Scientific paper

A Novel Crystal Form of Cabergoline, Form L

Zoran Ham,[1] Peter Svete,[1] Uroš Urleb[1] and Darko Kocjan[2,*]

[1] Lek Pharmaceuticals, Sandoz Development Centre Slovenia, Verovškova 57, 1526 Ljubljana, Slovenia

[2] EN-FIST Centre of Excellence, Dunajska 156; National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

* Corresponding author: E-mail: darko.kocjan@ki.si

Received: 03-05-2011

Dedicated to Professor Dušan Hadži on the occasion of his 90th birthday

Abstract

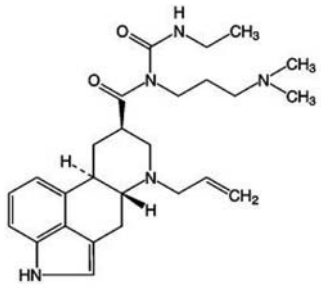
A new crystal form of cabergoline, form L, was discovered and characterized by thermal analyses, X-ray powder diffraction (XRPD), infrared spectroscopy (IR), Raman spectroscopy, dynamic vapor sorption (DVS), and comparative dissolution behavior. The morphology of rod-shaped crystals was investigated by scanning electron microscopy (SEM). Form L was compared to other non-solvated forms of cabergoline such as form I, II, VII and to the amorphous form. The thermodynamic stability of form L is supported by its long-term stability. Form L is a pharmaceutically applicable crystal form.

Keywords: Cabergoline polymorphism, X-ray powder diffraction, IR and Raman spectroscopy, thermal analysis and stability

1. Introduction

Cabergoline is the generic name of the compound 1((6-allylergolin-8β-yl)-carbonyl)-1-(3-dimethylaminopropyl)-3-ethylurea (Scheme), which belongs to the field of pharmaceutical agents for the treatment of hyperprolactinemia, prolactinoma, Parkinson's disease, and restless legs syndrome, as well as for the treatment of diseases like progressive supranuclear palsy and multisystematic atrophy.

The synthesis of the compound was first described in the 1980's.[1] The first crystal form of cabergoline was prepared as transparent plates from diethylether,[2] which became designated as crystal form I.



Scheme: Structural formula of cabergoline

It was later found that cabergoline exists in several different crystalline forms. Sheikh et al. reported on the preparation of a toluene solvate and the process of its conversion into the crystal form I.[3] Tomasi et al. described a crystallization in methyl tert-butyl ether for the preparation of crystal form II,[4] Candiani et al. described a crystallization in 1,4-dioxane for the preparation of crystal form VII.[5] The high degree of conformational freedom of cabergoline facilitates the existence of several different crystalline forms.[6,7] Form I, form II and form VII of cabergoline are unsolvated and are acceptable crystalline forms for the final dosage forms as they do not contain pharmaceutically unacceptable solvents.

Polymorphs with different crystalline characteristics have become the subject of extensive solid-state studies over the past few years, primarily due to their potential impact on the development of active pharmaceutical ingredients and the final dosage form.[8] In order to assure the right bioavailability and stability of active pharmaceutical ingredient in the final dosage form and to control the manufacturing process, both polymorphism and pseudo-polymorphism should be investigated during the preformulation phase of the development.[9]

We discovered a new crystal form of cabergoline, form L, which is thermodynamically stable, shows long-term stability and is applicable for using in final dosage forms. Form L was characterized by several analytical methods such as thermal analysis by DSC, IR and Raman spectroscopy, X-ray powder diffraction, SEM (Scanning Electron Microscopy), DVS (Dynamic Vapor Sorption), and solubility/dissolution measurements. Active pharmaceutical ingredients should be stable during technological processes for the preparation of a final dosage form and must also remain stable during storage.

2. Materials and Methods

2. 1. Materials

Cabergoline as a starting material for crystallization studies is a semi-solid, pitchy, oily or amorphous material. It was prepared by chemical synthesis and purified using chromatographic methods. [1]

Form I was prepared according to our novel procedure described in WO/2008/049884.[11] It starts with the crystallization of the chlorotoluene solvate below –10 °C. The chlorotoluene solvate is stirred in n-heptane at –10 °C, filtered at the same temperature, and then dried. Form II was obtained by a crystallization in methyl tert-butyl ether,[4] form VII by a crystallization in 1,4-dioxane.[5] The powdered amorphous form was prepared by further evaporating and drying of pitchy cabergoline until a dry material was formed.

Form L was prepared according to our novel procedure described in the patent application WO/2008/092881.[12] It is a precipitation from a mixture of aromatic solvents, preferably halogenated aromatic solvents, and aliphatic hydrocarbons as anti-solvents. For example, the first crystallization of the chlorotoluene solvate occurs at temperatures below –10 °C, followed by the addition of n-heptane and a slow desolvation while heating the solution up to 25 °C, and final crystallization of form L in n-heptane at 25 °C.

2. 2. Methods

2. 2. 1. Thermal Analysis

DSC was performed on a Mettler DSC821e. The sample (4–6 mg) was placed in an unsealed aluminum crucible with a hole and heated in an N_2 atmosphere at a heating rate of 5 K/min in the temperature range from 30 °C to 200 °C. Indium was used to calibrate the instrument. Endothermic transitions (e.g. melting in Fig. 4) are oriented downwards.

2. 2. 2. FT-IR spectroscopy

The infrared spectra were recorded within the wave number range of 4000–400 cm^{-1} with a Thermo Nicolet Nexus FT-IR spectrometer at a resolution of 2 cm^{-1} and with 16 scans. Samples were prepared in KBr pellets.

2. 2. 3. Raman Spectroscopy

The Raman spectra were recorded on an FT Raman spectrometer Nicolet Nexus Raman Module at a resolution of 2 cm^{-1} with 64 scans in the spectral range 4000–400 cm^{-1}.

2. 2. 4. X-ray Powder Diffraction (XRPD)

The powder x-ray diffraction patterns were obtained using a Panalytical X'Pert PRO diffractometer with an X'Celerator detector (RTMS; Real Time Multiple Strip) using CuKα radiation (tube operating at 45 kV and 40 mA) in the Bragg-Brentano (reflection) geometry. Data were recorded from 2 to 40° 2θ in steps of 0.033° 2θ with a measurement time of 50 seconds per step. Variable divergence and antiscatter slits were used to maintain 10 mm of sample length irradiated. Major diffraction peaks were extracted from the diffraction patterns using X'Pert HighScore Plus 2.0 software.

2. 2. 5. Solubility and Dissolution Rate

The solubility was determined in aqueous medium at room temperature (1 min of shaking vigorously and 15 min without shaking, repeated twice). In addition, the following method was used to prevent CO_2 from dissolving in the solution. 35±1 mg samples (3 batches, 3 replicates) were added to 30 mL of degassed demineralized water. Samples were kept in a closed nitrogen atmosphere. Suspensions were mixed for 24 hours at 22.3 °C, filtered and diluted. The concentration of dissolved cabergoline was determined spectrophotometrically using a Tecan Safire2 instrument (UV_{max} = 280 nm).

The dissolution profile was determined in aqueous medium at room temperature. Suspension was stirred and sampled at 15, 30, 60 and 180 minutes, filtered and diluted. The concentration of dissolved cabergoline was determined as described above.

2. 2. 6. Scanning Electron Microscopy (SEM)

Electron images of crystals were obtained using a scanning electron microscope (Jeol JXA-840A) operating at 18 kV. The magnification was 1000x. The specimens were mounted on a metal stub (with double-sided adhesive tape) and coated with gold under vacuum prior to observation.

2. 2. 7. Dynamic Vapor Sorption (DVS)

Hygroscopicity of the samples was determined using a dynamic vapor sorption instrument (DVS 1, Surface

Measurement Systems Ltd.) at a temperature of 25 °C. 3 to 5 mg of the sample was added to the micro balance in the DVS instrument and the relative humidity (RH) was varied from 0 to 90% and back again. Two cycles of sorption and desorption were carried out. The mass change of the sample was recorded.

3. Results and Discussion

Form L was prepared according to our novel procedure, which is simple and efficient.[12] In the following we compare the new crystal form of cabergoline (L) with other unsolvated forms. Form L is a well-defined anhydrous crystal form showing long-term stability. It exhibits high thermodynamic stability and is the first known cabergoline form that appears in the shape of rods/needles.

It is evident from the X-ray diffraction of form L that its diffractogram does not resemble the diffraction patterns of form I, form II or form VII (Fig. 1). All samples are well crystallized, have a similar degree of crystallinity, and no distinctive preferred orientation. Characteristic diffraction peaks with d-values and relative intensities of the polymorphic forms are presented in Table 1.

FTIR and Raman spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for different solid-state forms of organic compounds.[13] Differences in absorption bands in FTIR (Fig. 2) and Raman spectra (Fig. 3) of cabergoline polymorphic forms confirm differences in the crystal structure. Most probably, they indicate diferences in the conformation of cabergoline, intermolecular hydrogen bonds, and different crystal packing. Single crystal X-ray analysis of polymorphic forms I[2], VII[6], II[7] revealed different crystal packing. FTIR spectrum of the amorphous form manifests broad absorption bands as expected, whereas the crystal forms show more distinctive absorption bands. The N-H stretching absorption area between 3300 and 3600 cm^{-1} have contribution from indole and amide N-H, severely broadened due to the intermolecular (indole N-H) and intramolecular (amide N-H) hydrogen bond interactions. It should be noted that only form L has a strong absorption band at 3376 cm^{-1}. This is most probably connected to the indole N-H bond, which is characteristic for indole derivatives.[14] CO stretching region between 1600 and 1700 cm^{-1} have contributions from the C8-substituted carbonyl and urea group.

Fig. 3 shows characteristic Raman bands of the amorphous form, form I, form II, form VII and form L for the region between 1200 cm^{-1} and 700 cm^{-1}. It is clearly evident that form L has a different Raman spectrum than other forms, with the greatest difference appearing in the region between 800 cm^{-1} and 700 cm^{-1}. Raman spectroscopy could be introduced as a routine method for identifying cabergoline form L.

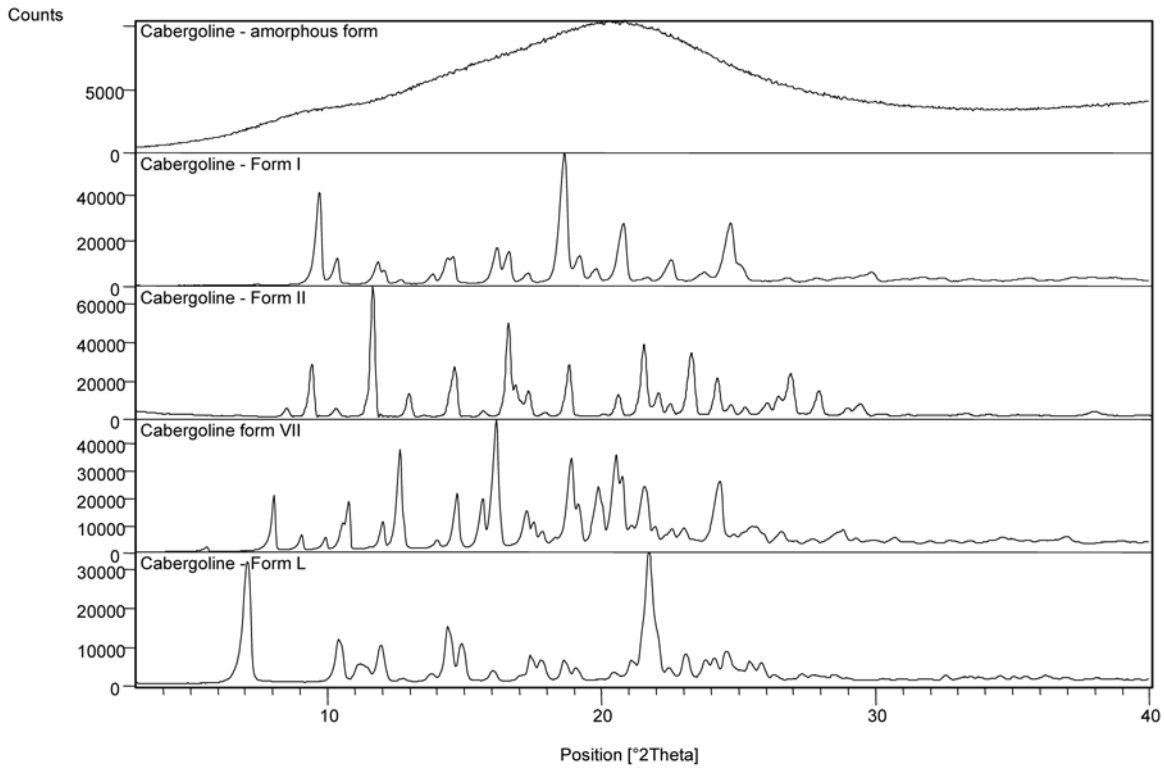


Fig. 1. X-Ray powder diffraction patterns of amorphous cabergoline, form I, form II, form VII, and form L in the 2θ range of 2°–40°.

Table 1. X-Ray powder diffraction data of cabergoline form I, form II, form VII, and form L

Form I			Form II		
d-values, Å	**2θ-values, °**	**Relative intensities, %**	**d-values, Å**	**2θ-values, °**	**Relative intensities, %**
9,09	9,725	74	10,37	8,522	7
8,53	10,365	21	9,36	9,444	40
7,47	11,841	18	8,58	10,304	7
7,31	12,101	11	7,58	11,668	100
6,97	12,693	4	6,81	12,993	18
6,38	13,873	8	6,14	14,418	17
6,17	14,347	20	6,03	14,682	37
6,06	14,609	21	5,65	15,676	5
5,46	16,225	29	5,34	16,592	74
5,32	16,655	25	5,25	16,879	25
5,11	17,345	8	5,11	17,345	21
4,75	18,670	100	4,71	18,830	40
4,61	19,243	21	4,31	20,596	18
4,47	19,851	11	4,12	21,557	57
4,26	20,841	45	4,02	22,100	19
4,09	21,717	4	3,95	22,497	11
3,93	22,613	18	3,81	23,335	48
3,74	23,778	8	3,67	24,238	31
3,60	24,717	46	3,60	24,717	11
3,54	25,143	12	3,53	25,215	9
3,31	26,922	3	3,42	26,040	13
3,20	27,865	3	3,37	26,433	17
3,10	28,783	3	3,31	26,922	35
2,99	29,866	7	3,19	27,954	21
			3,08	28,974	9
			3,03	29,463	11

Form I			Form II		
d-values, Å	**2θ-values, °**	**Relative intensities, %**	**d-values, Å**	**2θ-values, °**	**Relative intensities, %**
15,78	5,597	4	12,33	7,165	100
10,98	8,048	44	8,40	10,526	33
9,74	9,074	12	7,86	11,251	14
8,90	9,933	10	7,70	11,486	14
8,40	10,526	19	7,36	12,018	26
8,21	10,770	37	6,91	12,804	2
7,35	12,035	22	6,39	13,851	6
7,00	12,639	78	6,10	14,513	41
6,31	14,028	7	5,92	14,957	29
6,01	14,732	43	5,50	16,106	8
5,65	15,676	39	5,06	17,517	20
5,48	16,165	100	4,96	17,873	16
5,13	17,276	29	4,73	18,750	15
5,05	17,552	20	4,63	19,159	9
4,96	17,873	13	4,32	20,548	6
4,69	18,911	70	4,19	21,193	27
4,62	19,201	32	4,11	21,610	88
4,46	19,896	47	4,08	21,771	98
4,32	20,548	72	4,03	22,045	55
4,27	20,791	54	3,94	22,555	9
4,10	21,664	41	3,85	23,089	18
4,04	21,989	15	3,73	23,843	17
3,93	22,613	14	3,62	24,578	23
3,86	23,028	14	3,44	25,886	11
3,65	24,373	48	3,39	26,275	4
3,48	25,583	14	3,27	27,257	5
3,35	26,594	10	3,13	28,502	5
3,21	27,777	3			
3,09	28,878	11			

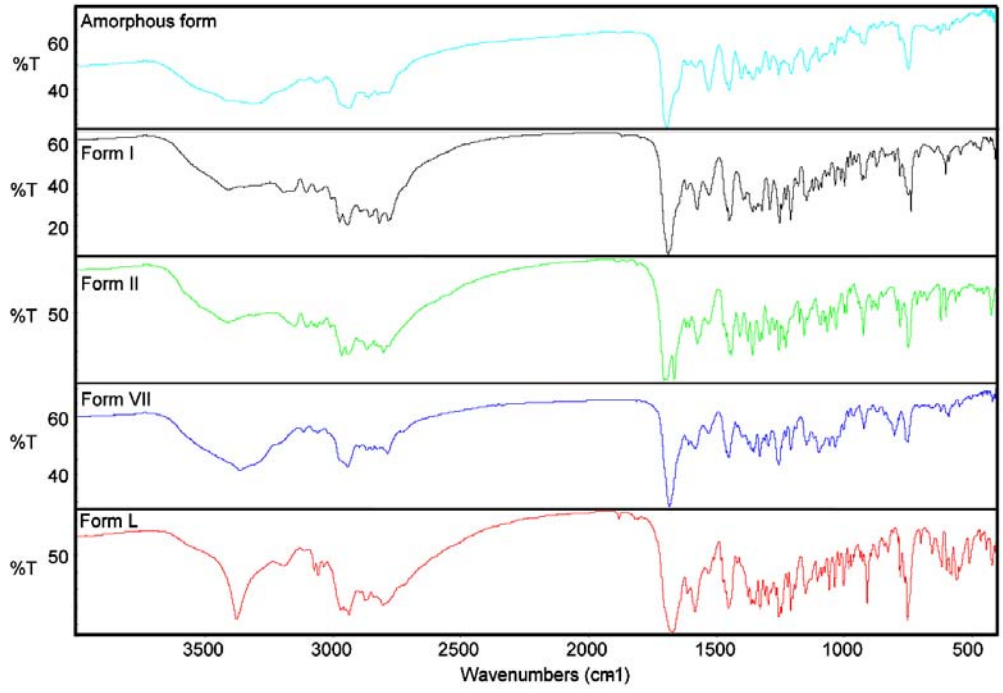


Fig. 2. IR spectra of form I, form II, form VII, amorphous form, and form L of cabergoline in the 4000–400 cm^{-1} region.

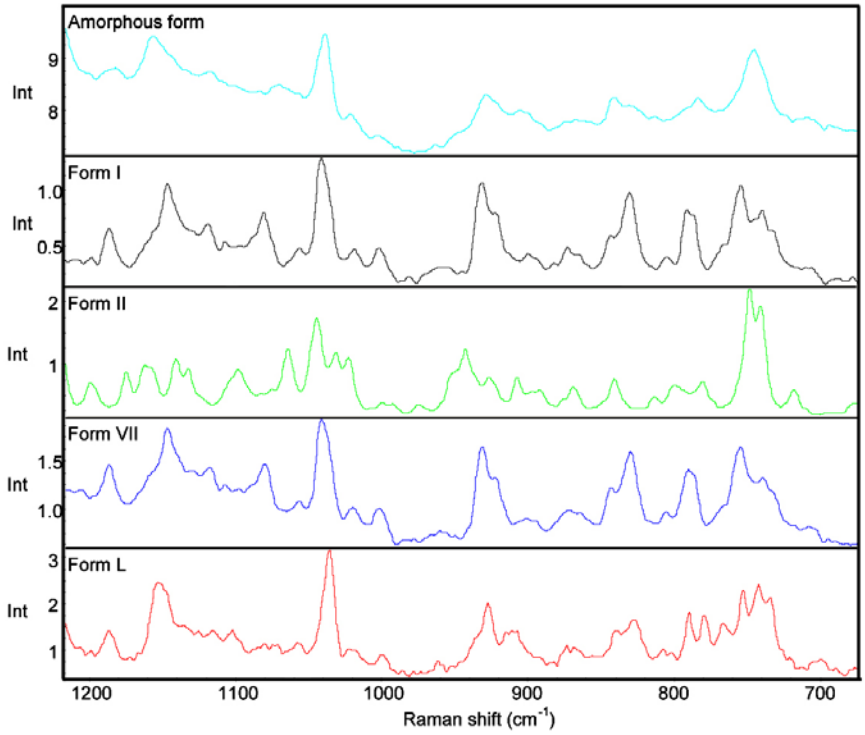


Fig. 3. Raman spectra of the amorphous form, form I, form II, form VII, and form L of cabergoline in the spectral range from 680–1220 cm^{-1}.

Fig. 4 shows DSC thermograms of the crystal forms and amorphous form of cabergoline. There are no endothermic peaks at temperatures below the melting points. It can be easily concluded from the DSC analysis that the crystal forms of cabergoline are unsolvated forms. The melting points and the melting enthalpies were obtained from the DSC thermograms and are presented in Table 2. Some variations and broadening of the melting range is

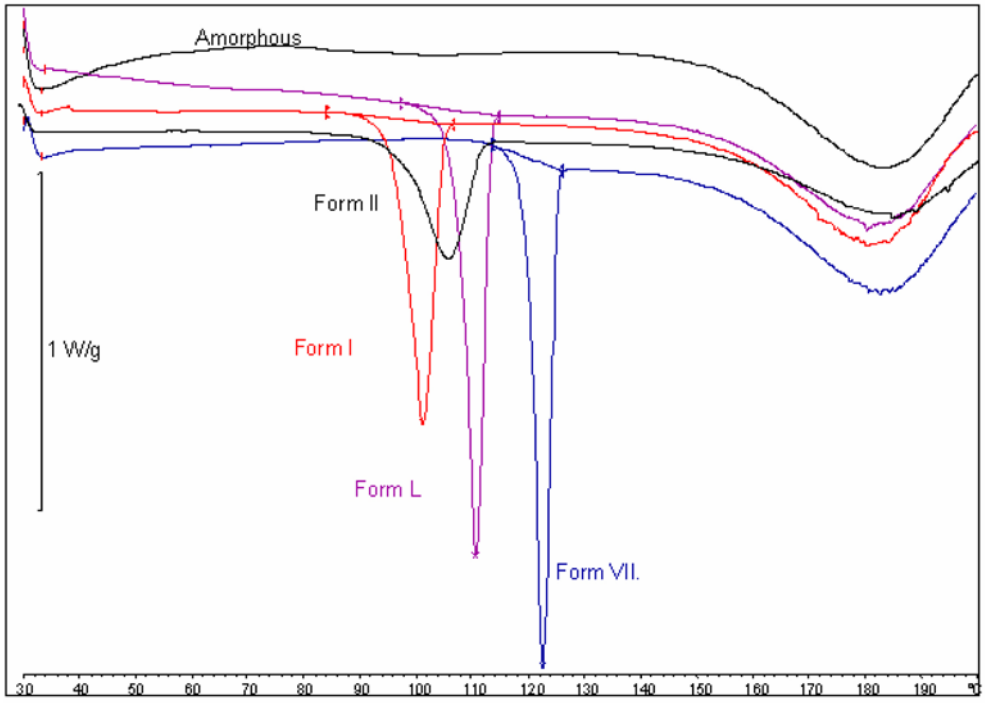


Fig. 4. DSC thermograms of amorphous form, form I, form II, form VII, and form L of cabergoline

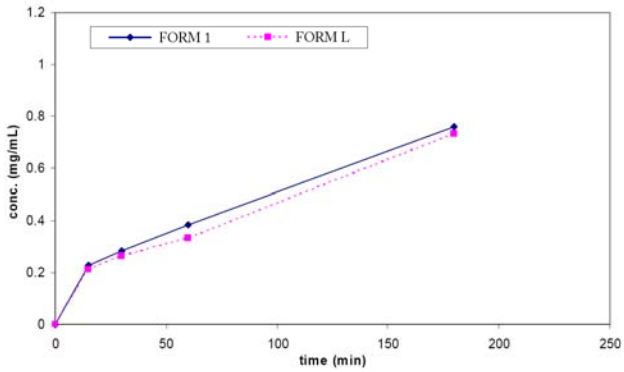


Fig. 5. Dissolution profile of form I and form L.

caused by differences in sample purity, particle size distribution, and the presence of residual solvents. Form VII has the highest melting point and form I the lowest. Forms II, VII and L exhibit the same melting enthalpy . Form L is thermodynamically the second most stable form of cabergoline. The melting enthalpy of form I is slightly lower (cca. 4 J/g) due to the lower crystallinity. A wide endothermic peak at the temperature above the melting point shows the decomposition of cabergoline. The glass transition of the amorphous form was not detected under the measuring conditions, most probably due to the low heating rate.

Table 2. Melting points and enthalpies of the crystal forms of cabergoline

Crystal form	Onset melting point (°C)	Peak (°C)	Melting enthalpy (J/g)
Form I	95.9	101.0	–58.1
Form II	98.0	105.6	–62.0
Form VII	120.0	122.3	–62.5
Form L	107.5	110.4	–62.6

The solubility of cabergoline form L was determined by the dissolution method. Cabergoline has very low solubility in water (0.078 mg/ml) due to the hydrophobic nature. Majority of ergoline drugs are prepared as salts in order to enhance the solubility in water. An additional experiment was done to confirm the saturated solubility of cabergoline with the aim of preventing CO_2 from dissolving in the solution. The saturated concentration of cabergoline was determined to be 1.06 mg/ml at pH 8.9. Form L is slightly less soluble than form I which correlates with their melting points and melting enthalpies. Form L is thermodynamically more stable than form I.

Cabergoline has slow dissolution kinetics. The dissolution profiles of form I and form L are presented in Fig. 5. It is evident that there are no significant differences in the solubility and dissolution rates between form I and form L.

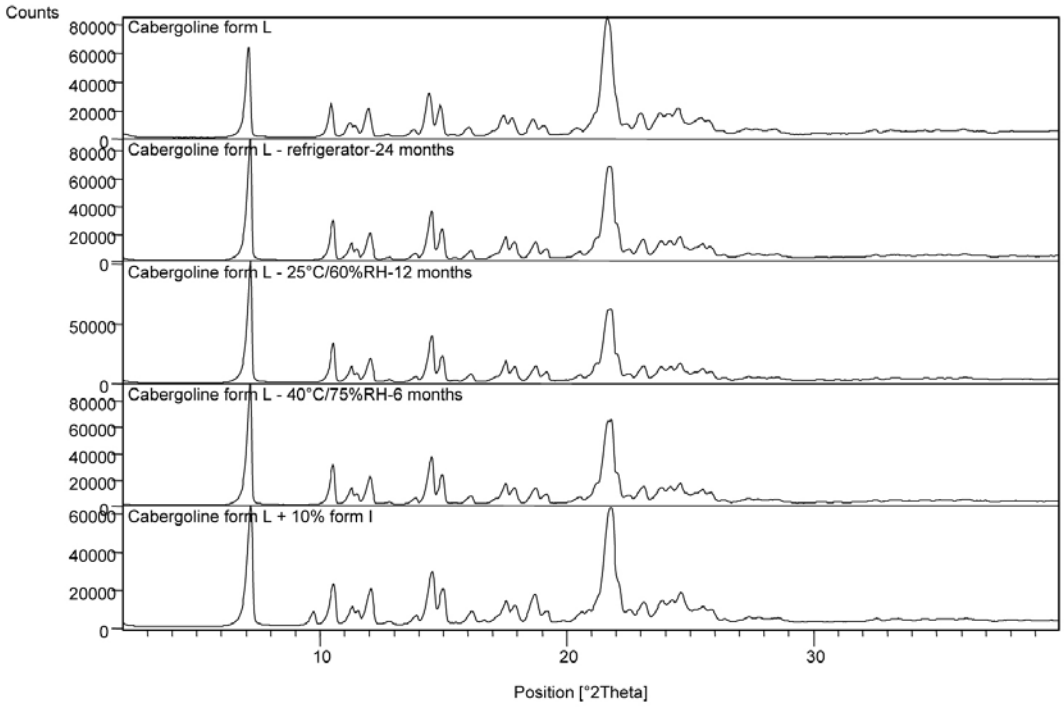


Fig. 6. X-ray diffractograms of cabergoline form L in stability testing, from top to bottom: initial sample, the sample after 24 months in refrigerator, the sample after 12 months at 25 °C and 60% relative humidity, the sample after 6 months at 40 °C and 75% relative humidity, and the mixture of form L and form I (10%).

* (We noted that when the crystallization procedure of form L was not done correctly a mixture of form L and form I appeared. The X-ray diffractogram was similar to that presented at the bottom in Fig. 6.)

The stability of form L was evaluated under long-term and accelerated stability testing conditions using XRPD analysis. The results are summarized in Fig. 6. A mixture of form I and form L was included for a comparison (10% of form I determined by the external standard method)*. Characteristic diffraction peaks of form I at 9.7°, 16.6°, 19.8°, 20.8° and 29.9° 2θ are observed in the lower diffractogram. The most intense form I peak (d = 4.75; 2θ = 18.68) is also apparent in the mixture diffractogram as an enhanced peak relative to the pure form L pattern. No polymorphic conversion was observed after 24 months of storage in the refrigerator (2–8 °C), after 12 months at 25 °C and 60% relative humidity, and after 6 months at an elevated temperature of 40 °C and 75% relative humidity. There are also no significant changes in the size of crystallites, the preferred orientation or the particle size.

The morphology of particles plays an important role in the process technology of the final dosage form. We compared morphological characteristics of form I, form L, and the amorphous form of cabergoline by means of scanning electron microscopy (SEM). The acquired photographs are presented in Fig. 7. Semicrystallized form I is also included for a comparison. It is clearly evident from the photographs that form L crystallizes in the shape of fine needles or as rod shaped crystals, whereas form I crystallizes as hard cloddy aggregates bound with some amorphous material. We found out that form I often crystallizes as a semicrystalline material (Fig. 7, bottom right). Form VII crystallizes in the shape of prisms.[6] However, cabergoline tends to form amorphous material when impurities are present (Fig. 7, bottom left). The SEM results showed that the size of the crystals of form I is comparable to form L. The average shorter axis of the needles is below 10 μm. Milling of cabergoline form L by a hammer mill gives the particle size distribution d (0,9) below 10 μm. The particle size distribution was measured by the Malvern method. Powdered and not agglomerated cabergoline is very suitable for the preparation of the final dosage forms because it can be homogeneously incorporated into excipients**,** which guarantees homogeneity and a repeatable dissolution profile.

The hygroscopicity of substances is very important for designing a final dosage form. A high water content can lead to a faster and more extensive decomposition of

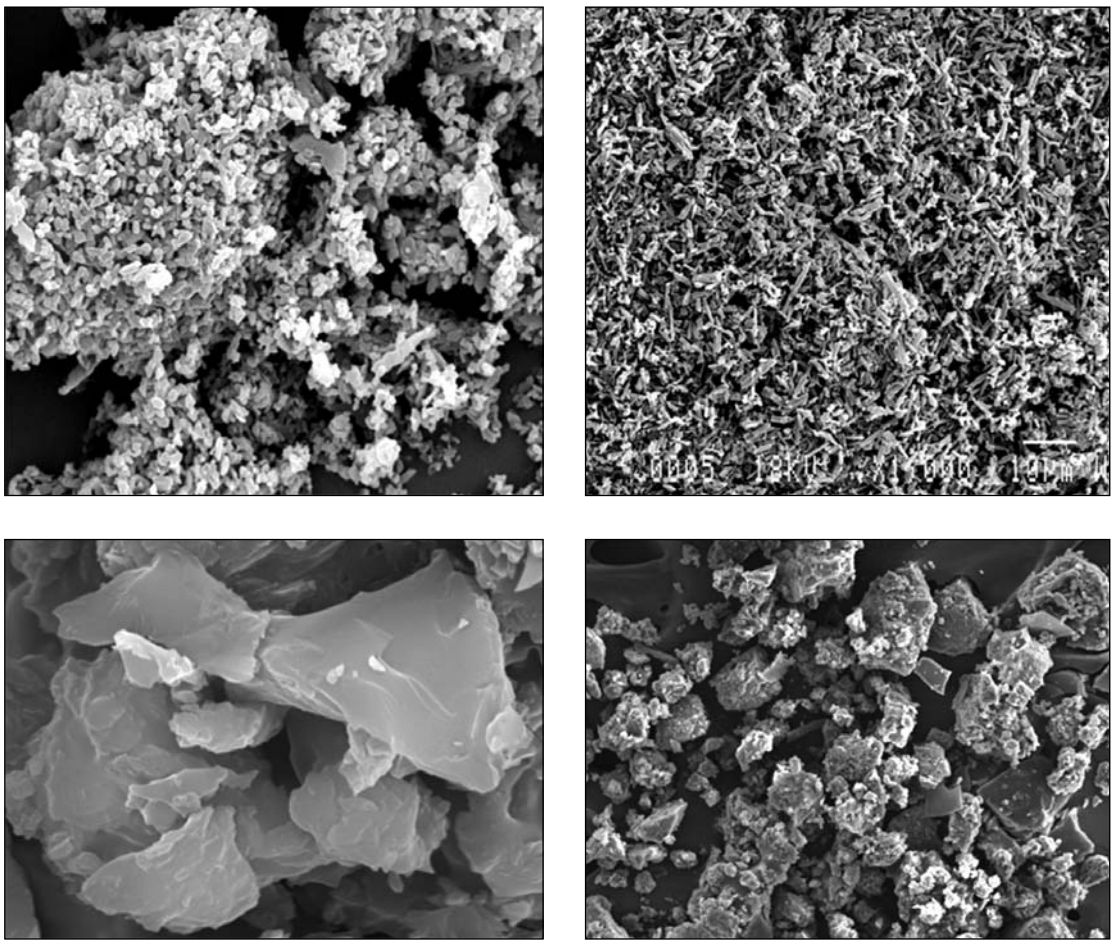


Fig. 7. SEM pictures of form I (above left), form L (above right), amorphous form (bottom left), and semicrystallized form I (bottom right) of cabergoline at a magnification of 1000x.

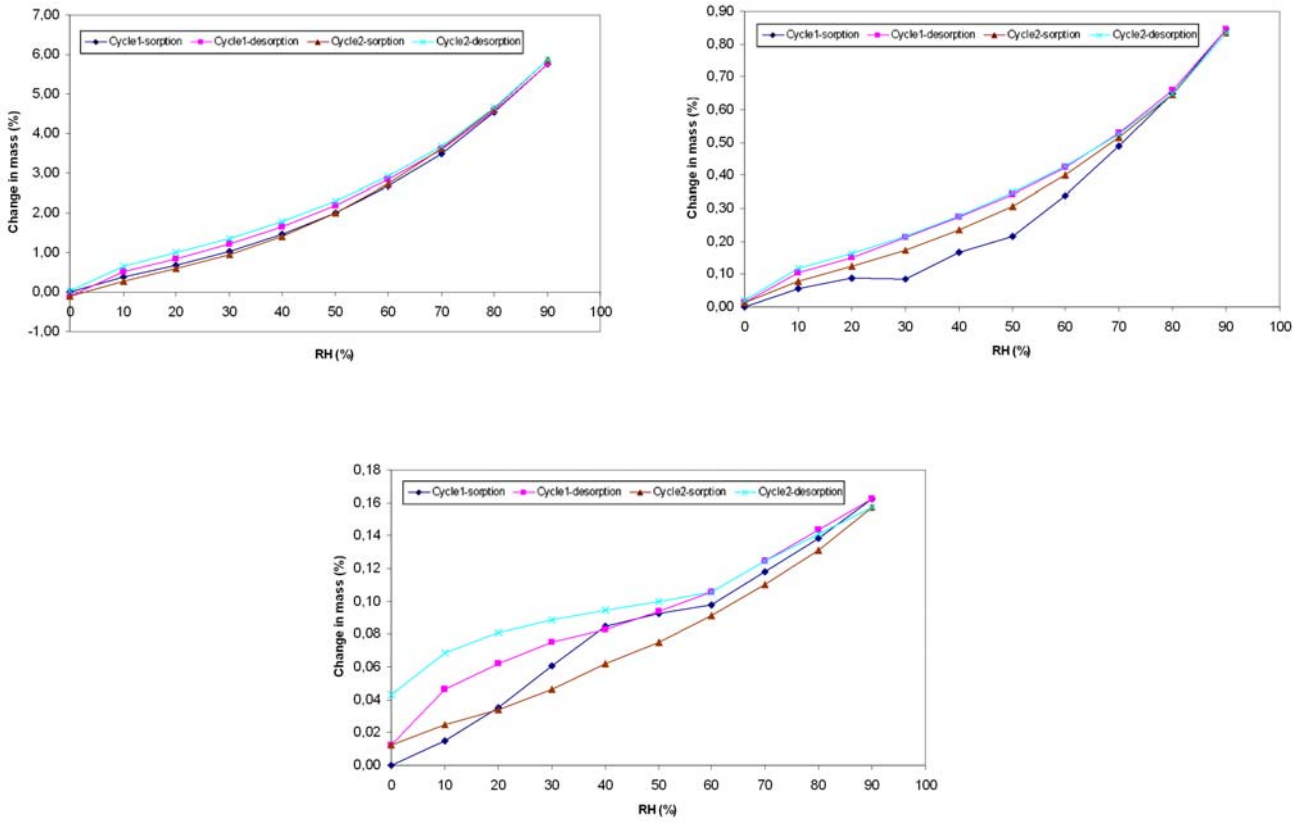


Fig. 8. DVS results for cabergoline forms: amorphous (top left), form I (top right) and form L (bottom)

the substance during ageing. To ensure a stable and safe composition, without high expenses for dry manufacturing or packaging conditions, it is highly desirable to handle non-hygroscopic substances. We analyzed the hygroscopic behavior of forms I and L and amorphous cabergoline using the dynamic vapor sorption (DVS) method. It is evident from the moisture sorption profiles (Fig. 8) that the amorphous form is the most hygroscopic. The amorphous form, form I, and form L absorb 5.8, 0.84, and 0.16% of water at 90% RH, respectively. Form L is not hygroscopic, so it is suitable for final dosage formulation.

4. Conclusion

A novel crystal form of cabergoline, form L, was discovered during the study of polymorphism of various crystal forms of cabergoline and its preparation from halogenated aromatic solvents (solvates). The new crystal form was characterized by various analytical methods (XRD, FTIR, Raman, DSC, SEM, and DVS). Form L is thermodynamically stable. Its long-term stability testing using several stability programs showed no solid-state transitions. Due to the crystal form, shape, size, and particle size distribution, it is easily transferable into the drug manufacturing process.

5. References

1. E. Brambilla, E. Disalle, G. Briatico, S. Mantegani, A. Temperilli, *Eur. J. Med. Chem.* **1989,** *24*, 421–426.
2. P. Sabatino, L. Riva di Sanseverino, R. Tonani, *Il Farmaco*, **1995**, *50*, 175–178.
3. A. Y. Sheikh, WO **2003**/078392.
4. A. Tomasi, S. Magenes, G. Ramella, M. Ungari, M. Pandolfi, US **2004**/0072855.
5. I. Candiani, R. Budelli, M. Pandolfi, M. Ungari, US 6, 680, 327.
6. R. Bednar, L. Cvak, J. Cejka, B. Kratochvil, I. Cisarova, A. Jegorov, *Acta Cryst*. Sect. E, **2004,** *60*, o1167–o1169.
7. A, Jegorov, Z. Horak, J. Cejka, B. Kratochvil, I. Cisarova, *Acta Cryst*., Sect. C, **2003**, *59*, o575–o576.
8. J. T. Carstensen, Advanced Pharmaceuticals Solids, Drugs and Pharmaceuticals Sciences. Marcel Dekker, New York, **2001**.
9. K. R. Morris, Structural aspects of hydrates and solvates, In: Polymorphism in Pharmaceutical Solids. Marcel Dekker, New York, **1999**.
11. Z. Ham, M. Črnugelj, WO **2008**/049884.
12. Z. Ham, A. Premrl, WO **2008**/092881.
13. H. G. Brittain, *J. Pharm. Sci.* **1997,** *86*, 405–412.
14. L. J. Bellamy, The infrared spectra of complex molecules. Chapman and Hall, London, **1975**.

Povzetek

Odkrili smo novo kristalno obliko kabergolina, obliko L. Ovrednotili smo jo s termično analizo, rentgensko praškovno difrakcijo, infrardečo spektroskopijo, Ramansko spektroskopijo, DVS analizo ter s testi topnosti in hitrostjo raztapljanja. Morfologijo paličastih kristalov smo določili z elektronsko mikroskopijo (SEM). Obliko L smo primerjali z ostalimi nesolvatnimi oblikami kabergolina, in sicer z obliko I, II, VII in amorfno obliko. Termodinamsko stabilnost oblike L smo podprli še z določitvijo dolgoročne stabilnosti. Kristalna oblika L je primerna za farmacevtsko uporabo.