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Determination of Residual Solvents in BCD — Is Ph.Eur. Method Up-to-date?

The Pharmacopoeias strictly regulate the residual solvent content in the pharmaceutical excipients. In the case of β-cyclodextrin (βCD) the residual toluene and trichloroethylene content, derived from the production, should be under the limit of 10 mg/kg.

Production processes

In general there are two different ways to produce βCD[1] (see Fig. 1): In "Solvent Processes" an organic complexing agent (solvent) precipitates one type of CD selectively and as such directs the enzyme reaction to produce mainly this type of CD. The ratio of CDs produced depends only on the CGTase used and on the reaction conditions. In the "Non-Solvent Process" no complexing agent is added and therefore a mixture of different CDs is formed. βCD is separated from this mixture by crystallization. This solvent-free method applied in Japan has low yield, cannot be applied to other CDs, and energy consuming (cooling is needed for crystallization). Outside Japan the manufacturers use the solvent processes[1]. In the case of βCD the complexing agent is toluene or trichloroethylene. That is the reason why the residues of these two solvents have to be determined according to the European Pharmacopoeia.

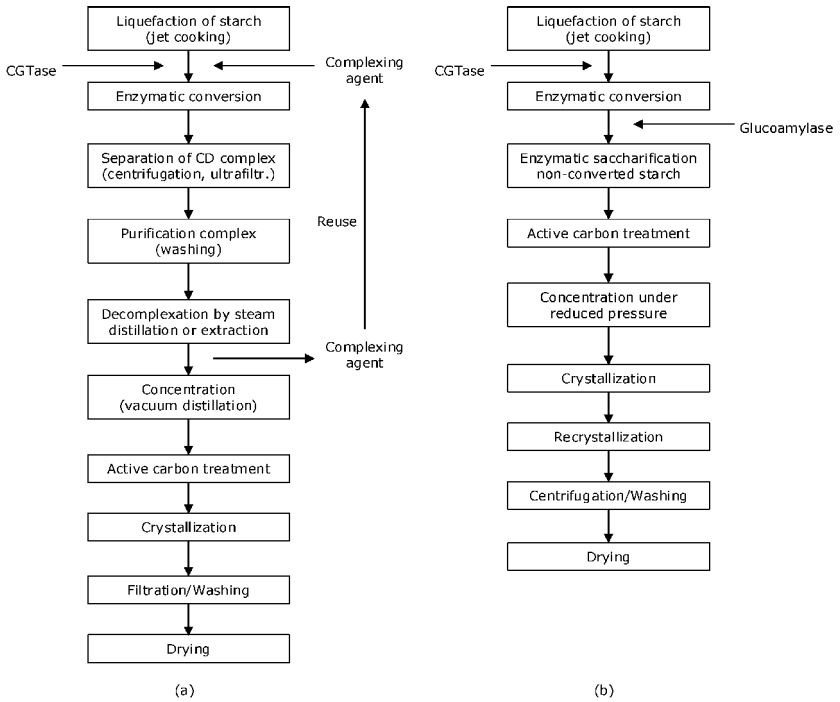

Figure 1. Solvent (a) and non-solvent (b) type of producing βCD[1]

Method in Pharmacopoeia

According to Betadex Monograph No. 1070 the residual toluene and trichloroethylene (TCE) should be determined in βCD samples with headspace gas chromatography (HS-GC) by standard addition method (Fig. 2 shows the method description scanned from the Pharmacopoeia).

These solvents can be found in the βCD samples in two different forms: as „free" (adsorbed) and included in complex. The principle of the method is that the solvents included in the CD cavity can be deliberated completely only in case the CD ring is destroyed by enzymatic cleavage. An α-amylase is added as a „ring-opener" to the sample solutions.

Residual solvents. Head-space gas chromatography (2.2.28): use the standard additions method.

Internal standard: ethylene chloride R. Test solutions. In each of 4 identical 20 mL flasks, dissolve 0.5 g of the substance to be examined in water R and add 0.10 g of calcium chloride R and 30 µL of α-amylase solution R. Add 1 mL of reference solutions (a), (b), (c) and (d), adding a different solution to each flask. Dilute to 10 mL with water R.

Reference solutions. Prepare a 10 µL/L solution of ethylene chloride R (reference solution (a)). Prepare reference solutions (b), (c) and (d) from reference solution (a) to contain respectively, per litre, 5 µL, 10 µL and 15 µL of both trichloroethylene R and toluene R

Static head-space conditions which may be used:

– equilibration temperature: 45 °C;

– equilibration time: 2 h.

Temperature:

– column: 50 °C;

– injection port: 140 °C;

– detector: 280 °C.

Detection: flame ionisation

Figure 2. Sample preparation and equilibration (incubation) conditions as described in the European Pharmacopoeia[2]

The method is based on the opening of the ring and then determination of the „free" toluene and trichloroethylene content.

It is well-known from the literature that the α-amylases can hydrolyze the β-cyclodextrin only in a small extent[3,4,5]. The relative activity of this enzyme is very low on α– and βCD (2 and 5 %, resp.); contrarily very high on γCD and starch (65 % and 100 %)[3]. This fact was proved by our HPLC measurements shown in Figure 3.

Figure 3 depicts an HPLC chromatogram of a β-cyclodextrin sample without (blue) and with enzyme treatment (red). There are degradation products, but in negligible amount. The evaporative light scattering detector (ELSD) is a non-linear detector, so the area % does not show the real ratio. The chromatogram shows unambiguously that βCD was far not destroyed by the enzyme.

The question comes up: why to use an enzyme, which does not work (does not open the βCD ring)?

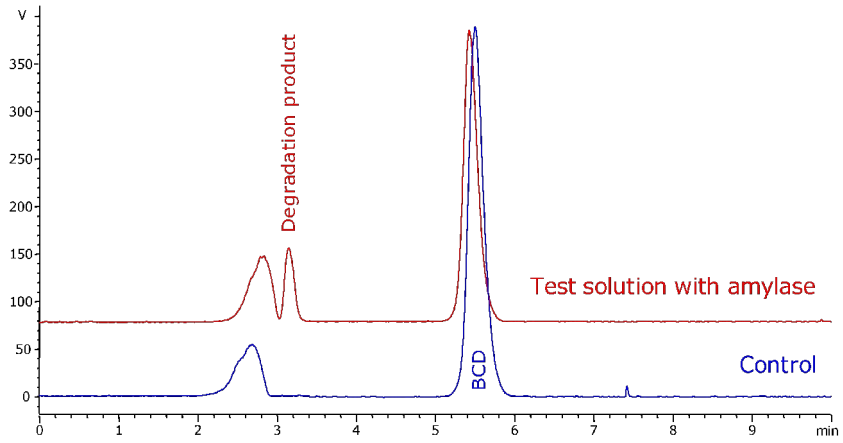

Figure 3. HPLC measurements of βCD samples without (blue) and with (red) enzyme treatment (incubation with α-amylase at 45 °C for 2 h)

(Chromatographic conditions: Column: CD-Screen, 5 µm, 4.0 x 250 mm (ChiroQuest Ltd, Budapest, Hungary), eluent: 43 % MeOH; flow: 1 ml/min; temperature: 30 °C; detection: ELSD)

We have decided to revise the official method.

Experimental

Various experiments were performed to improve the method described in Pharmacopoeia.

1. Enzyme was skipped from sample preparation but all the other parameters were the same as in the Ph.Eur method, to see the effect of enzyme (method I: incubation at 45 °C for 120 min)

2. Enzyme was skipped from sample preparation and the incubation time was reduced to 20 min keeping the same temperature (45 °C), to see the effect of incubation time (method II)

3. Enzyme was skipped from sample preparation and both the incubation time and temperature were changed (time = 20 min, temperature = 90 ^{o}C), to see the effect of temperature (method III)

4. Sodium benzoate was added to the sample as a competitive complexing agent for stripping the complexed solvents from the cavity. The incubation time was 20 min, the temperature was 45 ^{o}C (method IV)

5. Benzyl alcohol was added to the sample as competitive complexing agent at 45 ^{o}C, incubation time was 20 min (method V)

Competitive complex formation can help to expel the solvents from the cavity. For instance, sodium benzoate and benzyl alcohol were successfully applied to improve the deliberation of chamomile, menthol, eucalyptus oil, etc. from their complexes with βCD[7]. We assumed that these competitive guest molecules form complexes of higher stability than toluene and trichloroethylene so they were added to the samples to replace the solvents in the cavity.

The βCD sample with code number B018/14 was measured with all of the five methods.

The GC conditions were the same as described in Ph.Eur method (see Fig.2) and the typical chromatogram can be seen in Figure 4. The results are summarized in Table 1. As neither of the βCD samples contained trichloroethylene, only the results for toluene are shown.

Table 1.: Comparing the results from different methods for various β-CD samples

Sample code	B018/14	B023/14	B036/14	B050/14
	Toluene mg/g	Toluene mg/g	Toluene mg/g	Toluene mg/g
Ph.Eur method	23 ± 4.5	9.6 ± 0.6	22 ± 4	1.2 ± 0.2
method I	22 ± 4.2	n.m.r*	21 ± 3.7	n.m.r*
method II	2.2 ± 0.5	n.m.r*	n.m.r*	n.m.r*
method III	21 ± 4	11 ± 0.4	n.m.r*	1 ± 0.2
method IV	23 ± 4.6	n.m.r*	n.m.r*	n.m.r*
method V	14 ± 3	n.m.r*	n.m.r*	n.m.r*

* no measured results

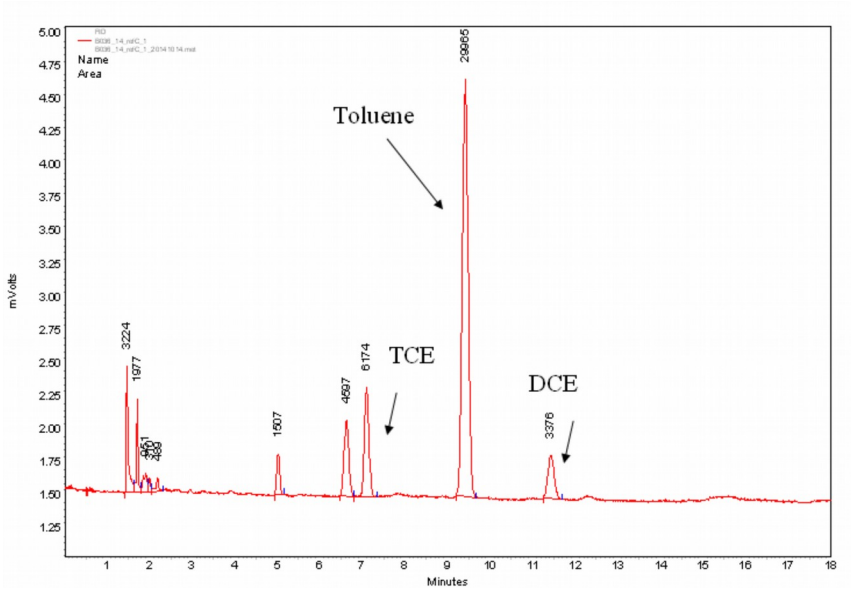

Figure 4. Typical GC-HS chromatogram of determination of the residual solvents in β-cyclodextrin according to Ph. Eur. method (DCE is dichloroethylene, the internal standard)

Sample is one of the calibration samples

Conclusion

1. As expected, the enzyme has not any influence on the determination. We got almost identical toluene content incubating the sample at 45 ^{o}C for 120 min with and without enzyme (Ph.Eur and method I.).

2. Incubation for 20 min at 45 ^{o}C without enzyme is not enough to reach the equilibrium head space concentration (method II). The measured toluene content is much less than the one obtained with Ph.Eur method.

3. Since the incubation at 45 ^{o}C for 20 min was not enough, the temperature was increased up to 90 ^{o}C (method III). Under these circumstances we got identical toluene content as with the Ph.Eur method.

4. When competitive guest sodium benzoate was added to the sample and incubated at 45 ^{o}C for 20 min (method IV) we got the expected toluene content.

5. Benzyl alcohol was tried as another competitor (method V), but it proved to be weaker complexing agent than sodium benzoate. The measured toluene content was less than that obtained with Ph.Eur method.

On the whole, there are three different ways to substitute the Ph.Eur. method. All of them skip the α-amylase, which has not any effect on the results. Incubation at 45 ^{o}C for 120 min (method I), incubation at 90 ^{o}C for 20 min (method III) and incubation at 45 ^{o}C for 20 min in

the presence of sodium benzoate (method IV) gave similar results. Method III was selected for further experiments. This method is the easiest to work with. There is no need either for enzymes or compexing agents, only the increased temperature ensures the dissociation of the complex and deliberation of the solvents. The method was tried with various β-cyclodextrin samples and the trend was the same: we measured toluene content identical with the official method (see Table 1).

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β- and γ-CD, Photoionization, Cycloreversion

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Flurbiprofen, Naproxen, Ethenzamide

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Antibodies, Immunoglobulin G (IgG)

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Viscosity decrease, Thixotropic index

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β-Cyclodextrin-ferrocene, Drug delivery systems, Star polymer 4-arm-poly(ε-caprolactone)-β-CD, Click reaction, β-CD with azide group

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Insent® taste sensing system, Orally disintegration tablet, Orodispersible tablet, Electronic tongue, Taste trial

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Freeze-drying, Liver cells

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Rutin-G, Hsp-G, Amorphous state, α-CD

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Antioxidant, Suppression of oxidative stress in mitochondria

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Coprecipitation method, Tissue revitalization, Wound healing, H1 NMR, FT-Raman, FT-IR/ATR

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Doxorubicin, γ-CD

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Gene trasfer, Condensation reaction, Transfection efficiencies, Rat alveolar macrophage cell line

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Carbodiimide activation, Inhibited fibrilization

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Semen samples, Methyl-β-cyclodextrin

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Semen sample, Cryopreservation

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β-CD, Antimicrobial activity, Electrospinning, Polyvinyl alcohol

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Superparamagnetic iron-oxide nanoparticles (SPION), Photodegradation, Nanocomposite, Bisphenol A

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Progesterone, Hot-melt polycondensation, Swelling capacity, Particle size

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Artificial photosynthetic solar cells, Gas sensors, Nonlinear optics, Molecular electronic devices, Cyclodextrin-based host-guest interactions

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TRIMEB, Screening of antioxidants, CD-DIPPMPO, Free radical, RAW macrophages

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Glassy carbon electrode, Single-walled carbon nanotubes, Recognition ability of β-cyclodextrin to Sudan I

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Attaching a β-cyclodextrin polymer, Analysis of glucose in human serum, Fluorescence, Magnetic polymer particle

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Glucose, Galactose, γ-CD

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Xenoestrogens, Spectrofluorimetry, Methyl-β-cyclodextrin, Unfolded partial least-squares

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Sensors for phosphoric acid derivatives, Coumarin as a fluorophore, Metal ion recognition, Anion recognition

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Ferrocene, Tandem duplex formation, Quencher, β-Cyclodextrin

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