School of Pharmacy[1], Tabriz University of Medical Sciences, Tabriz, Iran, and School of Pharmacy[2], University of Bradford, United Kingdom

Nonaqueous capillary electrophoretic analysis of hexetidine in a commercial liquid formulation

A. JOUYBAN[1], J. YE[2] and B. J. CLARK[2]

A non-aqueous capillary electrophoresis method has been proposed to analyse hexetidine and its degradation products in a commercial liquid pharmaceutical formulation. The separation was achieved using a 37 cm fused silica capillary with a 75 μm internal diameter, an electrolyte of 120 mM sodium acetate buffer in methanol, at 16 kV and 25 °C. The results show that the proposed method is able to resolve hexetidine from its degradation products and/or impurities.

1. Introduction

Hexetidine [1,3-bis (ethylhexyl)-5-methylhexahydropyridimidine] is a hexahydropyrimidine derivative with biocidal activity against pathogenic organisms. Its antibacterial and fungicidal activities have been explained by its interactions with vital compounds like purines, thiamine and uracil. The drug is available as 0.1% w/v mouthwash for local infections and oral hygiene.

Hexetidine is practically insoluble in water and a liquid formulation of hexetidine has been prepared using surface active agents and cyclodextrins and its *in vitro* availability has been studied [1]. Hexetidine is unstable in aqueous solution and in the presence of light. Its main hydrolytic degradation products are triamine [N_1,N_3-bis-(β-ethylhexyl)-2-methyl-propanetriamine-(1,2,3)] and hexedine [2,6-bis-(β-ethylhexyl)-hexahydro-7α-methyl-1H-imidazo (1,5-*c*) imidazole] [2]. Dehydrohexetidine is an oxidative degradation product of hexetidine [3].

Commercial hexetidine contains 70–85% of hexetidine, 5–10% of triamine and 5–15% of hexedine, quantities of dehydrohexetidine and an unknown compound [3]. The hexetidine as a bulk drug differs from commercial hexetidine in that it is not a mixture and contains not less than 98.0% hexetidine [4]. The published analytical methods for hexetidine are TLC [3], GLC [5], and HPLC [2]. However, no capillary electrophoresis (CE) method has been reported so far. Non-aqueous capillary zone electrophoresis methods provide advantages over aqueous methods particularly for materials which have aqueous solubility problems. As an example, hexetidine, which is insoluble in water and quite sensitive to hydrolytic degradation, a non-aqueous running buffer may be the best choice. Hexetidine is soluble and stable in methanol and, therefore, methanolic solutions have been used in this work which was done to develop a specific, rapid, easy and inexpensive CE method for the determination of hexetidine in liquid pharmaceutical formulations.

2. Investigations, results and discussion

The CE method was developed using a sample of hexetidine mixture and an expired commercial formulation (30 months before date used) in order to obtain easily measurable degradation products. The main emphasis was to obtain a good resolution between hexetidine and its degradation products and/or impurities. Because of the low water-solubility of hexetidine, a nonaqueous method was used in the development of the analytical method. The investigation included the examination of different concentrations of sodium acetate and acetic acid, and also the applied voltage. The best buffer concentration was sodium acetate 50 mM and acetic acid 70 mM and the applied voltage was 16 kV. Sample electropherograms of the separations at these conditions are shown in the Fig.

Table: Results obtained for expired and non-expired commercial hexetidine formulations (Oraldene™)

Expiry date	Claimed	Found	RSD (%) (n = 5)
30 months before date used	0.1% w/v	0.0688% w/v	1.4
14 months after date used	0.1% w/v	0.0983% w/v	1.2
16 months after date used	0.1% w/v	0.0969% w/v	1.3
18 months after date used	0.1% w/v	0.0987% w/v	1.2

Good resolution of hexetidine with other components was obtained, and comparison of the separation results indicated that component **4** was hexetidine and the component **1** was the component due to hexetidine degradation (hexedine). The components **2** and **3** were triamine and dehydrohexetidine, respectively.

The standard curve is linear in a range of 0.05 to 0.3 mg/ml (n = 6). The mean coefficient of determination (R^2) for calibration curve was 0.9995.

The method was applied to determine hexetidine in a liquid pharmaceutical preparation according to the above procedure. The results are shown in the Table. The separation time in this method is only 6 min, and the remaining components are washed in the next rinse step. The whole analysis procedure takes 9 min (pre-washing with sodium hydroxide 1 min and running buffer 2 min and run time 6 min). As it has been shown in the Fig. (electropherograms b and c), the method is able to detect the degradation products of hexetidine. These data show the suitability of the proposed method for pharmaceutical analysis and quality control of hexetidine preparations.

3. Experimental

3.1. Samples and reagents

The pure hexetidine for standard solutions was kindly provided by Warner Lambert. A hexetidine mixture sample was purchased from Sigma (Dorset, UK). Sodium acetate, glacial acetic acid and methanol were supplied by BDH (Poole, UK). Purified water was prepared with Milli-Q system. An Oraldene™ (Warner Lambert, UK) commercial formulation was purchased from a community pharmacy. A bottle of Oraldene™ (0.1% w/v hexetidine) that had expired 2.5 years before the study was used to demonstrate the separation between hexetidine and its degradation products in a pharmaceutical formulation.

The stock solutions of pure hexetidine and hexetidine mixture were prepared by dissolving the appropriate amount of substance (25 mg) in 25 ml of methanol in volumetric flasks, and stored in the dark.

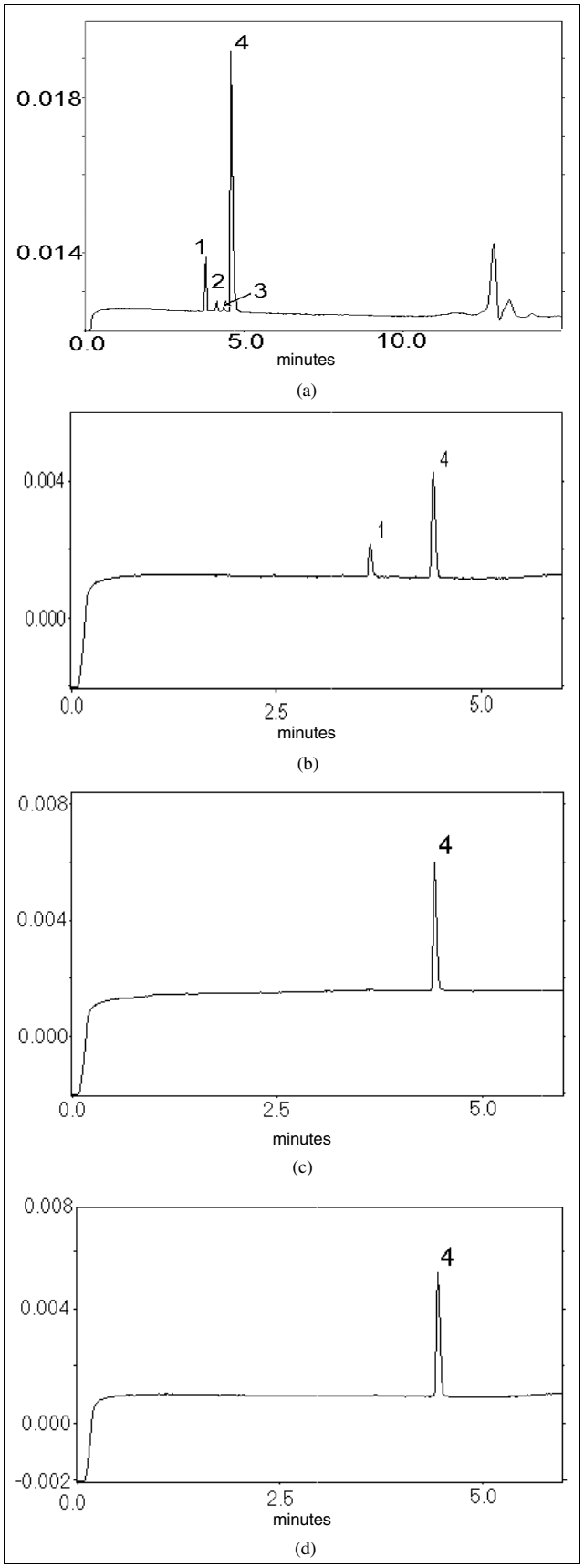

Fig.: Electropherogram of (a) hexetidine mixture, (b) an expired Oraldene™ sample (30 months before date used), (c) a fresh Oraldene™ sample (will expire 16 months after date used) and (d) a pure hexetidine solution. The component 1 is hexedine, 2 is triamine, 3 is dehydrohexetidine and 4 is hexetidine.
Operating conditions: Fused silica capillary 37 cm × 75 μm I.D., running buffer containing 50 mM sodium acetate and 70 mM acetic acid in methanol, voltage 16 kV, temperature 25 °C.

3.2. Instrumentation and CE conditions

Analysis was performed using a Beckman P/ACE System 2210 series instrument with Beckman P/ACE software (Beckman Instruments Europe, High Wycombe, UK). A plain fused silica capillary (75 μm i.d. × 37 cm length, 30 cm to detector) was used to separate the samples. Prior to use, the capillary was washed with sodium hydroxide (1.0 M) for 30 min and with running buffer for 30 min. All analyses were performed after prewashing with sodium hydroxide (0.1 M) for 1 min and running buffer for 2 min. The sample was injected by electrokinetic mode (10 kV) for 5 s and detected by a UV detector at 214 nm. The applied voltage was 16 kV and the temperature was 25 °C. The running buffer was sodium acetate 50 mM and acetic acid 70 mM dissolved in methanol.

3.3. Sample and standard solution preparations

The sample solution was prepared by diluting 1.0 ml Oraldene™ with 5.0 ml running buffer and 4.0 ml methanol. Calibration standards were prepared at concentrations of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg/ml standard hexetidine sample in the mixture of running buffer and pure methanol (50 : 50).

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A. Jouyban
School of Pharmacy
Tabriz University of Medical Sciences
Tabriz 51664
Iran