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Evaluation of a liquid chromatography method for quality control of methylated cyclodextrins

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ABSTRACT

Halo C18 column (fused core particles) and Chromolith RP18 column (monolith) were evaluated in liquid chromatography in order to analyze methylated-β-cyclodextrins (Me-β-CD) with various degrees of substitution, DS such as the number of methyl groups per cyclodextrin ring. Chromolith RP18 enables a performing analysis of Me-β-CD with low DS but is not suitable for dimethyl-β-cyclodextrins (DM-β-CD). On the other hand, Halo C18 column allows an improved fingerprint of CDs having a DS from 4.9 up to a value major than 14 and avoiding the use of various chromatographic systems. Thus, liquid chromatography performed with this column and an evaporative light scattering detector can be used as a generic system for methylated CD analysis. Moreover, fused core particles of Halo C18 column enables a rapid analysis and liquid chromatography coupled with electrospray-mass spectrometry appears as a powerful tool to determine co-elution and to characterize various isomers of complex methylated-β-cyclodextrin mixtures.

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1. Introduction

Cyclodextrins (CDs) are hydrophilic cyclic oligosaccharides with a conical cylinder shape presenting hydrophobic cavities and are used as hosts to obtain the formation of inclusion complexes to improve the solubility, stability and bioavailability of poorly soluble guest molecules in pharmaceutical formulations [1–4]. Moreover they exhibit enantiodiscriminating properties due to the chirality of the single glucose units [5,6]. Modified β-cyclodextrins are synthetized to increase these properties. In fact, the derivatization of the secondary and/or primary hydroxyl groups in native CDs induces a distortion in the CD structure and the presence of substituents on the rim of the CD cavity significantly changes their complexing and enantiodiscriminating properties. Methylations of the native β-CD are widely used in order to increase their complexation power and aqueous solubility. However the methylation is seldom selective then commercially available methylated-β-CDs are therefore mixtures containing various degrees of substitution (DS) defined by the number of substituents by cyclodextrin ring. For example, DS is about 14 for dimethyl-β-CDs (DM-β-CDs) that possess an average of 14 methyl groups while Me-β-CD named Kleptose® Crysmeb has an average DS in the range 2.8–4.9 (less than 7 methyl groups by CD).

Methylated CD derivatives as heptakis (2,6-di-O-dimethyl)-β-CD and randomly methylated β-CD (RAMEB) are considered safe and the best solubilizers for poorly water-soluble drugs [7]. RAMEB is a mixture of isomers/homologues sometimes preferred because it can be produced with more affordable prices [8]. If the 3 hydroxyl groups were quasi-equivalent on the 7 glucose units, there are 2^{21} i.e., 2,097,152 possible isomers. As the reactivity between the primary hydroxyl group, located on the wider opening of the conical cylinder and secondary one located on the narrower opening is different, the number of isomers is actually lower, for example, it is reduced at 16,384 isomers for 2 secondary hydroxyl groups.

Consequently, the nature of the methylated hydroxyl groups (primary or secondary), their position in the rim of the cavity and the number of potential isomers can induce a variability of the complexation properties. It is why the appropriate characterization of the methylated CD mixture is important. A good knowledge of the CD mixture composition is a prerequisite for a better control of the inclusion complex with many guest molecules when they are used in various fields as pharmaceutical drug carriers [2–4], or as chiral selector [5,6,9].

Mass spectrometry (electrospray or MALDI-TOF) enables only to determine the substitution pattern and the average DS of the Me-β-CD crude mixture [10] and all isomers of the same molecular weight are measured together. In the same manner, if ^{1}H NMR allows the exploration of the inclusion mode of CD–drug complexes, the analysis of various modified CD confirms only the DS by comparing the integration area of these protons [10,11]. Only 2D NMR allows

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the determination of their stereochemistry [12] but this technique becomes difficult to use as control of these complex mixtures from batch to batch.

If thin-layer chromatography with detection by derivatization is a simple and fast method for the separation of native cyclodextrins (α, β and γ) [13,14] and the comparison of patterns of methylated CDs [9,15], it remains less efficient for evaluating many isomers/homologues of these CDs. It is why liquid chromatography (LC) has been used to obtain a relatively detailed fingerprint of various substituted CD derivatives [16–18]. But a lot of chromatographic systems should be tested in order to improve the selectivity. Thus, for DM-β-CD (DS about 14), a comparison of various kinds of C8 and C18 stationary phases (Supelco ABZ+, HiPURITY Elite, Asahipak ODP 50, Lichrospher RP 18 and Lichrospher RP 18e, Lichrospher RP Select B, Spherisorb ODS 1 and ODS 2, Nucleosil 50-5 C8) has shown that the best fingerprint was obtained on Nucleosil 50-5 C8 phase that affords the most polar secondary interactions with acetonitrile–water as mobile phase [17]. Moreover the weakly substituted Me-β-CD Crysmeb presents a range of polarity of components larger than that of DM-β-CDs mixture and requires LC in gradient elution [16] while the components of DM-β-CDs are separated in isocratic mode [17]. Previously, Purospher Star RP-18 has been the most suitable for the analysis of the low methylated β-CDs [12] because its endcapping improves strong hydrophobic and decreases hydrophilic interactions whereas Nucleosil-50-5-C8 was preferred for DM-β-CDs [17] because both hydrophobic and polar interactions are reinforced. Finally, a specific stationary phase was used to analyze RAMEB [18].

The aim of this work was to investigate a generic chromatographic system enabling to replace the set of several columns required to analyze different methylated CDs with various DS. This system may enable to control the quality of these complex mixtures that can differ from manufacturer to manufacturer and sometimes from batch to batch.

In this paper the performances of Halo C18 and Chromolith RP18 columns were evaluated in order to bring out a new generic chromatographic system coupled with evaporative light scattering detection (ELSD) required by the lack of chromophore group for these compounds. In fact ELSD affords a suitable detection with a good sensitivity [19] to avoid overload injections which strongly influence the peak shape and the retention measurements [14]. The Halo material exhibits high efficiencies with its fused-core particle technology [20] while the second one, based on monolith, is known to enable high flow-rates without loss of performances. Then, the experimental conditions in LC-ELSD can be transposed in liquid chromatography–electrospray ionization mass spectrometry (LC–ESIMS) in order to control the co-elution and the characterization of each peak.

2. Experimental

2.1. Reagents

Analytes: dimethyl-β-cyclodextrin (DM-β-CD, Fluka, Saint Quentin Fallavier, France).

Me-β-CD (Kleptose® Crysmeb Exp, Lab 3487) was obtained from Roquette (Lestrem, France). Its low degree of substitution was produced through selective methylation of β-CD using a technology patented by Roquette.

RAMEB obtained from Wacker Chemie GmbH (Burghausen, Germany) was a complex mixture of dimethylated-β-cyclodextrins with under- and overmethylated homologues.

Stock standard solutions for each of the analytes were prepared by diluting the powders in an appropriate volume of deionized water to obtain the desired concentrations in the range of 0.75–2.0 g/L. All the solutions were stored at 4 °C in the darkness for up to 5 weeks.

HPLC-grade solvents were used, supplied by either Carlo Erba (i.e., acetonitrile, methanol). Deionized water was produced with Elga stat UHQ II system (Elga, Antony, France) under conductivity of 18.0 MΩ/cm.

2.2. Chromatographic column

The following columns were used: Chromolith RP 18 (100 mm × 4.6 mm I.D.) from Merck (Darmstadt, Germany), Halo C18 (50 mm × 4.6 mm I.D.) from Interchim (Montluçon, France) and Purospher Star RP18 (125 mm × 4 mm, 5 μm) from Merck. Specific surface area of Chromolith support was 300 m^2/g and similar to that of Purospher (330 m^2/g) with the same carbon content (17%) and coverage density (about 3 μmol/m^2). Halo C18 solid core particle (1.7 μm diameter) with a 0.5 μm porous silica layer fused to the surface had a smaller specific surface area (150 m^2/g) and the monomeric bonding had a coverage density 3.5 μmol/m^2.

2.3. Chromatographic conditions

The chromatographic system consisted of a model L-2130 Elite Lachrom quaternary pump (VWR), a Rheodyne model 7125 injection valve (Berkeley, CA, USA) equipped with a 20 μL loop, a CROCO-CIL™ column oven model CCIV (CIL-Cluzeau, Ste-Foy-la-Grande, France), a Sedex model 85 Low Temperature Evaporative Light Scattering Detector (LT-ELSD, S.E.D.E.R.E, Alforville, France). The LT-ELSD detector settings were as follows: drift tube temperature, 40 °C; nebulizer gas pressure, 2.2 bar; photomultiplier, 7. Data were performed using EZChrom Elite version 3.2.1 software (Merck, Darmstadt, Germany).

The ratio (36:64) of the acetonitrile–water mobile phase in isocratic elution was adjusted from previous works on several columns [17]. For Me-β-CD and RAMEB, experiments were achieved with an acetonitrile–water mixture in gradient elution. As various flow-rates were evaluated, gradient times were adjusted in order to keep a similar gradient slope.

2.4. Mass spectrometry

ESI-MS: experiments were carried out on a Sciex API 3000 triple quadrupole mass spectrometer, Applied Biosystems (Forster City, CA, USA) operating in positive mode with a TurboIonSpray source. Nitrogen was used as nebulizer and curtain gas. Spectrometer parameters were as follows: nebulizer gas flow-rate, NEB = 8 (1.2 L/min), curtain gas flow-rate, CUR = 8 (1.2 L/min), ionspray voltage, IS = 5000 V, source temperature, TEM = 200 °C, declustering potential, DP = 30 V, focusing potential, FP = 200 V, entrance potential, EP = 10 V. The dwell time and the pause time were 100 ms and 2 ms, respectively. Each spectrum was the sum of 50 scans in the range 1250–1450 amu for DM-β-CD, 1100–1330 amu for Me-β-CD and 1220–1420 amu for RAMEB with a step size of 0.5 amu. Sample solutions (1 mg/L) were pumped at 50 μL/min by a Harvard model 11 syringe pump (Instech Laboratories, Plymouth Meeting, PA, USA). Data were acquired with the Analyst version 1.4.2 software from Applied Biosystems (Forster City, CA, USA).

ESI-MS infusion: 1 mg/L in 36:64 acetonitrile–water + 5 mM ammonium acetate for DM-β-CD, 1 mg/L in 5:95 acetonitrile–water + 5 mM ammonium acetate for Me-β-CD and RAMEB.

LC–ESI-MS: analyses were carried out using an Agilent 1100 apparatus (Santa Clara, CA, USA) equipped with a sample loop of 20 μL. Mobile phase was a mixture of water, acetonitrile and 5 mM ammonium acetate. Addition of ammonium acetate does

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