

Original Research Article

Spectrodensitometric and ultra-performance liquid chromatographic quantification of dapagliflozin and saxagliptin in their dosage form and human plasma

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Abstract

Purpose: To simultaneously quantify dapagliflozin (DAPA) and saxagliptin (SAX) in a pharmaceutical product and human plasma.

Methods: Separation and quantification of DAPA and SAX were performed on pre-coated TLC plates in TLC-densitometric method using a solvent system of chloroform, ethyl acetate and methanol at a volume ratio of 8:1:1 as the mobile phase. The developed spots were scanned at 225 and 210 nm in absorbance mode. Moreover, the studied drugs were concurrently determined in human plasma using ultra-performance liquid chromatography (UPLC). The separation process was carried out in Waters™ Acquity C18 BEH column using a solvent system of 0.02 M KH₂PO₄ buffer, pH 4; MeOH and acetonitrile (2:1:1, v:v:v) isocratically at a flow speed of 0.5 mL/min. The absorbance of each eluent was read at 220 nm.

Results: Concurrent evaluation of DAPA and SAX was carried without separation using TLC-densitometric method, and it was successful in determination of DAPA and SAX in concentration ranges of 10 – 70 µg/band and 5 – 60 µg/band, respectively. In addition, retardation factor (R_f) values for SAX and DAPA were 0.17 and 0.31, respectively. Furthermore, the studied drugs were concurrently determined in human plasma using UPLC, which was sensitive enough to quantify DAPA and SAX in concentration ranges of 100 – 1000 and 20 – 200 ng/mL, respectively.

Conclusion: These methods can be utilized for sensitive monitoring of DAPA and SAX in pharmacokinetic and bioequivalence studies.

Keywords: TLC-densitometry, UPLC, Dapagliflozin, Saxagliptin, Simultaneous assay

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INTRODUCTION

Dapagliflozin (DAPA) (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol, is used for controlling blood glucose levels in patients having type 2 diabetes. It

enhances the elimination of blood glucose through the urine by inhibiting the protein involved in transport mechanism of sodium-glucose co-transporter (SGLT2) [1]. Saxagliptin (SAX), or (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0]

hexane-3-carbonitrile) is an oral hypoglycemic agent which inhibits dipeptidyl peptidase 4 (DPP-4), thereby increasing insulin secretion while decreasing glucagon release from pancreatic islet cells [2]. In recent times, two or more anti-diabetic drugs are frequently prescribed for type 2 diabetes patients, and these multi-targeted therapies are more effective in hyperglycemia control than monotherapy due to their synergistic effect on management of the disease [3].

Qtern® tablet (AstraZeneca Pharmaceuticals LP, Wilmington, UK) is a pharmaceutical co-formulation product of DAPA and SAX. It is generally prescribed for control of hyperglycemia in patients where metformin, sulphonylurea and/or DAPA or SAX fail to provide sufficient control of blood sugar [4]. Several spectrophotometric [5-7] and HPLC methods [8-12] have been reported for concurrent estimation of DAPA and SAX in their co-formulation products. However, there is only one reported HPTLC method that deals with the stability study of co-formulated DAPA and SAX [13]. This method was developed for an in-house co-formulation tablet of DAPA and SAX, rather than for the marketed product. A validated LC-MS/MS method has been reported for parallel investigation of DAPA or SAX with metformin in human plasma [14].

Human plasma contents of DAPA and SAX have also been simultaneously determined using HPLC, but the key drawback of this reported method is that its linearity range does not cover the maximum plasma concentration of either DAPA or SAX [15]. In addition, another HPLC method for quantitation of DAPA and SAX uses protein precipitation technique for the preparation of samples and drug extraction from human plasma [16]. Being a non-specific technique, this procedure results in loss of analytes due to co-precipitation of matrix proteins. Furthermore, the purification method is weak, causing low detection of analytes, poor sensitivity, and low reliability of the method [17].

In this study, TLC-densitometry was used to quantify DAPA and SAX simultaneously in marketed pharmaceutical products. This developed and validated procedure can be used in routine quality control since it is simple, economical, and accurate. To the best of our knowledge, no reports are available in the literature on any procedure for quantifying the two studied drugs using ultra-performance liquid chromatography (UPLC) coupled with UV-detector. Therefore, a simple, delicate, precise, and reproducible bioanalytical UPLC technique for the quantitation of DAPA and SAX in plasma

was established in the present study. The TLC-densitometric and UPLC methods were validated as per ICH-Q2B and US-FDA guidelines, respectively.

EXPERIMENTAL

Chemicals and materials

Deionized water from Milli-Q water purification unit was utilized for the preparation of samples, as required; DAPA in form of propanediol monohydrate bulk powder (purity 99.73%; CAS # 461432-26-8) was purchased from BioVision (USA), while SAX in form of hydrochloride salt bulk powder (purity 99.54%; CAS # 709031-78-7) was bought from Cayman Chemical Company (USA). Chloroform (CHCl₃), CH₃COOC₂H₅, CH₃OH, acetonitrile and *o*-phosphoric acid were of HPLC quality, and were purchased from Sigma-Aldrich.

Instrumentation

Thin layer chromatography (TLC) scanner (Model: 3 S/N 130319) run through *visionCATS* program, Linomat 5 autosampler and microsyringe from CAMAG® (Muttenz, Switzerland) were utilized for TLC-densitometric study. An ACQUITY UPLC setup from Waters Corporation (Elstree, UK), furnished with 500 nL flow cell and a rheodyne injector, as well as ACQUITY UPLC BEH C18 Column (1.7 μm, 2.1 mm × 100 mm) and UV-visible wavelength detector (Waters, 2489) were used in this study.

Human plasma samples

Human plasma samples were kindly supplied by King Khalid Hospital, Al-Kharj, KSA.

Pharmaceutical product

The pharmaceutical formulation was Qtern® tablets (NDC 0310-6770-30; AstraZeneca Pharmaceuticals LP, Wilmington, UK) containing 10 mg DAPA (12.3 mg dapagliflozin propanediol) and 5 mg SAX (5.95 mg saxagliptin HCL) per tablet.

Preparation of stock and working standard solutions

TLC-densitometric method

Standard solutions of DAPA and SAX (1 mg/mL) were made by precisely weighing 25 mg of each drug, dissolving in 5 mL of distilled water and vortexing separately, prior to making up the volume to 25 mL exactly using methanol.

UPLC method

Standard stock solutions of DAPA and SAX (100 µg/mL) were made by precisely weighing 10 mg respectively, dissolving in 5 mL of distilled water and vortex-mixing separately, and bringing up the final volume to exactly 100 mL using distilled water. A 1:10 dilution of the stock solution of DAPA in distilled water comprised the working standard. The SAX working standard solution (1 µg/mL) was made by mixing 1 mL of SAX stock solution with distilled water up to 100 mL in a volumetric flask. Calibration standard and quality control solutions were made in a biological matrix similar to that used for the samples to be analyzed. The calibration curves consisted of a blank sample (biological matrix sample only) and eight non-zero samples having the serial concentrations to be quantified. Plasma calibration standard solutions were made by spiking control human plasma with standard solutions of DAPA and SAX. Eight plasma calibration samples were made for DAPA (100, 200, 300, 400, 500, 600, 800 and 1000 ng/mL), and also for SAX (20, 40, 60, 80, 100, 140, 180 and 200 ng/mL). Samples for low quality control (LQC) of DAPA (200 ng/mL) and SAX (40 ng/mL), medium quality control (MQC) of DAPA (500 ng/mL) and SAX (100 ng/mL), and high-quality control (HQC) of DAPA (800 ng/mL) and SAX (180 ng/mL), were prepared. All calibration standard solutions were vortex-mixed for 5 min to ensure proper mixing.

Extraction procedure of plasma samples for UPLC

On the day of assay, samples to be extracted were taken out in their frozen forms and allowed to thaw for one hour at room temperature. To all tubes, HCl (40 µL, 1 M) was added and vortexed. Solid phase extraction cartridges (SPE) were positioned on the extraction manifold, and each cartridge was conditioned with 2 mL of methanol, equilibrated with 2 mL of 20 mM HCl, and washed with 2 mL of water. Plasma containing DAPA and SAX were added to the cartridges, and treated with 2 mL of 20 mM HCl, followed by 4 mL methanol. The eluents collected were evaporated to dryness at 50°C under nitrogen, mixed with the mobile phase (250 µL) and loaded in auto sampler.

Method validation

The TLC densitometric and UPLC methods were validated as per ICH-Q2B [18] and US-FDA [19] guidelines, respectively.

TLC densitometric method**Linearity**

Standard solutions of DAPA and SAX (1 mg/mL) were spotted as bands, with 10 mm spacing, on precoated TLC plates (silica gel 60, F254; depth: 0.25 mm; dimension: 20 cm × 20 cm), using an autosampler and micro-syringe to achieve 10 - 70 µg/band (DAPA) and 5 - 60 µg/band (SAX), respectively. The chromatographic tank was saturated for 1 h with the mobile phase (chloroform:ethylacetate:methanol; 8:1:1), and air-dried TLC plates were developed by ascending chromatography at room temperature over a distance of 15 cm. The air-dried, developed plates were then scanned at 225 and 210 nm at a scanning speed of 20 mm/sec and slit dimension of 4.00 × 0.45 mm. A calibration curve was plotted between the areas under peaks (AUPs) and drug concentrations. The respective regression equations were also calculated for DAPA and SAX.

Determination of accuracy and precision

These were assessed by evaluating three different concentrations of DAPA (10, 30 and 50 µg/band, and SAX (5, 20 and 50 µg/band) on a day (intra-day), and during 3 successive days (inter-day) as indices of quality control of samples.

Sensitivity

This was assessed using LOD and LOQ. The latter refers to the least amount of the drug that can be quantified from the background, while LOQ is the least amount of the drug that can be perfectly and precisely quantified (ICH 2005). Two of them were determined using Equations 1 and 2, as shown below.

$$\text{Limit of detection} = 3.3\sigma/s \dots\dots\dots (1)$$

$$\text{Limit of quantitation} = 10\sigma/s \dots\dots\dots (2)$$

(σ represents lowest SD, and S represents gradient of calibration curve).

Selectivity

It was determined by comparing results obtained for the mixture of DAPA, SAX and excipients with that for blank (excipients only in methanol) [18]. The selected excipients and drug-to-excipient ratio were similar as indicated in the monograph of Qtern® tablets. Selectivity was validated by analyzing in-house prepared

mixtures comprising different concentrations of the studied drugs.

Robustness

Robustness was validated by assessing the impact of little alterations in the assay settings, and it was evaluated by carrying out deliberate changes in the composition of the developing system.

UPLC method

Linearity

A total of nine samples (one blank and eight non-zero samples) of DAPA and SAX, ranging in concentration from 100 to 1000 ng/mL and 20 to 200 ng/mL, respectively were used. The sample solutions included samples for low quality control (LQC), medium quality control (MQC), high quality control (HQC), lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ). The separation of the analytes in samples was achieved using ACQUITY UPLC BEH C18 Column (1.7 μ m, 2.1 mm \times 100 mm) as stationary phase. The solvent system used consisted of a combination of potassium dihydrogen phosphate (0.02 M; adjusted to pH 4), methanol and acetonitrile at a volume ratio of 2:1:1. It was pumped at a speed of 0.5 mL per min in isocratic mode, with UV-Vis detector used at a wavelength of 220 nm. The linearity was measured using the peak area ratio of the analytes by plotting a graph of MQC (external standard method) against standard concentrations. The correlation was validated by calculating the % changes in back-calculated concentrations relative to the nominal concentrations (distribution of the residuals). Except for LLOQ, when the residuals were within 15% of calibration levels, the approval standard of the calibration model was applied. The calibration curve was analyzed using slope, intercept and correlation coefficient.

Accuracy and precision

These were determined for LQC, MQC, HQC, and LLOQ in 6 replicates for DAPA and SAX for intra-day, along with 3 successive days for inter-day, by means of quality control samples.

Sensitivity

Sensitivity is the least amount of the analyte that can be estimated with reasonable accuracy and precision. It was validated using 6 replicates of LLOQ so as to define the values of LOD and LOQ.

Specificity

Six plasma samples from different donors were processed as indicated under *Sample preparation* without the addition of the studied drugs. The final extract of each sample was spiked with standard solutions of DAPA and SAX at concentrations of 200 and 40 ng/mL, respectively. Standard solutions of the studied drugs at similar concentrations in mobile phase were prepared with the same approach. Peak area for analyte for each prepared sample was then measured, and matrix effect was calculated.

Recovery

Recovery of drugs after the extraction process was done for LQC, MQC, and HQC samples by measuring and matching the peak areas of extracted (spiked before extraction) and unextracted (spiked after extraction) samples.

Stability

The stability study of drugs in plasma was executed by keeping replicates for 24 h at room temperature. It was also carried out for 3 freeze-thaw phases by defrosting for 2 - 3 h at room temperature, and then re-freezing for 12 - 24 h. Long term stability study of drugs in plasma was also executed after storing at -28°C for 30 days. The amount of drug in plasma after each storage was compared with the original drug concentration. Drugs in plasma were labelled stable if the concentration was within the acceptable limits of accuracy and precision.

Application of the TLC densitometric method for analysis of Qtern® tablets

Ten Qtern® tablets were weighed precisely and the mean weight was noted. The tablets were crushed and mixed well to obtain a fine powder. Then, powder samples corresponding to 100 mg of DAPA and 50 mg of SAX were taken in a 250 mL beaker. Methanol (20 mL) and distilled water (20 mL) were added. The content was stirred for 20 min, filtered into a volumetric flask (100 mL), and the residue was washed with methanol (2 \times 10 mL). The volume of the flask content was adjusted to 100 mL by adding methanol, with extensive stirring. The amount of each drug was determined using the TLC-densitometric method. The concentrations of DAPA and SAX were determined from their respective regression equations. Furthermore, standard additions were made via addition of various amounts of bulk DAPA and SAX in the formulation, and then analyzing using the proposed analytical method.

RESULTS

Method validation

TLC densitometric method

The ICH-Q2B guidelines were adopted for the validation of TLC densitometric method. The separation pattern is shown in Figure 1.

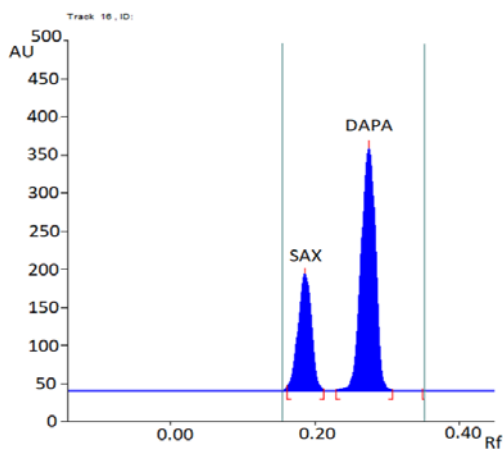


Figure 1: TLC-densitometric separation patterns of DAPA and SAX

Linearity

Linearity was evaluated by plotting peak area against drug concentrations of 10-70 µg/band for DAPA, and 5 - 60 µg/band for SAX. The respective regression equations are presented in Equations 3 and 4.

$$PADAPA = 1.762C + 198.48 \quad (r^2 = 0.9996) \quad \dots (3)$$

$$PASAX = 4.789C + 356.45 \quad (r^2 = 0.9997) \quad \dots (4)$$

where PA represents area under peak, and r^2 represents correlation coefficient.

Accuracy

Accuracy was analyzed with quality control samples consisting of 3 concentrations each of DAPA and SAX within the stated linearity range. The accuracy of the developed procedure was confirmed by determining recovery and % RSD which were found to be satisfactory (Table 1).

Precision

Three concentrations each of DAPA and SAX were evaluated using the proposed TLC-densitometric method for the duration of one day (intra-day) and 3 consecutive days (inter-day). The % RSD was below 2 for both, confirming

good precision of the developed procedure (Table 1).

Table 1: Indices for validation of DAPA and SAX estimation using the proposed TLC-densitometric procedure

Index	DAPA	SAX
Linearity		
Span	10 – 70 µg/band	5 – 60 µg/band
Gradient	1.762	4.789
Intercept	198.48	356.45
r^2	0.9996	0.9997
Accuracy		
Mean \pm SD* (n=9)	101.96 \pm 0.892	98.98 \pm 0.992
% RSD	0.875	1.002
Precision		
<i>Intra-day precision</i>		
Mean \pm SD* (n = 9)	99.84 \pm 0.765	100.84 \pm 0.674
<i>Inter-day precision</i>		
Mean \pm SD* (n = 9)	100.89 \pm 0.576	101.99 \pm 1.196
LOD		
(µg/band)	4.97	2.87
LOQ (µg/mL)		
	9.94	4.98
Robustness		
Mean \pm SD* (n=9)	101.23 \pm 0.811	99.74 \pm 0.547

*Standard deviation

Specificity and selectivity

The method was specific, since no interference of any excipients or formulation matrix was observed in the determination of analytes. Therefore, it can be applied in the determination of DAPA and SAX in marketed pharmaceutical formulations. This method was also evaluated for the analysis of in-house mixtures having different amounts of DAPA and SAX. Results with satisfactory % RSD confirmed the selectivity of the proposed procedure (Table 2).

Sensitivity

The sensitivity of the procedure was studied with LOD and LOQ, and their levels established that its sensitivity was acceptable.

Robustness

This was confirmed using minor alterations in settings of the method, which resulted in effects smaller than 1% RSD (Table 1).

UPLC method

The US-FDA guidelines were adopted for the validation of UPLC method.

Table 2: Application of the proposed TLC-densitometric method for the determination of DAPA and SAX in laboratory-prepared mixtures

Parameter	DAPA	SAX
Claimed concentration of DAPA/SAX mixture	10 mg	5 mg
Mean \pm SD* (n=3)%	102.52 \pm 0.997	98.99 \pm 0.8320
RSD	0.972	840
Claimed concentration of DAPA/SAX mixture	5 mg	5 mg
Mean \pm SD* (n=3)	100.86 \pm 0.542	101.83 \pm 1.234
% RSD	0.537	1.211
Claimed concentration of DAPA/SAX mixture	10 mg	20 mg
Mean \pm SD* (n=3)	100.56 \pm 0.682	98.66 \pm 0.764
% RSD	0.678	0.774

*Standard deviation

Linearity

The peak area ratio of each studied drug was used to plot the calibration curve. Linearity of the procedure was recognized by an 8-point calibration curve covering concentrations of 100 - 1000 ng/mL for DAPA, and 20 - 200 ng/mL for SAX. The respective regression equations are shown in Equations 5 and 6 for DAPA and SAX, respectively.

$$PARDAPA = 0.002C - 0.011 \quad (r^2 = 0.9983) \dots (5)$$

Table 3: Summary of accuracy and precision (inter-day and intra-day) for the proposed UPLC-method

Parameter		DAPA	SAX	
Day 1	LLOQ^a	%Mean \pm SD* (n=6)	102.48 \pm 1.459	101.88 \pm 1.341
		% RSD	1.424	1.316
	LOQ^b	%Mean \pm SD* (n=6)	101.69 \pm 1.231	99.18 \pm 0.769
		% RSD	1.211	0.775
	MQC^c	%Mean \pm SD* (n=6)	97.98 \pm 1.112	101.54 \pm 1.872
		% RSD	1.134	1.843
HQC^d	%Mean \pm SD* (n=6)	98.46 \pm 0.986	99.12 \pm 1.873	
	% RSD	1.001	1.890	
Day 2	LLOQ^a	%Mean \pm SD* (n=6)	101.56 \pm 1.119	102.12 \pm 1.241
		% RSD	1.101	1.215
	LOQ^b	%Mean \pm SD* (n=6)	98.56 \pm 0.765	99.57 \pm 1.855
		% RSD	0.776	1.863
	MQC^c	%Mean \pm SD* (n=6)	101.65 \pm 1.398	101.48 \pm 1.439
		% RSD	1.375	1.418
HQC^d	%Mean \pm SD* (n=6)	103.12 \pm 1.457	102.99 \pm 1.873	
	% RSD	1.413	1.819	
Day 3	LLOQ^a	%Mean \pm SD* (n=6)	99.39 \pm 0.896	99.36 \pm 0.876
		% RSD	0.901	0.881
	LOQ^b	%Mean \pm SD* (n=6)	103.12 \pm 1.652	102.89 \pm 1.824
		% RSD	1.602	1.773
	MQC^c	%Mean \pm SD* (n=6)	99.62 \pm 1.254	100.95 \pm 0.549
		% RSD	1.259	0.544
HQC^d	%Mean \pm SD* (n=6)	102.78 \pm 1.113	102.45 \pm 1.923	
	% RSD	1.083	1.877	

*Standard deviation; ^a100 ng/mL DAPA and 20 ng/mL SAX; ^b200 ng/mL DAPA and 40 ng/mL SAX; ^c500 ng/mL DAPA and 100 ng/mL SAX; ^d800 ng/mL DAPA and 180 ng/mL SAX

$$PARSAX = 0.010C - 0.006 \quad (r^2 = 0.9988) \dots (6)$$

where *PAR* represents peak area ratio and *r*² represents correlation coefficient. The high correlation coefficient values validated the linearity of the method.

Accuracy and precision

Six LLOQ, LQC, MQC, and HQC replicate samples were analyzed in the determination of accuracy and precision. The outcomes are displayed in Table 3.

Sensitivity

The LOD and LOQ were determined in blood samples, and it was found that 100 ng/mL of DAPA and 20 ng/mL of SAX were detected in all calibrators and consistently gave acceptable precision values when back-calculated. The signal-to-noise ratio at LLOQ was consistently more than 3. The calculated corresponding LOQs of DAPA and SAX were 100 ng/mL and 20 ng/mL, respectively.

Specificity

Matrix effect was determined to validate the specificity of the developed procedure. There

Table 4: Extraction recovery from plasma samples

Concentration	DAPA		SAX	
	% Recovery*	% RSD	% Recovery*	% RSD
LQC ^a	89.56	1.312	84.13	1.191
MQC ^b	88.34	0.823	85.43	1.135
HQC ^c	87.81	0.578	89.23	0.432

*Average of six readings; ^a200 ng/mL DAPA and 40 ng/mL SAX; ^b500 ng/mL DAPA and 100 ng/mL SAX; ^c800 ng/mL DAPA and 180 ng/mL SAX

Table 5: Stability data for DAPA and SAX in human plasma

Storage condition	DAPA			SAX		
	LOQ (200 ng/mL)	MQC (500 ng/mL)	HQC (800 ng/mL)	LOQ (40 ng/mL)	MQC (100 ng/mL)	HQC (180 ng/mL)
Bench-top stability	97.49 ±	98.94 ±	103.55 ±	98.86 ± 0.832	96.64 ±	102.46 ±
% Mean ± SD* (n = 6)	0.981	0.752	0.965		0.753	0.476
Freeze-thaw stability	103 ± 0.854	98.36 ±	104.43 ±	102.874 ±	97.13 ±	103.43 ±
% Mean ± SD* (n = 6)		1.125	0.915	0.549	1.094	0.913
Long term stability	97.78 ±	97.78 ±	102.99 ±	95.89 ± 1.876	98.98 ±	101.98 ±
% Mean ± SD* (n = 6)	1.387	0.987	1.298		1.732	1.274

*Standard deviation

was no interference from the biological matrix on the studied drugs. The calculated average matrix effects for DAPA and SAX were 105.50 and 1094.89 %, respectively.

Recovery

The recovery of DAPA and SAX were assessed by matching the average peak areas of six LQC, MQC and HQC replicate samples with corresponding average peak areas of unextracted samples. The results were within acceptable limits, as presented in Table 4.

Stability

This was investigated by means of 6 replicates of LQC, MQC and HQC samples, over 24 h at room temperature (bench-top stability). The concentrations were then matched with those of freshly prepared and processed samples, and the results suggested the stability of DAPA and SAX in plasma when stored at room temperature for 24 h. The stability was also tested for three consecutive freeze-thaw cycles. Moreover, long term stability was evaluated for 30 days at -28°C for same quality control concentration levels. All the acquired data indicated stability of the drugs, as shown in Table 5.

Use of TLC densitometric procedure for analyzing Qtern® tablets

In order to ensure content uniformity in Qtern® tablets, DAPA and SAX were quantified using the proposed method (Table 6). The pharmaceutical products showed satisfactory recoveries, within adequate content uniformity limits. The

consistency and applicability of the proposed method were certified by adding various known amounts of pure DAPA and SAX into the formulation, by standard addition method. The results showed significant recoveries of added drugs using the suggested method (Table 6).

Table 6: Determination of DAPA and SAX in Qtern® tablets and evaluation of recovery of added standard using the new UPLC procedure

Compound	Uniformity	Added standard
DAPA	Mean±SD* % RSD	102.67±1.764 1.718
SAX	Mean±SD* % RSD	101.89±1.231 1.208

*Standard deviation, average of three determinations

DISCUSSION

In this work, two sensitive and accurate analytical methods were adopted to simultaneously quantify DAPA and SAX in their dosage form (TLC-densitometric method) and in human plasma (UPLC-method). In the TLC-densitometric method, adequate separation of the studied drugs was done with a solvent system consisting of CHCl₃, CH₃COOC₂H₅ and CH₃OH at a volume ratio of 8:1:1. The retardation factor (R_f) for SAX and DAPA were found to be 0.17 and 0.31, respectively. This method has advantage in that it can be used to run several samples at the same time, making it very suitable for application in the quality control laboratories. The cited method was validated according to the ICH-Q2B guidelines regarding linearity, accuracy, precision, selectivity, sensitivity and robustness.

Moreover, a UPLC method was developed and optimized for excellent separation pattern of DAPA and SAX. This was done using Waters™ ACQUITY UPLC BEH C18 Column as stationary phase, and a mixture of potassium dihydrogen phosphate buffer (0.02 M; adjusted with o-phosphoric acid to pH 4), methanol and acetonitrile at a volume ratio of 2:1:1 as mobile phase (Figure 2). The UPLC technique has the advantage of using ultra-small size of the packing material to ensure excellent resolution of the studied drugs. The proposed procedure was fully validated according to the US-FDA standards regarding accuracy, linearity, precision, sensitivity, specificity, recovery and stability. The validation scheme showed good results as demonstrated in Tables 3, 4 and 5. The method can be best used in monitoring of these drugs in pharmacokinetic and bioequivalence studies.

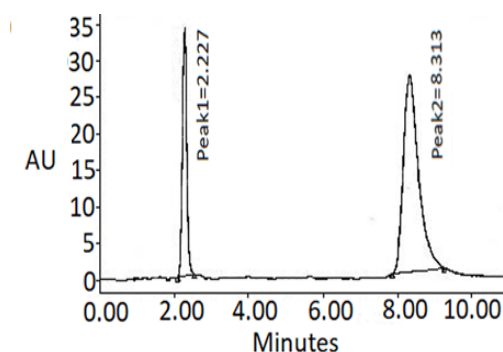


Figure 2: Typical UPLC profiles of DAPA (peak 2) and SAX (peak 1)

CONCLUSION

The results of this study indicate that the coupled TLC-densitometric method was well optimized, validated and can be used for simple, concurrent and accurate quantification of DAPA and SAX, either in bulk or dosage form. The merit of spectrodensitometry lies in its capacity to run multiple samples at the same time on a single plate, thereby reducing time and solvent volume per sample. Thus, this method can be utilized for regular analysis of formulations of DAPA and SAX. Moreover, a UPLC method for the sensitive and accurate measurement of these drugs in blood has been developed. The validation data of the proposed method, based on the US-FDA regulations, indicate that the method is associated with high accuracy, precision, sensitivity, selectivity, and stability. Thus, this method can be utilized for the sensitive monitoring of DAPA and SAX in pharmacokinetic and bioequivalence studies.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claim relating to the content of this article will be borne by the authors.

Data availability

All the data associated with this research have been included in the manuscript.

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