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Short communication

Determination of piperazine in pharmaceutical drug substances using capillary electrophoresis with indirect UV detection

Chris M. Denis, Nicole E. Baryla *

Lilly Analytical Research Laboratory, Eli Lilly Canada Inc., 3650 Danforth Avenue, Toronto, Ont., Canada M1N 2E8

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Abstract

A fast, selective capillary electrophoresis (CE) method was developed for piperazine counter-ion analysis and applied to the analysis of an active pharmaceutical ingredient (API) that exists as a hemipiperazine salt. Due to the poor chromophore, the detection method chosen was indirect UV detection using benzylamine as the UV absorbing probe. Piperazine quantitation was performed using diethylamine as an internal standard and the method was validated for specificity, linearity, precision, and accuracy. The results indicate the method is suitable for piperazine counter-ion analysis in support of salt form characterization.

Keywords: Piperazine; Indirect UV detection; Counter-ion analysis; Validation; Capillary electrophoresis

1. Introduction

Accurate determination of the purity of pharmaceutical compounds must account for the presence of the counter-ion from the salt form of the drug. HPLC with UV detection has been used for the analysis of such counter-ions as well as other molecules [1–3]. For ions that do not fluoresce or absorb at useable wavelengths, sensitive detection can present a challenge. A variety of techniques such as ion exchange chromatography with evaporative light scattering detection [4], gas chromatography/mass spectrometry, and HPLC/fluorescence of a derivatized analyte [5,6] can be used, but a simpler and less expensive method is always preferable. Capillary electrophoresis (CE) has the advantages of speed and cost versus HPLC and has been used for the counter-ion analysis of pharmaceutical compounds [7].

While CE with direct UV detection has been effectively used for molecules that absorb strongly at useable UV wavelengths [8,9], many counter-ions have poor UV chromophores. This obstacle can be overcome by using indirect UV detection [10]. A common counter-ion in API that could be analyzed by such a method is piperazine (Fig. 1).

The aim of this study was to develop a CE method with indirect UV detection for the quantitation of piperazine from an API hemipiperazine salt (Fig. 1). This method was developed using benzylamine (Fig. 1) as the probe, and quantitation of the piperazine was carried out using diethylamine (Fig. 1) as an internal standard. The method was validated for specificity, linearity, precision, and accuracy. Based on the validation results, the CE method developed was suitable for the quantitation of piperazine from an API hemipiperazine salt.

2. Experimental

2.1. Chemicals, materials, reagents, and solutions

The water used was de-ionized with a Milli-Q system (Millipore, New Bedford, MA, USA). The benzylamine probe and diethylamine internal standard were supplied by Aldrich (Milwaukee, WI, USA) and 99% piperazine was supplied by Sigma-Aldrich (St. Louis, MO, USA). The 1.0 M sodium hydroxide and 1.0 M HCl came from VWR (West Chester, PA, USA), the acetonitrile from EMD (Durham, NC, USA), the ethyl acetate from Burdick & Jackson (Muskegon, MI, USA), and the API hemipiperazine from Eli Lilly and Company (Indianapolis, IN, USA). The 1.0 μm filters were purchased from Pall (Ann Arbor, MI,

* Corresponding author. Tel.: +1 416 693 3621; fax: +1 416 693 3752.
E-mail address: baryla_nicole@lilly.com (N.E. Baryla).

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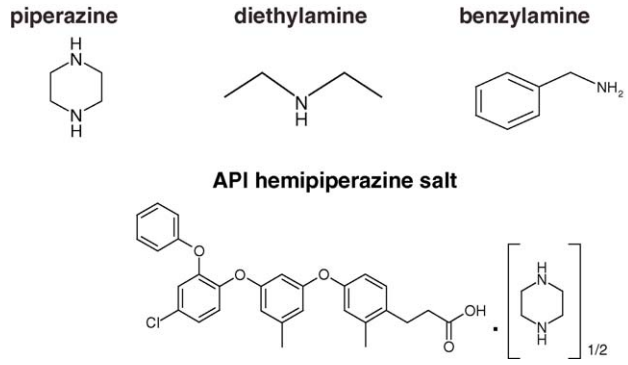

Fig. 1. Structure of the piperazine analyte, the diethylamine internal standard, the benzylamine probe, and the API hemipiperazine salt.

USA), and the bare fused silica capillary was from Polymicro Technologies (Phoenix, AZ, USA).

2.1.1. Preparation of solutions

The nominal concentration of the piperazine standard was 0.086 mg/mL. The sample solvent was 50% water and 50% acetonitrile containing 0.073 mg/mL diethylamine. The piperazine standard solutions were made by first preparing a stock solution of 0.8614 mg/mL piperazine and then diluting. Dissolution of the piperazine included sonication with a Branson 8200 sonicator (Danbury, CT, USA) for 2 min.

The separation buffer was a 50 mM solution of the benzylamine probe, prepared by adding an appropriate amount of benzylamine into water and adjusting the pH to 8.70 using 1.0 M HCl.

2.2. Sample preparation

In order to quantitate the amount of piperazine in the API hemipiperazine salt, which is theoretically 8.1% piperazine, a 1 mg/mL sample solution was made by dissolving 50 mg of API hemipiperazine in a 50 mL volumetric flask with the same solvent used for the piperazine solutions (Section 2.1.1), followed by sonicating for 4 min.

2.2.1. Preparation of API free acid

Hemipiperazine salt (1 g) was first mixed with 3.8 mL of 1.0 M HCl. The API free acid was extracted with 10 mL of ethyl acetate in a separatory funnel. The ethyl acetate layer was retained and nitrogen gas blown over it overnight to evaporate the ethyl acetate. The resulting viscous liquid was used for the accuracy experiments (Section 3.2.4).

2.3. Determination of piperazine mobility

The electrophoretic mobility of piperazine was estimated from a CE separation of 0.086 mg/mL piperazine prepared in water and run with a 5 mM chromate separation buffer. The sample was run at +5 kV on a bare fused silica capillary (internal diameter 77 μm, total length 32.8 cm, effective length 24.3 cm) and the piperazine peak eluted at 3.00 min, ahead of the water peak at 4.40 min.

2.4. Instrumentation and method conditions

Capillary electrophoresis separations were carried out using an Agilent Technologies ^{3D}CE system (Palo Alto, CA, USA). The data collection and analysis were carried out using Agilent Technologies ^{3D}CE ChemStation software (Palo Alto, CA, USA).

A new fused silica capillary (internal diameter = 77 μm, total length = 32 cm, effective length = 23.5 cm) was used to analyze piperazine solutions. The new capillary was pre-conditioned with 1.0 M NaOH for 15 min, water for 10 min, separation buffer for 5 min, and a +5 kV voltage was run across the capillary for 3 min prior to the first analysis. Prior to each injection, the capillary was flushed with separation buffer for 3 min. Following each separation, the capillary was flushed with 1.0 M NaOH for 3 min, followed by water for 3 min. The separation buffer was replenished every three runs.

Samples were injected hydrodynamically (50 mbar × 3 s) and a voltage of +7.5 kV was applied across the capillary, generating a current of approximately 30–35 μA. The capillary temperature was thermostatted to 25 °C and indirect UV detection was at 214 nm.

3. Results and discussion

3.1. Development of CE method

Initial method development involved choosing an appropriate probe to be used as the separation buffer. Piperazine is a weak base with two ionisable groups having pK_a values of 9.73 and 5.33 [11]. Therefore, a probe with a buffering capacity at a pH less than 8.8 was desired so that the piperazine molecules would be positively charged. In addition, a probe of similar charge and electrophoretic mobility to piperazine was required in order to minimize peak broadening and asymmetry of peak shape from electrodispersion. The effective mobility of piperazine was found to be approximately 28×10^{-9} m^2/V s. Comparison with the published mobilities of various potential probes suggested benzylamine as a good candidate for use with analytes of this mobility [12]. Using benzylamine at pH 8.7 as the background electrolyte, a piperazine peak with good shape was produced. For quantitation of piperazine using this method, a suitable internal standard was chosen. Diethylamine was used as the internal standard since it was sufficiently separated from piperazine.

In order to optimize the separation, the probe concentration was varied from 5 to 100 mM. The best peak shape for piperazine and diethylamine was achieved at a benzylamine concentration of 50 mM, with sensitivity negatively affected at a concentration of 100 mM (Fig. 2). At the extreme benzylamine concentrations, the peak shape has been impacted by the difference in conductivity between sample and separation buffer causing electrodispersion.

3.2. Validation

3.2.1. Specificity

Injections of sample solvent, internal standard (diethylamine), API hemipierazine, and piperazine were analyzed. No