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Synthesis and Morphology of Biological Active Compounds

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The objectives of this study were to investigate the effect of crystallization 5-H-dibenz(b,f)azepin-5-carboxamide and fexofenadine hydrochloride from different solvents commonly used for purification of drugs during their final stages of synthesis.
The synthetic pathway has presented the number of reactions in which the starting material is converted to the desired molecule structure. 5-H-dibenz(b,f)azepin-5-carboxamide is pharmaceutically active compound which is used in the treatment of epilepsy and trigeminal neuralgia. The synthesis 5-H-dibenz(b,f)azepin-5-carboxamide is performed in accordance with the mechanism of the nucleophylic substitution. When substances crystallize in a different but chemically identical crystal forms, then we talk about *polymorphism.* 5-H-dibenz(b,f)azepin-5-carboxamide is a very important pharmaceutically active compound which is present in at least four anhydrous polymorphic modifications, two of which are monoclinic, one is trigonal and the final one is triclinic. Fexofenadine hydrochloride, a piperidine derivative is H1 antihistaminic active compound. A number of polymorph modifications is present in the structure of molecule. Reaction of esterification, Friedel-Craft's alkylation, condensation, oxidation and reduction are included in the Fexofenadine hydrochloride synthesis pathway.

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Keywords: Biologically active compounds; 5-H-dibenz(b,f)azepin-5-carboxamide; fexofenadine hydrochloride; solubility.

1. INTRODUCTION

The synthesis implies a radical approach in which it is very important to know a final molecule in detail i.e., to define carbon skeleton, nature and orientation of functional groups in a desired molecule. It is important to define the main synthetic intermediate and stereo chemically amounts of reactants which is necessary to estimate the overall productivity of synthetic reactions. The ways of molecule synthesis with given structure may have a number of methods with a different efficiency rate. If the desired synthesis is reached there would be no possibility that even one method is wrong [1]. Also, the synthesis is used for preparing compounds that are not possible to be obtained from nature in the adequate amount (vitamins, penicillin etc) [2]. 5-H-dibenz(b,f)azepin-5-carboxamide is a tricyclic compound with azepin core (unsaturated seven-member ring with nitrogen as a heteroatom), associated with two benzene ring (Fig. 1).

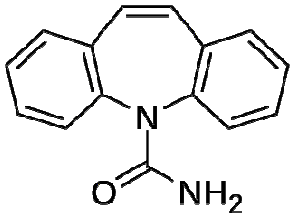

Fig. 1. Molecular structure 5-H-dibenz(b,f)azepin-5-carboxamide

Common sites of azepine core and benzene rings are marked with letters b (2,3 C-atom) and f (6,7 C-atom) which is shown as a three-dimensional structure of the molecule (Fig. 2).

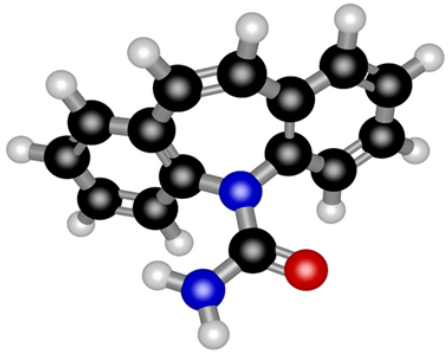

Fig. 2. Three-dimensional structure 5-H-dibenz(b,f)azepin-5-carboxamide

5-H-dibenz(b,f)azepin-5-carboxamide is biologically active compound based on the azepin molecule. This compound has found clinical use for the treatment of neuralgia and epilepsy. This important anticonvulsant has been found to crystallize into four different anhydrous polymorphs: C-monoclinic (form IV), P-monoclinic (form III), trigonal (form II) and triclinic (form I) [3]. The existence of four polymorphic forms of this compound has enabled this research to find a way to be reproducible, justified and reliable for giving a desired product of polymorphic form.

The possibility of identification and characterization of different polymorph forms of biologically active compounds, the process of the synthesis 5-H-dibenz(b,f)azepin-5-carboxamide is of great importance [4].

Acting with nucleophilic nitrogen compounds such as ammonia, ammonium hydroxide or amine, the chlorine atom is replaced with amino group in the structure of 5-H-dibenz(b,f)azepin-5-carbonyl chloride. Synthesized compound with a retained carbonyl group i.e., an appropriate amide 5-H-dibenz(b,f)azepin-5-carboxamide is obtained according to the synthetic reaction [5] in Fig. 3.

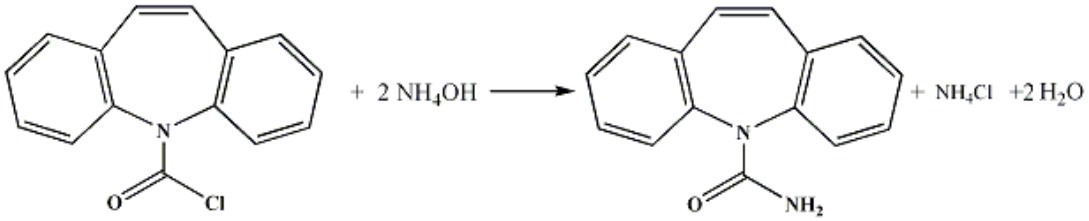

Fig. 3. Synthetic pathway 5-H-dibenz(b,f)azepin-5-carboxamide

Fexofenadine hydrochloride is a pharmaceutically active compound and antihistamine drug. Antihistamines are divided into two main groups of pharmaceutically active compounds such as: H1 antihistaminic drugs and H2 receptor antagonists [6]. In the group H1 of antihistaminic drugs there are also some pharmaceutically active compounds which actually represent derivatives of amino-alkyl ethers, ethylendiamines, alkylamines, piperazine, phenotiazine, piperidine. Fexofenadine hydrochloride belongs to this last sub-group i.e., it is a derivative of piperidine. This compound decreased activation of H1 receptors in cells over the histamine which is released in body through some other cells [7].

Piperidine is an organic compound which belongs to the heterocyclic amines and consists of a six-membered ring containing one nitrogen atom. Piperidine is widely used as a chemical reagent in the synthesis of organic compounds, including pharmaceutically active compounds. Piperidine prefers a chair conformation, similar to cyclohexane but unlike cyclohexane, piperidine has two distinguishable chair conformations. The first one has the N–H bond in an axial position and second one is the one where the N-H bond is in an equatorial position.

The equatorial conformation was found to be more stable in the gas phase. In polar solvents the axial conformer of the N-H bond may be more stable in comparison to the nonpolar solvents in which the equatorial conformation is preferred. The two conformers interconvert rapidly through nitrogen inversion [8].

A great number of single crystals are more often formed in polar and protic solvents. Also, crystallization with protic solvents provides better optimization for creating hydrogen bonds. Conformation flexibility helps to form pseudo-polymorph crystals of this compound [9].

Fexofenadine hydrochloride systematic name is 4-[1-Hydroxy-4-[4-(hydroxy-diphenyl-methyl)-1-piperidyl]butyl]-2,2-dimethylbenze ethane acid x HCl [10].

Fexofenadine hydrochloride contains several functional groups with a possibility of forming hydrogen bonds and in normal circumstances exists as a zwitter ion [11] and has the structure shown in Fig. 4.

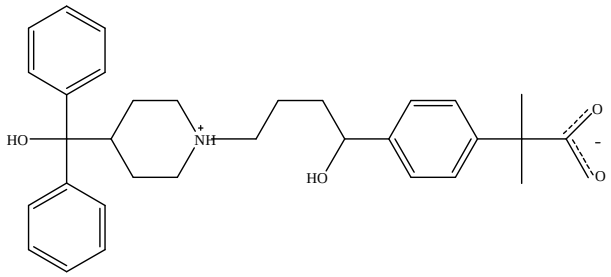

Fig. 4. Molecular structure of fexofenadine hydrochloride

Molecule structure provides the existence of more different polymorphic modifications: anhydrate polymorph forms (I and III) and hydrate forms (II and IV). There are also a number of crystal polymorphic modifications of fexofenadine hydrochloride which actually represent modifications of these four basic polymorphic forms that depend on the solvent used during their crystallization. These polymorphic forms are designated as Roman numerals as polymorphic form V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV [12]. Synthesis of fexofenadine hydrochloride is carried out in five stages that include reactions of esterification, Friedel-Craft's´ acylation, condensation, oxidation and reduction [13].

2. MATERIALS AND METHODS

Synthetically necessary chemicals and solvents of high purity are supplied by: Fluka (Switzerland) Merck (Germany), Chemos (Germany) and Sigma-Aldrich (USA).

2.1 Crystallization Procedures

The crude product (5-H-dibenz(b,f)azepin-5-carboxamide and fexofenadine hydrochloride) were dissolved with a suitable amount of different solvent at boiling point of solvents. Activated charcoal was added and such a hot supersaturated solution was filtered warm. The solution was kept at room temperature until its temperature reached 25℃. The precipitated crystals were collected by filtration through a Buchner funnel and dried 24 hours.

2.2 Methods

Methods of analysis and characterization synthesized biologically active compounds are:

- High-performance liquid chromatography *(HPLC)*
  Contents of 5-H-dibenz(b,f)azepin-5-carboxamide and Fexofenadine Hydrochloride were determined using high-performance liquid chromatography (Shimadzu chromatograph with UV-Vis fluorescent detector range 230 nm) and a column lichrospher 5μm, 250x4,0mm, 100 CN range; with flow of 1,5 per min. at 40ºC.

- Differential scanning calorimetry (*DSC*)
  The measurements were performed using the Perkin Elmer dsc pyris diamond differential scanning calorimeter. For the measurements, a 3-5 mg sample was heated at a rate of 40ºC /min. Thermal behavior of the samples 5-H-dibenz(b,f)azepin-5-carboxamide and Fexofenadine Hydrochloride was studied under a nitrogen purge covering a temperature range of 50–220ºC.
- Fourier transform infrared spectroscopy *(FTIR)*
  FTIR spectra of 5-H-dibenz(b,f)azepin-5-carboxamide and Fexofenadine Hydrochloride were recorded on a Perkin-Elmer Spectrum 1000 system, equipped with a deuterium triglycine sulfate detector. The scan range was 400–4000 cm^{-1}, using eight scans per spectrum with a resolution of 4 cm^{-1}. Spectra were obtained in the transmission mode in KBr pellets.
- Thermal microscopy (*HSM*)
  Thermomicroscopy was performed with a OLYMPUS BX 51 hot stage connected to an FP 90 control processor and viewed under polarized light with a Leica DMLP microscope. 5-H-dibenz(b,f)azepin-5-carboxamide and Fexofenadine Hydrochloride samples were heated from 20 to 220℃ at a rate of 4 0℃/min for initial observation.

3. RESULTS AND DISCUSSION

Synthesis of desired polymorphic form 5-H-dibenz(b,f)azepin-5-carboxamide is dependent on the recrystallization of crude compound.It is all about the complex interactions of the reactants, i.e., pure products and solvents for recrystallization. The results of the research have shown that use of methanol-water binary mixtures with higher water content, contribute to the form I 5-H-dibenz(b,f)azepin-5-carboxamide, while crystallization with more concentrated methanol solvate contributes to the form III [14].

Polymorphic form I, 5-H-dibenz(b,f)azepin-5-carboxamide has a number of hydrogen dimmers (R1, R2, C1) connected with short (1,97 Å) hydrogen bonds (O○○○H-N) in comparison to the polimorph form III [15]. As polar protic solvent methanol, in binary mixtures dissolves 5-H-dibenz(b,f)azepin-5-carboxamide which is hardly crystallized when mixture is cooled.

Water as a second component of the mixture decreases the solubility of 5-H-dibenz(b,f)azepin-5-carboxamide in supersaturated solution during the process of cooling which affects its crystallization and deposition of the polymorphic form I [16]. Water, compared to methanol, has the capacity of forming a number of hydrogen bonds (hydrogen bond networks), while the methanol affinity for creating hydrogen bonds is smaller due to the steric interference of the CH_3 group and inability to receive-give more hydrogen atoms. This is the reason why water in 60% methanol: water mixture represents the key factor in achieving supersaturation.

However, it is experimentally confirmed that the use of 90% methanol during the triple crystallization has an optimal effect in getting the desired polymorphic modification (form III) on 5-H-dibenz(b,f)azepin-5-carboxamide in comparison to quintuple crystallization with pure solvent (99,8% methanol) (Fig. 5).

5-H-dibenz(b,f)azepin-5-carboxamide is a pharmaceutically active compound which crystallizes in a hydrated, solvated, and four anhydride polymorphic modifications [3]: C-monoclinic (form IV), P-monoclinic (form III), trigonal (form II) and triclinic (form I). In form III,

it is detected that it crystallizes as a simple monoclinic cell and represents thermodynamically stable modification at room temperature. Already found trigonal modification is form II and recently discovered C-centered monoclinic form is form IV [17].

Long lasting problem for polymorphism 5-H-dibenz(b,f)azepin-5-carboxamide was identification of high-temperature modification (form I). Four energetically near anhydride polymorphic forms of 5-H-dibenz(b,f)azepin-5-carboxamide have the next order of stability at room temperature: P-monoclinic > triclinic > C-monoclinic > trigonal.

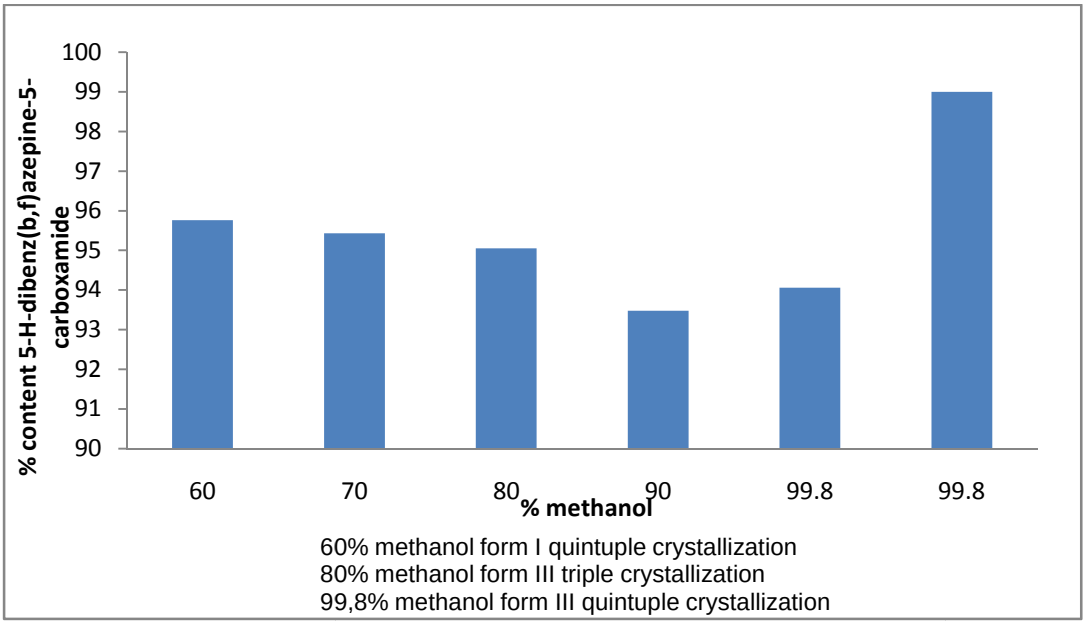

Fig. 5. HPLC analysis of synthesized 5-H-dibenz(b,f)azepin-5-carboxamide

Crystallization process of pharmaceutically active compounds is carried out in water solution of different solvents, so there is some space in their lattice on which hydrogen bonds associate with water molecules forming hydrated forms [18]. All the crystal structures of 5-H-dibenz(b,f)azepin-5-carboxamide show the presence of dimmers associated with hydrogen bonds [19] (Fig. 6).

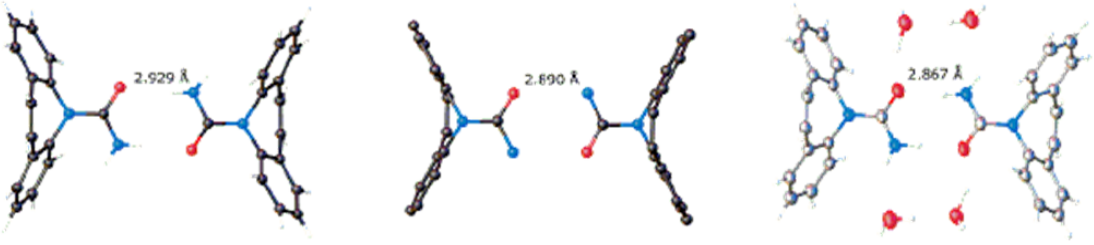

Fig. 6. Dimmers 5-H-dibenz(b,f)azepin-5-carboxamide in P-monoclinic form (left), trigonal form (in the middle) and dihydrate form (right)

Triclinic and trigonal forms with needle-like morphology have similar weak H-bonds [20] (Figs. 7 and 8).

Fig. 7. SEM picture of triclinic form 5-H-dibenz(b,f)azepin-5-carboxamide

Fig. 8. SEM picture of trigonal form 5-H-dibenz(b,f)azepin-5-carboxamide

Form III is characterized with more rigid structure with a compact arrangement of dimmers which are further connected by short van der Waals´ interactions (H⊙⊙⊙H) between phenyl and vinyl hydrogen of azepin ring [21] (Fig. 9).

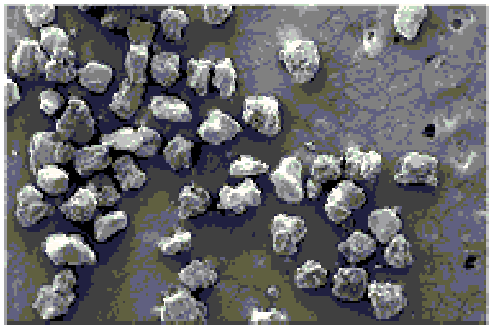

Fig. 9. SEM picture of form III 5-H-dibenz(b,f)azepin-5-carboxamide

When methanol and water is used in the form of binary mixtures of different percentage relations it results of getting polymorph form VIII of fexofenadine hydrochloride [22,23]. Values of FTIR and HPLC characterization do not show any deviations which vindicate that

with all mixture-solvents that are used during the experiment provide polymorphic form VIII fexofenadine hydrochloride. That is also confirmed with DSC analysis of the analyzed samples (Fig. 10).

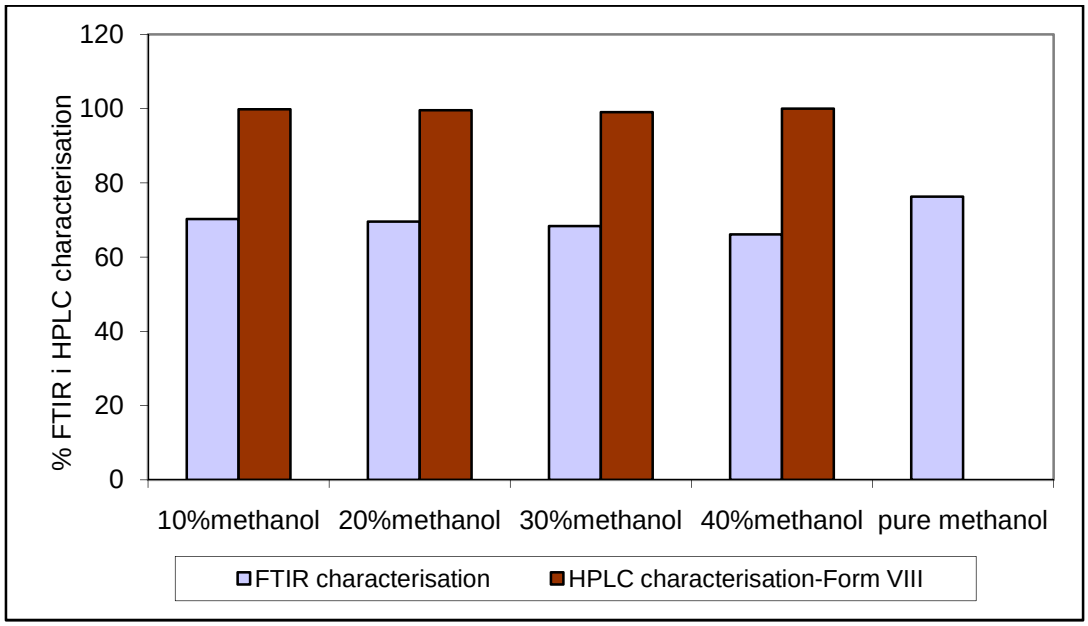

Fig. 10. The effect of different percentage composition of methanol and water of obtaining the corresponding polymorphous forms of fexofenadine hydrochloride

Changing solvents and using different concentrations of isopropanol-water mixture can be seen in the change of relation between molecules and crystals. The reason why is the fact that isopropanol is an alcohol which has longer and more branched hydrocarbon chain in comparison to methanol. Also, boiling point of isopropanol is higher than the methanol boiling point and crystallization process on different temperature heating can also lead to the conversion of pharmaceutically active compound into another polymorphic form.

It can be seen that use of 40% of isopropanol solution has led to the highest content of polymorphic form X (Fig. 11).

It can also be seen from the diagram that with the increase of the concentration of isopropanol the content of fexofenandine hydrochloride increases. On the other hand, with the increase of the concentration of methanol (10-30%) the content of a polymorph form VIII in the sample decreases. With the use of 40% of the methanol, the content of fexofenadine hydrochloride in the sample will be slightly increased (Fig. 12).

Isopropanol obtained samples show the highest percentage of compatibillity with the CRS standard using FTIR-database method characterization. It can be concluded that the increase in the percentage of FTIR-database matching is linear with increasing number of carbon atoms in hydrocarbon chain and alcohol branched.

Small value of FTIR-database matching, sample where acetone was used as a solvent can be interpreted with his partially polar character and with the presence of carbonyl functional

group which has no tendency of forming hydrogen bonds which are crucial when forming crystal order of most of the polymorphic forms of fexofenadine hydrochloride [24,25] (Fig. 13).

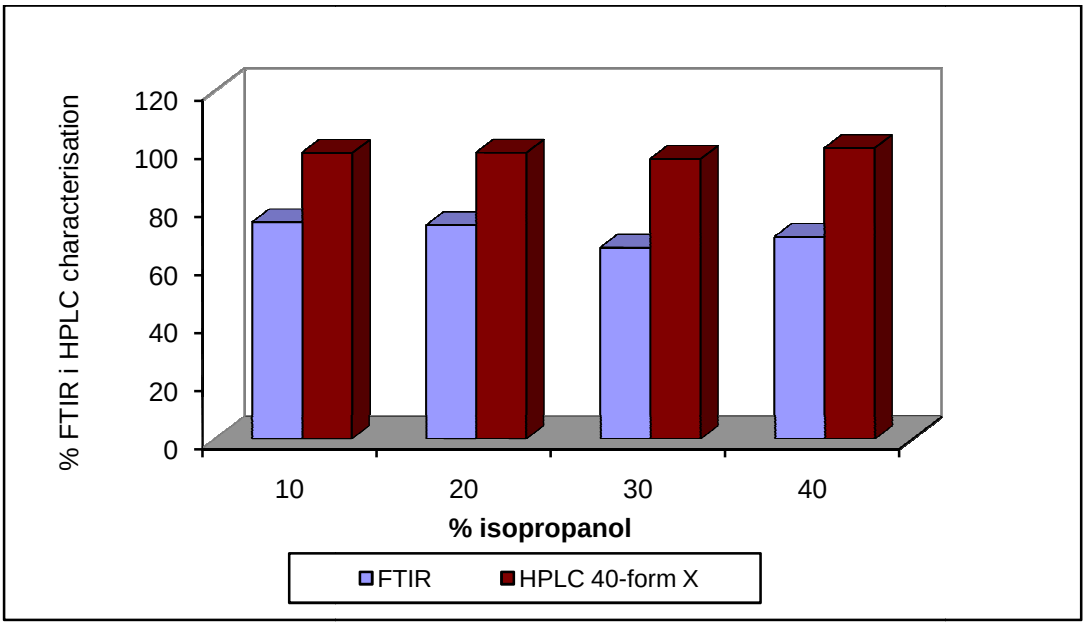

Fig. 11. The effect of different percentage mixture of isopropanol and water on FTIR and HPLCcharacterization and formation of a polymorphic form X

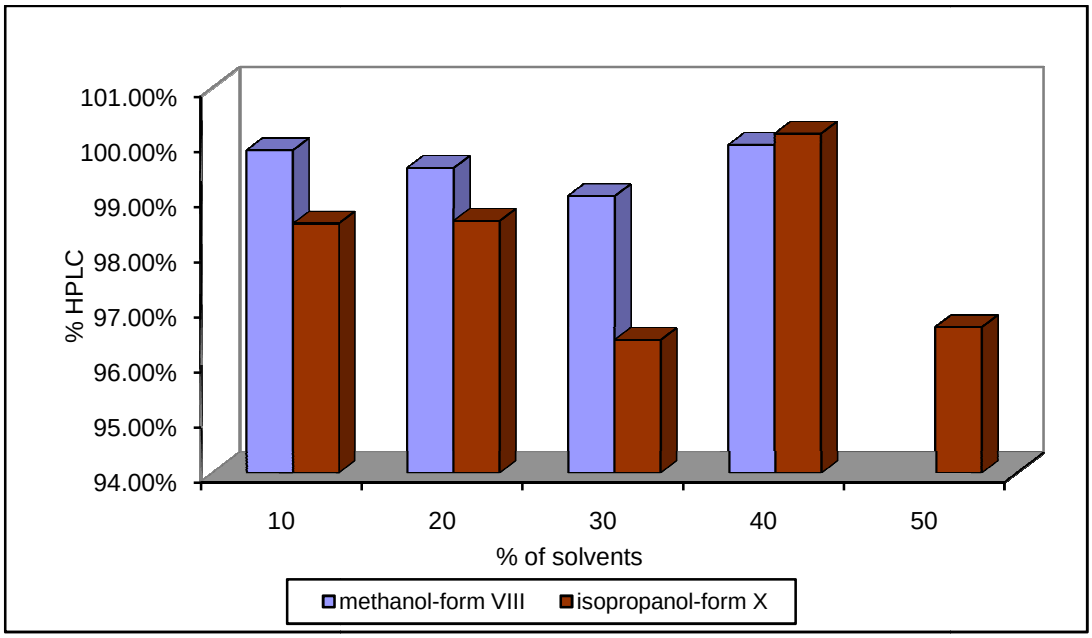

Fig. 12. The effect of different solute on the content of fexofenadine hydrochloride defined with HPLC method

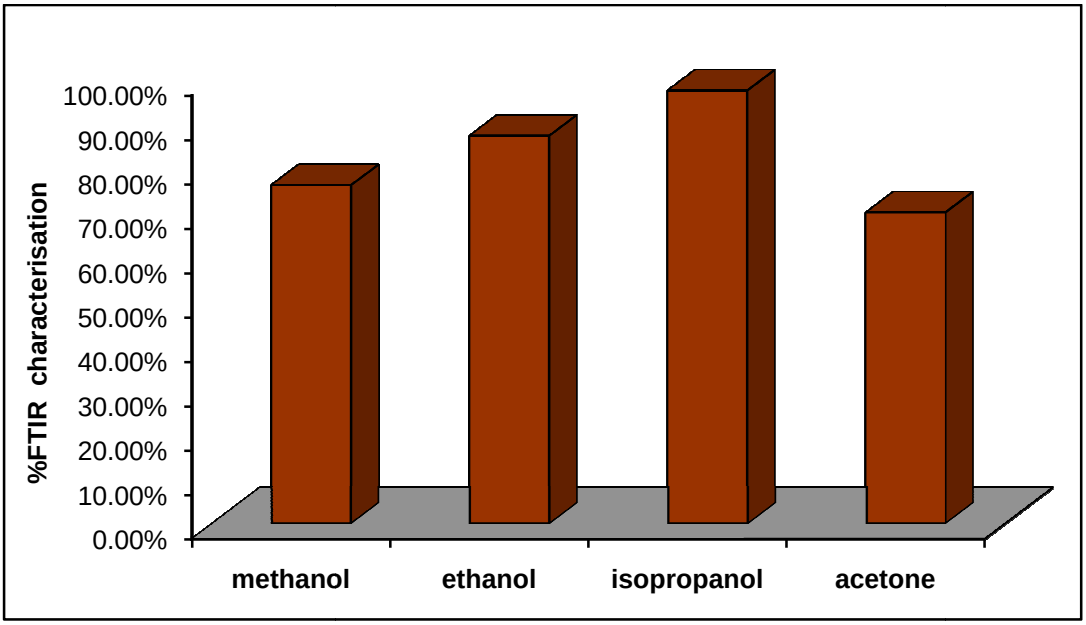

Fig. 13. The influence of the solvents and their structure on FTIR characterization of the synthesized fexofenadine hydrochloride

Fig. 14 show a SEM microscopic picture of synthetized polymorph form VIII.

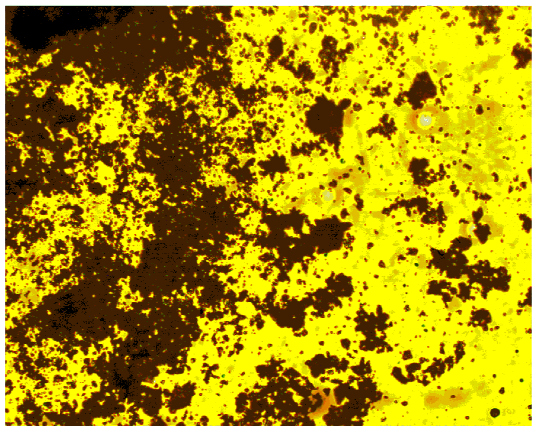

Fig. 14. Microscopic picture of the synthesized polymorph form VIII of fexofenadine hydrohloride

4. CONCLUSION

- Crystallization conditions can be modified to create a desirable occurrence of polymorph form of 5-H-dibenz(b,f)azepin-5-carboxamide.
- During the process of crystallization, use a binar mixture (methanol: water) with a higher water content leading to polimorphic form I 5-H-dibenz(b,f)azepin-5-carboxamide.

- According to the results of DSC and HSM analysis, obtaining of P-monoclinic form (form III) 5-H-dibenz(b,f)azepin-5-carboxamide requires the use of concentrated solvent such as methanol.
- Multiple crystallization (quintuple with 99.8% methanol) is one of the ways to increase yield and percentage content of P-monoclinic form (form III) 5-H-dibenz(b,f)azepin-5-carboxamide.
- By changing the solvent in the synthesis of fexofenadine hydrochloride it is possible to influence the final form of polymorphic synthetic compound;
- Using polar solvent favors of appropriate polymorphic forms (VIII and X);
- Binary mixtures of methanol: water in the final stage leads to the emergence of polymorphic form VIII;
- The use of a binary mixture of isopropanol: water favors the formation of polymorphic form X of fexofenadine hydrochloride.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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