

**Anlagenkonvolut K 63**

zum Schriftsatz vom 22.06.2018

i.S. Shire ./ CSL

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**HOYNG ROKH MONEGIER**  
**Partnerschaftsgesellschaft mbB**

**From:** [Broom, Colin](#)  
**To:** [Soland, Daniel](#); [Pietrusko, Robert](#); [Fletcher, Clayton](#); [Milano, Vincent J.](#)  
**Subject:** FW: Cinryze stability studies  
**Date:** Wednesday, March 11, 2009 7:49:07 PM  
**Attachments:** [036-xx in solution at higher concentration \(4\)4.doc](#)

So the fact that it is possible to concentrate 500U in 1.5ml has only taken decades to be determined!

Hopefully this 1.5 ml injection is not going to hurt too much.

Any volunteers?

Colin.

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**From:** Johnson, Judy  
**Sent:** Wednesday, March 11, 2009 2:01 PM  
**To:** Broom, Colin; Pietrusko, Robert  
**Subject:** Cinryze stability studies

Colin and Bob,

Here's the most recent draft report from Sanquin on the stability studies which seems to suggest that 1.5 mL would be the minimum volume for reconstitution of a vial of Cinryze for sc injection. When reconstituted in 1 mL, there was some material sticking to the vial walls.

Judy

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0 Limited distribution for:  
    0 Product registration  
    0 Publication  
    0 Third parties

Product: Cinryze  
No.: 036 - xxx  
Page: 1 - 6  
Date: 05Mar2009

Subject: Stability of Cinryze ( $\geq 100$  U/ml) in solution

Researcher: J. Bloem

#### Summary:

This report describes the investigation into the solubility and stability of reconstituted Cinryze containing  $\geq 100$  U/ml stored for up to 24 hours at  $20 \pm 2$  °C. It can be concluded that Cinryze containing the excipients as described in the BLA, can be solved in 1 - 5 ml of w.f.i within 10 min. No loss of activity, degradation or aggregation was detected after storage at room temperature for up to 24 hr in concentrations of up to 500 U/ml.

Approved by:		
Author: J. Bloem	Manager Product Development: A.H.L. Koenderman	
Dated:	Dated:	Dated:

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#### 1. Introduction

For the development of a C1-inhibitor product for subcutaneous administration a more concentrated product is preferred. As a first experiment it was tried to dissolve the current registered Cinryze product in a smaller volume to obtain a product with a higher C1-inhibitor concentration but also higher excipient concentrations. It was tested if the current product can be dissolved in 2.5, 2, 1.5 or 1 ml to obtain product containing 200, 250, 333 or 500 U/ml and higher concentrations of excipients (Table 1). Also, the stability of this concentrated product at room temperature up to 24 hrs was tested.

#### 2. Material and Methods

##### 2.1. Material

For the experiments vials of Cinryze batch 06C02L370A were used. Specifications of batch 06C02L370A at release were: C1-inhibitor activity 96 U/ml; protein 14.1 g/l; pH 7.0; sodium 106 mmol/L; citrate 10 mmol/L; sucrose 62 mmol/L; osmolality 302 mosmol/kg; valine 17 mmol/L; alanine 13 mmol/L; threonine 38 mmol/L.

##### 2.2. Methods

The solubility and stability at C1-inhibitor concentrations of 200, 250, 333 and 500 U/ml were tested. As a reference the product in a concentration of 100 U/ml was used. Per experiment several vials were used to have enough material to analyse the product at 0, 1, 3, 6 and 24 hr after dissolution and storage at  $20 \pm 2$  °C.

The solubility of the C1-inhibitor protein was determined using M066 (QC protocol). Subsequently, the vials were sampled at 5 time points, i.e.  $t=0$ ,  $t=1h$ ,  $t=3h$ ,  $t=6h$  and  $t=24h$ . The samples were transferred to Sarstedt tubes, frozen at -30 °C and despatched for the following determinations: C1-inhibitor activity (@0398), C1-inhibitor antigen (@0xxx) and HPSEC (PO method). It must be noted that the C1-inhibitor activity assay is not validated for products with high concentrations of C1-inhibitor and excipients (a.o. dilution effects).

#### 3. Results

##### 3.1 Stability of C1-inhibitor in solution

Cinryze can be dissolved in 1 - 5 ml within 10 min. (Table 2). Only if 1 ml of w.f.i. is used, some material is sticking to the wall, *showing that the dissolution procedure must be done carefully*. The



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Table 1: Theoretical concentrations of excipients in Cinryze in a smaller dissolution volume than described in the BLA

Volume ml	C1- inhibitor activity (U/ml)	Sodium mmol/L	citrate mmol/L	L-valine mmol/L	L-alanine mmol/L	L- threonine mmol/L	Sucrose mmol/L	%
5.0	100	100	10	17	13	38	60	2
2.5	200	200	20	34	26	76	120	4
2.0	250	250	25	43	33	95	150	5
1.5	333	333	33	57	43	127	200	7
1.0	500	500	50	85	65	190	300	10

Table 2: Solubility and appearance of the solution after storage for up to 24 hrs at room temperature;

conform release: no precipitate, no "flakes", not opalescent

Volume ml	Solubility	Appearance	
		at 3hrs	at 24 hrs
5.0	++	conform	conform
2.5	++	conform	conform
2.0	++	conform	conform
1.5	+(+)	conform	conform
1.0	-	conform	conform

++: within 5 min.

+ : 5 min.

- : 5 min., but some material stucked to the wall and did not dissolve completely.

stability of C1-inhibitor activity after reconstitution was determined at t=0 h, t=1 h, t=3 h t=6 h and t=24 h (Table 2) and no loss of C1-inhibitor activity was observed after storage at room temperature for up to 24 hrs. For the 200 U/ml experiments the measured C1-inhibitor concentration was lower than calculated, but the C1-inhibitor activity did not change after storage. It was decided to repeat the experiment with this concentraion and the results will be reported as an amendment to this report.

Also, an HPSEC analysis was performed showing that there was no aggregation or degradation after storage for up to 24 hrs, even for the highest concentration, 500 U/ml, tested.

*De analyses zijn klaar maar ik heb nu geen mogelijkheid om de figuren te maken. Zal moeten wachten tot Judith weer terug is.*

## 4 Conclusions

It can be concluded that dissolving the regular product in less w.f.i. to obtain a presentation containing up to 500 U/ml does not affect the stability of the product after reconstitution. However, due to the high concentration of the excipients more experiments are needed to find a formulation containing a high concentration of C1-inhibitor at lower concentrations of excipients.

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Table 3: C1-inhibitor activity after dissolution and storage for up to 24 hrs at room temperature

Cinryze	Activity	Yield (%)		Antigen	Specific activity
	U/ml	T = 0	theoretical	g/L	U/mg
<i>5 ml; 100 U/ml</i>					
T = 0	89		89	20	4.5
T = 1	91	102	91	20	4.6
T = 3	80	90	80	20	4.0
T = 6	84	94	84	21	4.0
T = 24	86	97	86	21	4.1
<i>2.5 ml; 200 U/ml</i>					
T = 0	127		64	29	4.4
T = 1	120	94	60	30	4.0
T = 3	114	90	57	29	3.9
T = 6	105	83	53	30	3.5
T = 24	121	95	61	29	4.2
<i>2 ml; 250 U/ml</i>					
T = 0	220		88	51	4.3
T = 1	210	95	84	51	4.1
T = 3	220	100	88	52	4.2
T = 6	210	95	84	52	4.0
T = 24	220	100	88	52	4.2
<i>1.5 ml; 333 U/ml</i>					
T = 0	280		85	70	4.0
T = 1	280	100	85	68	4.1
T = 3	290	104	88	69	4.2
T = 6	290	104	88	70	4.1
T = 24	280	100	85	68	4.1
<i>1 ml; 500 U/ml</i>					
T = 0	410		82	99	4.1
T = 1	410	100	82	99	4.1
T = 3	410	100	82	101	4.1
T = 6	430	105	86	101	4.3
T = 24	430	105	86	97	4.4

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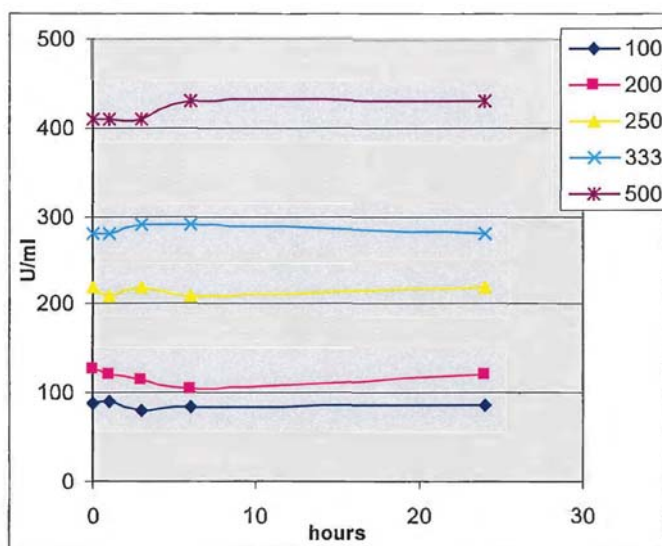


Figure 1: Stability of Cinryze after dissolution at high concentrations.

Figure 2: HPSEC analysis of Cinryze after dissolution in 5 ml and storage for up to 24 h at room temperature.

Samples were taken after storage for 10 min, 1 h, 3 h, 6 h and 24 h.

HPSEC overlay picture will be added: there were no changes during storage.

Figure 3: HPSEC analysis of Cinryze after dissolution in 2.5 ml and storage for up to 24 h at room temperature.

Samples were taken after storage for 10 min, 1 h, 3 h, 6 h and 24 h.

HPSEC overlay picture will be added: there were no changes during storage.

Figure 4: HPSEC analysis of Cinryze after dissolution in 2 ml and storage for up to 24 h at room temperature.

Samples were taken after storage for 10 min, 1 h, 3 h, 6 h and 24 h.

HPSEC overlay picture will be added: there were no changes during storage.

Figure 5: HPSEC analysis of Cinryze after dissolution in 1.5 ml and storage for up to 24 h at room temperature.

Samples were taken after storage for 10 min, 1 h, 3 h, 6 h and 24 h.

HPSEC overlay picture will be added: there were no changes during storage.

Figure 6: HPSEC analysis of Cinryze after dissolution in 1 ml and storage for up to 24 h at room temperature.

Samples were taken after storage for 10 min, 1 h, 3 h, 6 h and 24 h.

HPSEC overlay picture will be added: there were no changes during storage.