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Original article

A method for the determination of minoxidil in hair-regrowth formulations by micellar electrokinetic capillary chromatography

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Abstract

A method based on micellar electrokinetic capillary chromatography (MEKC) was developed for determination of minoxidil in Rogaine and competing products. The original intent of the work was to offer an orthogonal means to HPLC for testing illicit imitations of Rogaine. However, because the patent has since expired, we offer the procedure as a confirmatory measure to HPLC for assay of generic minoxidil products. The MEKC procedure complements an earlier method based on free solution capillary electrophoresis (FSCE), designed to the same end. Validation was carried out on both a Dionex CES-1, which utilizes gravity injection, and a PE-ABI 270HT, which employs vacuum injection. The procedure was validated for both active pharmaceutical ingredient and for minoxidil solutions. The run buffer is pH 7.0, 20 mM sodium phosphate, 20 mM sodium dodecyl sulfate, with 10% isopropanol; the internal standard is DL-tryptophan. The method bears the attributes of simplicity, ease of use, and short analysis time (12 min). It is selective with respect to known process and degradation impurities. High efficiency was achieved on the CES-1, with a plate count exceeding 200,000 for minoxidil at an elution time of 9 min. Although slight differences in performance were noted across the two instruments, results on both were in conformance with modern day validation expectations. Comparison of MEKC with HPLC resulted in slightly higher values for the former, but all results met registration specifications and internal targets.

Keywords: Minoxidil; Micellar electrokinetic capillary chromatography; MEKC; Method validation; Pharmaceutical analysis

1. Introduction

Capillary electrophoresis (CE) has been applied to virtually all types of pharmaceutical analysis. Because the concentration of a sample preparation is usually under the analyst's control, and hence can be adjusted to a suitably high level, CE is well suited to major component analysis, either for active pharmaceutical ingredient alone or for dosage forms. Free solution or capillary zone electrophoresis (FSCE, CZE) and micellar electrokinetic capillary chromatography (MEKC) together have been applied to virtually every class of drug [1–7]. The challenge is greater for impurities analysis, as the solution concentration sensitivity in CE is two or more orders of magnitude poorer than in HPLC [8]. Nevertheless, capillary electrophoretic techniques have been widely applied to analysis of impurities [1,3,7,9–13]. For impurities analysis it is essential that the areas be normalized through division by elution time so as to not bias the results due to differing rates of transport through the detection window [14]. For many ionizable compounds, one may have the choice of utilizing CZE on the charged species or operating at a pH where the analyte is either charged or neutral and employing MEKC. Minoxidil ((6-1-piperidinyl)-2,4-pyrimidine-3-oxide) is such a compound.

Minoxidil is the active ingredient in Rogaine®, indicated for treatment of hair loss. Although originally produced as a 2% (w/v) solution, it is now principally sold as a 5% solution. While it is no longer on patent, prior to the patent expiring, it faced an onslaught of illicit imitations. Possessing a pK_a of 4.6 and an intrinsic water solubility of 2.2 mg/ml, it should be a viable candidate for electrophoretic separation by either CZE or MEKC. We have earlier-developed a method based on CZE [15]. In that method a pH 3.5 lithium citrate

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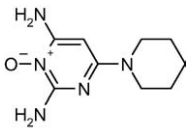
buffer is used to achieve a positive charge on the basic minoxidil. The goal in the present project was to separate minoxidil by MEKC at a pH where sodium dodecyl sulfate (SDS) or another surfactant could be used to advantage.



Minoxidil

In the earlier report [15] reference was made to the many illicit Rogaine imitations intercepted by the patent department and forwarded to the laboratory for forensic analysis, principally by HPLC. A number of those counterfeit preparations were subjected to confirmatory analysis using the earlier-developed FSCE procedure. Illicit imitations are no longer an issue for Rogaine since it is off patent. The new competition is from generic products. Confirmatory analysis of these has been the principal application of the MEKC method. Other than brief mention in generalized procedures for basic drugs in toxicological analysis [16,17], to our knowledge, only a single paper, one that was preliminary in nature, has appeared on CE applied to minoxidil [18]. A related paper utilizing capillary isotachophoresis was published by the same group [19]. None, to our knowledge, has been published on the use of MEKC.

2. Experimental

2.1. Chemicals

Sodium phosphate monobasic and dibasic were obtained from J.T. Baker, phosphoric acid from Mallinckrodt, sodium dodecyl sulfate from Aldrich (cat. no. 85,192-2), and 2-propanol (IPA) from either EM Science or Burdick and Jackson. All chemicals were used as received. The surfactant, although labeled dodecyl sulfate, sodium salt (SDS, sodium lauryl sulfate), was actually a mixture of 70% sodium dodecyl sulfate, ~25% sodium tetradecyl sulfate, and ~5% sodium hexadecyl sulfate. Sodium phosphate buffer was prepared by mixing equimolar concentrations of NaH_2PO_4 and Na_2HPO_4 and titrating to the desired pH. This solution was filtered prior to addition of SDS and IPA through a 0.45 μm filter, then degassed with vacuum and ultrasonication after addition of the SDS and IPA. The potential internal standards, including the one chosen, tryptophan, were purchased either from Sigma or from Aldrich. Minoxidil USP and minoxidil reference standard were obtained internally. The purity of the reference standard was 99.9%. Purified water (Nanopure® or Milli-Q®) was generated in-house.

2.2. Instrumentation/equipment

Procedures were developed on both Dionex CES-1 and PE-ABI 270HT Capillary Electrophoresis Systems. The methods differ slightly, as a method developed on one instrument is not directly transferable to a different model or make instrument. Neither of these instruments is still in production. Some of the method development was carried out on an ABI 270 A, an analogous instrument to the HT except that it contained only a four-position sampling carousel. The 270HT contains a 50-sample carousel and has provision for up to eight buffer vials. Hydrodynamic injection via gravity was used on the CES-1 whereas vacuum injection was used on the 270HT. The CES-1 had the drawback of having no forced cooling; the 270HT was air cooled. This required that currents be kept low on the CES-1.

The CES-1 contains three large buffer reservoirs, which allows for replenishment of buffer in both the source and destination vials after every sample. This was a feature unique to the CES-1. However, when the buffer contains surfactant, as in MEKC, it is preferable not to utilize this capability, as foaming may result. In the present case, in order to avoid exposing the buffer transfer lines to SDS surfactant, run buffer was filled into vials on the autosampler carousel. Each vial was filled with 100–200 μl of buffer and used only once. Capillaries for the 270HT were purchased with the detector window pre-exposed. The capillary was trimmed to the desired length (if necessary) and a short (0.5 cm) section of polyimide coating was burned off the inlet end. For the CES-1, capillary tubing was cut to the desired length and the window exposed using a flame and an implement furnished by Dionex. All capillaries were 50 μm inner diameter.

The final assay conditions were as follows. *PE-ABI 270H*. Capillary, length, 42 cm (20 cm to detector) × 50 μm id; run buffer, pH 7.0, 20 mM phosphate, 20 mM SDS, 10% 2-propanol (IPA); sample buffer, pH 7.0, 4 mM phosphate, 10% ethanol; internal standard solution, 167 μg/ml DL-tryptophan dissolved in sample buffer; rinsing solution, pH 7.0, 20 mM phosphate, 10% IPA, applied for 3 min followed by fresh run buffer for 3 min; injection, hydrodynamic (vacuum, 5 in Hg) for 2.5 s; temp., 30.0 °C; applied voltage, 12 kV (current, ~15 μA). *Dionex CES-1* (only differences noted). Capillary, length, 50 cm (45 cm to detector) × 50 μm id; rinse for 2 min followed by fresh run buffer for 3.3 min; injection, hydrodynamic (gravity), 100 mm × 10 s; temp., ambient (no temperature control); applied voltage, 15 kV (current, ~18 μA). Data collection and calculations were accomplished using an in-house data acquisition system in conjunction with internally developed and validated software. Raw areas were normalized by dividing by elution time.

3. Results and discussion

3.1. Method development

Minoxidil has a pK_a of 4.6. A buffer with pH > 6 would be expected to promote partitioning into SDS micelles and to achieve a high enough electroosmotic flow (EOF) so as to transport minoxidil past the detector. Because a buffer