Archives of Pharmaceutical Sciences Ain Shams University 2024; Vol. 8(2): 224-244

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Analytical Chemistry

Original Article

An Environmentally Friendly Chromatographic Technique for Estimating Four Co-Administered Medications in Solid Dosage Forms and Human Plasma for the Treatment of Benign Prostatic Hyperplasia

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ABSTRACT

Four frequently used medications—tadalafil, dapoxetine hydrochloride, tamsulosin hydrochloride, and solifenacin succinate— for the treatment of benign prostatic hyperplasia and its associated urological disorders, are determined in bulk, tablet dosage forms, and human plasma using an environmentally friendly, easy-to-use, affordable, and repeatable high performance liquid chromatographic method (HPLC). The separation by chromatography was carried out on an HILIC column (250 X 4.6 mm, 5 µm) at ambient temperature. Acetonitrile: water (80:20 v/v) was used to achieve separation, with a flow rate of 1.5 mL/min. The run lasted for twenty minutes. Tadalafil, Tamsulosin, and Solifenacin were detected at 204 nm and Dapoxetine at 210 nm, using a photodiode array detection. For Tadalafil, Dapoxetine, Tamsulosin, and Solifenacin, the corresponding linearity values were 0.05-100, 0.1-50, 0.2-80, and 0.1-60 µg/mL. The retention times were determined to be 1.683, 5.570, 9.191, and 16.545 minutes. The limits of detection for Tadalafil, Dapoxetine, Tamsulosin, and Solifenacin were determined to be 0.01, 0.02, 0.05, and 0.02 µg/mL, in that order. Low percentage RSD results show good precision in the procedure. It was discovered that the dosage forms recovery percentage fell between 98.50 and 100.61%. The procedure was deemed to be environmentally favorable after being evaluated using two distinct greenness evaluation indices. Thus, routine analysis of the recommended medications in their tablet dosage forms could be effectively conducted using this established and confirmed HPLC approach. Furthermore, a higher sensitivity is provided by the suggested method, making it possible to identify the medications under study in human plasma.

Keywords: *Benign prostatic hypertrophy, environmentally friendly HPLC evaluation, Tamsulosin, Dapoxetine, Tadalafil, and Solifenacin.*

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Citation | Zahra MH, Trabik YA, Hanaa NM, Abdel-Ghany MF, 2024. An Environmentally Friendly Chromatographic Technique for Estimating Four Co-Administered Medications in Solid Dosage Forms and Human Plasma for the Treatment of Benign Prostatic Hyperplasia. Arch Pharm Sci ASU 8(2): 224-244

DOI: 10.21608/aps.2024.291016.1172

Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.

Received 20 Mai 2024. **Accepted** 27 July 2024.

Published by: Ain Shams University, Faculty of Pharmacy

1. Introduction

Benign enlargement of the prostate (BEP) frequently referred to as benign prostatic hyperplasia (BPH), is a prostate adenoma. Benign growth of the prostate gland is attributed to uncontrolled hyperplastic proliferation of the fibromuscular as well as epithelial tissues in the periurethral region and transition zone (TZ). The average prostate size is 20 g, which is reached between the ages of 18 and 20. Studies on the histology of the prostate in autopsy samples have revealed that half of the male population aged 51 to 60 exhibits dysfunctional traits compatible with BPH. Globally, about 105 million people after the age of 40 are affected. One of the most common conditions affecting older men is BPH **[1]**. BPH results in an overactive bladder and can

hurt the quality of life as it is associated with low urinary tract symptoms (LUTS), including voiding symptoms, which occur after urination, nocturia, and occasional incontinence. Complications include sexual problems such as premature ejaculation (PE), erectile dysfunction (ED), and decreased libido **[2]**. Since BPH patients may also develop LUTS as well as sexual complications, there is an urge to co-administer drugs from different classes, such as Tadalafil, Dapoxetine, Tamsulosin, and Solifenacin to reveal all these combined symptoms. Several clinical studies showed that combination therapy using different classes of drugs, such as phosphodiesterase type 5 (PDE-5) inhibitors, alpha-blockers, 5α-reductase inhibitors, and antimuscarinics were effectively and safely used in the treatment regimen. Tamsulosin and Solifenacin are safely prescribed together for managing LUTS resulting from BPH **[3]**. The combination of Tamsulosin and Tadalafil was well tolerated and more effective in improving voiding symptoms (LUTS) due to BPH than monotherapy, as well as improving ED **[4, 5]**.

Tadalafil (TAD); hydro-2-methyl-6-[3,4-(methylenedioxy) phenyl] pyrazino-[1',2':1,6]pyrido[3,4-b]indole-1,4-dione **(Fig. 1.a)**

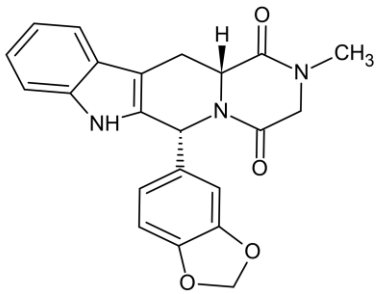

Fig. 1.a. Tadalafil chemical structure

Tadalafil is a type 5 phosphodiesterase (PDE-5) inhibitor. It serves to control ED by inhibiting particular PDE-5, which is in charge of cGMP degradation in the Corpus cavernosum **[6]**. It's the only PDE-5 inhibitor licensed to treat the signs and symptoms of BPH **[7]**. Various techniques have been documented in the literature for the analysis of TAD alone or combining other drugs as spectroscopic methods **[6, 8-17]**, chromatographic methods [**18-31]**, and electrochemical methods **[32–34]**.

Dapoxetine hydrochloride (DAP); (S)-N, N-Dimethyl-3-(naphthalen-1-yloxy)-1- phenylpropan-1-amine **(Fig. 1.b)**.

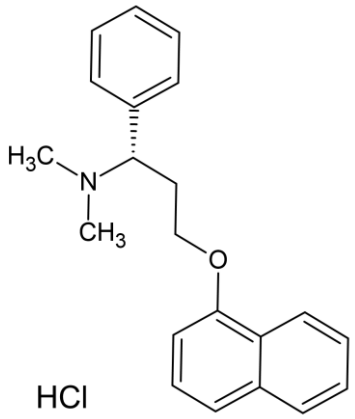

Fig. 1.b. Dapoxetine hydrochloride chemical structure

Dapoxetine hydrochloride is applied to male patients with PE. It causes a delay in ejaculation by blocking the transfer of serotonin **[6]**. Analytical methods for DAP analysis include spectroscopic methods **[35]**, chromatographic methods **[36–38],** and electrochemical methods **[39–41]**.

Tamsulosin hydrochloride (TAM); 5-[(2R)-2-[[2-(2-ethoxy phenoxy) ethyl] amino] propyl]-2- methoxy benzene sulfonamide hydrochloride **(Fig. 1.c)**, is an exclusive selective, potent and competitive α1–adrenoreceptor antagonist, predominantly present in the human prostate. It can lessen the uncomfortable and frequent urination, as well as the urgency and frequency of urination, symptoms associated with an enlarged prostate

[42]. Literature review reveals that techniques for analyzing TAM alone or in combination with other medications have been published as spectroscopic methods [**17], [43-45]**, chromatographic methods **[46–52],** and electrochemical methods **[53–55]**.

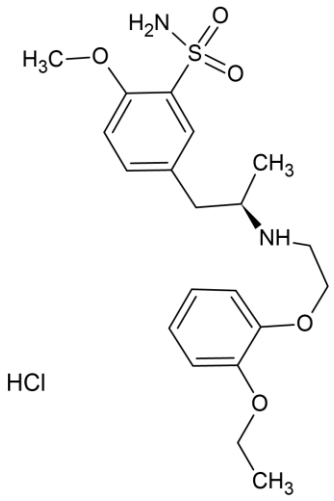

Fig. 1.c. Tamsulosin hydrochloride chemical structure

Solifenacin succinate (SFN); 1-azabicyclo [2.2.2] oct-8-yl (1S)-1-phenyl-3,4-dihydro1H-isoquinoline-2 carboxylate **(Fig. 1.d)**

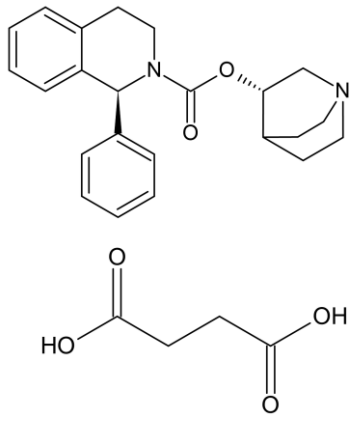

Fig. 1.d. Solifenacin succinate chemical structure

Solifenacin succinate is a muscarinic receptor antagonist that is competitive and used to treat overactive bladder with symptoms of LUTS. Literature review reveals that techniques for analyzing SFN alone or in combination with other medications have been published as spectroscopic methods **[56–59]**, chromatographic methods **[52, 60–63],** and electrochemical methods **[55, 64**]. Finally, this work was developed and validated for the first time to separate and estimate these four drugs with a lower concentration range.

2. Experimental

2.1. Apparatus HPLC equipment

Waters Alliance e2695 HPLC binary pump (Waters Technology, Milford, MA) equipped with an inline vacuum degassing, autosampler, heated column compartment with photodiode array detector (model 2998). Sample injection was done with a Rheodye 7725 injection valve via a 50 µL loop. Inerstil HILIC column (250×4.6 mm i.d×5 µm) packed with diol as a functional group was used for separation.

0.45 µm disposable syringe filters,0.22 µm disposable membrane filters, sonicator, vortex mixer, and Table-top centrifuge, Model PLC-012E (Gemmy Industrial Corp, Taiwan) were used for sample preparation.

2.2. Chemical and substances

All substances and solvents utilized in this project were of HPLC grade. Methanol, acetonitrile, and ammonium acetate buffer came from Mecca Pharmaceutical Chemicals Co. Cairo, Egypt. Acetic acid was purchased from ADWIC, Egypt. Water was acquired from Cornell Lab's Fisher Cairo, Egypt. The source of the frozen human plasma was VACSERA in Giza, Egypt.

2.2.1. Preparation of 10 mM Ammonium acetate buffer

0.77 g ammonium acetate was weighed and dissolved in 100 mL triple-distilled water to create an ammonium acetate solution (100 mM), which was then filtered through a 0.45 µm

membrane filter. From this stock, 10 mL was taken and diluted to 100 mL in a 100 mL volumetric flask to prepare 10 mM, which is used in the study **[65]**. PH was adjusted to 7 and 4 using acetic acid.

2.3. Pure Standards and Pharmaceutical Formulations

Tadalafil (certified to be 99.80%), Tamsulosin hydrochloride, and Solifenacin succinate (assay value was found to be 100.10%) were obtained as gift samples from EVA Pharma (Cairo, Egypt). Dapoxetine hydrochloride (certified to be 99.80%) was kindly donated by Hikma Pharmaceuticals company (Giza, Egypt).

Diamonrecta® 5mg 30 Tablets (EVA Pharma), Batch number 2306440. Joypox® 60 mg 3 Tablets (Sedico), Batch number 230803. Tamsulin Plus® 6/0.4 mg 20MR Tablets (Marcyl Co.) Solifenacin/Tamsulosin, Batch number 2331267, All were purchased from a local market pharmacy (Cairo, Egypt). Tadapox® 20/60 mg tablets were purchased from an Indian market pharmacy (produced by Sunrise Remedies Pvt.Ltd., India).

2.4. Standard solutions

2.4.1. Stock standard solutions

500 µg/mL standard stock solutions were prepared by dissolving 0.05 g of each drug separately and completing the final 100 mL volume with acetonitrile

2.4.2. Working solutions

Working reference solutions (10, 50, 100 µg/mL) were prepared for each drug by transferring (1, 5, 10 mL) into a 50 mL volumetric flask from each standard stock solution, and then adding acetonitrile to reach the final volume. Up to the time of analysis, the stock and working standard solutions were kept at 8 °C in amber bottles.

2.5. Procedure

2.5.1. Chromatographic conditions

Isocratic elution mode was adopted using a mobile phase of (water: acetonitrile) (20%: 80%) at 1.5 mL/min flow rate and an injection volume of 50 µL. The mobile phase underwent a 5-minute sonication to degas the mixture and then was filtered through a 0.22 µm membrane filter. 25 °C was the temperature specified for the column with a programmed UV detector (210 nm for DAP and 204 nm for TAD, TAM, and SFN). The column was washed and conditioned for at least 30 min before injection.

2.6.1. Application to Pharmaceutical Formulations

For Tadalafil: 20 tablets of Diamonrecta® 5 mg were precisely weighed; the mean weight was recorded and then ground into powder in a mortar. A quantity equal to 5 mg TAD was weighed precisely, transferred to a 100 mL volumetric flask, and methanol was added (50 mL). The drug was then completely extracted using sonication for 15 minutes with intermittent shaking. The solution was adjusted to the mark using methanol after being passed via a 0.45 µm filter to form a stock solution of 50 µg/mL. Then, different aliquots (1, 2, 4, and 5 mL) were exactly put into a set of 10 mL volumetric flasks, and the volume was filled with methanol to the last mark to get a final concentration of (5,10, 20, 25 µg/mL). Afterward, the suggested process was carried out after being injected into the HPLC apparatus for analysis. The associated regression equation was used to calculate the tablet's nominal content.

For Dapoxetine: 3 tablets from Joypox® 60mg were weighed, and the mean weight was recorded. The tablets were ground into powder in mortar. A quantity equal to 60 mg DAP was weighed precisely, transferred to a 100 mL volumetric flask, and methanol was added (50

mL). The drug was then completely extracted using sonication for 10 min with intermittent shaking. The solution was adjusted to the mark using methanol after being passed via a 0.45 µm filter to form a stock solution of concentration 600 µg/mL. Then, prepare a working solution of 100 µg/mL from the stock solution. Different aliquots of (1, 2, 3, and 6 mL) were exactly put into a set of 10 mL volumetric flasks, and the volume was filled with methanol to the last mark to get a final concentration of (10, 20, 30, and 60 µg/mL). Afterward, the suggested process was carried out after being injected into the HPLC apparatus for analysis. The associated regression equation was used to calculate the tablet's nominal content.

For Tadalafil and Dapoxetine: 3 tablets from Tadapox® containing 20/60 mg TAD and DAP, respectively, were weighed and ground. Amounts equivalent to 20 mg TAD and 60 mg DAP were weighed precisely, transferred to a 100 mL volumetric flask, and methanol was added (50 mL). The drugs were then completely extracted using sonication for 10 min with intermittent shaking. The solution was adjusted to the mark using methanol after being passed via a 0.45 µ filter to form a stock solution of 200 µg/mL for TAD and 600 µg/mL for DAP. Then, different aliquots of (0.25, 0.5, and 1 mL from stock) were exactly put into a set of 10 mL volumetric flasks, and the volume was filled with methanol to the last mark to get a final concentration of (5,10 and 20 µg/mL) for TAD,(15,30 and 60 µg/mL)for DAP. Afterward, the suggested process was carried out after being injected into the HPLC apparatus for analysis. The associated regression equation was used to calculate the tablet's nominal content.

For Solifenacin and Tamsulosin: 20 tablets from Tamsulin Plus® containing 6/0.4 mg SFN and TAM, respectively, were accurately weighed and ground. The amount equivalent to 6 mg SFN and 0.4 mg TAM was weighed precisely, transferred to a 100 mL volumetric flask, and methanol was added (50 mL). The drugs were then completely extracted using sonication for 10 min with intermittent shaking. The solution was adjusted to the mark using methanol after being passed via a 0.45 µ filter to form a stock solution of 60 µg/mL for SFN and 4 µg/mL for TAM. Then, different aliquots of (2.5, 5, and 10 mL from stock) were exactly put into a set of 10 mL volumetric flasks, and the volume was completed with methanol to get a final concentration of (15, 30, and 60 µg/mL) for SFN and (1,2 and 4 µg/mL) for TAM. The suggested process was carried out after being injected into the HPLC apparatus for analysis. The associated regression equation was used to calculate the tablet's nominal content.

The validity of the suggested methods was checked using the standard addition technique.

2.6.2. Application of the proposed method in human plasma samples spiked with studied drugs

Frozen human plasma was thawed at room temperature. One milliliter of human plasma was transferred into a set of falcon tubes and spiked with different aliquots of the stock standard solution of each of the four co-administered drugs, vortexed for 50 seconds. The protein precipitation technique was adopted to extract the four drugs from spiked plasma samples. Proteins were precipitated by the addition of 4 mL of acetonitrile. Followed by a 30-minute centrifugation at 6000 rpm to allow separation of the organic phase. The supernatant was then taken cautiously and allowed to evaporate at room temperature. The dried extract was then reconstituted using 1 milliliter of the mobile phase and thoroughly vortexed to obtain final concentrations of (0.1, 0.378, 0.5 µg/mL), (0.245, 0.349, 0.6 µg/mL), (0.2, 0.3, 0.6 µg/mL), and (0.138, 0.2, 0.5 µg/mL) for TAD, DAP, TAM,

and SFN, respectively an aliquot of 50µL of the reconstituted solution was injected for chromatographic analysis. Blank plasma was carried out.

2.7. Validation of the Method

The following parameters-linearity, accuracy, precision, specificity, limit of quantitation (LOQ), limit of detection (LOD), system suitability, and robustness-were used to validate the method: In accordance with ICH guidelines **[66]**.

2.7.1. Linear range

Calibration curves were created in the following manner:

Standard calibration curves: A 10 mL volumetric flask was filled with various portions of the working solution to prepare concentrations in the range of (0.05-100 µg/mL for TAD, 0.1-50 µg/mL for DAP, 0.2-80 µg/mL for TAM, and 0.1-60 µg/mL for SFN). Following the steps outlined in (2.5.1), 50 µL of the produced dilutions were injected three times into HPLC after being filtered using a 0.45 µm syringe filter.

The mean peak areas of three concentration determinations for each were plotted against the corresponding concentration to create calibration curves. For every medication, regression equations were calculated.

2.7.2. Accuracy

The preceding stated process in (2.5.1) was conducted three times with five different concentration settings (3, 10, 15, 25 and 80 µg/mL for TAD), (0.7, 3, 12, 25 and 40 µg/mL for DAP), (0.5, 5, 15, 20 and 70 µg/mL for TAM), and (0.5, 3, 15, 25 and 40 µg/mL for SFN) in pure form. The related regression equations were used to determine their concentrations, and each drug's mean recovery percentage was then computed.

2.7.3. Precision

Three concentration levels were used to repeat the process under (2.5.1) to perform intra- and inter-day precision measurements (3, 15 and 80 µg/mL for TAD), (0.7, 12 and 40 µg/mL for DAP), (0.5, 15 and 70 µg/mL for TAM), and (0.5, 15 and 40 µg/mL for SFN), in their pure form both on the same day and on three successive days. The percentage relative standard deviation was computed after the evaluation was conducted three times for each drug.

2.7.4. Specificity

Chromatograms of the four studied drugs were evaluated to determine whether they were coeluted together or contained any extra additive peaks. So, the marketed preparation was analyzed to find out how specific this optimized approach is when tablet excipients are present.

2.7.5. limit of Quantitation (LOQ) and Limit of Detection (LOD)

For the suggested method, the limits of quantitation (LOQ= 10* standard deviation of intercept/slope) and detection (LOD= 3.3* standard deviation of intercept/slope) were computed.

2.7.6. System Suitability Parameters

For method validation, a number of factors for system suitability assessment were determined, such as capacity factor, tailing factor, selectivity factor, resolution, number of theoretical plates, and height equivalent to theoretical plates.

2.7.7. Robustness

Little, purposeful adjustments to the chromatographic settings were made to evaluate the robustness of the suggested approach. Analysis of (20 µg/mL for TADA, 40 µg/mL for DAP and TAM, and 60 µg/mL for SFN) pure standards was completed with the aforementioned protocol under (2.5.1) with modest adjustments to the organic strength ± 2%,

detection wavelength ± 2 nm, and mobile phase flow rate -0.1 mL/min.

2.8. Greenness assessment:

Global sustainability is becoming more popular these days. Encouraging safer and more environmentally friendly analytical processes is the aim of green analytical chemistry. Different approaches were employed to evaluate the proposed method's environmental sustainability.

2.8.1. The Eco-Scale Method

A useful, semi-quantitative instrument for evaluating the greenness of an analytical procedure is the Analytical Eco-Scale. This metric is based on total points, which may indicate how eco-friendly the analytical process is. A penalty point reduces the overall score by emphasizing the negative effects of additives and excipients, such as hazardous solvents used in a procedure, as well as the effects on the environment and the amount of energy used. For each of the four key elements of the analytical process quantity, risk, energy consumption, and waste generation of the chemicals-penalties points are given **[67]**. The score starts at 100 points, which represents the highest eco-friendly degree with no penalties. If the end score is more than 75 points, the strategy is considered green; nevertheless, if the result is between 50 and 75 points, the approach is greenly acceptable. A score less than 50 suggests that the analytical method is not green enough. The non-hazardous category has zero penalty points **[68]**.

2.8.2. AGREE Software

A simple, adaptable, and free program called Analytical GREEnness yields results that are comprehensible and instructive. The criteria of this assessment are taken from the 12 green analytical chemistry tenets. It has a scale from 0 to 1. The straightforward red-yellow-green color scale represents each principal's technique performance, and the breadth of the segment that corresponds to each principle represents its weight.

When evaluating how environmentally friendly an analytical technique is, a variety of aspects are taken into account, including the amount and toxicity of reagents, waste output, energy requirements, the number of steps in the process, miniaturization, and automation **[69]**.

3. Results and Discussion

3.1. The chromatographic method's progress and enhancement for analysis of the studied drugs

We have devised a sensitive, accurate, and validated HPLC method in our study to determine the studied drugs in pure, dosage form, and human plasma. This is the first experiment for the determination of these four medications together utilizing a HILIC column, with the advantage of separating the four studied drugs with high resolution in a relatively short analysis time and achieving quantitative estimation of the studied drugs at low concentrations.

The following was how the chromatographic conditions had been optimized:

- In our investigation, we used two different columns: HILIC and C18. With C18, there was a weak resolution between the peaks, and the peak of SFN was forked. Otherwise, approximately 20 minutes of run time with a symmetric peak shape and sufficient resolution were appropriate for the separation of the four medicines using HILIC. HILIC is commonly used to separate polar to highly polar compounds that would be weakly retained in a traditional reversed-phase column [70]. Where SFN, TAM, and DAP are polar compounds, while TAD is the least polar one, so it is the first one eluted from the HILIC column.
- A variety of mobile phases, including

acetonitrile, methanol, water, and ammonium acetate buffer in various ratios, were investigated. The optimal mobile phase for the devised approach was determined to be acetonitrile: water (80:20 v/v) with a good baseline separation, the best resolution, and a symmetric peak shape.

Mobile phase	Comment
50 ACN:50Water	The drugs DAP, TAM, and SFN were retained in the column
60 ACN:40 Water	TAM and DAP peaks were overlapped
70ACN:30 Water	Weak separation between DAP and TAM
90 ACN:10 Water	Peaks were not symmetric, and their retention time was delayed.
95 ACN: 5 Water	Peaks were more retained, the run time was more than 35 min, and the first peak started at 4.6mins
80 ACN:20 Buffer pH 7	Peaks forked, Asymmetric
80 ACN:20 Buffer pH 4	Weak resolution, broad peak, and long run time
70 ACN:25 Water:5 Methanol	The drugs TAM and SFN were retained on column resulting in a long run time.
80ACN:20Water	symmetric peak shape, the best resolution between peaks in a suitable run time was achieved.

- The mobile phase's flow rate influences

Various flow rates were put to the test. The optimal flow rate for excellent elution of all the peaks in a brief analytical period with retention durations was 1.5 mL/min. With retention times of 1.683, 5.570, 9.191, and 16.545 min for TAD, DAP, TAM, and SFN, respectively in pure form, as shown in **Fig. 2**.

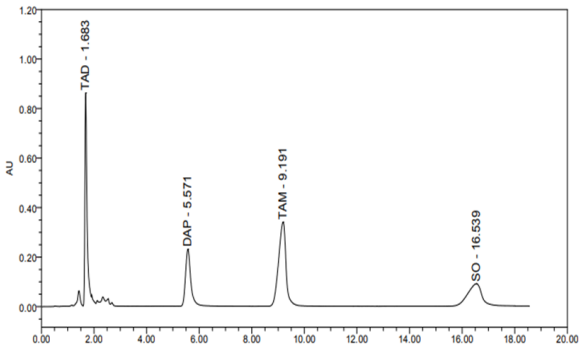

Fig. 2. HPLC chromatogram of the four pure drugs using our proposed method at a concentration of 20 µg/mL for TAD,40 µg/mL for DAP and TAM, and 60 µg/mL for SFN

- With the devised approach, the best option for improving the sensitivity of the medications under study was a 50 µL injection volume.

3.2. Method validation

The procedure was verified in compliance with ICH criteria **[66]**.

3.2.1. Linearity

The average peak area of the three measurements and the matching concentrations of the medicines in pure form were linearly correlated, according to the calibration curves **(Figs. 3-6)**. For each of the four medications, good linearity was found by computing regression equations and calculating correlation coefficients, as shown in **(Table 1)**.

3.2.2. Accuracy

In accordance with ICH criteria, three assessments of five concentration levels of TAD, DAP; TAM, and SFN in acetonitrile were used to assess the accuracy of the proposed approach. As can be seen in **(Table 1)**, the mean recovery percentages fell between 98.78% and 100.65%, demonstrating the accuracy of our approach.

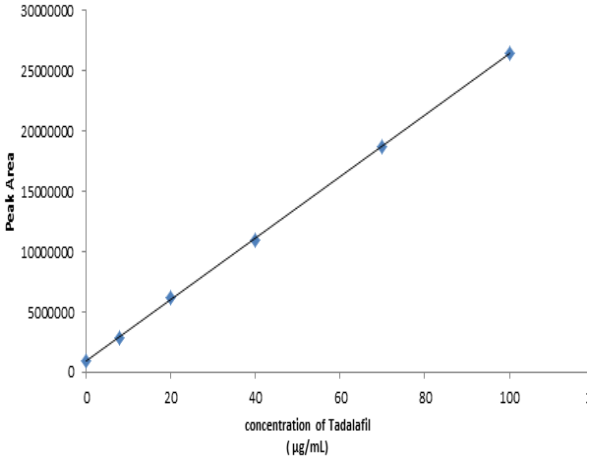

Fig. 3. Linearity of the peak areas versus the corresponding concentrations of TAD (0.05-100 µg/mL), at 204 nm using the proposed HPLC method

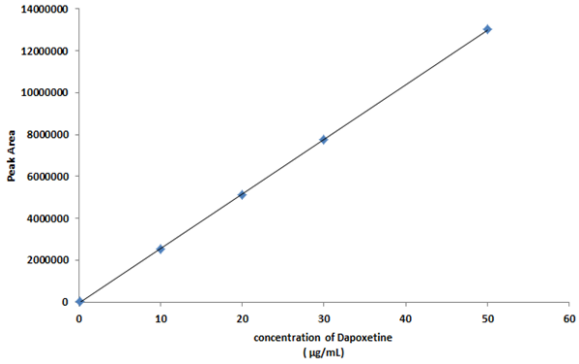

Fig. 4. Linearity of the peak areas versus the corresponding concentrations of DAP (0.10-50 µg/mL), at 210 nm using the proposed HPLC method

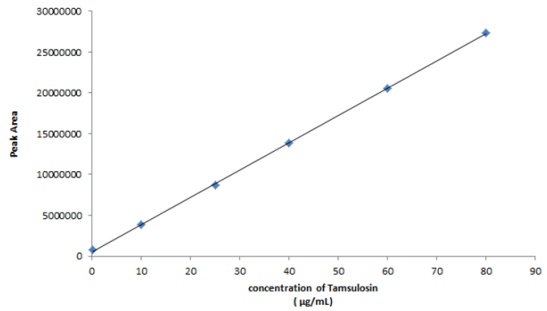

Fig. 5. Linearity of the peak areas versus the corresponding concentrations of TAM (0.20-80 µg/mL), at 204 nm using the proposed HPLC method

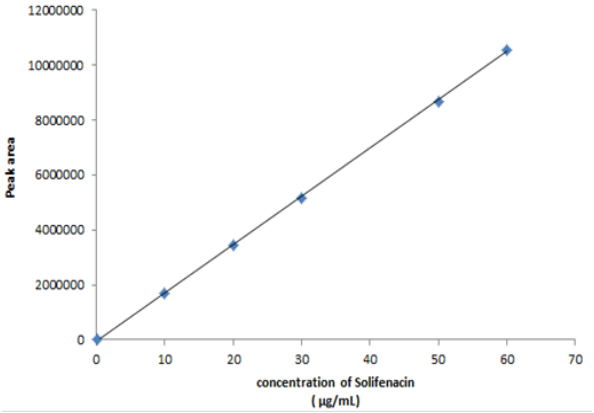

Fig. 6. Linearity of the peak areas versus the corresponding concentrations of SFN (0.10-60 µg/mL), at 204 nm using the proposed HPLC method

3.2.3. Precision

For three concentration levels of TAD, DAP, TMA, and SFN in acetonitrile on the same day and three days in a row, the percentage RSD was computed based on ICH guidelines. **Table 1** displays the numbers, which demonstrate the good precision of our method. The values ranged from 0.10% to 0.45%.

3.2.4. Specificity

The suggested approach is specific to the medications being measured. Similar to when additional inactive excipients are present in tablets, full separations of TAD, DAP, TAM, and SFN were observed. Furthermore, regarding the placebo solution's chromatogram, no interference was seen during the retention period.

3.2.5. limit of Quantitation (LOQ) and Limit of Detection (LOD)

For the four medications in pure form, the ranges for the limits of detection (LOD) and quantification (LOQ) were as follows: (0.01-0.05 µg/mL) and (0.03–0.15 µg/mL), respectively **(Table 1)**.

Table 1. Characteristic parameters and validation results for the determination of the studied drugs by the proposed method

Name of compound	Linear range	Regression equation	Correlation coefficient (r)	Accuracy*	Intraday precision** (RSD %)	Interday precision** (RSD %)	LOD	LOQ
TAD	0.05-100 µg/mL	y = 255789x + 859535	0.99995	99.73 ±0.75	0.19	0.44	0.01 µg/mL	0.03 µg/mL
DAP	0.1-50 µg/mL	y = 260963x – 62149	1.00	100.52 ± 0.55	0.28	0.45	0.02 µg/mL	0.06 µg/mL
TAM	0.2-80 µg/mL	y = 334697x + 522761	0.99995	99.71 ± 0.42	0.20	0.36	0.05 µg/mL	0.15 µg/mL
SFN	0.1-60 µg/mL	y =175843 x – 61108	0.9999	98.65 ± 0.39	0.10	0.14	0.02 µg/mL	0.07 µg/mL

*Mean of five concentrations (3, 10, 15, 25 and 80 µg/mL) for pure standard solutions of TAD, (0.7, 3, 12, 25 and 40 µg/mL) for pure standard solutions of DAP, (0.5, 5, 15, 20 and 70 µg/mL) for pure standard solutions of TAM and (0.5, 3, 15, 25 and 40 µg/mL) for pure standard solutions of SFN, each repeated three times.

**Mean of three concentrations (3, 15 and 80 µg/mL) for pure standard solutions of TAD, (0.7, 12 and 40 µg/mL) for pure standard solutions of DAP, (0.5, 15 and 70 µg/mL) for pure standard solutions of TAM and (0.5, 15 and 40 µg/mL) for pure standard solutions of SFN, each repeated three times (n= 9).

3.2.6. System suitability parameters

For the four medications in pure form, system suitability parameters were calculated for the suggested approach according to ICH **[71]**. The systems were found to be performing well and suitable for the four drugs in pure solvents as shown in **(Table 2)**.

3.2.7. Robustness

Applying tiny intentional adjustments to the chromatographic settings, such as adjusting the wavelength (±2nm), organic strength percentage (±2%), or mobile phase flow rate (-0.1 mL/min), did not show any discernible differences. **Table 3** displays the percentage relative standard deviation (%RSD) that was computed.

3.3.1. Application to Dosage Form for Pharmaceuticals and Standard Addition Technique

The suggested technique was effectively used for estimating TAD and DAP in their dose forms, in their combined dosage form, and for TAM, and SFN in their combined dosage form. The results indicated that the ranges for the recovery percentage and standard deviation are reasonable. The standard addition technique was applied to check the validity of the proposed method, by

adding different known concentrations of the pure drugs to a known concentration of each drug product. The concentrations were calculated as shown in **(Tables 4, 5, 6)**. Using the corresponding regression equations, it is evident that the approach can identify the medications under study without any interference from excipients.

Table 2. System suitability parameters for the analysis of TAD, DAP, TAM, and SFN in acetonitrile using the proposed HPLC method

Parameter	TAD	DAP	TAM	SFN	Reference value [69]
Capacity factor (K□)	2.5	4.570	8.190	15.544	**K□>2**
Tailing factor (T)	2.0	1.36	0.93	0.88	**T ≤ 2** **T =1 for the typical symmetric peak**
Selectivity factor (α)	----------	6.689	1.792	1.897	**1-10 is acceptable**
Resolution (Rs)	---------	16.55	8.66	10.12	**Rs ≥ 1.5**
Number of theoretical plates (N)	2357.587	4265.329	5364.188	4740.546	**>2000** **Increase with increasing the efficiency of the separation**
Height equivalent to theoretical plate (HETP)	0.10	0.058	0.04	0.052	**The smaller the value, the higher the column efficiency**

Table 3. Results of robustness testing of the proposed HPLC method

Parameter	TAD	DAP	TAM	SFN
Flow rate (-0.1mL/min) %RSD	**1.15**	**0.17**	**0.94**	**0.68**
Wavelength (+2nm) %RSD	**1.86**	**0.86**	**0.10**	**1.32**
Wavelength (-2nm) %RSD	**1.23**	**0.47**	**0.28**	**1.10**
Organic strength (+2%) %RSD	**0.63**	**0.035**	**0.16**	**0.32**
Organic strength (-2%)	**0.21**	**0.63**	**0.20**	**0.66**

Table 4. Determination of TAD and DAP each in their own formulated dosage form and application of standard addition technique using the proposed HPLC method

Dosage forms	pharmaceutical formulation			Standard addition			
	Taken (µg/mL)	Found (µg/mL)	% Recovery*	Taken (µg/mL)	Pure Taken (µg/mL)	Pure Found (µg/mL)	% Recovery*
TAD **Diamonrecta®5mg**	5	4.97	99.40	5	2.5	2.46	98.40
	10	9.83	98.30	5	5	4.95	99.00
	20	19.87	99.35	5	10	9.95	99.50
	25	25.07	100.28	-	-	-	-
Mean ± SD			99.33±0.81		**Mean ± SD**		98.97±0.55
DAP **Joypox® 60mg**	10	10.02	100.20	20	10	9.95	99.50
	20	20.02	100.10	20	20	19.91	99.55
	30	30.03	100.10	20	30	29.77	99.23
	60	59.91	99.85	-	-	-	-
Mean ± SD			100.06±0.15		**Mean ± SD**		99.43±0.17

*Mean of three determinations

Table 5. Determination of TAD and DAP in their combined formulated dosage form and application of standard addition technique using the proposed HPLC method.

Dosage forms	Commercial formulation			Standard addition			
	Taken (µg/mL)	Found (µg/mL)	% Recovery*	Taken (µg/mL)	Pure Taken (µg/mL)	Pure Found (µg/mL)	% Recovery*
TAD Tadapox® 20/60mg	5	4.93	98.60	5	2.5	2.50	100.00
	10	9.87	98.70	5	5	4.99	99.80
	20	19.91	99.55	5	10	9.99	99.90
Mean ± SD			98.95±0.52		**Mean ± SD**		99.90±0.10
DAP Tadapox® 20/60mg	15	14.88	99.20	15	10	10.07	100.70
	30	29.65	98.83	15	15	15.12	100.80
	60	59.95	99.92	15	30	30.10	100.33
Mean ± SD			99.32±0.55		**Mean ± SD**		100.61±0.25

*Mean of three determinations

Table 6. Determination of SFN and TAM in their combined formulated dosage form and application of standard addition technique using the proposed HPLC method

Dosage forms	Commercial formulation			Standard addition			
	Taken (µg/mL)	Found (µg/mL)	% Recovery*	Taken (µg/mL)	Pure Taken (µg/mL)	Pure Found (µg/mL)	% Recovery*
SFN Tamsulin plus® 6/0.4mg	15	15.00	100.00	15	5	5.00	100.00
	30	29.98	99.93	15	15	15.05	100.33
	60	59.96	99.93	15	20	20.15	100.75
Mean ± SD			99.95±0.04		**Mean ± SD**		100.36±0.38
TAM Tamsulin plus® 6/0.4mg	1	1.00	100.00	1	0.5	0.49	98.00
	2	1.99	99.50	1	1	0.98	98.00
	4	3.98	99.50	1	2	1.99	99.25
Mean ± SD			99.67±0.29		**Mean ± SD**		98.50±0.87

*Mean of three determinations

3.3.2. Application to human plasma samples spiked with studied drugs

The technique was effectively used to determine the amounts of the four co-administered medications under study in human plasma samples spiked with drugs without the influence of other endogenous substances. The %recoveries are shown in **(Table 7)**.

- Different methods of extraction were tested in our study, such as the Protein precipitation technique using acetonitrile as a precipitating agent for proteins, and the liquid-liquid extraction technique using 1 mL of 0.1 N NaOH, or 4 mL of organic solvents (a mixture of diethyl ether, ethyl acetate, hexane in equal volume and different ratios). It was found that using the protein precipitation technique is easier, greener, of better extraction efficiency, and has higher recovery % than the liquid-liquid extraction technique.

3.4. Assessment of Greenness

3.4.1. The Analytical Eco-Scale

One technique for evaluating greenness is the analytical eco-scale. It takes into account a variety of factors and procedures during the complete analytical procedure. Different reagent concentrations will result in different penalty points for the reagents. The classification and labeling of chemicals under the Globally Harmonized System (GHS) is the foundation for the penalty points for risks. Each chemical reagent has one or more of nine pictograms that graphically represent its dangerous characteristics. "Danger" and "warning" are the two signal words used by the GHS to indicate different types of risks. A higher risk is denoted by a two-point penalty, while a lower risk is indicated by a single point. Extra points are given based on the type and quantity of waste produced, the energy used, and the instruments utilized. Our method scores a total of 88 points, indicating that the method is of excellent greenness, as shown in **(Table 8)**.

Table 7. Determination of TAD, DAP, TAM, and SFN in human plasma using the proposed method

TAD			DAP			TAM			SFN		
Taken (µg/mL)	**Found (µg/mL)**	**% Recovery***	**Taken (µg/mL)**	**Found (µg/mL)**	**% Recovery***	**Taken (µg/mL)**	**Found (µg/mL)**	**% Recovery***	**Taken (µg/mL)**	**Found (µg/mL)**	**% Recovery***
0.1	0.10	100.00	0.245	0.26	106.12	0.2	0.19	95.00	0.138	0.12	86.96
0.378	0.37	97.88	0.349	0.37	106.02	0.3	0.29	96.67	0.2	0.17	85.00
0.5	0.49	98.00	0.6	0.62	103.33	0.6	0.57	95.00	0.5	0.45	90.00
Mean±SD		98.63± 1.19	**Mean±SD**		105.16± 1.58	**Mean±SD**		95.56± 0.96	**Mean±SD**		87.32± 2.52

*Mean of three determinations

Table 8: Analytical Eco-Scale penalty points for the proposed HPLC method

Hazard	Penalty points
Reagents	
Acetonitrile	6
Water	0
Instruments	
Energy (≤1.5 KW h per sample)	1
Occupational hazard	0
Waste	5
Total penalty points	12
Analytical Eco Scale total score	88

Analytical Eco-Scale total score 100- total penalty points.

3.4.2. AGREE-Analytical GREEnness Metric Approach and Software

SIGNIFICANCE, the twelve principles of green analytical chemistry, is used to transform the evaluation criteria into a single 0–1 scale. The principles of SIGNIFICANCE are used to determine the final score. When the total score, which is shown in the center of the pictogram, has values close to 1, the evaluated method is considered greener. As well as, how effectively the method performed in regard to each assessment criterion number is shown by the segment's color **[69]**. Our approach score is 0.66. **(Fig. 7)** displays the results.

3.5. Statistical analysis

The variance ratio F test and student's t-test were utilized to assess the viability of the suggested approach. **Table 9** shows the results of the statistical comparison between the proposed method and the reported method **[21, 56, 72, 73]** for TAD, DAP, TAM, and SFN. The calculated t and F values were less than the theoretical ones at p= 0.05 proving that the reported and suggested approaches are not significantly different from one another.

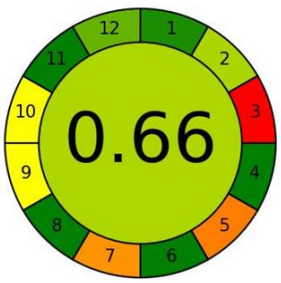

Fig. 7. Results of AGREE assessment for our proposed method

Table 9. Statistical comparison between the proposed HPLC method and the reported reference method for the determination of the four studied drugs in pure solvent

Parameter	TAD		DAP		TAM		SFN	
	Reported method** [21]	proposed method	Reported method** [72]	proposed method	Reported method** [56]	proposed method	Reported method** [73]	proposed method
Mean	99.74	99.73	100.46	100.52	99.82	99.71	98.52	98.65
SD	0.36	0.757	0.61	0.55	0.63	0.42	0.36	0.39
Variance	0.133	0.574	0.373	0.299	0.401	0.178	0.131	0.159
N	5	5	5	5	5	5	4	5
Student t-test *	'	0.0213 (2.306)	'	0.1635 (2.306)	'	0.323 (2.306)	'	0.497 (2.3646)
F *	'	4.316 (6.3882)	'	1.247 (6.3882)	'	2.252 (6.3882)	'	1.2137 (9.117)

*The theoretical values of student t-test and F at p= 0.05; ** Reference method is UPLC method for simultaneous estimation of TAD and its impurities in tablets using Acquity HSS T3 column (1.8 µm, 2.1 mm × 150 mm) using gradient conditions with methanol and ammonium acetate buffer (0.02 M; pH 4.0 adjusted with acetic acid) at a flow rate of 0.35 mL/min. UV detection was performed at 262 nm **[21]**; Liquid chromatography method with fluorescence detection for quantification of Tadalafil and Dapoxetine using C18 column and acetonitrile-0.15% triethylamine (40:60, v/v, pH 4) as mobile phase. The flow rate is 1 mL/min. Detection was time programmed at 330, and 410 nm for TAD, and DAP respectively, after excitation at 236 nm. **[72]**; spectrofluorimetric methods based on derivative and derivative ratio techniques. For the determination of tamsulosin hydrochloride (TAM) as a minor component with tolterodine tartrate (TOL) or solifenacin succinate (SFN) **[56]**; stability-indicating HPTLC method for quantitative analysis of solifenacin succinate both as the bulk drug and in formulations was performed on aluminum plates precoated with silica gel and the mobile phase n-butanol: acetic acid: water (4:1:1, v/v/v). Densitometric analysis of solifenacin succinate was performed in absorbance mode at 205 nm. **[73]**.

Conclusion

The HILIC-PDA approach was developed as a sensitive, quick, and precise way for the simultaneous analysis of four co-administered drugs in their pure form and finished pharmaceutical products with acceptable results, allowing their use for routine analysis in quality control labs, whether in bulk powder or commercial samples, without any interference from excipients. The proposed method is non-tedious, does not require pre-treatment, and is appropriate for a short analysis time (20 min) for daily drug monitoring. This study is the first to use an HPLC approach to simultaneously determine these four co-administered drugs in BPH cases. It represents the base for further clinical application to BPH patients, by knowing how to simultaneously analyze these potentially co-administered drugs. It is the first time to study the separation of these drugs using the HILIC technique. Our simple solvent system allows the separation of the drugs using HILIC in a short analysis time which makes it suitable for routine checking. Since the suggested method was also effective in determining the drugs under study in human plasma spiked with drugs without the interference of endogenous compounds, it could be used to determine TAD, DAP, TAM, and SFN simultaneously in both dosage forms for pharmaceuticals and spiked human plasma samples.

Declarations

Ethics Approval and Consent to Participate

Not Applicable.

Consent to Publish

Not applicable.

Availability of Data and Materials

This published article contains all the data produced or examined during this study.

Competing Interests

The authors declare that no competing interests exist.

Funding statement

The researchers didn't acquire any funding for the work they submitted.

Acknowledgments

The authors would like to acknowledge all colleagues in the analytical chemistry department, and faculty of pharmacy at Ain Shams University for their support

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