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Journal of Chromatography B, 720 (1998) 171–178

JOURNAL OF CHROMATOGRAPHY B

Determination of 2-hydroxypropyl-γ-cyclodextrin in plasma of cynomolgus monkeys after oral administration by gas chromatography–mass spectrometry

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Received 6 July 1998; received in revised form 15 September 1998; accepted 24 September 1998

Abstract

This report describes a specific and highly sensitive gas chromatography–mass spectrometry (GC–MS) assay for the analysis of industrially produced 2-hydroxypropyl-γ-cyclodextrin, a heterogeneous mixture of homologues and isomers, in plasma of cynomolgus monkeys. Instead of analyzing the polysaccharide mixture as a whole, in a first step the HP-γ-cyclodextrin mixture, together with the internal standard (2,6-di-*O*-methyl-β-cyclodextrin), was deuteromethylated, and in a second step hydrolyzed with hydrochloric acid to the respective monosaccharides. The resulting reaction mixture was trimethylsilylated to 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose (representative for HP-γ-CD) and 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose (representative for the internal standard), respectively, and analyzed by GC–MS. The limit of quantification of this assay was 20 nmol/l. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: 2-Hydroxypropyl-γ-cyclodextrin

1. Introduction

Cyclodextrins (CDs) are cyclic, toroidally shaped oligosaccharides formed from six to eight glucopyranose units. Their spatial conformation enables them to form inclusion complexes with lipophilic compounds. They can be applied in drug formulation for complexation. In liquid formulations cyclodextrin-complexation has the advantage that organic solvent free stable aqueous solution of water-insoluble drugs can be formed and secondly, that side effects as local irritations or hemolytic reactions can be reduced.

Industrially produced 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD), which is used as a complexing agent in new ophthalmic formulations [1], is not a single compound, but a heterogeneous, complex mixture of homologues and isomers, substituted by a variable number of 2-hydroxy-propyl groups at different positions of the sugar moieties (see Fig. 1). The average number of hydroxypropyl groups per CD unit can be determined by spectroscopic methods, but an unambiguous and sensitive quantitative detection of the polysaccharide mixture as a whole,

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PII: S0378-4347(98)00445-9

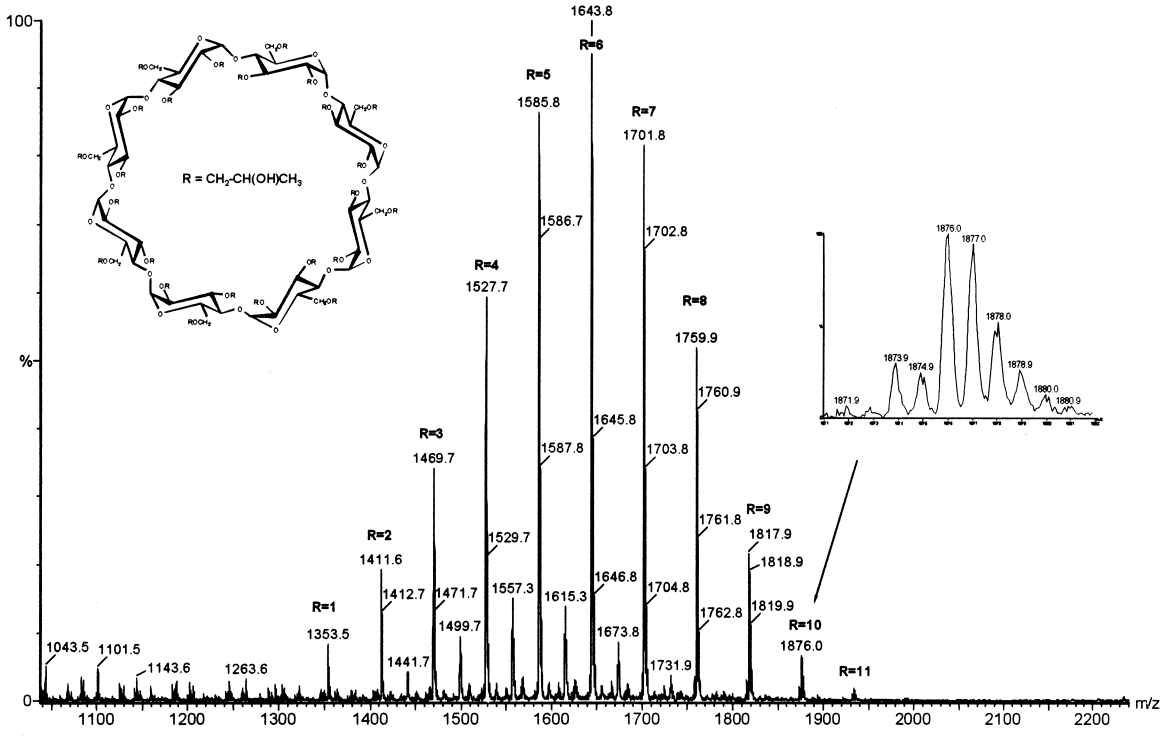


Fig. 1. Negative electrospray (ES) mass spectrum of technical HP-γ-CD.

in order to perform sensitive pharmacokinetic studies, was not feasible hitherto.

In this investigation we want to report an analytical method to determine HP-γ-CD by GC–MS in the plasma of cynomolgus monkeys after oral administration.

The limit of quantification (LOQ) of this assay was 20 nmol/l, depending on the sample volume. The GC–MS method was comparably sensitive than the size-exclusion chromatographic method reported on a similar analytical problem (determination of HP-β-CD [2]).

2. Experimental

2.1. Reagents

HP-γ-CD was purchased from Wacker, Burgdorf, Germany. The internal standard 2,6-di-*O*-methyl-β-CD, dry dimethylsulfoxid (DMSO), *N*,*O*-bis-(trimethylsilyl)-trifluroacetamide (BSTFA)+1% trimethyltrichlorosilane, acetonitrile, pyridine, chloroform and sodium hydroxide were purchased from Fluka, Buchs, Switzerland. Deuromethyliodide was purchased from Dr. Glaser, Basel, Switzerland.

2.2. Formulation

HP-γ-CD was dissolved in sterile isotonic (300 mOsm/l) saline solution. Ten ml of this solution (=200 mg HP-γ-CD/kg body mass) was administered orally by gavage to cynomolgus monkeys.

2.3. Sample preparation

2.3.1. Sample collection and storage

Blood samples from cynomolgus monkeys were taken 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24 and 48 h after oral administration and stored in polypropylene tubes containing lithium heparin as anticoagulant.

All samples were frozen at −20°C until derivatization and subsequent GC–MS analysis.

Prior to the sample preparation procedure the

samples were spiked with the internal standard (2,6-di-*O*-methyl-β-CD).

2.3.2. Preparation of plasma samples, derivatization of HP-γ-CD

One hundred and fifty μl of plasma sample was spiked with 25 ng of 2,6-di-*O*-methyl-β-CD and then mixed with 150 μl acetonitrile. The mixture was frozen at −5°C for 1 h in order to precipitate proteins. The opaque solution was then centrifuged (2800 *g*), the supernatant was evaporated to dryness and taken up in 100 μl DMSO. The insoluble residue was centrifuged again for 10 min, and the supernatant was mixed with 20 mg pulverized sodium hydroxide (NaOH) and 100 μl deuteromethyliodide (CD_3I). After stirring for 10 min the deuteromethylation was stopped by adding 350 μl water. The reaction mixture was extracted with 300 μl chloroform ($CHCl_3$). The separated organic phase was washed three times with 350 μl water and evaporated to dryness [3]. The perdeuteromethylated residue was hydrolyzed with 100 μl of 6 *M* hydrochloric acid (HCl) for 1 h at 70°C. The reaction mixture was then evaporated to dryness. The dry residue was dissolved in 10 μl acetonitrile, 10 μl pyridine and trimethylsilylated with 50 μl BSTFA. The mixture was kept at 70°C for 10 min. Aliquots of 1 μl were used for GC–MS analyses.

The general reaction scheme of the derivatization of 2,6-di-*O*-methyl-β-CD (=internal standard) and of HP-γ-CD is shown in Fig. 2.

2.3.3. Preparation of the calibration samples

Two ml cynomolgus monkey blank plasma was used to prepare the calibration samples. The respec-

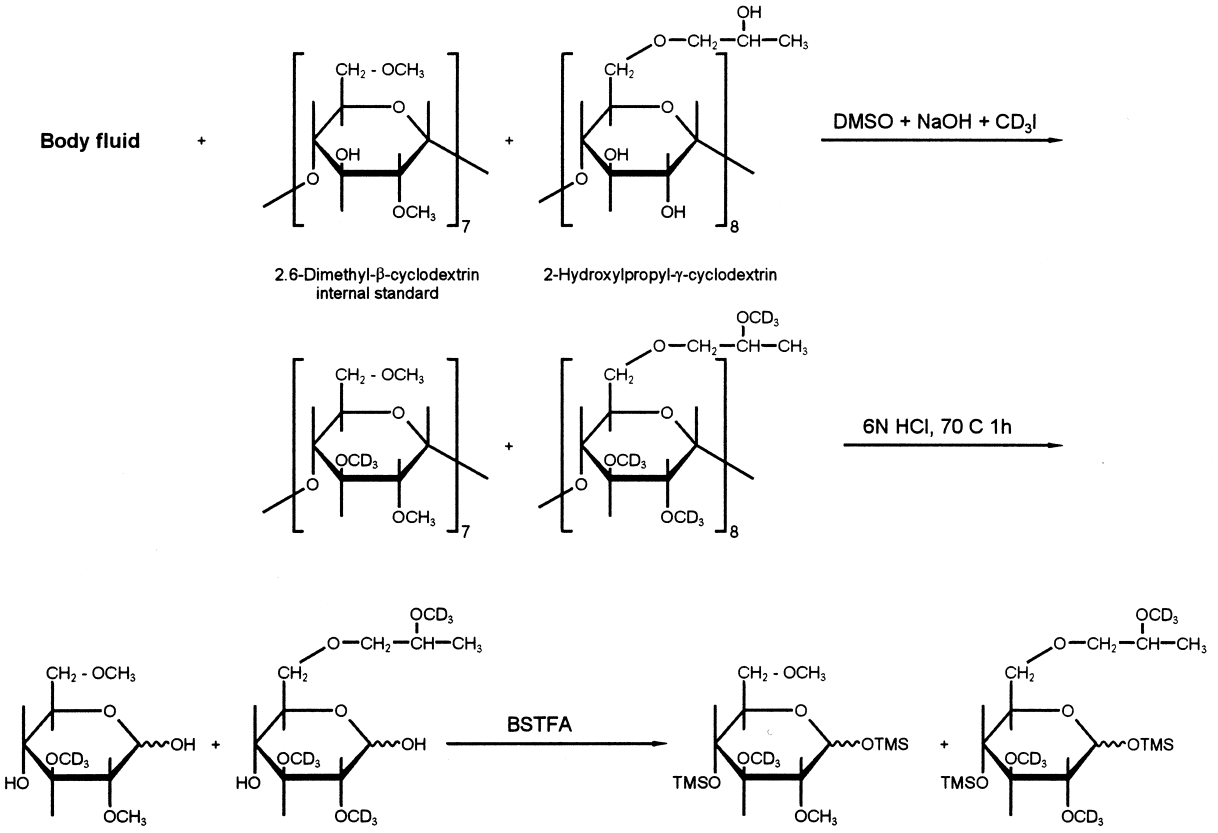


Fig. 2. Derivatization of the internal standard and of HP-γ-CD to 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose and 1,4-bis-(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose, respectively.

tive preparation methods were identical to those described for the plasma samples (see Section 2.3.2).

2.4. Gas chromatography–mass spectrometry

The gas chromatographic separation was performed on a Fractovap 4160 gas chromatograph (Carlo-Erba, Milan, Italy) and a laboratory-made 10 m×0.2 mm capillary column coated with the medium polar dimethyl/diphenylpolysiloxane stationary phase SDPE-08 as described previously [4]. All samples were introduced by cold on-column injection. In the analyses of HP-γ-CD, the column temperature was programmed from 70°C to 200°C at a rate of 8°C/min and from 200°C to 350°C at a rate of 20°C/min. Hydrogen was used as carrier gas.

The column was linked by a programmable, high-temperature coaxial glass interface [5] to a 4600 quadrupole mass spectrometer (Finnigan/MAT, San Jose, CA, USA). The ionization technique employed was chemical ionization (CI) with ammonia as reagent gas. The ion source temperature was 150°C. The transfer line was kept at 320°C.

The mass spectra of 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose and 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose are shown in Figs. 3 and 4. Quantitative analysis of HP-γ-CD was performed by selected ion monitoring over the molecular ion adducts at $m/z=387$ $[(M+NH_4)^+]$ and $m/z=451$ $[(M+NH_4)^+]$, respectively (see Fig. 5).

2.5. Method validation, recovery and linearity

Method validation was performed with each series by analysis of blank plasma samples spiked with known amounts of HP-γ-CD (see Section 2.3.3). The validation samples covered the concentration range between 20–1000 nmol/l.

The usual estimation of the recovery could not be realized due to the polymeric nature of both the parent compound and the internal standard. For the sample preparation it is unavoidable to hydrolyse and derivatize both compounds in the same manner.

Consequently, any fixed values get lost. Therefore, the calibration curve was prepared in blank plasma as well.

In the calibration curve shown in Fig. 6 the peak area ratios of 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose and 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-

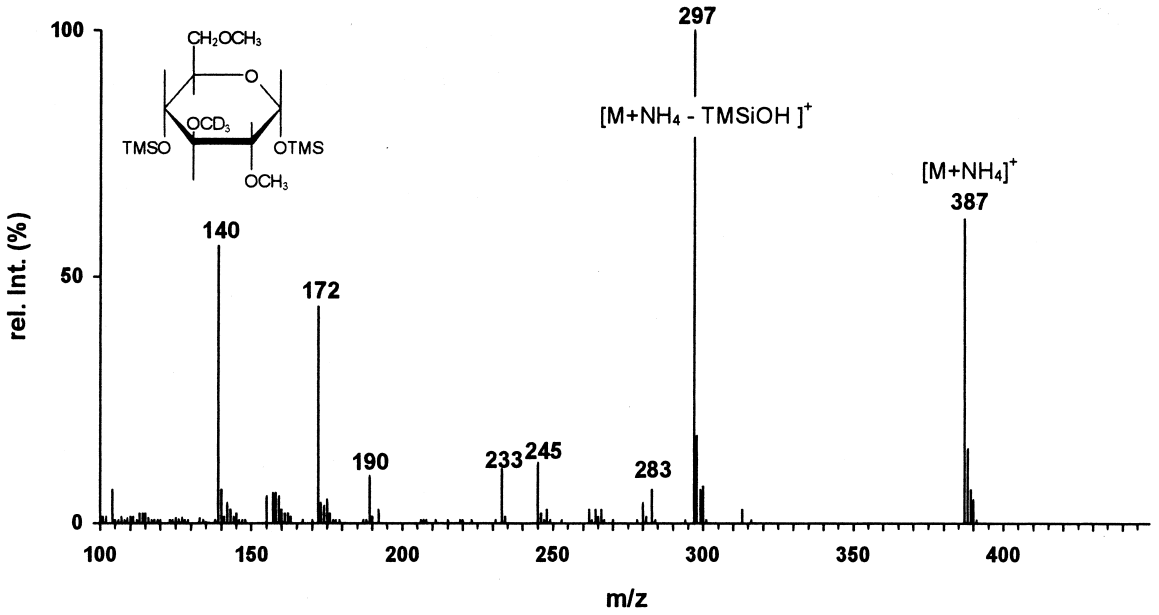


Fig. 3. CI/NH_3 mass spectrum of 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose.

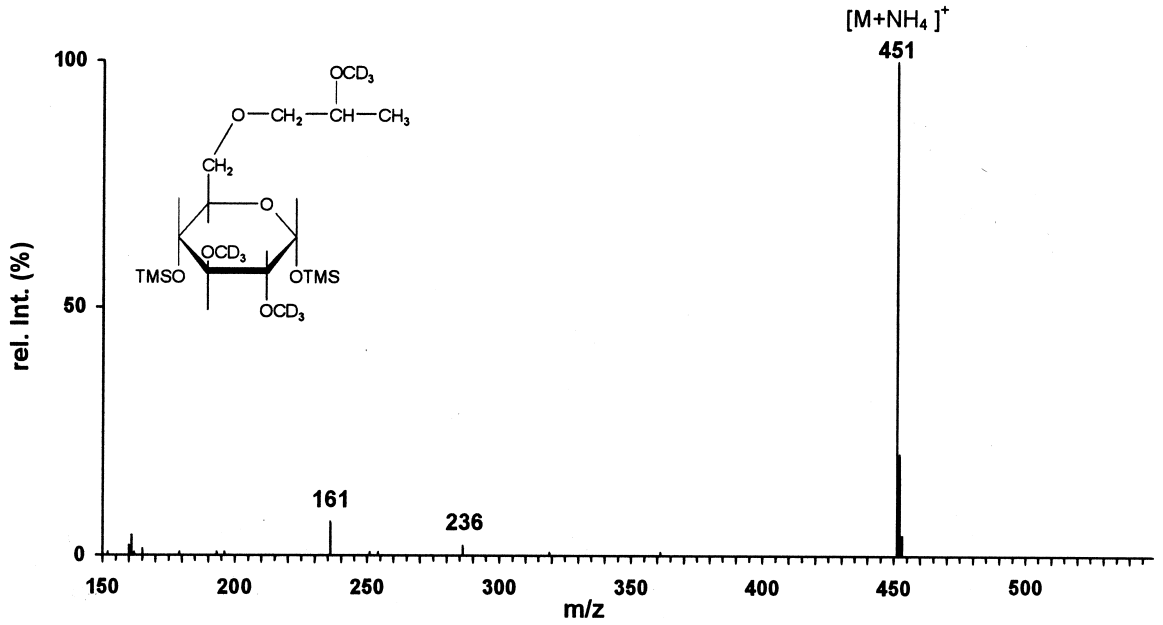


Fig. 4. CI/NH_3 mass spectrum of 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose.

deuteromethylglucose are plotted against the given concentrations of HP-γ-CD [measured as 1,4-bis-(*O*-trimethylsilyl)-2, 3-bis-*O*-deuteromethyl- 6 -*O*-2′-deutero-methoxypropylglucose]. The straight lines were calculated by least-square regressions between the respective minimal and maximal concentrations.

The linearity of the method is sufficient on condition that the amount of internal standard is adapted to the amount of the parent compound and does not exceed the amount of HP-γ-CD by a factor of 20.

The standard deviation was 2.5 nmol/l for a series of 11 samples spiked with 26 nmol/l HP-γ-CD.

3. Results and discussion

Several reports have been published on the determination of cyclodextrin in biological matrices by high-performance liquid chromatography (HPLC) using pulsed amperometric detection (e.g., Refs. [6,7]), inclusion complex formation and photometric detection (e.g., Refs. [8,9]) or fluorescence detection (e.g., Refs. [10–12]), respectively. All these papers describe pharmacokinetic studies of more or less well defined compounds, but none is dealing with the quantitative analysis of a complex mixture industrially produced HP-γ-CD a complex mixture of homologues and isomers. The negative electrospray (ES) mass spectrum of the technical product shows a series of molecular ions (and isotopomers) representative for the variable degree of hydroxypropylation (Fig. 1). The composition of the homologue mixture is listed in Table 1.

The analysis of a similar problem, the determination of hydroxypropyl-β-CD in blood and urine, was reported by Szathmary [2]. The used assay, size-exclusion chromatography with post-column complexation, reached a detection limit of 0.05 μg/ml.

The main experimental problem in this examination was the disappearance of 2′-hydroxy-propylglucose, created by acidic hydrolysis, when the concentration was reduced below 1 mg/l. This phenomenon, probably due to degradation by the treatment with concentrated hydrochloric acid, was not observed when the samples were peralkylated prior to acidic hydrolysis.

In order to protect the sugar moieties against uncontrolled degradation, permethylation of the hy-

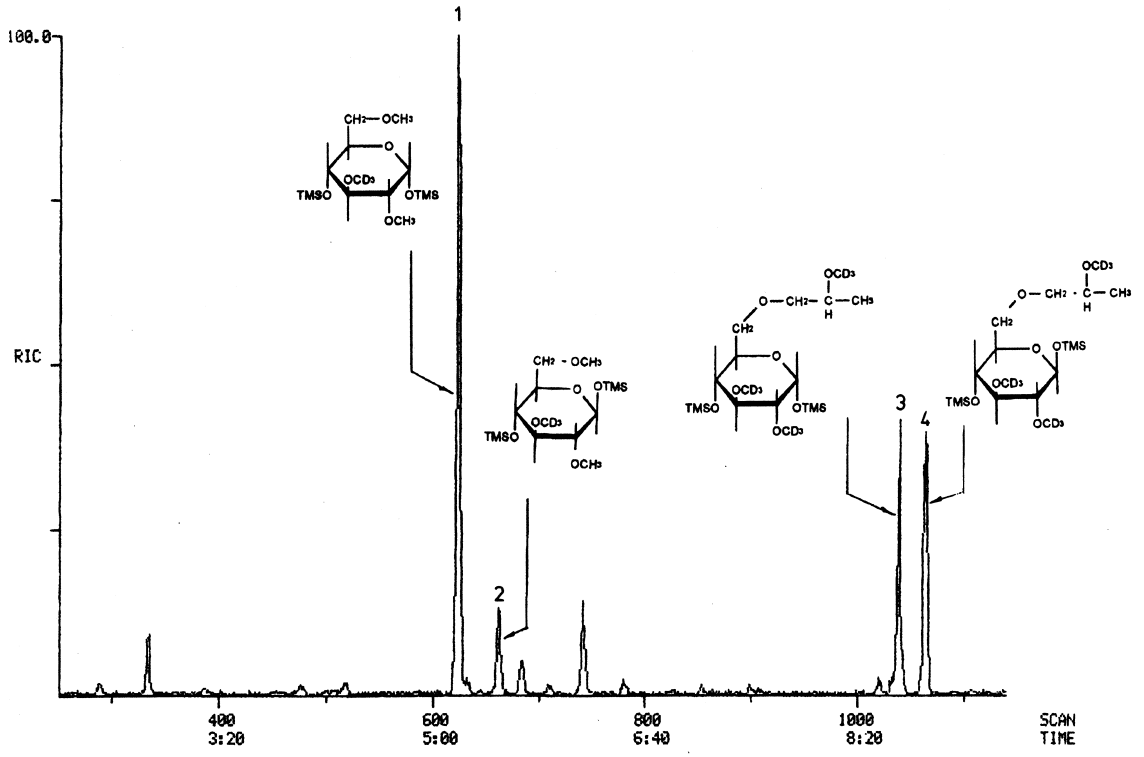


Fig. 5. Representative selected ion chromatogram over m/z 387 $[(M+NH_4)^+$ of 1,4-bis(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose] and m/z 451 $[(M+NH_4)^+$ of 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose] of a plasma sample after oral administration of 250 nmol HP-γ-CD.

droxy groups of the raw samples before acidic hydrolysis was introduced as additional step.

After methylation it is unavoidable that additional methylated glucose is formed resulting from hydrolyzed, partially hydroxyisopropylated-γ-CD and/or other polysaccharides in the biological matrices, interfering with the internal standard. In order to overcome this problem deuteromethylation of the samples was performed and partially methylated, 2,6-di-*O*-methyl-β-CD was used as internal standard.

The recovery of the method depends above all on the yield of the deuteromethylation reaction. Therefore, it is necessary to follow the derivatization procedure as reported by Ciucanu and Kerek [3] very carefully, and keep the reagents, in particular deuteromethyliodide and dimethylsulfoxide as dry as possible in sealed vials.

As shown in the ion chromatogram of plasma (Fig. 5) HP-γ-CD could be detected at low levels, despite the fact that both, the deuteromethylated/trimethylsilylated internal standard and the hydrolyzed deuteromethylated/trimethylsilylated HP-γ-CD appeared as α- and β-anomers, and despite the disturbing influence of complex biological matrices, particularly in plasma samples.

In the following calibration curves (see Fig. 6) the peak area ratios of 1,4-bis(*O*-trimethylsilyl)-2,3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose and 1,4-bis-(*O*-trimethylsilyl)-bis-2,6-*O*-methyl-3-*O*-deuteromethylglucose are plotted against the given concentrations of HP-γ-CD [measured as 1, 4-bis-(*O*-trimethylsilyl)-2, 3-bis-*O*-deuteromethyl-6-*O*-2′-deuteromethoxypropylglucose]. The straight lines were calculated by least-square regressions between the respective minimal and maximal concentrations.

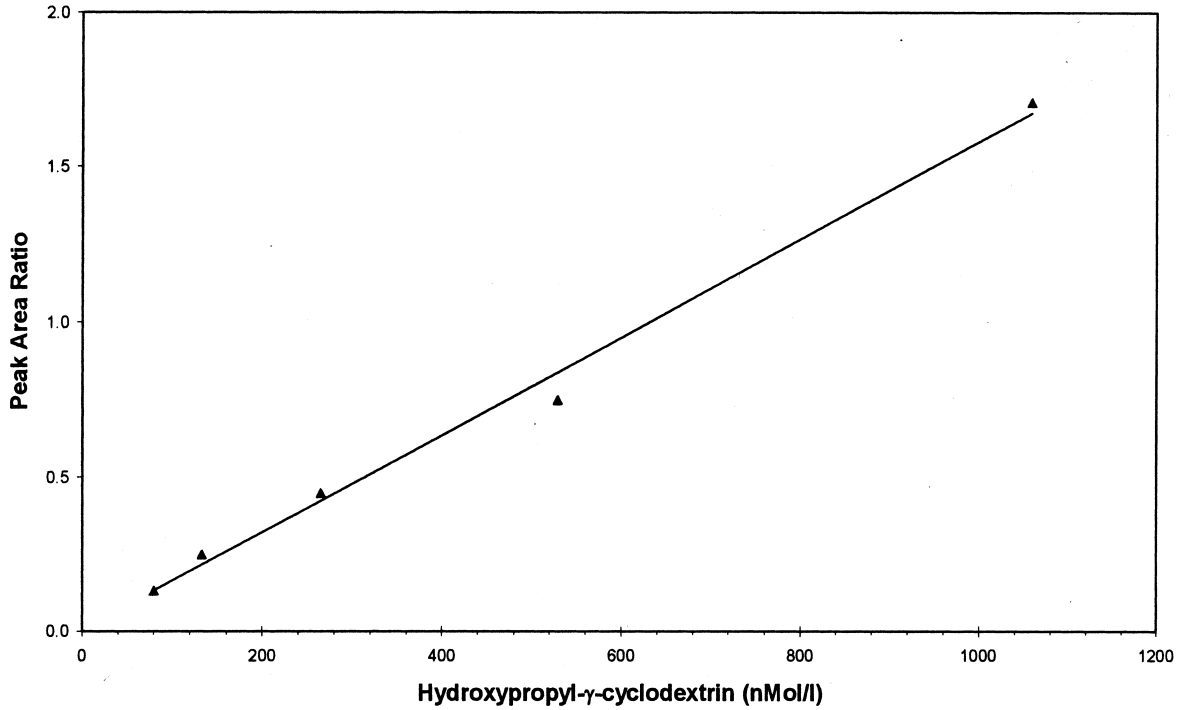


Fig. 6. Calibration for plasma samples of cynomolgus monkey 1 and 4, after oral administration, in the range 100 to 1000 nmol/l.

The time dependent concentrations of HP-γ-CD in plasma of two cynomolgus monkeys after oral administration are given in Fig. 7.

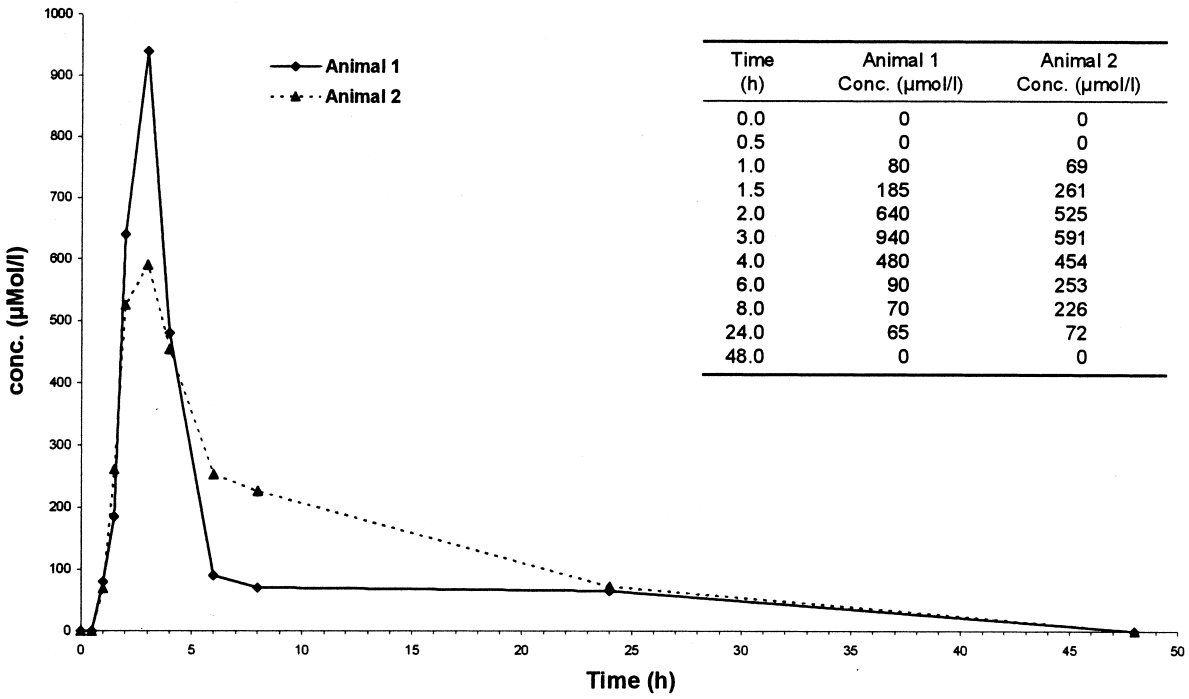
The concentrations rise within 3 to 4 h to maximal values. After approximately 8 h most of the cyclodextrin has been excreted by both animals. Excretion on a low level proceeds up to 24 h. After 48 h the HP-γ-CD concentration has fallen below the detection limit. The method has also been used successfully for the quantification of HP-γ-CD in urine of cynomolgus monkeys and in plasma and aqueous humor of rabbits

Table 1
Composition of HP-γ-CD homologues

m/z $(M-H)^-$	2-Hydroxypropyl groups	Amount (%)
1353.6	1	1.6
1411.6	2	3.8
1469.7	3	7.1
1527.7	4	12.5
1585.7	5	18.4
1643.8	6	21.2
1701.9	7	17.7
1759.9	8	11.1
1817.9	9	4.8
1876.0	10	1.5
1934.0	11	0.4

4. Conclusions

This work demonstrates for the first time the feasibility of sensitive pharmacokinetic studies on industrially produced 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD), a heterogeneous, complex mixture of homologues and isomers, in various body fluids, by GC–MS. The multistage sample preparation procedure needs some skill and carefulness, in particular the deuteromethylation reaction. However, the reliability and reproducibility of the method



Time (h)	Animal 1 Conc. (µmol/l)	Animal 2 Conc. (µmol/l)
0.0	0	0
0.5	0	0
1.0	80	69
1.5	185	261
2.0	640	525
3.0	940	591
4.0	480	454
6.0	90	253
8.0	70	226
24.0	65	72
48.0	0	0

Fig. 7. Time dependence of HP-γ-CD concentration in plasma of two cynomolgous monkeys, after oral administration.

could be demonstrated on several hundred analyzed plasma, urine and aqueous humor samples.

The chromatographic separation of analyte and internal standard needs no particular optimization. It can be performed on any medium polar capillary column.

The limit of quantification (LOQ) of 20 nmol/l, which was sufficient to measure this particular formulation project, can be improved.

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