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Short communication

Capillary electrophoresis for the determination of norfloxacin and tinidazole in pharmaceuticals with multi-response optimization

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Abstract

Capillary electrophoresis (CE) with UV photo-diode array detection technique was utilized to adopt new method for the analysis of norfloxacin and tinidazole in pharmaceuticals. Many CE aspects including separation, rapidity, sensitivity, ruggedness as well as the repeatability of qualitative and quantitative analyses were considered simultaneously for the purpose of optimization. Experimental design approach including factorial design and response surface methods were applied to optimize electrolyte concentration and the pH while injection time, voltage and column temperature were optimized using the univariate method. Successful results were obtained using 32.5 mmol l^{-1} phosphate electrolyte at pH 2.5, injection time 8.0 s, voltage 25 kV and column temperature 25 °C with detection at wavelength 301 nm. The analytical characteristics including recovery, intermediate precision, linear dynamic ranges, linearity and selectivity as well as limits of detection and quantification were demonstrated and the applicability to pharmaceuticals was studied. The newly provided method enjoys the advantages of CE over HPLC with respect to rapidity, ruggedness, simplicity in reagents and sample preparation as well as saving in reagents and samples.

Keywords: Capillary electrophoresis (CE); Experimental design; Pharmaceutical analysis; Norfloxacin; Tinidazole

1. Introduction

Norfloxacin (NFX) is a member of fluoroquinolones and it is chemically named as 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid (Fig. 1a). It is the first choice drug for the treatment of diseases caused by *Campylobacter*, *E. coli*, *Salmonella*, *Shigella* and *V. colera*. It is used for the treatment of gonorrhoea as well as eye and urinary tract infection [1].

Tinidazole (TNZ) is chemically named as (1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitro-1H-imidazole (Fig. 1b). It is a 5-nitroimidazole derivative used for the treatment of giardiasis and susceptible protozoal infections. It has also been used in regimens for the eradication of Helicobacter pylori in peptic ulcer disease [2,3].

Previously, fluoroquinolones and 5-nitroimidazole were given individually to treat chronic nonspecific inflammatory conditions of the gastrointestinal tract [2]. Recently, NFX and TNZ were prepared in a combination. Therefore, analytical methods for their separation and simultaneous quantification are required for quality control purpose. A survey of literature reveals that no capillary electrophoresis (CE) method for simultaneous determination of these two antibiotics is available in the literature while other techniques were utilized including high performance liquid chromatography (HPLC) [3,4], high performance thin layer chromatography [5] and spectrophotometry [6–12].

The United States and British pharmacopoeias [13,14] proposed liquid chromatographic (LC) and potentiometric methods for the individual assay of NFX and TNZ in the generic form and pharmaceutical formulations. In addition, other methods including HPLC [15,16], packed column super critical LC [17], spectrophotometry [18–22], fluorescence [23] and chemiluminescence [24–27] as well as voltammetry [28,29] and polarography [30] were also provided for individual assay of NFX and TNZ in pharmaceuticals.

Traditionally, pharmaceutical analysis relies heavily on LC. CE has many advantages over LC that are including greater separation efficiency, small sample and reagents volume, fast

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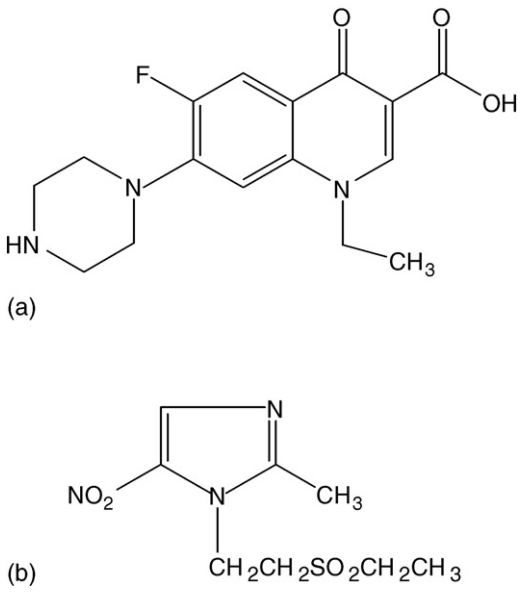

Fig. 1. Chemical structure of (a) NFX and (b) TNZ.

separation [31] and better ruggedness. Not least but more, unlike LC, reduction in sample preparation, simplicity in operating instrument and possibility of applying a single set of operating conditions to a wide variety of analytes all are available in CE analysis. These advantages empower CE with great utility to be successfully applied for routine pharmaceutical analysis.

Usually, experimental conditions affect the efficiency of chemical analysis and optimization of these conditions is always desirable. The univariate method is useful only for the optimization of irrelevant conditions. The experimental design, as multivariate methods, including factorial design and response surface are used to: (a) examine the main and interaction effects of conditions on the efficiency of analysis, (b) optimize simultaneously conditions regarding their interaction with minimum number of experiments and (c) reduce large amount of data that could be easily interpreted.

Analysis by CE generates large amount of data expressing selectivity, sensitivity, analysis time as well as precision; and many CE conditions potentially influence theses responses. This requires optimizing experimental conditions with considering CE responses simultaneously that can be performed using the multivariate methods. Unfortunately, the majority of the reported CE methods did not utilize the multivariate methods. This could be attributed to the fact that most of the CE practitioners have not yet discovered the inherent potential of the two scientific disciplines, i.e. CE analysis with multivariate optimization, working hand in hand.

The present work describes the development of a CE method for the analysis of NFX and TNZ in pharmaceutical formulations. Our attention was focused on the development of the major analytical aspects of the CE methods that are separation, rapidity, ruggedness and sensitivity as well as the repeatability of qualitative and quantitative analyses by optimizing the electrophoretic factors potentially controlling the analysis. Factorial design and response surface methods were utilized to optimize the relevant electrophoretic factors while other irrelevant electrophoretic factors were optimized using the univariate method.

2. Experimental

2.1. Chemicals and samples

All chemicals and reagents used in this study were of analytical grade. Cellulose, magnesium stearate, NFX, TNZ, starch and talc were supplied from Sigma–Aldrich (St. Louis, MO, USA). Phosphoric acid, sodium hydroxide and sodium phosphate were supplied from Merk (Darmstadt, Germany). Acetic acid, boric acid, sodium acetate and sodium tetraborate decahydrate were supplied from Sigma–Aldrich (Taufkirchen, Germany).

Conaz® tablets (400 mg NFX and 600 mg TNZ) was prepared by Pharaonia Pharmaceuticals (Cairo, Egypt).

2.2. Instrumentation and software

A P/ACE MDQ CE system coupled with a photo-diode array detector (PAD) supplied from Beckman (Fullerton, CA, USA) was used throughout the experiments. Separation was carried out in 31.2 cm long × 50 μm i.d. fused-silica capillary housed in a cartridge with a detector window 100 μm × 800 μm (10 cm to the detector, short way).

At each sequence experiment, the capillary was washed by 0.1 mol l^{-1} sodium hydroxide for 0.5 min and the separation electrolyte for 1.5 min. For sample loading, hydrodynamic injection mode was applied at the detector end of the capillary.

32 Karat Version 7.0 supplied from Beckman (Fullerton, CA, USA) was used for controlling the CE system as well as data acquisition and processing.

SigmaPlot® for Windows Version 8.0 supplied from Systat Software, Inc. (Point Richmond, CA, USA) was used for data interpolation and constructing response surface plots.

2.3. Preparation of standard solutions

Mixed primary standard solution was prepared by dissolving 100 mg NFX and 150 mg TNZ in about 80 ml of distilled deionized water with stirring in 100 ml volumetric flask. The volume was completed to the mark and stored protected from light at 4 °C, at this condition the solution is stable for at least 2 weeks. Working standard solutions were prepared from the primary standard solution by dilution in the appropriate way.

2.4. Preparation of samples

Twenty Conaz® tablets were powdered and amounts equivalent to 10 mg NFX and 15 mg TNZ were weighed and dissolved by stirring in about 80 ml of distilled deionized water. The solution was filtered in a 100 ml volumetric flask and filled to the mark. Other pharmaceutical samples were synthesized in our laboratories including NFX and TNZ in different concentrations with excipients (including cellulose, magnesium stearate, starch and talc) usually found in tablets formulation. In addi-