

STRUCTURES OF MILDRONATE POLYMORPHIC AND PSEUDOPOLYMORPHIC FORMS

A. Zvirgzdiņš, K. Veldre, A. Actiņš

Department of Chemistry, University of Latvia, Kr. Valdemāra 48, Rīga, LV-1013
e-mail: alvis_zvirgzdins@inbox.lv

Structures of mildronate polymorphic and pseudopolymorphic forms were determined using powder X-ray diffractometry data. Mildronate dihydrate structure was determined for the verification of methodology, which was applied for mildronate monohydrate and anhydrous form structure determination. All mildronate forms crystallize in primitive lattices – anhydrous form in $P2_1/n$, monohydrate in $\bar{P}1$ and dihydrate in $P2_1/c$. The structures of mildronate forms show similarities to carnitine and its derivatives.

Key words: *mildronate, hydrates, powder X-ray diffractometry, structure determination, Rietveld method.*

INTRODUCTION

Mildronate (3-(2,2,2-trimethylhydrazinium)propionate; MET-88; quaterine) is an antiischemic drug developed by I. Kalvinsh and his associates at the Latvian Institute of Organic Synthesis [1].

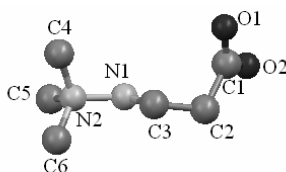


Fig. 1. Mildronate molecule.

In the last decade, software and computer evolution allows structure determination from powder X-ray diffractometry data. This feature is very useful when monocystals cannot be grown.

Structure determination from powder X-ray diffractometry data consists of indexing, space group determination, structure calculation and refinement of the calculated structure.

Indexing is a well-explored field in crystallography. The most widely used algorithms and software are described in literature [2]. Unit cell dimensions after calculation are refined by Pawley [3] or Le Bail methods [4].

The simulated annealing is a structure calculation method in which the molecular conformation of the asymmetric unit cell atoms is used. During the simulated annealing the atomic coordinates are calculated according to a genetic algorithm and cost function minimization [5].

The final structure refinement using powder X-ray data is performed by Rietveld method [6], which is well-known and adapted in the almost all software where the structures can be calculated.

Since the structure of mildronate dihydrate has been reported [7], this work was performed for examining the methodology of the structure determination from the powder X-ray diffractometry data.

EXPERIMENTAL

The initial sample of mildronate was its dihydrate supplied by JSC *Grindeks*. The stoichiometry of mildronate molecules and water was determined using thermogravimetric analysis by *SII Exstar 6000*.

Anhydrous mildronate was obtained by dehydration of dihydrate at 80 °C and 1% relative humidity.

Mildronate monohydrate was obtained from anhydrous form at 21% relative humidity and 75 °C.

Sample preparation. All samples were gently ground in an agate mortar and deposited in the glass sample holders. During the diffraction data collection, the sample holders of monohydrate and anhydrous forms were covered with a 12 µm sheet of polyethylene.

Diffraction data collection. Diffraction data were collected using a *Bruker D8 Advance* diffractometer, equipped with a secondary beam graphite monochromator and a scintillation detector. Data were collected in 2θ range of 3–50° with a counting step size of 0.007°.

Structure determination. The indexing of mildronate dihydrate and anhydrous form powder patterns was performed with *Dicvol*, *ITO*, *TREOR* and *McMaille* software, but for monohydrate only with *McMaille* and *Dicvol*, since these programs are the most powerful for the triclinic space group. All these programs are included in the software package *Fullprof*.

The determined unit cell dimensions were refined by Pawley method in *TOPAS*, and the space groups for the refined dimensions were determined using *EXPO 2009*, *Checkcell* and *TOPAS*.

Structures were calculated using a simulated annealing method in *EXPO 2009*. The necessary rigid bodies were constructed in *ChemSketch*. The structure calculations were performed from the different initial conditions by changing the cost function, cooling rate and other conditions of simulated annealing. The atomic coordinates were calculated only for nonhydrogen atoms.

Calculated atomic coordinates were refined using Rietveld refinement in *EXPO 2009*.

RESULTS AND DISCUSSION

The indexing and space group determination results of mildronate polymorphic and pseudopolymorphic forms are shown in the Table 1. The anhydrous form of mildronate and dihydrate crystallize in monoclinic space group, but monohydrate crystallizes in triclinic space group.

The determined structure of mildronate dihydrate was compared with a structure from literature [7]. The published structure [7] was determined at low temperature (–100 °C), so unit cell dimensions are smaller. In Fig. 2 one can see

that the supramolecular structure of mildronate dihydrate determined by us (Fig. 2 (1)) is similar with the published structure (Fig. 2 (2)). The structure of mildronate dihydrate was reported already in [7], the space group was found to be $P112_1/b$ since in the earlier work [7] and in this work it was $P2_1/c$.

Table 1. Unit cell dimensions of mildronate polymorphs and pseudopolymorphs

n	$a, \text{\AA}$	$b, \text{\AA}$	$c, \text{\AA}$	$\alpha, ^\circ$	$\beta, ^\circ$	$\gamma, ^\circ$	$V, \text{\AA}^3$	Z	$\rho, \text{g/cm}^3$
0	8.6897(6)	11.4105(8)	7.8455(5)	90	91.418(9)	90	777.66(12)	4	1.25
1	9.0230(3)	8.1186(5)	7.3835(3)	72.866(4)	66.881(3)	99.418(7)	450.71(6)	2	1.21
2	6.9417(5)	11.3273(8)	12.9063(11)	90	105.372(9)	90	978.52(5)	4	1.24
2	6.8927	11.2597	12.8390	90	105.418	90	960.56	4	1.24
2[7]	6.931(1)	12.885(2)	11.315(3)	90	90	105.36(2)	974.4(3)	4	1.24

Note: n – the number of water molecules per mildronate molecule;
 Z – the number of mildronate molecules in a unit cell

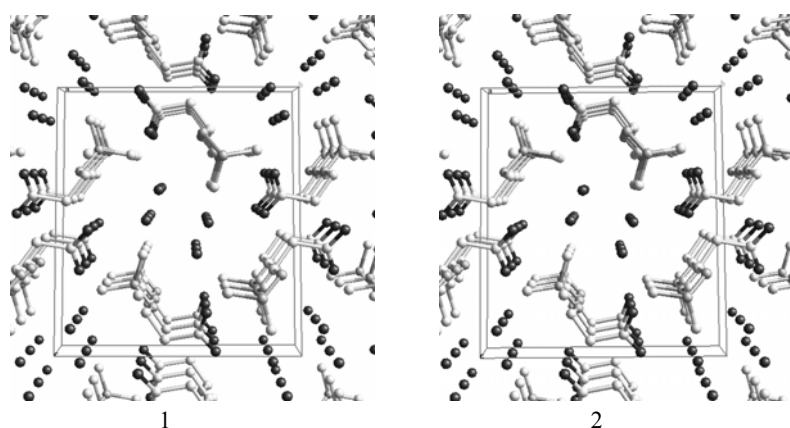


Fig. 2. Mildronate dihydrate structure.
View down the a axis, as determined in this work (1) and in [7] (2).

Table 2. Atomic coordinates of the determined mildronate dihydrate structure in this work and in [7].

Atom	x		y		z	
	*	[7]	*	[7]	*	[7]
O1	0.451 (2)	0.4498	0.1134 (13)	0.1165	0.672(9)	0.6742
O2	0.206(2)	0.2072	0.1509(12)	0.1494	0.5263(11)	0.5253
N1	0.023(3)	0.0214	−0.1756(14)	−0.1774	0.7620(12)	0.7601
N2	0.079(4)	0.0793	−0.100(2)	−0.1004	0.6807(13)	0.6807
C1	0.324(3)	0.3205	0.083(2)	0.0811	0.5930(15)	0.5923
C2	0.302(3)	0.3011	−0.051(2)	−0.0520	0.5723(14)	0.5702
C3	0.281(3)	0.2797	−0.1261(14)	−0.1270	0.6675(13)	0.6653
C4	−0.196(5)	−0.1967	−0.151(2)	−0.1521	0.745(3)	0.7470
C5	0.141(3)	0.1398	−0.141(2)	−0.1433	0.873(2)	0.8705
C6	0.046(3)	0.0450	−0.308(2)	−0.3076	0.743(2)	0.7429
O3	0.593(2)	0.5991	0.076(9)	0.0777	0.886(8)	0.8884
O4	0.3084(12)	0.3109	0.1231(11)	0.1249	0.993(8)	0.9945

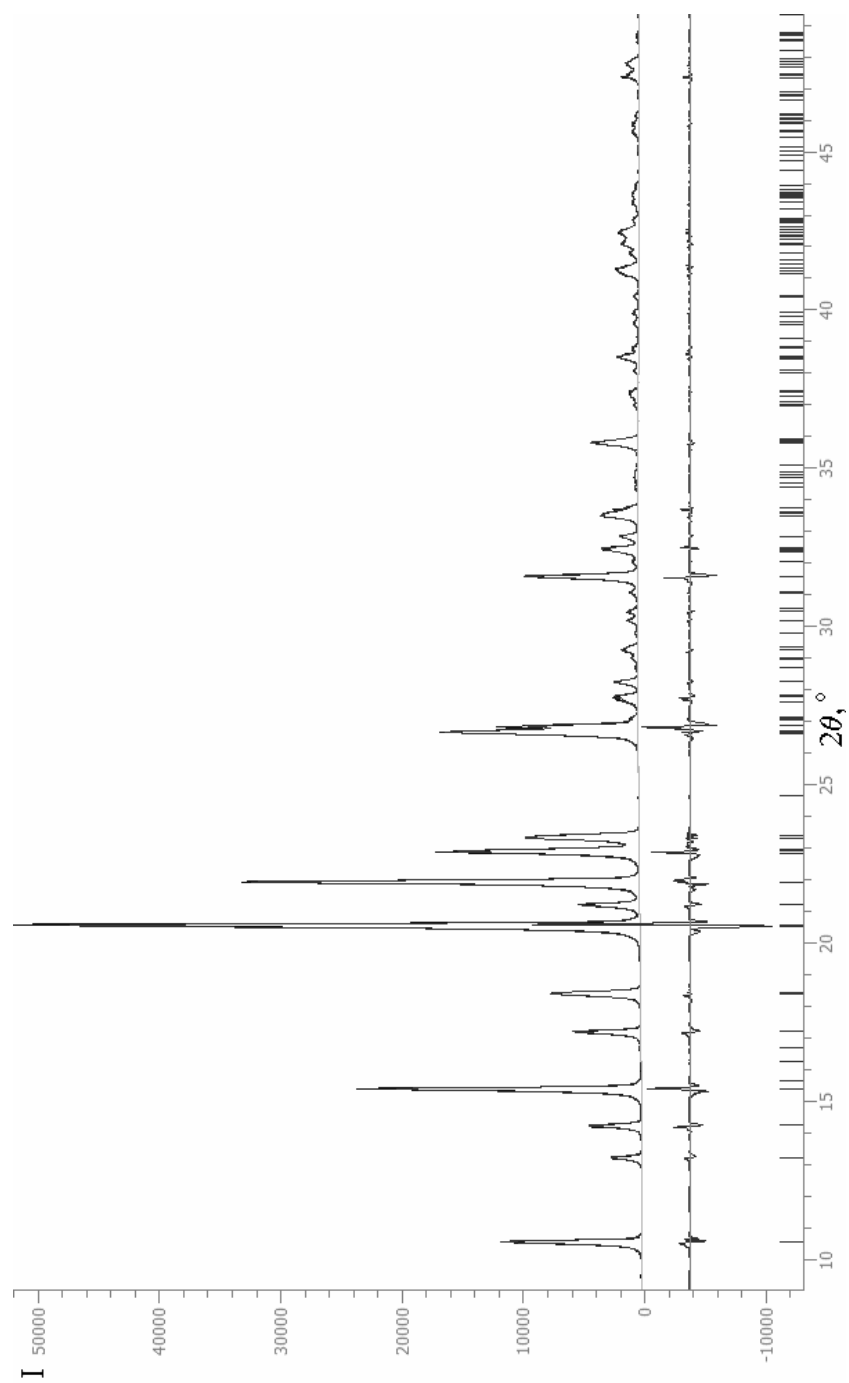


Fig. 3. The measured diffraction pattern of mildronate dihydrate, a theoretical diffraction pattern of the calculated structure and the difference between the both patterns.

From the Table 2 we can see that our calculated atomic coordinates conform well to the single-crystal data [7] within the range of standard deviations.

Our powder X-ray diffraction methodology was successfully used for structure determination of a crystal where the asymmetric unit cell consists of 12 nonhydrogen atoms, from which two are not connected with other atoms (water molecules).

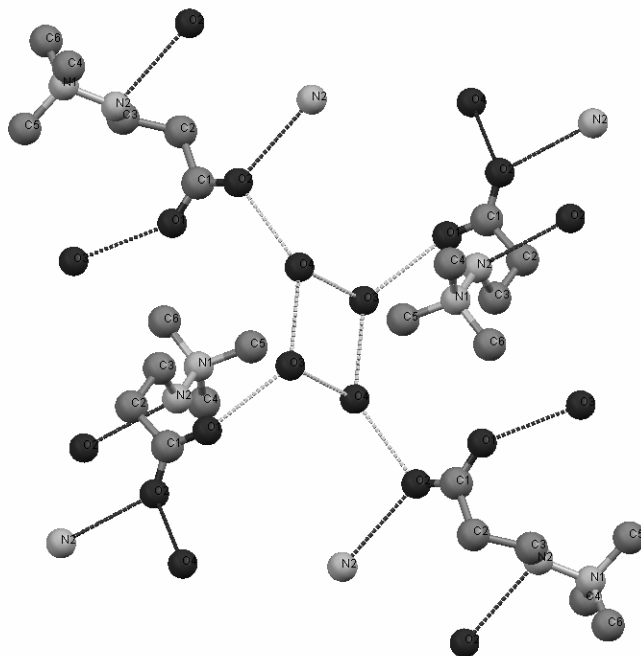


Fig. 4. Hypothetical hydrogen bonds in mildronate dihydrate structure.

The hypothetical hydrogen bonds in mildronate dihydrate molecule (Fig. 4) are assumed to connect the water molecules and the carboxyl group $O4-H\cdots O2$ and $O3-H\cdots O1$, also water and mildronate molecules $N(1)2-H\cdots O(2)2$ and $O(1)2\cdots H-N(2)2$, and water molecules $O3-H\cdots O4$ and $O4-H\cdots O3$. Although the hydrogen bond network appears to be well-defined, there are holes large enough for the extra water molecules in the calculated mildronate dihydrate structure.

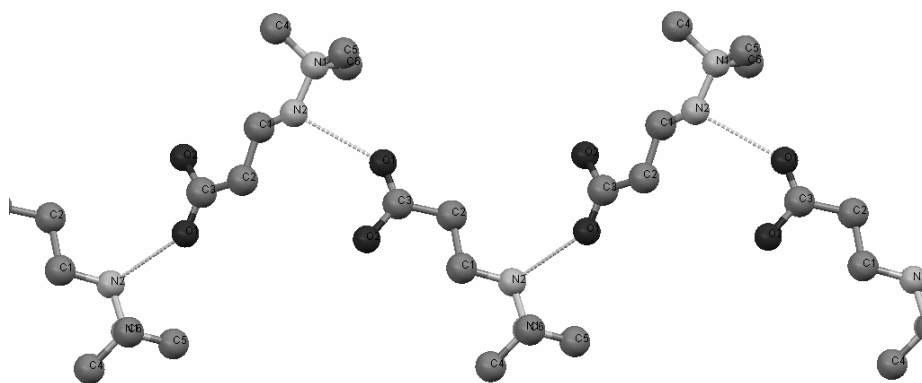


Fig. 5. Fragment of anhydrous mildronate structure.

The Fig. 5 illustrates zigzag type chains in the structure of anhydrous mildronate. These chains are formed of mildronate molecules, which are linked by hypothetical hydrogen bonds between O1...H–N2 atoms. This structure is similar to that of carnitine, where carnitine molecules are linked with single hydrogen bonds and form zigzag type chains. Molecules in mildronate chains are perpendicular to their neighbours, but in carnitine there is a parallel arrangement. The Fig. 6 shows larger scale structures of holes and layers in anhydrous mildronate crystals. These holes are large enough for water molecules, leading to the facile hydration of anhydrous mildronate.

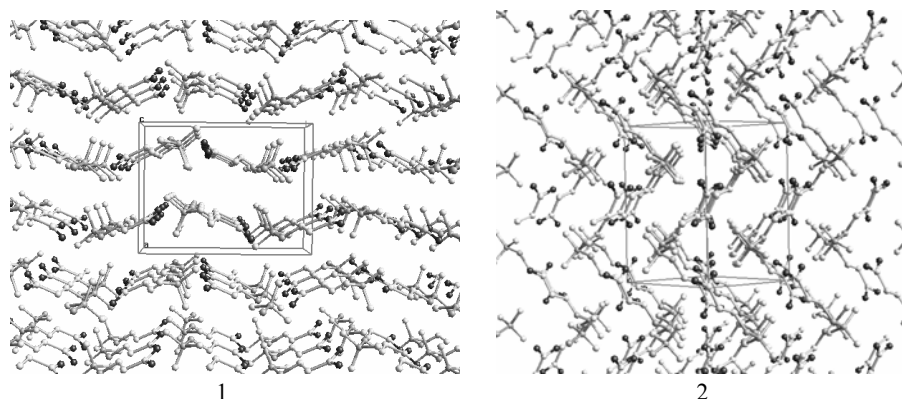


Fig. 6. Anhydrous mildronate structure view down *c* axis (1) and view down *c* edge (2).

Table 3. Atomic coordinates of anhydrous mildronate structure asymmetric unit

Atoms	<i>x</i>	<i>y</i>	<i>z</i>
C1	0.7640(2)	0.9139(12)	0.5040(2)
C2	0.7980(13)	0.9471(13)	0.6900(2)
C3	0.7270(2)	1.0650(2)	0.7260(3)
N1	0.8210(2)	0.7640(2)	0.2930(2)
N2	0.8530(12)	0.8080(10)	0.4610(2)
C4	0.8419(14)	0.8573(13)	0.1660(2)
C5	0.9279(13)	0.6676(12)	0.2578(15)
C6	0.6620(11)	0.7198(8)	0.2790(2)
O1	0.7309(11)	1.1040(14)	0.8700(12)
O2	0.6553(9)	1.1264(8)	0.5984(12)

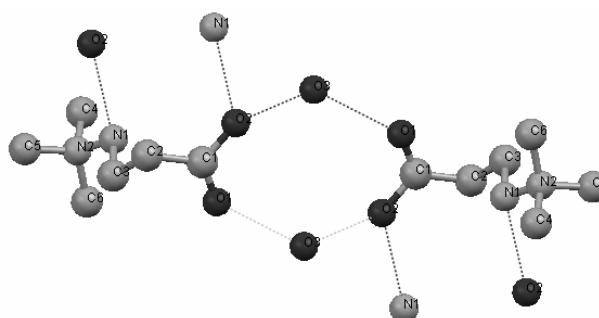


Fig. 8. Hypothetical hydrogen bonds in the structure of mildronate monohydrate.

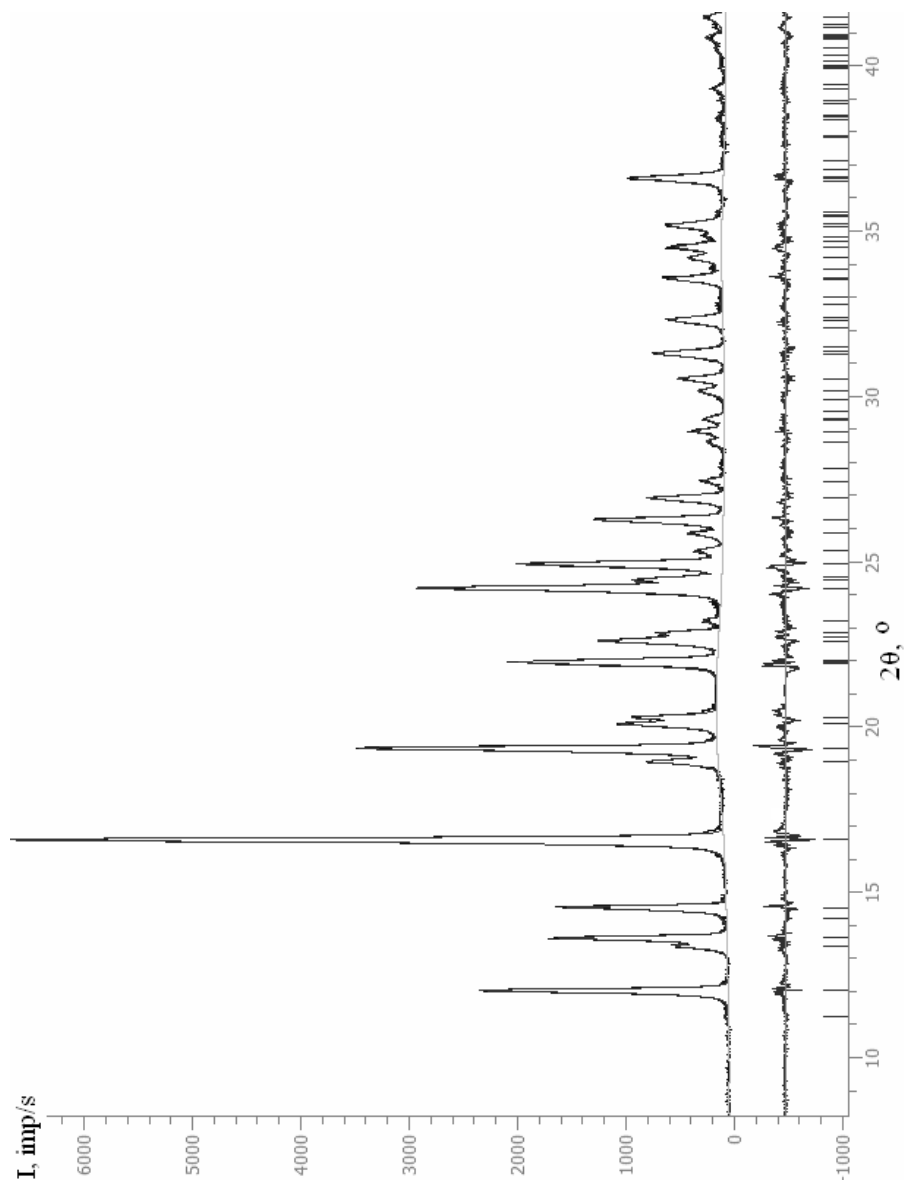


Fig. 7. The actual diffraction pattern of mildronate monohydrate, calculated diffraction pattern of the determined structure and the difference between both patterns.

As follows from the Fig. 8, in the mildronate monohydrate structure the water molecules are stabilized by the hypothetical hydrogen bonds between water and carboxyl groups – O(1)2···H–O3–H···O(2)1 and O(1)1···H–O3–H···O(2)2. Mildronate molecules are linked with the hypothetical hydrogen bonds O(1)2···N(2)1 and N(1)1···O(2)2. In the crystal of acetylcarnitine monohydrate the water molecules are bonded with the carboxyl groups in a similar way.

Table 4. Atomic coordinates of mildronate monohydrate structure asymmetric unit

Atoms	x	y	z
C1	0.6520(2)	0.3030(2)	0.5600(2)
C2	0.4690(2)	0.2250(2)	0.6140(2)
C3	0.3550(2)	0.1695(12)	0.8539(11)
N1	0.3398(10)	0.3331(13)	0.8986(12)
N2	0.2170(2)	0.2950(3)	1.1122(3)
C4	0.2160(13)	0.4677(14)	1.1369(14)
C5	0.0460(11)	0.1990(2)	1.1540(2)
C6	0.2600(2)	0.1840(2)	1.2700(2)
O1	0.7080(8)	0.2269(12)	0.6726(10)
O2	0.7533(9)	0.4605(12)	0.3853(12)
O3	0.9949(8)	0.7438(8)	0.3183(12)

Table 5. Torsion angles of mildronate molecules in mildronate polymorphic and pseudopolymorphic forms

Torsion angle	Mildronate form		
	anhydrous	monohydrate	dihydrate
C4 N1 N2 C1	54.0	179.2	169.2
N1 N2 C1 C2	175.5	171.5	172.1
N2 C1 C2 C3	171.6	74.6	69.4
C1 C2 C3 O1	175.1	45.3	51.4

Torsion angles (Table 5) show that the conformations of mildronate monohydrate and dihydrate are almost similar, but anhydrous mildronate molecule is more stretched. Since N2 atom hypothetically is linked with the oxygen atom O1 via a hydrogen bond, the linear chains are formed in this structure.

CONCLUSIONS

Mildronate polymorphic and pseudopolymorphic crystal structures of non-hydrogen atoms were calculated using powder X-ray diffractometry data. Although each step in this procedure was critical and time-consuming, this method can be used for structure determination when monocrystals cannot be grown or are too unstable for measurements, such as in the case of mildronate monohydrate and anhydrous mildronate.

Determined structures show that hypothetical hydrogen bond amount decrease from mildronate dihydrate to anhydrous form in which mildronate molecules are bounded with one hydrogen bond. Mildronate polymorphic and pseu-

dopolymorphic form stability agrees with observations in room temperature and humidity [8].

Mildronate monohydrate and anhydrous form structures showed similarities with the structures of carnitine and some of its derivatives.

REFERENCES

1. Sjakste, N., Gutcaits, A., Kalvinsh, I. (2005). Mildronate: an antiischemic drug for neurological indications. *CNS Drug Reviews*, 11 (2), 151.
2. Shirley, R. (2003). Commission of crystallographic computing, overview of powder-indexing program algorithms (history and strengths and weaknesses). *Newsletter*, 2, 48.
3. Pawley, G.S. (1981). Unit-cell refinement from powder diffraction scans. *J. Appl. Cryst.*, 14, 357.
4. Le Bail, A., Duroy, H., Fourquet, J.L. (1988). Ab-initio structure determination of LiSbWO_6 by X-ray powder diffraction. *Mater. Res. Bull.*, 23, 447.
5. Coelho, A. (2000). Whole-profile structure solution from powder diffraction data using simulated annealing. *J. Appl. Cryst.*, 33, 899.
6. Rietveld, H.M. (1969). A profile refinement method for nuclear and magnetic structures. *J. Appl. Cryst.*, 2, 65.
7. Kemme, A., Bleidelis, J., Kalvinsh I., Eremeyev, A. (1983). Молекулярно-кристаллическое строение дигидрата 3-(2,2,2-триметилгидразиний) пропионата C_6H_{14} . *Изв. АН Латв. ССРС, Сер. Хим.*, 2, 215.
8. Veldre, K., Actiņš, A., Kalniņa, A. (2010). Thermal stability of 3-(2,2,2-trimethylhydrazine) propionate. *Solid State Chem.*, 231

MILDRONĀTA POLIMORFO UN PSEIDOPOLIMORFO FORMU STRUKTŪRAS

A. Zvirgzdiņš, K. Veldre, A. Actiņš

KOPSAVILKUMS

Izmantojot pulvera rentgendifraktometrijas datus, noteiktas mildronāta polimorfo un pseidopolimorfo formu struktūras. Mildronāta dihidrāta struktūra noteikta, lai pārbaudītu metodi, kura tālāk izmantota monohidrāta un bezūdens formas struktūras noteikšanai. Visas mildronāta formas kristalizējas primitīvos kristālrežģos – bezūdens $\text{P2}_1/\text{n}$, monohidrāts $\bar{\text{P}}1$ un dihidrāts $\text{P2}_1/\text{c}$. Mildronāta formu struktūrās saskatāma līdzība ar karnitīna un tā atvasinājumu struktūrām.

Iesniegts 11.04.2011