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Analysis of cefaclor in novel chocolate-based camouflage capsules

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The determination of cefaclor in a new, complex chocolate matrix was performed by using a simple sample preparation (dispersion in dilute hydrochloric acid at 80 °C, centrifugation, washing with cyclohexane), followed by ion pair HPLC on a Kinetex™ pentafluorophenyl core-shell stationary phase with UV detection at 265 nm. We obtained good linearity ($R^2 = 0.9976$) and precision (average RSD 0.86%) for the relevant concentration range. The preparations, although hand-made in this pilot phase, showed good uniformity of content. After being stored for four weeks in a refrigerator the preparation did not contain recognizable amounts of decomposition products.

1. Introduction

Appropriate pharmaceutical forms are essential for successful pharmacotherapy in general, but particularly in the oral therapy of young children (European Medicines Agency 2006; Krause and Breitkreuz 2008). One of the major problems in the long-term medication of young children is the bitter taste of drugs like antibiotics, and much effort has been undertaken to cover bitter taste by the addition of selected flavors and sweeteners (Deepak et al. 2012). As chocolate can cover sweet as well as bitter taste (European Medicines Agency 2006), attempts have been undertaken to use chocolate as matrix of pharmaceutical formulations, with drugs either dispersed homogeneously (Lang 2001; Mayank and Kumar 2012) or embedded in microcapsules (Rotmann 1988). One drug containing the laxative sodium picosulfate in chocolate tablets (Darmol®) is available on the German market.

In this context a new pharmaceutical form named "camouflage capsule" for oral application has been developed (Pohl and Pohl 2012). Designed to conceal the displeasing taste and appearance of drugs these are coated in an inconspicuous mantle, which is made up of chocolate. In this investigation we prepared a camouflage capsule containing 500 mg of the bitter-tasting antibiotic cefaclor, a medication taken permanently by young patients suffering from cystic fibrosis (Adam 1979). For confirming the feasibility of this concept, we worked out a method for determination of cefaclor in this new pharmaceutical formulation, and had a first view on its stability in this formulation.

Quantification of cefaclor in bulk can be performed by reversed-phase HPLC following the respective Ph. Eur. monograph or literature (Huang et al. 1991), for determination in complex matrices like drug formulations (Nawaz et al. 2011; Patel et al. 2012), milk (Karageorgou et al. 2010), and plasma alternative HPLC (Granados-Soto et al. 2003) and HPLC-MS-MS methods (Chen et al. 2003) have been worked out. Stability of cefaclor in bulk (Lorenz et al. 1992; Medenecka et al. 2009) and preparations (Medenecka et al. 2009; Vilanova et al. 1996; Nakashima et al. 1985; Tarawneh et al. 2011) has been analyzed by HPLC. In tablets decomposition is most efficiently suppressed by exclusion of humidity (Medenecka et al. 2009).

To the best of our knowledge, analysis of cephalosporins in chocolate matrix has not been reported yet. However, HPLC methods have been published on the determination of phenylethylamines (Riederer and Burger 2009), fatty acid tryptamides (Janßen and Matissek 2002) and xanthines (Matissek1997) in chocolate. Very recently, we worked out a protocol for the determination of caffeine and nicotine in chocolate (Müller et al. 2014).

2. Investigations, results and discussion

Chocolate "camouflage capsules" were prepared as described in the utility model application (Pohl and Pohl 2012). Shortly, cefaclor was obtained from commercial hard gelatin capsules by opening the capsules and the content was collected and processed as it was. An aliquot of this powder, together with a low-melting whole milk chocolate mass, was melted at < 60 °C with continuous shaking to obtain a homogeneous, highly fluid dispersion. Aliquots of this dispersion corresponding to 500 mg cefaclor were filled into commercial hollow chocolate shells, and the remaining hollow space was filled up with molten whole milk chocolate. The preparations obtained this way were 19 mm in diameter and weighed about 4.1 g.

For determination of cefaclor in these preparations a workup procedure was worked out consisting of conversion of the drug into its amply water-soluble hydrochloride and separation of the lipophilic components of the chocolate mass. Melting the preparation with 1 M hydrochloric acid at 80 °C, followed by vortexing, centrifugation, and extraction of the aqueous supernatant with cyclohexane gave an acidic aqueous solution which was subjected to ion pair HPLC analysis after a filtration step. The eluent prescribed by the Ph. Eur. monograph "Cefaclor" (sodium pentanesulfonate, triethylamine-phosphoric acid buffer pH 2.5) was found to be suitable. The elution behavior of cefaclor is known to depend strongly on the pH of the eluent,

Table 1: Precision and recovery data at the corresponding concentration levels (n = 3)

Concentration (mg/mL)	0.40	0.45	0.50	0.55	0.60	Average
RSD (%)	1.13	0.46	0.23	0.57	1.90	0.86
Recovery (%)	70.0	74.5	75.9	77.0	77.3	74.9

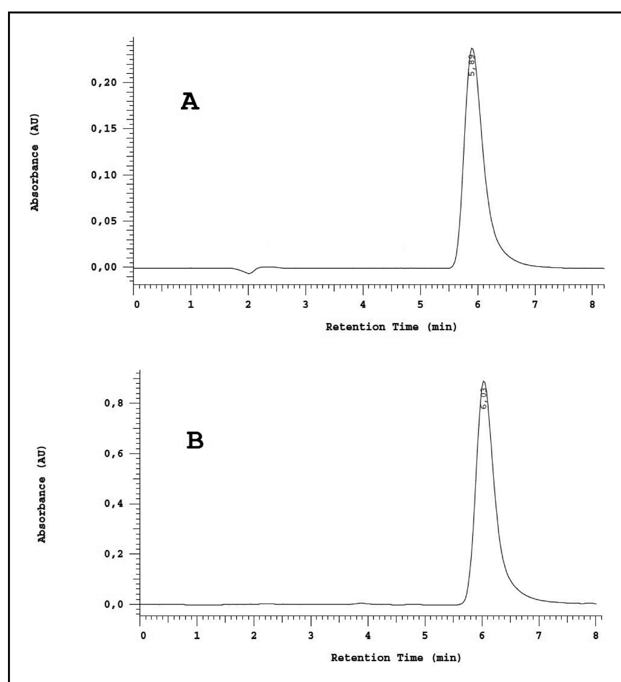

Fig. 1: HPLC chromatogram for (A) a reference solution of cefaclor *CRS* (0.1 mg/mL in the mobile phase), and (B) a sample obtained from a camouflage capsule by the workup procedure described in the Experimental.

and acidic eluents have been found to be superior in order to obtain reproducible elution (Lorenz et al. 1992). Whereas the RP-18 column used in the Ph. Eur. monograph gave only poor chromatograms, a Kinetex™ pentafluorophenyl core-shell stationary phase gave an acceptably shaped peak for cefaclor with a retention time of about 5.9 min (Fig. 1, B). This column performs like a fully porous sub-2 μm column at moderate backpressures, resulting in short analysis times and strongly reduced solvent usage. No matrix peaks were detected in a control experiment of drug-free preparation with the described workup and chromatography protocols.
Linearity and precision of the chromatographic system for the relevant concentration range were determined ($R^2 = 0.9976$) following the ICH criteria (ICH Secretariat, 2005), and the results are shown in Fig. 2. The average RSD was 0.86 %, with a maximum RSD of 1.9 % (n = 3, Table 1). To analyze the influence of the matrix on the extraction, the recovery was determined for five different concentration levels ranging from the lowest to the highest concentration used for linearity data. The minimum recovery rate was 70.0 %, the maximum 77.0 % (Table 1).
Last but not least we used the workup procedure described above on six "camouflage capsules". The content of cefaclor in the preparations was determined using the calibration curve and

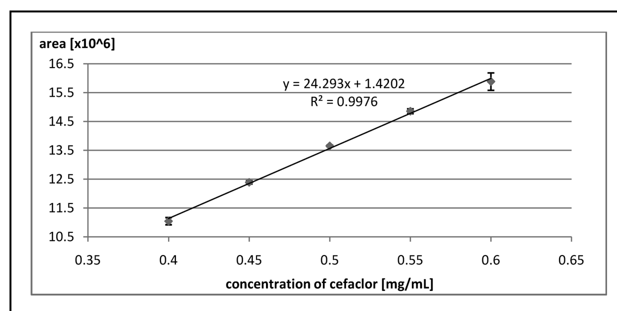

Fig. 2: Linearity data with a calculated linear regression curve and standard deviation (n = 3).

corrected with the average recovery. The results show that most of the measured masses were very close to the scheduled content of 500 mg cefaclor, and all of them are within 80 % and 120 % of the theoretical content (Table 2). The low content in capsule 1 might be due to the not yet optimized manual volumetric dosing step in the preparation of the "camouflage capsules".
In conclusion, we could demonstrate that even with the ordinary equipment of a pharmacy's laboratory, uniform formulations on the basis of chocolate can be prepared, which might enhance the compliance of little children who need a long-term therapy with cefaclor. Further we worked out a method for determination of cefaclor in this rather complex matrix by HPLC, which can be the basis for a full validation in strict coherence with relevant guidelines. Moreover, our investigations demonstrate that the antibiotic is very stable in this matrix. Chocolate is an almost anhydrous medium, which does not promote hydrolysis of water-sensitive active agents (Mayank and Kumar 2012).The HPLC analysis did not detect decomposition products after four-week storage in a refrigerator.
These first investigations evidence the practicability of this new dosage form, and justify further efforts on its development.

3. Experimental

3.1. Chemicals

Cefaclor *CRS* was purchased from EDQM (Strasbourg, France), Cefaclor-ratiopharm 500 mg Kapseln (PZN: 0632595, Ch.-B. L61152) were from Ratiopharm (Ulm, Germany). Hollow chocolate shells "Pralinen-Hohlkörper" (Art. Nr. 10222; 1.2 g each, composed of sugar, cocoa butter, milk powder, cocoa (35%), cream powder, emulgator: soya lecithin, vanilla) and whole milk chocolate filling material "Premo Pralinenmasse Vollmilch" (Art. Nr. 11020; composed of vegetable oil, sugar, hardened vegetable fat, cocoa butter, milk powder, humectant: glycerol, cocoa, de-oiled cocoa, emulgator: soya lecithin, native vanilla aroma, aroma) were purchased from Pati-Versand.de (Herzlake, Germany).
Chemicals for workup and analysis were of analytical grade and purchased from Sigma-Aldrich (Schnelldorf, Germany).

3.2. Preparation of the "camouflage capsules"

Chocolate "camouflage capsules" (scheduled content 500 mg cefaclor) were prepared as described in the utility model application (Pohl and Pohl 2012). Shortly, the content of 10 Cefaclor-ratiopharm® 500 mg hard gelatin capsules was collected, and placed in a beaker. In another beaker 40 g of the whole milk chocolate filling material was molten in a microwave oven (600 W, 15 s, final temperature: 58 °C), and 20 g of this hot, freely liquid mass were given rapidly to the cefaclor powder, and the mixture was stirred until it appeared homogeneous. The hot dispersion was taken up in a wide-bore Braun Injekt syringe (B. Braun, Melsungen) and filled in equal aliquots into 10 hollow chocolate shells. The remaining hollow space was filled up

Table 2: Content of cefaclor of six "camouflage capsules" determined by the calibration curve method

Capsule 1	Capsule 2	Capsule 3	Capsule 4	Capsule 5	Capsule 6	Average	RSD
405.0 mg	498.6 mg	492.5 mg	521.3 mg	503.5 mg	505.2 mg	487.7 mg	8.5 %

with molten, drug-free whole milk chocolate, and the preparations were kept in a refrigerator (2–8 °C) for 60 min for solidification of the whole milk chocolate. The preparations obtained this way were 19 mm in diameter and weighed about 4.1 g. The product was stored in a refrigerator.

3.3. Instrument and chromatographic conditions

The HPLC system was consisted of a LaChrom D-7100 pump (Merck Hitachi) and a LaChrom D-7455 detector (Merck Hitachi), separation was performed on a Kinetex-PFP column (2.6 μm, 100 × 2.1 mm, Phenomenex). The column oven was maintained at 30 °C, the flow rate was 0.15 μL/min, the UV detector was set at 265 nm. Injection volume: 10 μL, run time: 9.0 min.

The mobile phase was consisted of water (780 mL), methanol (220 mL), triethylamine (10 mL), sodium pentanesulfonate (1.0 g), adjusted to pH 2.5 with phosphoric acid.

3.4. Preparation of reference solution

A cefaclor stock solution was prepared by accurately weighing in 500 mg cefaclor *CRS* and dissolving it in the mobile phase to a concentration of 50.0 mg/mL. The reference solution was obtained by diluting the stock solution to a concentration of 0.1 mg/mL with the mobile phase.

3.5. Sample preparation

The capsules were stored in a refrigerator (2–8 °C) for 4 weeks before analysis. In a 100 mL Erlenmeyer flask one "camouflage capsule" was treated with 10.0 mL 1 M hydrochloric acid and placed in a hot-air cabinet at 80 °C for 10 min for melting the chocolate, then the mixture was vortexed until homogeneous, and centrifuged at 3000 x g in a Megafuge 1.0R Centrifuge (Heraeus, Kendro). Then 5.0 mL of the supernatant were taken off, and shaken vigorously with 5 mL cyclohexane. After phase separation 1.00 mL of the aqueous phase were taken off and diluted to 10.00 mL with water. 100 μL of this solution were mixed with 900 μL of the mobile phase (see 3.3.), the resulting solution was filtered through a syringe filter (PTFE syringe filter, 0.2 μm, 13 mm, VWR International, Darmstadt), and analyzed by HPLC as described under 3.3.

3.6. Determination of linearity and precision

Validation data was obtained by analyzing triplicates of five concentration levels (0.40 mg/mL, 0.45 mg/mL, 0.50 mg/mL, 0.55 mg/mL, 0.60 mg/mL). Linearity was evaluated using a linear calibration curve using the method of least square regression and calculating the coefficient of determination. To determine the precision, the relative standard deviation (RSD) of the measured areas of the analyzed chromatograms was calculated.

3.7. Determination of recovery rate

To determine the recovery rate the data of the linearity experiment was compared to the data obtained from spiked matrix samples. For this purpose 4.0 g samples of whole milk chocolate were spiked each with 40 mg, 45 mg, 50 mg, 55 mg, and 60 mg cefaclor *CRS*, and extraction was performed with 10.0 mL of 1 M hydrochloric acid as described under 3.5. Only the 1:10 dilution step after the cyclohexane extraction was omitted, so that the resulting analyte solutions had theoretical concentrations of 0.40 mg/mL, 0.45 mg/mL, 0.50 mg/mL, 0.55 mg/mL, and 0.60 mg/mL.

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