Formulation of Atovaquone Syrup by Hydrotropy Method

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Abstract

The aim of the present research study was to explore the possibility of employing Hydrotropy techniques in the formulation and evaluation of aqueous oral liquid formulation of a poorly water soluble drug Atovaquone. In the present study practically insoluble drug Atovaquone was tried to solubilize by employing the combination of physiologically compatible hydrotrope and solubilizer agents i.e. solubilizer to attempt its oral liquid formulations. Hydrotropy is a solubilisation process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. Hydrotropy method help to improve aqueous solubility of Atovaquone drug having very low aq. Solubility i.e.0.2µg/ml.Inthis method Atovaquone was solubilized up to 4680.95 mg/ml (increase in solubility).As there is no liquid formulation of Atovaquone available in the market. Atovaquone syrup is useful for the pediatric as well as geriatric to improve patient compliance. Hydrotropy approach used to improve the solubility of Atovaquone by using Sodium benzoates a solubilizer. The prepared syrup formulation was subjected to physical stability testing programmed as per ICH conditions at for a period of 60 days. The results showed that the formulations were unaffected in respect of color stability and precipitation on storage at room temperature & 75% RH.

Key words: ICH: International council for harmonisation, RH: Relative humidity,SA: Sodium acetate, SB: Sodium benzoate, SS: Sodium salicylate

Introduction

Hydrotrophy is a solubilisation process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. Solute consists of alkali metal salts of various organic acids. Hydrotropic agents are ionic organic salts. Additives or salts that increase solubility in given solvent are said to “salt in” the solute and those salts that decrease solubility “salt out” the solute. Several salts with large anions or cations that are themselves very soluble in water result in “salting in” of non-electrolytes called “hydrotropic salts” a phenomenon known as “hydrotropism”. Hydrotropic solutions do not show colloidal properties and involve a weak interaction between the hydrotropic agent and solute. Hydrotrophy designate the increase in solubility in water due to the presence of large amount of additives. The mechanism by which it improves solubility is more closely related to complexation involving a weak interaction between the hydrotropic agents like sodium benzoate, sodium acetate, sodium alginate, urea and the poorly soluble drugs. Additives may either increase or decrease the solubility of a solute in a given solvent. These salts that increase solubility are said to salt in‘the solute and those salts that decrease the solubility salt out‘ the solute. The effect of an additive depends very much on the influence; it has on the structure of water or its ability to compete with the solvent water molecules.Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the Solubility of drug molecules. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Currently only 8% of new drug candidates have both high Solubility and permeability. Orally administered drugs completely absorb only when they show fair solubility in gastric drug solubility in an aqueous environment and drug permeability through lipophilic membranes being the important ones

Advantages of hydrotropic solubilisation technique**:**

1) Hydrotropy is suggested to be superior to other solubilisation method, such as miscibility, micellarsolubilisation, cosolvency and salting in, because the solvent character is independent of pH, has high selectivity and does not require emulsification

2) It only requires mixing the drug with the hydrotrope in water.

3) It does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion system

4) It can replace organic solvents which are employed in various titrimetric and spectrophotometric estimations of poorly water soluble drugs. Also the hydrotropic solutions can be employed for TLC of poorly water soluble drug precluding the use of organic solvent. So it is economic, safe and user friendly method.

Hydrotropes used for present study

Hydrotropic agents used for the solubility enhancement and formulation development of syrup of Atovaquone are listed below with their LD50 values performed on animals

Table No. 1 Hydrotropes used for present study

Sr.No.	Hydrotropic agent code used for hydrotropic agent	Source
1	Urea U	LD 50 values calculated in animal
2	Sodium benzoate SB	15 gm/kg -Rat 11.5 gm/kg – Mouse
3	Sodium acetate SA	070 gm/kg-Rat, 1.600 gm/kg- Mouse 2.000 gm/kg- Rabbit
4	Sodium citrate SC	6.891 gm/kg – Mouse 3.530 gm/kg – Rat

Material & Method

Table No. 2 Chemicals Used During Research Work

Sr.No.	Name of chemicals	Source
1	Atovaquone	GSK.Pharmaceutical
2	Sodium salicylate	Research Fine Lab
3	Sodium benzoate	Research Fine Lab
4	Sodium acetate	Research Fine Lab
5	Sodium citrate	Research Fine Lab
6	Urea	Research Fine Lab

Table No. 3 Equipments Used During Research Work:

No.	Equipments	Manufacturer
1.	Weighing balance	Citizen(CY-220)
2.	Micropipette	SIMCO
3.	Melting point apparatus	LAB-TRONICS
4.	pH meter	EQUIP-TRONICS EQ-614A
5.	Hot air oven	Universal oven Chief Scientific
6.	U V Spectrophotometer	Schimadzu
7.	Infrared spectrophotometer	JASCO(IR-4100)
8.	Stability chamber	Thermo lab
9.	Differential Scanning Calorimeter	SCHIMADZU DSC-60
10.	Sieve	Alpine
11.	Ultrasonicator	APL

Selection of Hydrotropic Agents for Atovaquone

Solubility Determination:

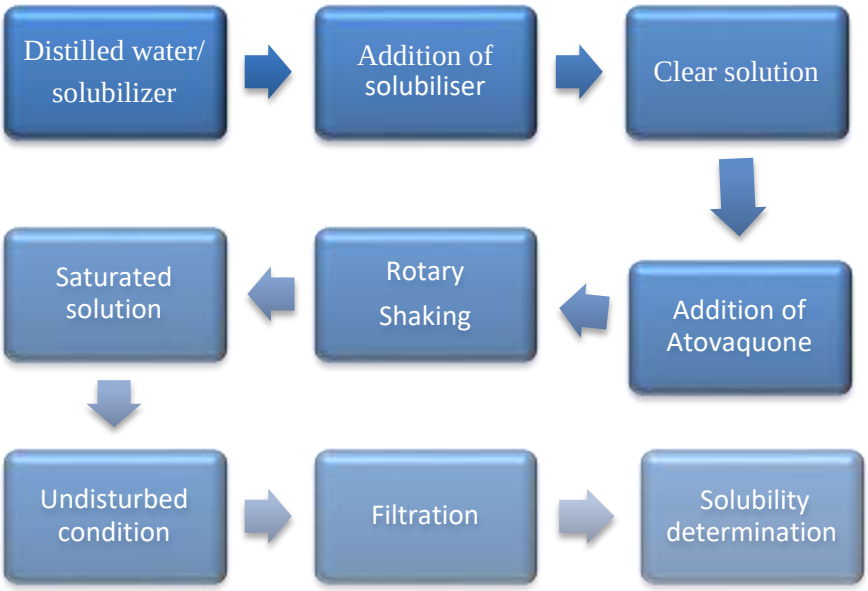


Figure No. 1 General procedure of solubility determination

Since, the present investigation is the beginning of the hydrotropic solubilisation phenomenon and no work on method and formulation development has been reported earlier using this technique so, the widely used hydrotropic agents, sodium benzoate, urea, sodium acetate, and sodium citrate, sodium salicylate were selected as model hydrotropic agents to solubilize the drug Atovaquone (antimalarial) which is practically insoluble in water.

Solubility Enhancement Ratio Was Calculated by Using Following Equation

Solubility enhancement ratio = Solubility in particular hydrotropic solution/solubility in water.

Solubilization of Atovaquone in Different Blends of Hydrotropic Agents

By using mixed Hydrotropy method the solubility of Atovaquone was determined. In this method used combination of three and two hydrotrope of concentration 10% given.

Preformulation Study

1. Flow Property of Drug

Table No. 4: Physical property of Atovaquone

Sr.No.	Physical property	Result	**Description**
1	Bulk Density (gm/ml)	0.24 gm/ml	
2	Tapped Density (gm/ml)	0.31	-
3	Angle of repose (degrees)	32	Passable
4	Carr's index	24.10	passable
5	Hausner's ratio	0.75	Poor

From above table it was concluded that Atovaquone drug having passable flow property

2. Drug Characterization:

Determination of Melting Point of Atovaquone

Table No. 5: Melting point of Atovaquone

Sr.No.	**Melting Point**	**Mean**	**Reference Value**
1	224°C		
2	223°C	**224**°C	**221-225**°C
3	226°C		

From above table it was concluded that Atovaquone drug having passable flow property

The melting point of Atovaquone was found to be **224°C** which is same as reported in literature **(221 – 225°C)**

3. UV Spectra for Atovaquone in Distilled Water:

The absorbance maxima found for Atovaquone is **251 nm**

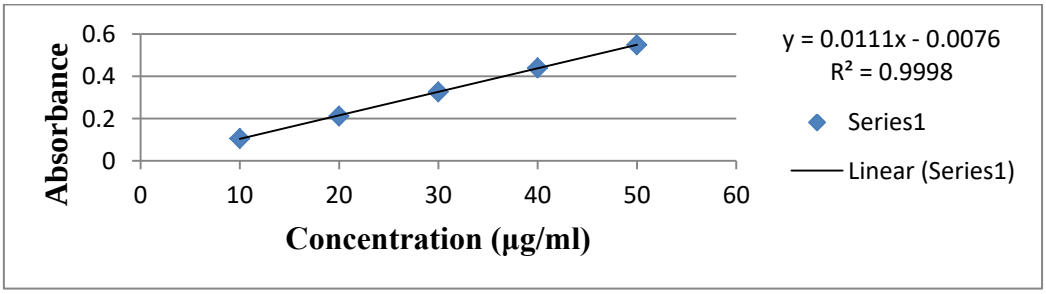


Figure No.2: Calibration curve of Atovaquone in distilled water at 251 nm

3. IR Spectra for Atovaquone:

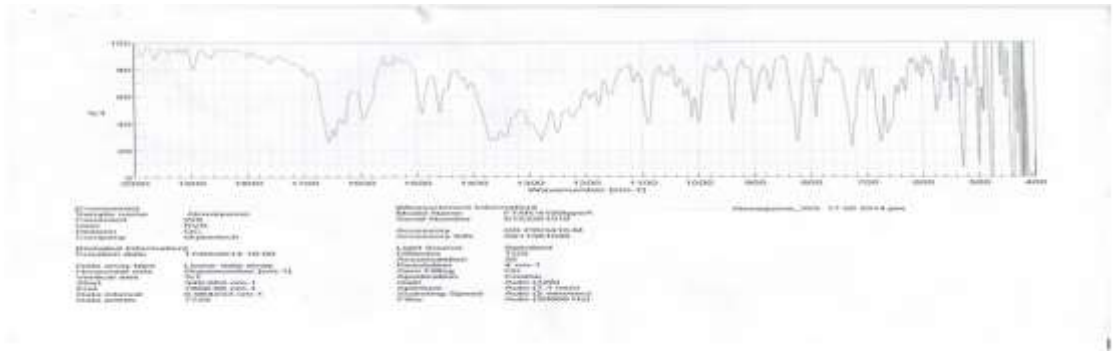


Figure No.3: IR spectra for Atovaquone

4. Interpretation for IR of Atovaquone

Sr.No.	Peak	Functional groups present	Range
1	1580	(C=C)	1500-1600
2	720.3	(C-Cl)	600-800
3	1660	(C=O)	1670-1820
4	1460	(C-H)	1300-1500
5	1640.4	(C -C)	1600-1700
6	1240.5	(O-H)	1200-1500

The FTIR spectrum of Atovaquone sample has shown identical peaks and all peaks are in range.

5. Differential Scanning Calorimetry (DSC) of Atovaquone: The thermal behaviour of Atovaquone was examined by DSC, using a SHIMADZU DSC- 60 differential scanning calorimeter. The system was calibrated with a high purity sample of Indium. Atovaquone were scanned at the heating rate 20°C /min over a temperature range of 100-250°C.

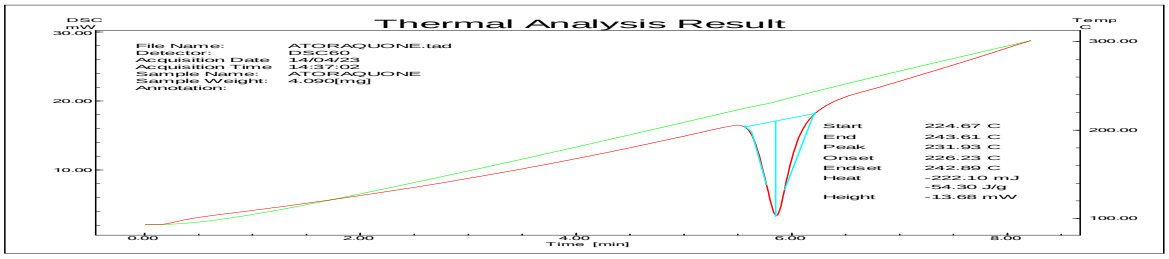


Figure No. 4: DSC of Atovaquone

The melting point of Atovaquone was found to be 224.6°C by DSC which is same as reported in literature (221-225°C). Result shown in Figure 4.

6. Drug: Excipients Physical Compatibility Study:

Table No. 6 : Drug: excipients physical compatibility study

Sr. No	Drug-excipients mixture	Initial appearance	Temperature conditions for 1months		
			2-8°C	20-25°C	40°C
1	Atovaquone	Yellow powder	No change	No change	Reddish brown
2	Atovaquone + Urea	Yellow powder	No change	No change	Reddish brown
3	Atovaquone + Sodium benzoate	Yellow powder	No change	No change	Reddish brown
4	Atovaquone + Sodium acetate	Yellow powder	No change	No change	Reddish brown
5	Atovaquone + sodium citrate	Yellow powder	No change	No change	Reddish brown
6	Atovaquone + sodium salicylate	Yellow powder	No change	No change	Reddish brown

From above table No 6 it is concluded that, Atovaquone is physically compatible with given Hydrotropes. There is no colour change and degradation of drug observed during storage of different temperature condition

Thus it was concluded that given Hydrotropes are compatible with Atovaquone drug.

Drug Excipient Chemical Compatibility Study:

IR Spectra for Atovaquone and Sodium Benzoate

Figure 5: IR spectra for Atovaquone and sodium benzoate

Table No. 7: Interpretation for IR of Atovaquone and sodium benzoate

Sr.No.	Peak	Functional groups present	Range
1	1560	(C=C)	1500-1600
2	720	(C-Cl)	600-800
3	1660	(C=O)	1670-1820
4	1240.5	(O-H)	1200-1500
5	1420	(C-H)	1300-1500
6	1620.2	(C-C)	1600-1700

From the IR graph of drug and (drug + sodium benzoate) it reveals that the selected Hydrotrope is chemically compatible with drug. **That means there was no any structural change in the drug when it is in contact with sodium benzoate**.

Analytical Method Development (UV Spectrophotometric):

The drug obeyed Beer– Lambert's law in the concentration range of 10- 50 µg/ml with regression 0.999 at 251 nm. The calibration curve is shown in Figure no.2

The developed method was validated as per ICH guidelines (ICH Q1B, 1996, ICH Q2 R1, 2005) for following parameters. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and Interday Precisions. Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Intraday precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily for three consecutive days..

Drug-Solubilizers Interference Study:

Table No. 8: UV spectral analysis data of solubilizers (cut-off wavelength)

Sr.No.	Solubilizers	Cut-off wavelength (nm)
1	UR	217
2	SB	248
3	SA	227
4	SC	224
5	SS	236

***SB = sodium benzoate, UR =urea, SC = sodium citrate, SA = sodium acetate, SS = Sodium Salicylate**

The aim of this study was to investigate the interference of solubilizers in Spectrophotometric estimation of Atovaquone. It is evident from the result that solubilizers didn't interfere in the

estimation of drug. The result are given in Table 6 and shows that, there is no shift in the λmax (251 nm) of drug in the presence of solubilizers. Also solubilizers have cut - off wavelengths less than 251 nm result shown in Table 27, hence not interfered in estimation of drug (λmax 251 nm).

Table No. 9 Solubilization of Atovaquone in Different Blends of Hydrotropic Agents:

Blend code	% concentration of blend			% Total concentration of blend
	Sodium acetate	Sodium benzoate	Sodium salicylate	10
B1	4	3	3	10
B2	4	4	2	10
B3	4	2	4	10
B4	3	4	3	10
B5	3	3	4	10
B6	2	4	4	10
B7	--	5	5	10
B8	--	6	4	10
B9	--	4	6	10
B10	--	8	2	10
B11	--	2	8	10
B12	--	7	3	10
B13	--	3	7	10

Method of Preparation of Atovaquone Syrup

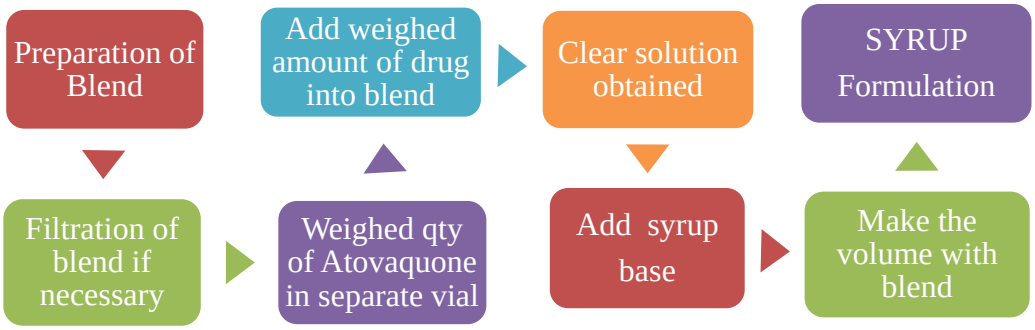


Figure No. 6 Method of preparation

Required amount of hydrotropic agents were dissolved in distilled water (40% of the total volume of syrup). Calculated quantity of Atovaquone (750 mg/5ml) was dissolved in the

prepared blend. Required amount of desired additive after dissolving in suitable volume of water was added to the prepared drug solution and properly mixed to get the syrup. The syrup was formulated according to the formulation details given in table 4, following the procedure given below.

The required quantities of solubilizer were transferred to a flask (50 ml capacity) containing 20 ml of distilled water and the flask was shaken until complete dissolution of solubilizer. Then Atovaquone drug (750 mg) was added and the flask was shaken to dissolve the drug completely. Then required amount of sucrose (was added to same flask, then add favoring agent as quantity sufficient and make up the volume 50 ml with distilled water. Finally, syrup was filtered through filter paper; first few ml of syrup was discarded and preserved in airtight container (amber coloured bottle). On the basis of the results obtained from the solubilization studies the syrup of poorly water-soluble drug Atovaquone has been developed to contain 750 mg of Atovaquone

Table No. 9 Formula of Developed Syrup

Sr.No.	Ingredients	Quantity
1	Atovaquone	7.5 gm
2	Sodium Benzoate	20 gm
3	Sucrose	33.35 gm
4	Rasbery Syrup	Q.S.
5	Water	Q.S. 50 ml

RESULT AND DISCUSSION:

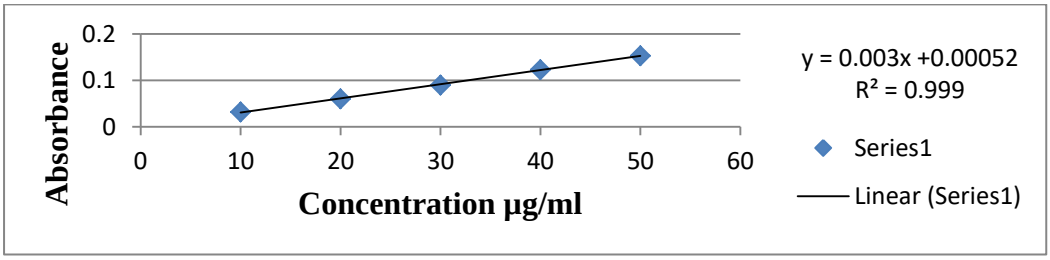


Figure No. 6: Calibration curve of Atovaquone in Sodium acetate at 251 nm

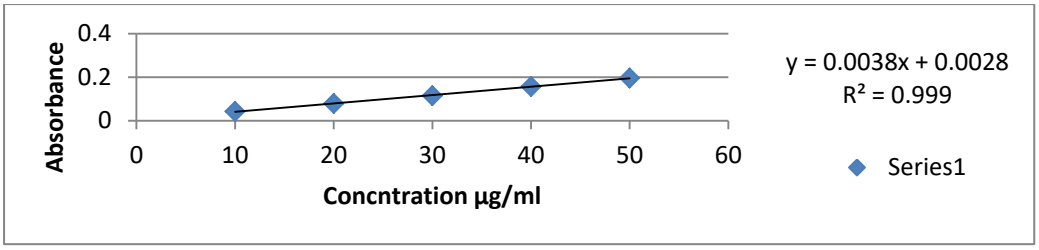


Figure No. 7: Calibration curve of Atovaquone in Sodium citrate at 251 nm

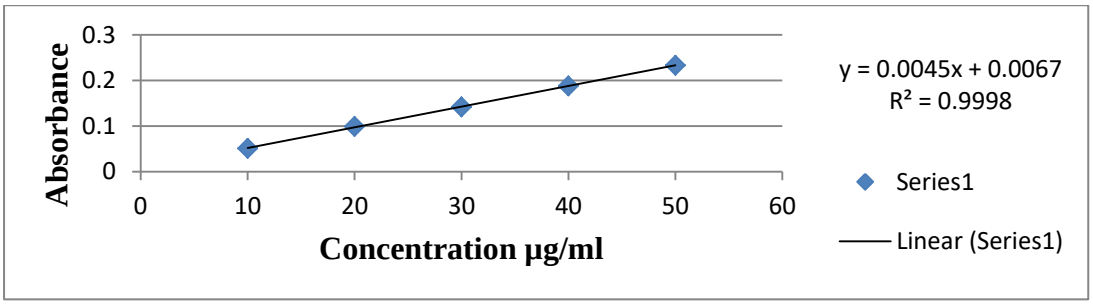


Figure No. 8: Calibration curve of Atovaquone in Sodium salicylate at 251 nm.

Calibration Curve of Atovaquone in Mixed Hydrotropic Agents:

Table No. 10: Calibration curve of Atovaquone in SA+SB+SS at 251 nm

Sr. No.	Concentration (µg/ml)	Absorbance
1	10	0.232
2	20	0.461
3	30	0.675
4	40	0.934
5	50	1.13

***SA= Sodium acetate, SB=Sodium benzoate, SS= Sodium salicylate**

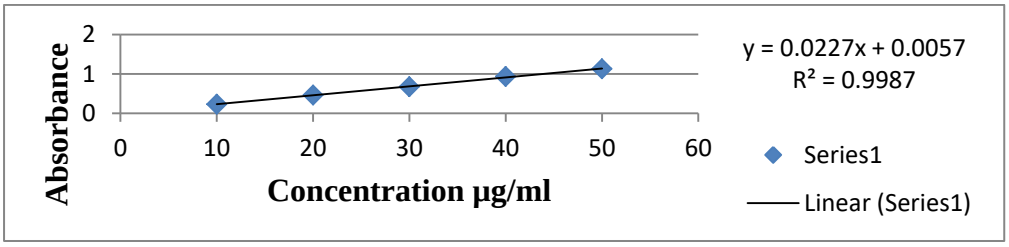


Figure No. 9: Calibration curve of Atovaquone in SA+SB+SS at 251 nm

Graph No. 6, 7, 8 & 9shows linear regression Equation and obtained linearity was with in range of ICH standard

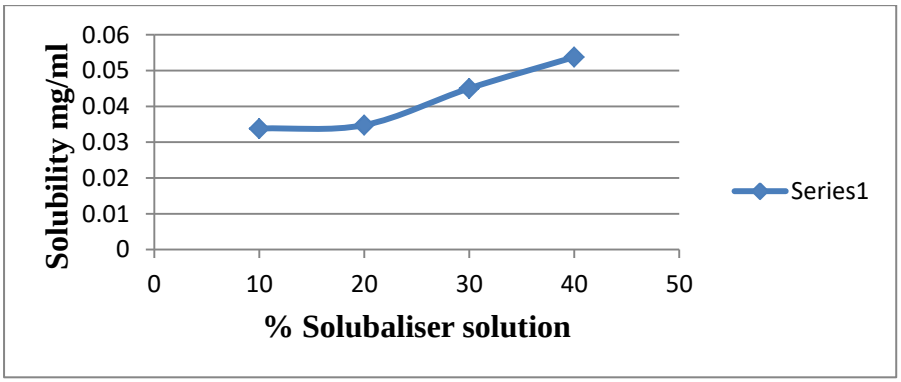
Solubilization studies:

Determination of solubility comprises of preparing a saturated solution of the given substance and finding out the amount present in a definite quantity of the solution.

Equilibrium solubility and Solubility enhancement ratio of Atovaquone in various solubilizers:

Table No. 11 .Equilibrium solubility and Solubility enhancement ratio of Atovaquone in Urea

Hydrotropic solution	Absorbance	Conc. µg/ml	Conc. mg/ml	SER
10%	0.107	33.80448	0.033804	7.13082
20%	0.110	34.76602	0.034766	7.33367
30%	0.142	45.02243	0.045022	9.49719
40%	0.173	53.75	0.05375	11.33822



FigureNo.10: Equilibrium solubility curve of Atovaquone in Urea solution

Table No. 12: Equilibrium solubility and Solubility enhancement ratio of Atovaquone in Sodium benzoate

Hydrotropic solution	Absorbance	Conc. µg/ml	Dilution factor	Conc. X dil.factor	Conc. mg/ml	SER
10%	0.761	45.42857	100	45442.8571	4.5428	958.28
20%	0.788	46.71428	100	4671.4286	4.6714	985.40
30%	0.405	28.47619	10000	284761.9	284.76	60068.74
40%	**0.790**	**46.80952**	**100000**	**4680952.4**	**4680.9**	**987417.71**

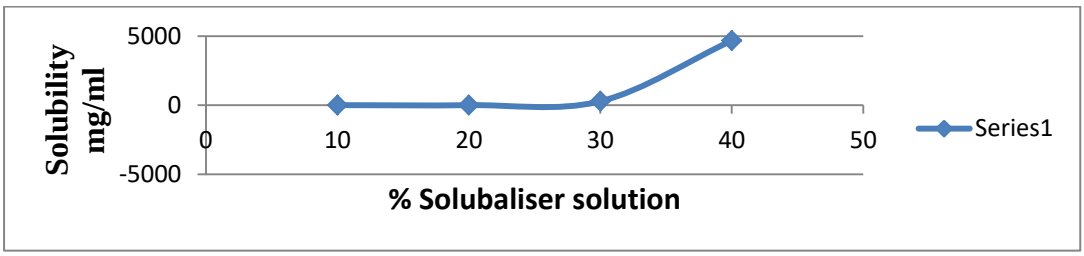


Figure 11: Equilibrium solubility curve of Atovaquone in Sodium benzoate solution

Table No.13:Equilibrium solubility and Solubility enhancement ratio of Atovaquone in Sodium acetate

Hydrotropic solution	Absorbance	Conc. µg/ml	Conc. mg/ml	SER
10%	0.272	89.3026	0.0893	18.8378
20%	0.596	195.8815	0.19588	41.3198
30%	0.710	233.3815	0.23338	49.23026
40%	0.801	263.3157	0.26331	55.5448

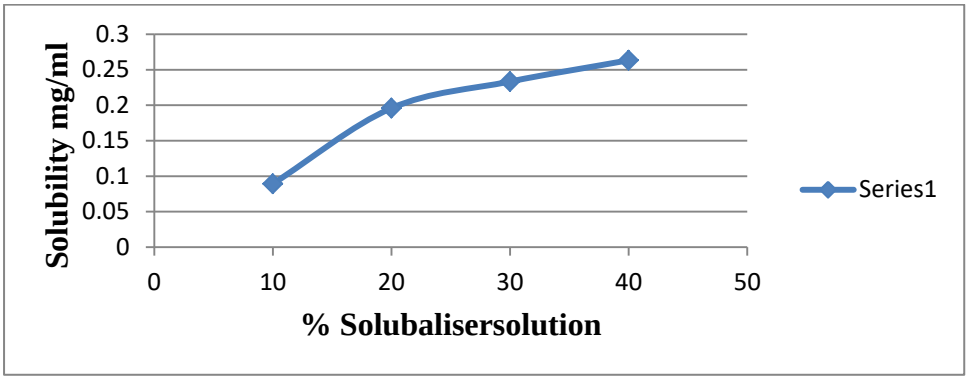


Figure No.12 : Equilibrium solubility curve of Atovaquone in Sodium acetate solution

Table No. 14: Equilibrium solubility and Solubility enhancement ratio of Atovaquone in Sodium citrate

Hydrotropic solution	Absorbance	Conc. µg/ml	Conc. mg/ml	SER
10%	0.108	27.43603	0.027436	5.78745
20%	0.444	115.1644	0.1151645	24.29323
30%	0.551	143.1018	0.143101	30.1864
40%	0.582	151.1958	0.1511958	31.8938

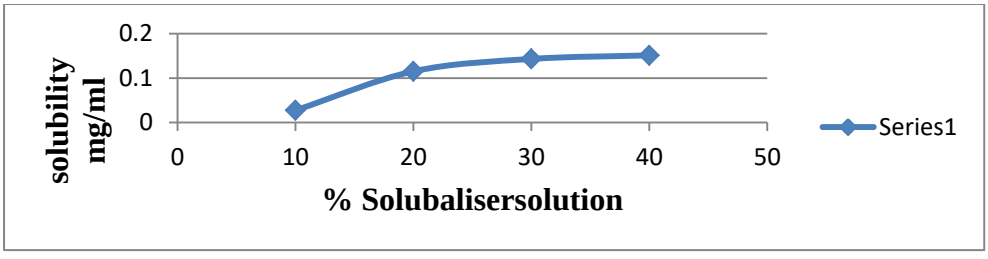


Figure No.13: Equilibrium solubility curve of Atovaquone in Sodium citrate solution

Table No.15: Equilibrium solubility and Solubility enhancement ratio of Atovaquone in Sodium salicylate:

Hydrotropic solution	Absorbance	Conc. µg/ml	Dilution factor	Con X dil.factor	Conc. mg/ml	SER
10%	0.214	45.8008	100	4580.088	4.58008	966.1411
20%	0.212	45.3584	10000	453584.07	453.58407	95680.73
30%	0.364	78.9867	10000	789867.2	789.8672	166617.56
40%	0.478	104.2079	10000	1042079.6	1042.0796	219820.19

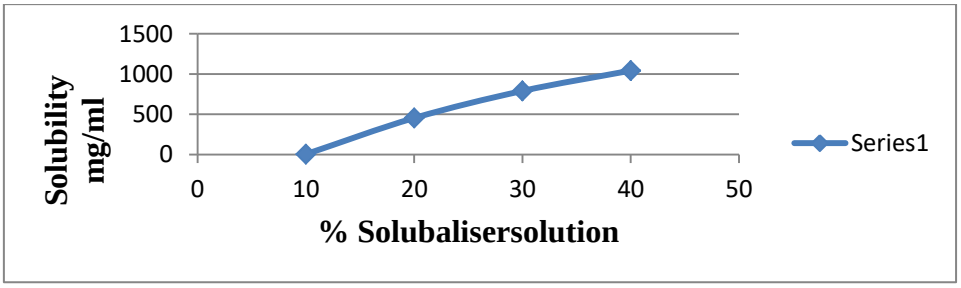


Figure No. 14: Equilibrium solubility curve of Atovaquone in Sodium salicylate solution

Solubility of Atovaquone in Mixed-Hydrotropy:

TableNo.16:Equilibrium solubility and Solubility enhancement ratio of Atovaquone in SA+ SB+ SS:

Hydrotropic solution	Absorbance	Conc. µg/ml	Dilution factor	Con X dil.factor	Conc. mg/ml	SER
B1	0.084	3.55954	10000	35595.455	35.59545	7573.501
B2	0.144	6.11991	10000	61199.115	61.19115	13021.088
B3	0.053	2.09336	10000	2.933.62	20.93362	4453.9634
B4	0.045	1.73938	10000	17393.8	17.3938	3700.8085
B5	0.159	6.783628	10000	67836.28	67.83628	14433.25
B6	0.054	2.129131	10000	21291.31	21.291318	4530.067

***SA= Sodium acetate, SB=Sodium benzoate, SS= Sodium salicylate**

*** SB=Sodium benzoate, SS= Sodium salicylate*SER= Solubility Enhancement Ratio**

SER= Solubility in particular hydrotropic solution/solubility in water

The solubility was determined using the corresponding regression equations given in above tables. The equilibrium solubility of Atovaquone in 40% concentration of various solubilizers was determined and result indicates that **maximum solubility of 4.6 gm/ ml was obtained with sodium benzoate with enhancement ratio of 987417.71 and least solubility of 0.53 x $10^4$was observed with Urea.**

Also solubility of Atovaquone was determined by using mixed Hydrotropy method. The result found that there was no increase in solubility of Atovaquone in mixed Hydrotropy method as compair to Sodium Benzoate. It is given in table no. 16. Thus mixed Hydrotropy method was not selected for formulation of Atovaquone syrup.

From above observation tables it is concluded that, the **solubility** of Atovaquone was **increased in 40% sodium benzoate solution**. Therefore Sodium benzoate 40% was selected as Hydrotrope for solubility improvement of Atovaquone in Formulation of Atovaquone syrup.

Comparative Equilibrium Solubility of Drug in Five Hydrotropes at Various Concentrations::

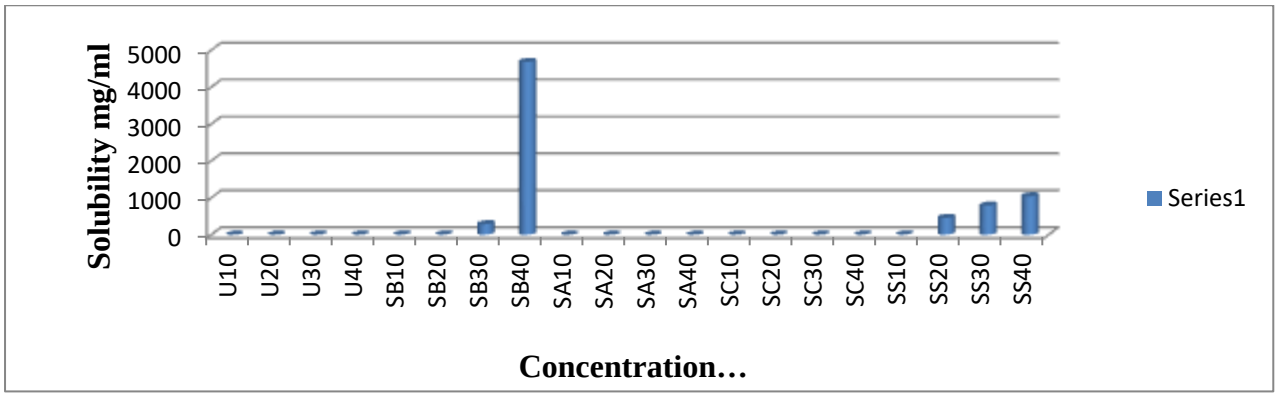


Figure No. 15: Comparative equilibrium solubility of drug in five Hydrotropes at various Concentrations

The results showed that the maximum equilibrium solubility was obtained in**Sodium benzoate**. The solubilising power of different hydrotropic agents could be ranked as: **sodium benzoate > sodium salicylate >Sodium acetate > Sodium citrate.**

Formulation and Development of Atovaquone Syrup: Atovaquone syrup was prepared by taken 7.5 gm of Atovaquone, it solubilise in hydrotropic solution of Sodium Benzoate (20 gm), add sucrose and flavouring agent and make up volume 50 ml with water.

Evaluation of Formulated Syrup:

Table No. 17: Evaluation parameter of Syrup

Sr. No	Evaluation parameter	Inference
1	Color	Yellowish orange
2	Clear	Homogeneous
3	Odour	Pleasant
4	pH	6.8
5	Density	1.351 gm/ml
6	Viscosity	0.024 poise
7	Drug content-	94.96%

The formulated Syrup was evaluated and the Evaluation parameters were given in Table no.17

Stability Batches:

Three stability batches were prepared and kept for stability testing. The study was undertaken to determine physical stability of formulations. Precipitation or colour change was not observed in the formulations (**FA1, FA2, and FA3**) up to two weeks. Therefore, all formulations were selected for further studies

Physical and Chemical Stability Testing Data for Syrup Formulation:

Table No. 18: Physical and chemical stability testing data for syrup formulation FA1

Condition	Time (days)	Colour	Clarity	Precipitation	pH	Drug content%
RT	0	Yellowish orange	Clear	No PPT	6.8	95.96
	15	Yellowish orange	Clear	No PPT	6.8	93.47
	30	Yellowish orange	Clear	No PPT	6.7	93.12
	45	Yellowish orange	Clear	No PPT	6.8	90.37
	60	Yellowish orange	Clear	No PPT	6.8	87.56
40°C/75% RH	0	Yellowish orange	Clear	No PPT	6.8	96.27
	15	Yellowish orange	Clear	No PPT	6.8	94.89
	30	Yellowish orange	Clear	No PPT	6.7	93.79
	45	Yellowish orange	Clear	No PPT	6.8	90.35
	60	Yellowish orange	Clear	No PPT	6.8	88.54
55°C	0	Yellowish orange	Clear	No PPT	6.8	95.98
	15	Yellowish orange	Clear	No PPT	6.8	93.47
	30	Yellowish orange	Clear	No PPT	6.7	87.34
	45	Yellowish orange	Clear	No PPT	6.7	83.16
	60	Yellowish orange	Clear	No PPT	6.8	80.78

Table No.19: Physical and chemical stability testing data for syrup formulation FA2

Condition	Time (days)	Colour	Clarity	Precipitation	pH	Drug content%
RT	0	Yellowish orange	Clear	No PPT	6.7	95.96
	15	Yellowish orange	Clear	No PPT	6.7	94.47
	30	Yellowish orange	Clear	No PPT	6.8	94.08
	45	Yellowish orange	Clear	No PPT	6.8	93.27
	60	Yellowish orange	Clear	No PPT	6.8	90.12
40°C/75% RH	0	Yellowish orange	Clear	No PPT	6.7	96.47
	15	Yellowish orange	Clear	No PPT	6.7	94.57
	30	Yellowish orange	Clear	No PPT	6.8	94.09
	45	Yellowish orange	Clear	No PPT	6.8	90.35
	60	Yellowish orange	Clear	No PPT	6.8	87.54
55°C	0	Yellowish orange	Clear	No PPT	6.7	95.98
	15	Yellowish orange	Clear	No PPT	6.7	93.37
	30	Yellowish orange	Clear	No PPT	6.8	87.64
	45	Yellowish orange	Clear	No PPT	6.8	83.56
	60	Yellowish orange	Clear	No PPT	6.9	80.98

Table No. 20: Physical stability testing data for syrup formulation FA3

Condition	Time (days)	Colour	Clarity	Precipitation	pH	Drug content(%)
RT	0	Yellowish orange	Clear	No PPT	6.7	95.96
	15	Yellowish orange	Clear	No PPT	6.8	93.47
	30	Yellowish orange	Clear	No PPT	6.7	90.98
	45	Yellowish orange	Clear	No PPT	6.7	90.37
	60	Yellowish orange	Clear	No PPT	6.8	89.12
40°C/75% RH	0	Yellowish orange	Clear	No PPT	6.7	96.52
	15	Yellowish orange	Clear	No PPT	6.7	94.57
	30	Yellowish orange	Clear	No PPT	6.8	94.09
	45	Yellowish orange	Clear	No PPT	6.8	90.35
	60	Yellowish orange	Clear	No PPT	6.8	87.54
55°C	0	Yellowish orange	Clear	No PPT	6.7	95.98
	15	Yellowish orange	Clear	No PPT	6.7	92.37
	30	Yellowish orange	Clear	No PPT	6.7	87.67
	45	Yellowish orange	Clear	No PPT	6.8	83.72
	60	Yellowish orange	Clear	No PPT	6.9	80.68

No physical changes were observedduring the stability study for two months. At the end of two months the syrup were since clear without precipitation at different temperature condition such room temperature,40°C/75% RH, 55°C.During the stability study period, syrup shows pH in the range 6 to 8 which is normal range. It is given in table no 18, 19 & 20.

The drug complied with the tests prescribed in the monograph. The Infrared spectra of the dug showed major peaks at wave numbers that are characteristic of Atovaquone. Preformulation study of the drug was carried out to determine the solubility of drug in water. Aqueous solubility of drug was found to be 0.0047406mg/ml. The approximate solubility of drug in Urea and Sodium acetate, Sodium salicylate, Sodium citrate were found to be very less. Therefore, they were excluded from the study. Also in mixed hydrotropic methodobserved less improvement in solubility of Atovaquone. Thus the single hydrotrope was selected to improve solubility of Atovaquone by hydrotropic method. The solubilizer sodium benzoate was selected for Solubilization studies on the basis of approximate solubility determination at 10, 20, 30 and 40% concentrations. The solubility determination of drug in hydrotropic solutions was carried out at room temperature. The equilibrium solubility studies in individual solubilizers are then taken at lower i.e. 10, 20, 30 and 40% concentrations. The solubility of drug in hydrotropes was found in decreasing order as

Sodium benzoate >Sodium salicylate > Sodium acetate > Sodium citrate > Urea

The linearity of calibration curve showed that the Beer Lamberts law was obeyed in the concentration range of 10-50 µg/ml at the λ_{max} of 251 nm. From the equilibrium

solubility curves of Atovaquone in solubilizer it was concluded that increase in the solubility was non linear function with respect to the hydrotrope concentration

The results should that the enhancement in solubility of the drug was not entirely due to pH effect but mostly due to solubilizer Solubilization phenomenon. From the results of solubility determination studies and considering the literature survey solubilizers was employed for developing aqueous liquid Syrup (750 mg/5ml) of Atovaquone, 33.35 % w/v sucrose was added to stabilize formulation.

On the basis of the results of solubility studies, to develop Atovaquone syrup. (each containing 750mg and 10% w/v sucrose). These syrups were coded as FA1, FA2, and FA3.

The prepared syrup formulation was subjected to physical stability testing programmed as per ICH conditions at for a period of 60 days. The results showed that the formulations were unaffected in respect of color stability and precipitation on storage at Room temperature. 40°C 75% RH and 55°C Further, the formulations were subjected to chemical stability

studies on storage at Room temperature. 40°C 75% RH and 55°C for a period of 60 days. The formulations were analyzed by the UV at the interval of 15 days. The results showed that there was no appreciable loss of drug after one 60 days at 40°C/75% RH and room temperature and 55°C which suggest the long term stability of the developed formulations at room temperature.

Summary and conclusion:

The present research study explores the possibility of employing hydrotropy techniques in the formulation and evaluation of aqueous oral liquid formulation of a poorly water soluble drug Atovaquone.

In the present study, practically insoluble drug, Atovaquone was tried to solubilise by employing the combination of physiologically compatible hydrotrope and solubilizer agents i.e. solubilizer to attempt its oral liquid formulations.

The melting point determination and spectrophotometric analysis showed purity of drug sample. The drug complied with the tests prescribed in the monograph. The Infrared spectra of the dug showed major peaks at wave numbers that are characteristic of Atovaquone.

In the preformulation study physical compatibility of the drug-excipient was carried out and compatibility was observed between Atovaquone and selected solubilizers. In the drug solubilizers interference study no interference was observed in UV Spectrophometric analysis of Atovaquone in presence of employed solubilizers.

Preformulation study of the drug was carried out to determine the solubility of drug in water. Aqueous solubility of drug was found to be 0.0047406mg/ml. The approximate solubility of drug in Urea and Sodium acetate, Sodium salicylate, Sodium citrate were found to be very less. Therefore, they were excluded from the study. Also in mixed hydrotropic method observed less improvement in solubility of Atovaquone. Thus the single hydrotrope was selected to improve solubility of Atovaquone by hydrotropic method. The solubilizer sodium benzoate was selected for Solubilization studies on the basis of approximate solubility determination at 10, 20, 30 and 40% concentrations. The solubility determination of drug in hydrotropic solutions was carried out at room temperature. The equilibrium solubility studies in individual solubilizers are then taken at lower i.e. 10, 20, 30 and 40% concentrations. The solubility of drug in hydrotropes was found in decreasing order as

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Conclusion:

Atovaquone poorly water soluble drug, having water solubility less than 0.0002 mg/ml. By the use of hydrotropy approach to improve the solubility of Atovaquone by using Sodium benzoates a solubilizer, in this method Atovaquone was solubilized up to 4680.95 mg/ml (increase in solubility).

Furthermore Atovaquone was effectively developed in liquid Syrup formulation; which shows desired formulation within limit and stability for two month as per ICH condition.

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