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Capillary electrochromatography–electrospray ionization mass spectrometry for the qualitative investigation of the drug etodolac and its metabolites in biological samples

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Abstract

On-line coupling of capillary electrochromatography (CEC) with electrospray ionization (ESI) mass spectrometry (MS) was used for the qualitative investigation of the biotransformation of the non-steroidal anti-inflammatory drug etodolac. The coupling of this electro-driven separation technique with mass spectrometry allowed us to demonstrate the presence of different metabolites of etodolac extracted from human urine.

Keywords: Electrochromatography; Mass spectrometry; Etodolac

1. Introduction

Capillary electrochromatography (CEC) is one of the most rapidly developing microanalytical separation techniques at present [1–3]. It is a hybrid technique between capillary electrophoresis and capillary high-performance liquid chromatography (capillary HPLC). In CEC the mobile phase is driven through the packed capillary by electroosmotic flow (EOF). Depending on the nature of the analytes and the pH of the mobile phase the separation can be achieved by differential partition between two phases, differential electromigration or a combination of both.

The increasing application of capillary separation techniques has led to a growing interest in detection methods, which are more sensitive, selective or structurally informative for the analytes. Especially in bioanalysis, UV detection often results in very complex chromatograms with overlapping peaks of metabolites and endogenous substances. This problem can be solved with mass spectrometry, which is recognized as the most selective and broadly applicable detector for analytical separations. Therefore the combination of CEC with mass spectrometry is of particular interest.

Electrospray ionization (ESI) interfacing is currently the method of choice to combine capillary separation techniques with MS due to its versatility and ease of implementation. Numerous designs have been developed for ESI interfacing [4] which differ in the way the separation and ESI voltage is applied to the end of the capillary. The most used method for ESI interfacing is the sheath liquid flow system which is illustrated in Fig. 1.

We started our work on CEC–ESI-MS coupling in

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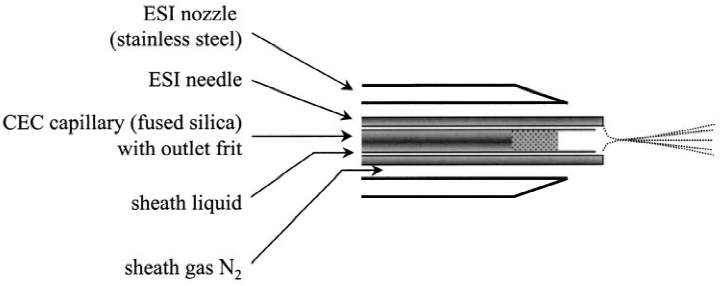

Fig. 1. CEC–ESI interface (sheath liquid flow system).

cooperation with Fanali and Desiderio et al. [5]. In this paper we demonstrated the separation of etodolac (ET) (Fig. 2) and the hydroxylated phase I metabolites 6-hydroxy etodolac (6-OH-ET), 7-hydroxy etodolac (7-OH-ET) and 8-(1′-hydroxyethyl) etodolac (8-OH-ET) as reference samples. In this technique even at pH 3.0 a high essential electroosmotic flow (EOF) was observed in C-18 packed capillary. Because of the shortcomings of most commercial instrumentation concerning the smallest required column length of typically more than 90 cm [6] a laboratory-made electrophoretic device [7] (Fig. 3) was used for the coupling of CEC with ESI-MS. In the present studies the prior investigations are extended to assays of metabolites from biological samples.

2. Experimental

2.1. Chemicals and reagents

Racemic etodolac (ET) and its phase (metabolites, 6-hydroxy etodolac (6-OH-ET), 7-hydroxy etodolac

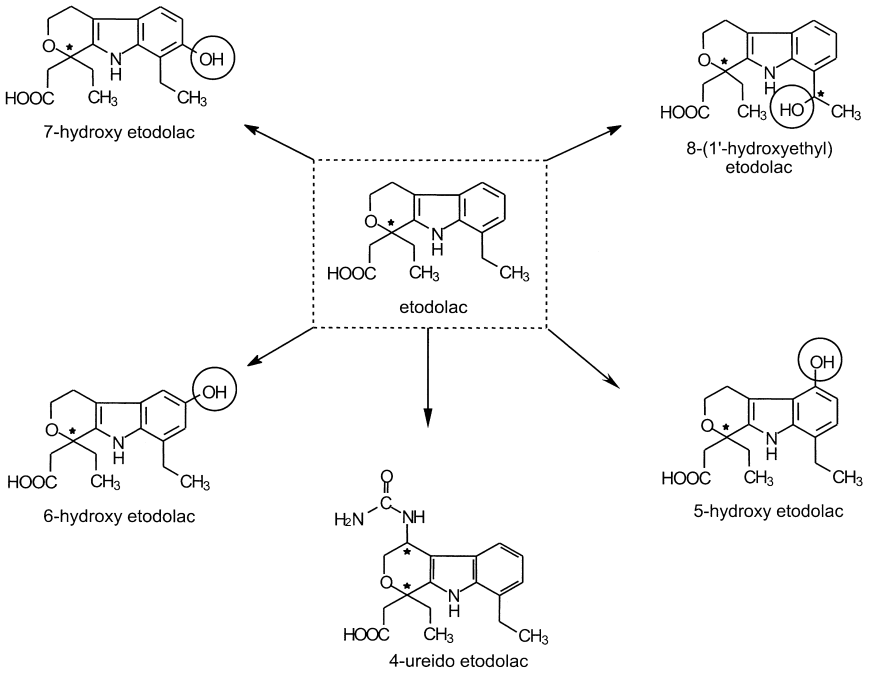

Fig. 2. Structure of etodolac (ET) and its metabolites 5-hydroxy etodolac (5-OH-ET), 6-hydroxy etodolac (6-OH-ET), 7-hydroxy etodolac (7-OH-ET), 8-(1′-hydroxyethyl) etodolac (8-OH-ET) and 4-ureido etodolac (4-UR-ET).

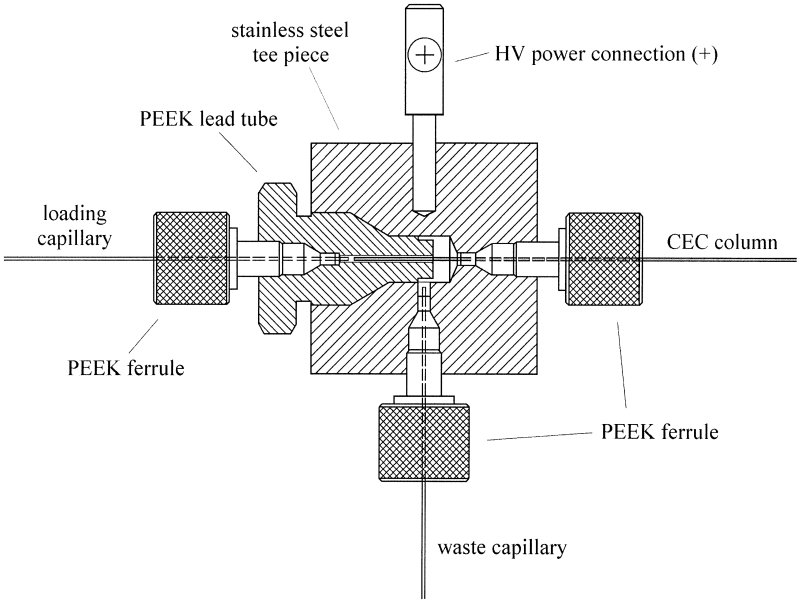

Fig. 3. CEC sample interface (laboratory-made).

(7-OH-ET) and 8-(1′-hydroxyethyl) etodolac (8-OH-ET) were gifts from Wyeth Pharma (Münster, Germany). Acetic acid, acetonitrile, ammonium formate, ammonium acetate, formic acid and methanol were from different commercial sources and used without further purification. LiChrospher® 100 RP-18 (5 μm) used for the preparation of packed capillaries was purchased from Merck (Darmstadt, Germany).

2.2. CEC–ESI-MS coupling

2.2.1. CEC–ESI-MS setup

A LCQ® ion trap mass spectrometer (Thermoquest, San Jose, CA, USA) equipped with an sheath flow electrospray interface was used for the on-line coupling. The buffer (10 m*M* ammonium formate pH 3.0/acetonitrile 50/50), which was also used for the separation, served as the sheath liquid. It was delivered at a flow-rate of 3 μl/min using a syringe pump. Separations in CEC were performed using a laboratory-made CEC sample interface consisting of a stainless steel tee piece and a restrictor similar to that described recently by Taylor and Teale (Fig. 3) [7]. The stream of mobile phase generated using a commercial HPLC pump flows coaxially passing the capillary column inlet through a PEEK lead tube and leaving the tee piece through a restriction capillary. A voltage of 20 kV was applied to the metal tee piece using a Grom Capillary Electrophoresis System 100 high-voltagesupply (Grom, Herrenberg, Germany). The outlet of the capillary was grounded. The sample was introduced into the mobile phase stream using the injection valve of the HPLC system and further loaded, pressure assisted, onto the separation capillary electrokinetically (Fig. 4).

2.2.2. Preparation of capillary columns

Fused-silica capillaries of 100 μm I.D. and 375 μm O.D. from Polymicro Technologies (Phoenix, AZ, USA) were used. The inlet-end of the capillary was connected to an HPLC-precolumn (4.6×50 mm) which served as reservoir for the slurry of the packing material in methanol. A commercially available HPLC column frit was connected to the outlet-end of the capillary in order to retain the packing material. The slurry of the packing material was ultrasonicated in a water-bath (15 min) and transfered into the reservoir. The system was closed tightly, a pressure of 400 bar was applied using a Knauer pneumatic pump (Knauer, Berlin, Germany) and maintained for 1 h. After release of the residual

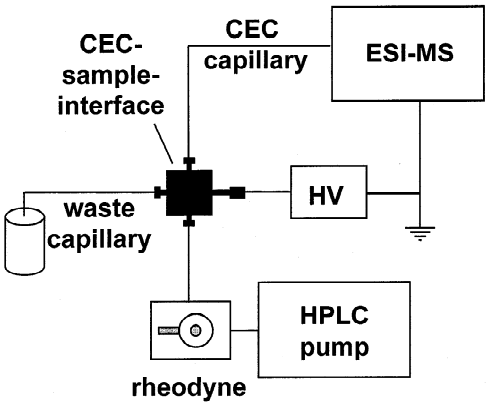

Fig. 4. CEC–ESI-MS (experimental setup).

pressure (2–3h), bidistilled water was pumped through the packed bed with a pressure of 200 bar for 30 min. The outlet and inlet frits were sintered by local heating of the packed bed for approximately 15 s using a heating coil (700–800°C). The packed capillaries prepared according to this technique were used for capillary HPLC, pressure assisted CEC (pCEC) and CEC separations.

2.2.3. Samples

ET, 6-OH-ET, 7-OH-ET and 8-OH-ET were dissolved in methanol/water (50:50) at a concentration of 75 μg/ml. Blank urine samples were spiked with 300 μg/ml ET, 6-OH-ET, 7-OH-ET and 8-OH-ET and then extracted with the same volume of ethyl acetate. The organic solvent was evaporated and the residue reconstituted in the original volume of buffer.

Urine samples were collected 1–3 h after oral administration of 1 tablet of 400 mg racemic etodolac (Lodine®). The samples were extracted with the same volume of ethyl acetate, the organic solvent was evaporated and the residue reconstituted in the original volume of buffer.

3. Results and discussion

The on-line CEC–ESI-MS coupling was performed using the CEC sample interface shown in Fig. 3. The overall set-up is illustrated in Fig. 4. The chromatogram resulting from the selected mass track ($m/z=302$) of the urine extract spiked with 6-OH-ET, 7OH-ET and 8-OH-ET is depicted in Fig. 5a. The three phase I metabolites are completely separated. Full scan mass spectra of each peak are shown in Fig. 5b–d. More detailed results into this investigation have been discussed in a previous paper [5]. In Figs. 6a–8a the selected mass tracks of an extracted urine sample, collected 1–3 h after oral administration of a standard dose of 400 mg racemic etodolac to a human volunteer are presented. In the mass track $m/z=302$ only 6- and 7-OH-ET are observed. The smaller signal at $t=6.8$ min has been recently identified as 5-hydroxy etodolac [8]. A signal for 8-OH-ET is not observable. This is in accordance with HPLC–ESI-MS investigations and earlier pharmacokinetic studies [9,10]. According to Ferdinandi et al. [11] the two peaks with $m/z=344$ could probably stem from the two diastereomers of 4-ureido etodolac (4-UR-ET) (Fig. 7). Furthermore signals for etodolac ($m/z=286$) (Fig. 7), hydroxylated etodolac glucuronides (OH-ET-GLUC) ($m/z=478$) and the signal for etodolac glucuronide (ET-GLUC) ($m/z=462$) (Fig. 8) are observable. The full scan mass spectra of each peak are shown in Fig. 6–8(b–d). From these qualitative data it is obvious that on-line CEC–ESI-MS coupling can be used for selective detection, identification and peak purity testing when analyzing complex biomedical samples.

4. Conclusions

As shown in this study, the coupling of CEC with an ESI-MS is quite easy to manage. Although limited to volatile buffers, CEC–ESI-MS proved to be a useful tool for bioanalytical investigations. CEC–ESI-MS offers the opportunity to gain structural information about the analytes. A simple laboratory-made CEC sample interface where the inlet buffer vial is replaced by a metal tee piece was used. This allows continuous replenishment of the inlet buffer during the run and the application of nearly any desired pressure and length of the capillary is enabled. In the capillaries packed with C-18 material even at pH 3.0 a sufficiently powerful electroosmotic flow (EOF) was generated to transport the mobile phase and the uncharged analytes through the packed bed.

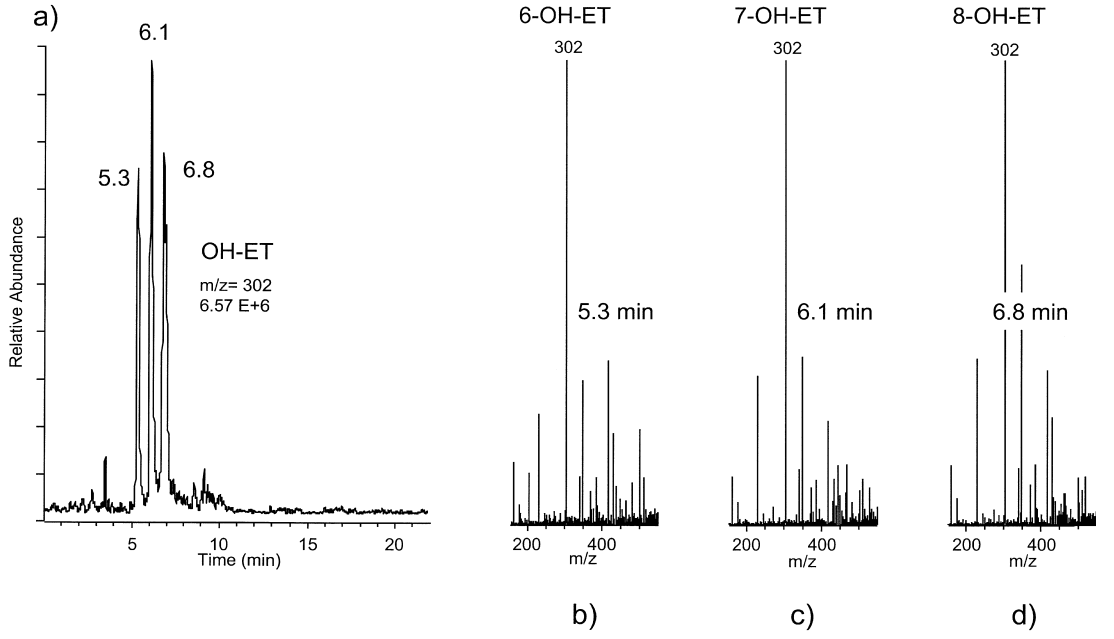

Fig. 5. CEC–ESI-MS analysis of a blank urine extract spiked with 6-OH-ET, 7-OH-ET and 8-OH-ET (300 μg/ml); (a) chromatogram: selected mass track of $m/z=302$; full scan mass spectra of the peaks at: (b) 5.3 min; (c) 6.1 min; (d) 6.8 min. Instrument: laboratory-made CEC sample interface in combination with Grom CE-100 system; capillary: fused-silica 100 μm I.D., 23 cm packed (LiChrospher® 100 RP-18, 5 μm); buffer: 10 m*M* ammonium formate pH 3.0/acetonitrile, 50:50; voltage: 20 kV; injection: electro-kinetically; detection: ESI-MS. ESI-MS conditions: polarity: negative; source voltage: 3.5 kV; sheath gas flow: 20 arbitrary units N_2; sheath liquid: separation buffer; sheath liquid flow: 3.0 μl/min.

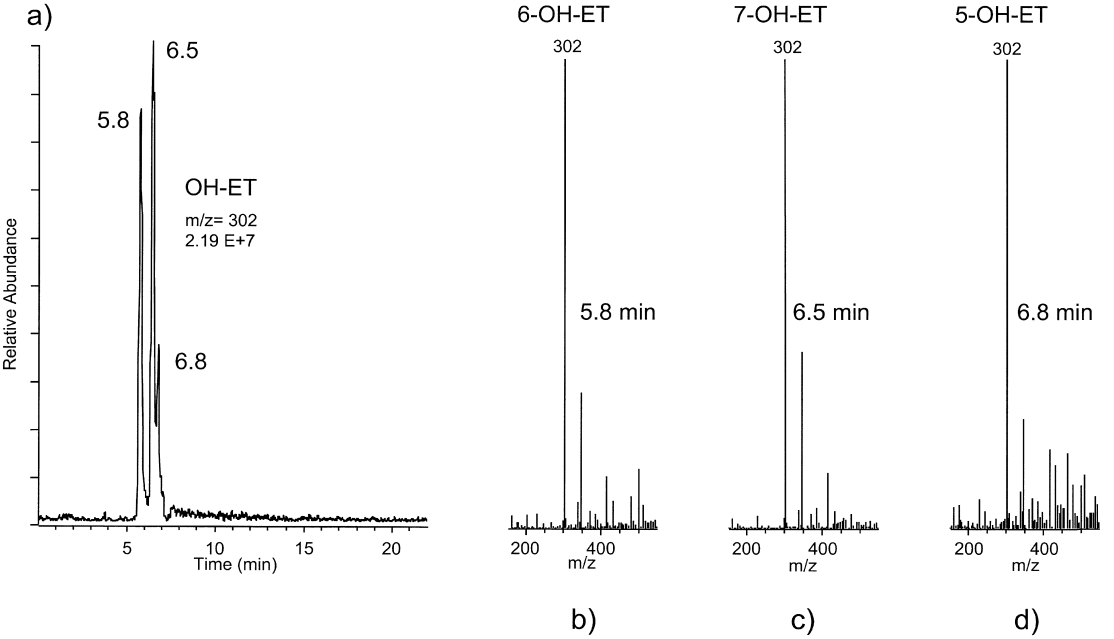

Fig. 6. CEC–ESI-MS analysis of an extracted urine sample (collected 1–3 h after oral administration of 400 mg racemic etodolac); selected mass track of (a) $m/z=302$, monohydroxylated etodolac metabolites (OH-ET); full scan mass spectra of the peaks at: (b) 5.8 min; (c) 6.5 min; (d) 6.8 min. Conditions as described in Fig. 5.

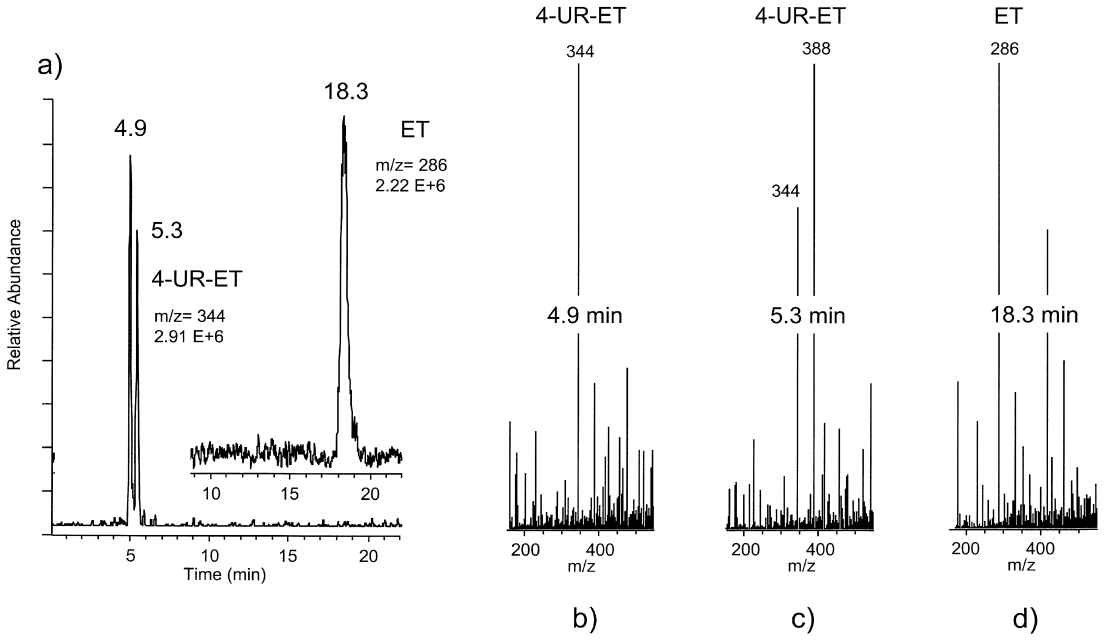

Fig. 7. CEC–ESI-MS analysis of an extracted urine sample (collected 1–3 h after oral administration of 400 mg racemic etodolac); selected mass track of (a) $m/z=344$, 4-ureido etodolac (4-UR-ET) and $m/z=286$, etodolac (ET); full scan mass spectra of the peaks at: (b) 4.9 min; (c) 5.3 min; (d) 18.3 min. Conditions as described in Fig. 5.

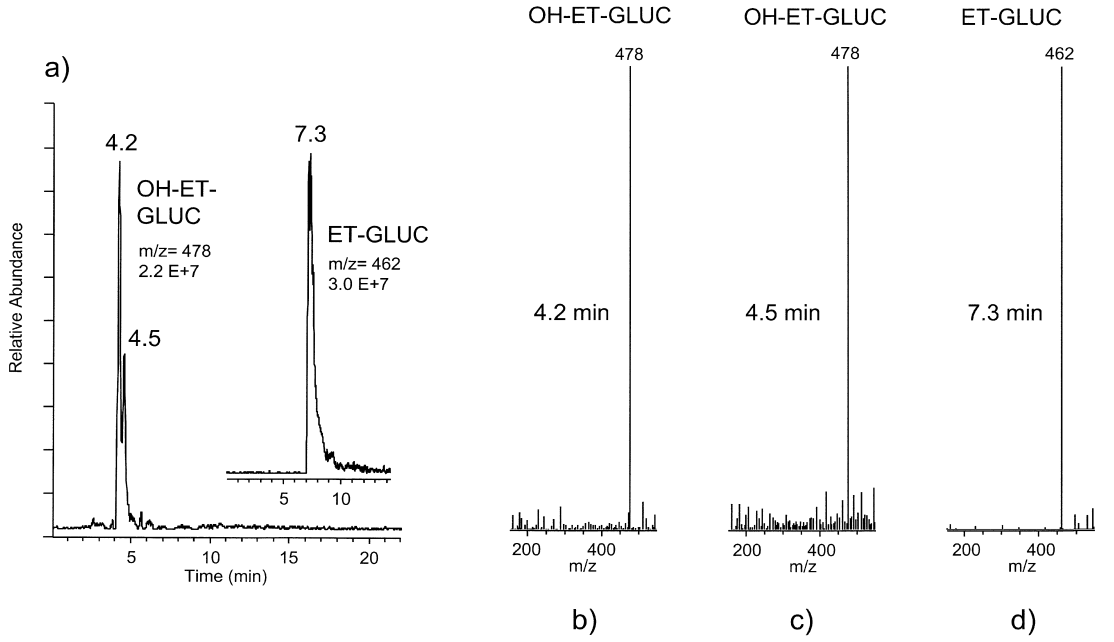

Fig. 8. CEC–ESI-MS analysis of an extracted urine sample (collected 1–3 h after oral administration of 400 mg racemic etodolac); selected mass track of (a) $m/z=478$, monohydroxylated etodolac glucuronides (OH-ET-GLUC) and $m/z=462$, etodolac glucuronide (ET-GLUC); full scan mass spectra of the peaks at: (b) 4.2 min; (c) 4.5 min; (d) 7.3 min. Conditions as described in Fig. 5.

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