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Validated Capillary Zone Electrophoretic Determination of Avanafil and Dapoxetine Hydrochloride in their Pure form and Pharmaceutical Preparation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, a simple, green, and rapid capillary zone electrophoresis (CZE) method coupled with a diode array detector (DAD) was applied for the analysis of avanafil (AVA) and dapoxetine hydrochloride (DAP) as a binary mixture using vardenafil (VAR) as an internal standard (IS) in pure form and pharmaceutical formulation.
Methodology: The separation was done using fused silica capillary (58.5 cm total length, 50 cm effective length, and 50 µm internal diameter) and the running background electrolyte (BGE) was 100 mM acetate buffer at pH 3.6. During the separation process, the applied voltage was 30 KV, while the temperature was 25 °C. The sample injection was applied at a pressure of 50 mbar for 10 s, and detection was carried out at 210 nm for DAP and 248 nm for AVA and VAR.

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Results: Analysis of the tested drugs and the internal standard was carried out in less than 6.5 min, where the migration times were 4.29, 4.90, and 6.02 min for IS, DAP and AVA respectively. The proposed method showed linearity in the concentration range 5-80 and 5-70 µg/mL with correlation coefficients 0.9996 and 0.9999 for AVA and DAP respectively. The limit of detection (LOD) was 0.523 and 0.531 for AVA and DAP respectively, while the limit of quantification (LOQ) was 1.585 and 1.608 in respective order. The Peak purity and identity in the proposed method were validated by DAD.

Conclusion: The proposed CZE method was validated according to ICH guidelines and applied successfully for the estimation of AVA and DAP in their combined pharmaceutical preparation.

Keywords: Avanafil; Dapoxetine hydrochloride; capillary zone electrophoresis; pharmaceutical preparation.

1. INTRODUCTION

AVA is a class of drugs called phosphodiesterase (PDE) inhibitors, and it works by improving blood flow to the penis during sexual stimulation leading to an erection [1]. AVA is absorbed quickly and has a faster onset of action than other PDE inhibitors [2]. It is chemically identified as: (S)-4-((3-chloro-4-methoxybenzyl) amino)-2-(2 (hydroxymethyl) pyrrolidin-1-yl)-N-(pyrimidin-2-ylmethyl) pyrimidine -5-carboxamide, as shown in Fig. 1. Dapoxetine hydrochloride is a drug that belongs to the selective serotonin reuptake inhibitors (SSRIs) family and it was employed for the treatment of premature ejaculation [3].After oral administration, dapoxetine is absorbed and eliminated rapidly[4]. It is Chemically recognized as: (S)-N, N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1- amine hydrochloride, as shown in Fig. 1. Recently, AVA was co-formulated with DAP to treat impotence and premature ejaculation. Several techniques were reported for the estimation of AVA in drug products including spectrophotometry [5,6], HPLC [7-9], LC-MS/MS [10] and voltammetry [11]. Different studies were developed for the determination of DAP alone or in a combination with other drugs including UV-Vis spectrophotometry [12-15], HPLC [16-22], capillary electrophoresis [23,24], screen printed electrode [25] and potentiometric ion-selective electrodes [26].The analytical techniques for determination of AVA and DAP in binary mixtures were reported including spectrophotometry [27,28], HPLC [29,30], HPTLC [31] and fluorimetry [32]. Capillary zone electrophoresis (CZE) has several advantages in drug analysis compared to other alternative techniques such as high separation efficiency, few waste products, short analysis time, ease of automation, and consuming small nanoliters injection volumes [33,34]. CZE methods are applied mostly in aqueous mediums and the concept of green chemistry can apply easily in opposed to the liquid chromatographic methods that need large amounts of organic solvents [35]. There is no reported electro-chromatographic method for the estimation of AVA and DAP in a binary mixture. Our objective in this study was to develop a CZE method for efficient separation, rapid, and selective analysis of AVA and DAP in a pure form and pharmaceutical preparation.

2. EXPERIMENTAL

2.1 CE System

The experimental study was carried out using Agilent 7100 CE device (Agilent Technologies Deutschland, GmbH, Hewlett-Packard-Str.8 Waldbronn, Germany) with Diode Array Detector (DAD) and Agilent ChemStation software for data analysis. In the same run, DAD can develop electropherograms at different wavelengths. As a result, each analyte can be detected easily at its maximum absorption wavelength that enhancing the sensitivity of the applied technique. DAP and AVA were detected using DAD at 210 nm and 248 nm respectively. Deactivated fused silica capillary (Agilent Technologies, Waldbronn, Germany) is the capillary that was used in the experiment with dimensions of: 50 cm effective length,58.5 cm total length, and 50µm internal diameter.

2.2 Materials and Chemicals

AVA was supplied by Andalous pharma, Cairo, Egypt. DAP and VAR were supplied by EVA Pharma, Cairo, Egypt. Methanol (HPLC grade) was purchased from Sigma-Aldrich Chemie GmbH, Switzerland. Analytical grade reagents including glacial acetic acid, sodium acetate, and sodium hydroxide were obtained from Oxford Company for Laboratory Reagents (Mumbai, India). commercial preparation containing AVA and DAP is super-avana® tablets claimed to contain 100 mg AVA and 60 mg DAP (Sunrise Remedies Pvt. Ltd., India).

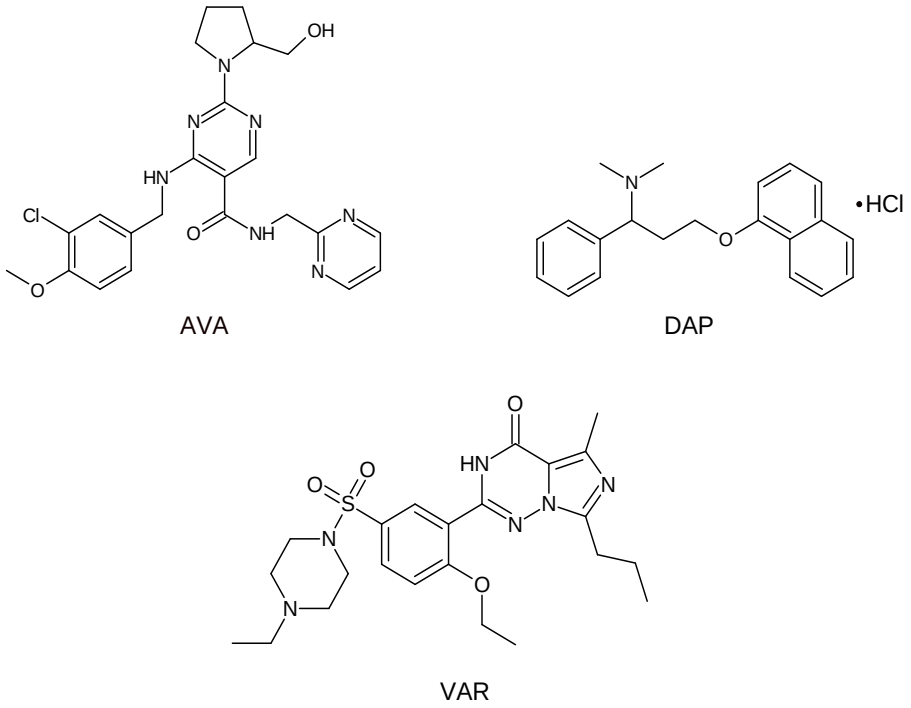

Fig. 1. Chemical structures of avanafil (AVA), dapoxetine hydrochloride (DAP) and vardenafil (VAR)

2.3 General Procedure

2.3.1 Preparation of background electrolyte (BGE)

Acetate buffer (100 mM at pH 3.6) was selected as the best buffer for the proposed experiment. The running buffer was prepared by mixing sodium acetate (0.572 g) and glacial acetic acid (0.55 ml), and the mixture was dissolved in 100 mL of distilled water. The pH of the final mixture was approximately3.6.After that, the final pH value was adjusted to be3.6 with 0.5 M sodium hydroxide or glacial acetic acid.

2.3.2 Capillary conditioning

The capillary was flushed using 0.5 M sodium hydroxide for 15 min at the beginning of each working day, and then with distilled water for 15 min. After that, the capillary was washed with 0.1 M sodium hydroxide for 5 min, waiting for 2.5 min to confirm that, the internal wall of the fused silica capillary was activated completely. The capillary was rinsed with the distilled water for 5 min then, the capillary was washed with the selected background electrolyte for 10 min. Between every two successive injections, The capillary was washed with the BGE for 2 min. The applied voltage was 30 KV, where the sample injection was applied for 10 s at a pressure of 50 mbar.

2.3.3 Preparation of standard solutions and construction of calibration graphs

AVA, DAP, and VAR stock solutions were prepared by dissolving 100 mg of each drug in 100 ml HPLC-grade methanol to produce 1000 µg/mL stock solutions and stored in the refrigerator. Working standard solution of each drug was prepared by accurately transferring aliquots of each drug into a series of volumetric flasks (10 mL), then diluted with distilled water to achieve the concentrations of the linearity range (5–80 and 5-70 µg/ mL for AVA and DAP respectively), while the concentration of the working solution of the internal standard was 30 µg/mL. Furthermore, each concentration was examined three times. Finally, the calibration curve for each drug was produced by plotting the peak area ratios against the respective concentrations.

2.3.4 Assay of tablets dosage form

Ten super-avana® tablets were accurately weighed, crushed and mixed. A suitable quantity of the powder equivalent to 1 tablet (100 mg AVA, and 60 mg DAP) was put into a 100 ml volumetric flask and dissolved in 60 mL of HPLC-grade methanol. The resulted solution was sonicated for 10 min, centrifuged at 5000 rpm for

10 min, and then filtered into a 100 mL volumetric flask. Methanol was added to reach the filtrate up to the final volume. The working solution was prepared by transferring a suitable portion of the filtrate solution into a 10 ml volumetric flask and the solution was spiked with 300 µL VAR stock solution, then diluted to 10 ml with distilled water to achieve the desired concentrations inside the linearity ranges. All procedures in the method were completed as described above.

3. RESULTS AND DISCUSSION

3.1 Method Optimization

3.1.1 Buffer type and Ph

The influence of the type of a buffer on the experiment was investigated by using borate buffer of different pH values (8, 8.5 and 9), phosphate buffer of different pH values (6, 7 and 7.5). It was found that borate buffer produced a distorted peak shape of DAP while phosphate buffer produced bad resolution and peak broadening. 100 mM Acetate buffer of different pH values (3.6, 4.2, 4.8 and 5.4) was examined. It was found that sharper peaks were obtained at a reasonable time by using the acetate buffer of pH 3.6.

3.1.2 Buffer concentration

As buffer concentration increased the resolution between AVA and DAP increased. A small alteration occurred in the migration times, but a significant improvement of peak symmetry and the resolution occurred by increasing buffer concentration. Different concentrations of BGE were examined (from 20 to 100 mM), 100 mM acetate buffer exhibit the best peak symmetry (Fig. 2) and the highest resolution (from 2.55 in 20 mM buffer to 7.35 in 100 mM buffer) with reasonable migration time.

3.1.3 Applied voltage

The influence of applied voltage on the experiment was studied. Different values of the applied voltage were examined (15, 20, 25 and 30 kV) using the optimized BGE. The results showed that the migration times increased as the applied voltage decreased (Fig. 3) because of decreasing in the electro-osmotic flow (EOF). The resolution was unaffected by changing voltage and its value remained around 7.3 in spite of changing the migration times. As applied voltage decreased the peak broadening increased. As a result, 30 kV (maximum applied voltage that can be reached by the CE device) was chosen to achieve effective separation with less migration time.

3.1.4 Sample injection and use of internal standard

Sample injection times affect the peak height and width so different injection times (4.0–18.0 s) were investigated at a pressure of 50 mbar. Generally, the peak area (signal response) increased by increasing the injection time. On the other hand, a further increase in the injection time resulted in a loss of linear response and peak tailing. The optimum results showed at 10 s injection time. Vardenafil is a drug that belongs to the phosphodiesterase (PDE) inhibitors class like AVA. VAR is used as an internal standard because of applying an internal standard in the proposed method is used to improve the quantitative estimation of the drugs and to decrease the possible errors caused by fluctuations in migration times and injection volumes [36].

3.1.5 Selection of detection wavelength

AVA and DAP were measured at their maximum absorption wavelength as DAD can measure samples at several wavelengths and you can choose the best one which results in greater sensitivity. DAP was measured at 210 nm, while AVA and internal standard (VAR) were measured at 248 nm. The proposed method exhibit effective separation between AVA, DAP and the IS (VAR) with a retention time less than 6.5 min. Fig. 4 showed electropherograms for VAR, DAP and AVA respectively at the two selected wavelengths. The proposed method showed well-defined peaks of VAR, DAP, and AVA with migration times 4.29, 4.90, and 6.02 min respectively. Table 1 describes system suitability parameters for the analyzed drugs such as selectivity (α), retention factors (k), number of theoretical plates (N) and resolution [37].

3.2 Method Validation

The applied technique was validated following the International Conference on Harmonization (ICH) guidelines. [38].

3.2.1 Linearity and ranges

From the proposed method described previously, the recorded responses (ratios of the peak area

of AVA or DAP against the IS) were observed to be proportional to concentrations of the analyzed drugs where the linearity was found to be 5–80 and 5-70 µg/mL for AVA and DAP respectively. The regression equations were produced using the least squares treatment of the calibration data. Table 2 describes the linearity data as well as other statistical parameters such as concentration ranges, regression equations, correlation coefficients, slope (Sb), standard deviations of the intercept (Sa), and standard deviations of residuals (Sy/x). Statistical parameters showed good linearity where the correlation coefficient values were 0.9996 and 0.9995 for AVA and DAP respectively. Moreover, the linearity was checked by measuring the RSD % value of the slope (Sb%) for each drug, which was less than 1%. High F values and low significance F in the analysis of variance (ANOVA) test indicate an increase in the mean of squares because of regression and a decrease in the mean of squares because of residuals.

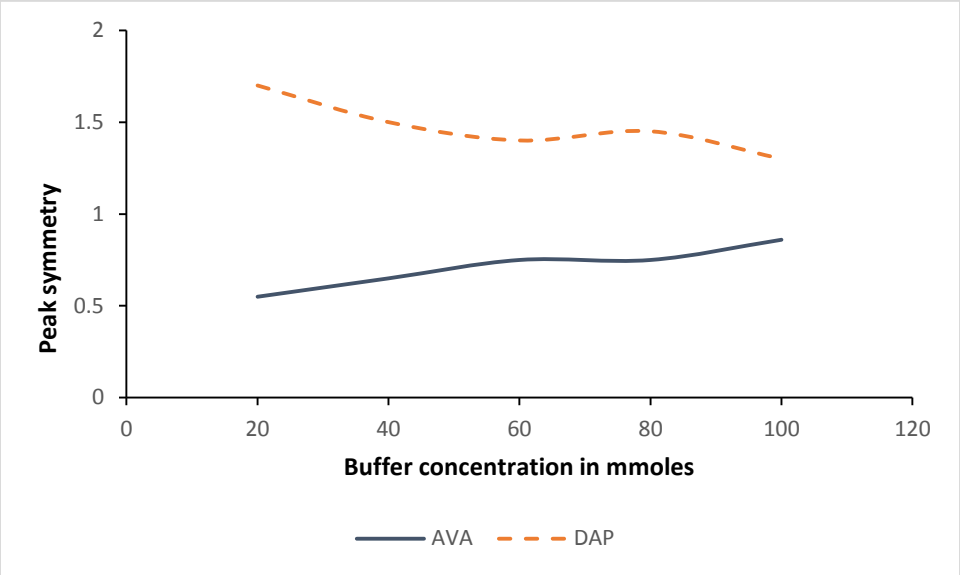

Fig. 2. Effect of buffer concentration on the peak symmetry of AVA and DAP

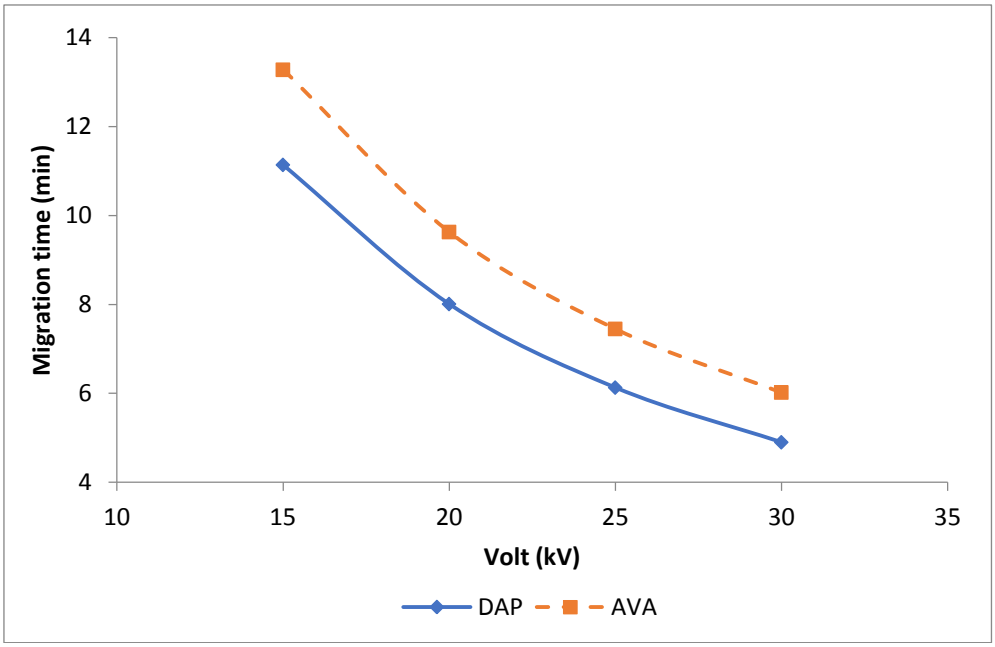

Fig. 3. Effect of applied voltage on the migration times of AVA and DAP

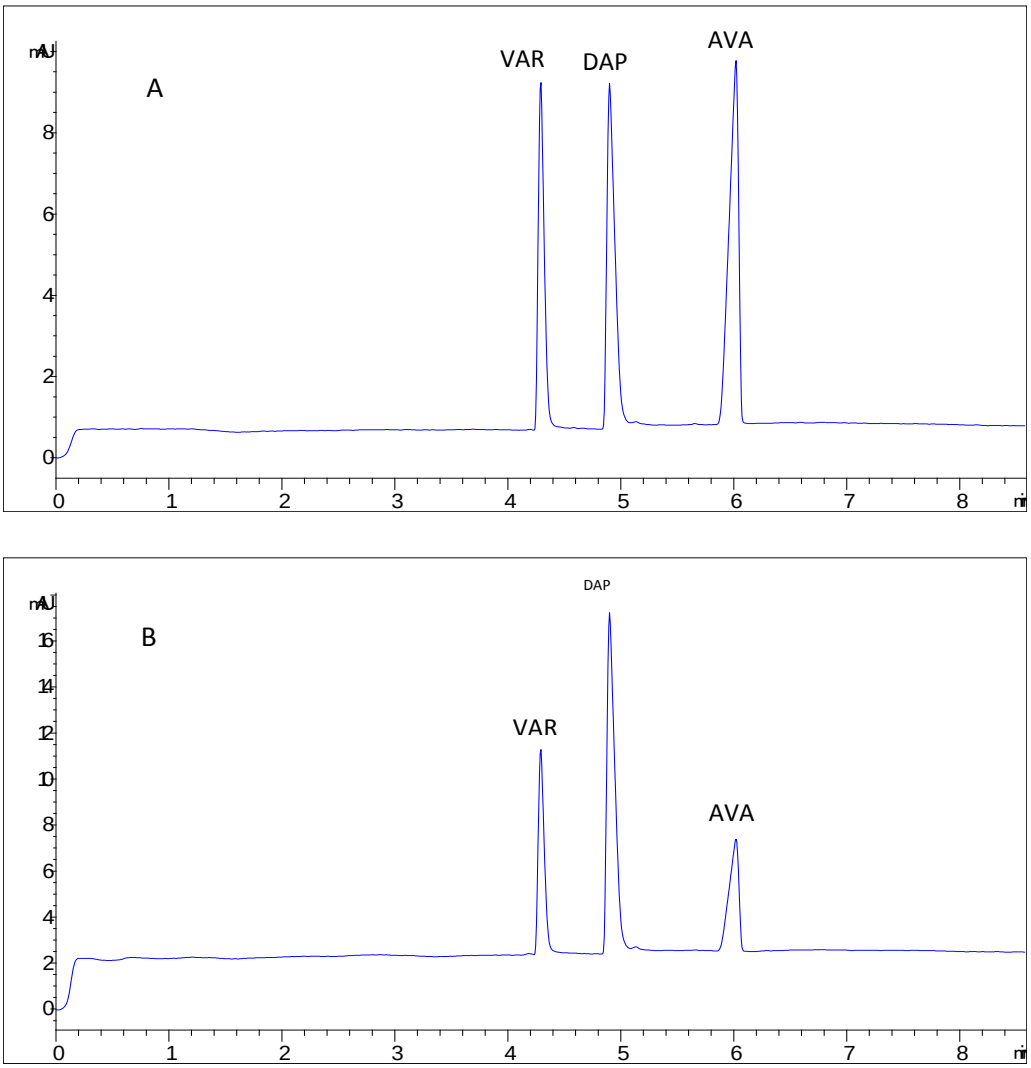

Fig. 4. Electropherograms of a mixture containing 30 µg/mL of AVA, DAP and VAR (IS) at (a) 248 nm for AVA and VAR and (b) 210 nm for DAP

Table 1. System suitability parameters for determination of AVA and DAP mixture using VAR as an internal standard

Parameter	VAR	DAP	AVA
tR ± SD (min)	4.29 ± 0.09	4.90 ± 0.15	6.02 ± 0.12
Retention factors (k')	1.86	2.27	3.01
Theoretical plates (N)	28366	20784	20531
USP tailing factor	1.18	1.30	0.86
Selectivity (α)	-	1.22	1.33
Resolution (Rs)	-	5.11	7.35

3.2.2 Limit of detection and quantification

The LOD and LOQ values for AVA and DAP were determined following the ICH recommendations, where LOD and LOQare defined as the sample concentration with a signal-to-noise ratio of 3:1 and 10:1 respectively. Table 2 represents the values of LOD and LOQ that indicate the sensitivity of the applied method.

3.2.3 Accuracy and precision

The accuracy and intra-day (within-day) precision of the applied method were investigated by

testing each compound three times at three concentration levels within one day, while the inter-day (between-day) precision was studied by testing each compound three times at three concentrations levels repeated on three different days. Statistical results of precision data of AVA and DAP (Table 3) exhibited low RSD% values, which were less than 2%, indicating the method's precision. The Er% values of AVA and DAP did not exceed 2%, indicating the accuracy of the applied method.

3.2.4 Selectivity

The selectivity of the applied method was investigated by analyzing several laboratory-prepared mixtures of the AVA and DAP at various concentrations ratios within their cited linear ranges. The mixtures of the analyzed drugs were prepared in different concentrations ratios lower or higher than those found in the commercial tablet. Table 4 demonstrates the ability of the proposed method in the separation and quantification of AVA and DAP in different concentrations ratios, where RSD% and Er% values proved the selectivity of the applied method. DAD can verify the purity of the resulted peaks, therefore, the proposed method when applied in commercial tablets didn't show co-eluted peaks from the inactive ingredients that indicate the selectivity of the proposed method. furthermore, the superimpositions of a set of spectra at five different time intervals across the peak confirm the purity of the peak and the selectivity of the method (Fig. 5 (A1, B1, C1)). Fig. 5 (A2, B2, C2) confirmed the high similarity of all peaks because the evaluation included all the spectra obtained throughout the migration or elution of the peak rather than only three or four spectra. The similarity curves did not exceed the noise threshold value that appeared as a red-colored area which indicates the reliability and the sensitivity of the peak purity evaluation.

3.2.5 Robustness

The robustness of the applied technique was investigated by monitoring the migration times and peak area ratios after small alterations in the method conditions. The studied conditions include buffer pH 3.6 ± 0.2, acetate buffer concentration 100 ± 2 mM, and wavelength 210 ± 2 nm and 248 ± 2 nm. Analysis was carried out by injection of 20 µg/mL mixture of AVA, DAP and 30 µg/ mL of VAR three times with only one alteration occurred at a time. The investigated parameters had no noticeable impact on migration times or peak area ratios. The RSD% values in Table 5 reflect the robustness of the applied method.

3.2.6 Stability of solutions

The stability of working sample solutions was examined using a 30 µg/mL mixture of AVA, DAP and IS within 24 hr at room temperature. The examined drugs showed good stability, and no noticeable change in peaks areas or migration times of the tested drugs as the %RSD values were not more than 1.9. Furthermore, no

Table 2. Analytical parameters for estimation of AVA and DAP mixture using the proposed CZE method

Parameter	AVA	DAP
Wavelength (nm)	248	210
Concentration range (µg/mL)	5 – 80	5 - 70
Intercept (a)	-0.018	0.048
S_a [a]	0.005	0.007
Slope (b)	0.035	0.041
S_b [b]	1.15×10^{-4}	1.74×10^{-4}
RSD% of the slope (Sb%)	0.332	0.421
Correlation coefficient (r)	0.9996	0.9999
R square	0.9992	0.9999
$S_{y/x}$ [c]	0.009	0.011
F [d]	90773	56546
Significance F	1.17×10^{-15}	3.73×10^{-13}
LOD [e] (µg/mL)	0.523	0.531
LOQ [f] (µg/mL)	1.585	1.608

[a] Standard deviation of the intercept; [b] Standard deviation of the slope; [c] Standard deviation of residuals; [d] Variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals); [e] Limit of detection; [f] Limit of quantification

Table 3. Precision and accuracy for estimation of AVA and DAP in bulk form using the applied method

Analyte	Nominal value (µg/ml)	Found ± SD[a] (µg/ml)	RSD(%)[b]	E_r(%)[c]
AVA	Within-day			
	20	19.96 ± 0.08	0.40	-0.20
	40	40.08 ± 0.30	0.75	0.20
	80	80.08 ± 0.81	1.01	0.10
	Between-days			
	20	20.04 ± 0.37	1.85	0.20
	40	39.60 ± 0.64	1.62	-1.00
	80	80.06 ± 0.66	0.82	0.08
DAP	Within-day			
	20	20.24 ± 0.17	0.84	1.20
	40	39.53 ± 0.49	1.24	-1.18
	70	69.82 ± 0.35	0.50	-0.26
	Between-days			
	20	20.27 ± 0.21	1.04	1.35
	40	39.95 ± 0.57	1.43	-0.13
	70	69.98 ± 0.47	0.67	-0.03

[a] Mean ± standard deviation for three determinations; [b] % Relative standard deviation; [c] % Relative error

Table 4. Analysis of AVA and DAP in laboratory-prepared mixtures using the proposed CZE method

Nominal value (µg/ml)		Found ± SD[a] (µg/ml)		RSD(%)[b]		Er(%)[c]	
AVA	**DAP**	**AVA**	**DAP**	**AVA**	**DAP**	**AVA**	**DAP**
30	50	29.46 ± 0.53	50.22 ± 0.77	1.81	1.53	-1.80	0.44
50	30	49.70 ± 0.41	30.13 ± 0.51	0.82	1.70	-0.60	0.42
10	70	10.01 ± 0.18	70.02 ± 0.97	1.75	1.38	0.14	0.03
70	10	70.14 ± 0.55	9.95 ± 0.20	0.78	1.98	0.20	-0.50
50	50	49.86 ± 0.85	50.18 ± 0.83	1.70	1.65	-0.28	0.36

a Mean ± standard deviation for five determinations; b % Relative standard deviation; c % Relative error

Table 5. Robustness evaluation for the determination of AVA and DAP mixture using the proposed CZE method

Parameter	**AVA***			
	Peak area ratio ± SD	**RSD%**	**Migration time ± SD**	**RSD%**
Buffer concentration 100 ± 2 mmole	0.72 ± 0.008	1.06	6.00 ± 0.015	0.25
Buffer pH 3 ± 0.2 pH unit	0.72 ± 0.010	1.44	6.03 ± 0.031	0.51
Wavelength 235 ± 2 nm	0.73 ± 0.013	1.73		
Parameter	**DAP***			
	Peak area ratio ± SD	**RSD%**	**Migration time ± SD**	**RSD%**
Buffer concentration 100 ± 2 mmole	0.86 ± 0.010	1.16	4.92 ± 0.025	0.51
Buffer pH 3 ± 0.2 pH unit	0.85 ± 0.012	1.35	4.89 ± 0.036	0.74
Wavelength 210 ± 2 nm	0.86 ± 0.015	1.78		

** Robustness parameters were determined for a mixture containing 20 µg/mL of each AVA and DAP with 30 µg/mL of VAR as internal standard*

chromatographic alterations were observed. The absorption spectra of the analyzed drugs were also investigated and showed no significant change. The stability of stock solutions was examined, and they were observed to remain stable for at least three weeks when kept in a refrigerator at 4 °C. The concentrations of the previously prepared solution (from two weeks) and the newly prepared one were calculated using the applied method, and the RSD% values were observed to be less than 2.0%.

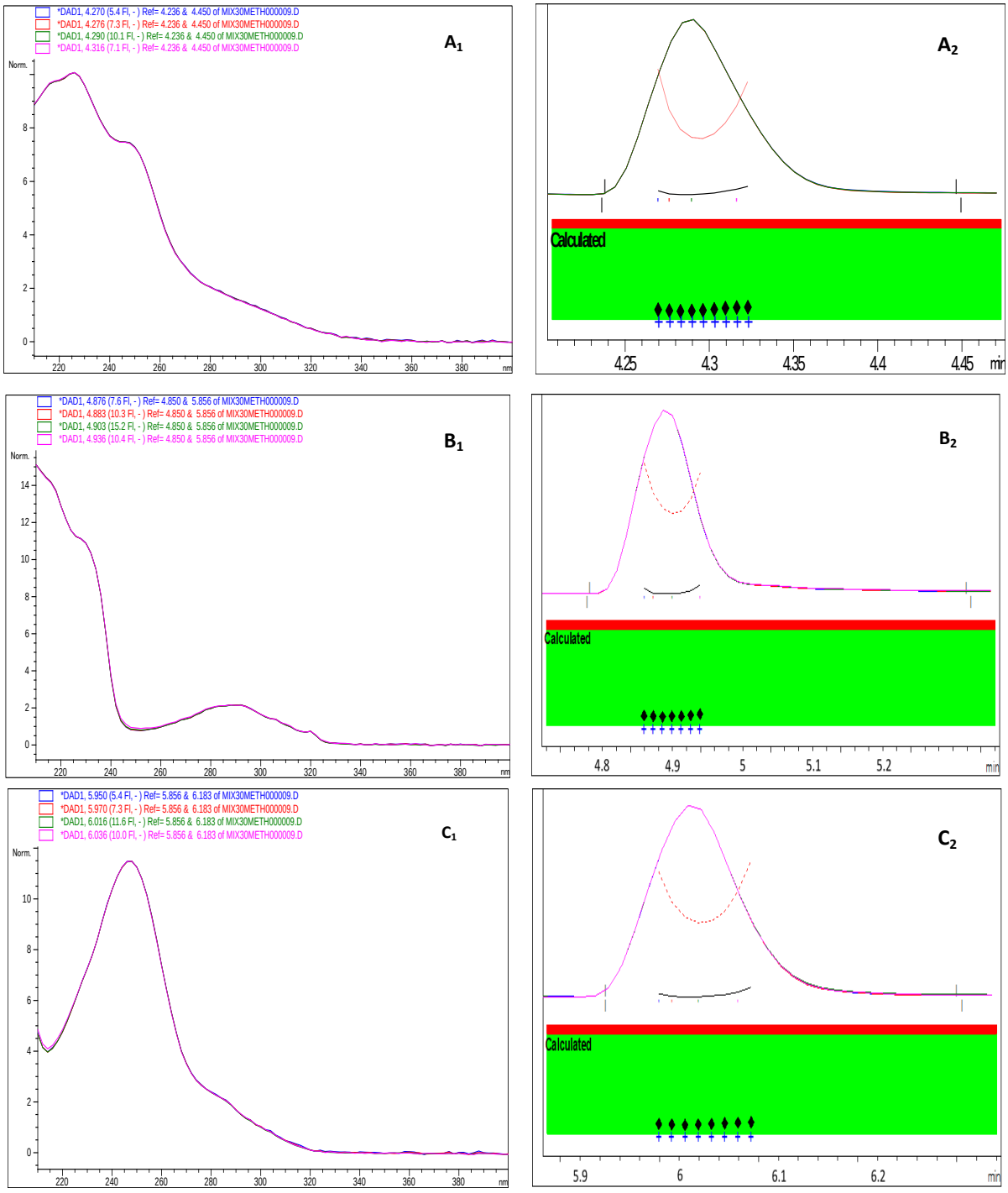

Fig. 5. Absorption spectrum of 30 µg/mL mixture of VAR(A_1), DAP(B_1), and AVA(C_1) measured at 5 different time intervals across the CE peak, while A_2, B_2, and C_2 represent the purity plot for VAR, DAP, and AVA peaks, respectively

3.3 Application of Dosage Forms

The ability of the applied method for the quantitative determination of AVA and DAP as a binary mixture in a tablet dosage form was examined. There were no interfering peaks from the inactive components and no change in migration time for the analyzed drugs (Fig. 6). Analysis results showed good Recoveries and low RSD% values as showed in (Table 6). DAD can check the peak purity, where the examined drugs exhibited homogenous and pure peaks. As a result, the applied method is successfully applied for the estimation of AVA and DAP in combined dosage form with good recovery and selectivity.

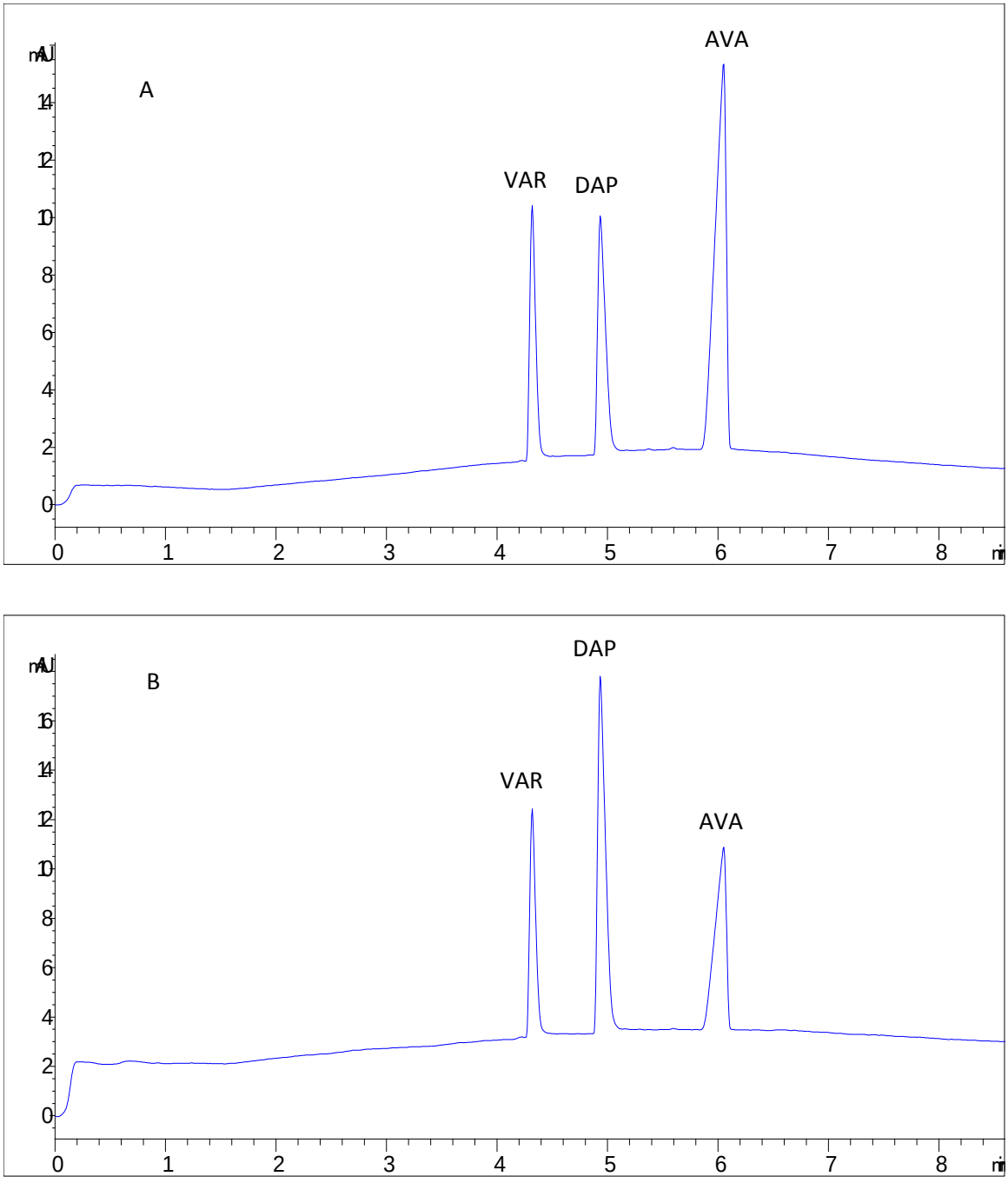

Fig.6. Electropherograms of a mixture containing 50 µg/ml AVA and 30 µg/mL DAP were obtained from super-avana® tablet and spiked with 30 µg/ml VAR (IS) (a) at 248 nm (b) at 210 nm

Table 6. Application of the proposed method to the estimation of AVA and DAP mixtures in the tablet dosage form

super-avana® tablets	External Standard		Standard Addition	
	AVA	DAP	AVA	DAP
%Recovery ± SD[a]	99.96 ± 0.42	100.03 ± 1.69	99.89 ± 0.85	100.41 ± 1.56
RSD%[b]	0.42	1.69	0.85	1.55

Quantification was carried out at the following wavelengths: 210 for DAP and 248 for AVA; [a] Mean ± standard deviation for five determinations; b % Relative standard deviation.

4. CONCLUSION

The validated CZE technique was applied and assessed for the quantitative estimation of AVA and DAP in a pure form and pharmaceutical preparation. The proposed method is fast, simple, robust and showed acceptable performance concerning all validation parameters for the analyzed drugs in pure form and pharmaceutical preparation. Furthermore, the use of DAD provides a significant benefit for the verification of peak purity. The proposed method is the first electro-chromatographic method applied for the binary mixture of AVA and DAP, and this method can be used as an alternative to HPLC reported methods because it is also effective for routine analysis with a lower cost and the proposed method applied the concept of green chemistry.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kedia GT, Ückert S, Assadi-Pour F, Kuczyk MA, Albrecht K. Avanafil for the treatment of erectile dysfunction: initial data and clinical key properties. Therapeutic Advances in Urology. 2012;5(1):35-41. Available:https://doi.org/10.1177%2F1756287212466282
2. Kyle JA, Brown DA, Hill JK. Avanafil for Erectile Dysfunction. Annals of Pharmacotherapy. 2013;47(10):1312-20. Available:https://doi.org/10.1177/1060028013501989
3. Modi NB, Dresser MJ, Simon M, Lin D, Desai D, Gupta S. Single- and Multiple-Dose Pharmacokinetics of Dapoxetine Hydrochloride, a Novel Agent for the Treatment of Premature Ejaculation. The Journal of Clinical Pharmacology. 2006;46(3):301-9. Available:https://doi.org/10.1177/0091270005284850
4. Buvat J, Tesfaye F, Rothman M, Rivas DA, Giuliano F. Dapoxetine for the Treatment of Premature Ejaculation: Results from a Randomized, Double-Blind, Placebo-Controlled Phase 3 Trial in 22 Countries. European Urology. 2009;55(4):957-68. Available:https://doi.org/10.1016/j.eururo.2009.01.025
5. Kashyap R, Srinivasa U. Development and validation of new colorimetric method for the estimation of Avanafil in bulk and dosage form. International Journal of Pharmacy and Technology. 2013;5:5811-26.
6. Darwish MK, Soliman MM, Abdel-Razeq SA. Validated stability indicating HPTLC and UV-spectrophotometric techniques for the determination of avanafil. Asian J Chem Sci. 2019:1-16. Available:https://doi.org/10.9734/ajocs/2019/v6i419005

7. Kumar N, Sangeetha D, Kalyanraman L, Sainath K. Stability-indicating HPLC method for simultaneous determination of degradation products and process-related impurities of avanafil in avanafil tablets. Acta Chromatographica Acta Chromatographica. 2018;30(3):158-63.
Available:https://doi.org/10.1556/1326.2017.00116
8. Patel M, Kothari C. Comprehensive stability-indicating method development of Avanafil Phosphodiesterase type 5 inhibitor using advanced Quality-by-Design approach. Journal of Analytical Science and Technology. 2020;11(1):29.
Available:https://doi.org/10.1186/s40543-020-00228-4
9. CAN NÖ. Development of Validated and Stability-Indicating LC-DAD and LC-MS/MS Methods for Determination of Avanafil in Pharmaceutical Preparations and Identification of a Novel Degradation Product by LCMS-IT-TOF. Molecules. 2018;23(7):1771.
Available:https://doi.org/10.3390/molecules23071771
10. ÖztürkEr E, Özbek B, Bakırdere S. Accurate and sensitive determination of sildenafil, tadalafil, vardenafil, and avanafil in illicit erectile dysfunction medications and human urine by LC with quadrupole-TOF-MS/MS and their behaviors in simulated gastric conditions. Journal of separation science. 2019;42(2):475-83.
Available:https://doi.org/10.1002/jssc.201800734
11. Ali MB, Abdel-Raoof AM, Hendawy HAM, Talaat W, Omran GA, Morshedy S. An Eco-Friendly Solid-State Electrode Modified WithZnO Nanoparticles Decorated With MWCNT as an Electrochemical Sensor for the Determination of Avanafil in Pure Form, Dosage Form and Human Plasma. Journal of The Electrochemical Society. 2021;168(8):087510.
Available:https://doi.org/10.1149/1945-7111/ac1d7d
12. Chabukswar AP, Kuchekar BS, Patil SL, Moon SA, Chate SG, Pagare BD. Spectrophotometric simultaneous determination of dapoxetine and sildenafil in combined tablet dosage form by absorbance corrected method. Der Pharma Chem. 2012;4:1404-7.
13. Savjiyani NB, Patel PB. Simultaneous estimation of vardenafil hydrochloride and dapoxetine hydrochloride in combined pharmaceutical dosage form by spectrophotometry and RP-HPLC. Indo Am J Pharm Res. 2013;3:3652-68.
14. Elama HS, Elsebaei FA, Ali FAA, El-Brashy AM. Validated spectrophotometric and spectrofluorimetric methods for determination of dapoxetine hydrochloride and dosulepin hydrochloride in their dosage forms using mercurochrome. Luminescence. 2018; 33(8):1306-13.
Available:https://doi.org/10.1002/bio.3566
15. Magdy MA, Anwar BH, Naguib IA, Abdelhamid NS. Quantitative determination of Dapoxetine Hydrochloride and Tadalafil using different validated spectrophotometric methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;226: 117611.
Available:https://doi.org/10.1016/j.saa.2019.117611
16. Hamilton CL, Cornpropst JD. Determination of dapoxetine, an investigational agent with the potential for treating depression, and its mono-and di-desmethyl metabolites in human plasma using column-switching high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications. 1993;612(2):253-61.
Available:https://doi.org/10.1016/0378-4347(93)80171-Y
17. Patil RB, Deshmukh TA, Patil VR. Stability indicating HPLC method for dapoxetine HCl in bulk and in formulation. Int J Pharm Pharm Sci. 2014;6:687-90.
18. Giri AD, Bhusari VK, Dhaneshwar SR. Validated HPLC method for simultaneous quantitation of tadalafil and dapoxetine hydrochloride in bulk drug and formulation. Int J Pharm Pharm Sci. 2012;4(2):654-58.
19. Liew KB, Peh KK. Stability indicating HPLC method for simultaneous quantification of sildenafil citrate and dapoxetine hydrochloride in Pharmaceutical products. Pakistan journal of pharmaceutical sciences. 2018;31(6).
20. Baker MM, Belal TS, Mahrous MS, Ahmed HM, Daabees HG. High-performance liquid chromatography with diode array detection method for the simultaneous determination of seven selected phosphodiesterase-5 inhibitors and serotonin reuptake inhibitors

used as male sexual enhancers. Journal of Separation Science. 2016;39(9):1656-65. Available:https://doi.org/10.1002/jssc.201501339

21. Darcsi A, Tóth G, Kökösi J, Béni S. Structure elucidation of a process-related impurity of dapoxetine. Journal of Pharmaceutical and Biomedical Analysis. 2014;96:272-7. Available:https://doi.org/10.1016/j.jpba.2014.04.002
22. Hegazy M, Kessiba A, Abdelkawy M, Emad El Gendy A. A novel liquid chromatographic method with fluorescence detection for quantitation of tadalafil and dapoxetine hydrochloride in pharmaceutical dosage form and human plasma. 2015. Available:https://doi.org/10.3724/sp.j.1123.2015.02063
23. Harnisch H, Scriba GKE. Capillary electrophoresis method for the determination of (R)-dapoxetine, (3S)-3-(dimethylamino)-3-phenyl-1-propanol, (S)-3-amino-3-phenyl-1-propanol and 1-naphthol as impurities of dapoxetine hydrochloride. Journal of Pharmaceutical and Biomedical Analysis. 2019;162:257-63. Available:https://doi.org/10.1016/j.jpba.2018.09.039
24. Neumajer G, Sohajda T, Darcsi A, Tóth G, Szente L, Noszál B, et al. Chiral recognition of dapoxetine enantiomers with methylated-gamma-cyclodextrin: A validated capillary electrophoresis method. Journal of Pharmaceutical and Biomedical Analysis. 2012;62:42-7. Available:https://doi.org/10.1016/j.jpba.2011.12.032
25. Mohamed MA, Atty SA, Yehia AM, Foster CW, Banks CE, Allam NK. Electrochemical Determination of the Serotonin Reuptake Inhibitor, Dapoxetine, Using Cesium–Gold Nanoparticles. ACS Omega. 2017;2(10):6628-35. Available:https://doi.org/10.1021/acsomega.7b01193
26. Aziz A, Khamees N, Mohamed TA-F, Derar AR. Comparative Study of PVC-Free All-Solid-State, PVC Membrane, and Carbon Paste Ion-Selective Electrodes for the Determination of Dapoxetine Hydrochloride in Pharmaceutical Formulation. Journal of AOAC International. 2016;99(6):1499-504. Available:https://doi.org/10.5740/jaoacint.16-0089
27. Kashyap R, Srinivasa U, Badodaria K. First Order Derivative and Dual Wavelength Spectrophotometry Methods Development and Validation For Simultaneous Determination of Avanafil and Dapoxetine Hydrochloride in Bulk and Dosage Form. Int J Pharm Technol. 2014;6:6418-38.
28. Patel MN, Kothari CS. Multivariate Approaches for Simultaneous Determination of Avanafil and Dapoxetine by UV Chemometrics and HPLC-QbD in Binary Mixtures and Pharmaceutical Product. Journal of AOAC INTERNATIONAL. 2016;99(3):649-63. Available:https://doi.org/10.5740/jaoacint.15-0259
29. Hegazy M, Kessiba A, Abdelkawy M, El-Gindy AE. RP-HPLC with Time Programmed Fluorescence Detection for Quantitation of Avanafil and Dapoxetine Hydrochloride: Application to Pharmaceutical Dosage Form and Biological Fluid. Journal of Liquid Chromatography & Related Technologies. 2015;38(18):1660-5. Available:https://doi.org/10.1080/10826076.2015.1087860
30. Kashyap R, Srinivasa U, Badodaria K. Development and validation of RP-HPLC method for simultaneous estimation of avanafil and dapoxetine hydrochloride in bulk and dosage form. World J Pharm Pharm Sci. 2014;3(7):1697-719.
31. Shah DA, Vegad KL, Patel ED, Prajapati HK, Patel RN, Patel YK. Analytical method validation for estimation of avanafil and dapoxetine hydrochloride tablet dosage form by HPTLC method. Pharm Biol Eval. 2017;4(3):171-9.
32. Mohamed TA, Atty SA. Native and synchronous fluorescence spectroscopy for determination of avanafil in presence of its co-formulated drug (dapoxetine hydrochloride): Application to pharmaceutical product, biological fluid and content uniformity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;229:117898. Available:https://doi.org/10.1016/j.saa.2019.117898
33. Elonsy SM, El Yazbi FA, Shaalan RA, Ahmed HM, Belal TS. Application of MEKC and UPLC with Fluorescence Detection for Simultaneous Determination of Amlodipine Besylate and Bisoprolol Fumarate. Journal

of AOAC International. 2021; 104(2):339-47.
Available:https://doi.org/10.1093/jaoacint/qsaa136

34. Ragab MAA, Abdel-Hay MH, Ahmed HM, Mohyeldin SM. Application of Capillary Zone Electrophoresis Coupled with a Diode Array Detector (CZE-DAD) for Simultaneous Analysis of Ibuprofen and Phenylephrine. Journal of AOAC INTERNATIONAL. 2019;102(2):473-9.
Available:https://doi.org/10.5740/jaoacint.18-0134
35. Ahmed HM, Belal TS, Shaalan RA, El Yazbi FA, Elonsy SM. Validated capillary zone electrophoretic method for simultaneous analysis of benazepril in combination with amlodipine besylate and hydrochlorothiazide. Acta Chromatographica Acta Chromatographica. 2020;32(4):219-27.
Available:https://doi.org/10.1556/1326.2019.00686
36. Salim M, El-Enany N, Belal F, Walash M, Patonay G. Simultaneous Determination of Sitagliptin and Metformin in Pharmaceutical Preparations by Capillary Zone Electrophoresis and its Application to Human Plasma Analysis. Analytical Chemistry Insights. 2012;7:ACI.S9940.
Available:https://doi.org/10.4137%2FACI.S9940
37. Ravisankar P, Anusha S, Supriya K, Kumar UA. Fundamental chromatographic parameters. Int J Pharm Sci Rev Res. 2019;55(2):46-50.
38. Guideline IHT. Validation of analytical procedures: text and methodology. Q2 (R1). 2005;1(20):05.

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