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Separation and quantitation of glycolipids as penetration modifiers in human skin using high-performance liquid chromatography–mass spectrometry with electrospray ionization

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Abstract

An high-performance liquid chromatography–mass spectrometry method is presented for the measurement of glycolipids used as modulators of the penetration of drugs into human skin. In methanol extracts from different skin layers a detection limit of 100–400 pg/ml could be achieved. A routine analytical procedure could be set up with good quantitation reliability (relative standard deviation 6.6%)

Keywords: Glycolipids; Lipids

1. Introduction

The increasing importance of skin diseases requires improvements in dermal administration of drugs. One efficient way is the incorporation of substances into dermal preparations influencing the penetration into and through the skin. From the pharmaceutical point of view glycolipids are considered as well-tolerated substances with interesting physicochemical properties. Their phase transition temperatures depend on the number of ethylene glycol units used as spacers between carbohydrate and lipophilic chain [1]. As amphiphilic molecules they can form lyotropic mesophases in aqueous systems corresponding to their concentration. Resulting from these properties glycolipids can be used for the modulation of the penetration of drugs into human skin because they are able to influence the structure of the lamellar lipid water bilayers of the stratum corneum (SC) which is the main barrier for drug penetration. Glycolipids may be used as penetration enhancers for hydrophilic drugs and as reducers for lipophilic drugs when it can be desirable to localize them in the epidermis. The influences of lipid chain length and inserted spacer groups on the penetration kinetics of drugs and glycolipids were investigated using the four glycolipids shown in Fig. 1 as model substances. However, for evaluating efficiency of penetration modifiers it is necessary to measure rate and extent of the penetration of the modifiers into the SC layers.

Several studies have discussed separation and determination of glycolipids by high-performance liquid chromatography (HPLC) [2–4] The investigated glycolipids mostly originate from plants or bacteria and differ more or less from these used in

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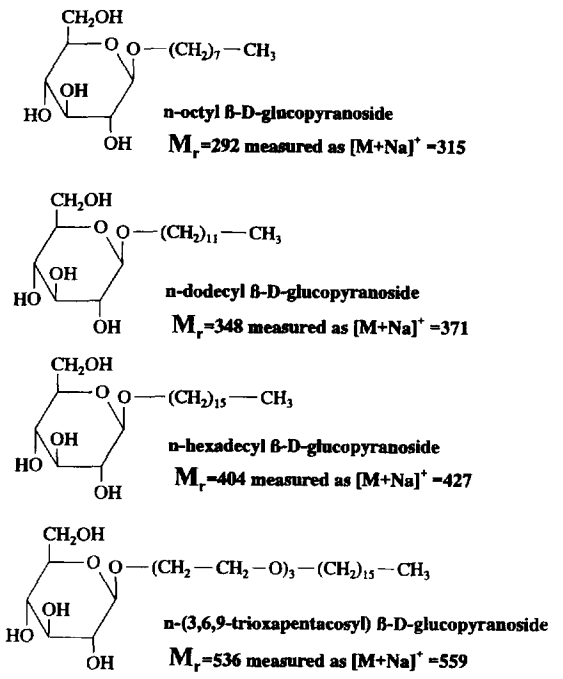

Fig. 1. Structures of the used glycolipids

our study. Gelpi gave an overview about biomedical and biochemical applications of LC–MS [5]. Cole describes MS with electrospray ionization (ESI) of glycolipids from bacteria [6]. The aim of the present work is to develop an efficient, sensitive and reliable analytical method for the measurement of glycolipids in different skin layers. A simultaneous determination of penetrated drug in the skin is desirable.

2. Experimental

2.1. Instrumentation

HPLC operations were carried out with a Waters 600 E system (Waters, Eschborn, Germany) with a Waters WISP 712 autosampler. Chromatographic separations were performed on a Nucleosil (120-$3C_{18}$ column (125×2 mm I.D., 3 μm particle size; Macherey–Nagel, Düren, Germany) without precolumn.

MS data were obtained from a quadrupole mass spectrometer Finnigan SSQ 710C (Finnigan MAT, Bremen, Germany) with an ESI interface.

2.2. Reagents

Methanol (gradient grade, LiChrosolv) which was used for sample extraction and as a component of the mobile phase was purchased from Merck (Darmstadt, Germany). For chromatographical purposes doubly distilled water was applied. *n*-Octyl β-D-glucopyranoside (lot No. 94H9005) and *n*-dodecyl β-D-glucopyranoside (lot No. 34H5037) were supplied by Sigma (Deisenhofen, Germany). *n*-Hexadecyl β-D-glucopyranoside and *n*-3,6,9-trioxapentacosyl β-D-glucopyranoside were synthesized according to Wilhelm et al. [7].

2.3. Sample preparation

Penetration experiments were carried out with the diffusion cell according to Franz [8]. The preparation of skin samples was described by Schmalfuss et al. [9]. Samples of the SC layers were prepared using the tape stripping method with Tesa (Beiersdorf, Hamburg, Germany), in the course of which twenty strips were made. From the epidermis samples two 20 μm cuts were made by the freezing microtome technique, the two dermis cuts had a thickness of 40 μm. Extraction was done with methanol at room temperature [10]. Extracts were measured by HPLC–MS without any further preparation.

2.4. Chromatography

As mobile phase a mixture of methanol–water (90:10, v/v; degassed with helium) was used. A 200 μl volume of the sample and 10 μl of an internal standard solution (20 μg/ml *n*-dodecyl β-D-glucopyranoside) were placed into each of the autosampler vials, from which 5 μl were injected. The applied flow ranged from 0.2 to 0.3 ml/min, depending on the retention times of the several glycolipids.

2.5. Mass spectrometry

ESI was carried out with a voltage of 4.5 kV and a temperature of 200°C at the heated capillary. Detection was performed in the positive mode. For collision induced dissociation a voltage of 20 V was applied. For quantitation of glycolipids the SIM (selected ion monitoring) mode was chosen because

of its higher sensitivity. A peakwidth of 0.5 u and a scan time of 0.25 s per scan were adjusted. The parameters of the lenses of the ion optics were optimized for each glycolipid.

2.6. Quantitation

Calibration curves with fourteen concentrations between 0.01 and 10 μg/ml for each glycolipid were set up. To control the system stability an internal standard (20 μg/ml *n*-dodecyl β-D-glucopyranoside) was used (10 μl+200 μl skin extract) and a calibration sample was set after every seventh measurement.

Quantitation refers to peak areas which were calculated using Microsoft Excel 7.0 software on an IBM type personal computer equipment.

3. Results and discussion

The mobile phase was optimized mainly with respect to MS requirements. The chosen ratio of methanol–water (90:10) provided shorter retention times and a better detection sensitivity (about 30%) for the $[M+Na]^+$ peak compared to methanol–water (80:20), while the resolution is sufficient. Under electrospray ionization conditions, glycolipids tend to form $[M+Na]^+$ adducts.

In the SIM mode the MS engine scans only a few (five at most) programmed mass ranges. Compared to the full scan mode the noise level is much lower so that up to 1000 times better detection limits are achieved. A major advantage is the fact that other substances from the complex matrix are scarcely detected and do not disturb the determination of the glycolipids. Fig. 2 shows a chromatogram of a skin extract. The two large peaks are surrounded by small peaks caused by the complex matrix. Even without chromatographical separation a simultaneous quantitation of several analytes is possible due to differences in the scanned masses.

The detection limits of the different glycolipids depend on the ionization ability of the substance. Increasing length of the lipid chain results in decreasing ionization ability and smaller peak areas at the same concentration (see Fig. 3). It is evident that insertion of further functional groups would improve

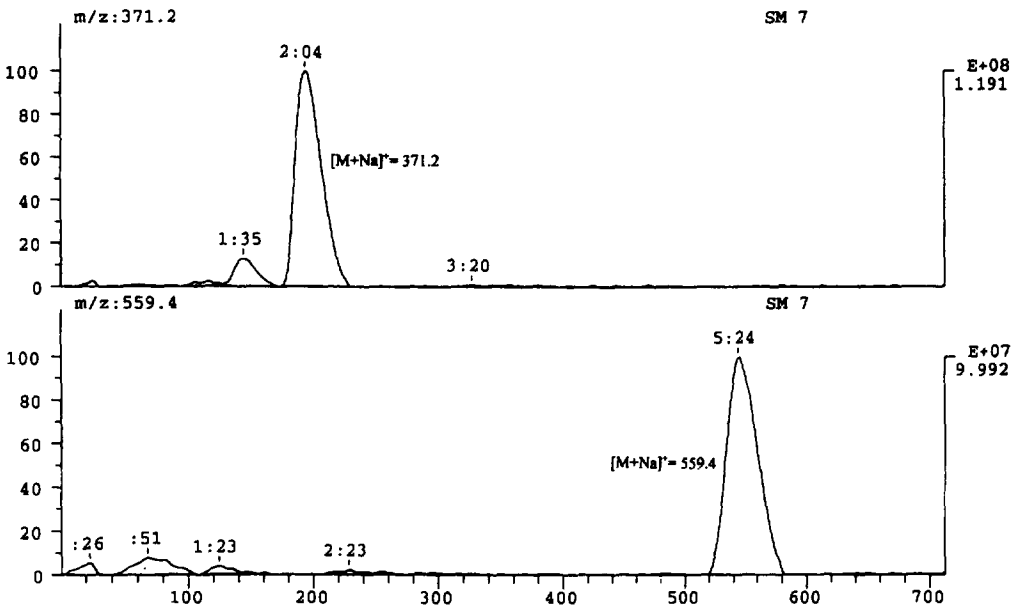

Fig. 2. Chromatogram of a sample prepared by a procedure described in Section 2.3. The different mass ranges stand for the internal standard *n*-dodecyl β-D-glucopyranoside (top) and the measured glycolipid *n*-3,6,9-trioxapentacosyl β-D-glucopyranoside (bottom). *x*-Axis: scan times in arbitrary units. *y*-Axis: normalized scan intensity in percent. Peak labelling: retention time (min), *m/z* for $[M+Na]^+$

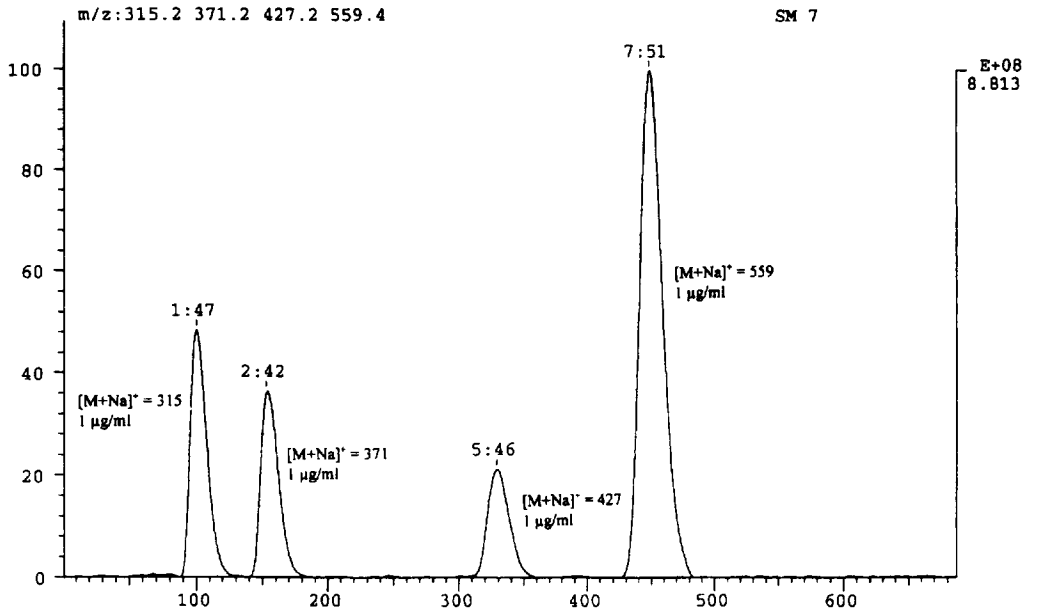

Fig. 3. Chromatogram of the used glycolipids (concentration 1 µg/ml in methanol); glycolipids were detected simultaneously at their specific *m/z* in the SIM mode. *x*-Axis: scan times in arbitrary units. *y*-Axis: normalized scan intensity in percent. Peak labelling: retention time (min), *m/z* for $[M+Na]^+$

the ionization which opens a promising perspective for analyzing more complex glycolipids such as e.g. glycosphingolipids.

Table 1 shows analytical parameters of the measured glycolipids. The wide range of quantitation and an excellent limit of detection indicate the analytical power of the described method. The relative standard deviation calculated from 50 measurements of *n*-dodecyl β-D-glucopyranoside taken from the calibration curve and from skin samples was 6.6%. Each sample including calibration values was measured twice. To analyze concentrations above the high limit of quantitation without sample dilution, the full scan mode should be applied, otherwise flattened peaks result and the linearity will be lost.

Table 1
Analytical parameters of the described method for the determination of glycolipids in skin extracts

Parameter	1	2	3	4
Limit of detection (pg/ml), *S/N* 3:1	250	250	400	100
Lower limit of quantitation (ng/ml)	0.5	0.5	0.8	0.5
High limit of quantitation (µg/ml)	5	5	10	2

1=*n*-octyl β-D-glucopyranoside, 2=*n*-dodecyl β-D-glucopyranoside, 3=*n*-hexadecyl β-D-glucopyranoside, 4=*n*-3,6,9-trioxapentacosyl β-D-glucopyranoside.

The SSQ software allows us to set up automatic procedures to control MS and chromatographical parameters. This makes it possible to use this technique for routine analytical determinations.

The described HPLC–MS method turned out to be suitable for measurements of glycolipids in samples extracted from human skin dissolved in methanol (SC, epidermis, dermis) as well as those dissolved in water (acceptor compartment of the Franz diffusion cell). In the authors opinion it is able to displace more expensive radioactive tracing experiments.

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