	16
Inlet temperature (°C) X2	45	50	55

Table 5: 3²factorial design batches

Batch	X ₁	X ₂	Standard deviation of coating uniformity	Coating process efficiency	%LOD
1	-1	-1	2.39	89.76	2.17
2	-1	0	2.34	87.55	1.99
3	-1	1	1.98	94.97	1.49
4	0	-1	2.49	74.43	2.4
5	0	0	2.37	77.68	2.33
6	0	1	2.29	84.67	2.12
7	1	-1	2.49	79.89	2.45
8	1	0	2.42	74.86	2.15
9	1	1	2.43	89.45	2.1

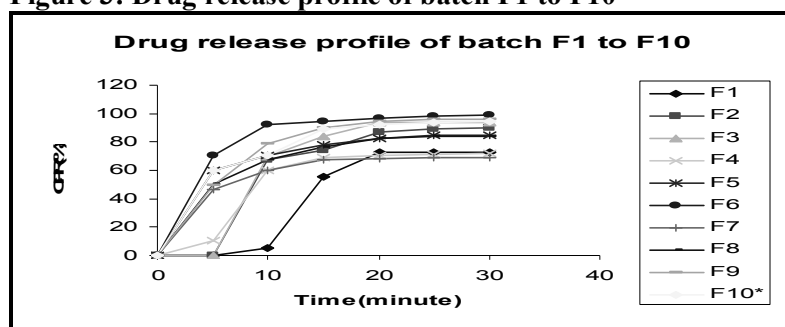
Figure 3: Drug release profile of batch F1 to F10

Figure 4: Contour plot

(a) Plot between crosscarmellose sodium, calcium carbonate and disintegration time (b) Plot between crosscarmellose sodium, calcium carbonate & drug release at 30 minute (c) Contour overlay

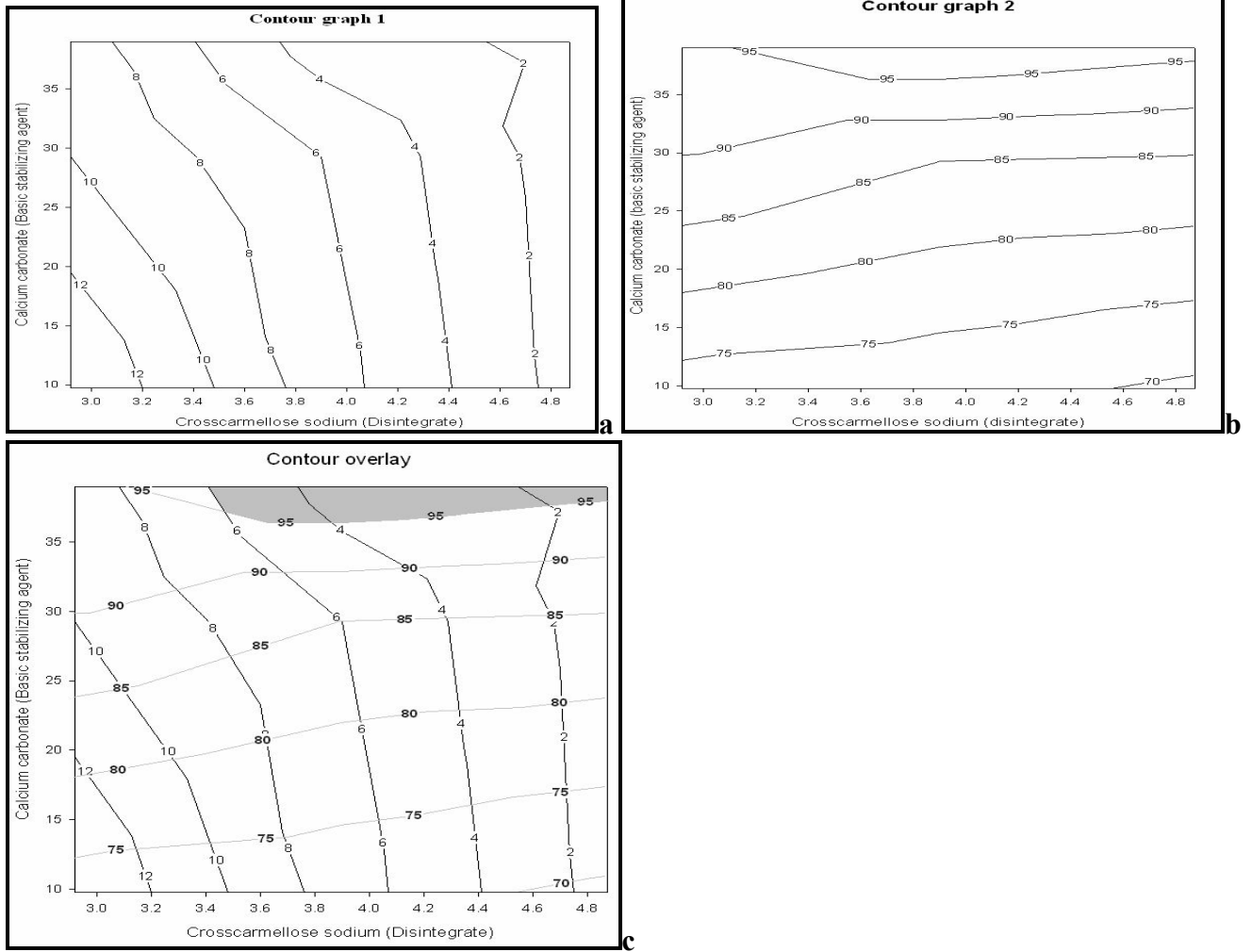


Figure 5: Amodiaquine hydrochloride drug release profile of batch G1 to G5

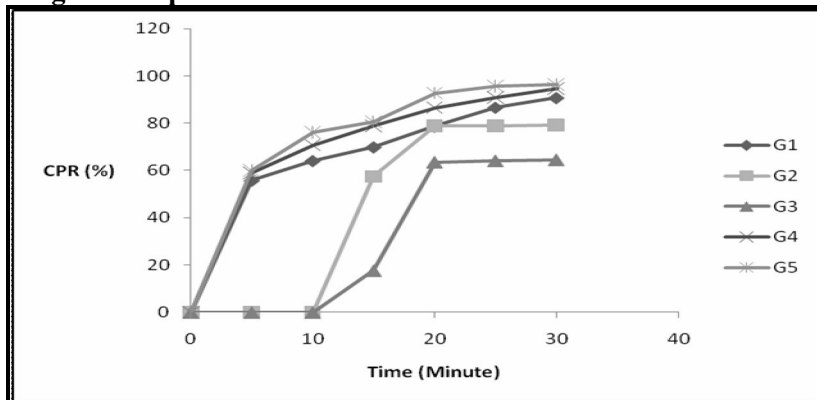


Figure 6: Dissolution profile of bilayer tablet

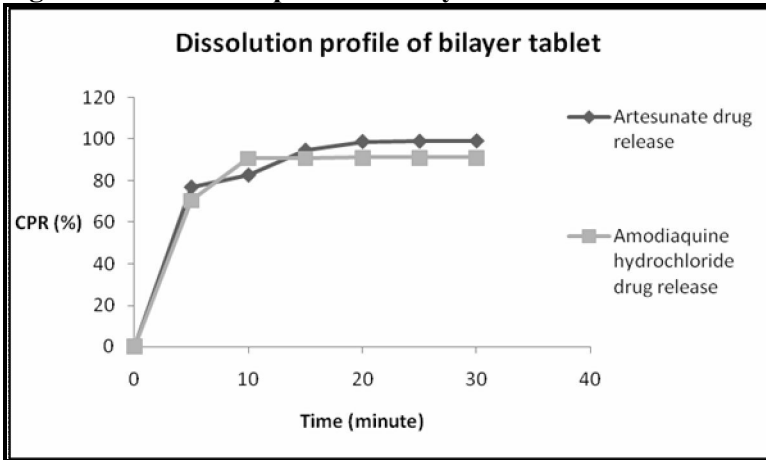
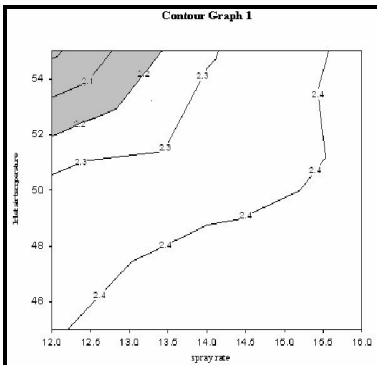
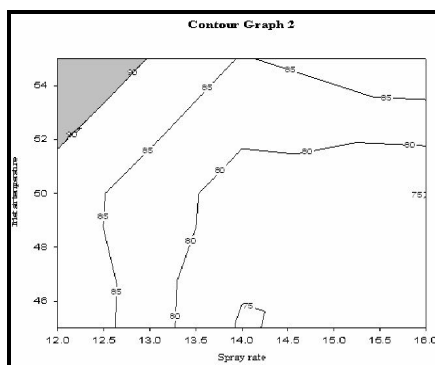


Figure 7: Contour plot

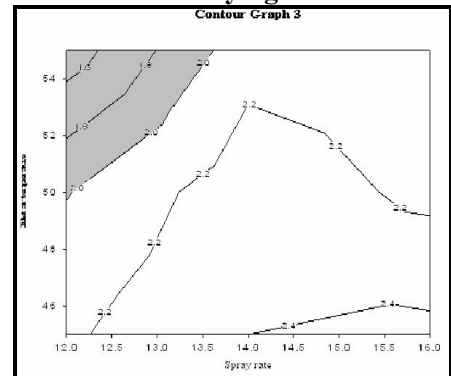
(a) Plot between spray rate & inlet air temperature on SD of coating uniformity



(b) Plot between spray rate & inlet air temperature on coating process efficiency



(c) Plot between spray rate & inlet air temperature on % loss on drying



(d) Contour overlay

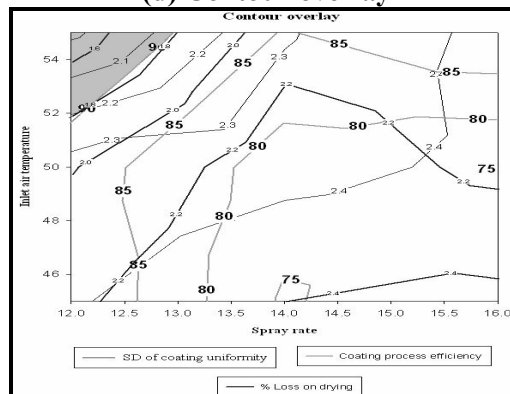
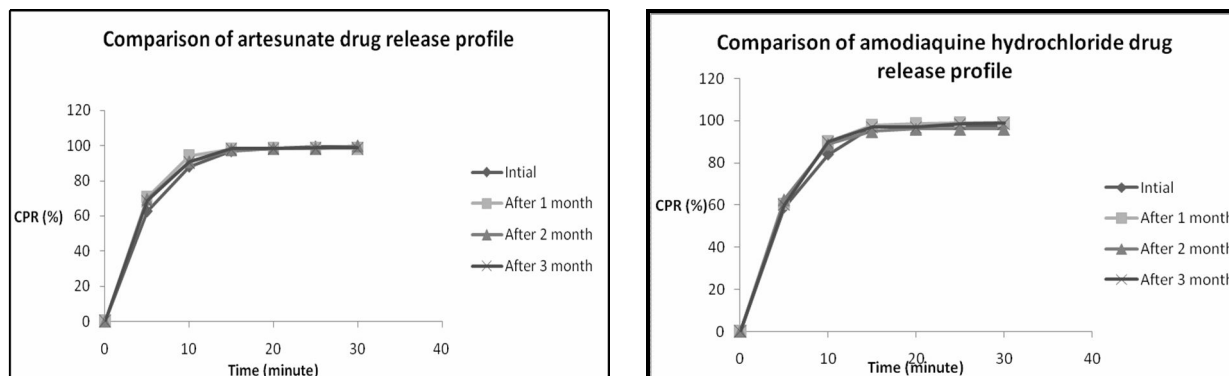


Figure 8; Drug release profile after accelerated stability study of 3 month

RESULT & DISCUSSION:

Preformulation study

The artesunate & amodiaquine hydrochloride have found poor flow property & compressibility. So tablets were prepared using granulation method. In drug-drug compatibility study after 2 week physical compatibility study of both drug dry powders mixture(1:4) showed no visible change but in wet mixture of two drug(1:4:0.05) was found to change colour from yellow to dark brown. So they were found stable in dry state. In non thermal analysis FT-IR study (figure 1) no interaction was found while in thermal analysis DSC study (figure2) interaction was found at glass transition temperature. So tablets were prepared in humidity & temperature controlled environment.

Factorial design batch F1to F9 &check point batch F10 for artesunate layer

The result of micromeritic properties & evaluation parameters were complied with in limit. The friability of batch F6 was found to be least & CPR (%) was found maximum than other batches. (figure3)

Contour plot: Contour plots of factorial design batches F1 to F9 using sigmastat software, version 12 were shown in figure 4. Contour plot between crosscarmellose sodium & calcium carbonate showed that the optimized concentration of crosscarmellose sodium & calcium carbonate were found to be 3.41 to 4.8mg & 35 to 40mg respectively. So F6 batch was considered as promising batch for further investigation.

Regression analysis: Regression analysis was done for X_1 & X_2 values taking the disintegration time as the response. The good correlation coefficient ($R^2=0.9822$) was found & P values were found to be 0.0012 and 0.0361 ($P<0.05$) for the X_1 & X_2 variables

respectively. X_1 variable has more significant effect than X_2 variable. The polynomial equation for Disintegration time

$$=5.133-4.866X_1-1.466X_2+1.300X_1X_2+0.800X_1^2+0.300X_2^2$$

Regression analysis was done for X_1 & X_2 values taking the cumulative drug release after 30 minute as the response. The correlation coefficient (R^2) was found to 0.9820. P value was found to be 0.0010 which clearly depicts that the X_2 variable has significant effect on drug release. The polynomial equation Drug release at 30 minute= $86.844-$

$$1.153X_1+12.578X_2+1.505X_1X_2-0.746X_1^2-2.501X_2^2$$

For the check point batch predicted value from equation & experimental value for disintegration time were found 2.43 & 2.10 respectively. Predicted value from equation & experimental value for drug release at 30 minute were found 95.14 & 94.17 respectively. So % error was found to be very less. So mathematical model was found to be validated.

Amodiaquine hydrochloride layer batch G1 to G5

In determining evaluation parameters hardness, disintegration time & % drug release of batch G1 complied the standard limit. So PVPK-30 selected as binder but in G1 batch friability was found more compare to other batch because the granules of G1 batch were soft & angle of repose of G1 batch was more. So for improving flow property & hardening of granules MCC (avicel) used as diluent in G4 & G5 batch. In G3 batch sticking problem was observed so solve this problem increase concentration of aerosil (1.5%) in G5 batch. The batch G5 was promising batch for further work. Drug release profile was described in figure 5.

Bilayer tablet of batch H:

The micromeritic & evaluation parameters of bilayer tablet complied with in limit.

Optimized coating parameter

The result of factorial design batches was shown in table 5. In factorial design batch no.3 standard deviation, % LOD were found be least & coating process efficiency was found to be maximum compared to other batches. Contour plots were shown in figure 7. Regression analysis was done for X_1 (Spray rate) & X_2 (Inlet air temperature) value for taking coating uniformity, coating process efficiency and %LOD as responses. The good correlation coefficient ($R^2= 0.9183$) was found for coating uniformity. The P value was found to be 0.0381 and 0.0325 ($P < 0.05$) for X_1 and X_2 variable respectively which clearly depict that both variables have significant effect. The polynomial equation for Coating uniformity = $2.404 + 0.105X_1 - 0.111X_2 - 0.087X_1X_2 - 0.041X_1^2 - 0.031X_2^2$

The good correlation coefficient ($R^2= 0.9433$) was found for coating process efficiency. The P value was found to be 0.02752 and 0.03689 ($P < 0.05$) for X_1 and X_2 variable respectively which clearly depict that both variables have significant effect. The polynomial equation for

Coating process efficiency = $75.2614 - 0.68X_1 + 4.168X_2 + 1.085X_1X_2 + 7.153X_1^2 + 5.498X_2^2$

The good correlation coefficient ($R^2= 0.9278$) was found for % LOD. The P value was found to be 0.0413 and 0.0233 ($P < 0.05$) for X_1 and X_2 variable respectively which clearly depict that both variables have significant effect on %LOD. The polynomial equation for

$$\%LOD = 2.306 + 0.175X_1 - 0.218X_2 + 0.0825X_1X_2 - 0.225X_1^2 - 0.035X_2^2$$

Accelerated stability study of best optimized batch

After 3 month accelerated stability study it was concluded that there was no significant change in all tablet evaluation parameters during accelerated stability study. Comparison drug release profile of initial & after 3 month accelerated stability study were described in figure 8. So the moisture barrier film coated tablet was found to be stable for 3 month.

CONCLUSION:

Attempts were made in the present investigation to prepare a stable fixed dose combination film coated tablet of artesunate & amodiaquine hydrochloride. These results clearly reflect that the prepared formulation offers desired release profile & stability. The stability & drug release profile of artesunate in bilayer tablet was improved using calcium carbonate as basic stabilizing agent & moisture barrier film coating for protection of moisture.

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