Original Article

FORMULATION OPTIMIZATION AND CHARACTERIZATION OF AQUEOUS INJECTION CONTAINING POORLY SOLUBLE DRUG USING MIXED HYDROTROPIC SOLUBILIZATION

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ABSTRACT

Objective: Mefenamic acid (MFA) is an NSAID that exhibits anti-inflammatory analgesic and antipyretic activity. Peak plasma levels are attained in 2-4 h and the elimination half-life approximates 2 h, repetitive administration of tablets for 3-5 times a day is desired. It is supplied only in the form of tablets for oral administration. In acute conditions drug administered parenterally could give rapid relief from severe symptoms like pain. Thus, formulation of injectable formulation of MFA could be better alternative compared to conventional tablet dosage form. The low aqueous solubility of MFA precludes its use in parenteral formulation development.

Methods: In this work attempt were made to enhance the aqueous solubility of mefenamic acid using mixed solvency technique. For that different hydrotropic agents such as Urea, Sodium acetate, sodium benzoate, sodium citrate and their blends were evaluated. Optimal concentration of hydrotropic agent in blend was determined using D-optimal mixture experimental design. The optimized bled was used to develop the aqueous injection of mefenamic acid. The developed injection was subjected for various quality control tests and stability of developed formulation was also evaluated.

Results: The aqueous solubility in optimized blend of hydrotropic agent batches (U: SA: SB: SC, 4:4:23:9 %w/v) showed 835.71-fold compared to MFA solubility in distilled water. The quality control tests for parenteral formulation and accelerated stability study were found to be within prescribed limits and stable.

Conclusion: The inadequate solubility of MFA was overcome, and aqueous injection was successfully developed which can be serve as cost effective treatment in various indications.

Keywords: Mixed solvency, Mefenamic acid, Solubility, Aqueous injection, Hydrotropy, Hydrotrops

INTRODUCTION

For poorly soluble drugs usually, dissolution becomes the decisive factor for its poor bioavailability which again can limit their therapeutic effectiveness [1]. Mefenamic acid (MFA) is an NSAID that exhibits anti-inflammatory analgesic and antipyretic activity. Peak plasma levels are attained in 2 to 4 h and the elimination half-life approximates 2 h, repetitive administration of tablets for 3-5 times a day (depending upon severity of pain) is desired. Also, the active site of absorption for mefenamic acid is stomach and upper part of small intestine. So, once emptied from stomach the passage through the small intestine is rapid, thus limiting the extent of absorption.

It is supplied only in the form of tablets and capsules for oral administration. Mefenamic acid is practically insoluble in water (0.004 mg/ml) [2, 3] which precludes its use in parenteral and oral solutions. Moreover, a drug administered parenterally is immediately available in systemic circulation and it is rapid as well as more effective in treatment of acute conditions like severe pain during excessively heavy periods caused by abnormal function and presence of a contraceptive coil. Due to low aqueous solubility of mefenamic acid resist the formulator to develop an aqueous intravenous injection. Therefore, aqueous solubility of mefenamic acid was need be enhanced. Solubility enhancement of insoluble drug mefenamic acid has been extensively studied to overcome difficulties that frequently come across during formulation development [4-6]. Hydrotropic agents and cosolvents have been observed to enhance the aqueous solubility of poorly water-soluble drugs [7-9]. Maheshwari *et al.* have demonstrated the synergistic solubilizing capability due to a mixed hydrotropy approach and this approach has been applied to enhance the solubility of various poorly soluble drug [10, 11].

In this present research, we have explored the application of mixed solvency technique in the formulation of aqueous injection dosage form of water-insoluble drug mefenamic acid and to reduce the concentration of individual hydrotropic agents to minimize the side effects. In hydrotropic solubilization, a high concentration of an additive is required to produce an appreciable increase in aqueous solubility of a poorly water-soluble drug. In this case, the hydrotropic agent employed to give a desirable solubility may produce its toxicity. However, if the same enhancement in solubility can be achieved by mixing, say five solubilizers (each in one-fifth concentration) then the toxic level of the five solubilizers can be reduced fivefold. In case of synergistic effect in solubility due to mixing of, say, five solubilizers (in one-fifth concentration), the toxic level of individual hydrotropes can further be lowered because still less concentration of the hydrotropic agents shall be sufficient for the desired solubility enhancement. In mixed solvency technique different hydrotropic agents, their blends were evaluated and optimized using D-optimal mixture experimental design.

MATERIALS AND METHODS

Materials

Mefenamic acid was obtained as a gift sample from Alembic Ltd., Vadodara, India. The hydrotropes like urea, sodium acetate, sodium benzoate, sodium citrate were purchased from Merk laboratories, Mumbai, India. Polyethylene glycol 400 and sodium lauryl sulfate were also procured from Merk laboratories, Mumbai, India. The other reagents and solvents were used of laboratory grade.

Methods

Development of UV calibration curve of mefenamic acid in hydrotropic solutions

For quantification of the drug during various stages of formulation development, the UV calibration curve was developed using UV visible spectrophotometer (Shimadzu® UV-1800, Kyoto, Japan). A 10 mg of pure drug mefenamic acid was accurately weighed and transferred into a 100 ml volumetric flask. It was dissolved in an adequate amount of various hydrotropic solvent systems (table 3) and volume was made up to 100 ml to obtain a stock solution of 100 μg/ml. From 100 μg/ml solution appropriate dilutions were prepared in appropriate ranges. The absorbance of resulting solutions was noted at 332 nm against the respective blank.

Saturation solubility study

The saturation solubility study was performed using the shake flask method. The excess amount of sample was added in 10 ml of the solvent system (distilled water and different solutions containing varying concentrations of hydrotropic agent/s) in a 10 ml volumetric flask. The flask was shaken for 24 h in wrist action shaking matching. Then, the solution was centrifuged at 3000 RPM for 5 min and then filtered through Whatman filter paper. An aliquot was suitably diluted and analyzed using UV spectrophotometer at 332 nm.

Selection of hydrotropic agent

To identify appropriate hydrotropic agent, the solubility of mefenamic acid was determined individually in four different hydrotropic agents namely urea (U), sodium acetate (SA), sodium benzoate (SB), sodium citrate (SC) at concentration of 10%, 20%, 30%, and 40% solutions. Accurately measured 5 ml of a particular blend of hydrotropic agent was taken in a 10 ml volumetric flask and an excess amount of drug was added and mechanically shaken until a saturated solution was formed. The volumetric flask was shaken on wrist action shaking matching for 12 h so that equilibrium solubility can be achieved, and the solution was allowed to equilibrate for 24 h. Then solution was centrifuged at 3000 RPM for 5 min in ultra-centrifuge and then solution was filtered through Whatman filter. An aliquot was suitably diluted with distilled water and analyzed using UV spectrophotometer at 332 nm.

Optimization of hydrotropic mixture using D-optimal design

The primary objective of optimization is to achieve highest solubility ($\geq$ 40 mg/ml) of 30% and 40% solutions, solubility of mefenamic acid was increasing with increasing mefenamic acid using mixture of various hydrotropic agents (mixed solvency technique). Then, to decrease the concentration of sodium benzoate, different combinations of above mentioned four hydrotropic agents in different ratios were tried to determine enhancement in saturation solubility so that the total concentration of hydrotropic agents was always 40% w/v (volume of aqueous phase is constant). Thus, in the present investigation, further optimization with these four identified factors (% w/v concentration of hydrotropes) was performed using a D-optimal design. Briefly, 16 mixture batches were prepared by varying the %w/v concentration of hydrotropes and evaluated for saturation solubility (mg/ml) of mefenamic acid (table 1).

Table 1: D-optimal design setup

Factor code	Factor name	Factor level	
		Low (-1)	High (+1)
Independent variables			
X_1	Concentration of Urea (U) (% w/v)	0	40
X_2	Concentration of sodium acetate (SA) (% w/v)	0	40
X_3	Concentration of sodium benzoate (SB) (% w/v)	0	40
X_4	Concentration of sodium citrate (SC) (% w/v)	0	40
Dependent variable			
Y_1	Saturation solubility of MFA		
Constrain: total concentration of hydrotropic agents in a blend must be 40% w/v			

Formulation of aqueous injection of mefenamic acid

Each ampule containing 100 mg mefenamic acid per 2.5 ml was prepared. The proposed formulations containing the amount of solubilizers and drug that will be administered through each mixed blend is shown in table 2. Three batches (Batch size 500 ml) of optimized aqueous injection formula were prepared in an aseptic area at the temperature NMT 25 °C and RH 45%±5%. All the utensils used were sterilized. To 450 ml of WFI, sodium citrate was added under continuous stirring, then sodium benzoate was added and stirred to get a clear solution. Then Urea and sodium acetate were added and dissolved. To this solution, mefenamic acid was added and stirred for 20 min to get a clear solution, and volume was makeup to 500 ml using WFI. The final injection solution was filtered through a 0.45 μ membrane filter using a sterilized vacuum filtration assembly. The 2.5 ml of final product was filled in type I clear glass ampules using a syringe and sealed by pull sealing method under aseptic condition.

Table 2: Injection composition for different batches

Ingredients	Concentration (%w/v)		
	MFI-OB1	MFI-OB3	MFI-OB5
Mefenamic acid	40	40	40
Urea	5.454	0.106	4
Sodium acetate	0.092	2.8	4
Sodium Benzoate	24.396	23.144	23
Sodium citrate	9.904	13.849	9
Water for injection	Up to 2.5 ml		

Benchtop stability of mefenamic acid in bulk solution

The stability of mefenamic acid in the bulk solution was studied for 24 h under room temperature and refrigerated conditions (2 to 8°C) before ampule filing. The % drug content, presence of turbidity, and pH were observed for each batch (MFI-OB, MFI-OB3, and MFI-OB5).

Leak test

To detect faulty sealing of ampules leak test was performed. After applying positive and reduced pressure in the leak test apparatus, the ampules were removed and washed with distilled water, and visually inspected for color change in ampule solution.

Clarity test

To detect visible particulate matter in sealed ampules clarity test was performed using white and black background under illuminating light.

Pyrogen test (LAL test)

The Limulus amebocyte lysate test (LAL test) was conducted to detect bacterial endotoxins in the formulation. The solution from four sealed ampules (batch MFI-OB5) was withdrawn in sterile test tubes containing LAL reagent and mixed well. Positive control spiked with control standard endotoxin (CSE) and negative control containing triple distilled water was also prepared to eliminate procedure error. The test tubes were incubated at 37 °C for 1 h. Then each test tube was visually inspected for turbidity and gel formation, firm gel formation indicates the presence of Pyrogen.

Sterility test

The sterility test of prepared injections was tested using direct Inoculation method. The liquid was removed from each test container (ten sample ampules) with a sterile syringe. Half of the ampule content (batch MFI-OB5) was transferred into a culture tube (containing fluid thioglycolate medium). The inoculated media was incubated for 14 d. All the cultures were observed several times during the incubation period. If the test specimen is positive before

14 d of incubation, further incubation is not necessary. If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

Dilution profile of formulated injection

Series of dilutions were done by diluting injection of mefenamic acid (batch MFI-OB5) with different diluents like normal saline (0.9% NaCl), 5% dextrose solution to observe the precipitation after dilution.

Accelerated stability study

The sealed ampules of the aqueous injections (batch OB-5) were inspected for color, turbidity, and pH of the formulation. The chemical stability of the formulation was assessed by the estimation of the percentage drug remaining in the formulation on storage at 2-8 °C in a refrigerator, room temperature, and 40±2 °C/75±5% RH in a humidity chamber at day 0, day 15 and day 30.

RESULTS AND DISCUSSION

UV calibration curve of mefenamic acid in hydrotropic solutions

The standard calibration curves for mefenamic acid were developed in the various hydrotropic solvent system to quantify the drug during different stages of formulation development. The regression equations and R^2 value for all calibration curves were given in table 3.

Table 3: Regression equations of MFA in different solvent systems

S. No.	Solvent system	Regression equation	R^2
1	Only DW	y = 0.0038x+0.0015	0.998
2	U (20% w/v)	y = 0.0298x+0.0193	0.9997
3	SA (20% w/v)	y = 0.0255x+0.0007	0.9954
4	SB (20% w/v)	y = 0.1453x+0.0494	0.9977
5	SC (20% w/v)	y = 0.0107x+0.0196	0.9962
6	U (40% w/v)	y = 0.0596x+0.0673	0.9995
7	SA (40% w/v)	y = 0.0787x+0.049	0.9923
8	SB (40% w/v)	y = 0.604x+0.4426	0.9984
9	SC (40% w/v)	y = 0.0406x+0.0418	0.9988
10	U+SA (20+20 % w/v)	y = 0.2047x+0.152	0.9996
11	U+SB (20+20 % w/v)	y = 0.9034x+0.6815	0.996
12	U+SC (20+20 % w/v)	y = 0.2226x+0.1781	0.9994
13	SA+SB (20+20 % w/v)	y = 0.8991x+0.6678	0.995
14	SA+SC (20+20 % w/v)	y = 0.0573x+0.0483	0.9998
15	SB+SC (20+20 % w/v)	y = 0.9769x+0.7456	0.9979
16	U+SA+SB+SC (8+8+8+8 % w/v)	y = 0.4175x+0.3185	0.9996

Where, DW= Distilled water, U= urea, SA= sodium acetate, SB = sodium benzoate, SC = sodium citrate

Selection of hydrotropic agent

Saturation solubility of MFA in different concentration of hydrotropic agents was performed and results were reported in table 4. From the results it was concluded that saturation solubility (SS) of mefenamic acid was increasing with increasing concentrations of hydrotropic agents, for example, solubility in 40 %

urea solution was found to be much higher than solubility in 10%, 20%, or 30% urea solutions. The highest solubility was obtained in 40% sodium benzoate solution. Then, in order to decrease the concentration of sodium benzoate, different combinations of above mentioned 4 hydrotropic agents in different ratios were tried to determine enhancement in solubility so that the total concentration of hydrotropic agents was always 40% w/v.

Table 4: MFA solubility in different concentration of hydrotrops

Hydro-tropic agents	Saturation solubility of MFA (mg/ml) (mean±SD)				Solubility enhancement (times or fold)			
	10%	20%	30%	40%	10%	20%	30%	40%
Only DW	0.0049±0.0004				1			
U	0.884±0.014	1.240±0.083	1.728±0.132	2.520±0.289	18.038	25.308	35.269	51.423
SA	0.172±0.018	1.029±0.047	1.873±0.113	3.153±0.154	3.500	21.000	38.231	64.346
SB	1.623±0.075	5.620±0.458	12.950±0.604	24.709±1.258	33.115	114.692	264.385	504.269
SC	0.198±0.007	0.449±0.012	0.792±0.078	1.702±0.288	4.038	9.154	16.154	34.731

Where, DW= Distilled water, U= urea, SA= sodium acetate, SB = sodium benzoate, SC = sodium citrate, n=3 for SD

Statistical analysis of D-optimal design

Sixteen batches were prepared by using a D-optimal experimental design, varying four independent variables, %w/v concentration of

X_1= urea (U), X_2= sodium acetate (SA), X_3= sodium benzoate (SB), X_4= sodium citrate (SC) as shown in table 5. All batches were evaluated for saturation solubility of mefenamic acid (Y). Results are recorded in table 5.

Table 5: D-optimal design layout with responses

Batch No.	Independent variables								Dependent variable
	Coded value				Actual value (%w/v)				SS (mg/ml)
	U	SA	SB	SC	U	SA	SB	SC	SS
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Y
1	-1	-1	-1	-1	0	0	0	0	0.05
2	0	-1	-1	-1	20	0	0	0	1.24
3	+1	-1	-1	-1	40	0	0	0	2.52
4	-1	0	-1	-1	0	20	0	0	1.03
5	0	0	-1	-1	20	20	0	0	8.47
6	-1	+1	-1	-1	0	40	0	0	3.15
7	-1	-1	0	-1	0	0	20	0	5.62
8	0	-1	0	-1	20	0	20	0	37.43
9	-1	0	0	-1	0	20	20	0	36.98
10	-1	-1	+1	-1	0	0	40	0	24.71
11	1/5	1/5	1/5	1/5	8	8	8	8	17.38
12	-1	-1	-1	0	0	0	0	20	0.45
13	0	-1	-1	0	20	0	0	20	9.42
14	-1	0	-1	0	0	20	0	20	2.39
15	-1	-1	0	0	0	0	20	20	41.02
16	-1	-1	-1	+1	0	0	0	40	1.70

U= urea, SA= sodium acetate, SB = sodium benzoate, SC = sodium citrate, SS = Saturation Solubility

Mathematical modeling was carried out as per Equation 1 to obtain a quadratic polynomial equation (full model) which describes the relationship of the dependent variable Y with X_1, X_2, X_3, and X_4 [12]. Full model Equation 1 for Saturation solubility described as bellow:

$$SS = 0.2128 + 0.0191X_1 - 0.0178X_2 - 0.0976X_3 - 0.0395X_4 + 0.0166\,X_1X_2 + 0.0775\,X_1X_3 + 0.0204\,X_1X_4 + 0.0769\,X_2X_3 + 0.0034X_2X_4 + 0.0885\,X_3X_4 + 0.0011X_1^2 + 0.0024\,X_2^2 + 0.0179\,X_3^2 + 0.0020\,X_4^2 \ldots\ldots\ldots (1)$$

The SS (dependent variables) obtained at various levels of four independent variables (X_1, X_2, X_3, and X_4) were subjected to multiple regression to yield a quadratic polynomial equation (full model). The main effects of X_1, X_2, X_3, and X_4 represent the average result of changing one variable at a time from its low to high value. The interactions (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4) show how the dependent variable changes when two or more independent variables were simultaneously changed.

A value of SS varies from 0.049 to 41.021 mg/ml among sixteen batches. This is reflected by the wide range of coefficients of the

terms in Equation 1. Small values of coefficients ($p>0.01$) are regarded as least contributing and non-significant in the optimization process. By omitting non-significant terms from the full model, reduced model equations for SS (Equation 2) as below:

$$SS = -0.3393 + 0.074X_1 + 0.083X_2 - 0.062X_3 + 0.048X_4 + 0.013X_1X_2 + 0.076X_1X_3 + 0.018X_1X_4 + 0.074X_2X_3 + 0.086X_3X_4 + 0.0179\,X_3^2 \ldots\ldots (2)$$

The predicted and observed values of the response parameter are shown in fig. 1 low values of residuals implied that there was a reasonable agreement between the predicted and observed values. This indicates the suitability of the model.

The significance of each coefficient of Equation 2 was determined by F-test and p-value which are listed in table 6. The larger the magnitude of the F value and smaller the p-value, the more significant is the corresponding coefficient [13]. In reduced quadratic model, main and interaction effect for concentration of different hydrotropic agents are significant ($p<0.05$) thus this model can be used for prediction of saturation solubility (SS) of mefenamic acid.

Table 6: Regressions analysis for saturation solubility (y)

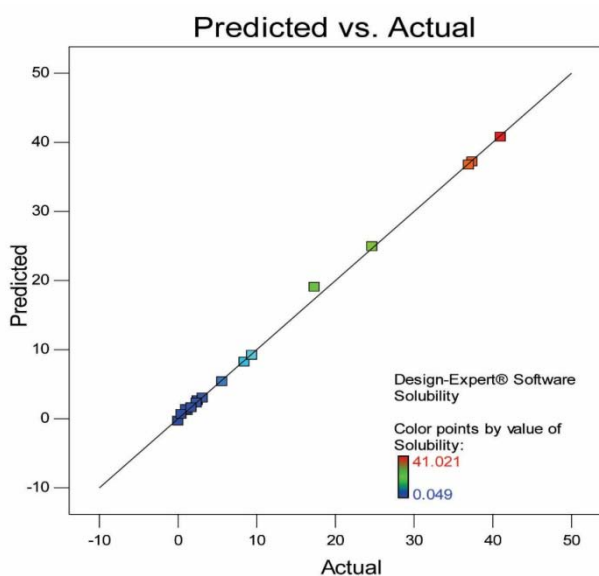| Model term	Full model			Reduced model		
	Coefficients	F-Value	p-value	Coefficients	F-value	p-value
Model	0.2128	93.496	0.0809	-0.339	461.519	<0.0001
X_1	0.0191	42.612	0.0968	0.074	552.174	<0.0001
X_2	-0.0178	31.272	0.1127	0.083	572.354	<0.0001
X_3	-0.0976	230.948	0.0418	-0.062	1194.07	<0.0001
X_4	-0.0395	40.269	0.0995	0.048	763.355	<0.0001
X_1X_2	0.0166	4.503	0.2804	0.013	30.3881	0.0027
X_1X_3	0.0775	98.536	0.0639	0.076	541.936	<0.0001
X_1X_4	0.0204	6.829	0.2327	0.018	51.8421	0.0008
X_2X_3	0.0769	97.023	0.0644	0.074	530.008	<0.0001
X_2X_4	0.0034	0.185	0.7414	-	-	-
X_3X_4	0.0885	128.361	0.0560	0.086	712.599	<0.0001
X_1^2	0.0011	0.051	0.8585	-	-	-
X_2^2	0.0024	0.255	0.7021	-	-	-
X_3^2	0.0179	14.300	0.1646	0.017	49.7446	0.0009
X_4^2	0.0020	0.183	0.7424	-	-	<0.0001

*Significant (p value<0.01)

Fig. 1: Observed vs predicted values of response (ss)

Validation of statistical design

The table 7 represents ANOVA of the full model and reduced model for SS. F-statistic value obtained from the results of ANOVA confirmed omission of non-significant terms of equations. Since the calculated F value, as shown in table 7, was less than the tabled F value for saturation solubility (SS), it was concluded that the neglected terms do not significantly contribute to the prediction, and hence reduced model can be applied.

For Equations 2, a sign of the coefficients explains the nature of the effect while magnitudes determine extent of effect for variables. Among the four independent variables value of coefficient of X_3X_4, X_2, X_1X_3, X_1, and X_2X_3 was found to be maximum in equation 3. This reveals that X_3X_4, X_2, X_1X_3, X_1, and X_2X_3 was major contributing variable for saturation solubility of mefenamic acid (SS). The goodness of fit of the model was checked by the determination coefficient (R^2). For SS, the values of the determination coefficients ($R^2 = 0.9992$ for full model and 0.9989 for reduced model) indicated that over 99 % of the total variations are explained by the model. The values of adjusted determination coefficients (SS: adjusted $R^2 = 0.9885$ for full model and 0.9967 for reduced model, are also very high which indicates a high significance of the model. All the above considerations indicate excellent adequacy of the regression model and it can be used for the optimization of dependent variable SS [14, 15].

Table 7: ANOVA for full and reduced model

Particulars	Model type	SS	DF	MS	F-value	P-value	R²	F-statistic
Regression	FM	3239.515	14	231.39	93.496	0.081	0.9992	F$_{cal}$=0.36
	RM	3238.481	10	323.84	461.519	9.01×10$^{-[07]}$*	0.9989	F$_{tab}$=2.60
Error	FM	2.47491	1	2.4749	-	-	-	(α=0.05)
	RM	3.50850	5	0.7017	-	-	-	

*Significant (p value<0.05). Where DF, Degree of freedom; FM, full model; F, Fischer ratio; MS, Mean squares; RM, reduced model; SS, Sum of squares

Optimization and checkpoint analysis

One can conclude the mathematical model itself if only the main terms are significant. Direct interpretation of equation 2 may lead to errors since interaction and polynomial terms are also significant. Therefore, contour plots and response surface plots were drawn (fig. 2). A nonlinear relationship is visible in all the plots fig. 2. Design space can be identified based on the highest and the lowest range of SS set by the user. The desirability function approach was used to search for the optimized mixture composition with desired responses. Selection from suggested mixture composition was done based on ease of mixture phase preparation and maximum saturation solubility of mefenamic acid was desired. Validation was performed by checkpoint analysis. For that, four selected mixture

compositions were used to analyze the saturation solubility experimentally.

The simulated results were validated experimentally by checkpoint analysis (table 8) and it was found that the saturation solubility of mefenamic acid has less than 10% Bias between experimentally and predicted results, thereby demonstrating the suitability of the proposed modeling approach and it can enable an efficient and thorough establishment of an optimal mixture formulation using different hydrotropic agents. Checkpoint batch OB-1 (U: SA: SB: SC, 5.454:0.092:24.396:9.904 %w/v), OB-3 (U: SA: SB: SC, 0.106:2.800:23.144:13.849 % w/v) and OB-5 (U: SA: SB: SC, 4:4:23:9 %w/v) were selected for final formulation of mefenamic acid injection.

Table 8: Checkpoint analysis results

Optimized blend	Blend composition (%w/v)				Desirability	Solubility of mefenamic acid (mg/ml)		% Bias
	U	SA	SB	SC		Predicted	Experimental mean±SD	
OB-1*	5.454	0.092	24.396	9.904	1.000	41.366	40.06±0.80	3.15
OB-2	9.871	0.009	21.474	8.643	1.000	41.041	39.01±1.19	4.94
OB-3*	0.106	2.800	23.144	13.849	1.000	41.041	42.01±0.40	2.36
OB-4	6.040	2.121	22.851	8.909	1.000	41.058	38.23±1.29	6.88
OB-5*	4	4	23	9	1.000	41.04	40.95±1.97	0.23

*Optimized blend selected for further formulation development of MFA injection. n=3 for SD

Benchtop stability of mefenamic acid in bulk solution

From table 9 it can be observed that the bulk solution of formulation batch MFA-OB5 shows no signs of turbidity and there was no change in assay compared to MFA-OB1 and MFA-OB3 during 24 h at room temperature (20-25 °C) and refrigerator condition. In batch MFA-OB1 and MFA-OB3, there were some losses of drug that may be due

to precipitation of drug in given condition. Additionally, the pH of prepared batches was found to be 7.53, 8.47, 7.86 for MFA-OB1, MFA-OB3, and MFA-OB5 respectively. The pH of prepared formulation bulk solutions was found to be within range of 7.5 to 8.5 which is accepted range for intravenous injection [16, 17]. Finally, MFA-OB5 was considered for further processing of ampule filling and quality control tests.
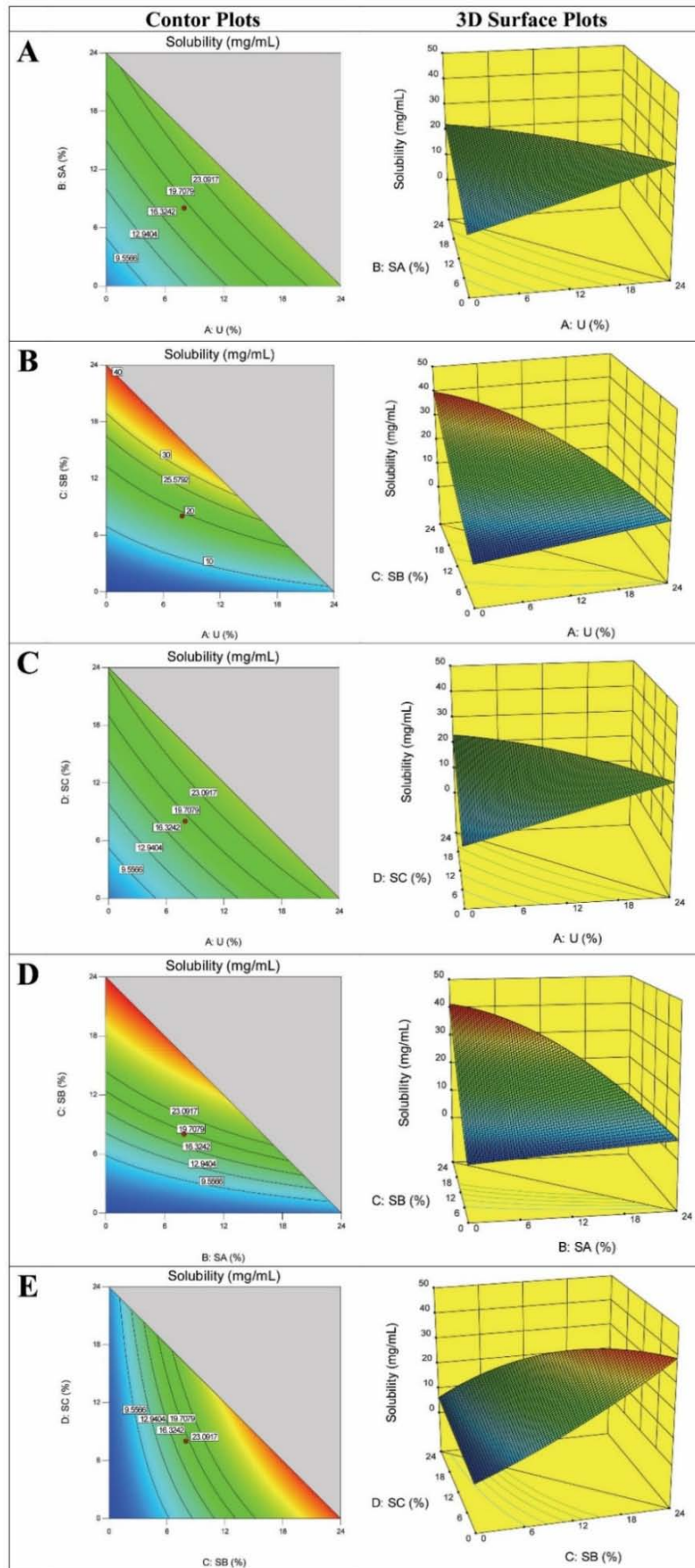
Fig. 2: Contour and 3D surface plots for saturation solubility

Table 9: Benchtop stability of mefenamic acid in bulk solution

Time (h)	MFA-OB1			MFA-OB3			MFA-OB5		
	Assay (%)		Turbidity (Yes/No)	Assay (%)		Turbidity (Yes/No)	Assay (%)		Turbidity (Yes/No)
	2-8 °C	20-25 °C		2-8 °C	20-25 °C		2-8 °C	20-25 °C	
0	100	100	-	100	100	-	100	100	-
4	100	100	-	100	100	-	100	100	-
8	100	100	-	99.87	100	-	100	100	-
12	100	100	-	99.65	99.97	+	100	100	-
16	100	100	-	98.97	99.85	+	100	100	-
20	100	100	-	98.43	99.23	+	100	100	-
24	99.98	100	+	97.93	98.93	+	100	100	-

(+) turbidity present, (-) turbidity absent

Clarity test, pyrogen test, and sterility test

Visual inspection of prepared mefenamic injection ampules reveals no suspended particulate matters which suggests that prepared formulation is fit for further quality control tests. The Limulus amebocyte lysate test (LAL test) was conducted to detect bacterial endotoxins in formulation. Each test tube under test, positive control, and negative control were visually inspected for turbidity and gel formation. There were no signs of turbidity and firm gel formation in the sample under test and negative control tube, while in positive control tube firm gel was formed. The results indicate the absence of pyrogen in prepared formulations. Furthermore, in sterility testing inoculated media was incubated for 14 d. All the cultures were observed several times during the incubation period.

There was no evidence of microbial growth in the specimen tube. The results suggest that the preparation under examination complies with the test for sterility.

Dilution profile of formulated injection

Series of dilutions were done by diluting injection of mefenamic acid (batch MFA-OB5) with different diluents like normal saline (0.9% NaCl), 5% dextrose solution for observing the precipitation in diluted solutions. The results shown in table 10 indicated that the formulation was stable in both dilution media. As the dilution ratio was increased, slight precipitate was observed, but after light steering, the precipitate was disappeared. This might be due to the redissolution of the precipitate.

Table 10: Dilution profile of injection formulation (batch MFA-OB5)

Dilution ratio (Formulation: Diluent)	Observation in different diluent	
	Normal saline solution (0.9% NaCl)	5% dextrose solution
1:1	-	-
1:5	-	-
1:10	-	-
1:20	-	-
1:30	-	-
1:40	-	-
1:50	-	-
1:100	-	-
1:500	-	-

(-) No precipitation, (+) Precipitation

Accelerated stability study

Results of the stability study of formulation showed that it remains unchanged in respect of pH and color table 11. Precipitate formation was not observed at different storage conditions, showing appreciable physical stability. The chemical stability was also noticed as there was no significant change in the assay of prepared batch at different storage conditions.

Table 11: Accelerated stability study of batch MFA-OB5

Physical stability parameter	Time	Conditions		
		Refrigeration (2-8 °C)	Room temperature	40 °C/75% RH
pH	Initial	7.86	7.86	7.86
	After 15 d	7.86	7.88	7.64
	After 30 d	7.77	7.91	8.03
Color	Initial	Clear	Clear	Clear
	After 15 d	Clear	Clear	Clear
	After 30 d	Clear	Clear	Clear
Precipitation	Initial	No ppt	No ppt	No ppt
	After 15 d	No ppt	No ppt	No ppt
	After 30 d	No ppt	No ppt	No ppt
% Assay (n=3)	Initial	100.00±0.021	100.00±0.021	100.00±0.021
	After 15 d	99.87±0.098	100.00±0.042	100.00±0.041
	After 30 d	99.76±0.145	99.98±0.045	98.67±0.222

CONCLUSION

The mixed hydrotropy technique was found to be a novel, safe and effective way to enhance the solubility of poorly aqueous soluble drugs. From the present studies, it can be concluded that the problem of inadequate aqueous solubility of mefenamic acid (0.049 mg/ml) overcame using mixed hydrotropic solubilization. Using the design of experiment the hydrotropic mixture composition was optimized which shows 835.71-fold (40.95±1.97 mg/ml: OB-5 batch) enhancement in solubility of mefenamic acid. There are many advantages of using optimization techniques while developing a formulation because it gives the researcher the ability to study interactions between factors. It is an excellent tool for developing the best possible formulation under a given set of conditions with minimum experimentation, saving considerable time, money, and effort. Moreover, it is strongly favored by regulatory agencies because it justifies the choice of ranges and finds a robust (optimum) region and thus should be used wherever possible to obtain a product with superior quality. Moreover, the aqueous injection was successfully developed which passes all quality control tests. The developed injection formulation was also found to be stable as per the accelerated stability study. The developed formulation can be served as a cost-effective treatment in various indications for rapid relief in different clinical conditions.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Authors have no conflict of interest.

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