DETERMINATION OF VARENICLINE BY CAPILLARY ZONE ELECTROPHORESIS

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A fast, sensitive and selective method for the detection and quantification of varenicline (VRC) in tablet dosage form was developed using capillary zone electrophoresis separation with DAD detector. The used capillary was uncoated standard bare fused-silica with 100 µm I.D, 40 cm total length and 31.5 cm effective length. The electrophoretic separations were carried out in 50 mM sodium phosphate buffer (pH 2.5) under normal mode (30 kV). Samples were filtered through a 0.22µm CME membrane filter and then injected hydrodynamically at a pressure of 50 mbar for 10 s. The calibration curve of VRC was linear (r=0.998) over the concentration range 1–16 µg/ml. The LOD and LOQ values were 0.2 and 0.6 µg/ml, respectively. Finally, the method proved to be suitable for quality control studies.

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Keywords: varenicline, capillary electrophoresis, quality control, smoking.

1. Introduction

Varenicline (VRC) is a partial selective agonist of the α4β2 subtype of the nicotinic acetylcholine receptor. It is used as an aid for smoking cessation[1,2]. In addition, it acts on α3β4 and weakly on α3β2 and α6-containing receptors[2]. A full agonist was displayed on α7- receptors[2]. The chemical name of VRC is 7,8,9,10–Tetrahydro-6*H*-6,10-methanoazepino[4,5-*g*] quinoxaline. It is a white powder and highly soluble in water. The chemical structure of VRC tartrate is shown in Figure 1.

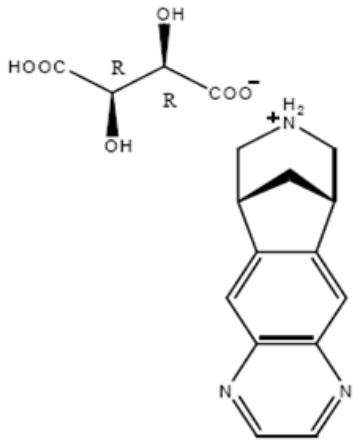

Fig.1: Chemical Structure of Varenicline tartrate

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Until now, VRC tartrate is not mentioned in any pharmacopoeia. Chromatographic HPLC[2,3] and UPLC[4] methods have been published for the determination of VRC from tablet dosage form. Furthermore, one UPLC method[5] has been published for its determination from plasma. Capillary electrophoresis (CE) is one of preferred techniques in quantitative analysis and particularly considered fast, more selective, economic and precise when comparing with other techniques such as HPLC [6].

1.1. Objectives

A scifinder literature search reveals the absence of capillary electrophoresis method for VRC. Aim of the work was to develop capillary electrophoresis method for determination of VRC in dosage forms. Developing CE method for VRC will reduced the consumption of references pharmaceuticals materials and solvents so it will be more applicable and economic in routine work as in quality control in pharmaceutical companies, governmental controlling labs, etc.

2. Experimental

2.1 Chemical

VRC Tartarate reference standard with claimed purity of 99% was purchased from Weihua Pharma Co. Ltd. (Zhejiang, China). Champix® tablets was distributed by Pfizer Inc. (New York, USA); labelled to contain 1 mg (as the anhydrous base) per tablet and obtained from the local market. Sodium hydroxide, 50 mM Sodium phosphate buffer (pH 2.5) and Ultra pure water were purchased from Agilent Tech. (Böblingen, Germany). The internal standard (Isoniazide) was obtained from Nile Pharmaceuticals and Chemical Ind. (Cairo, Eygpt)

2.2 Apparatus and Instrumental Conditions

Analysis was performed using an Agilent Technologies CE system (Waldbronn, Germany) model G1600A consisting of an automatic sampler, a diode array detector and an Agilent ChemStation software for data acquisition. The voltage of capillary was set to 30 kv with 150 µA as maximum current. The capillary used was uncoated standard bare fused-silica with 100 µm I.D, 40 cm total length, and 31.5 cm effective length (Agilent Technologies, Böblingen, Germany). The new capillary was conditioned by rinsing with 0.1 N sodium hydroxide for 20 min at 25°C, followed by water for 5 min and 50 mM sodium phosphate buffer (pH 2.5) for 5 min at 25°C and water for 10 min at 25°C. Before starting work every day and between consecutive analyses, the capillary was conditioned by rinsing with 0.1 N sodium hydroxide for 10 min at 25°C, followed by water for 5 min and 50 mM sodium phosphate buffer (pH 2.5) for 5 min at 25°C. At the end of daily used, the capillary was rinsed with 0.1 N sodium hydroxide for 10 min at ambient temperature followed by water for 10 min and then stored in water. The electrophoretic separations were carried out in 50 mM sodium phosphate buffer (pH 2.5) in the normal mode (30 kV). Samples were injected hydrodynamically at a pressure of 50 mbar for 10 s.

2.3. Preparation of Calibration Standards

Primary stock solutions of VRC (500 µg/ml) and INH (1000 µg/ml) were separately prepared in water. The working standards of VRC (2, 4, 8, 16, 32 and 64 µg/ml) and INH (0.9 µg/ml) were prepared by diluting primary solution for each drug with water.

2.4. Sample Preparation

Twenty 20 tablets of VRC were weighed and the average weight was calculated. Tablets were crushed to a fine powder and a quantity of the powdered tablets equivalent to 1 mg of VRC was transferred to 5 ml volumetric flasks. The 5 ml volumetric flask was filled with distilled water to mark then shacked handily for 5 min. This solution (theoretically, 200 µg/ml) was diluted with distilled water to give three solutions with different concentration 1 µg/ml, 4µg/ml and 16 µg/ml. These solutions were filtered through a 0.22µm CME membrane filter and the filtrate was introduced to CE instrument for analysis.

2.5. Validation

Method validation was performed according to current international regulations on analytical method validation[7]. The method was validated by using quality control samples (n=5) at 1, 4 and 16 µg/ml for VRC to determine the accuracy and precision of the method. Quality control values were calculated from a standard regression curve, constructed from the ratio of peak areas of analyte to internal standard peak areas.

2.6. Recovery

Absolute recovery of the analyte in tablet was determined in triplicates at high, medium and low concentrations. Recovery was calculated by comparison of the analyte peak ratios for VRC of the prepared samples with those of the analyte standards.

2.7. Stability of Stock Solutions

The stability of VRC and INH stock solutions were evaluated at room temperature, refrigerator and standard freezer for 2 months. Stability was calculated by comparing the pertinent responses obtained from the tested stock solution(s) with the responses of freshly prepared ones.

3. Results and discussion

The electrophoretic separation of VRC in presence of INH as internal standard was developed by evaluating several parameters, including running buffer (type, pH and electrolyte concentration), applied voltage. A capillary temperature was fixed in this studied to 25°C. In these experiments, water was used as a sample solvent to initiate stacking and improve peak shape**.** Both VRC and INH are basic compounds and the molecular weight of VRC is larger than INH which explains the faster migration of INH than VRC as shown in Figure 2**.**

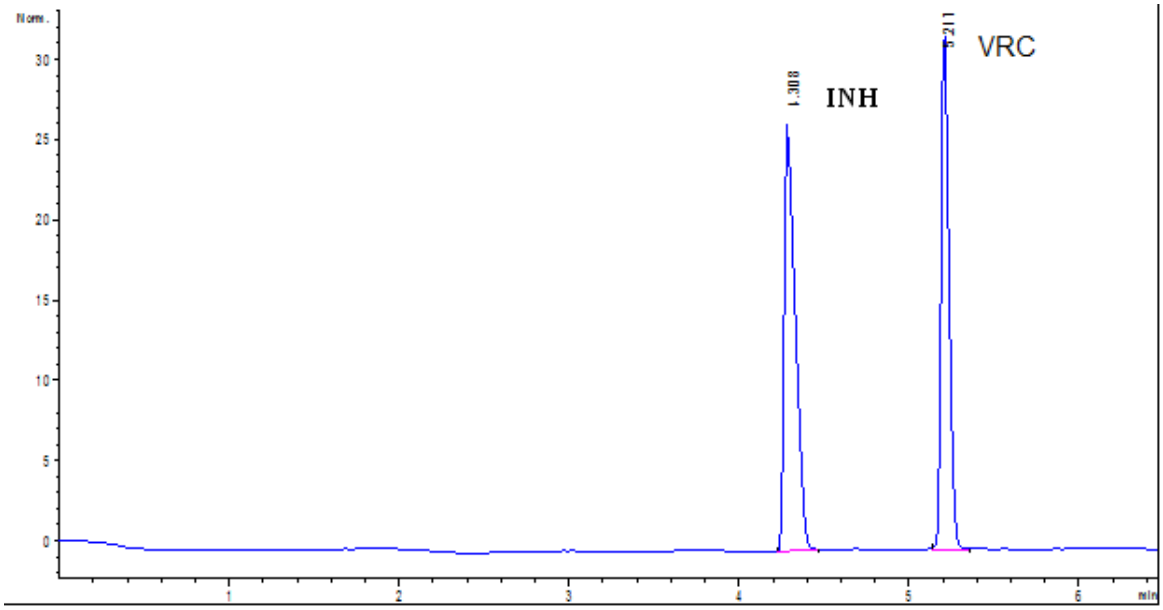

Fig. 2. Electropherogram of VRC and INH. Electrophoretic conditions: uncoated bare fused-silica with 100 µm I.D. , 40 cm total length, and 31.5 cm effective length; running buffer, 50mM sodium phosphate buffer (pH 2.5); injection (50mbar, 10s) at the anodic end of the capillary; applied voltage, 30kV; detection wavelength, 235nm; capillary temperature, 25°C.

During optimization of this method the purity of both VRC and INH were tested depending on diode array detector peak purity test. At least three points were selected of tested peak and a spectrum of each point was extracted and then overlay as shown in Figure 3 for INH

and Figure 4 for VRC. All extracted spectra for each compound were identical overlay indicating pure peaks. The appearance of colour below the peaks after DAD purity check as shown in Fig. 5 indicates pure peaks.

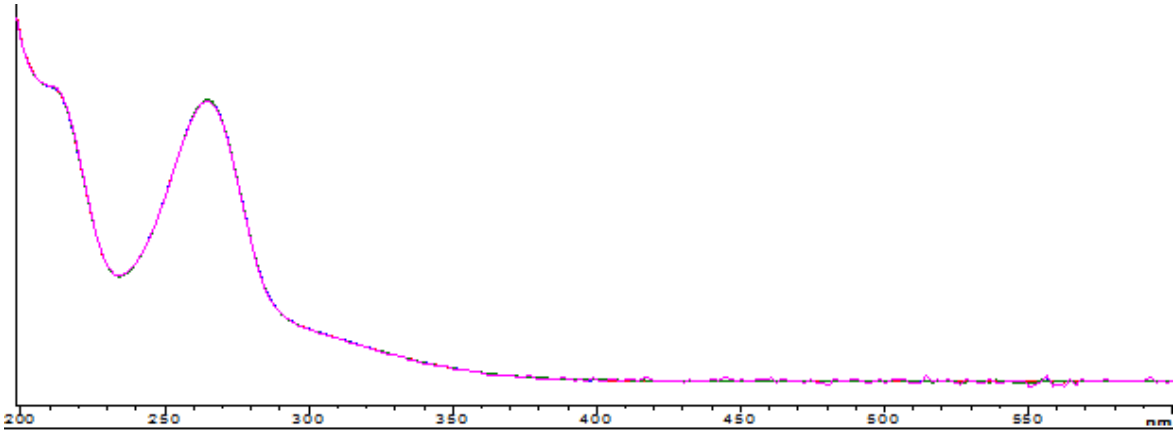

Fig. 3. Identical overlay spectra of INH peak for purity check by DAD.

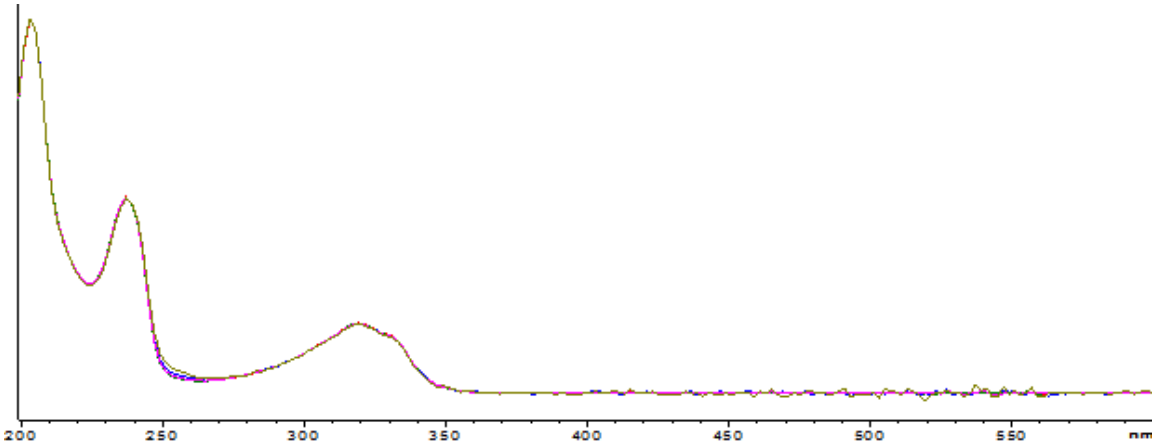

Fig. 4. Identical overlay spectra of VRC peak for purity check by DAD.

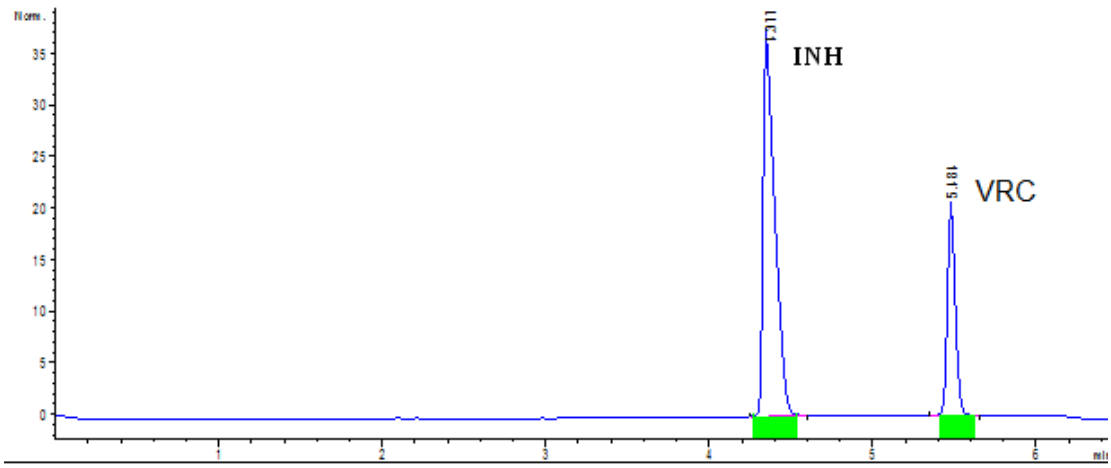

Fig. 5. Representative CE electropherogram showing the purity of VRC and its internal standard INH according to DAD.

The total analysis time was less than 6 min made it possible to analyse more samples per day.

The INH was used as internal standard to calculate the peak ratio of VRC. The calibration curve was generated by 5-concentration points; each concentration was injected in five replicates. Regression analysis for the results was carried out using the least-square method. The calibration curve of VRC was linear (r=0.998) over the concentration range 1–16 µg/ml as shown in Fig. 6.

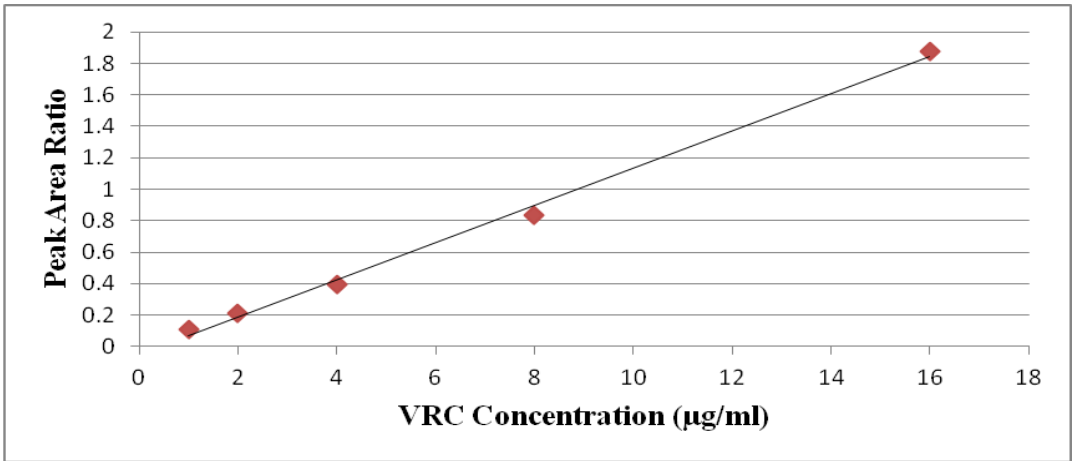

Fig. 6: Calibration curve of Varenicline tartrate for the developed CE method.

Table 2 shows the quality controls data obtained during the validation of the developed method while Table 3 shows the intra-day back calculated quality controls. Both intra-and inter-assay CV values ranged from 1.66–8.94% at three QC levels (i.e., 1, 4 and 16 µg/ml). Results from both intra-and inter-assay CV values (< 9) indicate a valid method. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated practically based on signal-to-noise ratio[7]. LOD was then defined as signal-to-noise ratio 3:1 and LOQ was defined as signal-to-noise ratio 10:1. The LOD and LOQ values were 0.2 and 0.6 µg/ml, respectively. The representing mean recovery of VRC from tablet was 105%. Data of stock solution stability for VRC and INH are presented in Table 4.

Table 2: Summary of intra-day quality control results for VRC (n=5).

VRC	QH*	QM	QL
Nominal (ng/ml)	16	4	1
Mean	15.71	3.81	1.25
%Nom	98.24	95.33	125.36
RSD (%)	7.85	6.11	1.36

* QH, QM and QL are abbreviations of high, medium and low quality controls, respectively.

Table 3: Summary of back calculated quality control concentrations of VRC (n=5) (inter-day variation) showing the repeatability of the method.

VRC	QH	QM	QL
Nominal (ng/ml)	16	4	1
Mean	15.40	3.75	1.26
Accuracy (%)	96.25	93.78	126.85
RSD (%)	8.94	6.43	3.54

Table 4: Summary of stability of VRC and INH in stock solution

Drug(n=3)	2 months at RT*	2 months at RF	2 months at FR
VRC			
Accuracy (%)	115.32	104.86	127.25
INH			
Accuracy (%)	86.29	88.29	88.07

* RT, RF and FR are abbreviations of room temperature, refrigerator and freezer, respectively.

4. Conclusion

A highly sensitive and fast method for the detection and quantification of VRC in tablet dosage form has been developed and validated by using CE technique. To the best of the authors' knowledge, this method is the first reported for quantitation of VRC using CE. The run time of 6 min made it possible to analyze more samples per day. This method is economic due to reduced consumption of references pharmaceuticals materials, solvents and sample.

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