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MULTI-RESPONSE OPTIMIZATION OF A CAPILLARY ELECTROPHORETIC METHOD FOR DETERMINATION OF VARDENAFIL IN THE BULK DRUG AND IN A TABLET FORMULATION

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SUMMARY

Capillary electrophoresis (CE) with diode-array detection has been used to develop a simple and rapid method for assay of vardenafil in the bulk drug and in a tablet formulation. Multi-response optimization of sensitivity, speed, repeatability of peak height and migration time, and their relative standard deviation (*RSD*; $n = 5$), was performed. Electrophoretic factors assumed to be independently affecting the analysis were optimized by use of factorial design and response-surface methods. Other factors were optimized by the univariant method. The optimum conditions were: running buffer 100 mM phosphate, pH 6, injection time 8 s, column temperature 25°C, separating potential 25 kV, column length 31.2 cm, and detection at 222 nm. The method was validated in the presence of the excipients identified by the manufacturer of the tablets (Levitra). Good analytical data were obtained, including linear range 0.5–75 μg mL^{-1}, recovery 98.8%, repeatability (as *RSD*) 1.3% (seven consequent injections on the same day), intermediate precision (as *RSD*) 3.4% (five injections over a week), and limits of detection and quantification 0.042 and 0.140 μg mL^{-1}, respectively. Selectivity was assessed in relation to the other, structurally related, drug commonly used to treat erectile dysfunction (sildenafil); sufficient separation was achieved.

INTRODUCTION

In 2003, vardenafil (1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-*f*][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethylpiperazine, monohydrochloride; Fig. 1) was approved by the US Food and Drug Administration (FDA) as the second choice for erectile dysfunction thera-

py after sildenafil. Vardenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP). cGMP is primarily responsible for increasing and reducing the size of the blood vessels carrying blood to and from the penis, respectively. Vardenafil prevents an enzyme called phosphodiesterase-5 from destroying cGMP, so cGMP persists longer [1–4]. Although vardenafil is safer than sildenafil [5], it can cause headaches, dyspepsia, diarrhea, chest pain, and other side-effects. In general, these drugs should be administered under medical supervision [4]. They have, however, become over-the-counter and have been extensively consumed.

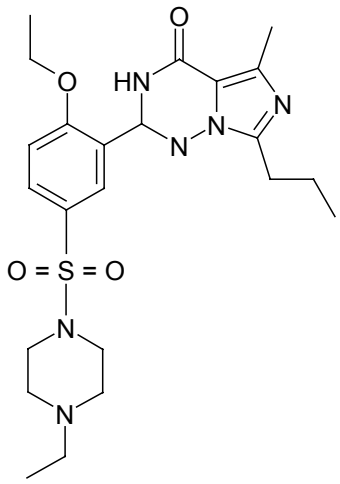


Fig. 1

The chemical structure of vardenafil

The availability of assay methods for vardenafil in dosage forms is rather limited. A literature survey revealed only four methods – HPLC [6], electrospray tandem MS [7], micellar electrokinetic capillary chromatography [8], and electrochemical [9].

In general, pharmaceutical analysis relies heavily on HPLC. Capillary electrophoresis (CE), however, another separation technique, has many advantages over HPLC, including speed and greater separation efficiency (an order of magnitude more theoretical plates). Unlike HPLC, reduction in sample pretreatment is possible in CE, in which uncharged molecules remain at the start of the capillary. Consumption of reagents and sample volumes, rather than capillary columns, is, moreover, relatively low in CE which is, therefore, relatively inexpensive. CE is, furthermore, a versatile

technique gaining widespread use in the separation and determination of ionic and neutral substances. Last, CE is a simple technique in which, often, a single set of operating conditions can be applied to a wide variety of analytes [10]. New CE methods continue to appear, therefore, and to complement and, eventually, replace HPLC methods.

In CE many electrophoretic factors (EF), e.g. electrolyte concentration (EC), electrolyte pH, injection time, separation voltage, etc., critically affect the analytical results, for example resolution, peak area (PA), and migration time (t_m). EF dependently or independently affect CE response at different levels. To develop CE methods successfully, therefore, it is advisable to optimize EF simultaneously, taking into consideration multi-CE response.

The common practical method for optimizing experimental conditions is the univariate method. In this approach, experimental conditions are optimized separately by varying the levels of a condition while keeping others constant at unspecified levels. Experimental design methods, as a multivariate optimization approach, including factorial design and use of response surfaces, are recommended for accomplishing three objectives:

1. reducing the large amount of data and enabling easy interpretation;
2. examining the main and the interaction effects of experimental conditions on the efficiency of analysis; and
3. simultaneous optimization of experimental conditions with regard to their interactions with each other by performing the minimum number of experiments [11].

The response surface is also a powerful tool for testing the ruggedness of analysis, i.e. the efficiency of a method under different experimental conditions [12].

This manuscript reports the multi-response optimization of a CE method for analysis of vardenafil in the bulk drug and in a tablet formulation. To achieve sensitivity, speed, and repeatability the CE responses considered were PA, t_m, and their repeatabilities. Although use of a highly efficient separation technique for analysis of a single active ingredient in pharmaceutical preparations is a relatively easy task, the univariate method is not always the ideal strategy for optimizing experimental conditions. Even if the univariate strategy seems the simplest approach, this is not the correct way to avoid pitfalls during experimental work. Therefore, both the univariate and multivariate methods were applied in this study. EF assumed not to interact in their effect on the CE method, i.e. injection time, separation potential, and column temperature were optimized by the uni-

variate method. Other relevant EF, i.e. EC and pH, were optimized using the factorial design and response-surface methods.

EXPERIMENTAL

Chemicals, Reagents, and Samples

All chemicals and reagents used in this study were of analytical grade. Water was double-distilled and deionized. Hydrochloric acid, phosphoric acid, sodium hydroxide, and sodium phosphate were from Merck (Darmstadt, Germany). Acetic acid, boric acid, sodium acetate, and sodium tetraborate decahydrate were from Sigma–Aldrich (Taufkirchen, Germany).

Levitra tablets (2.5, 5, 10, and 20 mg vardenafil) prepared by Bayer (Leverkusen, Germany) and Viagra tablets (50 mg sildenafil) prepared by Pfizer (New York, USA) were examined in this study.

Vardenafil standard and bulk material, and excipients present in vardenafil tablets (Levitra), as declared by the manufacturer, including microcrystalline cellulose, crospovidone, colloidal silicon dioxide, magnesium stearate, hypromellose, poly(ethylene glycol), titanium dioxide, yellow ferric oxide, and red ferric oxide were gifts from Samf (Khartoum North, Sudan).

Preparation of Standard Solutions, and Sample Preparation

Acetate–acetic acid, borate–boric acid, and phosphate–phosphoric acid buffers were prepared at different concentrations for optimization of electrolyte type and concentration.

Vardenafil primary standard solution (1000 μg mL^{-1}) was prepared in water. Working standard solutions were prepared by appropriate dilution. A series of mixed standard solutions containing vardenafil and excipients were prepared for method validation. Three placebo samples containing total excipients at concentrations of 1, 10, and 30 μg mL^{-1} were also prepared.

Ten tablets of each of vardenafil and sildenafil were weighed and triturated to a fine homogeneous powder. A mixed solution containing 10 mg L^{-1} of each drug was prepared. Solutions containing excipients were centrifuged at 4000 rpm for 10 min and the supernatant was used for analysis.

Instrumentation and Software Packages

A Beckman (Fullerton, USA) P/ACE MDQ CE system coupled with

a diode-array detector (DAD) was used throughout the experiments. Separations were performed in fused-silica capillary tubing 31.2 cm long (21.2 cm to the detector) × 50 μm i.d., housed in a cartridge, with a 100 μm × 800 μm detection window.

Before first use capillaries were conditioned by rinsing in sequence with methanol for 5 min, water for 2 min, 1.0 M hydrochloric acid for 5 min, water for 2 min, 0.1 M sodium hydroxide for 10 min, water for 2 min, and electrolyte for 5 min. Before each set of measurements the capillary was washed with 0.1 M sodium hydroxide for 0.5 min and electrolyte for 1.5 min. Samples were introduced hydrodynamically.

Instrument control, and data acquisition and processing were performed by use of Beckman Coulter (Fullerton, USA) 32 Karat version 7.0 software. SigmaPlot for Windows version 8.0 from Systat Software (Point Richmond, USA) was used for data interpolation and for constructing response-surface plots.

RESULTS AND DISCUSSION

Preliminary Investigation

The spectrum of vardenafil was acquired in the range 190–400 nm. Absorbance was maximum at 222 nm. The spectrum of the standard solution of vardenafil was examined for at least three weeks and remained unchanged, indicating good stability.

Vardenafil contains three basic functional groups (Fig. 1), pyrimidine, pyrazole, and piperazine. It will, therefore, be positively charged by hydrolysis and so separation by CE should be possible.

A standard solution containing 10 μg mL^{-1} vardenafil, and excipients at a total concentration of 10 μg mL^{-1}, was used in the optimization study. The effect of the type of electrolyte was examined. Phosphate–phosphoric acid buffer resulted in better electropherograms than the other buffers and was therefore used for further investigations. Sensitivity, speed, and the repeatability (*RSD*, $n = 5$) of both PA and t_m were evaluated throughout. Unless otherwise stated, the term 'responses' refers to PA, t_m, and their *RSD*.

The Univariate Optimization Method

The common practical range of injection times in CE (1–20 s [13]), was examined at a pressure of 0.5 pounds-force per square inch, p.s.i. Increasing the injection time improved the signal but some loss of resolution

and peak symmetry occurred. An injection time of 8 s resulted in good response and was thus selected for further studies. Different separation potentials ranging from 5 to 30 kV were examined. To achieve a good compromise between t_m and electric current, 25 kV was chosen as the optimum. The effect of column temperature was examined in the range 15–30°C [13]. Increasing the temperature resulted in lower migration time but some loss of resolution and 25°C was selected as an acceptable compromise between t_m and resolution.

Experimental Design Optimization Methods

When applying experimental design methods it is advisable to keep the number of variables as small as possible to avoid complex models and large variability [11]. The factorial design and response-surface methods were used to optimize EC and pH. Preliminary investigation revealed that 10–100 mM phosphate and pH 2.5–9.5 were suitable ranges. Although it improves peak shape, high EC is not usually possible because of the problem of internal heating within the capillary. Low EC means low conductivity. This increased analysis time and some loss in separation occurred. Investigation of the effect of pH revealed that reducing the pH increased the ionization of basic solutes whereas increasing the pH increased the electro-osmotic flow [13].

The Factorial Design Method

A 3^2 full-factorial design was conducted, where the base 3 stands for levels of the variables (low, medium, and high values) and the power 2 stands for the number of conditions to be optimized. The low and high values were obtained from the preliminary investigation whereas the medium values were calculated mathematically. A total of nine experiments, as the result of the adopted factorial design, were conducted. The responses were calculated and the results obtained are listed in Table I.

Unless otherwise indicated, the term 'interaction' refers to the interaction between EC and pH. The main and interaction effect factors for the responses were calculated by use of Eq. 1 [12]:

$$E_f = \frac{\sum y(+1)}{n} - \frac{\sum y(-1)}{n} \qquad (1)$$

where E_f is an effect factor, $y(+1)$ and $y(-1)$ are responses at the maximum and minimum levels, respectively, of the examined factor, and n is the number of variables at that level (in this design $n = 2$). The results obtained are

Table I

3^2 factorial design matrix of electrolyte concentration and pH, with the respective CE responses

No.	EC[a]	pH[b]	PA[c]	*RSD*[d] of PA (%)	t_m [e] (min)	*RSD* of t_m (%)
1	−1	−1	5200	0.80	3.53	0.64
2	−1	0	2188	0.87	3.53	0.64
3	−1	+1	2889	3.64	3.35	0.23
4	0	−1	8411	1.47	12.29	1.63
5	0	0	2654	3.94	5.02	2.62
6	0	+1	4562	4.13	5.03	0.42
7	+1	−1	8963	2.18	14.10	1.13
8	+1	0	8113	0.90	6.95	0.21
9	+1	+1	4570	4.57	6.12	0.20

[a]Level of electrolyte concentration
[b]Level of pH
[c]Peak area
[d]Relative standard deviation (n = 5)
[e]Migration time

listed in Table II. In general, pH has the dominant effect on all responses except t_m, possibly because pH has the potential to affect both the electrophoretic mobility of the solutes and the ionization of acidic silanols on the capillary wall [13]. The negative effect of pH on response is because of the ionization of vardenafil in acidic media, i.e. ionization increases the charge-to-size ratio and the mobility. The effect of EC on PA and its *RSD* is of second order. The ionic strength of the electrolyte, which is related to its concentration, has a substantial effect on the mobility of the solute and hence

Table II

Main effect and interaction effect factors on CE response

Effect factor	Condition	Response			
		PA[b]	*RSD*[c] of PA (%)	t_m [d] (min)	*RSD* of t_m (%)
Main	EC[a]	+2722	+1.155	+6.67	+0.23
	pH	−3352	−2.615	−4.08	−0.67
Interaction		−1041	−0.225	−3.90	−0.26

[a]Electrolyte concentration
[b]Peak area
[c]Relative standard deviation (n = 5)
[d]Migration time

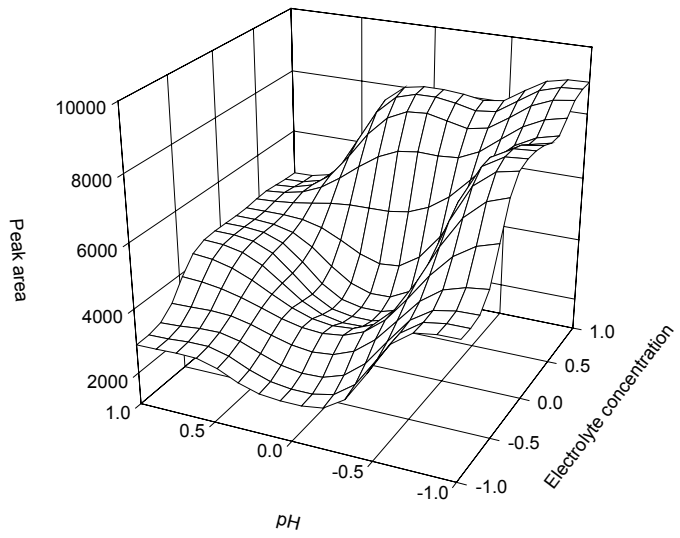
on the separation [13]. In general, it can be concluded that pH had the largest effect on sensitivity and repeatability for both PA and t_m whereas EC had the largest effect on speed. Interaction of EC and PA had a slight effect on all CE responses except the repeatability of t_m.

Response-Surface Plots

The three coded levels of the adopted factorial design, with their respective responses as listed in Table I, were interpolated and response surface plots were constructed as functions of EC and pH (Figs 2a–2d). As depicted in Fig. 2a, high PA could be obtained under three conditions:

- maximum level of EC with minimum level of pH;
- maximum level of EC with medium level of pH; and
- medium level of EC with minimum level of pH.

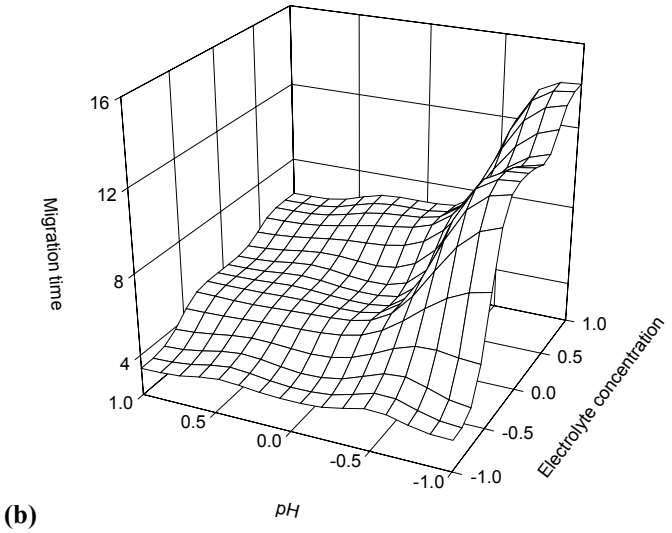
Otherwise, PA is substantially lower. Figure 2b reveals that t_m is more rugged than PA, i.e. shorter t_m could be obtained at all levels of both EC and pH except the maximum level of EC with the minimum level of pH. Although the ruggedness of the *RSD* of PA was relatively poor (Fig. 2c), repeatability is always acceptable (*RSD* < 5). Figure 2d also reveals that acceptable repeatability of t_m (*RSD* ≈ 1) is achieved for all levels of EC and pH.



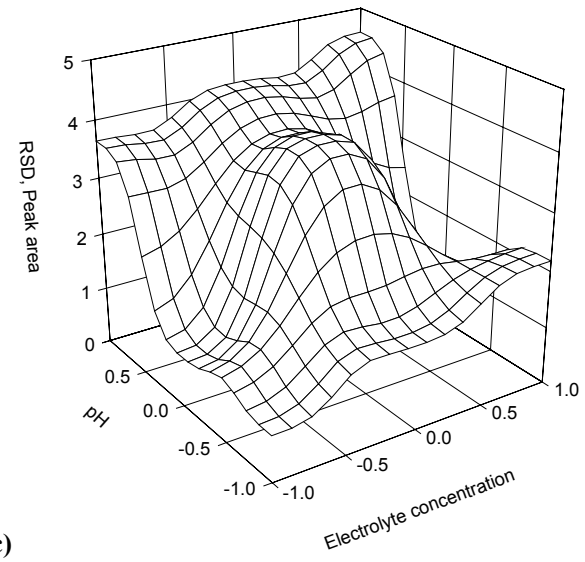
(a)

Fig. 2

Response-surface plots of (a) peak area as functions of electrolyte concentration and pH



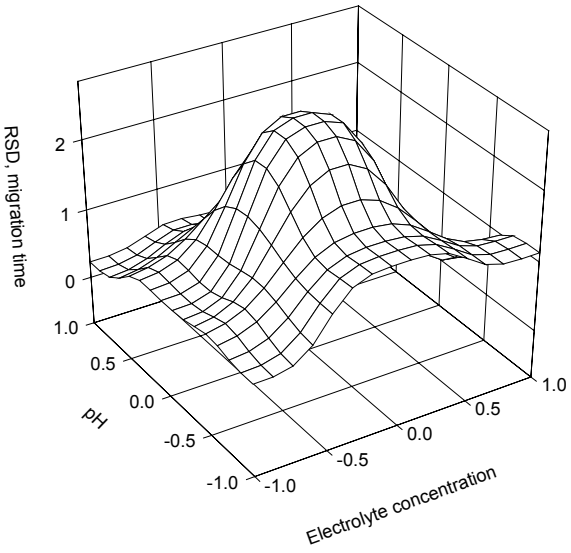
(b)



(c)

Fig. 2 (continued)

Response-surface plots of (b) migration time and (c) relative standard deviation (*RSD*) of peak area, as functions of electrolyte concentration and pH



(d)

Fig. 2 (continued)

Response-surface plots of (d) relative standard deviation (*RSD*) of migration time as functions of electrolyte concentration and pH

For a satisfactory fit between responses, 100 M phosphate buffer and pH 6 were regarded as optimum. Under these conditions the best sensitivity with short analysis time (t_m < 8 min) and acceptable repeatability of both PA (*RSD* ≈ 1.0%) and t_m (*RSD* < 0.5%) could be achieved.

Method Validation

The optimized CE method for assay of vardenafil in the presence of excipients certified by the manufacturer of the tablet dosage form Levitra was validated in respect of linearity, recovery, repeatability, intermediate precision, and limits of detection (*LOD*) and quantification (*LOQ*).

A series of standard solutions of vardenafil with concentrations in the range 0.1–100 μg mL^{-1} and with excipients at a total concentration of 10 μg mL^{-1} were subjected to the optimized CE method for calibration purposes. The method was found to be linear over a wide range – 0.5 to 75 μg mL^{-1}. The weighted regression equation was $y = 1.351x + 1.280$, where y is peak area and x is the concentration of vardenafil in μg mL^{-1}. The correlation coefficient was 0.9998.

Recovery was examined by analysis of three standard solutions containing 1, 10, and 50 μg mL^{-1} vardenafil, and excipients at a total concen-

tration of 10 μg mL^{-1}. Each solution was injected seven times in succession. Average recovery and average *RSD* were 98.4% and 1.7%, respectively, indicating good accuracy and repeatability. The intermediate precision was examined by analysis of the same standard solutions five times during the course of a week. Good intermediate precision was obtained (*RSD* 3.4%).

The *LOD* and *LOQ* were calculated as the concentrations of solute for which the peak heights were 3 and 10 times the baseline noise, respectively; the values obtained were 0.042 and 0.140 μg mL^{-1}. These low values are most probably because of successful optimization of peak height and the smooth baseline.

The selectivity of the method was examined in relation to sildenafil, the other, structurally related, drug commonly used to treat erectile dysfunction. When a mixed solution of vardenafil and sildenafil tablets was subjected to the CE method the two drugs were successfully separated (Fig. 3).

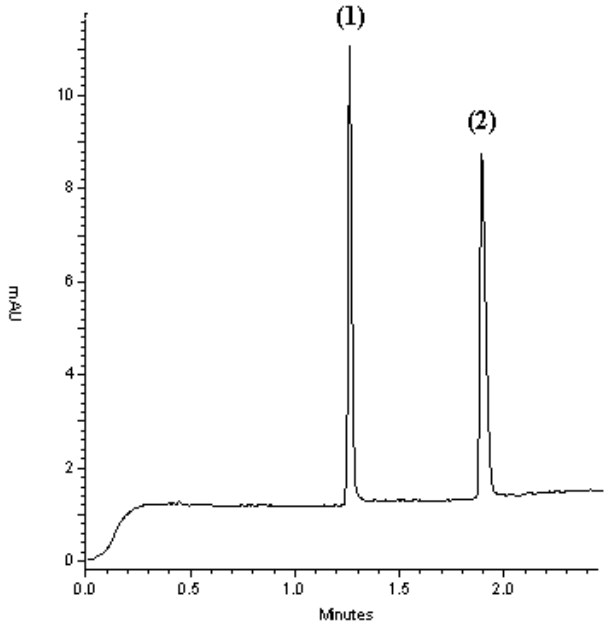


Fig. 3

Electropherogram obtained from (1) sildenafil and (2) vardenafil in a mixed solution of their dissolved tablets under the optimized conditions (100 mM phosphate, pH 6.0, column length 31.2 cm, injection time 8 s, column temperature 25°C, applied potential 25 kV, and detection at 222 nm)

CONCLUSIONS

A new simple, rapid, accurate, precise, and sensitive CE method has been established for assay of vardenafil in the bulk drug and in a tablet formulation. Several properties of the method were monitored, including sensitivity, rapidity, and repeatability as peak area (PA), retention time, t_m, and their *RSD* values, respectively. Univariate and multivariate strategies were successfully used to optimize the EF affecting the method both dependently and independently. The response-surface method was also used to examine the ruggedness of the method. By use of a simple electrolyte, vardenafil was successfully separated from excipients and another structurally and pharmaceutically related compound, sildenafil.

Because the method has the advantages of CE of rapidity, cost-effectiveness, and simplicity, it is suitable for routine quality control analysis in pharmaceutical laboratories.

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