An Efficient Procedure for Development of Levofloxain Hemihydrates Synthesis and Purification

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Abstract:

Levofloxacin is a broad spectrum synthetic fluoroquinolone antibiotic and is used to treat infections including: respiratory tract infections, cellulites urinary tract infections, prostates, endocarditic, meningitis, pelvic inflammatory disease, traveler's diarrhea, tuberculosis and plague. Levofloxacin is the S-enantiomer of a racemate, named Ofloxacin.

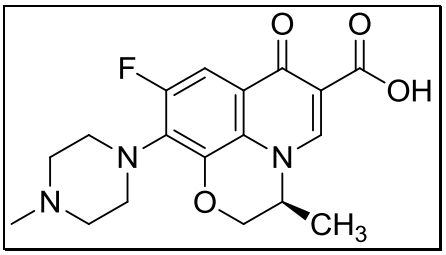
Many synthetic routes for preparing levofloxacin have been reported. Most of them involved using non-feasible reagents, high temperature during synthesis, non-recyclable, costly and poisonous solvents. Therefore, the selection of an inexpensive, recoverable and non-toxic medium for synthesis with high yield and also having minimum negative effect on the environment was of prime importance in industrial scale. Levofloxacin was produced by the reaction of (S)-(-)-9, 10-difluoro-3-methyl-7oxo-2, 3-dihydro7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid with N-methyl piperazine in a polar solvent.

The pure levofloxacin was used as hemihydrates, which had the empirical formula of $C_{18}H_{20}FN_3O_4$ ½ H_2O. Herein, we wish to investigate the effect of various solvent systems on the yield of reaction. Depending on the solvent used, different temperature and reaction time for synthesis was considered. The solvent selected, considering two criteias such as comparison of the measured yields and also the possibility of recovering by simple distillation in order to reuse for consecutive batches. Another aspect of the present research is to develop an efficient method for purification of crude levofloxacin and convert it to hemihydrates form, which is stable and can be used as an API (Active Pharmaceutical Ingredient). The selection of solvent and also the amount of water to be used in purification step in order to change crystalline structure is very important, which will be investigated in this section. The results show that using DMSO for synthesis and Ethanol/Water for purification step are the best choices.

Keywords: Antibiotic; Fluoroquinolone; Hemihydrate; Recoverable Solvent; Scale up; Eco-friendly

Introduction:

Levofloxacin, (S) -9-fluoro-2, 3-dihydro -3-methyl-10-(4-methyl-1- piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid has the formula as given below.



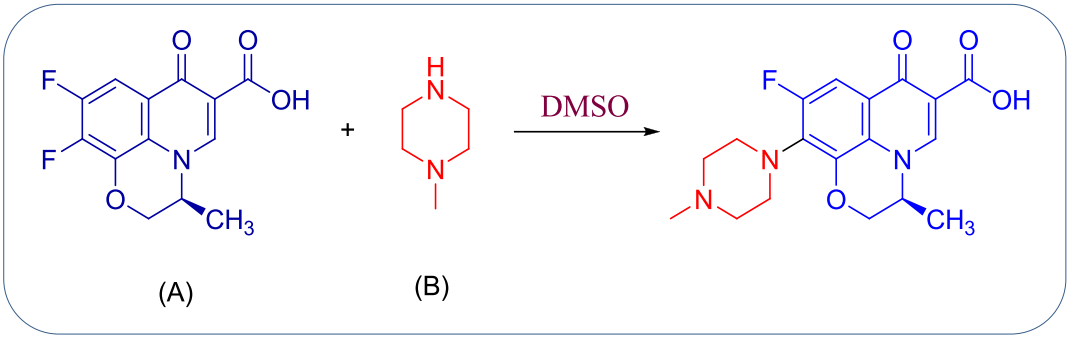
Levofloxacin is the S-enantiomer of Ofloxacin, a fluoroquinolone antibacterial agent. The mechanism of action of Levofloxacin and other fluoroquinolone antibacterials involves the inhibition of DNA gyrase (bacterial topolsomerase II), an enzyme required for DNA replication transcription repair and recombination. [1]

Many synthetic routes have been reported for preparation optically active fluoroquinolone derivatives such as use of the optically active pyridobenzoxazine derivatives, intermediates useful for preparation of pyridobenzoxazine derivatives. [2, 3]

Three polymorphic forms of levofloxacin i.e. anhydrous levofloxacin form α, β, γ and two hydrated forms i.e. hemihydrate and monohydrate are reported in the literature.[5] But levofloxacine hemihydrates (with 2.1-2.7 percent water) have maximum antibacterial effect among all structures.

One of the disadvantages of the prior methods for synthesis and purifying of levofloxacin is that they often produce an unsatisfactory yield, For example, 45-65% yields are typical. In addition usually they use much toxic solvents for synthesis and purification of levofloxacin hemihydrate without any possibility of recovering the solvents used. There remains a need for novel methods for purifying levofloxacin, particularly purified preparations having decreased percentage of impurities formed, such as anti-levofloxacin, desmethyl levofloxacin, N-oxide levofloxacin, deflour-levofloxacin and/or decarboxy-levofloxacin and using recoverable solvents for 2 steps synthesis and crystallization.

Assessment of various conditions in synthesis of levofloxacin led to a process comprising reacting (S)-(-)-9, 10-difluoro-3-methyl-7oxo-2, 3-dihydro7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (A) with N-methyl piperazine (B) in a polar solvent, preferably at a technically available temperature to form levofloxacin in an industrially feasible yield. [4]



Scheme 1. Synthesis of levofloxacin

On the other hand, one process is known for production Hemihydrates product which involves in crystallization or re-crystallization of crude levofloxacin in a solvent mixture of water and acetonitrile, diethyl ether and some alcohols with various portions. This proportion is very critical because with a minor change in the amount of water would lead to formation of other forms of levofloxacin.

Herein, we wish to present a versatile and new method for the recrystallization of Levofloxacin Hemihydrate with easy to get solvents. All the critical and effective factors investigated are presented in **table 2** in "Results and Discussion" section.

Experimental Section:

The chemicals used in the synthesis were supplied by Aldrich and Merck. Melting point was determined using a Reichert Kofler thermo pan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. 1H NMR spectra were recorded on a Bruker AMX spectrometer at 400 MHz, using TMS as internal standard (chemical shifts in δ values, J in Hz). Moisture of content was controlled using METTLER TOLEDO volumetric Karl Fischer compact titrators V20. Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

General Procedure for the preparation levofloxacin Hemihydrates

Assessment of various conditions in synthesis of levofloxacin led to a process comprising reacting (S)-(-)-9, 10-difluoro-3-methyl-7oxo-2, 3-dihydro7H-pyrido [1, 2, 3-de] [1, 4] benzoxazine-6-carboxylic acid (A) (1mol) with N-methyl piperazine (B) (2mol) in presence of 200 ml DMSO at 80 °C for the time represented in **table 1** in "Results and Discussion" section. The reaction is carried out into a 2 L round-bottom flask equipped with mechanical stirrer, thermometer and condenser until product is formed completely. The reaction progress is monitored by TLC. After completion of the reaction, the process will continue by adding 1200

ml Isopropyl alcohol into flask and mixing for about 60 min at 25 °C. The participated solid was filtrated and washed with 175 ml isopropyl alcohol and then dried at 60-70 °C until constant weight is obtained. The above-obtained crude of levofloxacin (150 g) is dissolved in a mixture of ethanol (810 ml) and water (90 ml). Activated carbon (10 g) is added and stirred for about 30 min at reflux temperature. Then filtrated reaction mass is cooled to 5-10 °C for 1 hour The Product is filtered and dried at 60-70 °C to constant weight.

Filtrate is distilled off under vacuum at temperature below 60 °C. Isopropanol used is recovered and could be used for the following batches.

Solvents recovery

Recover solvents for Synthesis step:

After completion of the reaction, in order to recover the used solvents, the filtrate is distilled under atmospheric condition at 80 °C and the two solvents, DMSO and isopropyl alcohol are separated due to difference in boiling points and , could be used for next batches. These solvents can be used for several times without any negative effect on quality of product. The Results of repeated batches shown in **diagram 1** in "Results and Discussion". Clearly indicates the efficacy of the designed process. If the color of DMSO has darkened charcoal should be used for de-coloration of it.

Recovery of solvents used for recrystallization:

After the crude product is collected, the filtrate which is a mixture of water and ethanol is distilled under vacuum at temperature below 60 °C after adjustment of ratios of ethanol/water the recovered solvent can be used for next bathes.

Results and Discussion:

In the present work we continue to exploit the synthesis of levofloxacin Hemihydrate in a series of solvents (see **Table 1**), with the aim to select an inexpensive, recoverable and non-toxic medium for synthesis with aiming to have high yield having minimum negative effect on environment. **Table 1** shows the effect of various conditions on synthesis of levofloxacin. Comparison of the results shows that using DMSO as a solvent for synthesis is the best choice.

Table 1. The effect of various conditions on synthesis of levofloxacin

Entry	Solvent	Yield[c] (%)	Temperature (°C)	Time (hr)
1	DMSO[a]	81	80	2.5
2	PGME[b]	62	118	23
3	Isobutanol	77	reflux	6
4	Dimethyl acetamide	89	110	1.5
5	Neat	65	98	0.7

[a] Dimethyl sulfoxide
[b] Propylene Glycol Methyl Ether
[c] The yields refer to the isolated product

Although DMSO is not considered as a green solvents but for redeeming this negative impact, its re-use could compensate this draw-back The recovered DMSO has been reused successfully in 5 subsequent batches with a little increase on the yield of reaction because of the existence of unreacted starting material and products in residue, The results of these batches is represented in the below diagram.

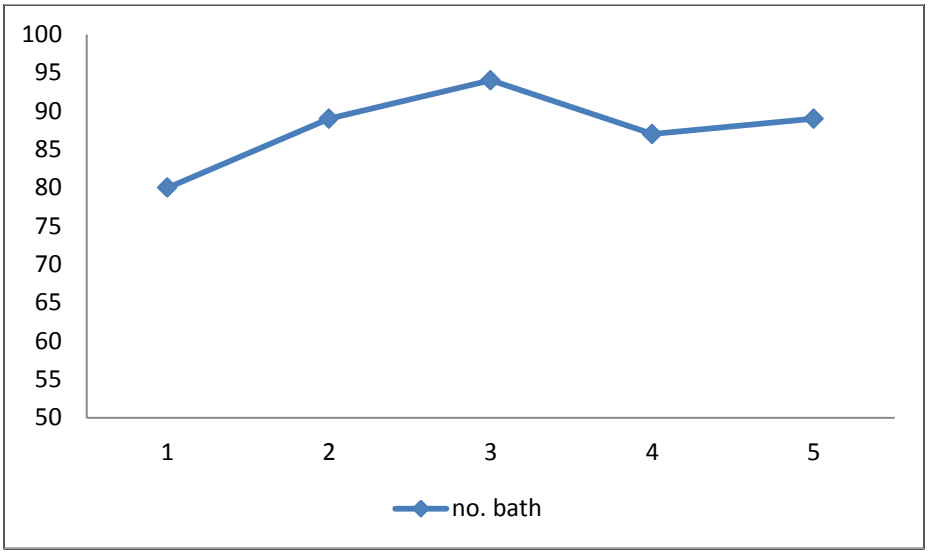


Diagram 1. Yield of levofloxacin with using recovered DMSO and Isopropyl alcohol

For the preparation of levofloxacin hemihydrate, several solvent systems were investigated, such as acetonitrile/water, isopropyl alcohol/water, ethyl acetate /water and Ethanol /water. The results moisture content of each sample is presented in **table 2**; indicating ethanol /water with 20% to about 30% (v/v) water is the best choice to make the crude product as hemihydrates.

Table 2. Effect of solvent system on moisture of levofloxacin crude

No	Solvent system	Portion	Moisture content
1	ACN : H_2O	70 : 30	0.57%
2	n-BuOH : H_2O	90:10	2.7%
3	Toluene : H_2O	70:30	3.76%
4	MEK[a]: H_2O	70:30	1.7%
5	MeOH : H_2O	70:30	85%
6	EtOH : H_2O	70: 30	2.6%

[a] Methyl ethyl ketone

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