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Formulation and *In-vivo* Evaluation of Atovaquone Solid Dispersions

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A B S T R A C T
In the present study Atovaquone solid dispersions were prepred in order to improve the bioavailability and solubility of the drug by using PVP, PEG 5000, Poloxamer 188 as hydrophilic carriers and Gelucire 44/14 and Gelucire 50/13 as lipophilic carriers. Aerosil 380 was selected as inert carrier in case of solid dispersions prepared with lipophilic carriers. Ratio of drug to polymer was varied from 1:1 to 1:5. Melting and Solvent evaporation method was followed. From the results of comparative dissolution studies conducted between optimized formulations, pure drug and marketed formulations, it was concluded that formulations prepared with gelucire 44/14 (ASD 24) have shown greater drug release than remaining formulations. From the results of *In vivo* studies conducted between final optimized formulation of each drug, pure drugs and marketed formulation of Atovaquone, it can be concluded that bioavailability of final optimized formulation was higher than the marketed formulation as well as pure drug.

Keywords: Atovaquone, Solvent evaporation method, solid dispersions, PVP, PEG 5000, Poloxamer 188

A R T I C L E I N F O

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1. Introduction

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles (clusters) or in crystalline particles (Chiou and Riegelman, 1971).

Atovaquone is a unique naphthoquinone with broad-spectrum antiprotozoal activity. It is effective for the treatment and prevention of Pneumocystis carinii pneumonia (PCP), Malaria and Babesiosis .In spite of this wide spectrum of pharmacological activity, its use in pharmaceutical field is limited because it suffers from low aqueous solubility (less than 0.0002mg/ml at 25˚C) and belongs to class II of the biopharmaceutical classification system (BCS). As a result it exhibits poor dissolution and insufficient oral bioavailability.

2. Materials and Methods

Atovaquone, PVP, PEG, Poloxamer, Gelucire, Aerosil 380, chemicals were Laboratory grade made of SD Fine chemicals Pvt Ltd

Formulation Development of Solid Dispersions of Atovaquone: Solid dispersions of Atovaquone were prepared by using different hydrophilic/lipid based carriers such as PVP, PEG, Poloxamer 188, gelucire 44/14 and gelucire 50/13 in different ratios such as 1:1,1:2 &1:3. These ratios were decided based on the results obtained in phase solubility studies. Composition of prepared solid dispersions are given in table 1

UV Visible Spectroscopic method to estimate Atovaquone from *in vitro* samples

Calibration curve of Atovaquone was plotted by using UV visible spectroscopic method at 234 nm. Linearity was observed between 2-16 µg/mL. Absorbance values obtained are given in table 6 and calibration curve plotted is shown in figure 1. Regression equation obtained was used to estimate the Atovaquone from in vitro samples.

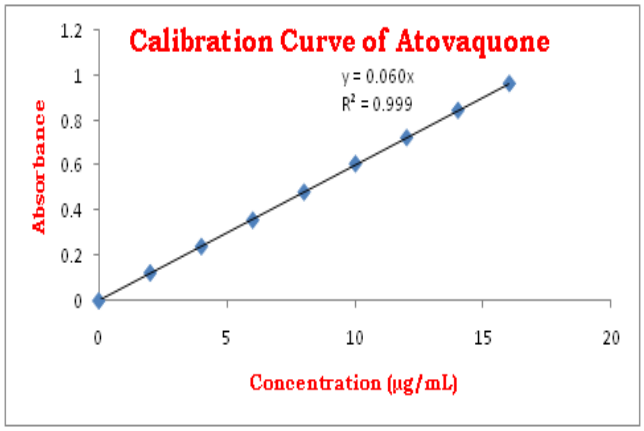

Figure 1: Calibration Curve of Atovaquone

RP-HPLC method to estimate Atovaquone from *in vivo* samples:

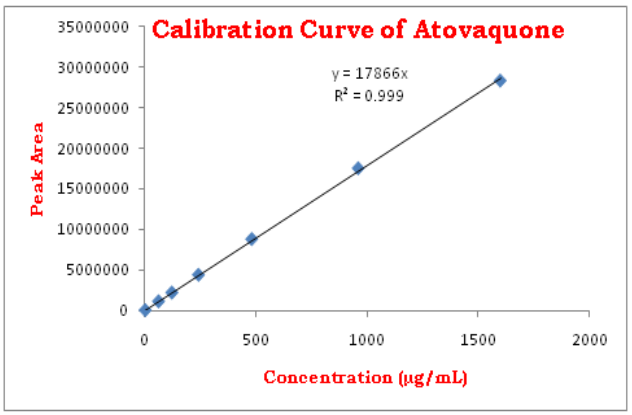

Figure 2: Calibration Curve of Atovaquone by HPLC

Calibration curve of Atovaquone was plotted by using RP-HPLC. Linearity was observed between 60-1600 µg/mL. Peak Area values obtained are given in table 7 and calibration curve plotted is shown in figure 2. Regression equation obtained was used to estimate the Atovaquone from in vivo samples.

3. Results and Discussion

Micromeritic evaluation: The prepared solid dispersions were characterized for their micromeritic properties. Results are tabulated in table no 8. It was observed that flow property was improved with all the formulations compared to pure drug.

***In Vivo* bioavailability studies:**

In Vivo bioavailability studies were conducted in rats for final optimized formulation, pure drug and marketed formulation. Balanced Incomplete Block Design was followed. Blood samples were collected at various time intervals for up to 4 hrs and assayed for parent drug by RP-HPLC method. Peak areas obtained for each formulation at each time intervals were shown in Tables 9. From the peak areas plasma concentrations at each time interval were calculated by using slope of the standard graph and shown in table 10. A graph was plotted by taking plasma concentration on Y- Axis and time on X-Axis and AUC values from o to t was calculated. Graphs were shown in Figures 3.

Two Way ANOVA was applied for AUC values (Tables 11) of all formulations. From the results (Tables 12) it was concluded that there is a significant difference in the bioavailability (AUC) of each formulation of both the drugs and from the average AUC values it can be observed that final optimized formulation have shown improved bioavailability than marketed formulation and pure drugs. Hence, it can be concluded that solid dispersion technique will improve the bioavailability of poorly soluble drugs.

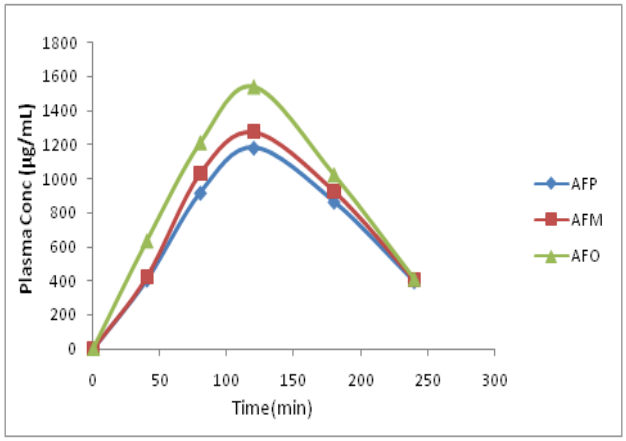

Figure 3: Mean Plasma Concentration-Time profile of Atovaquone Formulations

Various pharmacokinetic parameters like Cmax, t_{max} and elimination half-life were determined for pure drug, marketed formulation and optimized formulation and the results were shown in table 13. Relative bioavailability was determined and results obtained were tabulated in table 14 from the results obtained it was observed that the relative bioavailability of optimized formulation was higher than pure drug.

Table 1: Solid dispersions of Atovaquone prepared with PVP

Frmulation Code	Drug (mg)	Carrier (PVP) (mg)	Ratio	Method
ASD1	250	125	1:1	Melting
ASD2	250	250	1:2	Melting
ASD 3	250	370	1:3	Melting
ASD 4	250	125	1:1	Solvent Evaporation
ASD 5	250	250	1:2	Solvent Evaporation
ASD 6	250	370	1:3	Solvent Evaporation

Table 2: Solid dispersions of Atovaquone prepared with PEG 5000

Frmulation Code	Drug (mg)	Carrier (PEG) (mg)	Ratio	Method
ASD 7	250	125	1:1	Melting
ASD 8	250	250	1:2	Melting
ASD 9	250	370	1:3	Melting
ASD 10	250	125	1:1	Solvent Evaporation
ASD 11	250	250	1:2	Solvent Evaporation
ASD 12	250	370	1:3	Solvent Evaporation

Table 3: Solid dispersions of Atovaquone prepared with Poloxamer 188

Frmulation Code	Drug (mg)	Carrier (Poloxamer) (mg)	Ratio	Method
ASD 13	250	125	1:1	Melting
ASD 14	250	250	1:2	Melting
ASD 15	250	370	1:3	Melting
ASD 16	250	125	1:1	Solvent Evaporation
ASD 17	250	250	1:2	Solvent Evaporation
ASD 18	250	370	1:3	Solvent Evaporation

Table 4: Solid dispersions of Atovaquone prepared with Gelucire 44/14

Frmulation Code	Drug (mg)	Carrier (Gelucire) (mg)	Inert Carrier (Aerosil 380) (mg)	Ratio	Method
ASD 19	250	125	125	1:1	Melting
ASD 20	250	250	250	1:2	Melting
ASD 21	250	370	370	1:3	Melting
ASD 22	250	125	125	1:1	Solvent Evaporation
ASD 23	250	250	250	1:2	Solvent Evaporation
ASD 24	250	370	370	1:3	Solvent Evaporation

Table 5: Solid dispersions of Atovaquone prepared with Gelucire 50/13

Frmulation Code	Drug (mg)	Carrier (Gelucire) (mg)	Inert Carrier (Aerosil 380) (mg)	Ratio	Method
ASD 25	250	125	125	1:1	Melting
ASD 26	250	250	250	1:2	Melting
ASD 27	250	370	370	1:3	Melting
ASD 28	250	125	125	1:1	Solvent Evaporation
ASD 29	250	250	250	1:2	Solvent Evaporation
ASD 30	250	370	370	1:3	Solvent Evaporation

Micromeritic evaluation, Saturation solubility studies, and in-vitro dissolution studies are the various evaluation tests performed for the prepared solid dispersions

Table 6: Calibration curve of Atovaquone

Concentration (µg/mL)	Absorbance (at 234 nm)
0	0.000
2	0.124
4	0.241
6	0.358
8	0.482
10	0.608
12	0.724
14	0.845
16	0.964

Table 7: Calibration curve of Atovaquone (HPLC):

Concentration (µg/mL)	Peak Area
0	0
60	1086000
120	2174400
240	4358400
480	8767200
960	17500800
1600	28304000

Table 8: Micromeritic properties of Atovaquone Solid Dispersions

S.NO.	Angle of Repose (^{0})	Tapped Density (g/cc)	Bulk Density (g/cc)	Consolidation Index (%)	Hausner's Ratio
ASD1	$27^0 12'' \pm 12''$	0.89±0.01	0.74 ± 0.03	14.5± 0.11	1.16± 0.01
ASD2	$28^0 45'' \pm 18''$	0.84±0.03	0.72± 0.03	14.2± 0.34	1.18 ± 0.02
ASD3	$29^0 12'' \pm 32''$	0.82± 0.04	0.72± 0.05	12.5± 0.84	1.16± 0.03
ASD4	$29^0 54'' \pm 45''$	0.83±0.04	0.71± 0.06	12.1± 0.35	1.14 ± 0.04
ASD5	$30^0 12'' \pm 44''$	0.81±0.03	0.74± 0.01	12.3± 0.31	1.12 ± 0.03
ASD6	$27^0 19'' \pm 23''$	0.77±0.06	0.68± 0.03	12.7± 0.36	1.15 ± 0.09
ASD7	$26^0 12'' \pm 17''$	0.74±0.03	0.67± 0.09	13.2± 0.25	1.13 ± 0.05
ASD8	$29^0 52'' \pm 32''$	0.74±0.04	0.66 ± 0.09	13.3± 0.65	1.18± 0.07
ASD9	$26^0 18' \pm 15''$	0.77±0.06	0.68 ± 0.02	12.6± 0.76	1.15± 0.08
ASD10	$26^0 52'' \pm 14''$	0.72±0.05	0.62 ± 0.03	14.3± 0.77	1.14± 0.03
ASD11	$26^0 81'' \pm 12''$	0.71±0.07	0.58 ± 0.02	14.3± 0.57	1.18± 0.02
ASD12	$29^0 52'' \pm 52''$	0.68±0.03	0.57 ± 0.02	14.6± 0.98	1.17± 0.03
ASD13	$28^0 42'' \pm 61''$	0.66±0.04	0.54± 0.02	15.4± 0.87	1.18± 0.02
ASD14	$28^0 53'' \pm 60''$	0.63±0.05	0.53± 0.03	15.7± 0.75	1.19± 0.02
ASD15	$26^0 32'' \pm 32''$	0.62±0.03	0.52± 0.05	16.2± 0.65	1.19± 0.04
ASD16	$32^0 31'' \pm 53''$	0.69±0.03	0.57± 0.06	14.7± 0.64	1.19± 0.04
ASD17	$26^0 22'' \pm 34''$	0.68±0.03	0.58± 0.06	15.3± 0.98	1.16± 0.05
ASD18	$28^0 21'' \pm 15''$	0.66±0.05	0.56± 0.06	15.2± 0.85	1.16± 0.04
ASD19	$32^0 32'' \pm 11''$	0.85± 0.02	0.75± 0.02	14.6± 0.85	1.13± 0.06
ASD20	$2611'' \pm 19''$	0.62± 0.03	0.53± 0.03	14.9± 0.76	1.18± 0.05
ASD21	$27^0 11'' \pm 12''$	0.85±0.01	0.75 ± 0.02	14.7± 0.14	1.16± 0.01
ASD22	$29^0 52'' \pm 19''$	0.86±0.04	0.76± 0.04	14.1± 0.32	1.17 ± 0.02
ASD23	$26^0 21'' \pm 81''$	0.67± 0.03	0.56± 0.05	12.6± 0.76	1.13± 0.02
ASD24	$26^0 31'' \pm 17''$	0.63± 0.06	0.53± 0.08	12.3± 0.84	1.12± 0.06
ASD25	$26^0 52'' \pm 72''$	0.66± 0.04	0.58± 0.08	12.2± 0.85	1.16± 0.05

ASD26	$25^0 11'' \pm 11''$	0.76± 0.03	0.68± 0.02	12.9± 0.61	1.13± 0.04
ASD27	$29^0 31'' \pm 12''$	0.75± 0.06	0.69± 0.07	13.1± 0.75	1.15± 0.06
ASD28	$30^0 0'' \pm 81''$	0.72± 0.08	0.63± 0.09	13.1± 0.75	1.16± 0.08
ASD29	$28^0 3'' \pm 32''$	0.71± 0.08	0.66± 0.03	12.9± 0.96	1.16± 0.04
ASD30	$27^0 21'' \pm 53''$	0.67± 0.07	0.59± 0.04	14.3± 0.92	1.16± 0.05

Table 9: Peak Area Values of Atovaquone Formulations in the Rat Plasma

FORMULATION	SUBJECT	PEAK AREA AT				
		40 min	80 min	120 min	180 min	240 min
LFP (ATOVAQUONE PURE DRUG)	1	7149616	15875549	21026674	15185207	**6857864**
	2	7230192	15806586	20697940	15513941	**7022231**
	3	7214648	16450834	20771905	15678308	**7122638**
	4	7241804	17341633	21376669	14938656	**6858757**
	5	7116028	16368651	21355408	15596125	**7350966**
	6	7149616	16204283	21191041	15431758	**6857864**
LFM (ATOVAQUONE MARKETED)	1	7480137	18332839	22685889	16445117	**7151402**
	2	7488355	18341057	22694108	16535519	**7159621**
	3	7570539	18423241	22776291	16543737	**7184276**
	4	7605735	18447896	22833820	16593048	**7266460**
	5	7488355	18341057	22694108	16436899	**7150509**
	6	7496574	18505424	22693215	16510864	**7216256**
LFO (ATOVAQUONE OPTIMIZED FORMULATION8)	1	11303818	21735061	27764657	18236720	**7348643**
	2	11295600	20913225	27756439	18229216	**7356861**
	3	11303818	21817245	26942821	18154536	**7315770**
	4	11221635	21652877	27682474	18237434	**7349358**
	5	11295600	21737562	27749829	18234933	**7340425**
	6	**11312037**	**21735061**	**26934603**	**18237434**	**7350966**

Table 10: Plasma Concentration Values of Atovaquone Formulations in the Rat at Various Time Intervals

FORMULATION	SUBJECT	Plasma Concentration (ng/ml) at				
		40 min	80 min	120 min	180 min	240 min
AFP (ATOVAQUONE PURE DRUG)	1	400.18	888.59	1176.91	849.95	**383.85**
	2	404.69	884.73	1158.51	868.35	**393.05**
	3	403.82	920.79	1162.65	877.55	**398.67**
	4	405.34	970.65	1196.50	836.15	**383.90**
	5	398.30	916.19	1195.31	872.95	**411.45**
	6	400.18	906.99	1186.11	863.75	**383.85**
AFM (ATOVAQUONE MARKETED)	1	418.68	1026.13	1269.78	920.47	**400.28**
	2	419.14	1026.59	1270.24	925.53	**400.74**
	3	423.74	1031.19	1274.84	925.99	**402.12**
	4	425.71	1032.57	1278.06	928.75	**406.72**
	5	419.14	1026.59	1270.24	920.01	**400.23**
	6	419.60	1035.79	1270.19	924.15	**403.91**
AFO (ATOVAQUONE OPTIMIZED FORMULATION)	1	632.70	1216.56	1554.05	1020.75	**411.32**
	2	632.24	1170.56	1553.59	1020.33	**411.78**
	3	632.70	1221.16	1508.05	1016.15	**409.48**
	4	628.10	1211.96	1549.45	1020.79	**411.36**
	5	632.24	1216.70	1553.22	1020.65	**410.86**
	6	**633.16**	**1216.56**	**1507.59**	**1020.79**	**411.45**

Table 11: AUC Values of Atovaquone Formulations

FORMULATION	AUC (µg.hr/ml)					
	1	2	3	4	5	6
AFP	2.75	2.76	2.79	2.81	2.82	**2.78**
AFM	3.00	3.01	3.02	3.03	3.00	**3.01**
AFO	**3.54**	**3.51**	**3.50**	**3.54**	**3.54**	**3.50**

Table 12: ANOVA Two Way Method

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F
Between Subjects	0.00289	5	0.00057	1.598
Between Formulations	1.70831	2	0.85415	2358.09
Error	0.00362	10	0.00036	------
Total	1.71482	17	-----	------

Table 13: Pharmacokinetic Parameters (Mean ± SD) (n=6)

FORMULATION	Cmax (µg/ml)	Tmax (Hrs)	Half life (Hrs)
AFP	1.21 ± 0.01	2.1 ± 0.01	2.5 ± 0.02
AFM	1.56 ± 0.03	1.43 ± 0.02	2.6 ± 0.01
AFO	1.66 ± 0.04	1.35 ± 0.03	2.7 ± 0.03

Table 14: Relative Bioavailability of Optimized Formulation of Atovaquone (Mean ± SD) (n=6)

Formulation	Relative Bioavailability (%)
With Marketed Formulation	116 ± 2.3
With Pure Drug	126 ± 3.2

4. Conclusion

In the present study Atovaquone solid dispersions were prepared in order to improve the bioavailability and solubility of the drug by using PVP, PEG 5000, Poloxamer 188 as hydrophilic carriers and Gelucire 44/14 and Gelucire 50/13 as lipophilic carriers. Aerosil 380 was selected as inert carrier in case of solid dispersions prepared with lipophilic carriers. Ratio of drug to polymer was varied from 1:1 to 1:5. Melting and Solvent evaporation method was followed. From the results of comparative dissolution studies conducted between optimized formulations, pure drug and marketed formulations, it was concluded that formulations prepared with gelucire 44/14 (ASD 24) have shown greater drug release than remaining formulations. From the results of *In vivo* studies conducted between final optimized formulation of each drug, pure drugs and marketed formulation of Atovaquone, it can be concluded that bioavailability of final optimized formulation was higher than the marketed formulation as well as pure drug.

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