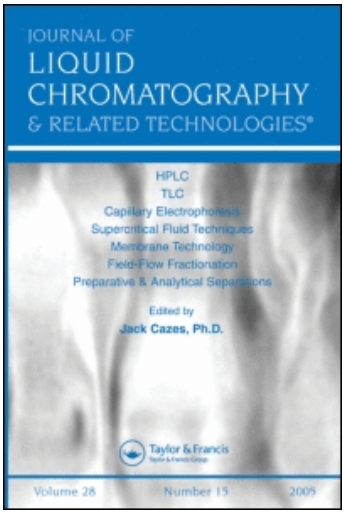


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Development and Validation of a Capillary Electrophoretic Method for the Determination of Degradation Product in Naphazoline HCl Bulk Drug Substance

A. Yesilada[a]; B. Tozkoparan[a]; N. Gökhan[a]; L. Öner[a]; M. Ertan[a]
[a] Department of Pharmaceutical Chemistry Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

To cite this Article Yesilada, A. , Tozkoparan, B. , Gökhan, N. , Öner, L. and Ertan, M.(1998) 'Development and Validation of a Capillary Electrophoretic Method for the Determination of Degradation Product in Naphazoline HCl Bulk Drug Substance', Journal of Liquid Chromatography & Related Technologies, 21: 17, 2575 — 2588
To link to this Article: DOI: 10.1080/10826079808003408
URL: http://dx.doi.org/10.1080/10826079808003408

J. LIQ. CHROM. & REL. TECHNOL., 21(17), 2575–2588 (1998)

DEVELOPMENT AND VALIDATION OF A CAPILLARY ELECTROPHORETIC METHOD FOR THE DETERMINATION OF DEGRADATION PRODUCT IN NAPHAZOLINE HCl BULK DRUG SUBSTANCE

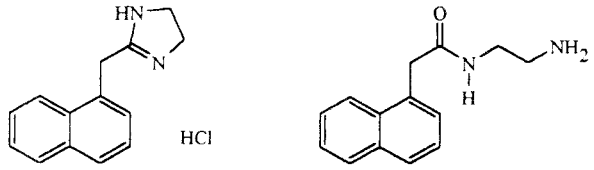
A. Yesilada,[1] B. Tozkoparan,[1] N. Gökhan,[1]
L. Öner,[2] M. Ertan[1,*]

[1] Hacettepe University
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
06100 Ankara, Turkey

[2] Hacettepe University
Department of Pharmaceutical Technology
Faculty of Pharmacy
06100 Ankara, Turkey

ABSTRACT

In this study a capillary electrophoretic method is described for the identification and quantitation of the main degradation product napthylacetyletylenediamine (NAED) in naphazoline HCl bulk drug. The effect of temperature, operating voltage, and electrolyte concentration on the resolution was determined by using a multivariate experimental design. The separation was achieved at 15 kV, on a 70 cm (62 cm effective) x 75 μm I.D. capillary at 20°C using 0.1 M , pH 3 phosphate buffer as background electrolyte. The method was validated and satisfactory specificity, linearity, precision, and accuracy results were obtained.



Naphazoline HCl 1- Naphthylacetylethylenediamine

Figure 1. Chemical structures of Naphazoline HCl and Naphthylacetylethylenediamine (NAED).

INTRODUCTION

Naphazoline (Figure 1) is an α-adrenergic and sympathomimetic agent used in topical nasal or ophthalmic pharmaceutical formulations.[1] The main possible degradative impurity is naphthylacetylethylenediamine (NAED) (Fig. 1). Determination of this compound in naphazoline bulk drug and preparations is an important issue for purity and stability investigations. Due to the similarity of the spectral profile of the two compounds, the methods reported for the determination of NAED in naphazoline salts include mainly chromatographic techniques, i.e., column chromatography followed by UV-assay and HPLC.[2,3]

As for the official methods, the British Pharmacopoeia (BP 93)[4] describes a TLC method based on visualization with ninhidrin and comparison of the intensity of the spots by eye sight for the determination of naphthylacetylethylenediamine in both naphazoline hydrochloride and nitrate. Recently, capillary electrophoresis has been used for the determination of drug-related impurities which may be present from synthetic or degradative sources.[5,7]

This work describes application of capillary zone electrophoresis for the separation and determination of validation parameters for side-by-side quantitation of naphazoline HCl and its primary degradation product.

MATERIALS AND METHODS

Reagents

Naphazoline HCl (USP) was from Merck (Schuchardt). Naphthyl-acetylethylenediamine (NAED) was synthesized and purified in our laboratory.[2] o-Phosphoric acid (97%), sodium hydroxide and sodium dihydrogenphosphate were from Merck (Schuchardt), water from Nanopure, Ultrapure water system.

Table 1

Capillary Electrophoresis Method of Separation

Conditions

Rinse cycle 1: 0.1N NaOH, 3 min.
Rinse cycle 2: Water 1 min.
Rinse cycle 3: pH: 3 0.1 M Phosphate Buffer, 5 min.
Wavelength: 220 nm
5 sec. hydrodynamic sampling
Operating temperature: 20°C
Operating voltage: 15 kV
Run time: 25 min.
Capillary: 70 cm (62 cm effective) x 75 μm i.d. fused silica

Instrument

Spectra PHORESIS 2000 CE instrument (Spectra Physics, Inc., San Jose, California, USA) with automated injection system was used. The fused silica capillaries were purchased from Spectra Physics. The separation conditions are given in Table 1.

Preparations of Standard and Samples

Naphazoline HCl and NAED standard solutions were prepared in water at 1 and 0.5 mg/mL concentrations, respectively. For standard addition, 0.5 mg/mL naphazoline HCl solution prepared in water was spiked with known amounts of NAED between 0.5-808 μg/mL concentration corresponding to 0.1%-16% Naphazoline HCl loading.

Peak area values were normalized with respect to their migration times. The solutions were analyzed in duplicate.

Multivariate Experimental Design

In this study a 3x3 factorial design was utilized. The two independent variables considered in this study were operating voltage (x) and operating temperature (y). The dependent variable include the resolution (z). The various combinations for the nine trials used are presented in Table 2.

Table 2

Results for 3x3 Factorial Experiment*

Parameters		Response (R)			
Voltage (kV)	Temperature (°C)	Electrolyte Concentration (M)			
		0.025	0.05	0.08	0.1
18	15	2.79	4.54	5.94	6.84
18	20	3.19	4.58	5.90	6.87
18	25	2.83	4.19	5.66	6.61
23	15	3.11	4.52	5.93	6.87
23	20	3.11	4.53	5.85	6.80
23	25	3.04	4.34	5.62	6.53
28	15	3.03	4.83	6.32	7.51
28	20	4.80	4.71	6.28	7.28
28	25	2.92	4.58	6.05	7.19

* The effect of operating voltage, temperature and electrolyte concentration on resolution of a solution containing 0.25 mg/mL NAED in 0.5 mg/mL naphazoline HCl.

Based on the observed data, quadratic equations were generated to establish the correlation between the independent variables (operating voltage, temperature and electrolyte concentration) and the dependent variable resolution.

The regression coefficients for the equations were calculated by the Statgraf, version 5.0 computer software program and this program was employed to produce the response surface diagrams.

RESULTS AND DISCUSSION

In this study a free solution capillary electrophoresis (FSCE) method was applied. Due to the basic character of the analytes, a low pH electrolyte was chosen[8] as the run buffer.

In BP 93, the minimum acceptable concentration for NAED in both naphazoline hydrochloride and nitrate, is given as 0.5% (w/w) of naphazoline salts. Thus, we aimed to develop a method with sufficient sensitivity to quantitate NAED in Naphazoline HCl at this level.

Optimization of the Method

In our preliminary study with CE,[9] an acetate buffer system with relatively large ions and low conductivity was used to control current generation for the separation and side by side quantitation of NAED and naphazoline HCl. Although this method was satisfactory with respect to precision and accuracy, the resolution was not enough to achieve a satisfactory sensitivity.

In CE, the main parameters affecting resolution directly or indirectly are the nature, pH and concentration of the running electrolyte, the applied voltage, and temperature. The pH and the ionic strength of the electrolyte are the main factors which alter electroosmotic flow (EOF). On the other hand, resolution is directly proportional to the square root of the voltage, the increase of which is limited by joule heating. Temperature is also another parameter which alters viscosity, EOF, and analysis time.[8,10] Thus, it can be concluded that the achievement of a satisfactory resolution can be fulfilled by optimizing the above mentioned parameters in order to reach a balance. In method development with CE this is still a challenge.

On the other hand, resolution is decreased by high concentrations of the analyte[11] and in some cases, although a resolution above 1 is achieved, this may not allow detection of one component in presence of the high concentration[12] of the other. In this study we aimed to detect and quantitate the degradation product (NAED) of naphazoline HCl at a limit of ≤ 0.5 % according to BP 93, where injection of a high concentration of naphazoline HCl is needed.

The resolutions (2.79-3.03) achieved by low electrolyte concentration (0.025 M) was not satisfactory for quantitation of the degradation product in naphazoline HCl, at ≤ 0.5 % level. Recently chemometrics has emerged as a method of evaluating experimental data multivariately, instead of scrutinizing the variables one at a time which is a COST (Consider One Separate variable at a Time) approach.

Since the instrument we used is capable of producing multivariate data, we used a chemometric approach for setting up a multivariate analysis in order to get an overview of all the data allowing an overall judgment and evaluation which will help to select the experimental conditions with maximum information content.[13]

The analytical experiments have been carried out according to the conditions stated in Table 2 where the pH adjustment was made by using 0.1 N NaOH in order to minimize the effects resulting from the nature and conductivity of the electrolyte.

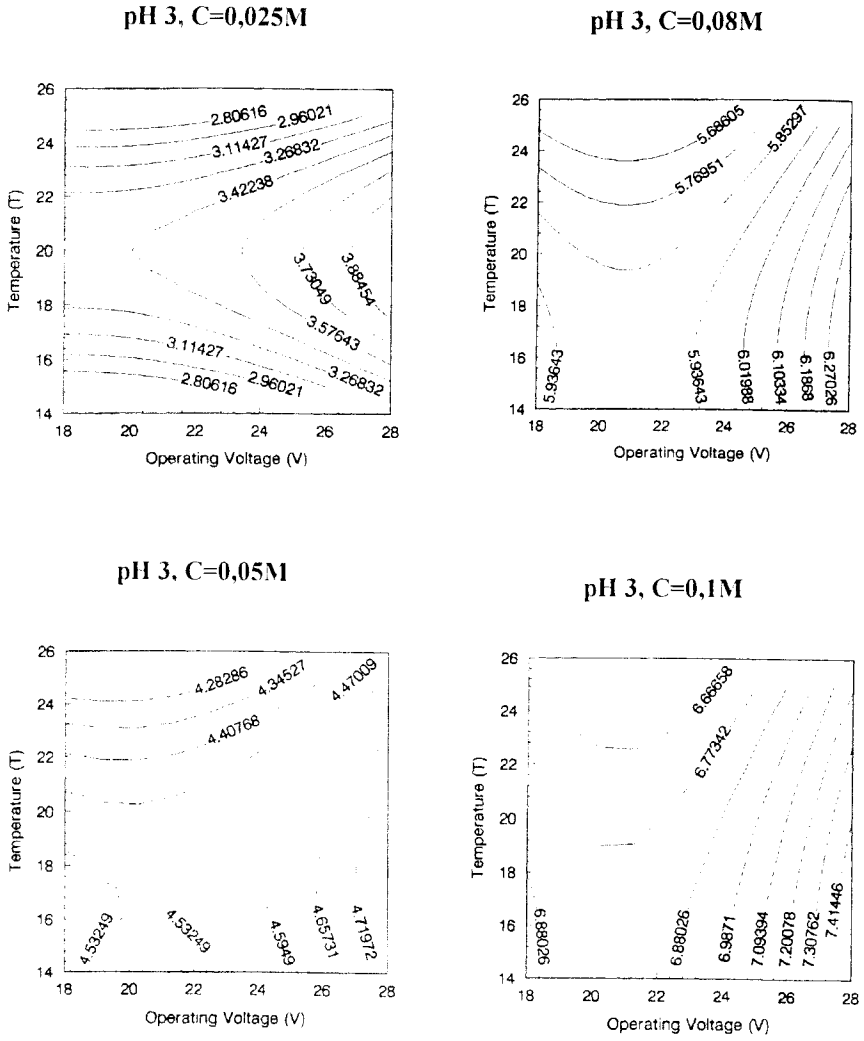


Figure 2. Counter diagram for the resolutions with variation in operating voltage and temperature.

As a result of these experiments, the response surface and counter graphics (Figures 2 and 3) demonstrated the effect of operating voltage, operating temperature, and electrolyte concentration on the resolution. The data in Table 2 indicate that resolution increases with increases in operating voltage and electrolyte concentration.

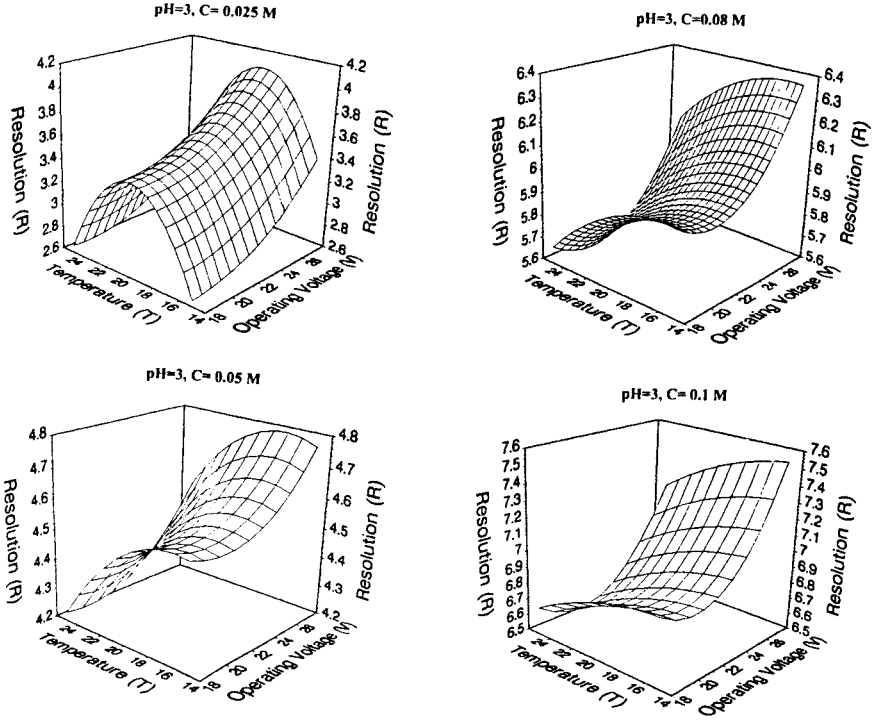


Figure 3. Response surface diagram for the resolutions with variation in operating voltage and temperature.

The quadratic equations relating to resolution, as a function of operating voltage and temperature were:

$$z = -6.779 - 0.2243x + 1.2245y + 0.0069x^2 - 0.0299y^2 - 0.0015xy$$
(C: 0.025, multiple R = 0.775)

$$z = 5.479 - 0.192x + 0.122y + 0.0043x^2 - 0.0043y^2 + 0.001xy$$
(C: 0.05, multiple R = 0.964)

$$z = 8.918 - 0.377x + 0.113y + 0.009x^2 - 0.0036y^2 + 1.10^{-4}xy$$
(C: 0.08, multiple R = 0.998)

$$z = 11.446 - 0.509x + 0.0844y + 0.0127x^2 - 0.0023y^2 - 9.10^{-4}xy$$
(C: 0.1, multiple R = 0.998)

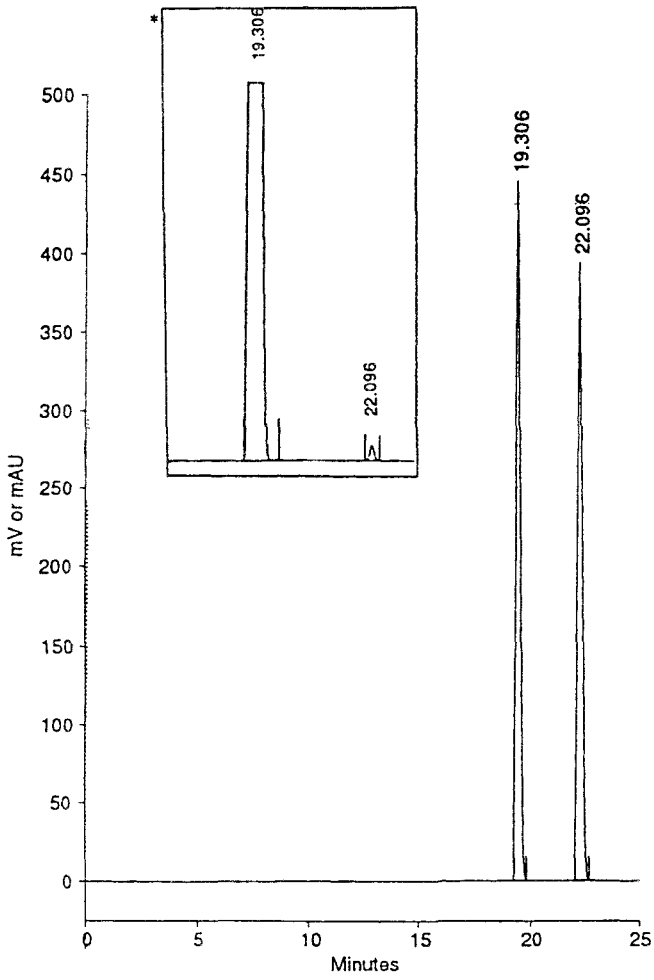


Figure 4. A typical electropherogram obtained by analysing a test mixture of Naphazoline HCl and NAED prepared in 0.5 mg/mL concentration in water. *The electropherogram of 0.5% mixture at the same scale.

where z is the resolution and x and y are the operating voltage and temperature, respectively. In conclusion, this study design allowed the investigation of effects on resolution by a range of operating voltage, temperature and electrolyte concentration for the development of a capillary electrophoretic method for the determination of degradation product in naphazoline HCl and the experimental conditions giving rise to the best resolution with current generation below 100

microamper was chosen (Table 2) which allowed the detection of NAED in naphazoline HCl even at 0.1 % level. At this point the basic component of electrolyte was changed to NaH_2PO_4 for pH adjustment, with a better and reproducible buffering capacity, and by lowering the applied voltage to 15 kV to prevent high current and heat generation, we obtained a resolution of nine and set up experimental conditions stated above. A typical electropherogram thus obtained is shown in Fig. 4.

The resolution was calculated according to the equation where t_1 and t_2 are the migration times of compounds, and $w_{1/2,1}$ and $w_{1/2,2}$ are their respective peak widths at half the peak heights.

$$R = \frac{t_2 - t_1}{w_{1/2.1} - w_{1/2.2}} \times 1.18$$

Validation of the Method

Specificity

Test mixtures prepared by spiking a known amount of naphazoline HCl with NAED at various levels (0.1%-16%) were analyzed under the conditions given in Table 1. Resolution was 9 (Figure 4) indicating freedom of interference of test components with respect to each other, and peak heights and normalized peak area values confirmed the theoretical values. The background electrolyte showed no interfering peaks at the detector wavelength.

Limit of detection (LOD)

The limit of detection was determined by injecting test solutions at various concentrations of NAED. LOD was 0.25 μg/mL with a peak height three times the level of baseline noise.

Limit of quantitation (LOQ)

The minimum value which could be measured precisely and accurately was 0.50 μg/mL (RSD= 3.99 %, n= 5).

Precision

Solutions of naphazoline HCl at 0.5 mg/mL concentration were spiked with 2.5 μg/mL NAED and analyzed five times. Satisfactory RSD values were obtained for peak area, peak height and migration time, indicating a good injection repeatability (Table 3).

Table 3

Analysis of Five Replicate Injection of Naphazoline HCl and NAED Synthetic Mixture in Water

Naphazoline 0.5 mg/mL	Normalized Peak Area Data	Peak Height Data	Relative Migration Time
Maxima	188822.117	226090	0.8593
Minima	186433.594	225756	0.8576
Mean	187644.289	225908	0.8585
R.S.D.%	0.49	0.053	0.08
NAED 2.5 μg/mL			
Maxima	943.836	2737	
Minima	925.376	2626	
Mean	937.457	2671	
R.S.D.%	0.77	1.58	

Table 4

Method Repeatability of 0.1 and 0.5 NAED in Naphazoline HCl

Sample No.	% Impurity	Sample No.	% Impurity
1	0.11	1	0.58
2	0.11	2	0.59
3	0.11	3	0.59
4	0.12	4	0.58
5	0.11	5	0.58
Average	0.112		0.584
Standard deviation	0.0044		0.0054
Relative standard deviation	3.99%		0.93%

Method repeatability was assessed by analyzing 5 synthetic samples containing 0.1% and 0.5% of NAED in naphazoline HCl successively. The RSD values thus obtained were in the acceptable ranges (Table 4).

Linearity

The calibration curve of NAED (Figure 5) showed good detector linearity in the practical concentration ranges (0.5-808 μg/mL); the regression equation and correlation coefficient given below:

$$y = -14.8653 + 403874.0143x \quad r^2 = 0.999 \text{ (For NAED)}$$

Calibration solutions were prepared by maintaining naphazoline HCl at constant concentration, and varying the content of NAED.

Accuracy

The agreement between the observed impurity level and the theoretical values was demonstrated by the determination of seven synthetic samples of NAED added to naphazoline HCl in the range 0.5 %-16 %. The theoretical concentration of impurity is plotted against experimentally determined concentration.

As can be seen in Figure 6 the curve obtained gave a regression equation with a slope of 0.94 and correlation coefficient $r^2 = 0.999$, indicating acceptable accuracy.

Quantitation

It is recognized[8] that in CE, peak areas is directly proportional to sample concentration and migration time. For the compensation of peak area difference resulting from migrating time differences, normalized peak areas were utilized in calculations.

The NAED content of synthetic solution containing a constant amount of naphazoline was calculated by internal standard (ISTD) procedure, where naphazoline HCl component served as an internal standard, according to the following equation:

Actual amount of NAED= (Response ratio x response factor) x Actual amount of naphazoline (Int.St.)

The naphazoline HCl concentration was corrected for counter ion content.

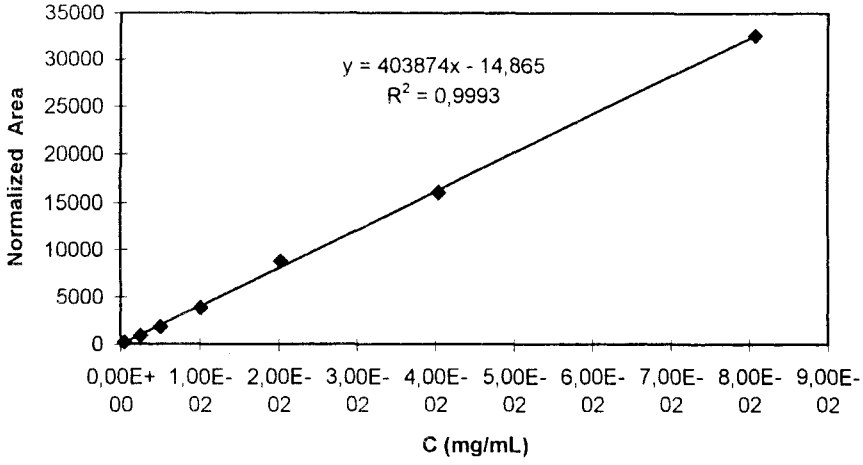


Figure 5. The calibration curve for NAED in 0.5 mg/mL Naphazoline HCl plotted in the concentration rage 0.5 - 808 μg/mL, corresponding to 0.1 - 16%.

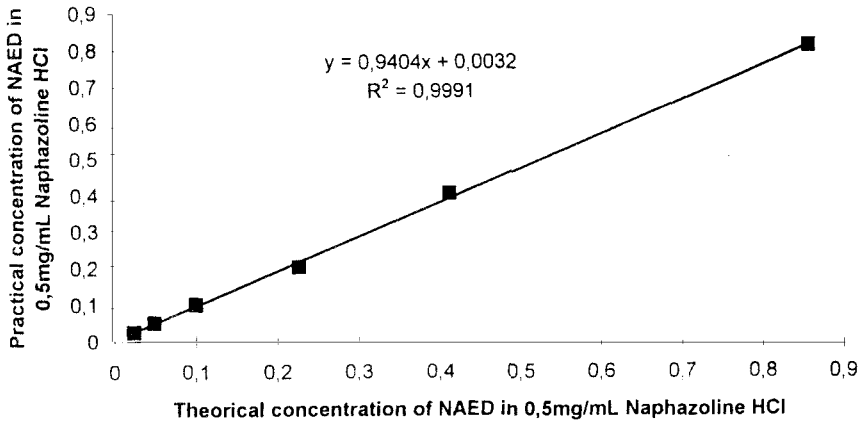


Figure 6. Accuracy plot. Theoretical concentration of impurity vs. practical concentration.

CONCLUSION

In this study free zone capillary electrophoresis was successfully applied for the impurity testing of naphazoline HCl bulk drug substance. The method development and optimization were achieved by a multivariate experimental design, which allowed investigation of the effect of the main parameters (temperature, operating voltage, and electrolyte concentration) on the resolution, and thus, it became possible to reach a high resolution where the degradation product (napthylacetylethylenediamine) could be quantitated in naphazoline HCl at 0.1% level. The method developed was validated; and showed satisfactory specificity, linearity, precision, and accuracy. The method may also be applicable to the stability studies and impurity testing of the preparations of the naphazoline HCl.

ACKNOWLEDGMENT

The authors would like to thank United Nations Development Programme for granting this work.

REFERENCES

1. Martindale, 29th edition, **The Extra Pharmacopoeia**, The Pharmaceutical Press, London, 1993, pp: 1712.

2. M. G. Wall, " Naphazoline Hydrochloride," in **Analytical Profiles of Drug Substances and Excipients**, K Florey, eds., Academic Press, Inc., Texas, 1992, pp.307-344.

3. J. Bauer, S. Krogh, J. Pharm. Sci., **72**, 1347-1349 (1983).

4. **British Pharmacopeia**, Vol.I, HMSO, London, 1993, pp: 441-442.

5. K. D. Altria, J. Chromatogr., 646, 245-257 (1993).

6. K. D. Altria, J. Elgey, P. Lockwood, D. Moore, Chromatographia, **42(5-6)**, 332-342 (1996).

7. K. D. Altria, J. Chromatogr., **634**, 323-328 (1993).

8. K. D. Altria, **Capillary Electrophoresis Guidebook**, Humana Press Inc., Totowa, New Jersey, 1996.

9. A. Yesilada, B. Tozkoparan, N. Gökhan, M. Ertan, 8th International Pharmaceutical Technology Symposium on Recent Advances in Peptide and Protein Delivery, Ankara, 1996, pp. 105.

10. N. A. Guzman, **Capillary Electrophoresis Technology**, Marcel Dekker, Inc., New York, 1993.

11. K. A. Altria, LC-GC Int'l, **6(3)**, 37-42 (1993).

12. C. S. Griend, K. Gröningsson, D. Westerlund, Chromatographia, **42**, 5-6 (1996).

13. S. Wold, J. Pharm. Biomed. Anal., **9(8)**, 589-596 (1991).

Received July 27, 1997
Accepted January 29, 1998
Manuscript 4551