Original Research Article

Signal processing techniques for the spectrophotometric quantitation of binary mixture of dapagliflozin and saxagliptin: A comparative study

Sherif A Abdel-Gawad[1,2]*, Hany H Arab[3], Said A Hassan[2]

[1]Pharmaceutical Chemistry Department, College of Pharmacy, Prince Sattam Bin-Abdul Aziz University, Al-Kharj, 11942, Kingdom of Saudi Arabia, [2]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, ET-11562, Egypt, [3]Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, PO Box 11099, Taif 21944, Saudi Arabia

*For correspondence: **Email:** sagawad@yahoo.com; **Tel:** 00966 540586921

Abstract

Purpose: To investigate the advantages and drawbacks of four signal processing methods for spectrophotometric quantitation of mixtures of dapagliflozin and saxagliptin.
Methods: The methods studied were numerical differentiation (ND), Savitzky-Golay filter (SG), discrete Fourier transform (DFT) and continuous wavelet transform (CWT). The resolution powers of the methods were compared via quantitative determination of dapagliflozin (DAP) and saxagliptin (SAX) in laboratory prepared mixtures. Furthermore, a new approach for validating robustness in spectrophotometric methods was developed, and the methods were compared using their robustness.
Results: Continuous wavelet transform (CWT) produced the best results regarding the analysis of the two drugs in different ratios. It also showed a lower limit of quantification (LOQ), when compared to each of the other methods. When the four methods were used for quantitation of pharmaceutical drug formulations, and subjected to validation in line with ICH regulations, they were found to be satisfactorily specific, accurate, precise and robust.
Conclusion: These results show that CWT technique is superior to the other three methods for analysis of drug mixtures with regard to sensitivity and resolving power. Thus, CWT can be used in the routine spectrophotometric analysis of pharmaceuticals in quality control laboratories.

Keywords: Dapagliflozin, Derivative spectrophotometry, Fourier transform, Numerical differentiation, Savitzky-Golay, Saxagliptin, Wavelet transform

INTRODUCTION

Derivative spectrophotometry (DS) is a well-established analytical technique for processing of UV-VIS spectra. The derivative calculation is used to remove background interference, resolve spectra, and perform quantitative analysis [1].

Derivative spectrophotometry has a significant impact on resolving overlapping UV-VIS spectra [2,3]. The usual technique for simultaneous quantitation of single or multi-component mixtures using DS is the zero-crossing method [4,5]. In spectral analysis, the use of traditional DS for absorption spectra is associated with

numerous drawbacks such as decrease in peak intensity, and the additional smooth function and scaling factor processes required [6]. As a result, other signal processing methods have emerged as strong substitutes for derivative calculations like wavelet transform, Fourier transform, and mean centering.

Wahbi *et al* [7] applied Fourier functions for the spectrophotometric quantitation of mixtures, and the values of the combined trigonometric Fourier functions were tabulated and used for calculation of discrete Fourier transform (DFT). This transform was utilized in processing raw and ratio spectra for resolution of binary and ternary blends [8,9]. In addition, wavelet transform was developed to decompose a signal into uncomplicated, and set building units at various scales and locations [10]. Continuous wavelet transform (CWT) has been applied in UV-VIS for the simultaneous quantitation of compounds in binary and ternary mixtures [11-14]. Finally, mean centering has been established as a processing procedure for ratio spectra by Afkhami and Bahram [15], and it has been successfully applied for the spectrophotometric analysis of binary and ternary mixtures [16,17].

Dapagliflozin propanediol monohydrate (DAP; Figure 1) is a sodium–glucose cotransporter 2 inhibitor, while saxagliptin hydrochloride (SAX) is a dipeptidyl peptidase-4 inhibitor [18]. The product QTERN® is a combination of DAP and SAX which is indicated to enhance glycemic control in patients with type 2 diabetes mellitus [18]. Many methods have been reported for the quantitation of DAP and SAX such as HPLC [19-21], LC-MS [22] and spectrophotometry [23], while no signal processing method has been developed for the determination of this mixture.
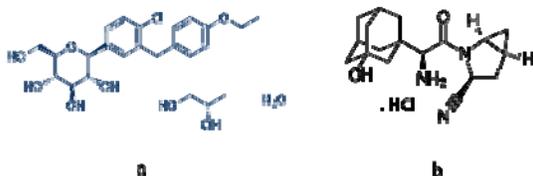
Figure 1: Structural formulae for (a) dapagliflozin and (b) saxagliptin

The aim of this work was to compare different techniques applied for processing UV-VIS spectra, such as ND, SG, DFT and CWT. A binary mixture of DAP and SAX was used as a model for overlapped spectra to facilitate this comparison. The objective was also to develop a fast and simple spectrophotometric method for the analysis of the drugs in their pharmaceutical formulation.

EXPERIMENTAL

Chemicals and materials

Pure DAP (CAS Reg. No. 461432-26-8) and SAX (CAS Reg. No. 709031-78-7) bulk powders were purchased from Cayman Chemical Company, Ann Arbor, United States of America (certified purity: 99.9 %). Distilled water was bought from "Aquatron" Automotive Water Still A 4000 [Bibby Sterillin Ltd., Staffordshire-UK).

The equipment comprised an IBM-compatible computer linked to HP 680 inkjet printer (Hewlett Packard, USA) connected to UV-VIS spectrophotometer (JASCO, Japan).

Software

Microsoft Excel® 2019 was used for ND and DFT calculations, while SG and CWT were calculated with Matlab® ver 7.12.

Pharmaceutical formulations

QTERN® film coated tablets (NDC 0310-6780-30) containing 10 mg dapagliflozin and 5 mg saxagliptin were manufactured by AstraZeneca Pharmaceuticals LP, Willington, DE 19850.

Standard solutions

Stock standard solutions of DAP and SAX (1 mg/mL) were prepared by accurately weighing and transferring bulk powders of DAP (50 mg) and SAX (50 mg) separately into two 100-mL volumetric flasks. Each drug was dissolved in 50 mL distilled water using a vortex mixer, and the solutions in the flasks were made up to volume using the same solvent. With distilled H_2O as diluent, a working standard solution of each drug (100 µg/mL) was prepared.

Procedures

Spectral characterization of dapagliflozin and saxagliptin

The zero-order spectra of DAP and SAX at concentrations of 20 and 10 µg/mL, respectively, were scanned, with distilled H_2O as blank, in wavelength spanning 200 - 300 nm.

Construction of standard curves

Volumes of standard solutions of DAP and SAX (100 µg/mL) corresponding to 20 – 250 µg (DAP) or 70 - 400 µg (SAX) were accurately and separately transferred into a series of 10-mL volumetric flasks and made up to volume with

distilled water. The absorption spectra were scanned, with distilled H_2O as blank.

Numerical differentiation

Second derivative spectra were computed with Δλ of 8 nm and scaling factor of 1000. Calibration curves were obtained by plotting peak amplitudes at 274.2 and 225.6 nm against the respective concentrations of DAP and SAX.

Savitzky-Golay filter

The first derivative spectra were calculated for the two drugs using SG filter of window width (i) = 21, scaling factor = 100 and cubic polynomial. Amplitude at 288.8 nm was used for DAP determination, while SAX was quantified by measuring the amplitude at 224.2 nm.

Discrete Fourier Transform (FT)

The FT of both drugs was got via 6 points, T' = [Cos x+ Cos (x+60)], pooled Fourier function, and the coefficients were computed based on the corresponding absorbance values, with 4 nm interval over a span of 206-300 nm. Calibration curves were created by plotting coefficient of DAP at λ_m = 283.0 nm, and SAX at λ_m = 234.0 nm, against drug concentration.

Continuous Wavelet Transform

The CWT spectra of both DAP and SAX were calculated using Gaussian-3 wavelet family (gaus-3) with scale = 50. Plots of the amplitudes at 233.8 and 225.0 nm were made against concentrations of DAP and SAX, respectively.

Use of the signal processing procedures for quantitation of DAP and SAX in laboratory-prepared mixtures

Different volumes of DAP and SAX were mixed in 10-mL volumetric flasks. The formed mixtures contained diverse ratios of the two drugs. Then, the concentrations of DAP and SAX contained in the mixtures were calculated as indicated earlier.

### Use of the new methods for quantitation of DAP and SAX in QTERN® tablets

The QTERN® film coated tablets (n = 10) were finely powdered and an amount of powder equivalent to 20 mg DAP/10 mg SAX was stirred for about 20 minutes with 50 ml of distilled H_2O in a 250-mL beaker. The resultant solution was subjected to filtration into 100-mL volumetric flask. Thereafter, the solution was made up to volume with distilled H_2O. Then, 1 mL was taken

up into a new 10 mL measuring flask and made up to mark with distilled water, followed by spectra scanning and computation of concentrations of DAP and SAX as outlined before.

RESULTS

UV-VIS spectrophotometry is a technique well-known for its speed, simplicity, and accuracy in quantitation of components of a mixture form without prior resolution. Interference in spectra can be easily eliminated using several mathematical techniques, the most common of which is signal processing. Signal processing techniques eliminate the interference via spectral transformation into novel forms showing zero crossing points.
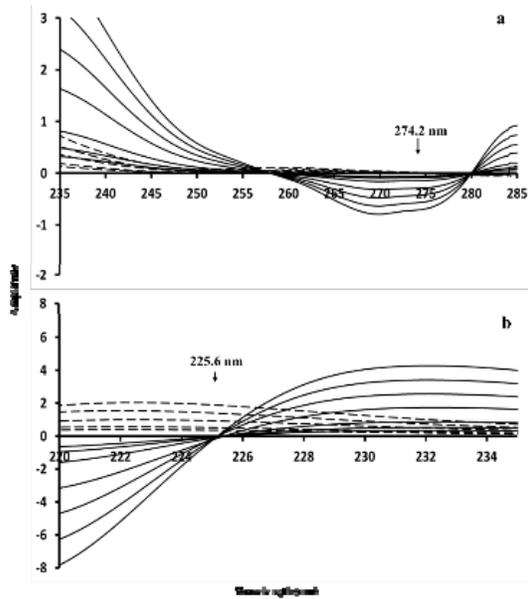
The objective of this work was to do a comparative study on four common signal processing techniques, so as to reveal the advantages and weaknesses of these techniques in analysis of binary mixtures. A binary mixture of DAP and SAX was chosen for demonstration of this comparison. As shown in Figure 2, the spectra of DAP and SAX overlapped in the range 200 - 240 nm, and absorptivity of DAP was higher than that SAX. These features deter the determination of SAX, because its spectrum is completely enclosed within that of DAP. The difficulty in SAX determination makes this combination an ideal mixture for carrying out the comparative study. Being the component with lower absorptivity, less extended spectrum and lower ratio in the dosage form, SAX recovery will be the critical factor that indicates the resolving power of the applied algorithms.
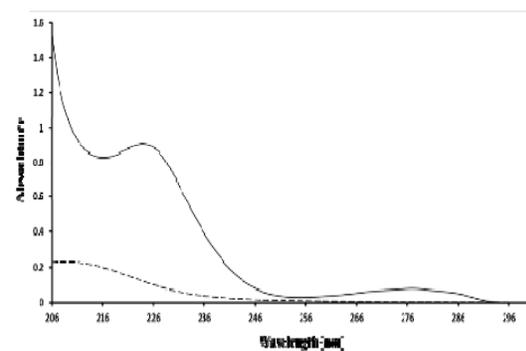
Figure 2: Zero-order absorption spectra of DAP (20 µg/ml; ——) and SAX (10 µg/ml SAX; - - -) against distilled H_2O

In ND method, the zero-crossing points (ZCPs) obtained in first order derivative did not show good recoveries with laboratory prepared mixtures. Therefore, the second derivative was

calculated, resulting in ZCPs at 274.2 and 225.6 nm for DAP and SAX, respectively Figure 3). For optimization of the derivative spectra, different Δλ (4 and 8) with scaling factors of 10, 10^2 and 10^3 were applied. Scaling factor of 1000 and Δλ = 8 were the best parameters, regarding linearity, spectral shapes, and recovery.

and with various wavelength differences (2, 4, 6 and 8 nm). The best parameters were six points combined Fourier function at 4 nm interval in 206 - 300 nm span. Fourier transform with the aforementioned parameters showed zero-crossing points at λ_m = 283.0 and 234.0 nm for DAP and SAX, respectively (Figure 5).

Figure 3: Second derivative (ND) of DAP (2-25 µg/ml; ——) and SAX (7-40 µg/ml; - - -) depicting ZCPs for DAP (a) and SAX (b)

Figure 4: First derivative (SG) of DAP (2-25 µg/ml; ——) and SAX (7-40 µg/ml; - - -) depicting ZCPs for DAP (a) and SAX (b)

For SG method, ZCPs were obtained in first order derivative at 288.8 and 224.2 nm for DAP and SAX, respectively (Figure 4). Several polynomials were applied, and no significant differences were observed between them. Cubic polynomial was used for calculation of the derivative. Several window widths (i) were applied, and the best width was 21 with a scaling factor of 100.
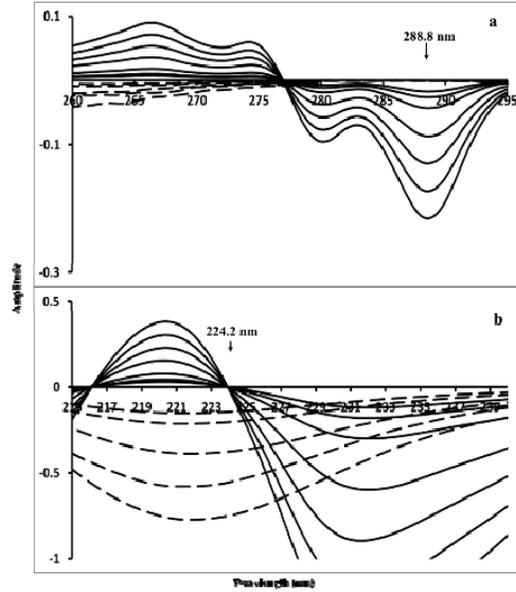
For DFT method, the parameters associated with calculation of Fourier coefficients (FCs) were modified. Sine (Sin) and cosine (cos) functions were examined, and where they were comparable, cos was chosen for calculation of the Fourier transforms. Values of FC, t^r_{rj}, were calculated from absorbance readings at 6 and 8 points, respectively, as shown in Eqs 1 and 2.

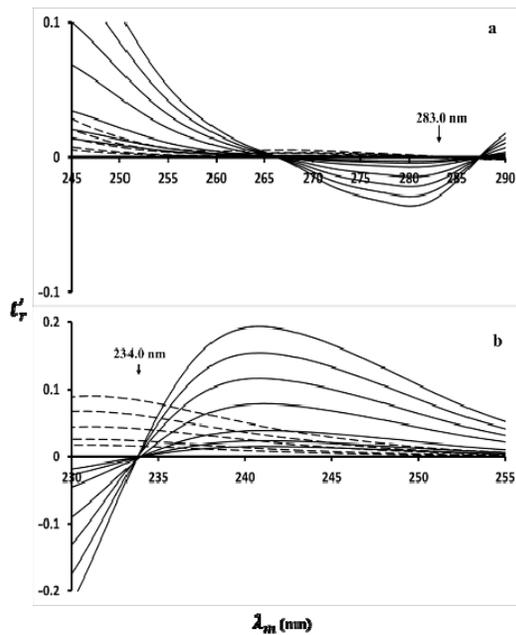$$T' = [\cos x + \cos (x + 60°)], \quad .... (1)$$

and at 8 points,

$$T' = [\cos x + \cos (x + 45°)] \quad .... (2)$$

Figure 5: Convoluted spectra (DFT) of DAP (2 - 25 µg/ml; ——) and SAX (7- 40 µg/ml; - - -) showing ZCPs for DAP (a) and SAX (b)

In CWT method, and to obtain zero-crossing for the two drugs, various wavelet families were applied: Daubechies (db), Coiflets (coif), Symlets (sym), Mexican hat (mexh), Meyer (meyr), Morlet (morl) and Gaussian (gaus). These were tested in their various orders and under changing scaling parameters. Zero crossing points were obtained with many families. The best outcome as regards linearity and recovery were obtained at 233.8 and 225.0 nm for DAP and SAX, respectively, with CWT calculated using gaus-3 family with scale = 50 (Figure 6).
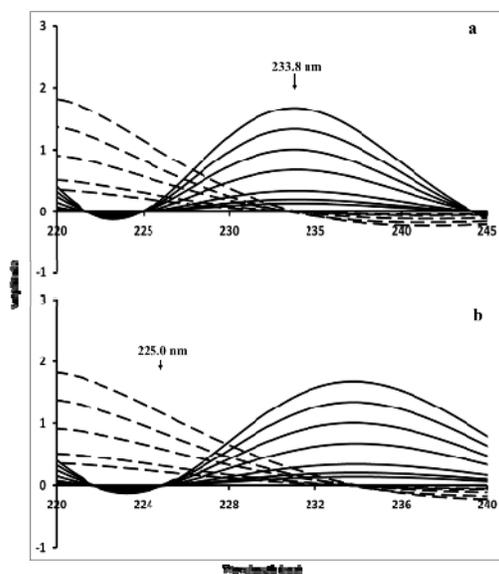
Figure 6: Continuous Wavelet Transform (gaus-3) of DAP (2-25 µg/ml; —) and SAX (7-40 µg/ml; - - -) depicting ZCPs for DAP (a) and SAX (b)

The resolution power of the compared algorithms was evaluated via quantitation of mixtures of DAP and SAX at various ratios (Table 1). The four methods were validated according to ICH guideline Q2(R1) [24]. The validation parameters are shown in Table 2. The robustness of each method was investigated by measuring the absorbance at wavelengths near the zero-crossing point (± nm). Different ranges were observed for each method (Table 3). The new procedures were used for the analysis of DAP and SAX in QTERN® tablets. The results are shown in Table 4.

DISCUSSION

As mentioned earlier, SAX was used for the comparison because it was the drug that indicated differences amongst the applied algorithms. The linearities of the studied algorithms were compared through their LOQs, which w calculated despite not being a validation requirement by ICH for active constituents [24]. As shown in Table 2, both DS methods (ND and SG) showed the highest LOQ for SAX (10 µg/mL), and DFT showed slightly better LOQ (8 µg/mL), while CWT gave the lowest LOQ (7 µg/mL). This might be due to the finding that derivative calculation diminishes S/N, when compared to other signal processing techniques [6]. While DFT does not degrade the signal by derivatization, CWT is associated with the merit of signal amplification, in contrast to the other three algorithms. In DS, when the first order derivative is ineffective in resolving overlapped spectra, higher order derivatives are used which reduce S/N drastically. In the drug mixture, the 1st derivative in ND was ineffective in introducing zero-crossing points for the two drugs. Therefore, the 2nd order was calculated. In contrast, SG gave zero crossing points for both drugs in the 1st order. This influenced the scaling factor used for each derivative, where 2nd order ND required a scaling factor of 1000, and the 1st order SG required only 100 as scaling factor.

Table 1: Analysis of DAP and SAX in laboratory mixtures using the proposed procedures

Mix. no.	Concentration (µg/mL)		ND		SG		DFT		CWT	
			Recovery (%) [a]							
	DAP	SAX	DAP	SAX	DAP	SAX	DAP	SAX	DAP	SAX
1	2	10	97.33	100.69	101.28	99.74	97.29	101.40	101.87	101.00
2	3	30	98.22	99.27	102.2	100.77	99.79	100.11	100.42	99.64
3	5	10	100.12	99.38	101.01	98.44	99.36	102.15	101.44	101.38
4	10	20	97.74	100.58	99	100.6	98.60	101.35	101.54	99.07
5	20	10	97.27	101.5	97.9	99.17	97.49	102.68	101.26	100.54
6	16	8	100.62	--	99.31	--	99.99	99.03	101.77	98.46
7	14	7	99.58	--	97.65	--	97.47	--	100.29	101.65
Mean ± SD			*98.70±1.39*	*100.28±0.94*	*99.77±1.76*	*99.74±0.98*	*98.57±1.16*	*101.12±1.34*	*101.23±0.63*	*100.25±1.22*

[a]Mean of three measurements

Table 2: Validation sheet of the proposed procedures for the simultaneous determination of the drugs in binary mixture

Parameter	ND		SG		DFT		CWT	
	DAP	*SAX*	*DAP*	*SAX*	*DAP*	*SAX*	*DAP*	*SAX*
Accuracy [a]	100.44	98.98	99.19	99.47	98.8	99.96	100.77	99.43
Specificity [b]	98.7±1.39	100.28±0.94	99.77±1.76	99.74±0.98	98.57±1.16	101.12±1.34	101.23±0.63	100.25±1.22
Precision								
Repeatability [c]	0.69	0.71	1.00	1.23	0.72	1.04	0.29	0.60
Intermediate precision [d]	1.38	1.31	1.36	1.52	1.41	1.46	0.69	1.56
Robustness [e]	1.63	1.85	0.76	1.56	1.20	1.30	1.95	1.13
(nm interval)	(± 0.6)	(± 0.2)	(± 0.8)	(± 0.2)	(± 0.6)	(± 0.2)	(± 0.8)	(± 0.4)
LOQ [f]	1.97	9.92	1.99	9.98	2.03	8.11	1.98	7.04
Linearity								
Slope	-0.0282	0.0452	-0.0086	-0.0183	-0.0012	0.0020	0.0669	0.0287
Intercept	-0.0023	0.0333	-0.0002	-0.0139	-0.0002	0.0016	0.0029	0.0185
Correlation coefficient (r)	0.9999	0.9992	0.9999	0.9993	0.9999	0.9993	0.9999	0.9991
Range (µg/ml)	2-25	10-40	2-25	10-40	2-25	8-40	2-25	7-40

[a] Accuracy (n=3), mean recovery of 3 concentrations of DAP (5, 10, 15 µg/mL) and SAX (10, 20, 30 µg/mL). [b] Specificity of the methods (mean ±SD) measured in drug mixtures. [c] Intra-day (n = 3) RSD of 3 concentrations of DAP (5, 10, 15 µg/mL) and SAX (10, 20, 30 µg/mL) repeated thrice within 24 h. [d] Inter-day (n = 3) RSD of 3 concentrations of DAP (5, 10, 15 µg/mL) and SAX (10, 20, 30 µg/mL) repeated thrice in 3 days. [e] Robustness (n = 3) RSD of recovery calculated at different wavelengths (indicated in parentheses) for 3 concentrations of DAP (5, 10, 15 µg/mL) and SAX (10, 20, 30 µg/mL). [f] LOQ was computed using the following equation: $LOQ = \frac{10\sigma}{S}$ (σ is the residual standard deviation, S is the slope)

Table 3: Robustness of the proposed spectrophotometric methods

Wavelength range (nm)	ND		SG		DFT		CWT	
	RSD (%) [a]							
	DAP	SAX	DAP	SAX	DAP	SAX	DAP	SAX
± 0.2	1.02	1.85	0.45	1.56	0.78	1.30	0.68	0.78
± 0.4	1.34	2.13	0.56	2.45	0.95	2.56	0.92	1.13
± 0.6	1.63	2.67	0.68	3.28	1.20	2.98	1.58	2.04
± 0.8	2.45	3.12	0.76	3.78	2.23	3.24	1.95	2.87

[a] Robustness (n = 3), RSD of the recovery calculated at different wavelengths (indicated in the parentheses) for 3 concentrations of DAP (5, 10, 15 µg/mL) and SAX (10, 20, 30 µg/mL)

Table 4: Analysis of DAP and SAX in QTERN® tablets using the proposed procedures

Drug	ND	SG	DFT	CWT
	Recovery % ± SD [a]			
DAP (20 µg/mL)	98.61±1.19	99.78±0.69	98.00±0.73	100.39±0.86
SAX (10 µg/mL)	100.32±1.08	98.89±0.40	101.89±0.95	101.30±0.72

[a] Mean of three determinations

In ND, the only factor to consider in derivative calculation is Δλ, while in SG the polynomial order is another parameter that needs optimization. This can be used to obtain derivative spectra with different shapes using the SG algorithm. In DFT, the parameters to be optimized are the number of points and the wavelength interval. These parameters can be varied to obtain different shape transforms. In CWT, there are several wavelet families that can be used for transform calculations, and each family has many orders. Moreover, the scale parameter contributes effectively to the shape of the calculated wavelet transforms, since it contracts or dilates the wavelet. The large number of wavelet families and their orders, together with the scale parameter, represent a big source of variation in the shape of spectra calculated using CWT, when compared to limited parameters in other algorithms. This gives CWT the flexibility in producing transformed spectra in different shapes and with higher possibility of zero crossing points than other methods, without the need for calculation of higher order derivatives. A new concept for calculating robustness in zero crossing point spectrophotometric methods was introduced in this study. Since determinations in these methods are done at the zero-crossing point, any variation in the measured wavelength might

severely affect recovery. In this work, robustness was determined by measuring the recoveries at different wavelength ranges from the zero-crossing point (± nm), and % RSD below 2 % was the accepted robustness. This study showed different ranges of flexibility for the four methods investigated. The robustness of DAP showed good % RSD at ± 0.6 nm in ND and DFT, but showed robustness at a wider range (± 0.8 nm) in SG and CWT. For SAX, CWT method was the only method that was robust in the range of ± 0.4 nm, while other methods showed their robustness at ± 0.2 nm only. For this concept of robustness, it is preferable to choose the zero-crossing wavelength at which absorbance of the analyte will not significantly change if the wavelength is slightly changed, i.e., $\Delta A / \Delta \lambda$ is minimum. This can be achieved at a broad horizontal band on the spectrum, in contrast to a steep portion of the spectrum. If the signal processing algorithm shows many different shapes of transformed spectra, this will increase the possibility of finding a zero-crossing point of the interferent that corresponds to a broad portion of the analyte spectrum, thereby increasing the robustness of the method.

In summary, CWT has many advantages over other signal processing methods in spectrophotometric determination of the two drugs. These include: (a) higher flexibility in calculating the transformed spectra via different wavelet families and scale parameters; (b) better S/N ratio and LOQ without the need for calculating higher derivatives; and (c) better robustness by increasing the chances of finding zero-crossing points at a broad portion of the spectrum.

CONCLUSION

Four signal processing methods (ND, SG, DFT and CWT) have been developed and applied successfully for the determination of DAP and SAX in a binary mixture. The findings indicate that CWT shows better sensitivity, resolution power and robustness than any of the other three methods. The methods have been successfully applied for the analysis of the two drugs in their pharmaceutical formulation, suggesting the suitability, validity and applicability of the methods in quality control laboratories lacking liquid chromatographic instruments.

DECLARATIONS

Acknowledgement

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 claims relating to the content of this article will be borne by the authors.

Open Access

REFERENCES

1. Talsky G, Derivative spectrophotometry: low and high order. California: VCH; 1994.

2. Ojeda CB, Rojas FS. Recent applications in derivative ultraviolet/visible absorption spectrophotometry: 2009–2011: a review. Microchem J 2013; 106: 1-16.

3. Rojas FS, Ojeda CB, Pavon JC. Derivative ultraviolet—visible region absorption spectrophotometry and its analytical applications. Talanta 1988; 35: 753-761.

4. Korany M, Wahbi A, Elsayed M, Mandour S. Application of first derivative spectrophotometry to the determination of certain drugs in single component dosage forms. Farmaco Ed Prat 1984; 39: 243-252.

5. Korany MA, El-Din AAS, Abael-salam NA. Application of Sacond Derivative Ultraviolet Spectrometry Part III: Determination of Eugenol, Thymol and Anethole in Volatile Oils. Anal Lett 1984; 17: 483-495.

6. Dinç E, Baleanu D. A new fractional wavelet approach for the simultaneous determination of ampicillin sodium and sulbactam sodium in a binary mixture. Spectrochim Acta A 2006; 63: 631-638.

7. Wahbi A, Abdine H, Korany M. The use of Fourier functions to eliminate interferences in spectrophotometric analysis (theory and application). Pharmazie 1978; 33: 278-282.

8. Hassan SA, Abdel-Gawad SA. Application of wavelet and Fuorier transforms as powerful alternatives for derivative spectrophotometry in analysis of binary mixtures: A

comparative study. Spectrochim Acta A 2018; 191: 365-371.

9. *Youssef RM, Maher HM. A new hybrid double divisor ratio spectra method for the analysis of ternary mixtures. Spectrochim Acta A 2008; 70: 1152-1166.*

10. *Daubechies I, Ten lectures on wavelets. Pennsylvania: SIAM; 1992.*

11. *Dinç E, Baleanu D. A zero-crossing technique for the multidetermination of thiamine HCl and pyridoxine HCl in their mixture by using one-dimensional wavelet transform. J Pharm Biomed Anal 2003; 31: 969-978.*

12. *Dinç E, Özdemir A, Baleanu D. An application of derivative and continuous wavelet transforms to the overlapping ratio spectra for the quantitative multiresolution of a ternary mixture of paracetamol, acetylsalicylic acid and caffeine in tablets. Talanta 2005; 65: 36-47.*

13. *Dinç E, Kanbur M, Baleanu D. Comparative application of wavelet approaches to absorption and ratio spectra for the simultaneous determination of diminazene aceturate and phenazone in veterinary granules for injection. Pharmazie 2005; 60: 892-896.*

14. *Elzanfaly ES, Hassan SA, Salem MY, El-Zeany BA. Continuous Wavelet Transform, a powerful alternative to derivative spectrophotometry in analysis of binary and ternary mixtures: A comparative study. Spectrochim Acta A 2015; 151: 945–955.*

15. *Afkhami A, Bahram M. Mean centering of ratio kinetic profiles as a novel spectrophotometric method for the simultaneous kinetic analysis of binary mixtures. Anal Chim Acta 2004; 526: 211-218.*

16. *Elzanfaly ES, Hassan SA, Salem MY, El-Zeany BA. Different signal processing techniques of ratio spectra for spectrophotometric resolution of binary mixture of bisoprolol and hydrochlorothiazide; a comparative study. Spectrochim Acta A 2015; 140: 334-343.*

17. *Hassan SA, Elzanfaly ES, Salem MY, El-Zeany BA. Mean centering of double divisor ratio spectra, a novel spectrophotometric method for analysis of ternary mixtures. Spectrochim Acta A 2016; 153: 132-142.*

18. *Coppenrath VA, Hydery T. Dapagliflozin/Saxagliptin Fixed-Dose Tablets: A New Sodium-Glucose Cotransporter 2 and Dipeptidyl Peptidase 4 Combination for the Treatment of Type 2 Diabetes. Ann Pharmacother 2018; 52: 78-85.*

19. *Padmaja BR, Sivagami B, Chandrasekar R. A Highly Validated RP-HPLC Method Development for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Tablet Dosage Forms. Int J Pharm Sci Drug Res 2018; 10: 372-378.*

20. *Godge RK, Shinde GS, Joshi S. Simultaneous Estimation and Validation of Dapagliflozin and Saxagliptin in Bulk Drug and Dosage Form by RP-HPLC. Res J Sci Technol 2019; 11: 59-63.*

21. *More SS, Sonawane SS, Chhajed SS, Kshirsagar SJ. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Saxagliptin and Dapagliflozin in Tablets. Asian J Pharm Technol 2018; 8: 145-148.*

22. *El-Zaher AA, Hashem HA, Elkady EF, Allam MA. A validated LC-MS/MS bioanalytical method for the simultaneous determination of dapagliflozin or saxagliptin with metformin in human plasma. Microchem J 2019; 149: 104017.*

23. *Lotfy HM, Mohamed D, Elshahed MS. Novel univariate spectrophotometric determination of the recently released solid dosage form comprising dapagliflozin and saxagliptin via factorized response spectra: Assessment of the average content and dosage unit uniformity of tablets. Spectrochim Acta A 2019:*

24. *Validation of Analytical Procedures: Text and Methodology Q2(R1), in: International Conference on Harmonization (ICH), 2005.*