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Preparation, Characterization of Hydrates and Anhydrous Forms of Anti migraine Drug - Frovatriptan Succinate

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Abstract

Frovatriptan succinate (FS) is administrated in monohydrate form. A new dihydrate form of FS is crystallized using acetone and water solvent combination. Interestingly, FS can be crystallized as monohydrate and dihydrate using acetone and water solvent combination. The process conditions are studied and optimized w.r.t. temperature of crystallization and % concentration of water, to get the desired hydrate. The result showed that the concentration of water is deciding factor for the formation of monohydrate and dihydrate of FS. Two different anhydrous forms were produced from two hydrates, by solvent mediated and solid-state transformation techniques. Effect of relative humidity on the anhydrous forms is also investigated. On exposure to humidity both the anhydrous forms are found to be unstable and convert to their corresponding hydrated form. Two hydrated forms and the two anhydrous forms obtained are characterized using X-Ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Fourier Transform Infra-red (FTIR).

Keyword: Frovatriptan succinate, polymorphism, hydrate, crystallization, polymorph characterization, thermal analysis.

Introduction

Polymorphism is a solid-state phenomenon and is defined as the ability of a compound to exist in different crystalline phases that have different arrangements and /or conformations of the molecules in the crystal lattice[1-3]. When two forms differ either due to different packing or different conformation of molecule within the crystal lattice then it is called as packing or conformational polymorphism respectively. Many times the drug substances are also isolated as solvate or hydrate. Drug substance can also be isolated in the amorphous state. More than 50% of the drugs marketed today exhibits polymorphism[4-5]. Polymorphism being a solid-state phenomenon, polymorphs are characterized by various solid-state characterization techniques[6].

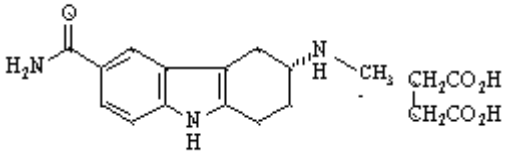
Polymorphs and hydrates differ in internal solid-state structure and therefore, possess different physical properties, including melting point, crystal habit, colour, density, compressibility, packing, thermodynamic, spectroscopic, kinetic, interfacial, and flow properties[1]. These differences in physical properties make polymorphism special, as they can have direct impact on drug substance processibility, drug product manufacturability and drug product quality/performance such as stability, dissolution and bioavailability[7]. Appearance of unwanted polymorph may affect the product development and in turn delay or interrupt commercial production[8].

For this reason it is now essential and important to give close attention to drug polymorphism during drug product development and ANDA regulatory review [9-10]. Regulatory agencies are asking to develop the process which will give the pure polymorphic form by following quality by design (QbD) aspects where in the condition are studied and established to get the desired form using process analytical techniques (PAT) [11-12]. Principle regulatory concern with regard to drug polymorphism is based upon the potential effect that it may have on drug product bioavailabiity/ bioequivalence (BA/BE). Polymorphs of drug substance may have different apparent aqueous solubility and dissolution rates; greater the difference more will be effect on BA[1,13-14]. The pervasiveness and relevance of polymorphism, polymorph screening is to select the best solid form for development was highlighted by many reviewers[15-16]. The U.S. regulatory agency, Food and Drug Administration (FDA), that oversees the pharmaceutical industry, published guidance for drug developer in 2000. Today polymorph screening of not only new drugs but also of old drug molecules has been initiated to explore the advantage of the new form over the existing form[17- 22].

Thus it becomes very important to carry out polymorph screening in the early stage of product development. Frovatriptan Succinate (FS) [structure 1] is a (R)-(+)-6-carboxamide-3-N-methylamino-1,2,3,4-tetrahydrocarbazole is 5HT1 receptor agonist. It is marketed under the brand name of FROVA® as a monosuccinate salt monohydrate, for the treatment of migraine. From the information available on drug label it is believed that it exist in monohydrate form[23].

To date, apart from mono-hydrate, additional hydrates or solvates of FS have not been reported in the public domain. The present article describes the preparation and characterization of FS hydrates, a monohydrate and a dihydrate by crystallization

and their conversion to anhydrous forms. The aqueous solubility of all the forms was studied. The two hydrates are formed using same solvent combination. The present article describes the optimization of crystallization parameters such as volume of water and temperature to get the desired hydrate. Effect of relative humidity on all the forms is studied. Polymorphs and pseudo polymorphs of FS are characterized by DSC, XRPD, FT-IR, TGA, microscopy and NMR. Monohydrate form is prepared by the process disclosed in US patent 5, 616, 603 [24].



Structure-1

Material and Methods

Materials: Chemically pure FS, in the solid state was prepared by following the process disclosed in the US Patent 5,616, 603. The crude FS monohydrate obtained was further purified using acetone + water solvent combination and used for preparation of other forms.

Designation of FS Polymorphs: Two hydrates of FS are obtained in stoichiometric and are monohydrate and dihydrate. For convenience, anhydrous form obtained from monohydrate is designated as Form I and another anhydrous form obtained from dihydrate is designated as Form II.

Isolation of Monohydrate FS: 5g of crude FS was suspended in 40ml of Acetone. The suspension was heated to reflux temperature and to this hot solution 17.5 mL of water was added drop wise to obtain clear solution. The Solution was filtered and cooled to 25-30°C under stirring. The solid obtained was isolated by filtration and dried at 50-60°C.

Isolation of Dihydrate FS: 1g of FS monohydrate FS was suspended in 60ml of Acetone. The suspension was heated to reflux temperature and to this hot solution 12 mL of water was added drop wise to obtain clear solution. The solution was filtered and cooled to 25-30°C under stirring. The solid obtained was isolated by filtration and dried at 50-60°C.

Isolation of Form I: 2g of FS monohydrate was suspended in 25 mL of Toluene. The suspension was heated to reflux temperature and was maintained at same temperature for 5 h. The solution was cooled to 25-30°C under stirring. The solid obtained was isolated by filtration and dried at 50-60°C.

Isolation of Form II: 2g of FS dihydrate was suspended in 25 mL of Toluene. The suspension was heated to reflux temperature and was maintained at same temperature for 5 h. The solution was cooled to 25-30^{0}C under stirring. The solid obtained was isolated by filtration and dried at 50-60^{0}C.

Alternate procedure for making Form II: 0.5g of FS dihydrate was heated at 160°C in air tray drier and was maintained at same temperature for 30 min. The solid obtained was isolated as Form IV.

Microscopy analysis: Microscopic images were captured with a Nikon Eclipse 80i Optical Microscope, with CF160 infinity optical system using 10× zoom, equipped with a Nikon digital camera. The software used to record and analyze the images is Q IMAGING.

Thermal Analysis: Differential scanning calorimetry (DSC) measurements were made on a Perkin Elmer, Diamond DSC instrument using aluminum sealed pans in a nitrogen atmosphere. The scans were recorded between 40 and 250˚C at a constant heating rate of 10˚C/min. The enthalpy of fusion values (ΔH_{fus}) was estimated by drawing a straight line connecting the baseline before and after the melting peak, and integrating the area under the peak. Thermogravimetric analysis (TGA) measurements were made on a Perkin Elmer, Pyris 1 TGA instrument. The scans were recorded between 40 and 250˚C at a constant heating rate of 10˚C/min. The % weight loss was calculated using Pyris 1 TGA software.

Single crystal X-Ray Diffraction: Prepared crystals of Form II using acetone + water solvent combination. However the crystals were obtained as polycrystalline material and hence found unsuitable for single crystal analysis.

X-ray Powder Diffraction (XRPD): The XRPD were recorded at room temperature using the Panlytical diffractometer with Cu Kα radiation (λ = 1.5418 Å), running at 45Kv and 40Ma. The 2-theta range covered from 4.00 to 50.00 degrees with a step size of 0.02 degrees and a count time of 1.00 second per each step.

IR Spectroscopy: FT-IR spectra were recorded on Schimadzu. The solids were ground with KBr and discs were prepared under compressed pressure. The spectra were collected on the fused discs in the spectral range, 400-4000 cm^{-1}, by averaging 32 scans with a resolution of 4 cm^{-1} with DTGS detector.

Results and Discussion

FS is insoluble in acetone. A dilute solution of FS in acetone and water is made to get the single crystals of monohydrate. Water is added at reflux temperature to the suspension of FS in acetone to get clear solution and cooled to 25°C to isolate the solid. 1g. FS was dissolved in acetone: water 60: 12 w/v ratio of FS and the solution was kept undisturbed. Light flaky crystals separate out. These crystals being polycrystalline are not suitable for single crystal analysis. The XRPD of these crystals was found to be different from the monohydrate, which was obtained by the process disclosed in US patent 5, 616, 603**.** The water content of these crystals was 8 %, corresponding to dihydrate of FS.

Effect of solvent: Since now we know that FS exists in two hydrated states, polymorph screening of FS was carried out using different solvent and solvent combinations. FS crystallized using methanol, ethanol, isopropyl alcohol (IPA), acetonitrile gave monohydrate. When anti solvent like di iso propyl ether, hexane were used to precipitate FS from its solution in methanol, ethanol and IPA, monohydrate is isolated. A dihydrate of FS is obtained when acetone and water solvent combination used for crystallization. Dihydrate of FS was obtained when more volume of water was added to acetone. When volume of water is reduced to increase the yield, it resulted in monohydrate.

Effect of concentration of water: It is very well known that same solvent can give multiple forms depending on the concentration, ratio of solvent and their combination as well as mode of addition[25]. In case of FS, when crystallization is carried out using acetone and temperature is maintained at 25°C, volume of water becomes critical factor for isolation of monohydrate or dihydrate. Thus it is necessary to establish the volume of water required for production of both the forms separately.

To study effect of concentration of water, 8 volume of acetone w.r.t. weight of FS was kept constant and volume of water was changed. The experiments were planned and results are summarized in table 1.

When acetone and water are used in the ratio 8:3.5 w/v w.r.t. weight of FS, monohydrate is obtained, whereas when the ratio has changes to 8:5.0 w/v w.r.t. weight of FS dihydrate is obtained.

For a given weight of FS, as the volume of acetone is kept constant and that of water is increased, the effective % of acetone is decreases and that of water is increases accordingly. Increased concentration of water, facilitate the formation of higher hydrate i.e. dihydrate over monohydrate. Up to 30% concentration of water generates pure monohydrate; as the % of water increases to 33% major monohydrate is obtained with some contamination of dihydrate, which is reversed, with further increase of water % to 37.5. Pure dihydrate is obtained from 38% and above concentration of water. The study confirms that concentration of water is critical in isolation of monohydrate or dihydrate FS.

However when, FS crystallized from pure water it produces monohydrate instead of dihydrate. Dihydrate is obtained only when acetone and water is used for crystallization hence volume of water in acetone and this solvent combination is deciding the preference for generation of the hydrate of FS.

Effect of temperature: To study the effect of temperature, all the experiments listed in table 1 are carried out at 10°C and 0°C. At both the temperature monohydrate is obtained even at higher volume of water. The study showed that the formation of monohydrate is dependent on the temperature of crystallization.

Solvent mediated transformation: When monohydrate and dihydrate of FS are refluxed in toluene, they transform into an anhydrous form I and form II respectively. In toluene at reflux, both the hydrates undergo solvent mediated polymorphic transformation. At reflux toluene removes the water molecule from the crystal of hydrated forms and converts it into an anhydrous form. Such polymorphic phase transformations are discussed in the literature, eletriptan HBr monohydrate undergo solvent mediated transformation to give anhydrous beta form[26-27].

Solid-state transformation: Figure-1 and figure-2 shows DSC and TGA of FS hydrates and anhydrous forms respectively. DSC of FS dihydrate show two endothermic events. The first event, at 136°C corresponds to a weight loss of 8% and transforms the dihydrate to the anhydrate of FS. The endotherm at 241°C is accompanied by melting of FS, Form II. Giron et. al noted that upon dehydration, the crystal packing may retain more or less its original organization. However, it may also rearrange to a lower hydrate or collapse to an amorphous form[28]. Albers et al observed similar behaviour: hydrates initially formed the anhydrous state followed by a phase transformation at higher temperature[29].

Dihydrate FS on heating at 140°C loses water molecule and gets converted into anhydrous form. The loss of 8% water produces anhydrous form designated as form II is also characterized by XRPD**.**

This shows that dihydrate FS undergoes solid-state polymorphic transformations with loss of water molecule producing form II. Monohydrate form I does not show such solid-state transformation. Figure-3 shows XRPD of hydrates and anhydrous forms of FS.

Table-1
Effect of concentration of water on isolation of FS hydrate

Wt of FS (g)	Volume of acetone (mL)	% of acetone	Volume of water (mL)	% of water	Form
1	8	69.6	3.5	30.4	monohydrate
1	8	66.7	4	33.3	monohydrate (major)+ dihydrate
1	8	64	4.5	36	monohydrate + dihydrate (major)
1	8	61.57	5	38.5	dihydrate
1	8	59.3	5.5	40.7	dihydrate

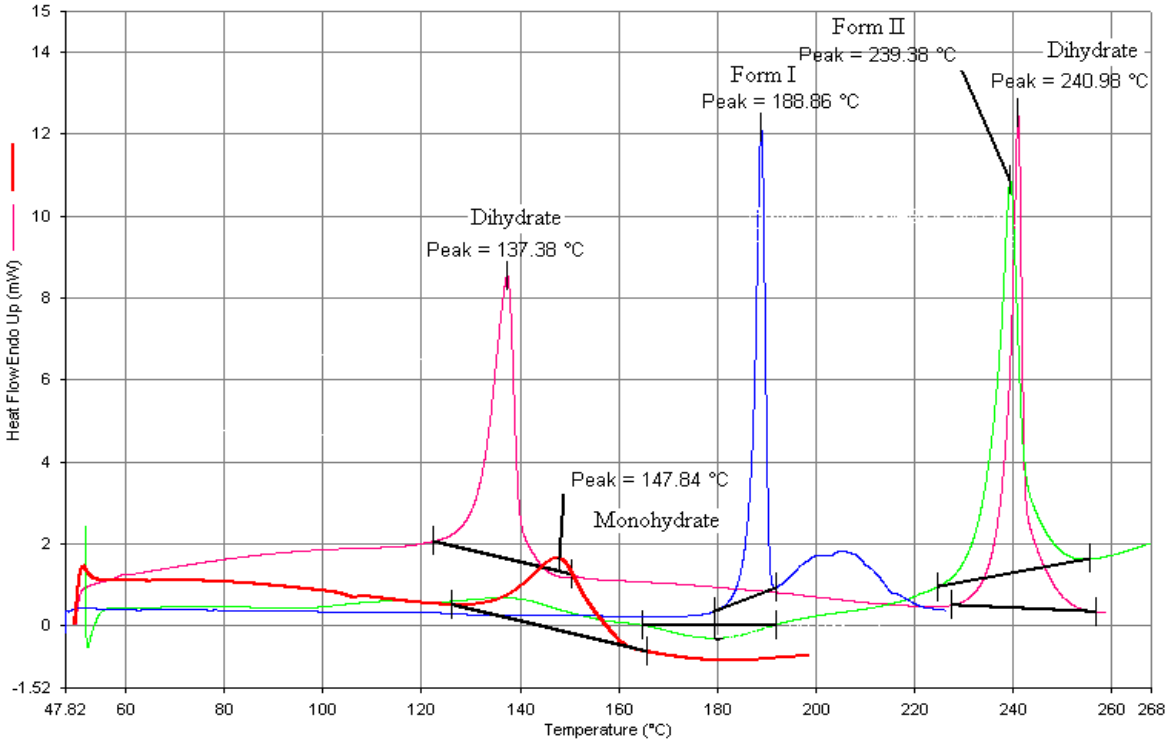


Figure-1
DSC of Frovatriptan Succinate hydrate and polymorphs

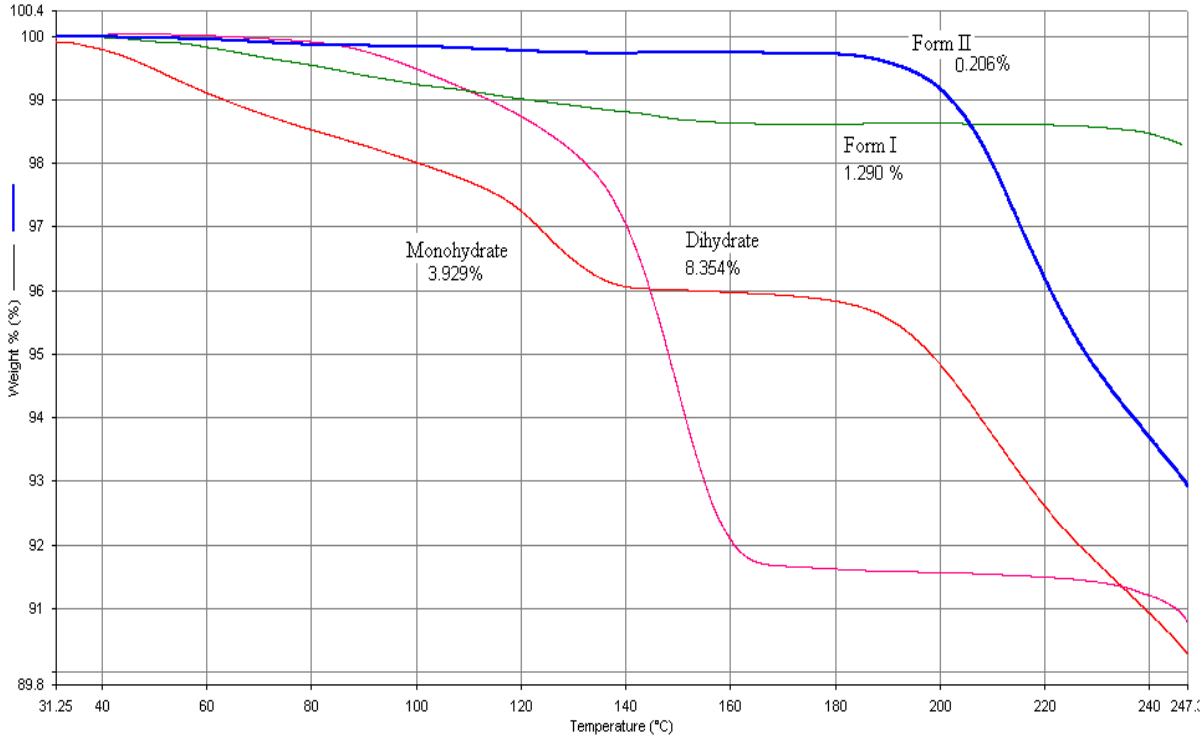


Figure-2
TGA of Frovatriptan Succinate hydrate and polymorphs

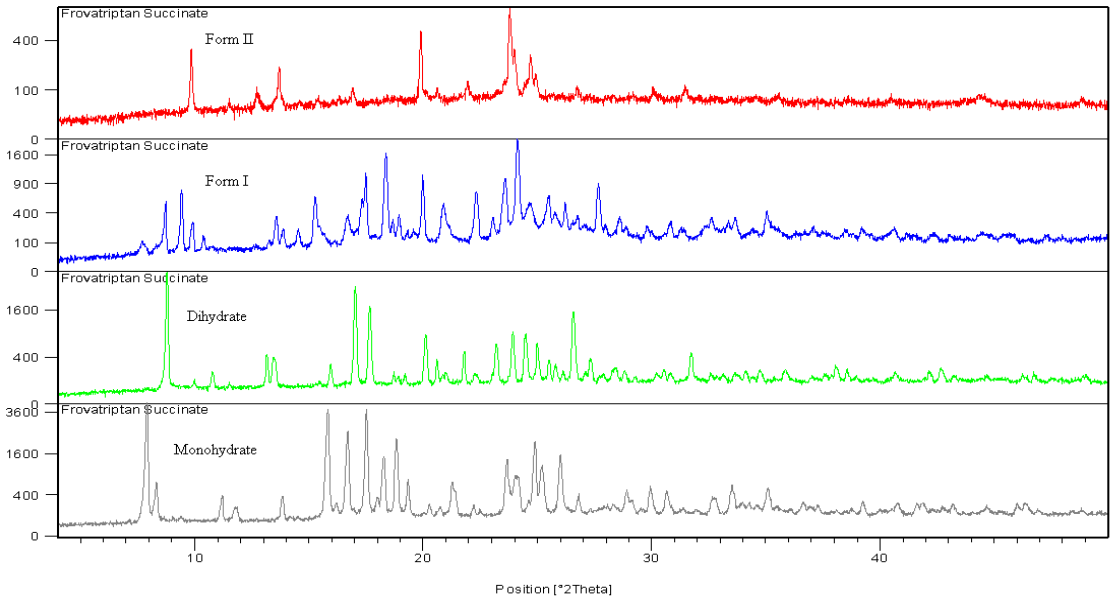


Figure-3
XRPD of Frovatriptan Succinate hydrate and polymorphs

Stability with respect to humidity: Both monohydrate and dihydrate forms are found to be stable at 30°C and 65% humidity for 12 months. Form I and form II, when exposed to relative humidity of 65%, converts back to monohydrate and dihydrate respectively.

The anhydrous form I and form II of FS are unstable and absorb moisture to regain their stable hydrated crystal structure. The conversion of form I is more rapid and completes in 24 Hrs whereas that of form II takes more than 3-5 days for complete conversion at 30°C and 65% relative humidity. The reversible conversion of anhydrous form I and form II to corresponding hydrates is shown in figure-4.

Thus in case of FS both the hydrated forms are stable and amongst the hydrates, crystal structure of dihydrate is more stable.

The inter conversion amongst the polymorphic forms of FS can be represented in figure-5.

Optical microscopy: Microscopic images of all the four forms show birefringence and are shown in figure- 6. The crystals of monohydrate are needle shape, where as that of dihydrate are elongated hexagon or bladed. Dihydrate crystals on drying maintain their habit but loses the transparency[30]. Crystals of form I are blocks.

Thermal Analysis: All crystal forms of FS are clearly distinguished by DSC and TGA as shown in figure-1 and 2. DSC and TGA data are recorded by following identical heating rates are given in table- 2.

TGA of monohydrate and dihydrate shows weight loss of 4% and 8% respectively, which clearly establishes their monohydrate and dihydrate nature.

Dihydrate shows two endothermic events, first event corresponds to loss of water molecule and the second corresponds to melting of the form. Monohydrate, Form I and form II shows single endotherm corresponding to melting. No phase transitions for monohydrate, I and II is observed and form simply melted at different temperature.

X-ray Powder Diffraction Pattern: XRPD pattern of all the forms are recorded and it shows clear distinction amongst all the forms. The comparison of 2θ values are given in table-3. Figure-3 shows crystalline nature of monohydrate, dihydrate, Form I and II of FS.

Fourier Transform Infrared Spectroscopy: Thermal analysis and XRPD study confirms that FS exist in two anhydrous forms along with, monohydrate and dihydrate state. When a compound exists in such multiple forms infrared spectroscopy is extremely valuable. For hydrates, observations within the region 3100-3600 cm^{-1} are most fruitful for identification purpose 1. Figure-7 is FT-IR spectra of all the forms of FS and are different from each other.

FS is having primary amide group and two secondary amino groups. Two N-H stretching bands resulting from symmetrical and asymmetrical stretching in 3400-3520 cm^{-1} corresponds to primary amide group. Monohydrate shows strong absorption at 3183 cm^{-1} and 3398 cm^{-1} where as Form I shows absorption at 3189 cm^{-1}.

The sharp peak at 3398 cm^{-1} in monohydrate is weak at 3395 in dihydrate, which indicates that this peak is due to hydrated water in the crystal of monohydrate which absent in form I. Dihydrate also shows sharp peak at 3422 cm^{-1}.

Table-2
DSC and TGA of FS hydrate and polymorphs

Form	Onset Temp (°C)	Peak Temp (°C)	Heat of fusion (j/g)	Weight loss (%)
Monohydrate	135.74	149.26	77.04	4
Dihydrate	133.25	136.63	136.67	8
	239.19	240.50	148.87	--
Form I	185.51	187.87	91	0.2
Form II	235.36	239.38	179	1.2

Table-3
XRPD values of FS hydrate and polymorphs

Form I	Form II	Form III	Form IV
Pos. [2θ]	**Pos. [2θ]**	**Pos. [2θ]**	**Pos. [2θ]**
7.93	8.74	7.74	9.85
8.34	10.75	8.74	12.75
11.21	13.13	9.46	13.7
11.80	13.40	9.91	19.88
13.87	13.53	10.39	21.93
15.86	15.92	13.58	23.8
16.20	17.01	13.88	24.69
16.71	17.61	14.54	24.94
17.54	18.68	15.30	30.17
18.00	20.06	16.69	31.48
18.30	20.11	17.31	44.43
18.87	20.56	17.49	
19.35	21.73	18.38	
20.28	22.22	18.67	
20.72	23.20	18.95	
21.29	23.92	19.99	
21.43	24.47	20.87	
22.21	24.98	22.34	
23.69	25.48	23.06	
24.02	25.75	23.60	
24.17	26.07	24.13	
24.90	26.51	24.72	
25.21	26.61	25.49	
26.00	27.29	25.78	
26.80	28.24	26.22	
28.24		26.75	

The C=O bond have strong absorption at 1650 cm^{-1}. In hydrated forms, water molecule forms a stronger hydrogen bond, which lead to shift of carbonyl group to a lower wavenumber [31]. Form I has absorption at 1724 cm^{-1}, which shifts to 1707 cm^{-1} in monohydrate and shifts further lower wavenumber 1656 cm^{-1} in dihydrate and again shifts to 1660 cm^{-1} after losing water of hydration in form II.

Solubility: The solubility of all the crystal modifications were studied in water at 25°C. Generally aqueous solubility of anhydrous form is higher than the hydrate[15,32]. Monohydrate of lisinopril possesses far greater solubility than dihydrate[33]. The hydrates of FS dissolve freely in water whereas anhydrous forms remains suspended and then dissolve. Dihydrate has highest solubility compare to monohydrate, form I and form II. The solubility of dihydrate form is nearly 3 times higher than the monohydrate form. The solubility data is tabulated in table-4.

Table-4
Solubility of FS hydrate and polymorphs

Form	Monohy-drate	Dihydrate	Form I	Form II
Solubility (mg/mL)	78.5	208	48.5	62.5

The order of solubility is dihydrate > monohydrate > form II > Form I.

The lower solubility of monohydrate is responsible for more stability. Due to higher stability of monohydrate, during crystallization at lower temperature, more stable monohydrate crystallizing out predominantly over dihydrate. On adding water both anhydrous forms do not dissolve immediately but remain suspended for 30-45 seconds and then start dissolving. It is now known that the anhydrous forms of FS convert to their corresponding hydrates, on exposure to humidity. It is likely that, in water anhydrous forms of FS convert to their respective hydrates and then undergo dissolution. The solubility of form II is high compare to form I. The probable reason for this could be the conversion of form II to highly soluble dihydrate.

Conclusion

Frovatriptan succinate is isolated in four crystalline forms of which two are hydrate and two are anhydrous. The hydrates are monohydrate and dihydrate. Both hydrated forms are obtained by same solvent combination. Concentration of water in acetone and temperature of crystallization are the driving factors for isolation of monohydrate and dihydrate of FS. Anhydrous form I and II are obtained by solvent mediated polymorphic technique, form II is also obtained by solid-solid transition technique. Different hydrate produces different anhydrous form. Anhydrous form I and form II are unstable and shows reversible transition to corresponding hydrate. The conversion of form I is more rapid than form II. Hydrated forms have more aqueous solubility than anhydrous form. The order of aqueous solubility is dihydrate > monohydrate > form II > Form I.

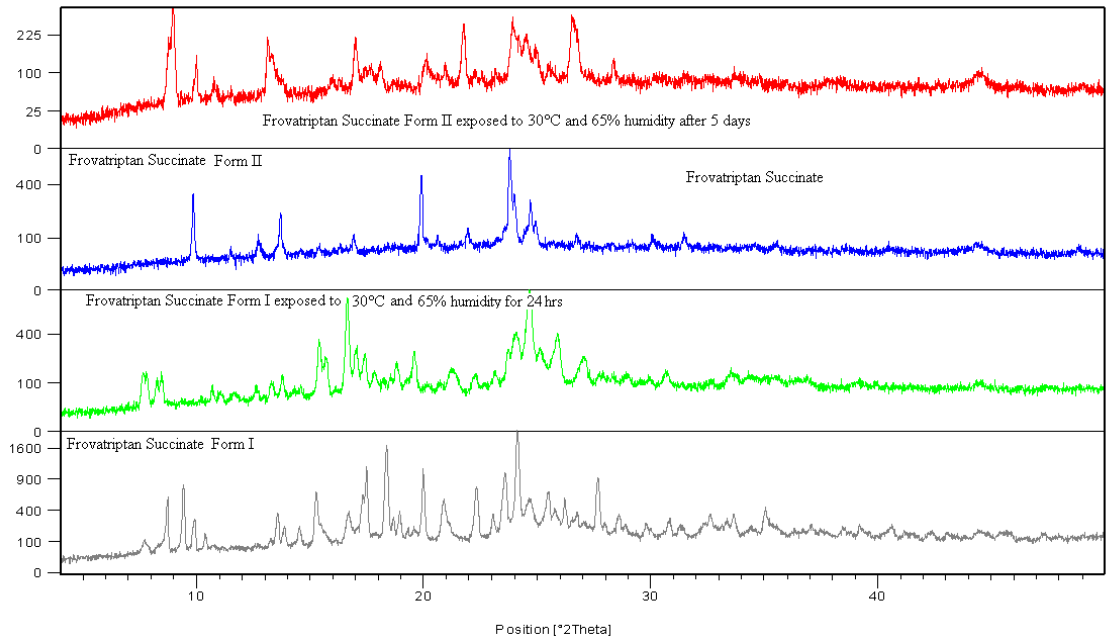


Figure-4
Effect of humidity on Frovatriptan Succinate polymorphs

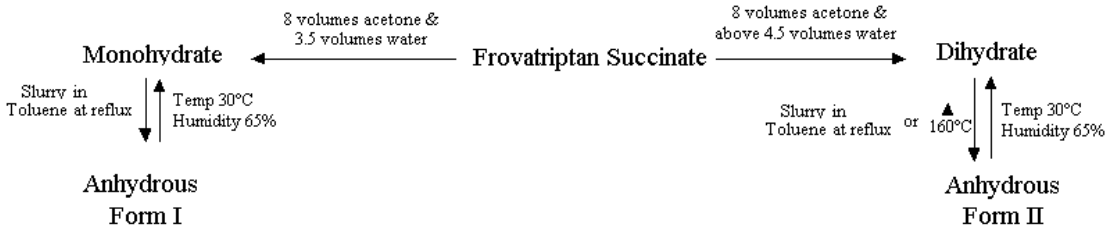


Figure-5
Schematic representation of Frovatriptan Succinate polymorphs and hydrates and their inter conversion

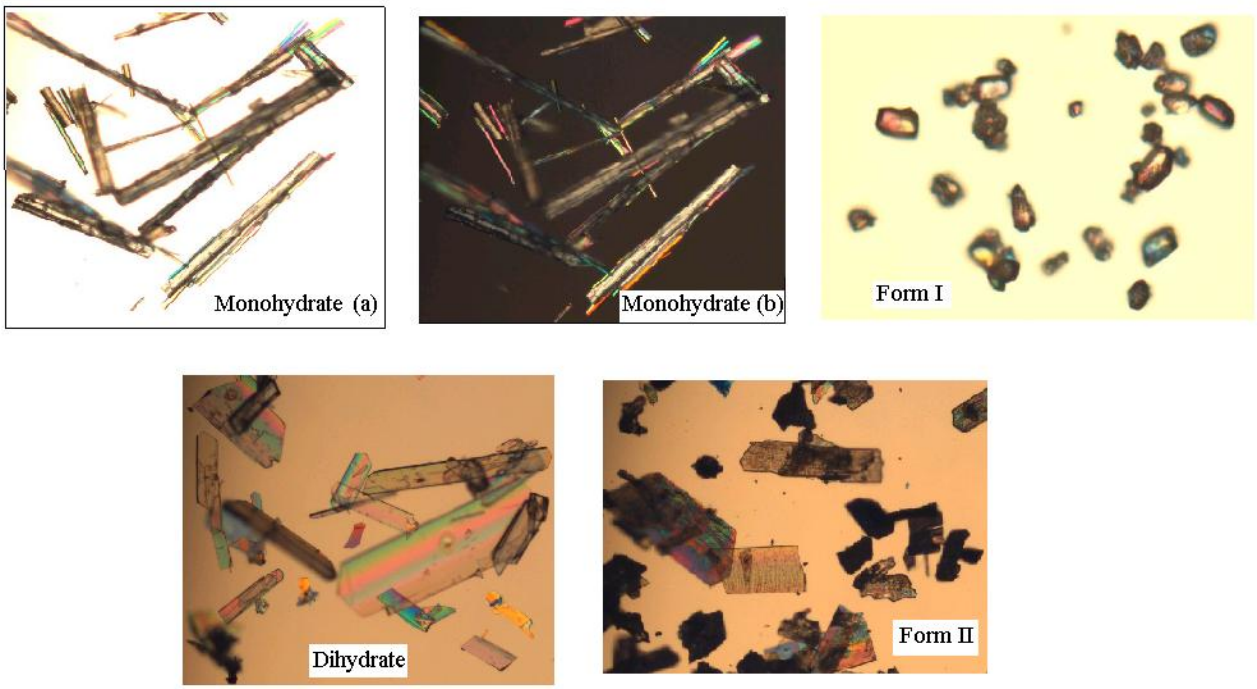


Figure-6
Microscopic images of Frovatriptan Succinate polymorphs and hydrates

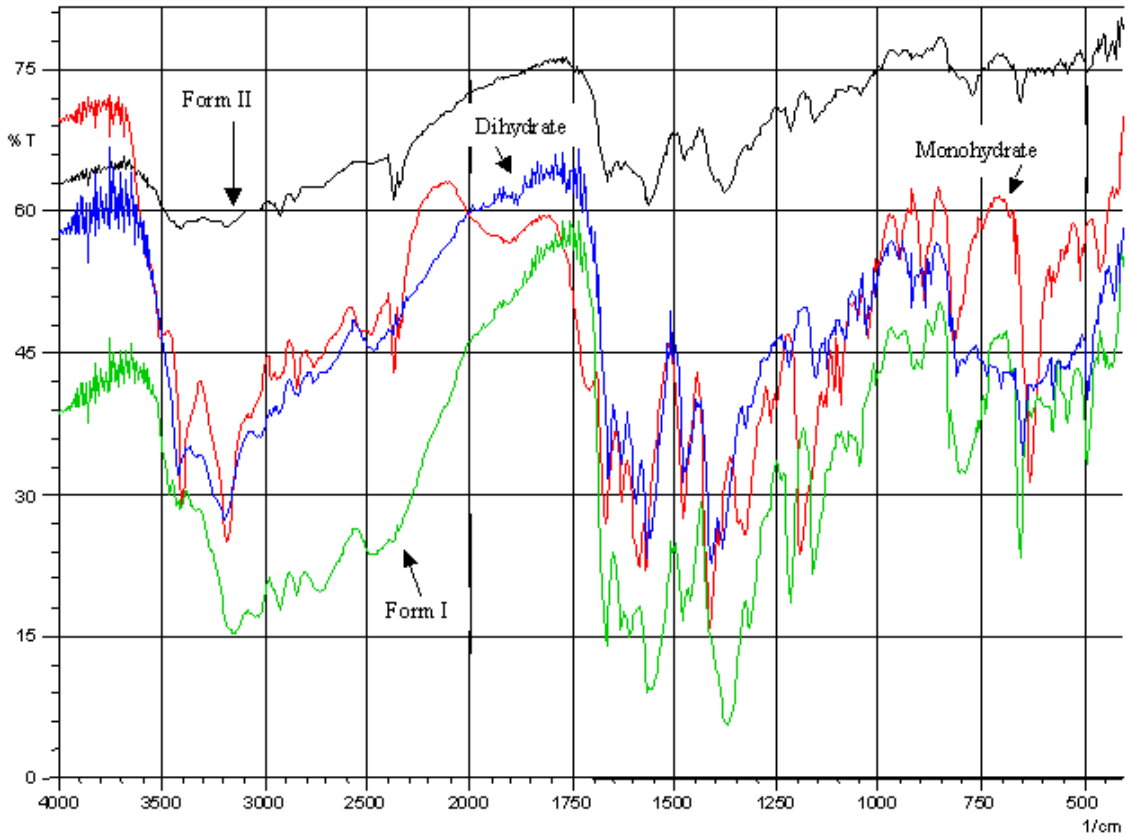


Figure-7
FT-IR of Frovatriptan Succinate polymorphs and hydrates

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