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Validation of a simple method for the determination of oxytetracycline in ointment by non-aqueous capillary electrophoresis

Jette Tjørnelund *, Steen Honoré Hansen

Department of Analytical and Pharmaceutical Chemistry, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark

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Abstract

A non-aqueous capillary electrophoresis method for the determination of oxytetracycline in an ointment have been validated. The oxytetracycline (OTC) is separated from related impurities and degradation products as metal chelates with magnesium ions. Thus, the method show high selectivity. The sample preparation is performed as a single extraction step of OTC from the melted ointment. The test for linearity in the range from 0.2-3.0 mg ml^{-1} gave a straight line with a coefficient of correlation greater than 0.999. Precision and accuracy were investigated using standard addition at three different concentration levels and six separate determinations at each level. The precision is good and may be expressed as the coefficient of variation which was lesser than 3.6%. The recovery is close to 100%. © 1997 Elsevier Science B.V.

Keywords: Oxytetracycline; Non-aqueous capillary electrophoresis; Ointment; Validation

1. Introduction

Extraction of a drug substance from ointment is often a tedious procedure as it may consists of several extraction steps in order to isolate the drug substance from the greasy matrix and recover it into a more convenient aqueous matrix suitable for reversed-phase HPLC or capillary electrophoresis (CE). A previously described procedure [1] for analyzing chlortetracycline in ointment include dissolution of the ointment in diethylether followed by repeated extractions with diluted hydrochloric acid. Non-aqueous capillary electrophoresis has been successfully applied to the analysis of pharmaceutical substances and products [2–5] but the use of the principle for the analysis of ointments has not previously been described. The use of non-aqueous capillary electrophoresis media makes it possible to inject the solvent used for a single extraction step with an organic solvent thus providing a very simple method. In this paper we describe the analysis of the widely used antibiotic oxytetracycline contained in a drug 'Hydrocortisone with Terramycin

* Corresponding author.

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ointment'. The method has been validated according to the ICH3 guidelines for the validation of analytical procedures [6].

2. Experimental

2.1. Chemicals

N-methylformamide (NMF) was purchased from Aldrich, Steinheim, Germany. Magnesium acetate tetrahydrate was obtained from E. Merck, Darmstad, Germany. Samples of oxytetracycline hydrochloride were kindly donated by Nycomed DAK (Roskilde, Denmark) and Dumex Ltd. (Copenhagen, Denmark). 4-Epianhydrotetracycline (EATC) hydrochloride, 4-epitetracycline (ETC) hydrochloride, 4-epioxytetracycline (EOTC) hydrochloride were CRS-standards from The Counsil of Europe. β-Apooxytetracycline (β-APO) and anhydrotetracycline (ATC) hydrochloride were purchased from Agros Chemicals (Beerse, Belgium). The ointment used for the experiments was Hydrocortisone with Terramycin (Pfizer, New York, USA). All other chemicals were of analytical grade.

2.2. Capillary electrophoresis

CE experiments were performed using Beckman P/ACE 5010 (Beckman, Fullerton, CA, USA) equipped with a UV-detector. The detector was operated at 280 nm. The samples were loaded in a fused-silica capillary (Polymicro Technologies Phoenix, AZ, USA) or (Beckman, Fullerton, CA, USA) 75 μm $\times$ 27 cm (20 cm to the detector) by applying a pressure (0.5 psi; ca. 3.5 kPa) for 3 s. The separations were carried out at 20°C by applying a voltage gradient of 15 kV and by using an electrophoresis medium consisting of 500 mM magnesium acetate tetrahydrate in NMF. The flow of air over the samples in the autosampler carousel was reduced in order to minimize evaporation of sample as well as of the electrophoresis medium during the experiments. Furthermore, the part of the leverarm opening the caps was replaced by parts made of teflon as NMF harms the material of the original leverarm. Data acquisition was accomplished with a NEM personal computer 486 with System Gold software.

Prior to use the capillary was conditioned with 1 M sodium hydroxide for 1 h, 0.1 M sodium hydroxide for 20 min, distilled water for 20 min, methanol for 20 min and finally the electrophoresis medium for 20 min. The capillary was flushed with the electrophoresis medium for 1 min between runs.

It was found very important to trim and clean the parts of the injection system with regular intervals in order to obtain a good precision of the injections as contamination of the sealing makes the system vulnerable to leaks resulting in variations of the injection pressure and thereby of the amount injected.

The electrophoresis medium was found to be stable and may be used for at least 15 runs without replacement.

2.3. Sample preparation

Of the ointment, 500 mg, was extracted with 25.0 ml NMF at 61.5°C for 15 min. By cooling on an ice bath the ointment matrix was solidified and was then easily removed by centrifugation. The clear extract was injected into the capillary. The calibration standard were prepared every day in NMF and protected against light. Calibration standards solutions were treated equivalent to the samples. Analysis should be performed within the same day.

2.4. Validation procedures

The method developed was validated with respect to specificity by looking for interfering impurities from the ointment matrix and from degradation products. Linearity and range were tested using a calibration standard of OTC. Repeatability and accuracy were investigated at three different concentration levels. The accuracy was determined using a standard addition procedure. The importance of using capillaries from different suppliers as well as the temperature of the surrounding of the capillaries were investigated.

3. Results and discussion

3.1. The electrophoresis system

Tetracyclines are known to form chelates with different metal ions and this has recently been used to obtain non-aqueous capillary electrophoretic separations of tetracyclines with high efficiency and selectivity [7]. In the present method chelation with magnesium ions is used as magnesium acetate is easily soluble in NMF.

3.2. Sample preparation and stability

Tetracyclines and related substances is known to have a limited stability as they are susceptible to both dehydration, epimerization, isomerization and oxidation [8] depending on pH, temperature as well as exposure to light. OTC can epimerize at C-4, resulting in formation of 4-epioxytetracycline (EOTC). The presence of a hydroxyl group at C-6 enables acid degradation and formation of anhydrooxytetracycline (AOTC). AOTC is quite unstable and rearranges in acid to the isomers α-apooxytetracycline (α-APO) and β-apooxytetracycline (β-APO) [9]. These isomers can further react to form terrinolide (TL). Furthermore, oxytetracycline may contain fermentation impurities such as 2-acetyl-2-decarboxamidooxytetracycline, tetracycline (TC) and its impurities. The known degradation products of OTC are shown in Fig. 1. Thus, it is very important to perform the extraction procedure under as mild conditions as possible. The ointment base consists of adeps lanae and vaselinum album, which both melt at approximately 55°C. In this procedure the matrix is melted in order to extract the tetracyclines in a single extraction step. NMF is used as the extraction solvent in order to resemble the composition of the electrophoresis medium as much as possible. The importance of the extraction temperature as well as the time of extraction is demonstrated in Fig. 2 showing the electropherograms of oxytetracycline after extraction of the ointment performed using a steam bath (100°C) or a water bath termostated to 61.5°C. No attempt was made to identify the degradation products formed. The OTC calibration standard is treated equivalent to the sample in order to compensate for the minor degradation taking place during extraction at 61.5°C.

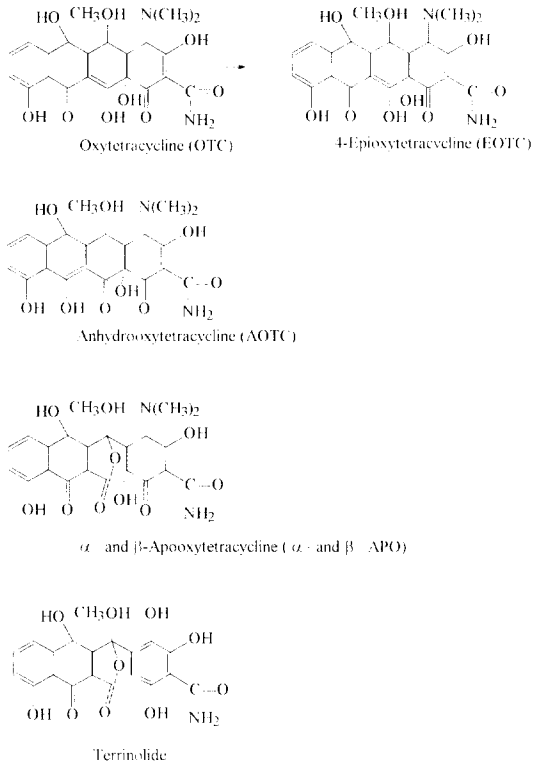

Fig. 1. Structures of oxytetracyclines and its major degradation products.

3.3. Validation

3.3.1. Selectivity

A number of aqueous CE methods and two non-aqueous CE methods demonstrating selectivity towards separation of tetracyclines and related

Fig. 2. Extraction of OTC from ointment using NMF. A, 61.5°C for 15 min; B, Adeps lanae extracted at 61.5°C for 15 min; C, 100°C for 1 min.; D, 100°C for 5 min.; E, 100°C for 10 min. Capillary: 27 cm × 75 μm I.D. (20 cm to detector). Electrophoresis medium: 500 mM magnesium acetate tetrahydrate in NMF. Temperature: 20°C. Voltage: 15 kV. Detection: 280 nm.

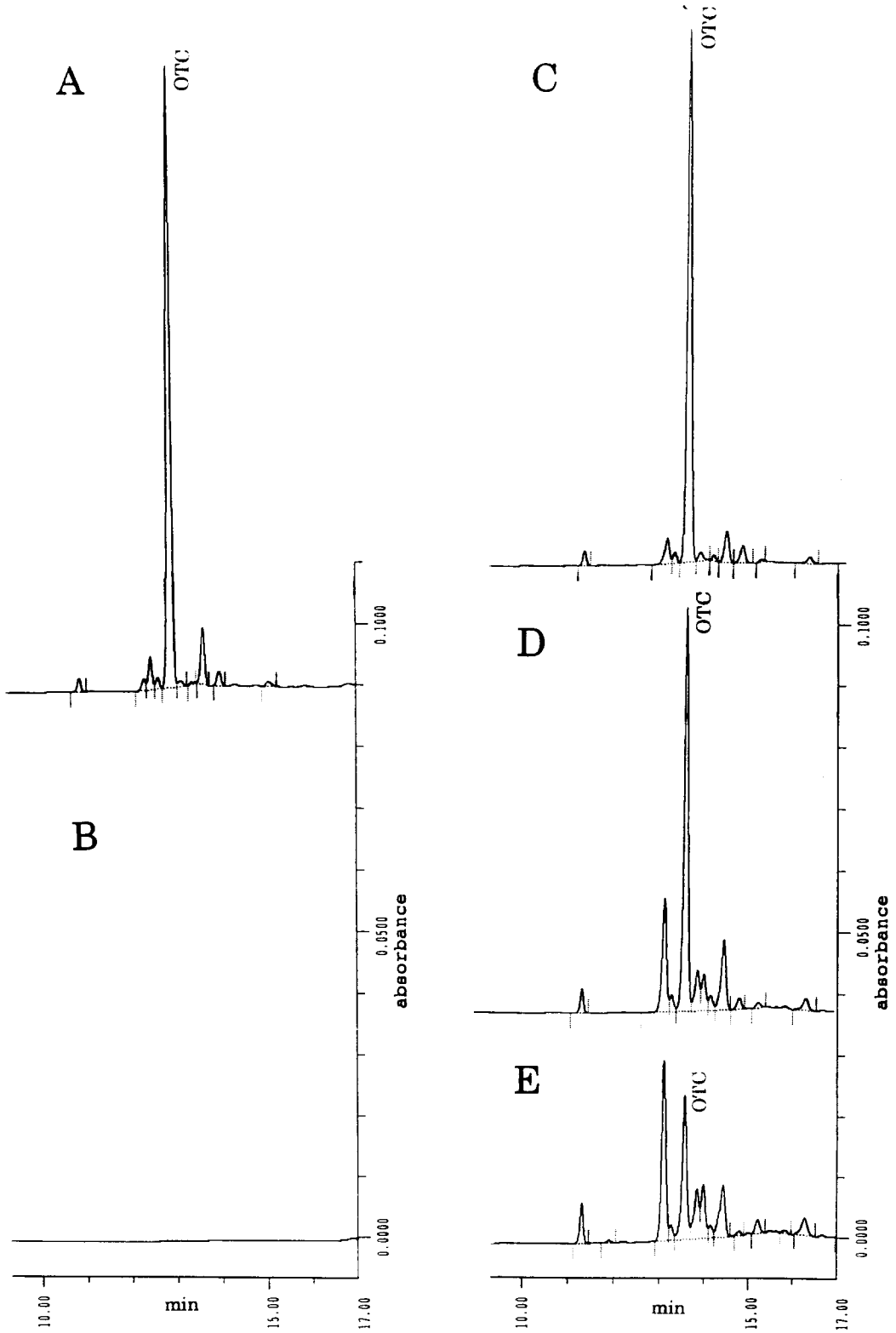
A
OTC
B
C
OTC
D
OTC
E
OTC
0.1000
0.0500
0.0000
absorbance
10.00
15.00
17.00
min

Fig. 2.

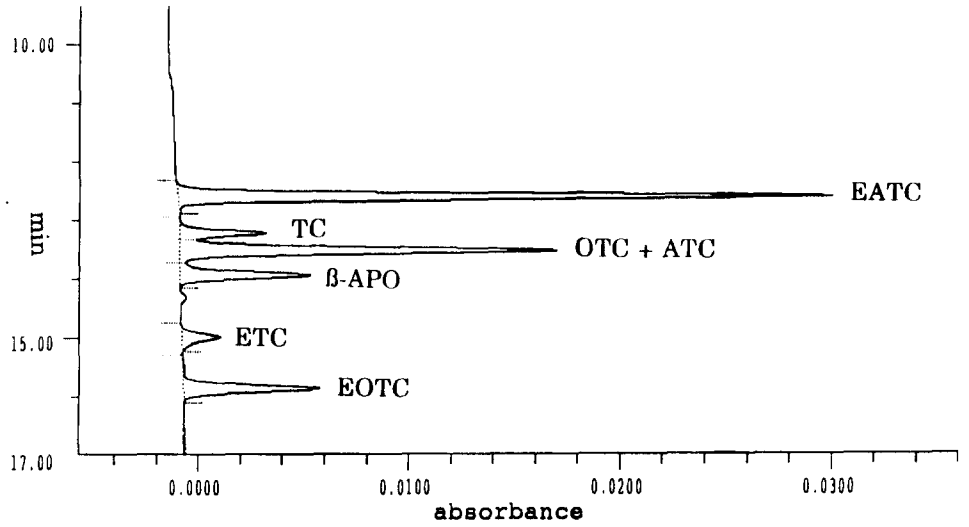

Fig. 3. Separation of OTC and a number of related substances. Electrophoretic conditions as given in Fig. 2. Peak identification: EATC, 4-epianhydrotetracycline; TC, tetracycline; ATC, anhydrotetracycline; β-APO, β-apooxytetracycline; ETC, 4-epitetracycline; EOTC, 4-epioxytetracycline.

substances has been presented [5,7,10–13]. The use of metal chelates gives a very excellent selectivity between tetracyclines. Fig. 3 shows the separation of OTC and a number of related substances separated as chelates with magnesium ions. OTC is well separated from TC, ETC, EATC, EOTC, β-APO but co-migrate with ATC. The constituents of the ointment matrix were analyzed according to the method described. Both components gave an electropherogram absolutely free of any peaks. An example is given in Fig. 2. Degradation products formed during analysis are also separated from OTC (Fig. 2). Thus, the selectivity of the method must be concluded to be high, and a mixture of tetracyclines may be used for a system suitability test.

3.3.2. Linearity

Calibration curves in the range 0.2–3.0 mg ml^{-1} gave straight lines which could be extrapolated through the origin. The correlation coefficients were greater than 0.999. At three concentrations (0.6, 1.0 and 1.5 mg ml^{-1}) repeatability studies were performed ($n = 6$) based on area measurements. The coefficients of variation (CV in percentage) obtained were 2.8, 4.3 and 3.1, respectively.

Precision and accuracy of the overall method were determined by standard addition at three concentration levels with six repeated assays at each level. The figures obtained are given in Table 1.

3.3.3. Migration times

The repeatability of the migration times for OTC was validated within and in-between days and was found to vary with a CV greater than 0.8% within day ($n = 8$) and less than 3.3% in-between day ($n = 6$).

3.3.4. Capillaries

Several fused silica capillaries from two different suppliers were compared. All capillaries had the same dimensions (27 cm × 75 μm I.D.). The migration times for OTC varied within 13.4–13.7 min. The efficiencies obtained were also comparable (ca. 300 000 plates m^{-1}).

3.3.5. Temperature

The temperature surrounding the capillary was varied between 20 and 40°C without any significant change in selectivity.

Table 1
Precision and accuracy of the determination of OTC in hydrocortisone with terramycin ointment (30 mg g^{-1})

Precision and accuracy ($n = 6$), (CV)				
Initial concentration mg g^{-1}	OTC added mg g^{-1}	OTC found mg g^{-1}	Recovery in %	Bias in %
Expected 30.0	0.00	29.4 (3.6%)	98.1 of declared amount	
Initially found 29.4	15.2	43.4 (3.2%)	97.3	−2.7
Initially found 29.4	30.2	57.3 (2.0%)	96.1	−3.9
Initially found 29.4	45.2	72.1 (1.5%)	96.7	−3.3

Ointment was extracted with 25.0 ml NMF for 15 min at 61.5°C.

4. Conclusion

A simple method for the assay of oxytetracycline in an ointment, hydrocortisone with terramycin, has been developed. The method involve capillary electrophoretic separation using a non-aqueous electrophoresis medium. The method has been validated according to the guidelines in ICH3 and has been found to be suitable for the purpose it was intended for.

Acknowledgements

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