

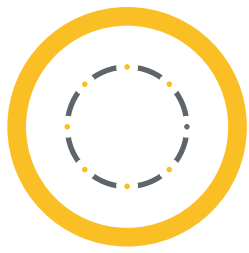


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Testing formulations at CarboHyde

How can we ensure the quality of our product?



CarboHyde's analytical portfolio

What analytical tests can be provided by CarboHyde for formulation purposes

	Complex stability (guest-CD)	Other Complex tests (guest-CD)	Purity inpurity	Structure assay	Degredation tests	Crystallinity	Amorf, polimorf tests	Guest quantity	Secondary force tests	Residual solvent content
HPLC	X		X					X		
NMR	X	X		X						
ESI-MS	X									
IR									X	
TGA										X
CE	X	X								
ICP-MS ICP-OES			X							

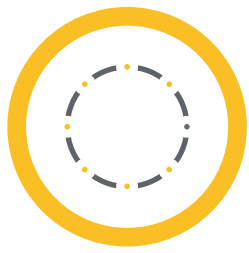


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Analytical methods

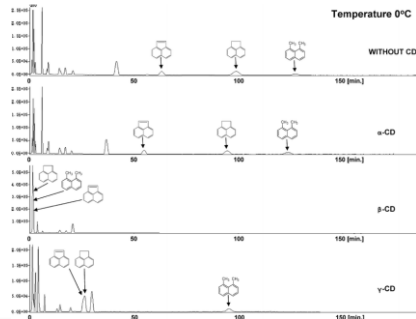
How can we ensure the quality of our product?



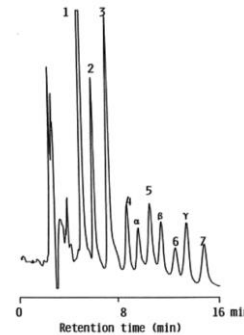
Chromatography methods

CarboHyde can provide services by HPLC

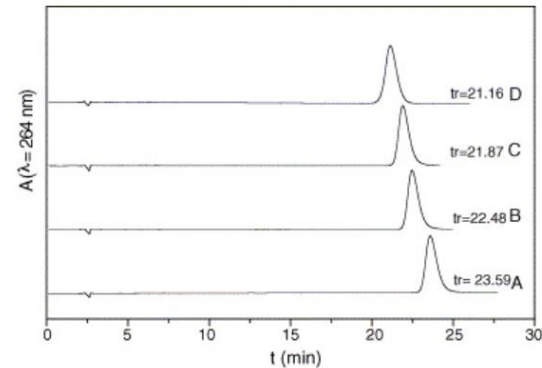
- Qualitative and quantitative determination of CD complexes
- CD content in biological samples or drug formulations
- Determination of impurities, purity, and assay in cyclodextrin samples
- API degradation and API degradation product monitoring



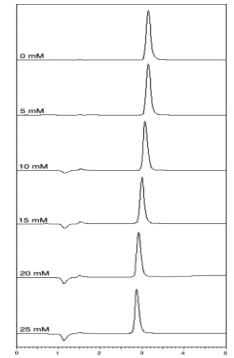
Separation of 1,8-dimethylnaphthalene, acenaphthene and acenaphthylene using mobile phases unmodified and modified with 10 mM native cyclodextrins at 0 °C



Chromatogram of the separation of malto-oligosaccharides and cyclodextrins.



Decrease in the retention time of pindolol in the presence of increasing concentrations of β -CD in the mobile phase



Decrease in Hidroxymethylnitrofurazone retention time in the presence of increasing concentrations of dimethyl- β -cyclodextrin (0, 5, 10, 15, 20, 25 mM)



HPLC in real life

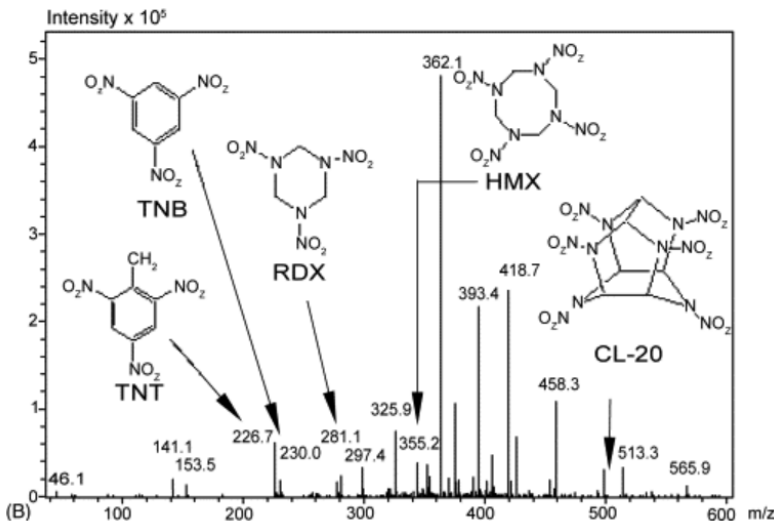
- 1) Zarzycki, Paweł Konrad, et al. "Interaction of native α -cyclodextrin, β -cyclodextrin and γ -cyclodextrin and their hydroxypropyl derivatives with selected organic low molecular mass compounds at elevated and subambient temperature under RP-HPLC conditions." *Analytical and bioanalytical chemistry* 391.8 (2008): 2793-2801.
- 2) Gazpio, Carmen, et al. "HPLC and solubility study of the interaction between pindolol and cyclodextrins." *Journal of pharmaceutical and biomedical analysis* 37.3 (2005): 487-492.
- 3) Berthod, Alain, et al. "Practice and mechanism of HPLC oligosaccharide separation with a cyclodextrin bonded phase." *Talanta* 47.4 (1998): 1001-1012.
- 4) <https://noguchi.ug.edu.gh/services/hplc-analysis/>
- 5) de Melo, Nathalie FS, et al. "Interaction between nitroheterocyclic compounds with β -cyclodextrins: phase solubility and HPLC studies." *Journal of pharmaceutical and biomedical analysis* 47.4-5 (2008): 865-869.



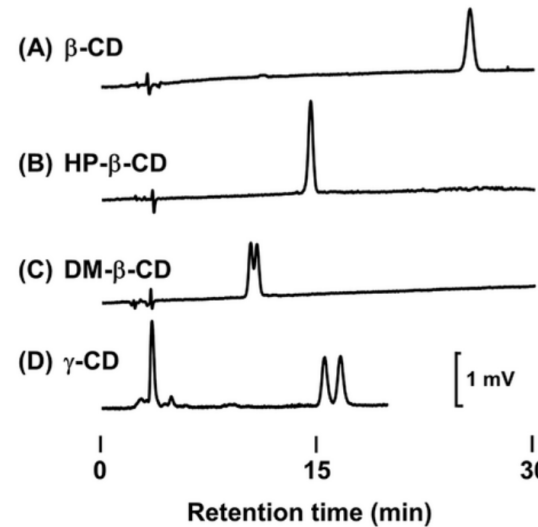
Chromatography methods

CarboHyde provides loads of different types of HPLC detectors

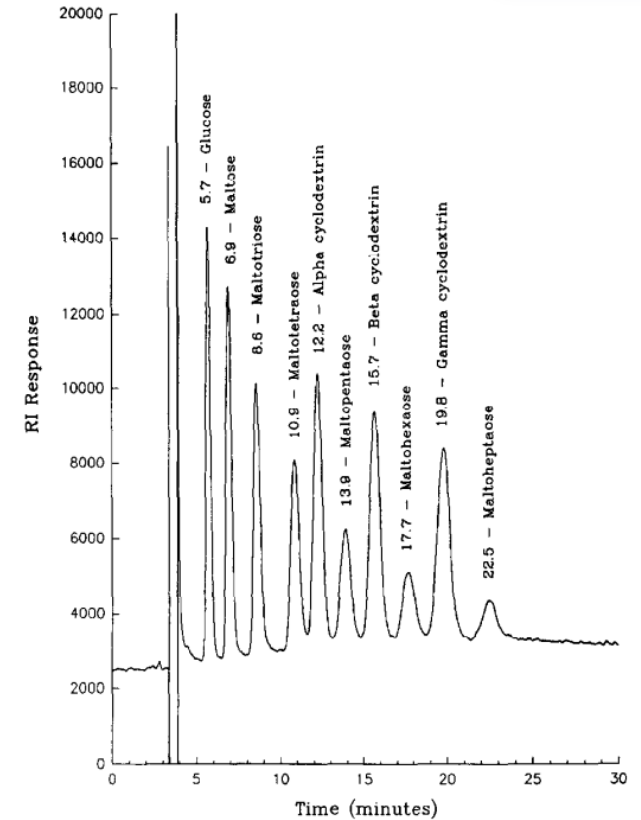
- Diode Array Detector (DAD)
- Refractive Index (RI)
- Mass Spectrometry (MS) detectors



Mass spectrum for the above infusion containing 10 mg/l TNT, TNB, RDX, HMX, and CL-20 (structures shown as inset)



Effect of the type of CD on the retention time and the resolution of HPLC analysis of racemic 2-cis,4-trans-abscisic acid (ABA)



RI detector made chromatogram. Chromatogram of γ -CD and nine potential synthetic pathway impurities

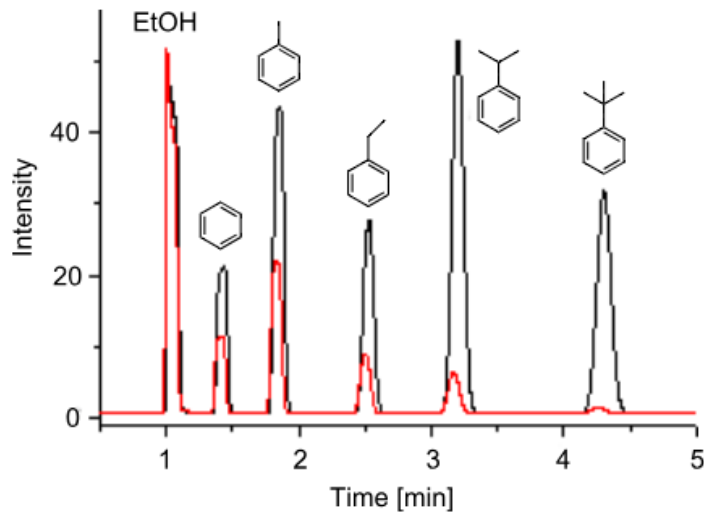
1) White, Gregory, et al. "Determination of the impurity profile of γ -cyclodextrin by high-performance liquid chromatography." *Journal of Chromatography A* 625.2 (1992): 157-161.
 2) Groom, Carl A., et al. "Detection of nitroaromatic and cyclic nitramine compounds by cyclodextrin assisted capillary electrophoresis quadrupole ion trap mass spectrometry." *Journal of Chromatography A* 1072.1 (2005): 73-82.
 3) Terashima, Hiroyuki, et al. "Direct chiral separation of abscisic acid by high-performance liquid chromatography with a phenyl column and a mobile phase containing γ -cyclodextrin." *Journal of Separation Science* 46.6 (2023): 6269-6277.



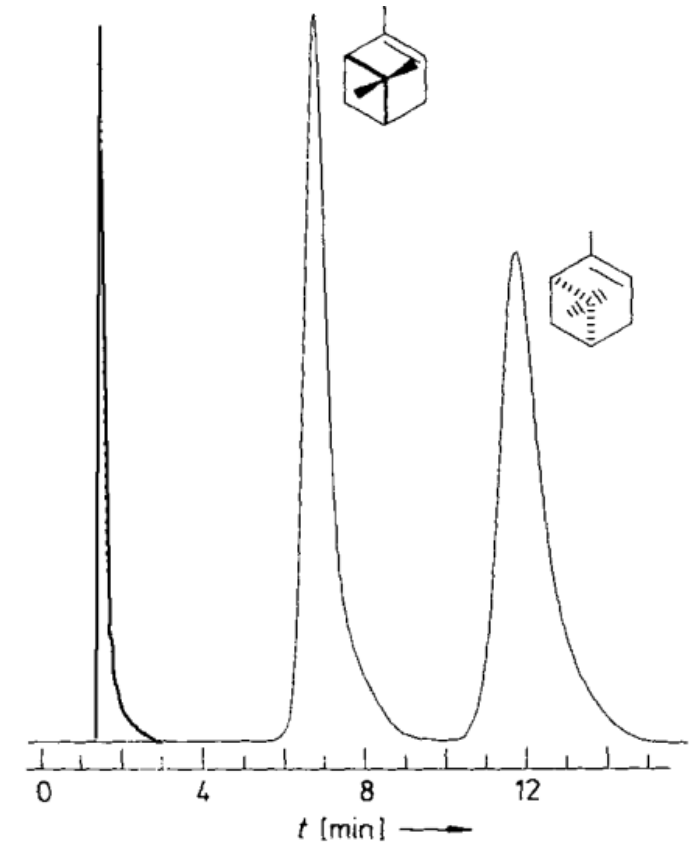
Chromatography

Gas chromatography (GC)

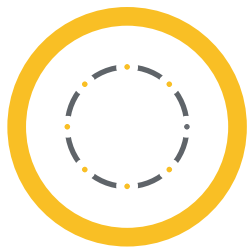
- Determination of volatile organic components, residual solvents, and volatile active ingredient content in cyclodextrin complexes
- Assessment of interaction strength of volatile guests with CDs



1) Chromatogram obtained by HS-GC for a mixture of aromatic guests (1 ppm) in water (black) and 10 mM solution of β -CD 1 (red).



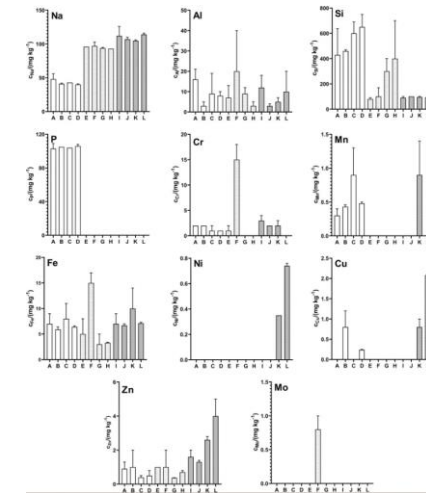
The GC-MS detector made chromatogram. Separation of the enantiomers of α -pinene on α -CD in formamide solution



Mass spectrometry and ICP methods

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

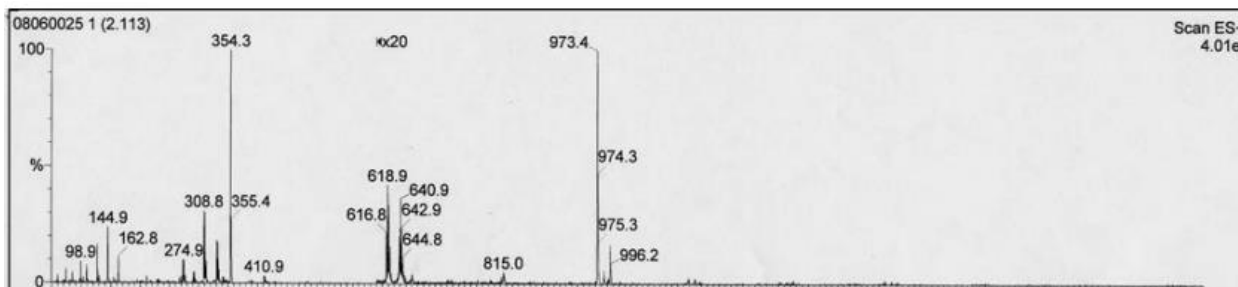
- Elemental impurities tests



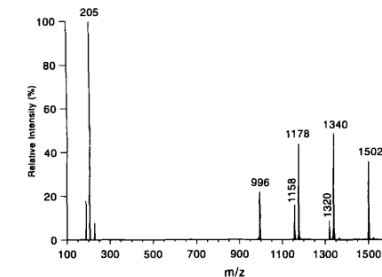
Elemental impurities detected by ICP-MS in the co-ground products prepared, where E) F) G) H) settings is containing HPβCD

Electron Spray Ionization - Mass spectrometry (ESI-MS)

- Identification of novel CD derivatives
- CD - guest complexes decomposition can be measured

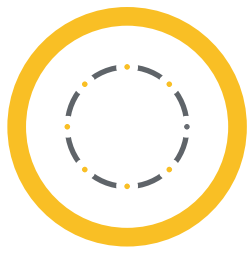


ESI mass spectrum of α -CD- α -chloralose complex



ESI mass spectrum of a solution that contained tryptophan α -, β -, γ -CDs

1) Ramanathan, Ragulan, and Laszlo Prokai. "Electrospray ionization mass spectrometric study of encapsulation of amino acids by cyclodextrins." *Journal of the American Society for Mass Spectrometry* 6.9 (1995): 866-871.
2) Jablan, Jasna, et al. "Product contamination during mechanochemical synthesis of praziquantel co-crystal, polymeric dispersion and cyclodextrin complex." *Journal of pharmaceutical and biomedical analysis* 238 (2024): 115855.
3) Jankowska, Agnes, Christopher K. Jankowski, and Julien B. Chiasson. "On chloralose-cyclodextrin complexes by ESI-mass spectrometry." *Journal of inclusion phenomena and macrocyclic chemistry* 52.3 (2005): 213-221.



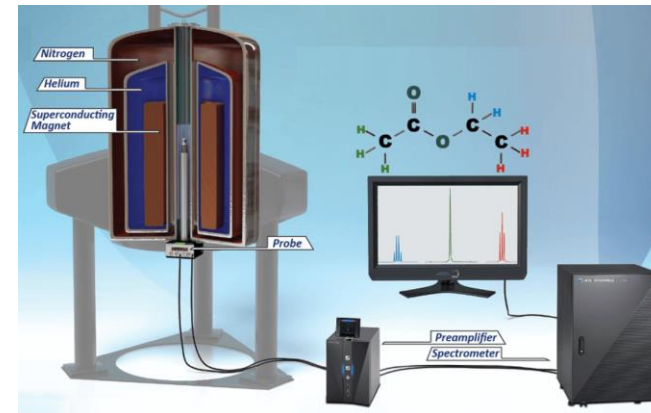
Nuclear Magnetic Resonance (NMR)

CarboHyde can provide services by NMR

- Structure identification <- chemical shifts (ppm) + peak height, types
- Determination of interaction strength between API and cyclodextrins
- Complexation efficiency
- Complex structure analysis
- Complex stoichiometry
- Complexation stability constant



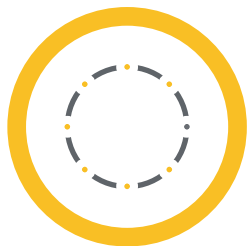
Same NMR, but they have different magnetic power (GHz)



The main parts of NMR

1) <https://www.bruker.com/en/products-and-solutions/mr/nmr/ascend-nmr-magnets.html>

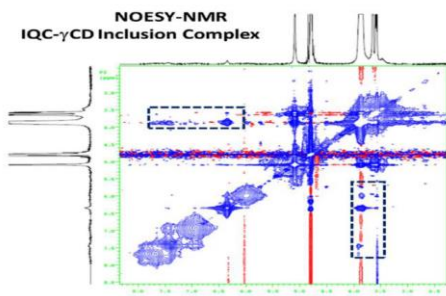
2) <https://www.jeolusa.com/RESOURCES/Analytical-Instruments/NMR-Basics>



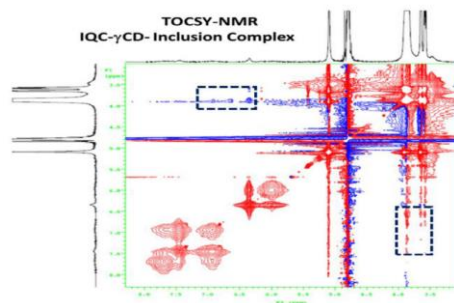
Nuclear Magnetic Resonance (NMR)

CarboHyde provides different type of NMR spectroscopy

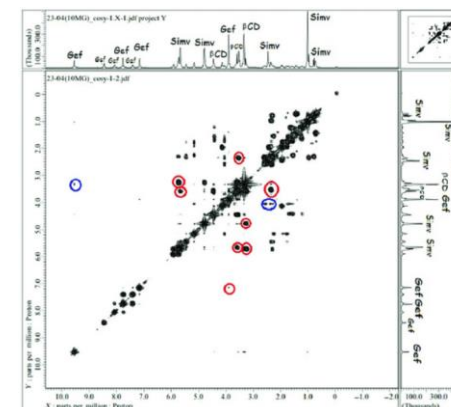
- Provided NMR spectroscopies:
 - *TOCSY (Total Correlation Spectroscopy)*
 - *ROESY (Rotating-frame nuclear Overhauser effect correlation spectroscopy)*
 - *COSY (Correlation Spectroscopy)*
 - (NOESY (only for small, or big molecules CarboHyde usually does not have to use it))



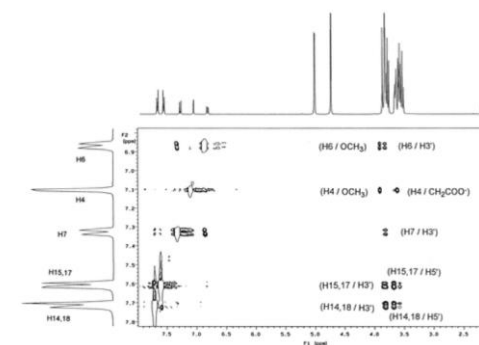
Profile display of 2D-NOESY nuclear magnetic resonance spectra of isoquercitrin- γ CD inclusion complex.



Profile display of 2D-TOCSY nuclear magnetic resonance spectra of isoquercitrin- γ CD inclusion complex.

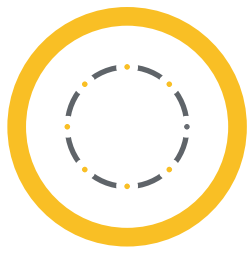


2D NMR (COSY) spectra of the inclusion complex. The red circles indicate β CD and simvastatin coupling while blue circles represent β CD-gefitinib and gefitinib-simvastatin coupling.



2D ROESY spectrum of indomethacin and β CD

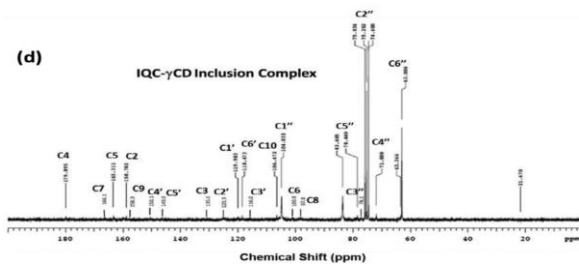
- 1) Kapoor, Mahendra P., et al. "Structural elucidation of novel isoquercitrin- γ -cyclodextrin (IQC- γ CD) molecular inclusion complexes of potential health benefits." *Carbohydrate Polymer Technologies and Applications* 2 (2021): 100046.
- 2) https://www.researchgate.net/figure/D-NMR-COSY-spectra-of-the-inclusion-complex-The-red-circles-indicate-b-cyclodextrin_fig2_317784033
- 3) Fronza, G., et al. "1H NMR and molecular modeling study on the inclusion complex β -cyclodextrin- indomethacin." *The Journal of Organic Chemistry* 61.3 (1996): 909-914.



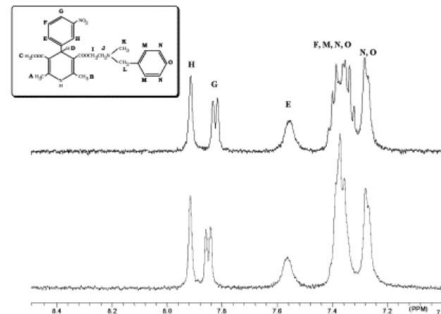
Nuclear Magnetic Resonance (NMR)

CarboHyde provides different type of NMR spectrum

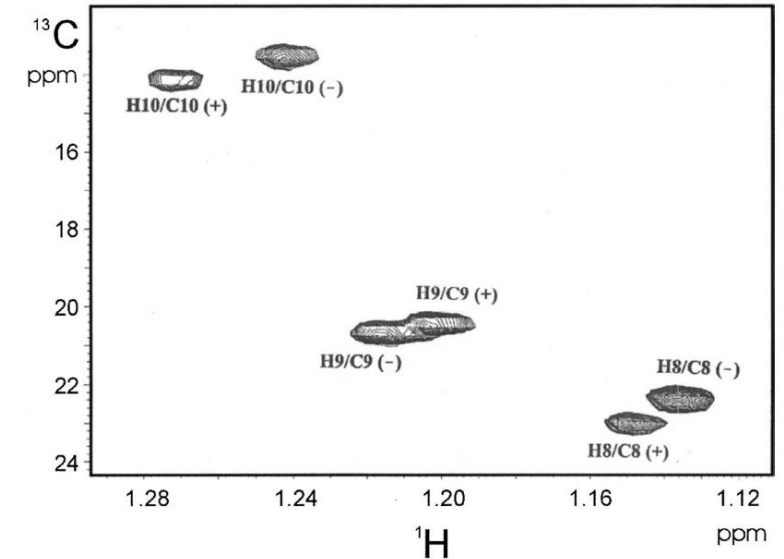
- Provided NMR spectrums:
 - ^1H (Hydrogen-1 spectrum): 1D NMR technique
 - ^{13}C (carbon-13 spectrum): 1D NMR technique
 - HSQC (Heteronuclear Single Quantum Coherence): 2D NMR technique
 - HMBC (Heteronuclear Multiple Bond Correlation): 2D NMR technique



^{13}C -NMR spectra of isoquercitrin- γ CD inclusion complex.



^1H NMR spectra of nicardipine hydrochloride in the absence (above) and presence of β CD (below).



Part of the 2D spectrum of ^1H and ^{13}C chemical shifts for methyl groups in complexes of camphor enantiomers with α CD

- 1) Kapoor, Mahendra P., et al. "Structural elucidation of novel isoquercitrin- γ -cyclodextrin (IQC- γ CD) molecular inclusion complexes of potential health benefits." *Carbohydrate Polymer Technologies and Applications* 2 (2021): 100046.
- 2) Fernandes, Catarina M., et al. "Multimodal molecular encapsulation of nicardipine hydrochloride by β -cyclodextrin, hydroxypropyl- β -cyclodextrin and triacetyl- β -cyclodextrin in solution. Structural studies by ^1H NMR and ROESY experiments." *European Journal of Pharmaceutical Sciences* 18.5 (2003): 285-296.
- 3) Dodziuk, Helena, Wiktor Koźmiński, and Andrzej Ejchart. "NMR studies of chiral recognition by cyclodextrins." *Chirality: The Pharmacological, Biological, and Chemical Consequences of Molecular Asymmetry* 16.2 (2004): 90-105.

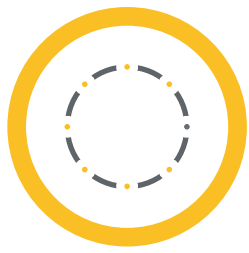


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Analytical other useful method results

How can we ensure the quality of our product?



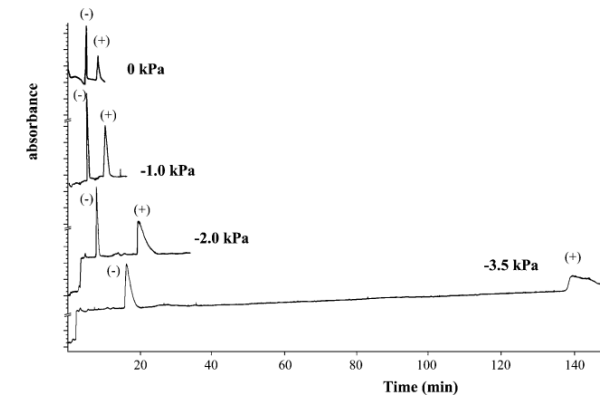
Other analytical tools

Capillary electrophoresis (CE)

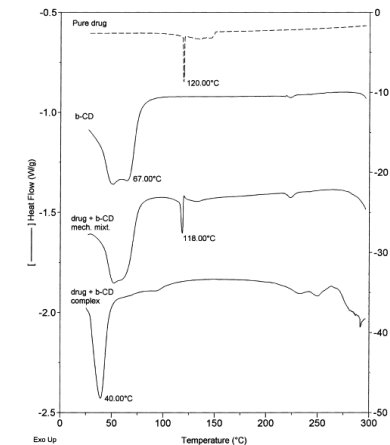
- Determination of interaction strength between API and cyclodextrins
- Residual ionic compounds
- Average degree of substitutions of charge cyclodextrins
- Chiral separations

Thermogravimetric analysis (TGA)

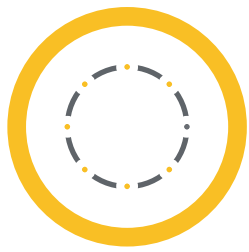
- Residual solvent content <- mass decrease from temperature rise
- Thermal degradation
- Content of hydrates



Effect of increasing counterpressure on the separation of (±)-chlorpheniramine in the presence of CM-β-CD.



Thermoanalytical profile of a drug-β-CD system. From top: pure components, mechanical mixture and inclusion compound.



Other analytical tools

Infrared spectroscopy (IR)

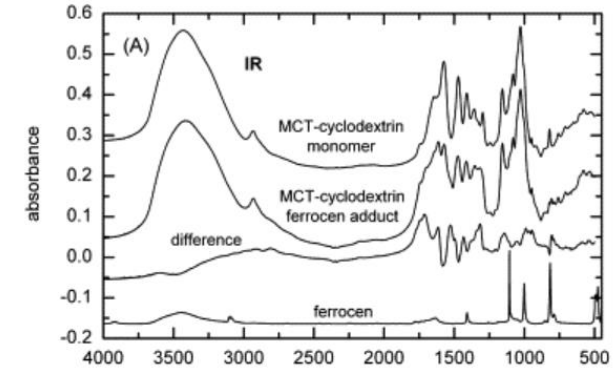
- Quality and reaction control
- Validation

Karl Fischer titration

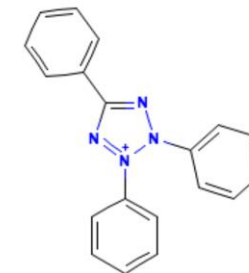
- It is substantial for all of the CDs complexes water containment measurements
- Precise and reliable

Reducing sugar

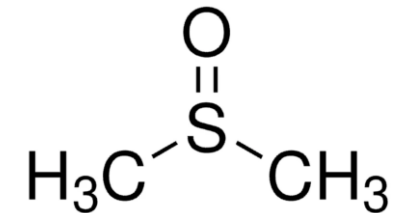
- Testing for degradation
- Maximum limitation: 0,05%
- test solution: triphenyltetrazolium chloride R
methanol R, dimethyl sulfoxide R, sodium hydroxide R ¹



Infrared Raman spectra of a reactive cyclodextrin derivative monomer (monochlorotriazinyl-β-cyclodextrin sodium salt) and its ferrocene inclusion complex..

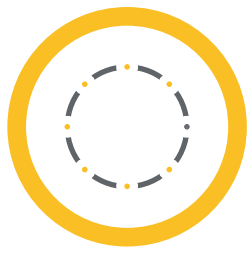


Triphenyltetrazolium chloride



Dymethyl-sulfoxid

1) ¹ Monograph of Sulfbuthylbetadex Sodium in Ph.Eur.11.4.
2) https://www.sigmaaldrich.com/HU/hu/product/sigma/d2438?utm_source=google&utm_medium=cpc&utm_campaign=21891572337&utm_content=171292948458&gad_source=1&gad_campaignid=21891572337&gclid=CjwKCAjw7fzDBhA7EiwAOqJkhz4nfNFoZkR99mY4TquBaXsY7mspTswcPgnWWj9KcGt7EJoIAsdBUBoCk-cQAvD_BwE
3) <https://pubchem.ncbi.nlm.nih.gov/compound/9283#section=2D-Structure>
4) Heise, H. M., et al. "Infrared spectroscopy and Raman spectroscopy of cyclodextrin derivatives and their ferrocene inclusion complexes." *Vibrational Spectroscopy* 53.1 (2010): 19-23.



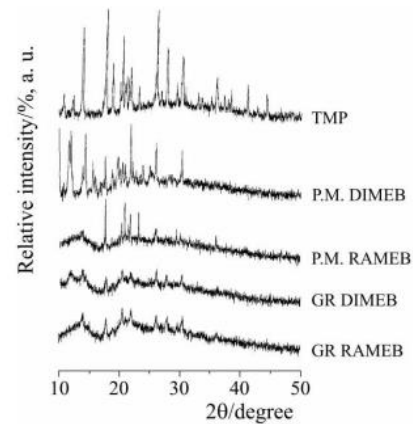
Other analytical tools

Isothermal Titration Calorimetry (ITC)

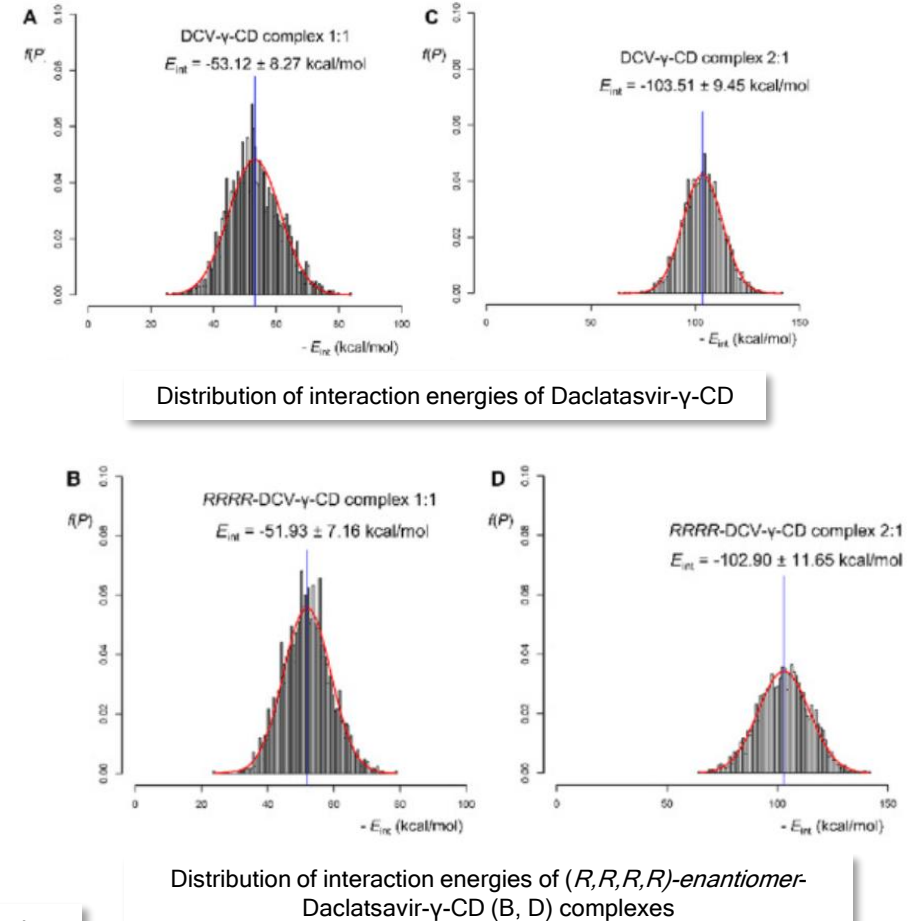
- Complex stability constant
- Complexation efficiency
- Complex stoichiometry

Powder X-Ray Diffraction (XPRD)

- Crystallinity
- Amorphisation of the complexes



Powder X-ray diffraction patterns of pure trimethoprim (TMP) and its equimolar physical mixtures (P.M.) and ground mixtures (GR) with DIMEB and RAMEB

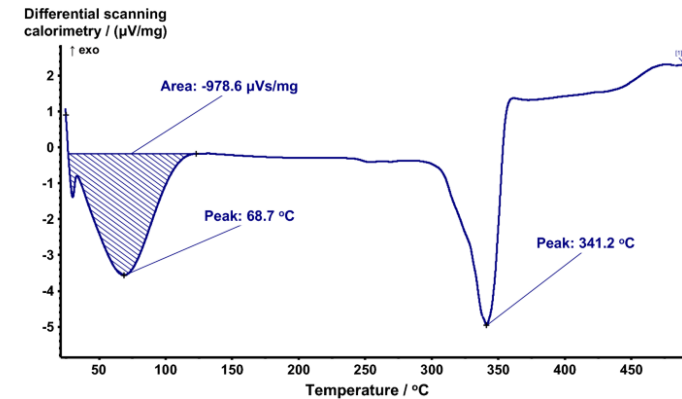




Other analytical tools

Differential Scanning Calorimetry (DSC)

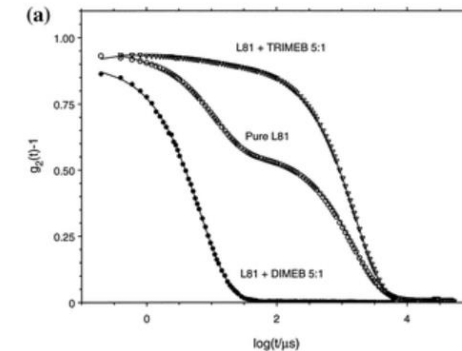
- Crystallinity
- Polymorph of the complex structure



Differential scanning calorimetry analysis of 2-hydroxypropyl- β -cyclodextrin.

Dynamic, Static Light Scattering (DLS/SLS)

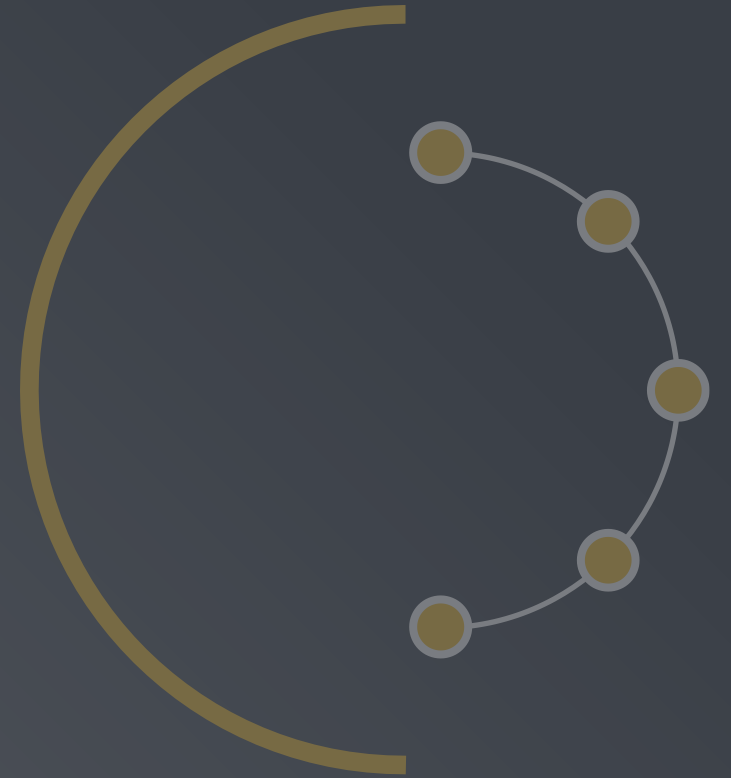
- Complex diameter
- Complex diameter distribution



DLS correlograms for L81 (surfactant) 1% at 16 °C in presence of DIMEB and TRIMEB at molar ratio 5:1.



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For any questions:

balazs.kondoros@carbohyde.com